A unique wheat disease resistance-like gene confers toxin-induced susceptibility to necrotrophic pathogens

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One sentence summary: The wheat *Tsn1* gene, which has protein kinase, nucleotide binding, and leucine-rich repeat domains, confers sensitivity to a proteinaceous host-selective toxin produced by necrotrophic fungal pathogens to cause effector triggered susceptibility in wheat.

Abstract

Plant disease resistance is generally conferred by genes with nucleotide binding and leucine-rich repeat (NB-LRR) or protein kinase (PK) domains. However, little is known regarding the mechanisms of resistance to necrotrophic pathogens. Here, we isolated the wheat *Tsn1* gene, which governs sensitivity to the proteinaceous host-selective toxin ToxA produced by two necrotrophic pathogens. *Tsn1* harbors PK, NB, and LRR domains, all of which are required for ToxA sensitivity. *Tsn1* is unique to ToxA-sensitive genotypes and arose in a diploid progenitor of polyploid wheat through genome shuffling. The Tsn1 protein does not interact directly with ToxA, and *Tsn1* transcription is regulated by the circadian clock and light. This work provides strong evidence that necrotrophic pathogens exploit the resistance mechanisms acquired by plants to combat other pathogens.

Plants have evolved sophisticated innate immune systems to protect themselves from invading pathogens and pests. The (in)direct recognition of pathogen-produced effectors by host resistance (R) genes leads to a resistance response known as effector triggered immunity (ETI). ETI is an active response by the host, and includes undergoing localized programmed cell death (PCD) known as the hypersensitive response (HR) to restrict pathogen growth (1). Most plant R genes belong to the nucleotide binding and leucine-rich repeat (NB-LRR) class of proteins, but some also involve protein kinases (PKs) (2). For example, in the tomato-*Pseudomonas syringae* system, the *Pto* gene (PK) and *Prf* (NB-LRR) are both required for ETI (3). NB-LRRs serve to recognize effectors either through direct interaction or as guards for target molecules and are known to confer resistance to bacteria, viruses, nematodes, oomycetes, insects, and biotrophic fungi. Much less is known about plant interactions with necrotrophic fungal pathogens, and because necrotrophs complete their life cycle on dead tissue, the mechanisms associated with ETI may not be effective against them.

Wheat provides more than 20% of the calories consumed by humans and is prone to substantial yield losses due to disease. *Stagonospora nodorum* and *Pyrenophora tritici-repentis* are devastating necrotrophic fungal pathogens of wheat that cause the diseases Stagonospora nodorum blotch (SNB) and tan spot, respectively, which can cause devastating yield losses (4,5). Both pathogens produce ToxA, a proteinaceous host-selective toxin (HST) (6) that causes necrosis in wheat lines harboring the dominant sensitivity gene *Tsn1* (7). The ToxA gene was laterally transferred from *S. nodorum* to *P. tritici-repentis* prior to 1941, which resulted in tan spot becoming an economically significant disease (8). Compatible *Tsn1*-ToxA interactions, which result in susceptibility

(Fig. 1), require both the toxin and the host gene, and have been shown to account for up to 95% of the variation in disease (9). The absence of either the toxin or the host gene results in an incompatible (resistant) interaction. Therefore, the active response of a plant to a compatible host-toxin interaction results in the opposite of ETI and may instead be referred to as effector triggered susceptibility (ETS). Here, we isolated and characterized the *Tsn1* gene to gain understanding of the mechanisms associated with toxin-induced susceptibility.

We previously reported the development of bacterial artificial chromosome (BAC) (10) contigs flanking *Tsn1* on chromosome 5B and anchored to a high-resolution genetic linkage map (Fig. 2A,B) based on the durum wheat variety Langdon (LDN) (11). Two genes, one with homology to a potassium transporter (PT) and another with homology to a U2 snRNP auxiliary factor (RNP), at the proximal end of the distal contig (ctg548) cosegregated with *Tsn1* in the mapping population. Beginning with marker *Xfcg26(PT)* derived from the PT gene, we performed multiple chromosome walking steps by screening the LDN BAC library, identifying and sequencing selected positive clones, and developing new markers for subsequent walking steps to extend ctg548 approximately 375 kb (Fig. 2C) (12). Marker *Xfcg30(WK95)*, which was developed from the middle of the fourth BAC, mapped 0.02 cM on the proximal side of the *Tsn1* locus, thus indicating the BAC contig spanned *Tsn1*.

The candidate gene region encompassing *Tsn1* spanned 0.11 cM of genetic distance and about 350 kb of physical distance. Sequence analysis indicated the candidate region contained six open reading frames (ORFs) (Fig. 2D). To further narrow the candidate region, we screened 386 *Triticum* accessions for reaction to ToxA and

evaluated haplotypes using markers *Xfcp1* and *Xfcp394* (13) (table S1). Accessions with apparent recombination within the candidate gene region were then evaluated with markers *Xfcp620(WK35)*, *Xfcg32(HP)*, *Xfcp623*, and *Xfcg26(PT)* to determine the relative positions of crossovers. This allowed us to further narrow the candidate gene region to a 120 bp segment containing four ORFs, which included a gene with similarity to PK and NB-LRR genes, along with hypothetical protein (HP), PT, and RNP genes (table S2, Fig. 2D).

To determine which of the candidate genes was *Tsn1*, we treated seed of LDN and the hexaploid wheat variety Bobwhite (BW) with the chemical mutagen ethylmethane sulfonate (EMS) and identified 13 ToxA-insensitive mutants (12). Sequence analysis of the four candidate ORFs of mutant LDNems937 indicated that the PK-NB-LRR-like gene harbored a missense mutation (table S3), whereas the other three ORFs were identical to the wild type. The PK-NB-LRR gene was then sequenced from the remaining 12 mutants along with three mutants previously developed in the hexaploid variety Kulm (12), and the analysis revealed that all 15 harbored mutations consisting of missense, nonsense, and splice site mutations. These results validated that the PK-NB-LRR-like gene was *Tsn1*. The mutations occurred in each of the three major domains indicating that all are essential for *Tsn1* to confer toxin sensitivity.

Tsn1 has eight exons and is 10,581 bp from start to stop codon with a coding sequence of 4,473 and 5' and 3' untranslated regions (UTRs) of 161 and 391 bp, respectively. Approximately 2.8 kb of the fourth intron has 80% similarity to a LINE retrotransposon (RIX_Yvonne_AY146588-1). The predicted protein of 1,490 amino acids harbors three highly conserved domains. The first five exons constitute the PK

domain, the sixth exon encompasses the NB domain, and the seventh exon is made up of LRRs (Fig. 2E). The last exon has no obvious conserved domains, and unlike most monocot NB-LRRs, *Tsn1* does not appear to contain a coiled-coil domain. In addition, *Tsn1* does not contain any apparent transmembrane domains and is therefore likely located in the cytoplasm.

Tsn1 had no significant similarity to any sequence in the NCBI nr database at the nucleotide level. At the amino acid level, the PK and NB-LRR portions of *Tsn1* had the most significant similarity to rice homologs (table S2). Phylogenetic analysis using the amino acid sequences of the PK and NB domains separately was conducted to determine relationships with other known plant genes. We found that the PK domain of *Tsn1* is closely related to those of the barley stem rust resistance gene *Rpg1* (14) and its homologues in monocots and may have a common ancestor with the dicot cysteine-rich receptor-like protein kinase (CRK) subfamily in the serine/threonine-specific protein kinase family (Fig. 3). The NB domain of *Tsn1* had similarity to the maize *Rp3* rust resistance gene (15) and its homologues suggesting that the NB domain of *Tsn1* diverged from the monocot *Rp3*-like genes that may have a common origin with certain dicot NB-LRR genes such as the tomato *I2C* gene (16) (Fig. 3). The barley stem rust R gene, *Rpg5*, is the only other gene reported to date that includes PK, NB, and LRR domains together in the same transcript (17), but *Tsn1* has no significant similarity to any of the domains in *Rpg5*. *Rpg5* also differs from *Tsn1* in that the PK domain is C-terminal relative to the NB and LRR domains rather than N-terminal as it is with *Tsn1*. The PK domain of *Rpg5* clusters with members of the tyrosine-specific protein kinases (TyrK) represented by the tomato *Pto* gene (3) and its NB domain is found in the Arabidopsis *RPM1*/*RPP8* group

(Fig. 3). Therefore, *Tsn1* and *Rpg5* do not appear to share a common ancestry, but both cases likely reflect the result of independent genome shuffling events that led to unique functional structures.

Comparative analysis of the *Tsn1* genomic region of wheat chromosome 5B with rice and *Brachypodium* indicated a conserved level of colinearity with rice chromosome 9 and *Brachypodium* chromosome 4, but *Tsn1* homologs were not present in the colinear segments of either species (table S4, fig. S2). Separate homologs of the PK and NB-LRR regions of *Tsn1* were present on rice and *Brachypodium* chromosomes 11 and 2, where they are separated by 8.5 kb and 2.1 Mb, respectively. Southern and PCR analysis of 24 wheat cultivars indicated that *Tsn1* is specific to ToxA-sensitive genotypes and that a null allele occurs in insensitive lines (fig. S1). This analysis also indicates that copies on wheat homoeologous chromosomes 5A and 5D do not exist, and the sequencing of a chromosome 5A BAC contig confirmed its absence on LDN chromosome 5A (fig. S2). Furthermore, genotyping of 386 *Triticum* accessions with the PCR marker *Xfcp623(Tsn1)* indicated that, with six exceptions, only ToxA-sensitive lines harbored *Tsn1*. We sequenced *Tsn1* from these six lines and found that Novo, Puseas, and Huo Mai all had a nonsense mutation at the same position within the LRR domain, which was the same position as that observed for the EMS-induced mutant Kems37-5 (table S3) indicating that this position is highly vulnerable to mutation. The lines Siu Mak, Ching Feng, and TA2601 all had frameshift mutations at different positions within *Tsn1* (table S3).

The screening of the 386 *Triticum* accessions indicated that *Tsn1* is present in B genome-containing tetraploids and hexaploids (fig. S1). This suggests that *Tsn1* arose in tetraploid wild emmer wheat (*T. turgidum* ssp. *dicoccoides*, AABB genomes), which is

the most primitive of the polyploids evaluated (18), or in the diploid B-genome progenitor, which is thought to be a close relative of *Aegilops speltoides* (SS genome) (19). To determine if *Tsn1* was present in *Ae. speltoides*, we screened 127 accessions for reaction to ToxA (table S5). Two accessions (PI369623 and PI542238) were sensitive to ToxA, and Southern analysis using probes from the LRR (FCG34) and PK (FCG35) domains indicated that both *Ae. speltoides* accessions harbored *Tsn1* homologs (fig. S3). Further amplification and sequencing of *Tsn1* from both accessions and the cDNA from PI542238 indicated they harbor functional copies. However, the 2.8 kb retrotransposon fragment was not present in either accession.

To evaluate the level of nucleotide variation in *Tsn1*, we sequenced the gene from a total of 42 diverse *Tsn1*-containing lines including the two *Ae. speltoides* accessions, bread and durum wheat varieties, and accessions of wild and domesticated emmer. The *Tsn1* alleles of the two *Ae. speltoides* accessions were much more diverse compared to the *Triticum* accessions. Among the *Triticum* accessions, wild emmer accessions were the most diverse with as many as 24 single nucleotide polymorphisms, but nucleotide variation among the durum and bread wheat varieties was nearly non-existent (fig. S4). This result, together with the finding that *Tsn1* homologs are not present in other members of the grass family, suggests that a common ancestor of the grasses likely harbored separate PK and NB-LRR genes that diverged extensively in the different grass lineages before combining as a single functional ORF in the B-genome progenitor of polyploid wheat.

Tsn1 is transcriptionally expressed in the leaves, stems, and immature spikes, but not in roots (Fig. 4A). Compatible *Tsn1*-ToxA interactions are dependent on light, i.e.

Tsn1 genotypes are insensitive to ToxA under darkness (20). Our study indicated that *Tsn1* expression was significantly down- and then up-regulated when plants were exposed to several hours of darkness followed by light, respectively (Fig. 4C). In a separate experiment, we evaluated the levels of *Tsn1* transcription under 12 hr light/dark regimes and under continuous darkness every three hours for three days and found *Tsn1* transcription to be tightly regulated by the circadian clock (Fig. 4B). In addition, transcription of *Tsn1* is not up-regulated by ToxA infiltration, but expression patterns of *Tsn1* in ToxA-infiltrated samples over time mimicked that of samples exposed to continuous dark (Fig. 4B,D). In both cases, expression was down regulated throughout the first 12 hours of the first day, just as it was in the control plants. However, *Tsn1* expression in both the dark-treated and the ToxA-infiltrated plants increased to only half the level of the control plants at 24 hrs, presumably due to rhythmic entrainment. Expression in both cases was again down regulated at 36 hr just as in the control, but little subsequent change was observed beyond this time point where expression levels remained low. There is considerable evidence indicating that the *Tsn1*-ToxA pathway is associated with photosynthetic pathways (22), including the observation that ToxA is located to the chloroplast (20) and directly interacts with plastocyanin (23), a vital component of electron transport in photosystem II. Therefore, it is likely that ToxA confers photosystem alterations, which would affect photosynthesis and lead to disruptions in circadian rhythms and perturbed regulation of *Tsn1* transcription similar to that observed under continuous darkness. These results also provide an explanation for the light dependency of compatible *Tsn1*-ToxA interactions. Others have demonstrated that the HR and defense response associated with other host-pathogen interactions are

also influenced by the circadian clock and light (21). It is possible that the expression of R genes governing ETI in other systems may also be regulated by these factors, but this has not been addressed.

