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# A revised nomenclature for *ToxA* haplotypes across multiple fungal species

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**Abstract**

ToxA is one of the most studied proteinaceous necrotrophic effectors produced by plant pathogens. It has been identified in four pathogens (*Pyrenophora tritici-repentis*, *Parastagonospora nodorum*, *Parastagonospora pseudonodorum* (formerly *Parastagonospora avenaria* f. sp. *tritici*) and *Bipolaris sorokiniana*) causing leaf spot diseases on cereals worldwide. To date, 24 different *ToxA* haplotypes have been identified. Some *Py. tritici-repentis* and related species also express ToxB, another small protein necrotrophic effector. We present here a revised and standardized nomenclature for these effectors, which could be extended to other poly-haplotypic genes found across multiple species.

**Keywords**

Allelic variation, haplotypes, necrotrophic effectors, tan spot, yellow leaf spot, septoria nodorum blotch, spot blotch, common root rot, *ToxA*, *ToxB*

52 ***Introduction***

53 ToxA is a major necrotrophic effector produced by a number of fungal pathogenic species
54 in the order Pleosporales. It is the first proteinaceous effector identified as a host-specific toxin
55 from a fungal species. It was originally discovered in *Pyrenophora (Py) tritici-repentis*, the
56 pathogen causing tan spot disease of wheat (Ballance et al. 1989; Tomas et al.1990; Tuori et al.
57 1995; Zhang et al. 1997). The ToxA effector causes strong necrosis only in sensitive wheat
58 genotypes carrying the dominant sensitivity gene *Tsn1* (reviewed in Faris et al. 2013). The coding
59 gene, *ToxA*, was found as a single copy in *Py. tritici-repentis Parastagonospora (Pa) nodorum*
60 and its sister species *Parastagonospora (Pn) pseudonodorum* (both species cause septoria
61 nodorum blotch mainly of wheat); and in *Bipolaris sorokiniana* (the pathogen causing common
62 root rot and spot blotch of wheat and barley) (reviewed in Hafez et al. 2022). The *ToxA* genes are
63 95.3-100% similar in these species. *ToxA* has been reported to have been horizontally transferred
64 between species as a gene embedded within a large transposon (Friesen et al. 2006; McDonald et
65 al. 2019; Gourlie et al. 2022) or presumably through rare gene introgression events between *Pa.*
66 *nodorum* and *Pa. pseudonodorum* (McDonald et al. 2013; Croll et al., 2021). Interestingly, a
67 *ToxA*-like gene (*ChToxA*) was identified from another Pleosporales species, *Cochliobolus*
68 *heterostrophus*, the causal agent of southern corn leaf blight in maize; the coded proteins shared
69 64% similarity. ChToxA has not been shown to possess ToxA like necrotic activity (Lu et al.
70 2015).

71

72 ToxA is the most common known *Py. tritici-repentis* effector worldwide, as annual surveys
73 of *Py. tritici-repentis* in North America over the past 30 years showed that over 98% of these
74 isolates were ToxA-producers (Aboukhaddour et al. 2013; Aboukhaddour et al. 2021). The
75 predominance of ToxA has been explained by the widespread cultivation of *Tsn1*-carrying wheat
76 (Lamari et al. 2005; Tran et al. 2007; Wei et al. 2021), and a recent gain of the *ToxA* gene into the
77 *Py. tritici-repentis* genome through horizontal gene transfer (Friesen et al. 2006). Whereas in *Pa.*
78 *nodorum,* the *ToxA* gene has been reported at a high percentage in isolates from Australia and
79 South Africa (Friesen et al. 2006), its distribution in the USA varied considerably among certain
80 regions (Richards et al. 2019). Over 95% of *Pa. nodorum* isolates were *ToxA*-coding in the Upper
81 Midwest of the USA, where *Tsn1* cultivation is dominant. In comparison, less than 6% of isolates
82 in the Southern, Eastern, and Pacific Northwest regions were *ToxA*-coding. These regions are

