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Reactive oxygen species in host-plant are required for an early defense response against attack of *Stagonospora nodorum* Berk. necrotrophic effectors SnTox

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Abstract

Reactive oxygen species (ROS) play central role in plant immune responses. The most important virulence factors of the *Stagonospora nodorum* are multiple fungal necrotrophic effectors (NEs) (SnTox) that affect the redox-status and cause necrosis and/or chlorosis on wheat lines possessing dominant susceptibility genes (*Snn*). However, the effect of NEs on ROS generation in the early stages of infection has not been studied. In this study, our aim was to research the effect of *S. nodorum* effectors SnToxA, SnTox1, SnTox3 on development of disease symptoms, generation of hydrogen peroxide and enzymes activity of redox-metabolism at early stage of infection in various wheat genotypes infected with isolates of *S nodorum* - Sn4VD, SnB and Sn9MN, carrying a various set of NEs genes. Our result indicates that all three NEs of SnToxA, SnTox1, SnTox3 played an important role in inhibition of ROS at the initial stage of infection. The *Tsn1*-SnToxA and *Snn3*-SnTox3 inhibited ROS production in wheat by affecting on NADPH-oxidases, peroxidases, superoxide dismutase and catalase. The *Snn1*-SnTox1 inhibited the production of ROS in wheat by mainly affecting the peroxidase. NEs suppress of ROS production only in the presence of the susceptibility genes *Tsn1*, *Snn1*, *Snn3*.

Keywords: *Stagonospora nodorum*; *Triticum aestivum*; necrotrophic effectors; reactive oxygen species; peroxidase.

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Zigzag model of the plant immune system

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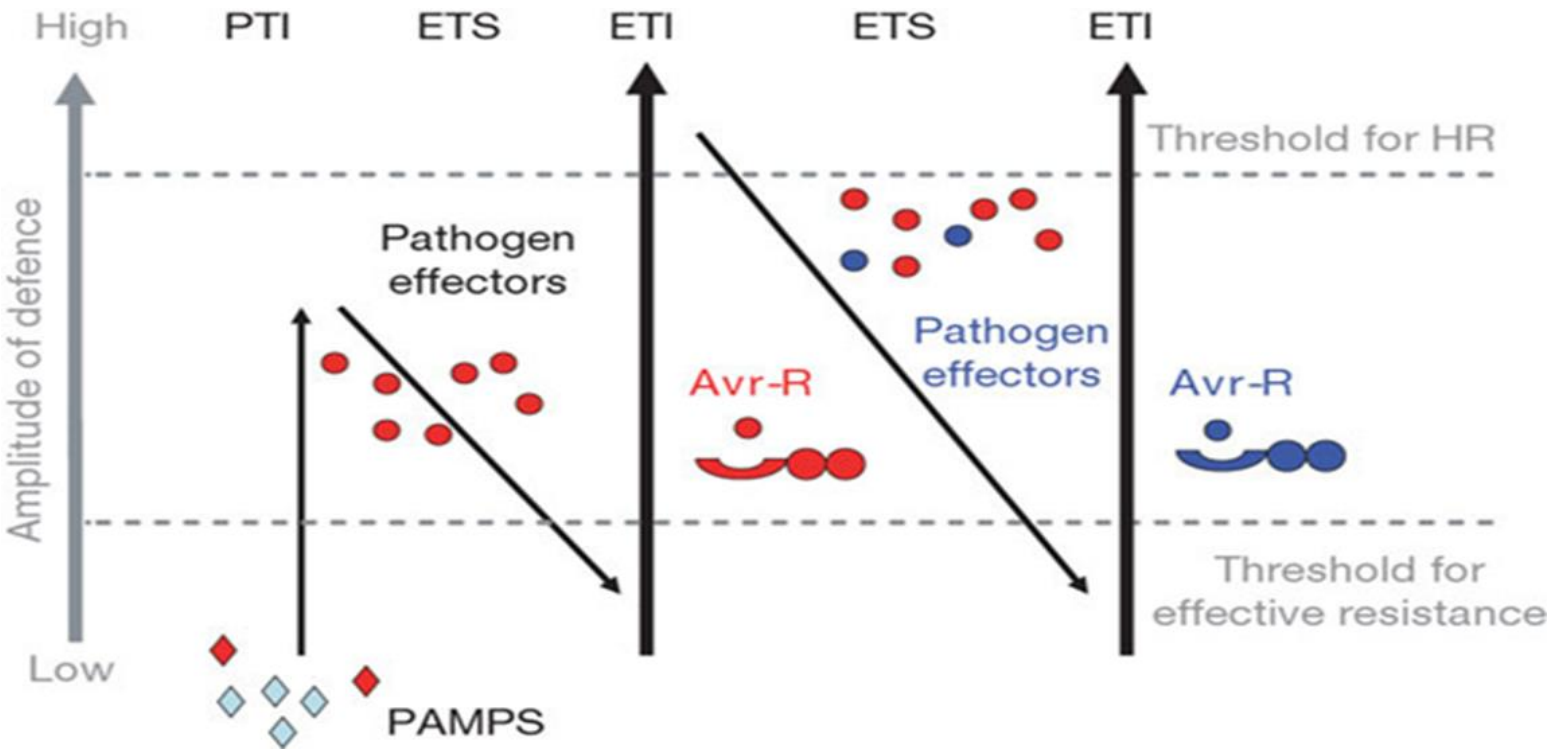

Plants have developed several levels of defense against microbial pathogens, which have been described in the «zig-zag» model of the plant immune system. The first line of defense in plants is through the perception of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), which leads to development of basal immunity, known as PAMP-triggered immunity (PTI). However, the pathogen can suppress PTI using effectors which leads to the development of effector-triggered susceptibility (ETS). The second line of defense in plants is called effector-triggered immunity (ETI) and develops when an effector is recognized by products of effector-specific resistance genes, which leads to the development of hypersensitive cell death (HR).