Previous work by others demonstrated that ToxA is imported within the cell in *Tsn1* lines, but not in lines lacking *Tsn1* (20). However, intracellular expression of ToxA resulted in cell death in both *Tsn1* and non-*Tsn1* lines indicating that the intracellular site of action is present in both, and suggesting that *Tsn1* may be the ToxA receptor. We conducted yeast-two hybrid and *in vitro* co-immunoprecipitation experiments (12) to determine if Tsn1 and ToxA interact directly, and the results indicated that they do not (fig. S5). Therefore, *Tsn1* likely acts as a guard to monitor the ToxA receptor or other associated target, and the sensing of ToxA-induced perturbations of the target by *Tsn1* likely triggers events that lead to the importation of ToxA.

The work reports for the first time the cloning and characterization of a gene conferring sensitivity to a proteinaceous HST (ToxA) and hence susceptibility to tan spot and SNB, two of the most economically important foliar diseases of wheat. Both pathogens produce multiple HSTs, all of which interact with single dominant host genes, and these interactions are genetically the inverse of the classic gene-for-gene model (24). The fact that *Tsn1* contains multiple R-gene signatures provides strong evidence that host response mechanisms associated with toxin-driven ETS to necrotrophic pathogens are the same as ETI to other pathogens. Furthermore, other research provides evidence that common signaling pathways are associated with both ETI to biotrophs and ETS to necrotrophs (25) including *P. tritici-repentis* (26,27). Most pathogen effectors cloned to date are small proteins. It is logical to presume that necrotrophic HSTs (virulence

effectors), and avirulence effectors function in the same manner and, when recognized by the cognate sensitivity/R gene, trigger similar responses including PCD. The differences in the outcomes (resistance vs. susceptibility) can be attributed to the biology of the pathogen, i.e. necrotrophic pathogens are equipped to exploit and thrive in an environment generated by the host that would be detrimental to pathogens with biotrophic lifestyles.

Given the prevalence of *Tsn1* and other toxin sensitivity genes in wheat cultivars grown world wide, they may have alternate functions, possibly serving to confer resistance to biotrophic pathogens as in the case of the oat *Pc-2* gene (28), which could provide a means for surviving selection. Breeding for resistance to both may pose a challenge because the acquisition of resistance to one could result in susceptibility to the other.

References and Notes

1. J.L. Dangl, R.A. Deitrich, M.H. Richberg, *Plant Cell* **8**, 1793 (1996).

2. G.B. Martin, A.J. Bogdanove, G. Sessa, *Ann. Rev. Plant Biol*. **54**, 23 (2003).

3. K.F. Pedley, G.B. Martin, *Annu. Rev. Phytopathol*. **41**, 215 (2003).

4. J.E. King, R.J. Cook, S.C. Melville, *Ann. Appl. Biol*. **103**, 345 (1983).

5. R.G. Rees, G.J. Platz, *Aust. J. Agric. Res*. **34**, 39 (1983).

6. G.M. Ballance, L. Lamari, C.C. Bernier, *Physiol. Mol. Plant Pathol*. **35**, 203 (1989).

7. J.D. Faris, J.A. Anderson, L.J. Francl, J.G. Jordahl, *Phytopathology* **86**, 459 (1996).

8. T.L. Friesen *et al.*, *Nat. Genet*. **38**, 953 (2006).

9. J.D. Faris, T.L. Friesen, *Phytopathology* **99**, 906 (2009).

10. A. Cenci *et al*., *Theor. Appl. Genet*. **107**, 931 (2003).

11. H.J. Lu, J.D. Faris, *Funct. Integr. Genomics* **6**, 90 (2006).

12. Information on materials and methods is available on Science Online.

13. Z. Zhang *et al., Mol. Breeding* **23**, 35 (2009).

14. R. Brueggeman *et al., Proc. Natl. Acad. Sci. USA* **99**, 9328 (2002).

15. C.A. Webb *et al., Genetics* **162**, 381 (2002).

16. N. Ori *et al., Plant Cell* **9**, 527, (1997).

17. R. Brueggeman *et al., Proc. Natl. Acad. Sci*. USA **105**, 14970 (2008).

18. Tetraploid wild emmer wheat (*T. turgidum* ssp. *dicoccoides*, 2n=4x=28, AABB genomes) arose through hybridization of two diploid species, *T. urartu* (2n=2x=14, AA genome) and what is thought to be a close relative of *Aegilops speltoides* (2n=2x=14, SS genome). Wild emmer gave rise to domesticated emmer wheat (*T. turgidum* ssp. *dicoccum*, 2n=4x=28, AABB genomes), which was later fully domesticated in the form

of durum (macaroni) wheat (*T. turgidum* ssp. *durum*, 2n=4x=28, AABB genomes). Hexaploid wheat (bread or common wheat; *T. aestivum*, 2n=6x=42, AABBDD genomes) arose under cultivation from a spontaneous hybridization between an AB-tetraploid and the diploid goatgrass *Ae. tauschii* (2n=2x=14, DD genomes).

19. J. Salse *et al., BMC Genomics* **9**, 555 (2008).

20. V.A. Manning, L.M. Ciuffetti, *Plant Cell* **17**, 3203 (2005).

21. L.C. Roden, R.A. Ingle, *Plant Cell* **21**, 2546 (2009).

22. V.A. Manning *et al. Mol. Plant-Microbe Interact*. **22**, 665 (2009).

23. Y.-S. Tai, J. Bragg, S.W. Meinhardt, *Am. J. Plant Physiol*. **2**, 76 (2007).

24. T.L. Friesen, J.D. Faris, P.S. Solomon, R.P. Oliver, *Cell Microbiol*. **10**, 1421 (2008).

25. J.J. Rudd, J. Keon, K.E. Hammond-Kosack, *Plant Physiol*. **147**, 802 (2008).

26. T.B. Adhikari *et al., Mol. Plant-Microbe Interact*. **22**, 1056 (2009).

27. I. Pendelova *et al., Mol. Plant* **2**, 1084 (2009).

28. J.M. Lorang, T.A. Sweat, T.J. Wolpert, *Proc. Natl. Acad. Sci. USA* **104**, 14861 (2007).

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and _, respectively.

Figure Legends

Figure 1. Leaves of Kulm (*Tsn1*) (A, C) and Kems103 (*Tsn1* mutant) (B, D) inoculated with *Stagonospora nodorum* isolate Sn2000 (A and B), and infiltrated with ToxA (C and D).

Figure 2. Map-based cloning of the *Tsn1* gene. A) The genomic region containing the *Tsn1* gene on the long arm of wheat chromosome 5B is shown in red. B) The genetic linkage map of the *Tsn1* region (13). C) BAC-based physical maps of the *Tsn1* region anchored to the genetic map. Langdon BACs previously described (11) are shown in grey. Glenlea and Langdon-derived BACs reported in the current work are shown in green and purple, respectively. Chromosome 5A BACs used in this work are shown in orange. Genetic markers are indicated below the BACs. D) Predicted genes (ovals) and markers (yellow triangles) used for genotyping the 386 *Triticum* accessions (table S1) within the *Tsn1* candidate and flanking regions. The blue and red lines indicate the candidate gene regions as defined by recombination in the mapping population and the haplotype analysis of the 386 *Triticum* accessions, respectively. E) The molecular structure of *Tsn1*. Exons and UTRs are shown in purple and grey, respectively. Blue, red, and orange circles indicate positions of missense, nonsense, and splice mutations in the EMS-induced ToxA-insensitive mutants. Yellow and red triangles indicate positions of naturally occurring frameshift and nonsense mutations, respectively.

Figure 3. Unrooted phylogenetic trees of the protein kinase (A) and nucleotide binding (B) domains of *Tsn1* and other plant disease resistance or defense response-related genes.

Numbers on the branches indicate bootstrapping values (from 1,000 replicates). Species abbreviations: *Arabidopsis thaliana* (At), *Brassica oleracea* (Bo), *Glycine max* (Gm), *Hordeum vulgare* (Hv), *Linum usitatissimum* (Lu), *Lycopersicon esculentum* (Le), *Nicotiana benthamiana* (Nb), *N. glutinosa* (Ng), *N. tabacum* (Nt), *Oryza sativa* (Os), *Phaseolus vulgaris* (Pv), *Solanum tuberosum* (St), *Solanum verrucosum* (Sv), *Sorghum bicolor* (Sb), *Triticum aestivum* (Ta), and *Zea mays (*Zm). Protein names or GenBank accession numbers are given after species abbreviations. The PK and NB domains of *Rpg5* (HvRpg5, GenBank no. ACH69774) are underlined. CRK: cysteine-rich receptor-like protein kinase.

Figure 4. Transcriptional expression of *Tsn1*. A) *Tsn1* expression survey by reverse transcription-PCR in leaves, stems, and roots at seedling stage and in immature spikes. *GAPDH* was used as an endogenous control. B) *Tsn1* expression levels in two-week old plants entrained with a 12 hr light/dark cycle evaluated every three hours over a 72 hr period using relative quantitative (RQ)-PCR. *Tsn1* expression under the 12 hr light/dark cycle is indicated in orange and *Tsn1* expression in plants subjected to continuous dark beginning with the first time point is indicated in green. C) RQ-PCR evaluation of *Tsn1* expression in two-week old plants entrained with a 12 hr light/dark cycle (control; blue bars) and plants subjected to three hours of dark (10:00 am to 1:00 pm) followed by two hours (1:00 pm to 3:00 pm) of light (red bars). (D) RQ-PCR evaluation of *Tsn1* expression in ToxA-challenged plants; blue bars: ToxA infiltrated; red bars: H_2O infiltrated; yellow bars: no infiltration.

Figure 1.

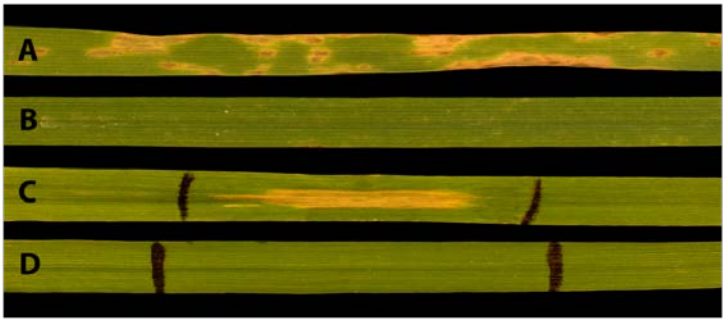
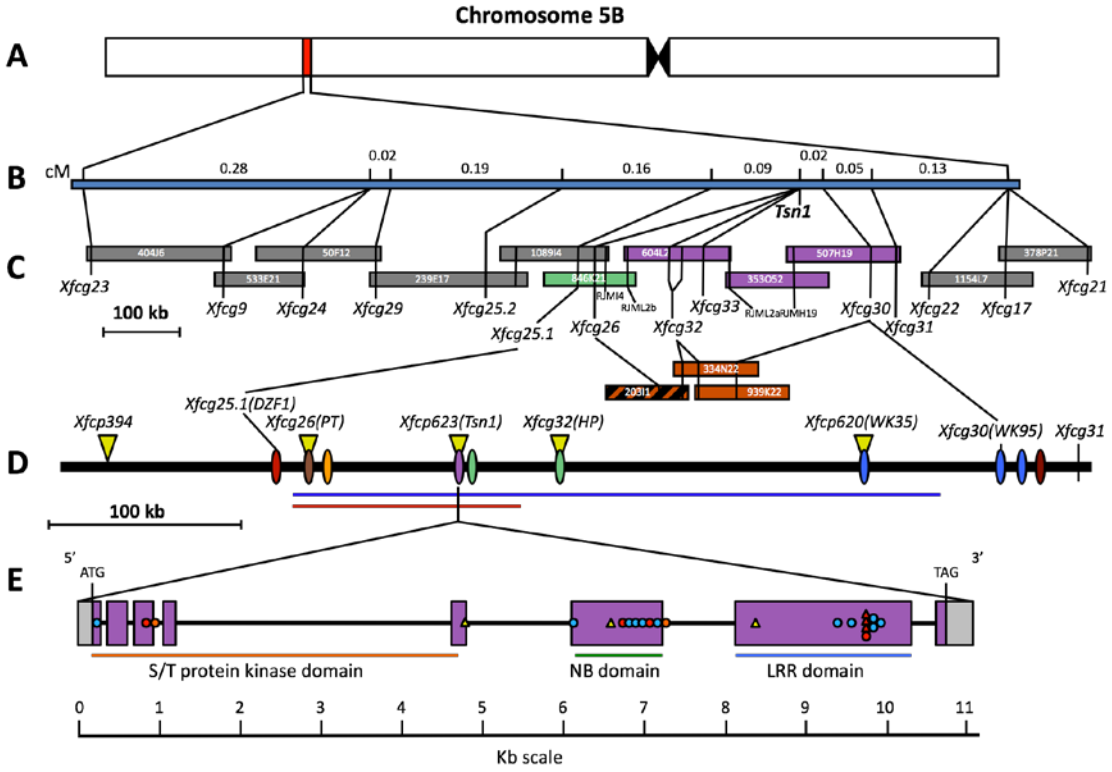


Figure 2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84G22
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Figure 3

