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*

Introduction

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

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

dominated by wheat cultivars lacking *Tsn1* (Richards et al. 2019). In *B. sorokiniana* and *Pa. pseudonodorum*, *ToxA* has been reported more recently, and more studies are needed to determine its prevalence in these species worldwide.

Why do we need a revised nomenclature system for ToxA haplotypes?

The last agreed on nomenclature for *Py. tritici-repentis* effectors (Ciuffetti et al. 1998), followed discussions at the 3rd International Tan Spot Workshop (Winnipeg, Canada) and the 3rd Tottori International Symposium on Host-Selective Toxins (Tottori, Japan). Since 1998, however, several fungal species have been reported to possess homologs to these effectors, and allelic variation in the coding genes of *ToxA* and *ToxB* (haplotypes) became evident (reviewed in Hafez et al. 2020 & 2022).

The ability of *Py. tritici-repentis* isolates to secrete necrosis-inducing toxins in culture filtrate was first established at Kansas State University (Tomas and Bockus, 1987). Subsequently, several research groups working around the same time in Canada and the USA purified the protein toxin, known today as the ToxA effector, which was initially named Ptr necrosis toxin (Ballance et al. 1989) or Ptr toxin (Tomas et al. 1990). Tuori et al. (1995) purified ToxA from the same isolate (a sub-culture) used by Tomas et al. (1990), and Zhang et al. (1997) purified ToxA from the same isolate used by Ballance et al. (1989). This was followed by cloning its coding gene (Ballance et al. 1996; Ciuffetti et al. 1997; Zhang et al. 1997).

Currently, 24 different *ToxA* haplotypes have been reported in the literature from four fungal species across diverse geographical origins (Stukenbrock & McDonald. 2007; McDonald et al. 2013; Friesen et al.2018; McDonald et al. 2018; Navathe et al. 2020; Ghaderi et al. 2020; Hafez et al. 2020 & 2022), with additional reports of new haplotypes to be released (Aboukhaddour, personal communications) (Table 1; Figure 1). These haplotypes code for various isoforms of the ToxA protein (Hafez et al. 2020 & 2022), which vary in activity and affect pathogen sporulation levels in planta (Tan et al. 2012). To date, the haplotypes have been named randomly, with different haplotypes often given the same code or, conversely, the same haplotype given different names. A consistent and standardized approach to haplotype naming is of value, particularly when tracing them in pathogen population studies. For example, McDonald et al. (2013) used H14, H15, and H16 to describe *ToxA* haplotypes in *P. nodorum*, while these same codes (H14, H15, H16) were used to describe *ToxA* haplotypes in *Py. tritici-repentis* (Stukenbrock

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

Suggested nomenclature

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

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

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

McDonald et al. 2013) (Table 1). The presence of premature stop codons (nonsense mutations) in the *toxa4 and toxa18* open reading frames indicates the non-functionality of these haplotypes.