Jones, Dangl. 2006, Nature

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Hypothesis of gene-for-gene relationships

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classical gene-for-gene relationships

Classical Host *R* Gene-Biotrophic Effector (*Avr*) Gene Interaction

Pathogen Genotype	Host Genotype *R_*	Host Genotype *rr*
+Avr	Resistant	Susceptible
-Avr	Susceptible	Susceptible

Host Sensitivity Gene-Necrotrophic Toxin Interaction (i.e. wheat-*P. tritici-repentis*)

Pathogen Genotype	Host Genotype *S_*	Host Genotype *ss*
+Tox	Sensitive	Insensitive
-Tox	Insensitive	Insensitive

inverse gene-for-gene interactions

Until recently, necrotrophic pathogens were considered universal and non-host specific. However, recent studies have revealed that about 20 necrotrophic fungal species in the Dothideomycete class produced effector proteins also known as host-selective toxins (HSTs) or necrotrophic effectors (NEs) that interact either directly or indirectly with dominant sensitivity/susceptibility gene products to induce disease. When a specific NE is recognized by the corresponding host gene, a host response follows that allows necrotrophs to penetrate, grow and sporulate. The miss of NE recognition by the host leads to resistance. Therefore, these host–pathogen interactions control in an inverse gene-for-gene manner, and the dominant alleles of the host NEs recognition genes are considered susceptibility genes. Classical HST pathogens include species of *Cochliobolus*, *Alternaria*, *Pyrenophora* and *Stagonospora*.

Faris et. al., 2013, Theor Appl Genet

Stagonospora nodorum, *Parastagonospora nodorum*, *Septoria nodorum*

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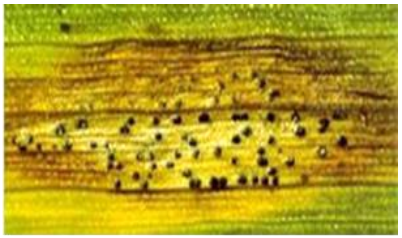

Рис. 331. *Septoria*.
А — пикнида; *Б* — конидии

Stagonospora nodorum (Parastagonospora nodorum, Septoria nodorum) is the causal agent of Septoria nodorum blotch (SNB). SNB is a major disease of durum and common wheat in many parts of the world. For many years causal agent of wheat leaf blotch such as *Stagonospora nodorum* have remained poorly understood, due to complexity of resistance to *S. nodorum* in seedling and adult plants. The most important factor of virulence of the *S. nodorum* are multiple fungal necrotrophic effectors (NEs) coded by *SnTox* genes that interact with the matching products of host susceptibility genes (*Snn*).

Effectors SnToxA, SnTox1, SnTox3 are considered the main ones in the pathogen *S. nodorum* and are quite widespread among strains and isolates. Effectors SnToxA, SnTox1, SnTox3 cause necrosis and chlorosis in susceptible wheat genotypes, it follows from this that they have an impact on the redox metabolism of the host plant.

Proteinaceous necrotrophic effectors	Corresponding host sensitivity genes
SnToxA	***Tsn1***
SnTox1	***Snn1***
SnTox3	***Loci Snn3-B1, Snn3-D1***

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The role of NEs SnToxA, SnTox1, SnTox3 in the suppression of PTI and the development of ETS

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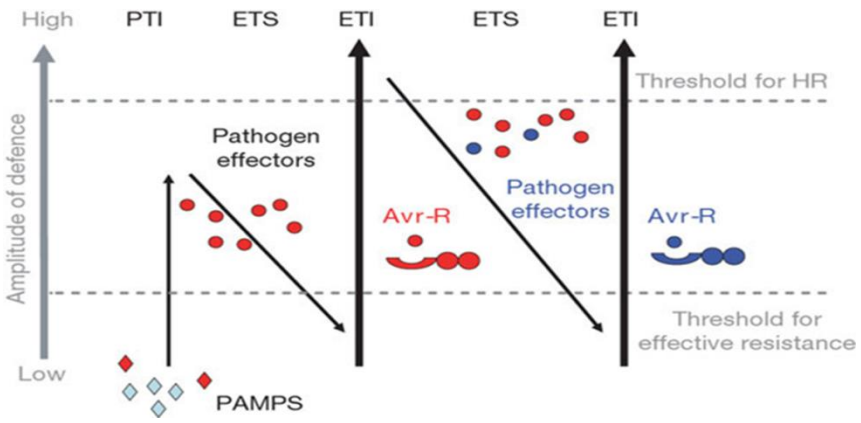

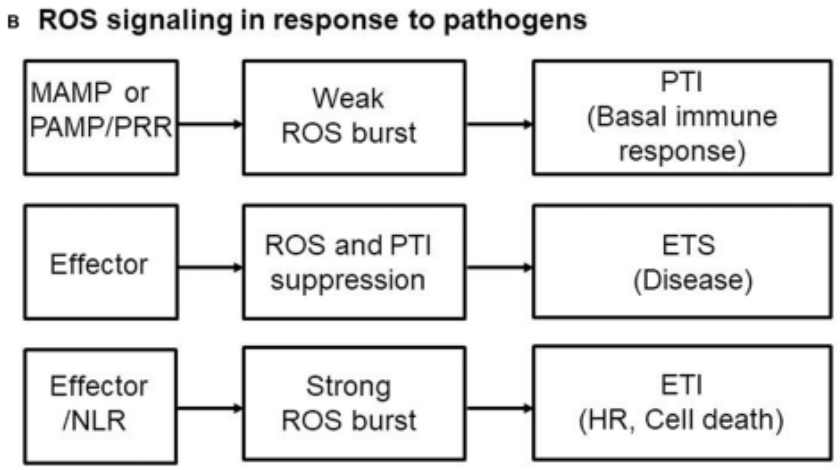

- The development of PTI and ETI induces similar responses in plants: both lines of defense can be separated in time and space, but both are closely related to the production of reactive oxygen species (ROS). PTI develops in the first minutes and hours of infection. PAMP-triggered ROS perform two functions. The apoplastic ROS are cytotoxic to kill pathogens, ROS act as signaling molecules to activate of plant defenses. ETI develops later and is a specific gene-for-gene response. The development of ETI leads to oxidative burst and formation of necrosis and restriction of the biotrophic pathogens growth.