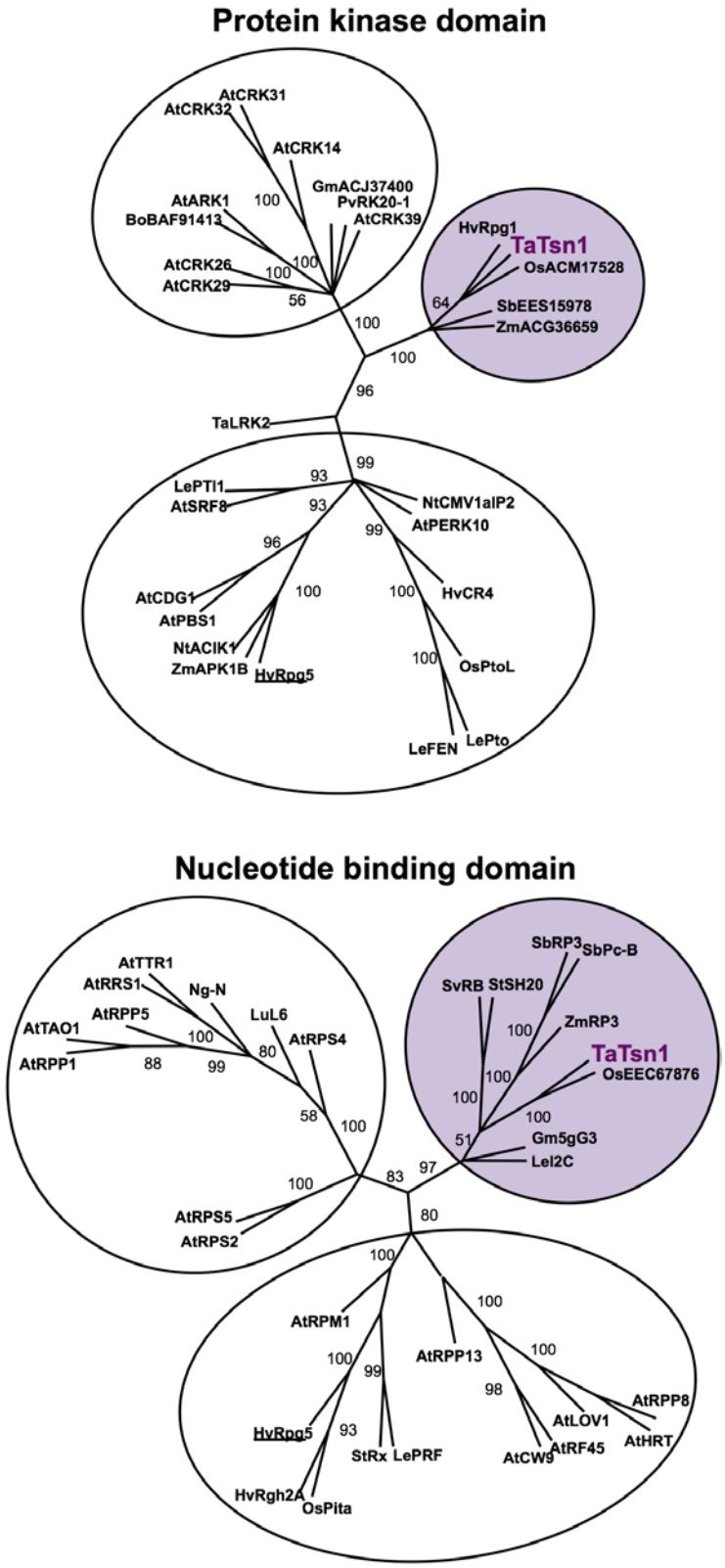
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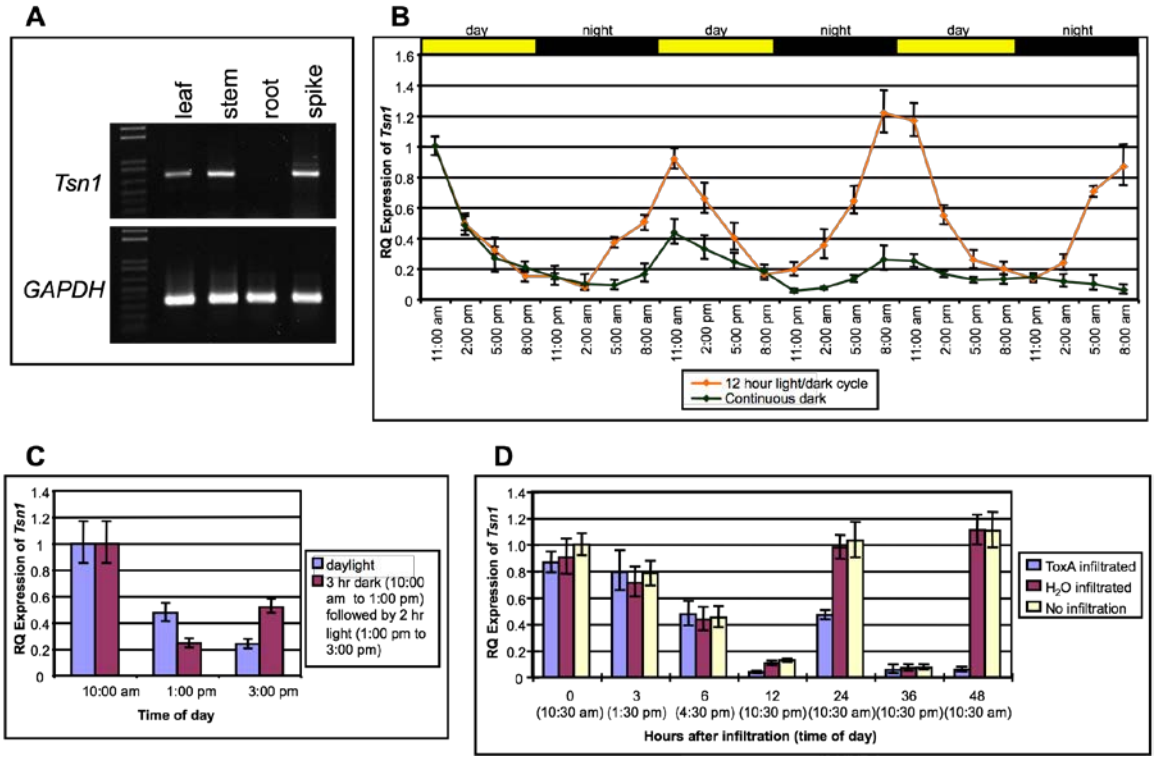
Figure 4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leaf
stem
root
spike
Tsn1
GAPDH
B
day
night
day
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day
night
RQ Expression of Tsn1
12 hour light/dark cycle
Continuous dark
C
RQ Expression of Tsn1
daylight
3 hr dark (10:00 am to 1:00 pm) followed by 2 hr light (1:00 pm to 3:00 pm)
10:00 am
1:00 pm
3:00 pm
Time of day
D
RQ Expression of Tsn1
ToxA infiltrated
H2O infiltrated
No infiltration
0 (10:30 am)
3 (1:30 pm)
6 (4:30 pm)
12 (10:30 pm)
24 (10:30 am)
36 (10:30 pm)
48 (10:30 am)
Hours after infiltration (time of day)

Supporting Online Material

Materials and Methods

Plant Materials: A high-resolution mapping population was developed from a cross between the durum wheat (*Triticum turgidum* ssp. *durum* L., 2n=4x=28, AABB genomes) cultivar Langdon (LDN) and a genetic stock where a pair of *T. turgidum* ssp. *dicoccoides* 5B chromosomes was substituted for LDN 5B chromosomes in the LDN background (LDN-DIC 5B). The population consisted of 2,719 ToxA-insensitive F_2 plants and was used to map *Tsn1* and anchor the BAC contig to the genetic map (1). LDN and the common wheat (*T. aestivum* ssp. *aestivum* L., 2n=6x=42, AABBDD genomes) varieties Kulm and Bobwhite (BW) were used for mutagenesis. A total of 386 tetraploid and hexaploid *Triticum* accessions were used for haplotype analysis to identify recombination within the *Tsn1* genomic region (table S1). A total of 127 accessions of *Aegilops speltoides* were evaluated for reaction to ToxA (table S5).

Chromosome Walking and Physical Mapping: BAC contigs shown in grey in Fig. 2C were previously described (1). To extend the larger distal contig (ctg548), probe FCG26 (table S6) was used to screen the LDN BAC library (2) using the methods described in (3) but no positive clones for chromosome 5B were identified. However, BAC 203I1 was detected and found to belong to the homoeologous region of chromosome 5A based on hybridization fingerprints. Low pass sequencing was performed on BAC 203I1 using the methods described in (3). Sequences were subjected to BLASTx searches of the NCBI database as described in (1), and segment with strong similarity to hypothetical proteins was used to develop probe FCG32 (table S6). *Xfcg32(HP)* cosegregated with *Tsn1* (Fig. 2B, C) and was used to screen the LDN BAC library. This led to the

identification of the chromosome 5B BAC 604L2 and the chromosome 5A BACs 334N22 and 939K22. Probe FCG33 was developed from BAC 604L2 (table S6), found to cosegregate with *Tsn1* on the genetic map (Fig. 2B, C), and used to rescreen the LDN BAC library and extend the 5B contig, but again, no new positive BACs were identified. Therefore, probe FCG30 (table S6) was developed from 334N22 and used to rescreen the library, which resulted in the identification of the 5B BAC 507H19. The mapping of *Xfcg30(WK)* indicated that it mapped 0.02 cM on the proximal side of *Tsn1*, and marker *Xfcg31*, which was developed from the most distal end of 507H19, mapped 0.07 cM proximal to *Tsn1* (Fig. 2B, C). This indicated that the BAC contig spanned the *Tsn1* locus, but two gaps – one between BACs 1089I4 and 604L2 and the other between BACs 604L2 and 507H19 – existed within the 5B contig and were spanned by 5A BACs.

To fill the gaps on the 5B contig, we developed PCR-based repeat junction markers (RJMs) (table S7) from BAC sequences flanking the gaps and obtained the LDN library in the form of pools from Dr. Jorge Dubcovsky, Univeristy of California-Davis. Screening the pools with primer sets RJMH19 and RJML2a resulted in the identification of BAC 353O5 which spanned the gap between BACs 604L2 and 507H19, but no positive BACs were identified using primer sets RJMI4 and RJML2b, which flanked the gap between 604L2 and 1089I4. Therefore, we screened the Glenlea BAC library (4) with RJMI4 and RJML2b and identified the Glenlea BAC 846K21, which spanned the gap. Thus, complete physical maps of the *Tsn1* region on chromosome 5B and the homoeologous region of chromosome 5A were obtained. All BACs were sequenced by the Washington Genome Sequencing Center, Washington University, St. Louis, MO and

annotated using the Rice Genome Automated Annotation System (RiceGAAS; http://ricegaas.dna.affrc.go.jp/) as described in (1).

Haplotype Analysis of* Triticum *and* Aegilops *Accessions: Initially, 104 *Triticum* accessions were surveyed for reaction to ToxA and genotyped with markers *Xfcp1* and *Xfcp394*, which flank *Tsn1* and define the locus to a 1.0 cM interval (5) (table S1). Then, the remaining 278 accessions were genotyped with *Xfcp394* and all 386 were genotyped with *Xfcp620(WK35)* and *Xfcp623(Tsn1)* using the primers in table S7. Accessions with apparent recombination events within the *Tsn1* candidate gene region were subsequently genotyped with RFLP markers *Xfcg26(PT)* and *Xfcg32(HP)* using the methods described in (3) to further resolve the crossover intervals.

Aegilops speltoides accessions were tested for reaction to ToxA infiltration (5). Southern analysis of *Ae. speltoides* accession PI542238 was done as described in (3) using probes FCG34 and FCG35 (table S6), which were derived from the LRR and PK domains of *Tsn1*, respectively.

Mutagenesis and Validation of Candidate Genes: Seed of LDN and BW were treated with EMS as described in (6), and M_2 generation plants were infiltrated with ToxA as described in (7). Plants were scored for presence/absence of necrosis three days after infiltration. M_2 plants showing insensitive reactions were selfed to obtain M_3 generation plants and screened with ToxA again to confirm the reaction. Nine ToxA-insensitive mutants were identified in the LDN background and four in the BW background (table S3). In addition to these, three previously identified ToxA-insensitive mutants in the Kulm background (8) were used for candidate gene validation.

Seed of LDN was also subjected to fast-neutron bombardment (5Gy) at the International Atomic Energy Agency, Vienna, Austria. One ToxA-insensitive mutant (LDNfn2411) was identified from the M_2 generation, and molecular marker-based analysis of this line indicated that it contained an interstitial chromosome deletion of at least 2 Mb in size encompassing the *Tsn1* locus (data not shown). LDNfn2411 was used as a negative control for multiple experiments in this work.

Recombination-based mapping followed by marker genotyping of *Triticum* accessions narrowed the *Tsn1* candidate region to a segment containing four genes (table S2, Fig. 2D). We obtained the DNA sequences of all four genes from the ToxA-insensitive mutant LDNems937. Comparisons with the wild type LDN sequences indicated that only the PK-NB-LRR-like gene harbored a disruption (table S3). The sequence of the PK-NB-LRR-like gene was then obtained from the remaining 15 mutants along with wild type BW and Kulm for comparison. The PK-NB-LRR-like gene was amplified in eleven overlapping fragments using the primers indicated in table S8, and three independent PCR reactions for each fragment were sequenced to eliminate PCR errors. Sequence comparisons were done using the software Sequencher v4.8 (Gene Codes Corporation, Ann Arbor, MI).

Comparative sequence analysis of the PK-NB-LRR-like gene from the 16 EMS-induced mutants indicated that all but two (LDNems230 and Kems103) had either missense or nonsense mutations. LDNems230 had a point mutation in the third intron at position 778 five bp from the spice site junction. Kems103 had a point mutation at position 7,074, which was the first base of intron 6. To determine if the mutations in LDNems230 and Kems103 affected splicing, total RNA was isolated from LDN,

LDNems230, Kulm, and Kems103 and used to make cDNA as described in (6). Reverse transcriptase (RT)-PCR was conducted using primers PK.F17 (5'-TATACCGTTCGCAACTTTGG-3') and PK.R899 (5'-ATTCCGGTGGCATGTACTTC-3') on LDN and LDNems230 cDNA, and primers NB.F959 (5'-TCACCGGTCCATCTGGAATA-3') and LRR.R1357 (5'-TGCATCACCGCGAAGTAGTA-3') were used to amplify Kulm and Kems103 cDNA. The fragment amplified in LDNems230 was 226 bp smaller than the fragment amplified in LDN (fig. S6). Subsequent sequence analysis indicated this was due to the absence of exon 3 in the transcribed sequence. The fragment amplified in Kems103 was larger than that of Kulm indicating that the splicing of intron 6 was alternated. Sequence analysis revealed that the exon 6/intron 6 wild type splice site was abolished and instead splicing occurred at position 7,116, which was 43 bp downstream of the wild type splice site.