83 dominated by wheat cultivars lacking *Tsn1* (Richards et al. 2019). In *B. sorokiniana* and *Pa.*
84 *pseudonodorum*, *ToxA* has been reported more recently, and more studies are needed to determine
85 its prevalence in these species worldwide.
86
87 ***Why do we need a revised nomenclature system for ToxA haplotypes?***
88 The last agreed on nomenclature for *Py. tritici-repentis* effectors (Ciuffetti et al. 1998),
89 followed discussions at the 3rd International Tan Spot Workshop (Winnipeg, Canada) and the 3rd
90 Tottori International Symposium on Host-Selective Toxins (Tottori, Japan). Since 1998, however,
91 several fungal species have been reported to possess homologs to these effectors, and allelic
92 variation in the coding genes of *ToxA* and *ToxB* (haplotypes) became evident (reviewed in Hafez
93 et al. 2020 & 2022).
94 The ability of *Py. tritici-repentis* isolates to secrete necrosis-inducing toxins in culture
95 filtrate was first established at Kansas State University (Tomas and Bockus, 1987). Subsequently,
96 several research groups working around the same time in Canada and the USA purified the protein
97 toxin, known today as the ToxA effector, which was initially named Ptr necrosis toxin (Ballance
98 et al. 1989) or Ptr toxin (Tomas et al. 1990). Tuori et al. (1995) purified ToxA from the same
99 isolate (a sub-culture) used by Tomas et al. (1990), and Zhang et al. (1997) purified ToxA from
100 the same isolate used by Ballance et al. (1989). This was followed by cloning its coding gene
101 (Ballance et al. 1996; Ciuffetti et al. 1997; Zhang et al. 1997).
102 Currently, 24 different *ToxA* haplotypes have been reported in the literature from four
103 fungal species across diverse geographical origins (Stukenbrock & McDonald. 2007; McDonald
104 et al. 2013; Friesen et al.2018; McDonald et al. 2018; Navathe et al. 2020; Ghaderi et al. 2020;
105 Hafez et al. 2020 & 2022), with additional reports of new haplotypes to be released
106 (Aboukhaddour, personal communications) (Table 1; Figure 1). These haplotypes code for various
107 isoforms of the ToxA protein (Hafez et al. 2020 & 2022), which vary in activity and affect
108 pathogen sporulation levels in planta (Tan et al. 2012). To date, the haplotypes have been named
109 randomly, with different haplotypes often given the same code or, conversely, the same haplotype
110 given different names. A consistent and standardized approach to haplotype naming is of value,
111 particularly when tracing them in pathogen population studies. For example, McDonald et al.
112 (2013) used H14, H15, and H16 to describe *ToxA* haplotypes in *P. nodorum*, while these same
113 codes (H14, H15, H16) were used to describe *ToxA* haplotypes in *Py. tritici-repentis* (Stukenbrock

114 and McDonald, 2007), and were cited as such in Kamel et al. (2019); Hafez et al. (2020);
115 Aboukhaddour et al. (2021). Careful examination of these haplotypes (Table 1) indicated that H14,
116 H15, and H16 from *Py. tritici-repentis* were identical and, therefore, were re-designated as PtrA1
117 in Hafez et al. (2022). Ghaderi et al. (2020) reported a *ToxA* haplotype termed H21 in *Py. tritici-*
118 *repentis*, which is identical to the previous haplotype H15, renamed as PtrA1 by Hafez et al.
119 (2022). Moreover, H21 was also used to describe a novel *ToxA* haplotype from *Pa. nodorum* in
120 Canada (Hafez et al. 2020). Two haplotypes are characterized in *B. sorokiniana*, denoted as
121 BsToxA1 and BsToxA2, or AusBsToxA and TexBsToxA to differentiate among *ToxA* haplotypes
122 in Australia vs. Texas, respectively (McDonald et al. 2018; Friesen et al. 2018). A standardized
123 nomenclature to trace these haplotypes among various species will help to avoid confusion in the
124 literature.