- The role of NEs SnToxA, SnTox1, SnTox3 in the suppression of PTI and the development of ETS is expected. Unfortunately, the whole signal transduction pathway from recognition of the effector by the receptor to the necrosis development is unknown. It is believed that suppression of primary ROS burst during PTI by effector proteins as virulence factors is a common adaptation of many virulent pathogens. Much data has been accumulated on the effectors of various pathogens that suppress ROS burst during PTI. However, there is no such data on NEs SnToxA, SnTox1, SnTox3.

Scheme of experiment

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Pathogens

SnB – *S. nodorum* Bash – Virulent isolate
Sn9MN- *S. nodorum* 9MN – Virulent isolate
Sn4VD – *S. nodorum* 4VD - Avirulent isolate

Plants

Zhnitsa - Susceptible variety
Kaz10 - Kazahstanskaya 10 - Susceptible variety
Om35 – Omskaya 35 – Resistant variety

In this study, our aim was to research the effect of *S. nodorum* effectors SnToxA, SnTox1, SnTox3 on the development of disease symptoms, generation of hydrogen peroxide, expression of oxidoreductase genes and the activity of their protein products at an early stage of infection in various cultivars of soft spring wheat infected with isolates of *S nodorum* - Sn4VD, SnB and Sn9MN, carrying a various set of NEs genes. Here, we evaluated the roles of each compatible interaction *Tsn1*-SnToxA, *Snn3*-SnTox3, *Snn1*-SnTox1 in suppressing ROS production at the initial stage of infection.

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Results and Discussion

Our results suggest that all three NEs of *S. nodorum* SnToxA, SnTox1, SnTox3 played an important role in inhibition of ROS during PTI at the initial stage of infection

Identification of alleles of susceptibility genes *Snn1*, *Tsn1* and *Snn3-B1* locus

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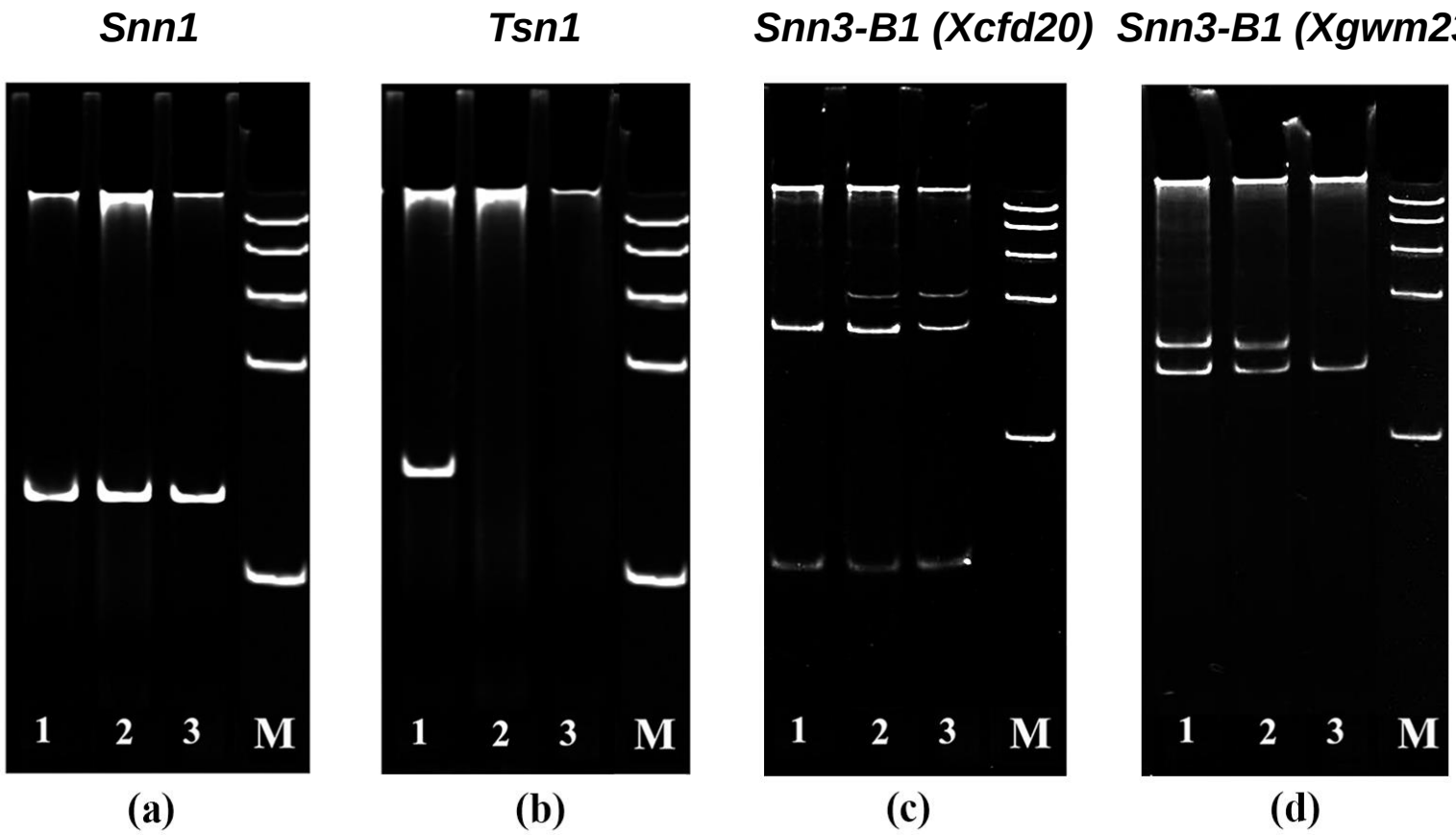

The Om 35 (*tsn1/Snn1/snn3*) was sensitive to NE of SnTox1.
The Kaz10 (*tsn1/Snn1/Snn3*) was sensitive to NE of SnTox1 and SnTox3.
The Zhnitsa (*Tsn1/Snn1/Snn3*) was sensitive to all three effectors.