Tsn1 ***Characterization***: Total RNA was isolated from leaf tissue of LDN and used to make cDNA as described in (6). The nearly full-length cDNA was amplified in 5 fragments using the primer pairs in table S9, sequenced, and compared to the genomic sequence to determine the intron/exon boundaries. 5' and 3' rapid amplification of cDNA ends (RACE) was performed as described in (6) using cDNA based primers PK.5RACE3 (5'-CCAGATCATCATGAAGCAACTTCACAGC-3') for 5' RACE and LRR.F2659 (5'-TAGAAACGAACTCTTGTTCCCTAAG-3') for 3' RACE to determine the 5' and 3' UTRs. Coding and deduced amino acid sequences were used in BLAST searches of the NCBI database, the rice genome (http://www.gramene.org/), and the *Brachypodium distachyon* genome (http://blast.brachybase.org/) to identify homologous sequences.

The isolation of total RNA and production of cDNA from *Ae. speltoides* accession PI542238 was done as described above. cDNA obtained from PI542238 was amplified in five overlapping fragments using the primers in table S10, sequenced, and assembled using Sequencher v.4.8 (Gene Codes Corporation, Ann Arbor, MI). The cDNA sequence was aligned with the *Tsn1* cDNA sequence from LDN and the amino acid sequence deduced using MacVector v.10.6 (Cary, NC) to verify that PI542238 contained an intact and functional *Tsn1* ORF (fig. S3).

Comparative Analysis: Putative ORFs within and flanking the *Tsn1* candidate gene region were subjected to BLASTx searches of the NCBI database as described (1) to assign putative function (table S2). The sequences were then subjected to tBLASTx searches of the rice (http://www.gramene.org/) and *Brachypodium* (http://blast.brachybase.org/) genomes to identify putative orthologues (table S4, fig. S2). Southern analysis (3) of 24 wheat lines in fig. S1 digested with the restriction enzyme *Xba*I was done using probe FCG34, which was derived from the LRR domain of *Tsn1*, to determine if homoeoalleles existed on chromosomes 5A or 5D. Deduced amino acid sequences of the PK and NB-LRR domains were subjected to BLASTp searches of the NCBI database to identify putative proteins with high similarity.

***Phylogenetic analysis*:** *Tsn1* was sequenced from the 40 wheat accessions shown in bold with asterisks in table S1. *Tsn1* phylogenetic trees were constructed from CLUSTALW alignments of the complete coding regions including the UTRs of *Tsn1* (minus a ~2.8 kb segment of intron 4 with about 80% similarity to the LINE retrotransposon RIX_Yvonne_AY146588-1) using the UPGMA method and multiple distance-based

methods available in MacVector v10.6 (Cary, NC). Confidence values for nodes were calculated using 1000 bootstraps.

The conserved NB domains or PK domains encoded in *Tsn1* and related plant genes were determined using the RPS-BLAST program (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and aligned using the CLUSTALX program (9). Phylogenetic analysis was performed using the neighbor-joining method with PHYLIP 3.61 package (10, http://evolution.genetics.washington.edu/phylip.html) and the unrooted tree (from 1,000 bootstrap replicates) was drawn using the TreeView software (http://taxonomy.zoology.gla.ac.uk/rod/ treeview.html).

Transcriptional Expression: The durum wheat cultivar LDN was used for all *Tsn1* transcriptional expression analyses. Plants were grown in growth chambers at a temperature of 21°C. A 12/12hr light/dark cycle was provided for all the experiments with lights turning on and off at 9:00 am and 9:00 pm, respectively, except for continuous dark treatment, which was carried out in a growth chamber with identical conditions except without light.

RNA samples were collected from leaves, stems, and roots at the seedling stage, and immature spikes at Feekes wheat growth stage 8 to evaluate *Tsn1* transcription in different tissues. Total RNA was extracted from plant tissues using the RNeasy Plant Mini Kit (Qiagen). The first-strand cDNA was synthesized from 2µg of total RNA using the TaqMan Reverse Transcription Reagents (Applied Biosystems). Reverse transcription (RT)-PCR was conducted using primers LRR.F2659 (5'-TAGAAACGAACTCTTGTTCCCTAAG-3') and LRR.R3346 (5'-TCGAATCCTCAAAGCCTACC- 3') for *Tsn1*, and GAPDH.F152 (5'-

CAACGCTAGCTGCACCACTAACT-3') and GAPDH.R338 (5'-GCTGCTTGGAATGATGTTGA-3') for *GAPDH* on cDNAs from different LDN plant tissues.

To evaluate the effects of the circadian clock on *Tsn1* transcription levels, RNA samples were collected from leaves of two-week old plants every three hours for three days from plants grown under the normal 12 hr light/dark regime and from plants placed under continuous darkness at the time of collection of the first sample.

Specific effects of light and dark on *Tsn1* transcription were evaluated by collecting RNA from leaves of two-week old plants at 10:00 am, then turning off the lights for three hours and collecting samples at 1:00 pm, followed by turning the lights back on for two hours and collecting samples at 3:00 pm. Samples were collected at the same time points from control plants under the 12 hr light/dark regime.

Three treatments including ToxA infiltration, water infiltration, and non-infiltrated controls were used to study the effects of toxin infiltration on *Tsn1* expression. Infiltrated regions of LDN seedling leaves and leaf samples of the non-infiltrated controls were collected at 0, 3, 6, 12, 24, and 36 hr time points, which correspond to 10:30 am (light), 1:30 pm (light), 4:30 pm (light), 10:30 pm (dark), 10:30 am (light), and 10:30 pm (dark), respectively. All expression studies were conducted using three biological replicates.

Relative quantitative (RQ)-PCR was used to evaluate *Tsn1* expression in the circadian clock experiment, the light/dark experiment and in the ToxA-challenge experiment. RQ-PCR was performed on a 7500 Real-Time PCR System (Applied Biosystems). The wheat *GAPDH* gene was used as an endogenous control. The PCR primers, LRR.F3182 (5'- CTCTTTGCCGGAGAGCATAC -3') and LRR.R3346 (5'-

TCGAATCCTCAAAGCCTACC -3') for the *Tsn1* gene, and GAPDH.F152 (5'-CAACGCTAGCTGCACCACTAACT-3') and GAPDH.R338 (5'-GCTGCTTGGAATGATGTTGA-3') for the wheat *GAPDH* gene were used for *Tsn1* gene expression assays. Each experiment was repeated three times and all reactions were done in quadruplicate. The 20 μL reaction contained 1X SYBR PCR Master Mix, 0.25 μM each primer, and 5 μL 15-fold diluted cDNA. The thermocycler program used for *Tsn1* gene expression was as follows: 10 minutes of pre-incubation at 95°C followed by 40 cycles for 15 seconds at 95°C and one minute at 60°C. A dissociation program was performed after each RQ-PCR assay to confirm specific amplification. The LDN *Tsn1* deletion mutant LDNfn2411 was used as a negative control.

Templates to determine the amplification efficiency of wheat *GAPDH* and *Tsn1* consisted of five 2-fold dilutions of LDN cDNA (1:4, 1:8, 1:16, 1:32, and 1:64) in quadruplicate. Raw C_T values were averaged for each dilution. Ct numbers were plotted against logarithm cDNA input, and linear regression was used to draw the best-fit line. Amplification efficiency were calculated based on slope where efficiency = 100% * $\tan^{-1}$ ($slope^{-1}$)/45. Amplification efficiencies were higher than 95% for both gene systems. Sample CT values were averaged after omitting outlying CT values for each gene. Sample averages were linearized using the $2^{-\Delta\Delta CT}$ method (11).

Yeast-Two Hybrid: A wheat cDNA library was constructed and screened using the Matchmaker Library Construction and Screening Kit (Clontech, Mountain View, CA) following the manufacturer's instructions. *ToxA* coding region (minus the putative signal peptide) was cloned in frame with the GAL4 DNA-binding domain (BD) of the bait vector pGBKT7 and *Tsn1* coding regions (full length or individual domains)

were cloned in frame with the GAL4 activation domain (AD) of the prey vector pGADT7. The bait and prey constructs were co-transformed into yeast strain AH109. Standard positive (pGBKT7-53 and pGADT7-RecT, Clontech) and negative (pGBKT7-ToxA and pGADT7-RecT) were included. Yeast transformants were selected on SD/–Leu/–Trp, SD/–Leu/–Trp/–Ade/–His and SD/-LTAH plus X-α-gal agar plates to detect the activation of reporter genes *HIS3*, *ADE2* and *MEL1*(for α-galactosidase activity).

Co-immunoprecipitation: *In vitro* translation of the bait and prey proteins and immunoprecipitation assays were performed using the TNT® T7 Coupled Reticulocyte Lysate System (Promega, Madison, WI) and Matchmaker Co-IP Kit (Clontech), respectively, following the manufacturer's instructions. Plasmid DNA of the corresponding yeast two-hybrid constructs were used as template for *in vitro* translation to generate cMyc (for ToxA) or HA (for Tsn1)-tagged proteins. The bait and prey proteins were mixed and incubated with anti-cMyc antibody (Clontech). The antibody-bound protein complex was captured with protein A beads and the co-precipitated proteins were subjected to Western blot analysis with the anti-HA-peroxidase conjugate as probe. Signals were detected using the Immobilon Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA).

Supplemental References and Notes

1. H.J. Lu, J.D. Faris, *Funct. Integr. Genomics* **6**, 90 (2006).

2. A. Cenci *et al*., *Theor. Appl. Genet*. **107**, 931 (2003).

3. J.D. Faris, J.P. Fellers, S.A. Brooks, B.S. Gill, *Genetics* **164**, 311 (2003).

4. S.D. Nilmalgoda, S. Cloutier, A.Z. Walichnowski, *Genome* **46**, 870 (2003).

5. Z. Zhang *et al., Mol. Breeding* **23**, 35 (2009).

6. K.J. Simons *et al*., *Genetics* **172**, 547 (2006).

7. T.L. Friesen, S.W. Meinhardt, J.D. Faris, *Plant J*. **51**, 681 (2007).

8. T.L. Friesen *et al.*, *Phytopathology* **92**, 38 (2002).

9. J.D. Thompson *et al., Nucleic Acids Res*. **25**, 4876 (1997).

10. J. Felsentein, *Cladistics* **5**, 184 (1989).

11. K.J. Livak, T.D. Schmittgen, *Methods* **25**, 402 (2001).

Supplemental Tables

Table S1. The 386 *Triticum* accessions evaluated for reaction to ToxA and genotyped with markers derived from the *Tsn1* genomic region.

Accession[a]	**PI/CI**	**Ploidy**	**Genus**	**Species**	**Subspecies/type**	***Xfcp1***[b]	***Xfcp620 (WK35)***	***Xfcg32 (HP)***	***Xfcp623 (Tsn1)***	**ToxA**[c]	***Xfcg26 (PT)***	***Xfcp394***
Glenn	PI639273	6X	*Triticum*	*aestivum*	*aestivum*/spring	+	+		+	+		+
Katepwa*	N/A	6X	*Triticum*	*aestivum*	*aestivum*/spring	+	+		+	+		+
Amery*	N/A	6X	*Triticum*	*aestivum*	*aestivum*/spring	+	+	+	+	+	+	+
Bobwhite*	PI520554	6X	*Triticum*	*aestivum*	*aestivum*/spring	+	+		+	+		+
Kulm*	PI590576	6X	*Triticum*	*aestivum*	*aestivum*/spring	+	+	+	+	+	+	+
Grandin*	PI531005	6X	*Triticum*	*aestivum*	*aestivum*/spring	+	+		+	+		+
Chinese Spring	CItr14108	6X	*Triticum*	*aestivum*	*aestivum*/spring	-	-		-	-		-
Fielder	CItr17268	6X	*Triticum*	*aestivum*	*aestivum*/spring	+	+		+	+		+
Salamouni	PI182673	6X	*Triticum*	*aestivum*	*aestivum*/spring	-	-		-	-		-
BR34	N/A	6X	*Triticum*	*aestivum*	*aestivum*/spring	null	+	-	-	-	-	-
Glenlea*	Cltr17272	6X	*Triticum*	*aestivum*	*aestivum*/spring	+	+		+	+		+
Hope*	CItr8178	6X	*Triticum*	*aestivum*	*aestivum*/spring	+	+		+	+		+
Sumai3*	PI481542	6X	*Triticum*	*aestivum*	*aestivum*/spring	+	+		+	+		+
ND2709*	N/A	6X	*Triticum*	*aestivum*	*aestivum*/spring	+	+		+	+		+
ND495*	N/A	6X	*Triticum*	*aestivum*	*aestivum*/spring	+	+		+	+		+
Timstein*	PI168688	6X	*Triticum*	*aestivum*	*aestivum*/spring	+	+		+	+		+
Selkirk	CItr13100	6X	*Triticum*	*aestivum*	*aestivum*/spring	+	+	-	-	-	-	-
Jagger*	PI593688	6X	*Triticum*	*aestivum*	*aestivum*/winter	+	+	+	+	+	+	-
TAM105	CItr17826	6X	*Triticum*	*aestivum*	*aestivum*/winter	+	+	+	+	+	+	-
Renan	PI564569	6X	*Triticum*	*aestivum*	*aestivum*/winter	-	-		-	-		-
Arina	N/A	6X	*Triticum*	*aestivum*	*aestivum*/winter	-	-		-	-		-
Atlas 66	CItr12561	6X	*Triticum*	*aestivum*	*aestivum*/winter	+	-		-	-		-
Cheyenne	PI192268	6X	*Triticum*	*aestivum*	*aestivum*/winter	+	+	+	+	+	+	-
Forno*	N/A	6X	*Triticum*	*aestivum*	*aestivum*/winter	null	+	+	+	+	+	-
Norstar	CItr17735	6X	*Triticum*	*aestivum*	*aestivum*/winter	+	+	+	+	+	+	-
Termok	PI41023	6X	*Triticum*	*aestivum*	*compactum*	+	+		+	+		+
Moco de Espiga Quadrada	PI56213	6X	*Triticum*	*aestivum*	*compactum*	-	-		-	-		-
Sinai No. 3	PI60740	6X	*Triticum*	*aestivum*	*compactum*	+	+		+	+		+
Gluclub	PI114638	6X	*Triticum*	*aestivum*	*compactum*	+	+		+	+		+
Premier	PI278581	6X	*Triticum*	*aestivum*	*compactum*	+	-		-	-		-