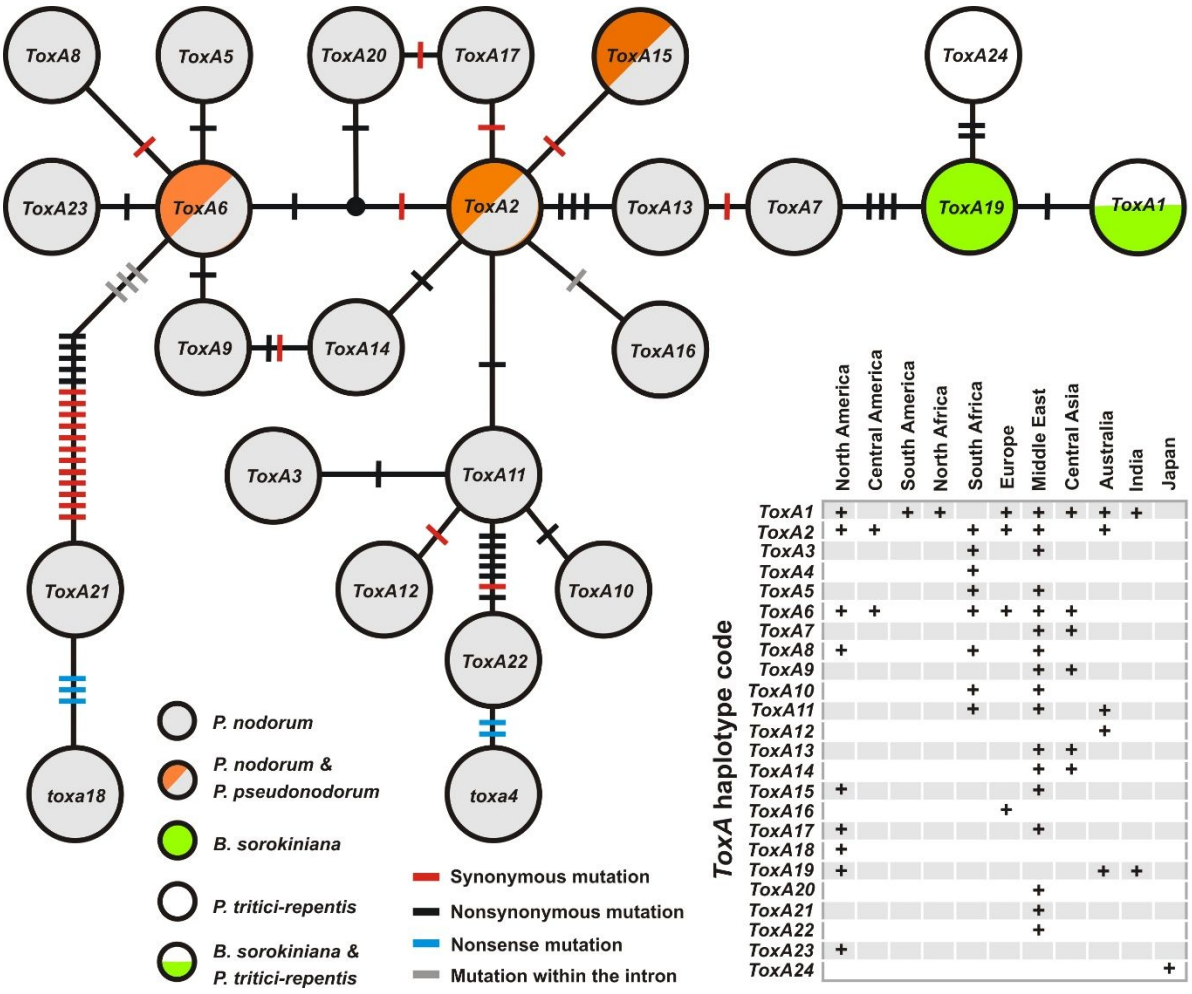
Extending the nomenclature to *ToxB*:

Here, we suggest extending the revised nomenclature system for *ToxB* haplotypes (and the homolog, the *toxb* gene) in a manner similar to that proposed for *ToxA* haplotypes: the use of "*ToxB*/or *toxb"* followed by the haplotype number (#). In total, 11 *ToxB*/*toxb* haplotypes have been identified to date, five of which occur in *Py. tritici-repentis* and are denoted here as *ToxB1, toxb2, toxb3, toxb4* and *ToxB5*, and six in its sister species *Pyrenophora bromi*, the causal agent of brown spot on bromegrass, and are denoted as *ToxB6* to *ToxB11* (Table 1).

ToxB was the second necrotrophic effector identified in *Py. tritici-repentis* and is a chlorosis-inducing effector encoded by the multi-copy *ToxB* gene (Strelkov & Lamari 2003; Strelkov et al. 2005). The *ToxB* gene is not as well explored as *ToxA*, simply due to the lack of ToxB-producing isolates in North America and Australia, where much of the work on *Py. tritici-repentis* and its effectors has been carried out. Six homologs of *ToxB* have been identified in *Py. bromi* and named *PbToxB* to differentiate their species of origin (Andrie et al. 2008). Here, we designated these six homologs as *ToxB6* to *ToxB11* (Table 1). The *ToxB* haplotypes identified in *P. bromi* possess toxic activity toward *ToxB*-sensitive wheat genotype, 6B662, but lack this activity on its original host, the bromegrass (Andrie et al. 2011). These names would replace the ambiguous *ToxB* and *toxb* used previously for the active and inactive *ToxB* haplotypes, respectively

A remaining challenge will be how to keep haplotype naming accurate. This will require a collective effort and open communication, yet some obstacles are to be expected. We have created a GitHub repository (Aboukhaddour et al. 2023) of described *ToxA* and *ToxB* haplotypes, and alleles. We are seeking input on how to enhance the nomenclature and keep it updated. We hope that publishing this as an open-access article and circulating it amongst the plant pathology community actively involved or interested in the subject matter will facilitate discussions. Open communication ahead of time with respect to denoting names may help to maintain a proper 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).



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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-1C-BFP	AF004369	Ciuffetti et al. 1997	H15 [a, b, c] H21[d] H23[e] PtrH1[f]
	B. sorokiniana	BRIP10943	KX816408	McDonald et al. 2018	BsToxA1[g] AusBsToxA[h] 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] TexBsToxA [h] H2 [d] 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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