Identification of alleles of susceptibility genes in different wheat varieties by PCR. 1 - Zhnitsa; 2 - Kazakhstanskaya 10; 3 - Omskaya 35; M – DNA molecular weight ladder 100–1000 bp.

Identification of NEs SnToxA, SnTox3 and SnTox1 in *S. nodorum* isolates

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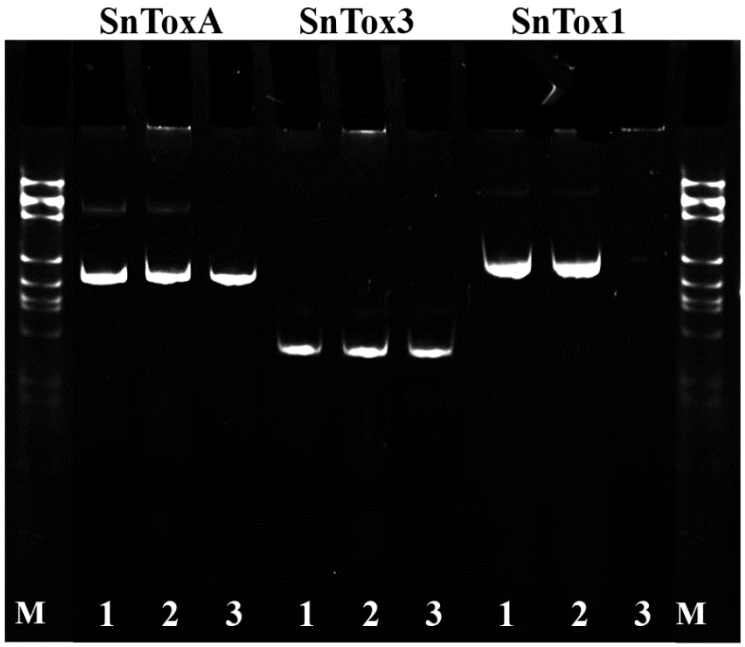
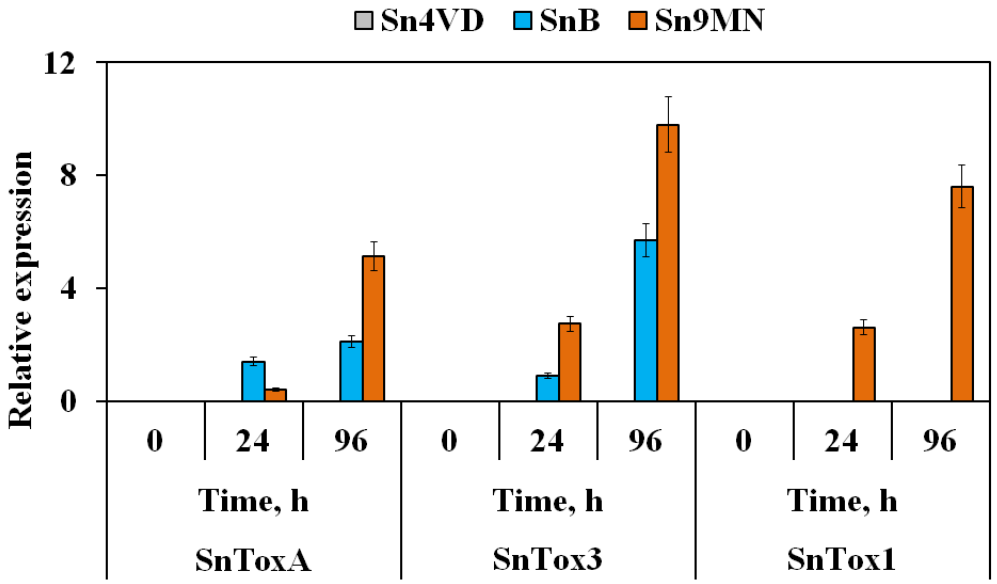


Identification of the SnToxA, SnTox3 and SnTox1 genes by PCR in tree isolates of *S. nodorum*: 1- Sn4VD; 2 - Sn9MN; 3 – SnB; M - DNA molecular weight ladder 100–1000 bp



Transcriptional activity of SnToxA, SnTox1, and SnTox3 effector genes (in planta) during inoculation of the susceptible genotype of the Zhnitsa (*Tsn1/Snn1/Snn3*) with water (0) and three isolates of *S. nodorum* SnB, Sn9MN, Sn4VD

The isolates Sn4VD and Sn9MN contained three NEs genes SnToxA, SnTox3 and SnTox1 in their genom. The isolate SnB contained only two NEs genes SnToxA and SnTox3 in the genome. In isolate Sn4VD, expression of all three NEs genes *in planta* was not detected. The SnB isolate expressed only two NEs genes, SnToxA and SnTox3. In isolate Sn9MN, expression of three NEs genes SnTox1, SnToxA, and SnTox3 was found.