Tincurrin	PI434642	6X	*Triticum*	*aestivum*	*compactum*	+	+		+	+		+
TA2601*	N/A	6X	*Triticum*	*aestivum*	*compactum*	+	+	+	+	-	+	+
Novo*	N/A	6X	*Triticum*	*aestivum*	*aestivum*/spring	+	+	+	+	-	+	+
Puseas*	N/A	6X	*Triticum*	*aestivum*	*aestivum*/spring	+	+	+	+	-	+	+
Ching Feng*	N/A	6X	*Triticum*	*aestivum*	*aestivum*/spring		+	+	+	-	+	+
Siu Mak*	N/A	6X	*Triticum*	*aestivum*	*aestivum*/spring		+	+	+	-	+	+
Huo Mai*	N/A	6X	*Triticum*	*aestivum*	*aestivum*/spring		+	+	+	-	+	+
Shagke Huomai*	N/A	6X	*Triticum*	*aestivum*	*aestivum*/spring		+	+	+	+		+
132	PI42014	6X	*Triticum*	*aestivum*	*sphaerococcum*	-	-		-	-		-
52	CItr8610	6X	*Triticum*	*aestivum*	*sphaerococcum*	+	+		+	+		+
219	PI70711	6X	*Triticum*	*aestivum*	*sphaerococcum*	-	-		-	-		-
I12	PI83402	6X	*Triticum*	*aestivum*	*sphaerococcum*	+	+		+	+		+
971	PI278650	6X	*Triticum*	*aestivum*	*sphaerococcum*	+	-		-	-		-
TA2605	N/A	6X	*Triticum*	*aestivum*	*sphaerococcum*	-	-		-	-		-
Sears 407a*	N/A	6X	*Triticum*	*aestivum*	*spelta*/Iranian	null	+		+	+		+
ts199	PI367199	6X	*Triticum*	*aestivum*	*spelta*/Iranian	null	+		+	+		+
I-1-3544	PI272555	6X	*Triticum*	*aestivum*	*macha*	-	-		-	-		-
Letshchumicum	PI352466	6X	*Triticum*	*aestivum*	*macha*	-	-		-	-		-
69Z5.193	PI355514	6X	*Triticum*	*aestivum*	*macha*	-	-		-	-		-
DN-2378	PI361862	6X	*Triticum*	*aestivum*	*macha*	-	-		-	-		-
G532	PI428146	6X	*Triticum*	*aestivum*	*macha*	-	-		-	-		-
G1260	PI428148	6X	*Triticum*	*aestivum*	*macha*	null	-		-	-		-
G866	PI428178	6X	*Triticum*	*aestivum*	*macha*	null	-		-	-		-
P78-81-1	N/A	6X	*Triticum*	*aestivum*	*spelta*/European	null	-		-	-		-
TA2603	N/A	6X	*Triticum*	*aestivum*	*spelta*/European	-	-		-	-		-
ts469	PI378469	6X	*Triticum*	*aestivum*	*spelta*/European	null	-		-	-		-
ts573	PI272573	6X	*Triticum*	*aestivum*	*spelta*/European	-	-		-	-		-
ts060	PI286060	6X	*Triticum*	*aestivum*	*spelta*/European	-	-		-	-		-
ts651	PI355651	6X	*Triticum*	*aestivum*	*spelta*/European	+	-		-	-		-
swedish	PI428342	6X	*Triticum*	*aestivum*	*vavilovii*	-	-		-	-		-
Langdon*	CItr13165	4X	*Triticum*	*turgidum*	*durum*	+	+	+	+	+	+	-
Ben	PI596557	4X	*Triticum*	*turgidum*	*durum*	+	+	+	+	+	+	-
Altar84	N/A	4X	*Triticum*	*turgidum*	*durum*	-	-		-	-		-
Mountrail	PI607530	4X	*Triticum*	*turgidum*	*durum*	-	-		-	-		-
Scoop1	N/A	4X	*Triticum*	*turgidum*	*durum*	-	-		-	-		-
Mexicali	PI433760	4X	*Triticum*	*turgidum*	*durum*	-	-	-	-	-	-	-
tp182/836	CItr191826	4X	*Triticum*	*turgidum*	*polonicum*	-	-		-	-		-
tp334	CItr225334	4X	*Triticum*	*turgidum*	*polonicum*	+	+		+	+		+
TA2801	N/A	4X	*Triticum*	*turgidum*	*carthlicum*	+	+		+	+		+
tc738	PI70738	4X	*Triticum*	*turgidum*	*carthlicum*	+	+		+	+		+
tc471	PI182471	4X	*Triticum*	*turgidum*	*carthlicum*	+	-		-	-		-

TA106	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-		-	-		-
td30	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-	-	-	-	-	+
td582	PI272582	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-		-	-		-
td328	PI352328	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-		-	-		-
15-26	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-		-	-		-
16-29	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-	-	-	-	-	+
18-1*	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-	+	+	+	+	+
18-10*	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-		+	+		+
18-20*	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-		+	+		+
18-56*	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-		+	+		+
36-12*	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-		+	+		+
A-33	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-		-	-		-
A-35	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-		-	-		-
B-16	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-		-	-		-
B-6	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	+	+	-	-	-	-	-
C-19	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-		-	-		-
C-36	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-		-	-		-
G-11*	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-		+	+		+
I-50	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-		-	-		-
L-1	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-	-	-	-	-	+
L-43	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	+	+	-	-	-	-	-
T-7-1*	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-		+	+		+
T-7-30*	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-		+	+		+
T-7-45*	N/A	4X	*Triticum*	*turgidum*	*dicoccoides*	null	-		+	+		+
TA10435	N/A	4X	*Triticum*	*turgidum*	*dicoccum*	-	-		-	-		-
tdom621	CItr14621	4X	*Triticum*	*turgidum*	*dicoccum*	null	-		-	-		-
tdom454	CItr14454	4X	*Triticum*	*turgidum*	*dicoccum*	+	-		-	-		-
tdom151	PI74108	4X	*Triticum*	*turgidum*	*dicoccum*	+	-		-	-		-
tdom680	PI94680	4X	*Triticum*	*turgidum*	*dicoccum*	+	-	-	-	-	-	+
tdom919	PI40919	4X	*Triticum*	*turgidum*	*dicoccum*	-	-		-	-		-
tdom664	PI94664	4X	*Triticum*	*turgidum*	*dicoccum*	-	-		-	-		-
tdom648	PI94648	4X	*Triticum*	*turgidum*	*dicoccum*	-	-		-	-		-
tdom94641	PI94641	4X	*Triticum*	*turgidum*	*dicoccum*	-	-		-	-		-
tdom824	CItr14824	4X	*Triticum*	*turgidum*	*dicoccum*	-	-		-	-		-
tdom747	PI94747	4X	*Triticum*	*turgidum*	*dicoccum*	-	-		-	-		-
tdom971	PI101971	4X	*Triticum*	*turgidum*	*dicoccum*	-	-		-	-		-
tdom013*	CI4013	4X	*Triticum*	*turgidum*	*dicoccum*		-	-	+	+	+	+
tdom024	PI41024	4X	*Triticum*	*turgidum*	*dicoccum*		-		-	-		-
tdom025	PI41025	4X	*Triticum*	*turgidum*	*dicoccum*		-		-	-		-
tdom041	PI194041	4X	*Triticum*	*turgidum*	*dicoccum*		-		-	-		-
tdom042	PI194042	4X	*Triticum*	*turgidum*	*dicoccum*		-		-	-		-

tdom085	CI14085	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	-	-	-	+
tdom086	CI14086	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	-	-	-	+
tdom091	PI191091	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom098	CI14098	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom099	PI196099	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom100	PI196100	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom101	PI196101	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom104	PI74104	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom106	PI74106	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom134	PI133134	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom135	CI14135	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	-	-	-	+
tdom14639	CI14639	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom14919	CI14919	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom14971	CI14971	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom154	PI154582	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom168637	PI168673	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom213	CI12213	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom214	CI12214	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom217637	PI217637	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom234	PI56234	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom259	PI197259	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom260	PI197260	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom3686	CI3686	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom386	PI191386	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom387	PI191387	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom388	PI73388	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom390	PI191390	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	-	-	-	+
tdom400	PI221400	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	-	-	-	+
tdom437	CI14437	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom481	PI197481	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom482	PI197482	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom483	PI197483	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom484	PI197484	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom485	PI197485	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom486	PI197486	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom487	PI197487	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom488	PI197488	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom489	PI197489	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom490	PI197490	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom491	PI197491	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom492	PI197492	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-

tdom493	PI197493	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom494	PI197494	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom495	PI197495	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom496*	PI197496	4X	*Triticum*	*turgidum*	*dicoccum*	+		+	+		+
tdom536	PI57536	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom578	PI164578	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom582	PI164582	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom58789*	PI58789	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	+	+	+	-
tdom592	CI14592	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom613*	PI94613	4X	*Triticum*	*turgidum*	*dicoccum*	+		+	+		+
tdom614	PI94614	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom615	PI94615	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom616	PI94616	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	-	-	-	+
tdom617	PI94617	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom618	PI94618	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom620	PI94620	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom623	PI94623	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom624	PI94624	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom625	PI94625	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom626	PI94626	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	-	-	-	+
tdom627	PI94627	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	-	-	-	+
tdom628	PI94628	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom630	PI94630	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom631	PI94631	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom632	PI94632	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom633	PI94633	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom634	PI94634	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom635	PI94635	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom636	CI14636	4X	*Triticum*	*turgidum*	*dicoccum*	-					-
tdom637	PI94637	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	-	-	-	+
tdom638	CI14638	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom639	PI217639	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom640	PI217640	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom641	PI193641	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom642	PI193642	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom643	PI193643	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom644	PI193644	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom649	PI94649	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom650	PI94650	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	-	-	-	+
tdom654	PI94654	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom655	PI94655	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	-	-	-	+

tdom656	PI94656	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom657	PI94657	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom659	PI94659	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom660	PI94660	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom661	PI94661	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom662	PI94662	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	-	-	-	+
tdom663	PI94663	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom665	PI94665	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom666	PI94666	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom667	PI94667	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom668	PI94668	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom669	PI94669	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom670	PI94670	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom671	PI94671	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom673	PI94673	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom674	PI94674	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom675	PI168675	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom676	PI168676	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom677	PI168677	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom678	PI168678	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom679	PI168679	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom681	PI94681	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom682	PI94682	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom683	PI94683	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	-	-	-	+
tdom686	CI7686	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	-	-	-	+
tdom687	CI7687	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom704	PI60704	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom706	PI60706	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom721	PI195721	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom722	PI195722	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom723	PI195723	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom738	PI94738	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom751	CI14751	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom752	CI14752	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom779	CI7779	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom787	CI14787	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom788	PI58788	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom789*	PI2789	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	+	+	+	+
tdom822	CI14822	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom834	CI14834	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom838	CI14838	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-

tdom867	CI14867	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom868	CI14868	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom873	PI193873	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom877	PI193877	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom878	PI193878	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom879	PI193879	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom880	PI193880	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom882	PI193882	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom883	PI193883	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom899	PI79899	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	-	-	-	+
tdom904*	PI196904	4X	*Triticum*	*turgidum*	*dicoccum*	+		+	+		+
tdom905*	PI196905	4X	*Triticum*	*turgidum*	*dicoccum*	+		+	+		+
tdom916	CI14916	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom917	CI14917	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom920	PI190920	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom921	PI190921	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom922	PI190922	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom923	PI190923	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	-	-	-	+
tdom926	PI190926	4X	*Triticum*	*turgidum*	*dicoccum*	-	-	-	-	-	+
tdom94621	PI94621	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom94638	PI94638	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom94640	PI94640	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom94642	PI94642	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom94675	PI94675	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom94676	PI94676	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom94677	PI94677	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom94678	PI94678	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom94679	PI94679	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom961	PI113961	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom962	CI7962	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom963	PI113963	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom966	CI7966	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom970	CI14970	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tdom972	CI14972	4X	*Triticum*	*turgidum*	*dicoccum*	-		-	-		-
tt011	PI295011	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt012	PI295012	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt015	PI191015	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt016	PI265016	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt043	PI295043	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt071	PI295071	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt104	PI191104	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-

tt134951	PI134951	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt134952	PI134952	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt134953	PI134953	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt145	PI191145	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt203	PI191203	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt204	PI191204	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt221	PI278221	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt258	PI331258	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt300	PI341300	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt306560	PI306560	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt308	PI225308	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt309	PI225309	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt3270	CI3270	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt353	PI191353	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt391	PI341391	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt413	PI345413	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt423	PI221423	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt424	PI221424	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt425	PI221425	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt440	PI323440	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt4577	CI4577	4X	*Triticum*	*turgidum*	*turgidum*	-	-	-	-	-	+
tt482	PI341482	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt484	PI166484	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt495	PI166495	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt502	PI167502	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt503	PI167503	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt520	PI192520	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt526	PI290526	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt527	PI290527	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt558	PI306558	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt559	PI306559	4X	*Triticum*	*turgidum*	*turgidum*	-	-	-	-	-	+
tt560	PI330560	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt561*	PI306561	4X	*Triticum*	*turgidum*	*turgidum*	+	+	+	+	+	-
tt564	PI306564	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt568	PI294568	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt572	PI167572	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt574	PI294574	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt579	PI191579	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt584	PI272584	4X	*Triticum*	*turgidum*	*turgidum*	-	-	-	-	-	+
tt588	PI272588	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt591	PI166591	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+