125 ***Suggested nomenclature***

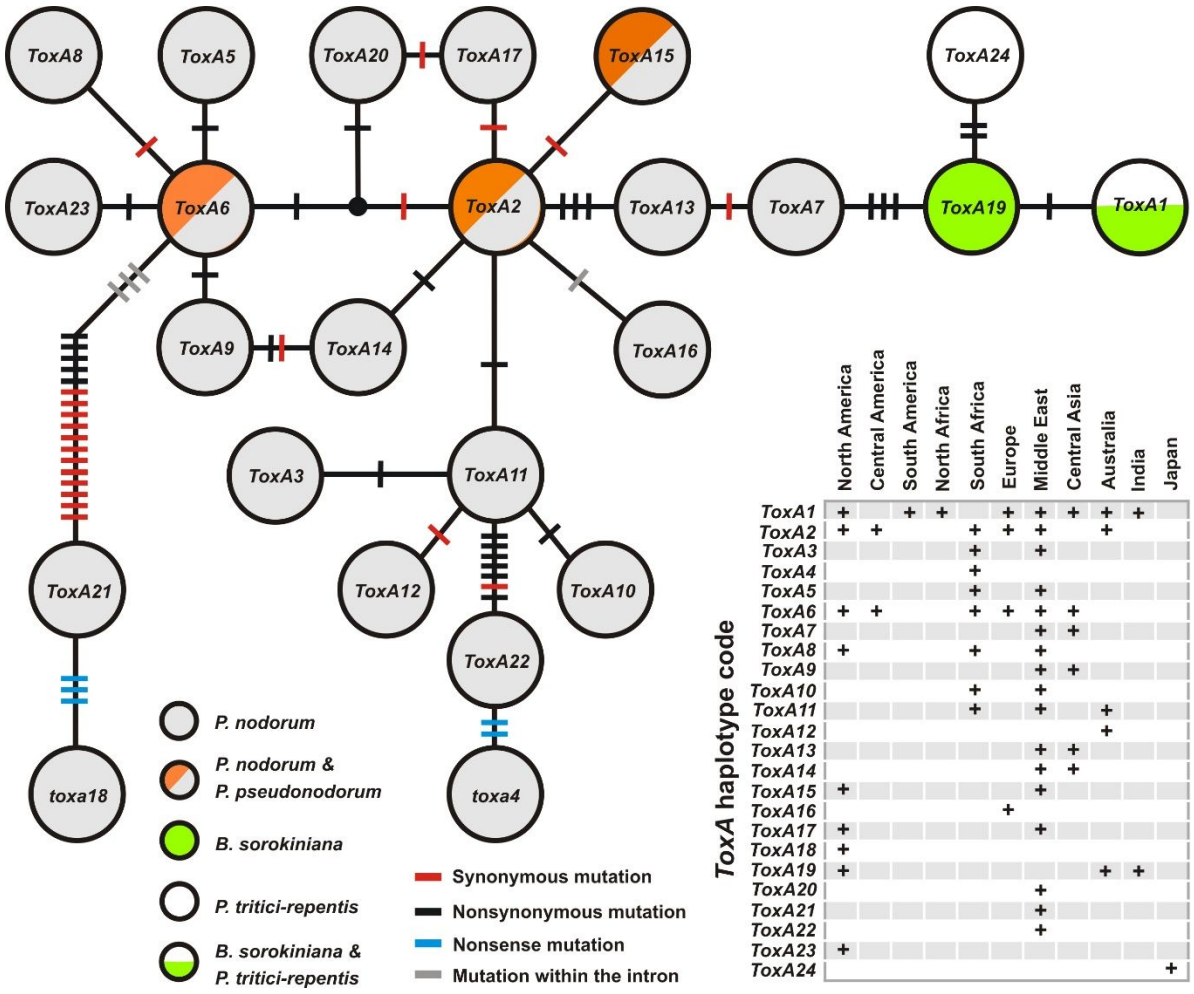
126 Here, we suggest using the "*ToxA*" abbreviation followed by the haplotype number (#)
127 assigned in the chronological order of haplotype's identification. *ToxA* is used for haplotypes that
128 induce necrosis and *toxa* for inactive ones. The first *ToxA* haplotype was identified in *Py. tritici-*
129 *repentis* (Ballance et al. 1996; Ciuffetti et al. 1997) and is denoted here as *ToxA1*. The second
130 haplotype was discovered 10 years later in *Pa. nodorum* (Friesen et al. 2006), and we denote it
131 here as *ToxA2*. This was followed by the identification of 21 further haplotypes in various species
132 that we name here as *ToxA3* to *ToxA24* (Table 1).

133 The septoria nodorum blotch pathogen, *Pa. nodorum* exhibits a high diversity of *ToxA*. So
134 far 21 haplotypes have been identified in *Pa. nodorum*, of which 18 are unique to *P. nodorum,* and
135 three are shared with *Pa. pseudonodorum* (Stukenbrock & McDonald 2007; McDonald et al. 2013;
136 McDonald et al. 2018; Ghaderi et al. 2020; Hafez et al. 2020). In *Py. tritici-repentis*, two
137 haplotypes have been found; the first one *(ToxA1*) is widely present in a worldwide collection of
138 isolates. Recently, a second haplotype was identified in isolates from Japan (Hafez et al. 2022) and
139 is denoted here as *ToxA24.* In *B. sorokiniana*, two haplotypes have been reported (McDonald et
140 al. 2018; Friesen et al. 2018), with one identical to *ToxA1* and the second unique to *B. sorokiniana*
141 and denoted here as *ToxA19* (McDonald et al. 2018; Friesen et al. 2018; Hafez et al. 2022).