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The role of compatible interactions in causing disease

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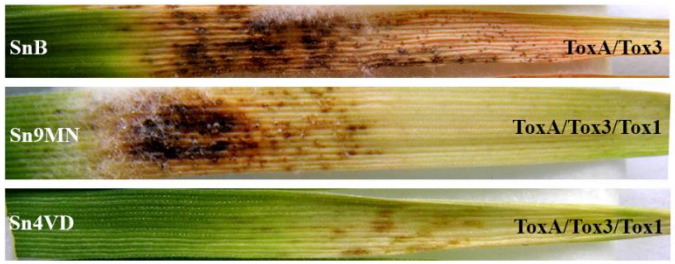
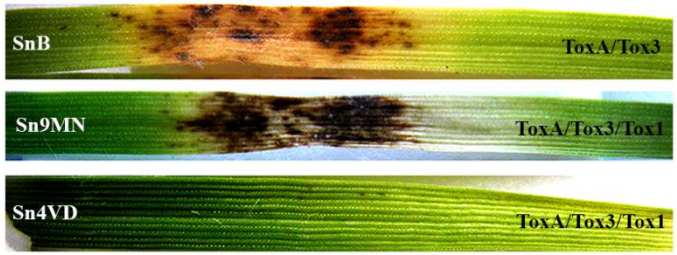
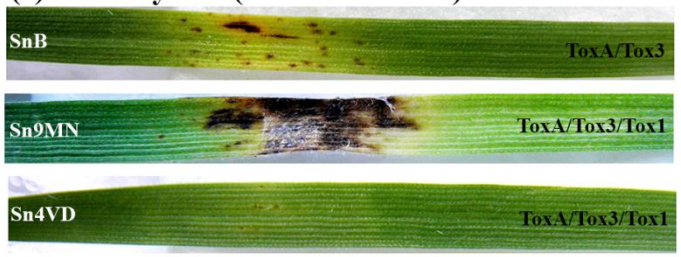
(a) Zhnitsa (Tsn1/Snn3/Snn1)



(b) Kazahstanskaya10 (tsn1/Snn3/Snn1)



(c) Omskaya 35 (tsn1/snn3/Snn1)



Reaction to damage of wheat varieties to infected with *S. nodorum* SnB, Sn9MN and Sn4VD isolates.

Variety	Reaction to damage	Isolate *S. nodorum*		
		Sn4VD (*toxa/tox3/tox1*)	SnB (*ToxA/Tox3/tox1*)	Sn9MN (*ToxA/Tox3/Tox1*)
Omskaya 35 (*tsn1/snn3/Snn1*)	Necrosis, %	0.05 ± 0.002	5 ± 0.7	23 ± 2
	Chlorosis, %	0	3 ± 0.5	0
	Damage zone, %	0.05 ± 0.001	8 ± 1	23 ± 2
	Damage score	1	2	3
	Resistance group*	RR	R	M
Kazahstanskaya 10 (*tsn1/Snn3/Snn1*)	Necrosis, %	0.05 ± 0.002	16 ± 2	31 ± 3
	Chlorosis, %	0	35 ± 3	25 ± 2
	Damage zone, %	0.05 ± 0.001	51 ± 5	56 ± 4
	Damage score	1	4	4
	Resistance group*	RR	S	S
Zhnitsa (*Tsn1/Snn3/Snn1*)	Necrosis, %	1 ± 0.1	18 ± 2	27 ± 2
	Chlorosis, %	2 ± 0.2	55 ± 4	57 ± 5
	Damage zone, %	3 ± 0.3	73 ± 6	84 ± 6
	Damage score	1	5	5
	Resistance group*	RR	SS	SS

Note:*RR (0 - 5%) - varieties with very high and high resistance; R (up to 10 - 15%) - resistant varieties; M (up to 25%) - slightly susceptible varieties; S (up to 40 - 65%) - susceptible varieties; SS (up to 90 - 100%) - varieties with very high and high susceptibility.

The minimal damage areas were observed in all incompatible interactions in variants of Om35/Sn4VD, Kaz10/Sn4VD, Zh/Sn4VD, Om35/SnB. The *Snn1*-SnTox1 interaction was observed in Om35/Sn9MN. The *Snn3*-SnTox3 interaction was observed in Kaz10/SnB. The *Snn3*-SnTox3 and *Snn1*-SnTox1 interactions were observed in Kaz10/Sn9MN. The *Tsn1*-SnToxA and *Snn3*-SnTox3 interactions were observed in Zh/SnB. The *Tsn1*-SnToxA, *Snn3*-SnTox3, *Snn1*-SnTox1 interactions were observed in Zh/Sn9MN.

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Apoplastic Reactive Oxygen Species Metabolism

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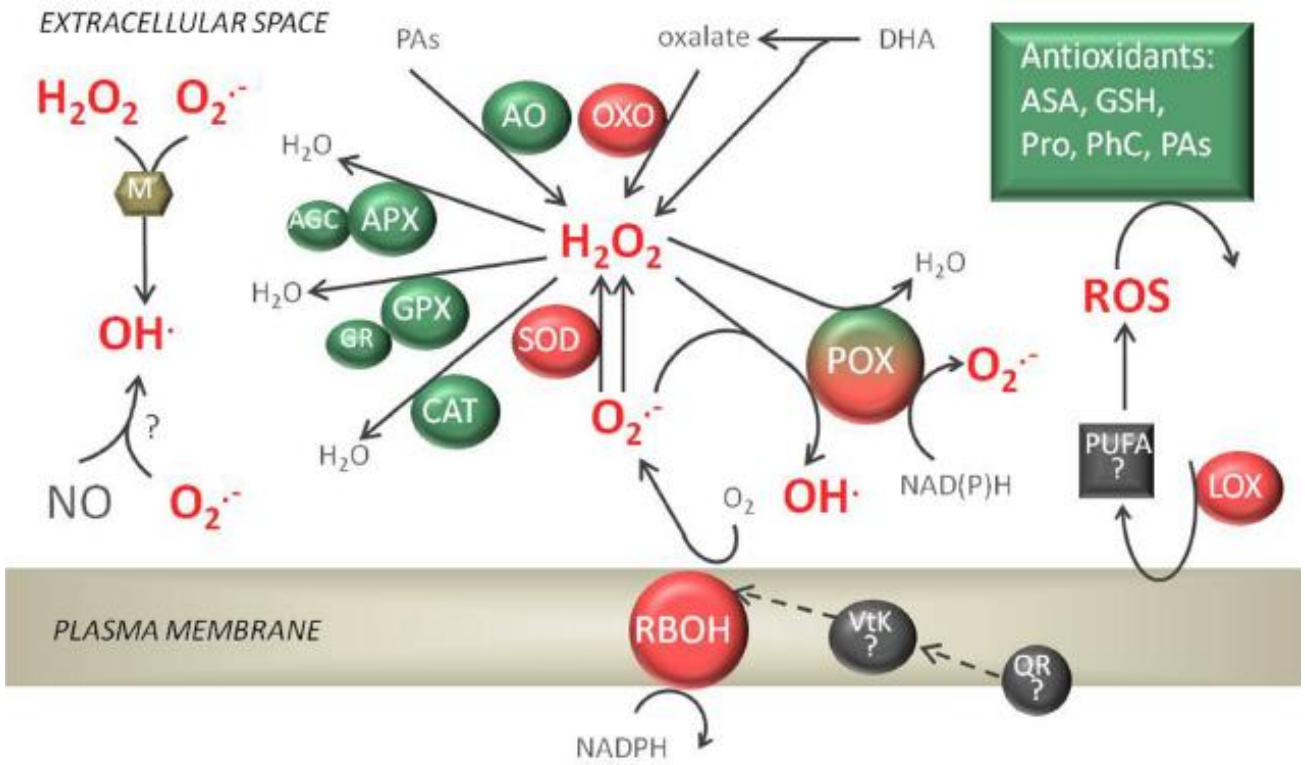