tt593	PI272593	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt596	PI278596	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt597	PI278597	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt666	PI245666	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt689	PI94689	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		null
tt712	CI13712	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt714	CI17714	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt723	PI185723	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt724	PI185724	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		null
tt726	PI185726	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt728	PI185728	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt734	PI185734	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt743	CI14743	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt7505	CI7505	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt751	PI245751	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt7519	CI7519	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt7688	CI7688	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt7772	CI7772	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		-
tt7774	CI7774	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt7778	CI7778	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt7785	CI7785	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt7786	CI7786	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt7795	CI7795	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt7796	CI7796	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt7798	CI7798	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt7833	CI7833	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt7839	CI7839	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt7840	CI7840	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt7859	CI7859	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt7863	CI7863	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt7864	CI7864	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt7871	CI7871	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt7875	CI7875	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt7881	CI7881	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt795	CI14795	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt8000	CI8000	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt8055	CI8055	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt8098	CI8098	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt8099	Ci8099	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt8115	CI8115	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt812	PI149812	4X	*Triticum*	*turgidum*	*turgidum*	-	-	-	-	-	+

tt8155	CI8155	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt835	PI212835	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt8481	CI8481	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt851	PI266851	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt863*	CI14863	4X	*Triticum*	*turgidum*	*turgidum*	-	+	+	+	+	-
tt867	PI167867	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt871	PI191871	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt885	PI191885	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt904	PI191904	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt912	PI208912	4X	*Triticum*	*turgidum*	*turgidum*	+		+	+		+
tt932	PI190932	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt946	PI134946	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt947	PI134947	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt948	PI134948	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt949	PI134949	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt951	PI191951	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt952	PI191952	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt953	PI191953	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt956	PI134956	4X	*Triticum*	*turgidum*	*turgidum*	+	-	-	-	-	+
tt957	PI134957	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt959	PI134959	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt960	PI134960	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt961	PI134961	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt962	PI134962	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt978	PI190978	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt981	PI191981	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt985	PI157985	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-
tt995	PI264995	4X	*Triticum*	*turgidum*	*turgidum*	-		-	-		-

[a]The *Tsn1* genomic coding region sequences were obtained from the accessions shown in bold and with asterisks. GenBank accession numbers are given in fig. S4.

[b]For molecular markers, which begin with "*X*," plus (+) and minus (-) signs indicate alleles associated with the sensitive and insensitive haplotypes based on Kulm and Mexicali, respectively.

[c]Plus (+) and minus (-) indicate sensitive and insensitive to ToxA, respectively.

Table S2. Predicted genes within the *Tsn1* candidate and flanking regions.

Gene	BLASTx hit	e-value	BAC clone(s)	Strand	No. exons	No. amino acids
SP	subtilase; SP1 [*Oryza sativa*]	0	507H19	+	10	739
WK106	wall-associated kinase-like 1, putative, [*Oryza sativa*]	1e-127	507H19	-	4	546
WK95	putative wall-associated kinase 4 [*Oryza sativa*]	0	507H19	-	5	1071
WK35	putative wall-associated kinase 4 [*Oryza sativa*]	2e-161	507H19, 353O5	-	5	1079
HP2	hypothetical protein [*Oryza sativa*]	3e-82	604L2	-	1	473
HP1[a]	hypothetical protein [*Oryza sativa*]	1e-90	604L2	+	1	473
Tsn1 (NB-LRR)[a,b]	Os11g0211300, Leucine Rich Repeat family protein, expressed [*Oryza sativa*]	0	604L2	+	8	1,490
Tsn1 (PK)[a,b]	Os11g0212300, Protein kinase domain containing protein, expressed [*Oryza sativa*]	1e-80				
RNP[a]	U2 snRNP auxiliary factor large subunit A (*Triticum aestivum*)	8e-180	846K21, 1089I4	+	11	539
PT[a]	Potassium transporter 18 [*Oryza sativa*]	0	846K21, 1089I4	+	9	785
DZF1	DHHC-type zinc finger domain-containing protein -like [*Oryza sativa*]	8e-165	846K21, 1089I4	-	6	547

[a]Within the *Tsn1* candidate gene region.

[b]Database searches were performed using BLASTp.

Table S3. Descriptions of induced and natural mutations identified within the *Tsn1* gene.

Induced mutant	Mutation type	Position[a]	Exon	Domain	Codon change	Amino acid change
LDNems114	Missense	86	1	PK	GGG->GAG	Gly->Glu
Kems37-5[b]	Missense/Nonsense	602/9,767	3/7	PK/LRR	TGT->TAT/TGG->TGA	Cys->Tyr/Trp->stop
BWems123	Nonsense	625	3	PK	GGA->TGA	Gly->stop
LDNems230[c]	Splice	778	-	-	-	-
Kems103[b]	Missense/splice	5,983/7,074	6/-	NB/-	AGG->AAG/-	Arg->Lys/-
LDNems391	Nonsense	6,663	6	NB	CAA->TAA	Gln->stop
LDNems346	Missense	6,705	6	NB	GGT->AGT	Gly->Ser
BWems687	Missense	6,721	6	NB	GCT->GTT	Ala->Val
Kems322	Missense	6,847	6	NB	TCC->TTC	Ser->Phe
LDNems299	Nonsense	6,959	6	NB	TGG->TGA	Trp->stop
LDNems138	Missense	7,008	6	NB	CTC->TTC	Leu->Phe
LDNems355	Missense	9,184	7	LRR	ACT->ATT	Thr->Ile
LDNems403	Missense	9,552	7	LRR	CCT->TCT	Pro->Ser
LDNems937	Missense	9,792	7	LRR	CTC->TTC	Leu->Phe
BWems952	Missense	9,792	7	LRR	CTC->TTC	Leu->Phe
BWems258	Missense	9,817	7	LRR	TGC->TAC	Cys->Tyr
Natural mutant						
TA2601	Frameshift	4,610	5	PK	-	-
Ching Feng	Frameshift	5,983	6	NB	-	-
Siu Mak	Frameshift	8,144	7	LRR	-	-
Huo Mai	Nonsense	9,767	7	LRR	TGG->TGA	Trp->stop
Novo	Nonsense	9,767	7	LRR	TGG->TGA	Trp->stop
Puseas	Nonsense	9,767	7	LRR	TGG->TGA	Trp->stop

[a]Base pair position counting from the translation start site.

[b]Kems37-5 and Kems103 each contained two mutations. RT-PCR and sequence analysis indicated that the splice mutation in Kems103 results in a product 43 bp larger than the wild type (fig. S6)

[c]RT-PCR and sequence analysis of LDNems230 showed that exon 3 is eliminated from the coding sequence (fig. S6).

Table S4. Similarity between predicted open reading frames within the *Tsn1* candidate and flanking regions and genes in rice and *Brachypodium*.

Gene	Rice				*Brachypodium*			
	Chromosome	tBLASTx	e-value	Position	Chromosome	tBLASTx	e-value	Position
SP	5	Os05g36010	1.3e-91	21,242,313	5	Bradi5g03790	0	4,759,668
WAK106	9	Os09g38910	1.4e-130	22,348,258	4	Bradi4g38010	0	42,996,968
WAK95	9	Os09g38910	8.0e-223	22,348,258	4	Bradi4g38010	0	42,996,968
WAK35	9	Os09g38910	4.2e-187	22,348,258	4	Bradi4g38010	0	42,996,968
HP2	9	Os09g39000	4.0e-77	22,390,829	4	Bradi4g38050	3e-34	43,032,352
HP1	9	Os09g39000	2.7e-85	22,390,829	4	Bradi4g38050	1e-48	43,032,352
Tsn1 (NB-LRR)	11	Os11g10610	3.2e-179	5,816,301	2	Bradi2g36180	0	36,611,574
Tsn1 (PK)	11	Os11g10640	1.4e-42	5,830,697	2	Bradi2g38510	2e-73	38,794,009
RNP	11	Os11g41820	5.0e-129	24,647,349	4	Bradi4g38060	0	43,041,754
PT	9	Os09g38960	4.3e-282	22,367,307	4	Bradi4g38070	0	43,046,331
DZF1	9	Os09g38970	3.8e-184	22,376,407	4	Bradi4g38080	0	43,056,076

Table S5. *Aegilops speltoides* (SS genome) accessions evaluated for reaction to ToxA.

PI/CI	Taxon	ToxA[a]
CIae 45	*Aegilops speltoides*	-
CIae 57	*Aegilops speltoides*	-
CIae 61	*Aegilops speltoides*	-
PI 170203	*Aegilops speltoides*	-
PI 170204	*Aegilops speltoides*	-
PI 172685	*Aegilops speltoides*	-
PI 173614	*Aegilops speltoides*	-
PI 174010	*Aegilops speltoides*	-
PI 219867	*Aegilops speltoides*	-
PI 254865	*Aegilops speltoides*	-
PI 266817	*Aegilops speltoides*	-
PI 315853	*Aegilops speltoides*	-
PI 330488	*Aegilops speltoides*	-
PI 369581	*Aegilops speltoides*	-
PI 369582	*Aegilops speltoides*	-
PI 369583	*Aegilops speltoides*	-
PI 369584	*Aegilops speltoides*	-
PI 369585	*Aegilops speltoides*	-
PI 369586	*Aegilops speltoides*	-
PI 369587	*Aegilops speltoides*	-
PI 369588	*Aegilops speltoides*	-
PI 369589	*Aegilops speltoides*	-
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PI 369592	*Aegilops speltoides*	-
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PI 369594	*Aegilops speltoides*	-
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PI 369596	*Aegilops speltoides*	-
PI 369597	*Aegilops speltoides*	-
PI 369598	*Aegilops speltoides*	-
PI 369599	*Aegilops speltoides*	-
PI 369600	*Aegilops speltoides*	-
PI 369601	*Aegilops speltoides*	-
PI 369602	*Aegilops speltoides*	-
PI 369603	*Aegilops speltoides*	-
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PI 369605	*Aegilops speltoides*	-
PI 369606	*Aegilops speltoides*	-
PI 369607	*Aegilops speltoides*	-
PI 369608	*Aegilops speltoides*	-
PI 369609	*Aegilops speltoides*	-
PI 369610	*Aegilops speltoides*	-
PI 369611	*Aegilops speltoides*	-
PI 369613	*Aegilops speltoides*	-
PI 369614	*Aegilops speltoides*	-
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PI 369616	*Aegilops speltoides*	-

PI 369617	*Aegilops speltoides*	-
PI 369618	*Aegilops speltoides*	-
PI 369620	*Aegilops speltoides*	-
PI 369621	*Aegilops speltoides*	-
PI 369622	*Aegilops speltoides*	-
PI 369623	*Aegilops speltoides*	+
PI 369624	*Aegilops speltoides*	-
PI 369625	*Aegilops speltoides*	-
PI 369626	*Aegilops speltoides*	-
PI 369660	*Aegilops speltoides*	-
PI 369661	*Aegilops speltoides*	-
PI 369662	*Aegilops speltoides*	-
PI 369663	*Aegilops speltoides*	-
PI 369664	*Aegilops speltoides*	-
PI 369665	*Aegilops speltoides*	-
PI 369666	*Aegilops speltoides*	-
PI 393492	*Aegilops speltoides*	-
PI 393494	*Aegilops speltoides*	-
PI 393495	*Aegilops speltoides*	-
PI 422448	*Aegilops speltoides*	-
PI 449338	*Aegilops speltoides* var. *speltoides*	-
PI 449339	*Aegilops speltoides*	-
PI 449340	*Aegilops speltoides*	-
PI 449341	*Aegilops speltoides*	-
PI 487231	*Aegilops speltoides*	-
PI 487232	*Aegilops speltoides*	-
PI 487235	*Aegilops speltoides*	-
PI 487238	*Aegilops speltoides*	-
PI 542238	*Aegilops speltoides*	+
PI 542239	*Aegilops speltoides* var. *ligustica*	-
PI 542240	*Aegilops speltoides* var. *ligustica*	-
PI 542241	*Aegilops speltoides* var. *ligustica*	-
PI 542242	*Aegilops speltoides* var. *ligustica*	-
PI 542243	*Aegilops speltoides* var. *ligustica*	-
PI 542244	*Aegilops speltoides* var. *ligustica*	-
PI 542245	*Aegilops speltoides* var. *ligustica*	-
PI 542246	*Aegilops speltoides* var. *ligustica*	-
PI 542247	*Aegilops speltoides* var. *ligustica*	-
PI 542248	*Aegilops speltoides* var. *ligustica*	-
PI 542249	*Aegilops speltoides* var. *ligustica*	-
PI 542250	*Aegilops speltoides* var. *ligustica*	-
PI 542252	*Aegilops speltoides* var. *ligustica*	-
PI 542253	*Aegilops speltoides* var. *ligustica*	-
PI 542255	*Aegilops speltoides* var. *ligustica*	-
PI 542256	*Aegilops speltoides* var. *ligustica*	-
PI 542261	*Aegilops speltoides* var. *speltoides*	-
PI 542262	*Aegilops speltoides* var. *speltoides*	-
PI 542265	*Aegilops speltoides* var. *speltoides*	-
PI 542266	*Aegilops speltoides* var. *speltoides*	-
PI 542267	*Aegilops speltoides* var. *speltoides*	-
PI 542269	*Aegilops speltoides* var. *speltoides*	-

PI 542271	*Aegilops speltoides* var. *speltoides*	-
PI 542272	*Aegilops speltoides* var. *speltoides*	-
PI 542273	*Aegilops speltoides* var. *speltoides*	-
PI 542274	*Aegilops speltoides* var. *speltoides*	-
PI 542276	*Aegilops speltoides* var. *speltoides*	-
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PI 554292	*Aegilops speltoides*	-
PI 554296	*Aegilops speltoides*	-
PI 554297	*Aegilops speltoides*	-
PI 554298	*Aegilops speltoides*	-
PI 554299	*Aegilops speltoides*	-
PI 554300	*Aegilops speltoides*	-
PI 554303	*Aegilops speltoides*	-
PI 554304	*Aegilops speltoides*	-
PI 554305	*Aegilops speltoides* var. *ligustica*	-
PI 560527	*Aegilops speltoides* var. *ligustica*	-
PI 560529	*Aegilops speltoides* var. *speltoides*	-
PI 560530	*Aegilops speltoides* var. *speltoides*	-
PI 560747	*Aegilops speltoides* var. *speltoides*	-
PI 560749	*Aegilops speltoides* var. *speltoides*	-
PI 560750	*Aegilops speltoides* var. *speltoides*	-
PI 560752	*Aegilops speltoides* var. *speltoides*	-
PI 573449	*Aegilops speltoides*	-
PI 573450	*Aegilops speltoides*	-
PI 573452	*Aegilops speltoides*	-

[a]Plus (+) and minus (-) indicate sensitive and insensitive to ToxA, respectively.