142 All *ToxA* haplotypes reported were found to have active toxicity and induced necrosis on
143 sensitive wheat genotypes that express *Tsn1* Two haplotypes reported in *Pa. nodorum*, *toxa4* and
144 *toxa18* are not known to cause necrosis in any wheat line (Stukenbrock & McDonald 2007;

145 McDonald et al. 2013) (Table 1). The presence of premature stop codons (nonsense mutations) in
146 the *toxa4 and toxa18* open reading frames indicates the non-functionality of these haplotypes.
147
148 **Extending the nomenclature to *ToxB*:**
149 Here, we suggest extending the revised nomenclature system for *ToxB* haplotypes (and the
150 homolog, the *toxb* gene) in a manner similar to that proposed for *ToxA* haplotypes: the use of
151 "*ToxB*/or *toxb"* followed by the haplotype number (#). In total, 11 *ToxB*/*toxb* haplotypes have been
152 identified to date, five of which occur in *Py. tritici-repentis* and are denoted here as *ToxB1, toxb2,*
153 *toxb3, toxb4* and *ToxB5*, and six in its sister species *Pyrenophora bromi*, the causal agent of brown
154 spot on bromegrass, and are denoted as *ToxB6* to *ToxB11* (Table 1).
155 ToxB was the second necrotrophic effector identified in *Py. tritici-repentis* and is a
156 chlorosis-inducing effector encoded by the multi-copy *ToxB* gene (Strelkov & Lamari 2003;
157 Strelkov et al. 2005). The *ToxB* gene is not as well explored as *ToxA*, simply due to the lack of
158 ToxB-producing isolates in North America and Australia, where much of the work on *Py. tritici-*
159 *repentis* and its effectors has been carried out. Six homologs of *ToxB* have been identified in *Py.*
160 *bromi* and named *PbToxB* to differentiate their species of origin (Andrie et al. 2008). Here, we
161 designated these six homologs as *ToxB6* to *ToxB11* (Table 1). The *ToxB* haplotypes identified in
162 *P. bromi* possess toxic activity toward *ToxB*-sensitive wheat genotype, 6B662, but lack this
163 activity on its original host, the bromegrass (Andrie et al. 2011). These names would replace the
164 ambiguous *ToxB* and *toxb* used previously for the active and inactive *ToxB* haplotypes,
165 respectively
166
167
168 A remaining challenge will be how to keep haplotype naming accurate. This will require a
169 collective effort and open communication, yet some obstacles are to be expected. We have created
170 a GitHub repository (Aboukhaddour et al. 2023) of described *ToxA* and *ToxB* haplotypes, and
171 alleles. We are seeking input on how to enhance the nomenclature and keep it updated. We hope
172 that publishing this as an open-access article and circulating it amongst the plant pathology
173 community actively involved or interested in the subject matter will facilitate discussions. Open
174 communication ahead of time with respect to denoting names may help to maintain a proper
175 naming system and keep it in order.

**Fig. 1:** Twenty-four *ToxA* haplotypes were previously described. Among these, 21 haplotypes were identified in *Parastagonospora nodorum* (*ToxA2-ToxA18* and *ToxA20-ToxA23*) and three in *Pa. pseudonodorum* (*ToxA2, ToxA6,* and *ToxA15*). The three *Pa. pseudonodorum* haplotypes were shared between *Pa. nodorum* and *Pa. pseudonodorum*. A total of three *ToxA* haplotypes were identified outside *Pa. nodorum*/*Pa. pseudonodorum*, with one haplotype unique to *Bipolaris sorokiniana* (*ToxA19*), a second one unique to *Pyrenophora tritici-repentis* (*ToxA24*), and a third haplotype shared between *B. sorokiniana* and *Py. tritici-repentis* (*ToxA1*). Each circle represents a unique *ToxA* haplotype, and the hatch marks along the network branches indicate the number of mutations. Red, black and blue hatch marks along the network branches represent synonymous, nonsynonymous, and nonsense mutations, respectively. Grey hatch marks represent mutations located in the intron. The geographical origins of different *ToxA* haplotypes are also indicated. The non-functional haplotypes indicated by lower case *toxa4* and *toxa18* contain nonsense mutations and are unlikely to translate into functional proteins. Detailed information for reference sequences used to construct the *ToxA* haplotype network is provided in Table 1. This figure was adapted from Stukenbrock and McDonald (2007); McDonald et al. (2013); McDonald et al. (2018); Kamel et al. (2019); Ghaderi et al. (2020); Hafez et al. (2020); and Hafez et al. (2022).