Podgórska et al., 2017, Front. Plant Sci.

It is known that one of the earliest responses to the invasion of pathogen is the local generation of ROS - oxidative "burst", which plays an important role in the development of systemic resistance and is controlled by enzymes of the pro-/antioxidant system. It is believed that H_2O_2 is a secondary messenger in signaling systems and activates transcriptional regulation factors, which leads to the expression of defensive genes and the synthesis of pathogenesis related proteins (PR proteins).

NADPH oxidases, also known as Respiratory Burst Oxidase Homologs (RBOHs), are responsible for production of ROS in plants during pathogen infection. In addition, the apoplastic peroxidases play an important role in production of ROS in PTI. There is some evidence on the catalysis of this process by the enzymes SOD and oxalate oxidase. Moreover, peroxidases are involved both in the generation processes and in the processes of ROS utilization in the apoplast, and catalase activate the decomposition reaction of H_2O_2 molecules.

The content of hydrogen peroxide in the leaves of wheat varieties with contrasting resistance, Zhnitsa and Omskaya 35 after infection with *S. nodorum*

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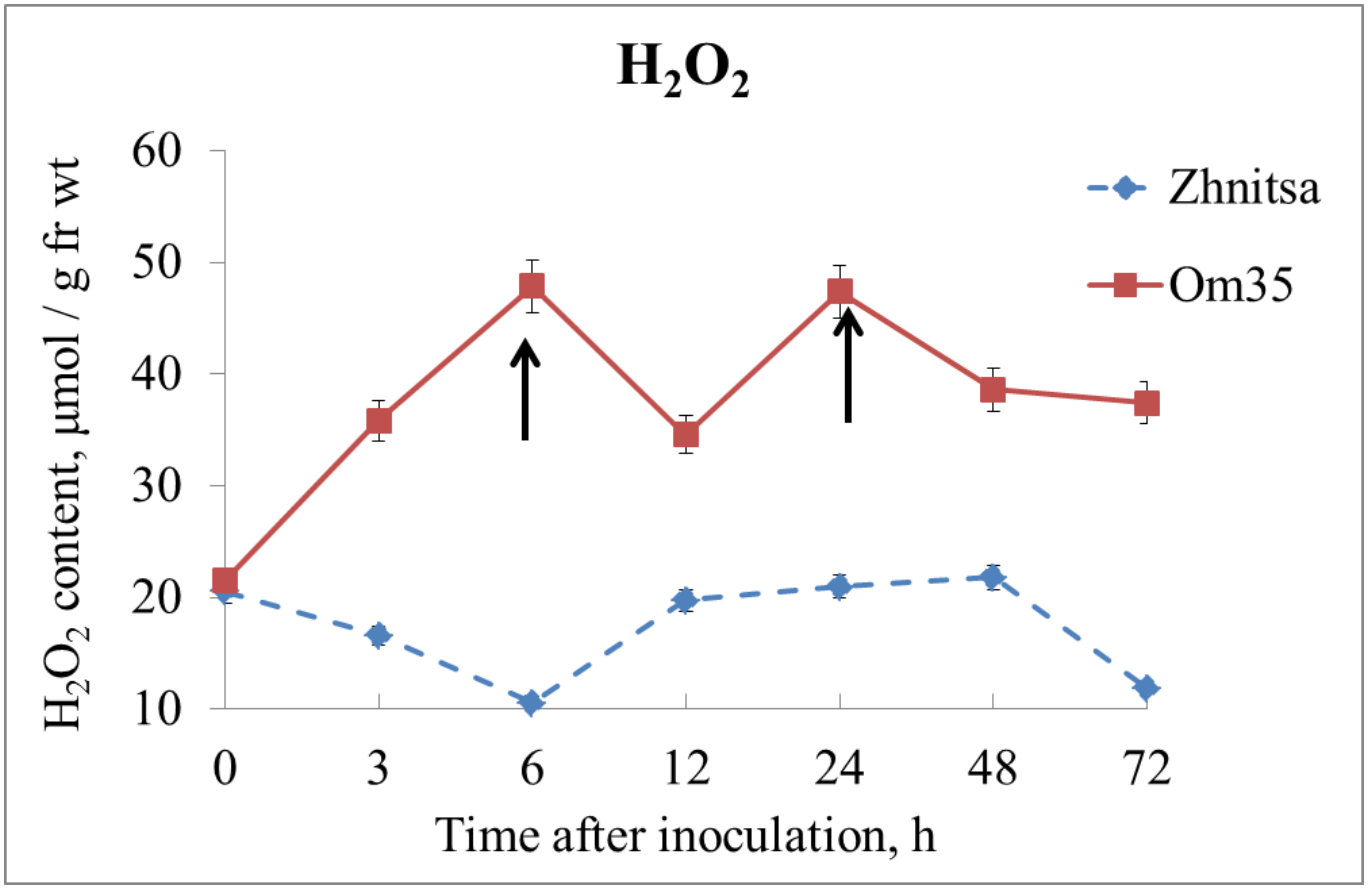

Earlier, we found that the resistance of the *T. aestivum* to the pathogen *S. nodorum* was determined by the intensive generation of ROS, mainly by the H_2O_2 at the initial stage of infection (6, 24 hours).