Table S6. Primers used to amplify fragments of BAC clones for use as restriction fragment length polymorphism probes.

Probe	Marker designation	BAC template	Forward primer	Reverse primer	Annealing temp.
FCG26	*Xfcg26(PT)*	1089I4	GACATGCCAATTGCTGGGAAGTCCA	CAGGTGAAGTGTCCTGACATGTTAT	55
FCG30	*Xfcg30(WK)*	507H19	CTACTTCATTAACATCCTTTCCTCC	TGAGGTTGCCATACTATCATAGGTC	55
FCG31	*Xfcg31*	507H19	CAGACAAGAAACATCATCACAGTCC	GTCACATAAAGGTACCAATGGATTGGG	55
FCG32	*Xfcg32(HP)*	604L2	TGAAGAAGAAGATCCTGCTGGCGAC	GGTGGTAGAGTATGTTTGCATGTGA	55
FCG33	*Xfcg33*	604L2	CTCAGGAACATTGCCTTCTTCG	AGTCAAATCTGCTGCCGACATC	55
FCG34[a]	*Xfcg34(Tsn1)*	604L2	TTCCTCGGCGCCTCTCACTTTGGGC	GCTATCTCCTGGCATGCAATTAAAC	55
FCG35[b]	*Xfcg35(Tsn1)*	604L2	GCATTCCGTTTCCTCCAAGTTAGCG	GTACTTCCTGCGTGATACAAAGACAC	55

[a]Probe FCG34 was derived from the LRR domain of *Tsn1*.

[b]Probe FCG35 was derived from the PK domain of *Tsn1*.

Table S7. PCR markers used for haplotype analysis of *Triticum* accessions (*Xfcp*) (table S1) and for screening BAC pools (RJM).

Marker	BAC template	Forward primer	Reverse primer	Annealing temp.
Xfcp394[a]	1089I4	GTAGCCTGCAGGTACAAACTGGA	CAGTGTTAAGAAGTGTGTTCTGGTC	60
Xfcp620[a]	507H19	CATAACCTTCATACGGACTTGCTCAC	TATTCTTGCCAGTGTTGGGAGGG	65
Xfcp623(Tsn1)	604L2	CTATTCGTAATCGTGCCTTCCG	CCTTCTCTCTCACCGCTATCTCATC	60
RJMI4	1089I4	GGTTTAACACCAATCTCGATGGTAGAGG	TTTTGCCAAGTGTGTGTGCCAGGAGG	60
RJML2a	604L2	TCCACTCTTGGTTATTCCGTGC	CGTTTTGACATCCATCTGCCAG	60
RJML2b	604L2	TGTGTCGATATTGTTCGGCTACTG	TACACCTGTGGGTCATCAAGGC	60
RJMH19	507H19	TGTGGCGTATGGGATAAAGGG	TGCCTTCCTTCACTTGTTATGTCC	60

[a]Markers previously described in (5).

Table S8. PCR primers used to amplify *Tsn1* fragments for sequencing genomic DNA.

Fragment	Forward primer	Reverse primer
1	PK.5U239F: TCCCTCTTGTTCCTCGTCTG	PK.1160R: ACTGCCGGTCCTGTCATAAA
2	Pk.F893: CCGGAATTCATCAAAGATGG	PK.Fr8.B1: CCAAAATGGAGATGGTGCTAGATCC
3	PK.lastex.F11: TTTGACCGCCTGCCAGAATG	PNL.B6: TGAAGAAGCAAAGCCCAAAGTG
4	NB.F400: TAAGCCTACCGCGCGACATTGCTCC	LRR.R2400: AGTAGGACCCATATCCACGATCAGG
5	LRR.F2300: TCCTCAAATGCATATGCCTGTGCAA	LRR.R3900: ATGCTCAAGGTTGGAAAGGGTACTG
6	LRR.F3100: AAGCAGTTGTCACTATGCATTGCT	LRR.4700: ATGTCCGAGGGCAGCGTGCTCTCAG
7	LRR.F4600: TAGAAACGAACTCTTGTTCCCTAAG	LRR.R6100: GTAACTGCTGGTATCAGCAACTTACC

Table S9. PCR primers used to amplify the *Tsn1* cDNA fragments from Langdon for sequencing.

Fragment	Forward primer	Reverse primer
1	PK.F17: TATACCGTTCGCAACTTTGG	PK.end2R: TCGTAGAGGAGGGCATATGAG
2	PK.3pr.1F: TCCAGCAGAGGAACTACATCAAGTGAA	NB.5pr.3R: TGCCAAGGTTGTCTTGCCAATTCC
3	NB.F24: TATCGCGAACAAACACCAAC	LRR.R1357: TGCATCACCGCGAAGTAGTA
4	NB.F959: TCACCGGTCCATCTGGAATA	LRR.R4300: ACAAACCTCCTAGCAGCCACAGTC
5	TILL. F2781: CAGTACCCTTTCCAACCTTGAGCAT	LRR.R3346: TCGAATCCTCAAAGCCTACC

Table S10. PCR primers used to amplify the *Tsn1* cDNA fragments from *Aegilops speltoides* accession PI542238 for sequencing.

Fragment	Forward primer	Reverse primer
1	PK.5U121F: TTCACTCTGCAACCATGCTC	PK.end2R: TCGTAGAGGAGGGCATATGAG
2	PK.3pr.1F: TCCAGCAGAGGAACTACATCAAGTGAA	NB.5pr.3R: TGCCAAGGTTGTCTTGCCAATTCC
3	NB.F24: TATCGCGAACAAACACCAAC	LRR.R1357: TGCATCACCGCGAAGTAGTA
4	Sp.LRR.F3100: AAGCAGTTGTCACTATGCATTACT	Sp.LRR.R4700: ATGTCCGAGGGCAGCGTGCTGTCAG
5	LRR. F2659: TAGAAACGAACTCTTGTTCCCTAAG	TILL.R4694: ATGTGAAAGGGTTCAGCCATTGAT

Supplemental Figure Legends

Figure S1. Southern and PCR analysis of 24 selected wheat lines. A: Southern hybridization of DNA digested with restriction enzyme *Xba*I and probed with FCG34, which is derived from the LRR region of *Tsn1*. B: PCR amplification with primers for marker *Xfcp623(Tsn1)* derived from intron five of *Tsn1*. The wheat genotypes labeled in black and blue are sensitive and insensitive to ToxA, respectively.

Figure S2. Colinearity of genes (colored ovals) within the *Tsn1* region of wheat chromosome 5B, the homoeologous region of wheat chromosome 5A, *Brachypodium* chromosome 4, and rice chromosome 9. Gene descriptions are presented in table S2. Note that *Tsn1* is present only in wheat 5B, the RNP gene (orange) is present in wheat 5B, wheat 5A, and *Brachypodium*, but not in rice, and the CP (cysteine protease-like) gene (yellow) is present in rice, *Brachypodium*, and wheat 5A, but not wheat 5B.

Figure S3. Southern and deduced amino acid sequence analysis of *Tsn1* in *Aegilops speltoides*. A: Southern blot of probes FCG34 and FCG35 (table S6) hybridized to Langdon durum, LDNfn2411 (fast neutron-induced deletion mutant), and ToxA sensitive *Ae. speltoides* accessions PI542238 and PI369263. The arrows indicate hybridizing *Tsn1* fragments. B: Alignment of Tsn1 deduced amino acid sequences from Langdon durum and *Ae. speltoides* PI542238. Blue, red, and green underlined regions indicate the protein kinase domain, nucleotide binding domain, the region containing leucine rich repeats, respectively.

Figure S4. Unrooted phylogenetic tree of 42 *Triticum* genotypes (table S1) based on full-length genomic DNA sequences (minus a ~2.8 kb segment of intron 4 with about 80% similarity to the LINE retrotransposon RIX_Yvonne_AY146588-1) of the *Tsn1* gene calculated by the UPGMA method. Blackened circles indicate nodes supported by bootstrap values >80%. GenBank accession numbers are indicated in parentheses.

Figure S5. *Tsn1* does not interact directly with ToxA. (A) Yeast two-hybrid assays. Agar plates containing selective medium (SD/-LT or SD/-LTHA) were inoculated with yeast transformants (four in one line) co-expressing bait and prey constructs as indicated. p53 and RecT, standard positive control (Clontech, CA), PRA, a newly-identified wheat protein that interacts with ToxA (Lu, S., unpublished). Transformants co-expressing ToxA and Tsn1 or the individual domains (PK , NB or LRR) of Tsn1 grew normally on SD/-LT (which selects for the expression constructs only) but failed to grow on SD/-LTHA (which selects for activation of the reporter genes *HIS3* and *ADE2*) and did not produce blue colors on X-α-gal plate (which selects for α-galactosidase activity). (B) Co-immunoprecipitation (Co-IP) assay. The *in vitro* translated bait and prey proteins (indicated on the top of each lane) were co-precipitated and analyzed by Western blot analysis as described in the text. Signals were detected from the protein samples containing ToxA and PRA but not from Tsn1 (represented by its PK domain). The *in vitro* translation of the full length Tsn1 was unsuccessful probably due to the large size of the protein (>168 KD) thus not included in the Co-IP tests.

Figure S6. RT-PCR of splice site mutants. A: cDNA of Langdon and LDNems230 amplified with primers PK.F17 and PK.R899, which flank the point mutation in LDNems230 at position 778. B: cDNA of Kulm and Kems130 amplified with primers NBS.F959 and LRR.R1357, which flank the point mutation in Kems103 at position 7,074.

Supplemental Figures

Figure S1

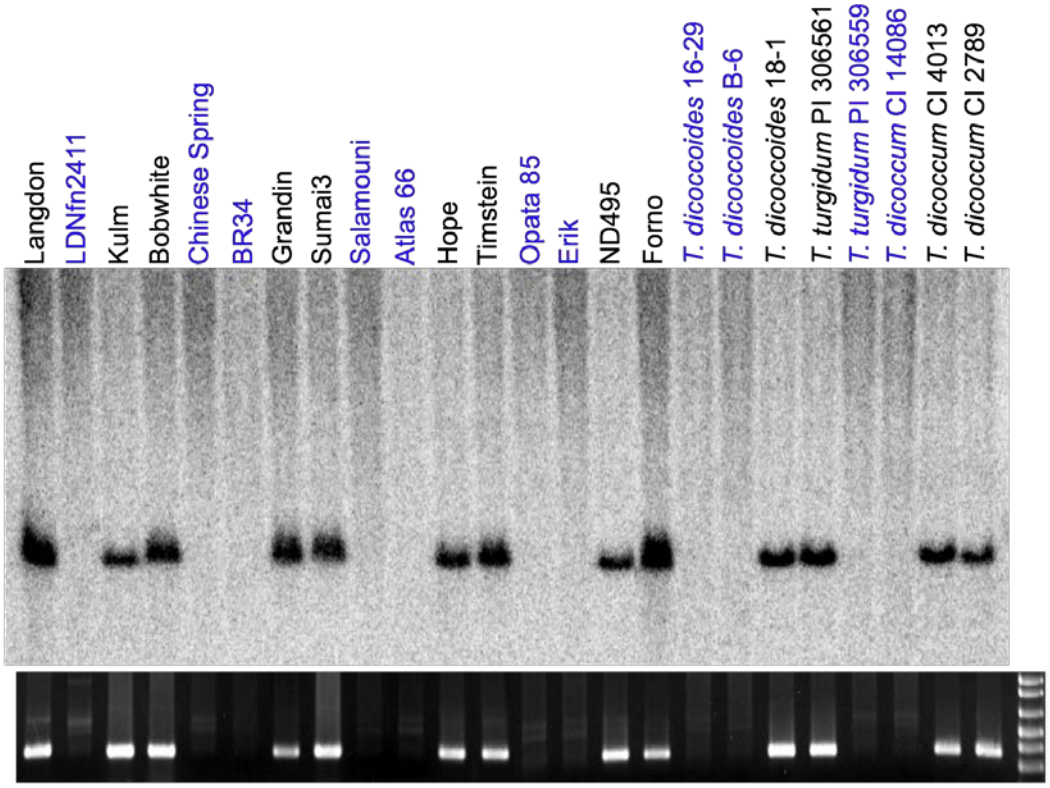
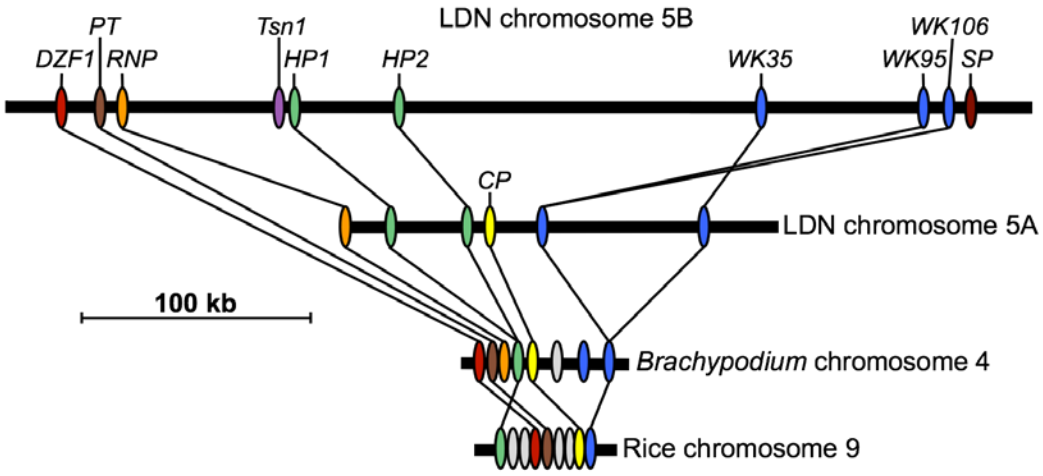