196

197 **Table 1:** Effector haplotypes in necrotrophic fungal pathogens. *ToxA* haplotypes were reported in
198 *Parastagonospora nodorum* (*ToxA2-ToxA18* and *ToxA20-ToxA23*); *Parastagonospora*
199 *pseudonodorum* (*ToxA2, ToxA6* and *ToxA15*); *Bipolaris sorokiniana* (*ToxA1* and *ToxA19*); and
200 *Pyrenophora tritici-repentis* (*ToxA1* and *ToxA24*). *ToxB* haplotypes were reported in *Py. tritici-*
201 *repentis* (*ToxB1-ToxB5*) and its sister species *Pyrenophora bromi* (*ToxB6-ToxB11*). GenBank
202 accession numbers and reference isolates are also indicated for each haplotype. Old names and
203 associated references for *ToxA* and *ToxB* were also indicated.

| Effector haplotype | Species | Reference isolate | Accession number | Reference | Old name |
|---|---|---|---|---|---|
| ***ToxA1*** | *Py. tritici-repentis* | Pt-lC-BFP | AF004369 | Ciuffetti et al. 1997 | H15 [a, b, c]<br>H21[d]<br>H23[e]<br>PtrH1[f] |
| | *B. sorokiniana* | BRIP10943 | KX816408 | McDonald et al. 2018 | BsToxA1[g]<br>AusBsToxA[h]<br>BsH1[f] |
| ***ToxA2*** | *Pa. nodorum* | Sn01Aus.A1 | EF108451 [(*)] | Stukenbrock and McDonald 2007 | H1 |
| | *Pa. pseudonodorum* | AI829 | JX997420 | McDonald et al. 2013 | |
| ***ToxA3*** | *Pa. nodorum* | SnSa95.8 | EF108458 [(*)] | Stukenbrock and McDonald 2007 | H2 |
| ***toxa4*** | *Pa. nodorum* | SnSA95.113 | EF108456 [(*)] | Stukenbrock and McDonald 2007 | H3 |
| ***ToxA5*** | *Pa. nodorum* | Sn95SA.103 | EF108455 [(*)] | Stukenbrock and McDonald 2007 | H4 |
| ***ToxA6*** | *Pa. nodorum* | NNDKXE02-1 | EF108454 [(*)] | Stukenbrock and McDonald 2007 | H5 |
| | *Pa. pseudonodorum* | AP1156 | JX997421 | McDonald et al. 2013 | |
| ***ToxA7*** | *Pa. nodorum* | SnTJ1-3 | EF108463 [(*)] | Stukenbrock and McDonald 2007 | H6 |
| ***ToxA8*** | *Pa. nodorum* | SnSA95.134 | EF108457 [(*)] | Stukenbrock and McDonald 2007 | H7 |
| ***ToxA9*** | *Pa. nodorum* | SnCA1-3 | EF108461 [(*)] | Stukenbrock and McDonald 2007 | H8 |
| ***ToxA10*** | *Pa. nodorum* | SnSA95.23 | EF108459 [(*)] | Stukenbrock and McDonald 2007 | H9 |
| ***ToxA11*** | *Pa. nodorum* | Sn01AUS.A2 | EF108452 [(*)] | Stukenbrock and McDonald 2007 | H10 |
| ***ToxA12*** | *Pa. nodorum* | Sn01AUS.B2 | EF108453 [(*)] | Stukenbrock and McDonald 2007 | H11 |
| ***ToxA13*** | *Pa. nodorum* | SnKZ30-5 | EF108462 [(*)] | Stukenbrock and McDonald 2007 | H12 |
| ***ToxA14*** | *Pa. nodorum* | SnKZ3-1-6 | EF108460 [(*)] | Stukenbrock and McDonald 2007 | H13 |
| ***ToxA15*** | *Pa. pseudonodorum* | AI825 | JX997416 | McDonald et al. 2013 | H15 |
| | *Pa. nodorum* | IRAN_FN313 | NA | Ghaderi et al. 2020 | |
| ***ToxA16*** | *Pa. nodorum* | AS1298 | JX997419 | McDonald et al. 2013 | H14 |
| ***ToxA17*** | *Pa. nodorum* | AD260 | JX997418 | McDonald et al. 2013 | H16 |
| ***toxa18*** | *Pa. nodorum* | AF385 | JX997417 | McDonald et al. 2013 | H17 |
| ***ToxA19*** | *B. sorokiniana* | WAI2674 | KX816409 | McDonald et al. 2018 | BsToxA2 [g]<br>TexBsToxA [h]<br>H2 [d]<br>BsH2 [f] |
| ***ToxA20*** | *Pa. nodorum* | IRAN_Fdez15 | NA | Ghaderi et al. 2020 | H18 |
| ***ToxA21*** | *Pa. nodorum* | IRAN_FN14 | NA | Ghaderi et al. 2020 | H19 |
| ***ToxA22*** | *Pa. nodorum* | IRAN_FKBG_4 | NA | Ghaderi et al. 2020 | H20 |
| ***ToxA23*** | *Pa. nodorum* | G211-5 | MT052949 | Hafez et al. 2020 | H21 |
| ***ToxA24*** | *Py. tritici-repentis* | K1 | MZ508320 | Hafez et al. 2022 | PtrH2 |
| ***ToxB1*** | *Py. tritici-repentis* | Alg3-24 | AF483831.1 | Strelkov and Lamari 2003 | *ToxB* [g] |
| ***toxb2*** | *Py. tritici-repentis* | 90-2 | AF483832.1 | Strelkov and Lamari 2003 | *toxb* [g] |
| ***toxb3*** | *Py. tritici-repentis* | D308 | AY243461.2 | Strelkov et al. 2005 | *toxb* [g] |