Veselova et al., 2018. Izvestija ufimskogo nauchnogo centra RAS. (In Russian) doi: 10.31040/2222-8349-2018-1-3-66-74

The H_2O_2 content, peroxidase – POX and catalase – CAT activity in contrast to resistance of wheat genotypes infected with various isolates of *S. nodorum*

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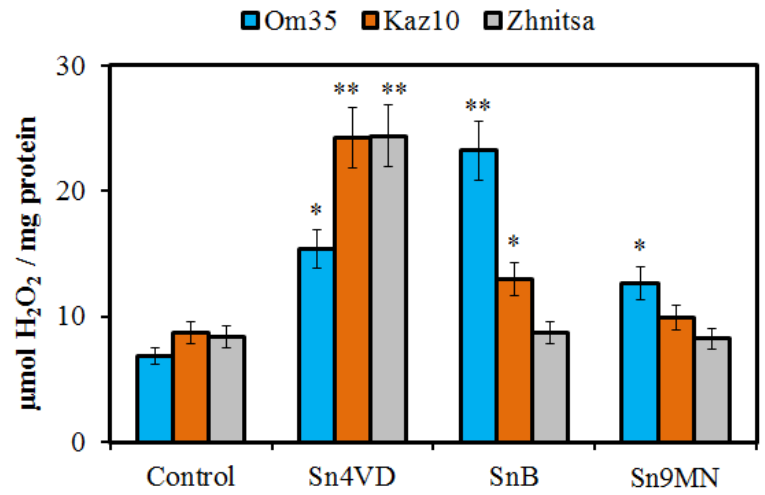
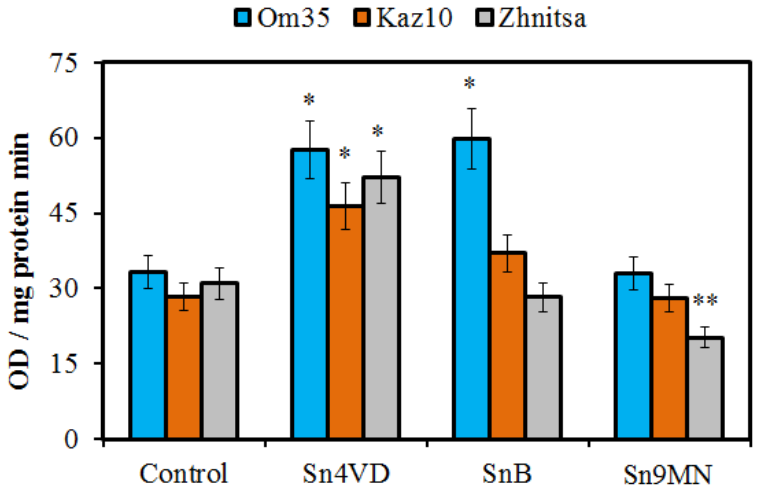
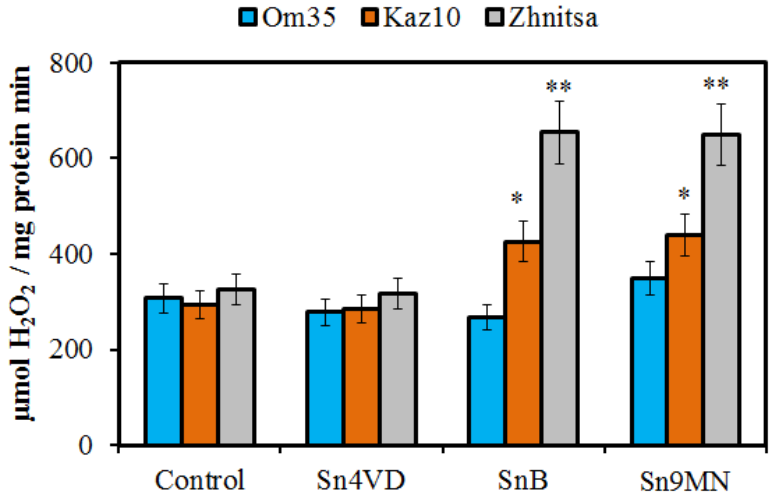
H_2O_2



POX



CAT



The incompatible interactions were characterized by an increase in hydrogen peroxide content due to a decrease or absence of increase in catalase activity and a sharp increase in peroxidase activity at the early stage of infection (24 hours). The compatible interactions were characterized by a decrease in hydrogen peroxide generation due to an increase in catalase activity and low peroxidase activity.

It should be noted that the *Snn1*-SnTox1 (Om35/Sn9MN) interaction did not lead to an increase in the activity of catalase compared to the incompatible interaction in variant Om35/SnB.

The interactions *Snn1*-SnTox1 (Om35/Sn9MN); *Snn3*-SnTox3 (Kaz10/SnB); *Snn3*-SnTox3 and *Snn1*-SnTox1 (Kaz10/Sn9MN); *Tsn1*-SnToxA and *Snn3*-SnTox3 (Zh/SnB); *Tsn1*-SnToxA, *Snn3*-SnTox3, *Snn1*-SnTox1 (Zh/Sn9MN). The incompatible interactions are Om35/Sn4VD, Kaz10/Sn4VD, Zh/Sn4VD, Om35/SnB.