Figure S2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DZF1
RNP
Tsn1
HP1
HP2
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WK106
WK95
SP
CP
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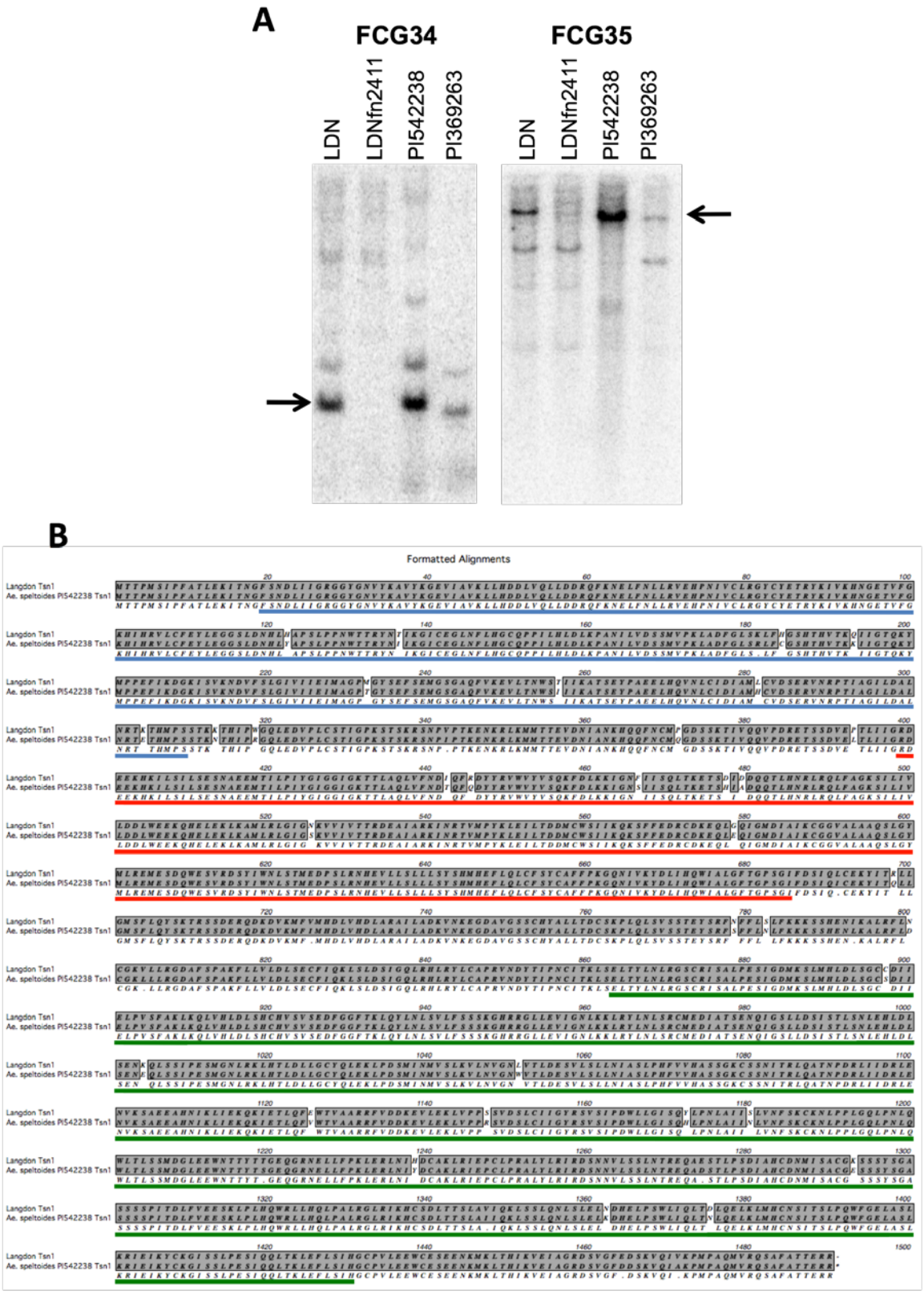
100 kb
Brachypodium chromosome 4
Rice chromosome 9

Figure S3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FCG35
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PI369263
LDN
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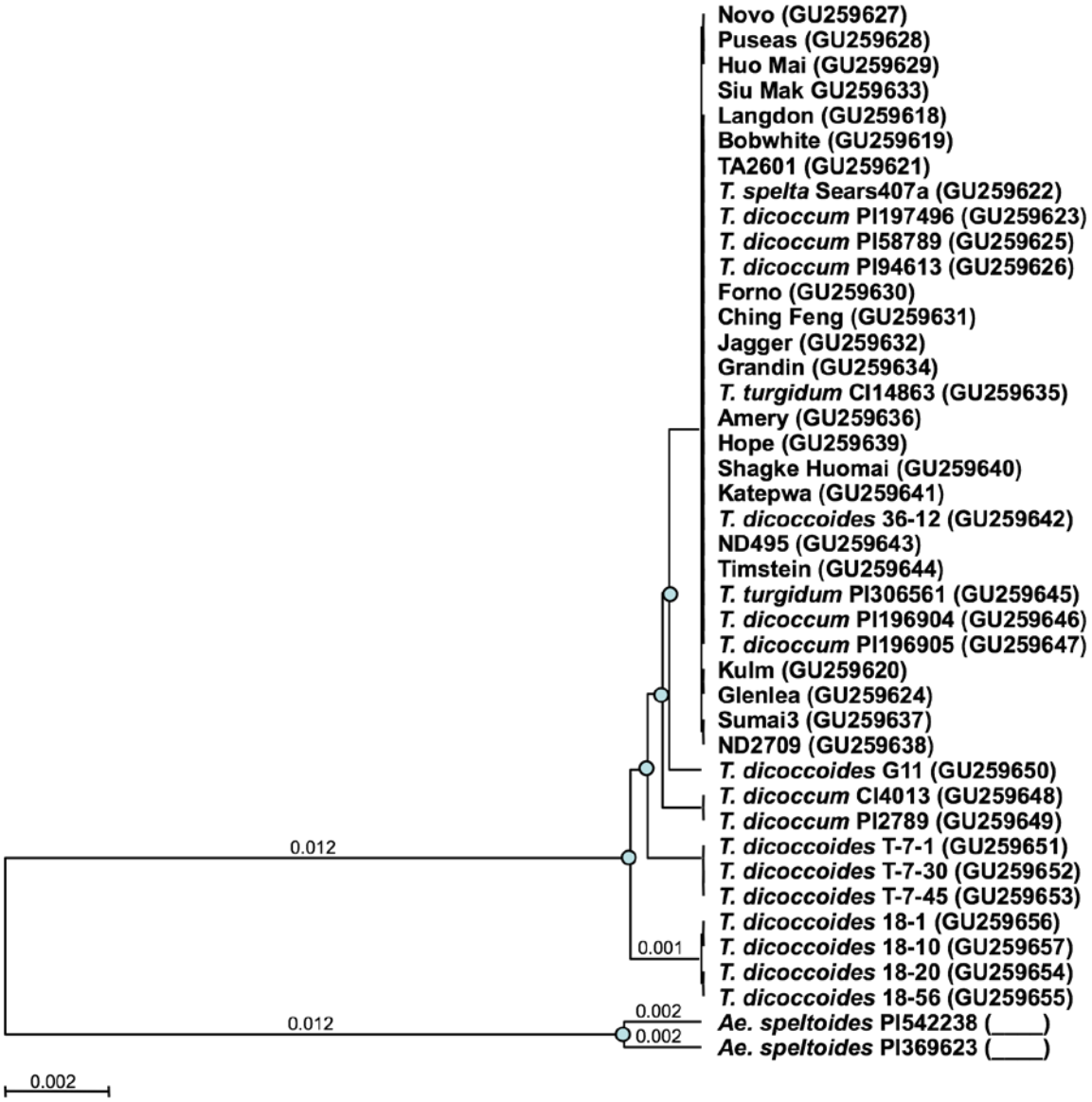
PI542238
PI369263
B
Formatted Alignments

Figure S4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Siu Mak GU259633)
Langdon (GU259618)
Bobwhite (GU259619)
TA2601 (GU259621)
T. spelta Sears407a (GU259622)
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T. dicoccum PI58789 (GU259625)
T. dicoccum PI94613 (GU259626)
Forno (GU259630)
Ching Feng (GU259631)
Jagger (GU259632)
Grandin (GU259634)
T. turgidum CI14863 (GU259635)
Amery (GU259636)
Hope (GU259639)
Shagke Huomai (GU259640)
Katepwa (GU259641)
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ND495 (GU259643)
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Kulm (GU259620)
Glenlea (GU259624)
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ND2709 (GU259638)
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Ae. speltoides PI542238 (____)
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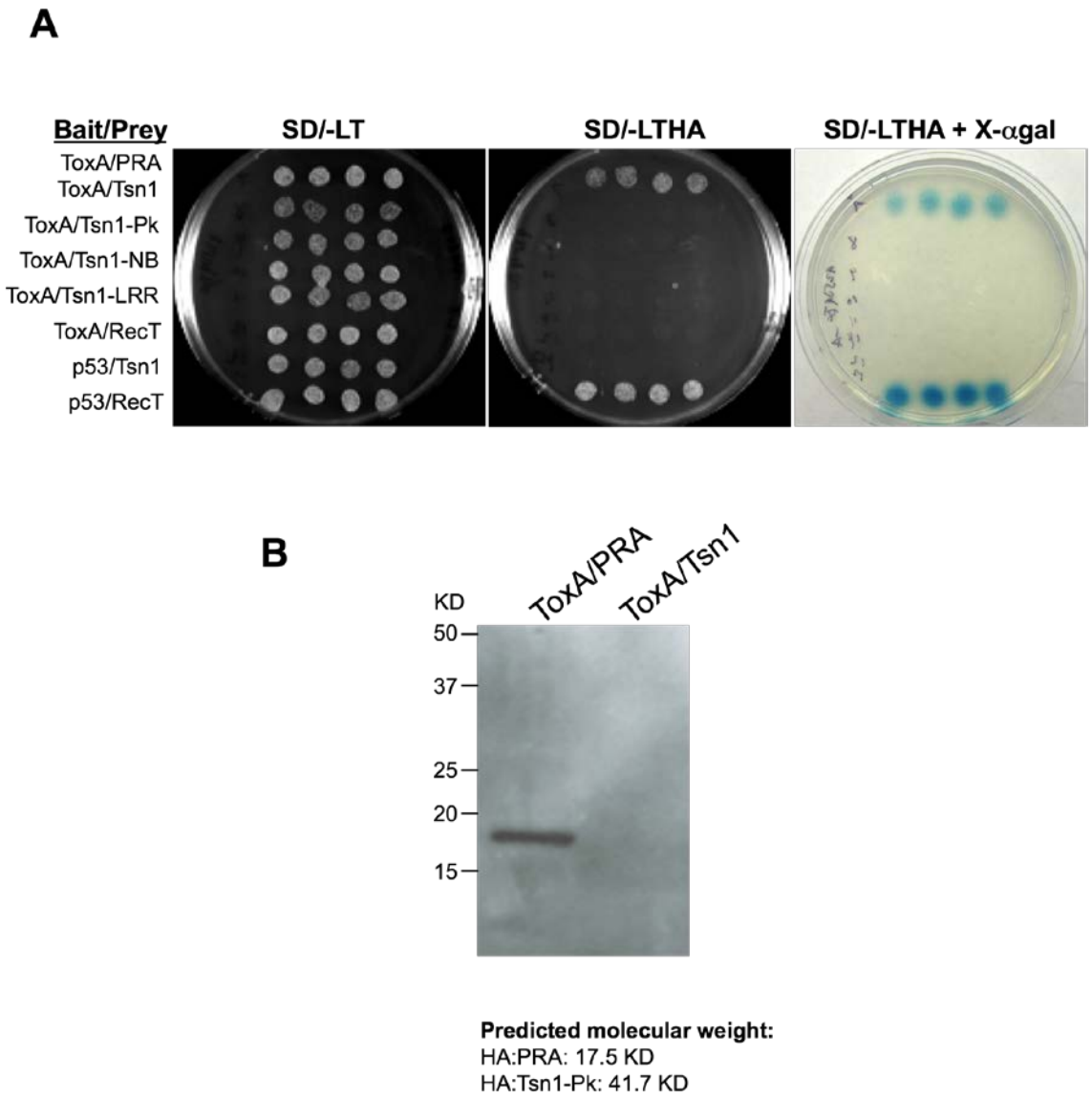
0.002
0.012
0.002
0.002

Figure S5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SD/-LT
SD/-LTHA
SD/-LTHA + X-αgal
ToxA/PRA
ToxA/Tsn1
ToxA/Tsn1-Pk
ToxA/Tsn1-NB
ToxA/Tsn1-LRR
ToxA/RecT
p53/Tsn1
p53/RecT
B
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37
25
20
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Figure S6