Interaction of the *Tsn1* and *Tsc2* sensitivity genes with the *ToxA* and *ToxB* effector genes in *Triticum* L. species

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Abstract: The present study was aimed at determining the presence of the *Tsn1* (5BL) and Tsc2 (2BL) sensitivity genes in *Triticum* L. species and at assessing the response of wheat accessions to infection with *Pyrenophora tritici-repentis* isolates with the complementary effector genes *ToxA* and *ToxB*. It was shown that the diploid species *T. urartu*, *T. boeoticum* and *T. monococcum* (2*n* = 2*x* = 14, A genome) had no *Tsn1* and *Tsc2* genes and showed no susceptibility reaction. The gene-for-gene interaction of *Tsc2-ToxB* was observed for the tetraploid species *T. aethiopicum* and *T. turgidum* (2*n* = 4*x* = 28, AB). Despite the presence of the *Tsc2* gene, the accessions of *T. dicoccoides* and *T. dicoccum* were resistant to the *ToxB*$^+$ isolate. Different types of *Tsc2-ToxB* and *Tsn1-ToxA* interactions were detected for accessions of other tetraploid and hexaploid wheat species (2*n* = 6*x* = 42, ABD). The paper discusses possible causes of discrepancies between the observed and expected plant phenotypic reactions, which were determined by the interaction of the *Tsn1* and *Tsc2* sensitivity genes with the isolates containing the *ToxA* and *ToxB* effector genes.

Key words: wheat species; tan spot; effectors; *Tsn1* and *Tsc2* sensitivity genes; gene-for-gene interactions.

1. Introduction

Tan spot caused by *Pyrenophora tritici-repentis* is a harmful disease spread worldwide. The three known genes of sensitivity to PtrToxA, PtrToxB, and PtrToxC toxins in *P. tritici-repentis* are *Tsn1, Tsc2* and *Tsc1*, respectively. The Tsn1-Ptr ToxA, Tsc2-Ptr ToxB, and Tsc1-Ptr ToxC interactions have all been shown to play a significant role in the development of tan spot in common wheat (*Triticum aestivum* L.) (Faris et al., 2013; Kariyawasam et al., 2016).

The wheat sensitivity genes *Tsn1* and *Tsc2*, as well as the *ToxA* and *ToxB* effector genes encoding PtrToxA and PtrToxB toxins, have been cloned and used for developing gene-specific primers (Faris et al., 2010; Abeysekara et al., 2010; Andrie et al., 2007).

The purpose of the present study was to identify the presence of the *Tsn1* (5BL) and *Tsc2* (2BL) sensitivity genes in *Triticum* L. species and to reveal their response to infection with isolates possessing the *ToxA* and *ToxB* complementary effector genes.

2. Materials and methods

All the 72 accessions representing 16 *Triticum* L. species from the VIR wheat collection were assessed for resistance to two isolates with *ToxA* (ToxA$^+$) originating from Kazakhstan and Russia, and one ToxB$^+$ from Greece. Pathogen specific primers were used for PCR detection of *P. tritici-repentis* isolates (Antoni et al., 2010). *ToxA* and *ToxB* identification in fungal isolates was performed using gene-specific primers (Andrie et al., 2007). The Tsn1 sensitivity gene was identified using functional allele-specific primers (Faris et al., 2010), and *Tsc2*, by using the XBE444541 marker (Abeysekara et al., 2010).

The procedure of resistance determination was based on determining the size of necrotic and chlorotic spots that formed after inoculation of seedling leaves with a conidial suspension. The detached leaf assays in benzimidazole (40 mg/l) were used. In the present work, the reaction of wheat accessions to the inoculation with ToxA$^+$ isolates (necrosis) and to the ToxB$^+$ isolate (chlorosis) below 2 points were considered as resistance.