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The mRNA accumulation of genes encoding NADPH-oxidase - *RbohF*, superoxide dismutase - *Sod*, anionic peroxidase – *Prx* in contrast to resistance of wheat genotypes infected with various isolates of *S. nodorum*

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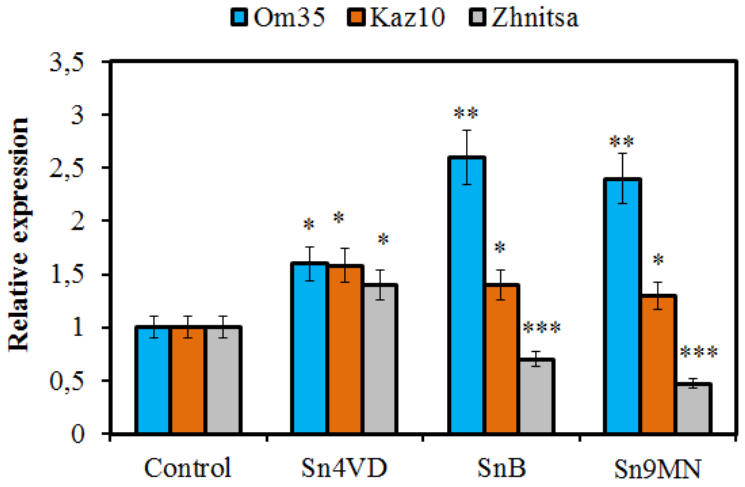
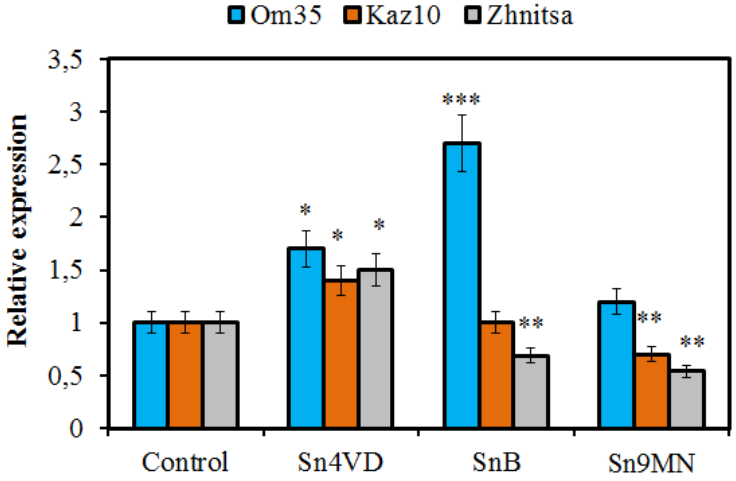
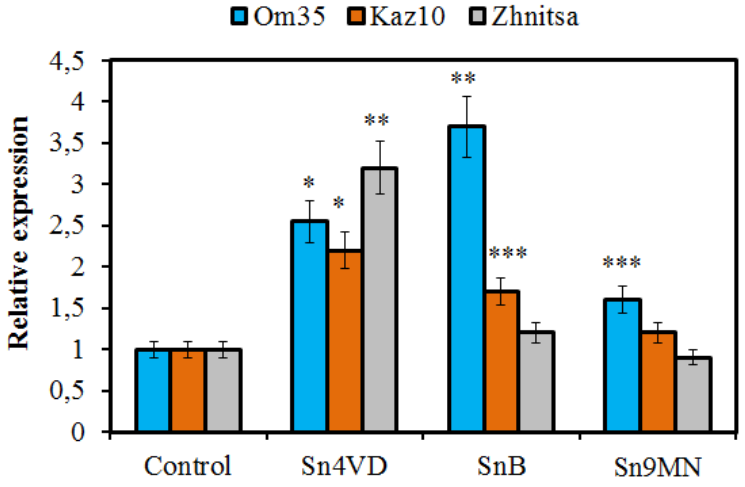
RbohF



Sod



Prx



The increase in hydrogen peroxide generation in the incompatibility reaction was also due to the accumulation of transcripts of the NADPH oxidase, peroxidase and SOD genes at early stage of infection (24 hours), which led to the development of a hypersensitive reaction, oxidative burst and restriction of pathogen growth. The decrease in hydrogen peroxide generation in the compatibility reaction was also due to a decrease in the transcriptional activity of the NADPH oxidase (*Rboh*), peroxidase (*Prx*) and superoxide dismutase genes (*Sod*) in the early stage of infection (24 hours). It should be noted that the *Snn*1-SnTox1 interaction did not lead to a decrease in the transcript level of gene *RbohF*.

The interactions *Snn1*-SnTox1 (Om35/Sn9MN); *Snn3*-SnTox3 (Kaz10/SnB); *Snn3*-SnTox3 and *Snn1*-SnTox1 (Kaz10/Sn9MN); *Tsn1*-SnToxA and *Snn3*-SnTox3 (Zh/SnB); *Tsn1*-SnToxA, *Snn3*-SnTox3, *Snn1*-SnTox1 (Zh/Sn9MN). The incompatible interactions are Om35/Sn4VD, Kaz10/Sn4VD, Zh/Sn4VD, Om35/SnB.

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Conclusions

- The *Tsn1*-SnToxA and *Snn3*-SnTox3 interactions inhibited H_2O_2 production in wheat at the early stage of infection by affecting on four enzymes of redox metabolism: NADPH-oxidases, peroxidases, superoxide dismutase and catalase.

- The *Snn1*-SnTox1 interaction inhibited the production of H_2O_2 in wheat by mainly affecting the peroxidase activity and the transcript level of gene encoding anionic peroxidase (*TaPrx*).

Conclusions

- The inhibition of ROS in PTI by NEs occurred only in the presence of the susceptibility genes *Tsn1*, *Snn1*, *Snn3*.

- The effector–host sensitivity gene interactions have the ability not only to hijack the host's own ETI pathway, but also suppress the host's own PTI pathway, resulting in NE-triggered susceptibility (NETS).

Acknowledgments

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РФФИ

Иннопрактика

Эврика
Конкурс прорывных идей

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Thank you for your attention!