| | | | | | |
|---|---|---|---|---|---|
| ***toxb4*** | *Py. tritici-repentis* | Ls13-14 | MN864562.1 | Guo et al. 2020 | *toxb* [g] |
| ***ToxB5*** | *Py. tritici-repentis* | Alg215 | RXHK00000000 | Moolhuijzen et al. 2022 | *ToxB* [g] |
| ***ToxB6*** [h] | *Py. bromi* | SM101 | EF452437.1 | Andrie et al. 2008 | Pb(SM101) ToxB1 |
| ***ToxB7*** [i] | *Py. bromi* | TW123 | EF452442.1 | Andrie et al. 2008 | Pb(TW123) ToxB |
| ***ToxB8*** [i] | *Py. bromi* | SM106 | EF452439.1 | Andrie et al. 2008 | Pb(SM106) ToxB1 |
| ***ToxB9*** [j] | *Py. bromi* | SM106 | EF452440.1 | Andrie et al. 2008 | Pb(SM106) ToxB2 |
| ***toxb10*** [k] | *Py. bromi* | Bf-1 | EF452435.1 | Andrie et al. 2008 | Pb(Bf-1) ToxB1 |
| ***ToxB11*** [j] | *Py. bromi* | SM101 | EF452438.1 | Andrie et al. 2008 | Pb(SM101) ToxB2 |

[a] Stukenbrock and McDonald (2007)
[b] Kamel et al (2019)
[c] Aboukhaddour et al (2021)
[d] Ghaderi et al. (2020)
[e] Hafez et al. (2020)
[f] Hafez et al. (2022)
[g] McDonald et al. (2018)
[h] Friesen et al. (2018)
[g] No haplotype numbers were previously assigned to *ToxB* or its homolog (*toxb*).
[h-k] Heterologously expressed protein from these haplotypes was infiltrated at different concentrations (9.5, 19 & 38 ng/µl.) into the 6B662 wheat line, and chlorotic symptoms were observed as reported by Andrie and Ciuffetti 2011 and summarized below:
[h] Induce chlorosis similar to that induced by Ptr ToxB at concentrations 9.5, 19 & 38 ng/µl.
[i] Only induce chlorosis when infiltrated at a concentration of 38 ng/µl.
[j] Gave weak chlorosis at 9.5 ng/µl, but chlorosis symptoms intensified at the higher concentrations of 19 and 38 ng/µl, but never reached the levels of chlorosis caused by Ptr ToxB.
[k] Gave no chlorosis symptoms at any concentration.
[*] Intron-exon junctions for *ToxA* sequences submitted to GenBank from Stukenbrock and McDonald (2007) were corrected here. These sequences should contains "T" (not "A") at position 405 in relation to the start codon of *ToxA* intron-less ORF.

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