3. Results and discussion

According to the gene-for-gene model, necrosis and/or chlorosis are observed on wheat leaves when both the plant and pathogen have the dominant genes *Tsn1/ToxA* and/or *Tsc2/ToxB*, respectively. Neither *Tsn1* nor *Tsc2* genes nor sensitivity reactions have been detected in all accessions of the diploid species *T. urartu, T. boeoticum* and *T. monococcum*. The gene-for-gene *Tsc2-ToxB* interaction was observed in the tetraploid wheats *T. aethiopicum* and *T. turgidum*, as well as in most accessions of the hexaploid wheats *T. sphaerococcum* and *T. compactum*, which had *Tsc2* and were sensitive to the ToxB$^+$ isolate.

Out of 14 *T. dicoccoides* and *T. dicoccum* accessions, 12 were resistant to the ToxB$^+$ isolate despite the presence of the *Tsc2* gene. Such a 'gene/plant reaction' link can be explained either by a mutation in the gene that disrupted its expression or by the presence in accessions of other gene(s) homologous to *Tsc2*.

When evaluating the *Tsn1-ToxA* interaction, 8 accessions of different tetra- and hexaploid wheats (*T. aethiopicum, T. durum, T. sphaerococcum*) aroused particular interest. The dominant allele *Tsn1* was not detected in these accessions, but strong necrosis was observed after infection with the ToxA$^+$ isolate, which is possibly due to the existence of other unknown susceptibility and effector genes. A number of accessions, in constrast, displayed the presence of *Tsn1* and the absence of susceptibility reactions. The reasons may be the same as in the case with Tsc2/ToxB interaction in *T. dicoccoides* and *T. dicoccum*. The studied accessions of *T. macha* and *T. spelta* were resistant to the ToxA$^+$ and ToxB$^+$ isolates,

while no *Tsn1* or *Tsc2* genes were identified in them, with one exception.

Deviations from the expected gene-for-gene relationships in common wheat, which were observed among the cultivars of *T. aestivum* (Mironenko, Kovalenko, 2018), can have an explanation that the *Tsn1-ToxA* interaction may be epistatic to the production of other necrotrophic effectors, depending on the genetic background of the sensitive host (Manning, Ciuffetti, 2015). The authors believe that perhaps some wheat genotypes possess factors that lead to altered expression levels of the *ToxA* gene through epistasis, or in some way inhibit the recognition of *ToxA* by *Tsn1* in plants inoculated with fungal spores.

The *Tsc2-ToxB* interaction is known to play a significant role in conferring susceptibility in tetraploid (Virdi et al., 2016) and hexaploid wheats (Abeysekara et al., 2010), while the *Tsn1-ToxA* interaction is not a significant factor for the development of tan spot in durum cultivars (Virdi et al., 2016). According to some researchers, the *Tsn1-ToxA* interaction can play a major or a minor role, or have no effect at all for common wheat, depending on the genetic background (Faris et al., 2013).

The analyzed accessions of *T. timopheevii* and *T. araraticum* ($2n = 4x = 28$, GA genome) have *Tsc2*, but not *Tsn1*; they were found to be resistant to the $ToxA^+$ and $ToxB^+$ isolates, which suggests the presence of a gene (genes) homologous to *Tsc2* in these accessions.

4. Conclusions

It is believed that the *ToxA* gene appeared in *P. tritici-repentis* as a result of horizontal transfer from another wheat pathogen, *Stagonospora nodorum* (Friesen et al., 2006). Therefore, *Tsn1* is the major determinant for sensitivity to both *S. nodorum* blotch (SNB) and tan spot. The *ToxA* gene in *P. tritici-repentis* is less expressed than the identical gene *SnToxA* in *S. nodorum* during wheat infection (Virdi et al., 2016). The high role of the *Tsn1-ToxA* interaction was shown for the SNB manifestation in both hexaploid and tetraploid wheat (Liu et al., 2006).

According to our data, the *Tsn1-ToxA* interaction does not always play a significant role in conferring susceptibility to tan spot in hexaploid wheats in the wheat-*P. tritici-repentis* pathosystem, and has no effect on this disease manifestation in durum wheat, in contrast to the *Tsn1-ToxA* interaction in the wheat-*S. nodorum* pathosystem. We believe that one of the reasons for these differences may be the low expression of the *ToxA* gene in *P. tritici-repentis*, which was introduced into this pathogen as part of the alien translocation from another wheat pathogen, *S. nodorum* (Friesen et al., 2006).

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