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Induced resistance to the greenbug aphid *Schizaphis graminum* Rond. in species of the genus *Triticum*

S.D. Rumyantsev ✉, S.V. Veselova, G.F. Burkhanova, I.V. Maksimov

Institute of Biochemistry and Genetics – Subdivision of the Ufa Federal Research Centre, RAS, Ufa, Russia
✉ e-mail: Rumyantsev-Serg@mail.ru

The greenbug aphid *Schizaphis graminum* Rond. causes a significant loss of the grain harvest. Therefore, to improve plant resistance to aphids is one of the topical tasks. The problem of creating varieties resistant to phloem-feeding insects is quite urgent, since the mechanisms procuring the resistance of plants to insects are not fully understood. Nevertheless, modern literature describes some mechanisms associated with changes in the redox state of colonized plants. Besides, attention is being increasingly focused on the study of mechanisms that underlie inducible resistance to aphids in wheat and are regulated by hormonal signaling systems. To detect connections among the redox status, indicators of resistance (antibiosis and endurance) of wheat plants to the pest, and induction of the jasmonate (JA) and salicylate (SA) signaling pathways, we studied accessions of three species of wheat plants – *Triticum aestivum* L., *T. monococcum*., and *T. timopheevii* Zhuk.– infested with *S. graminum* greenbugs by physiological, biochemical, and molecular methods. Analysis of antibiosis and endurance showed that *T. timopheevii* k-58666 and *T. monococcum* k-39471 were resistant to *S. graminum*, the latter accession being the most enduring. High hydrogen peroxide contents and high peroxidase activities were detected in the resistant plants. We investigated the expression of genes encoding PR proteins, including markers and regulators of the salicylate (*TaRboh*, *TaPAL*, *Tapr1*, *TaPrx*) and jasmonate (*TaPI*, *TaLOX*, *TaPrx*) signaling pathways. At the early stage of infestation in the susceptible *T. aestivum* variety Salavat Yulaev, the expression of only jasmonate-dependent genes was activated in response to plant damage. In the resistant *T. timopheevii* accession k-58666, expression of only salicylate-dependent genes was activated, while the aphid reproduction was practically absent. In the resistant *T. monococcum* accession k-39471, expression was activated in both the salicylate-dependent and jasmonate-dependent gene groups. We assume that the oxidative burst in the resistant forms of wheat was induced via the activation of the SA signaling pathway, which was of crucial importance in the further cascade of chemical reactions leading to the development of resistance.

Key words: *Schizaphis graminum* Rond.; *Triticum aestivum* L.; *T. monococcum* L.; *T. timopheevii* Zhuk.; polymerase chain reaction; plant immunity; redox metabolism; hormonal signaling systems; gene expression.

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Индуцированная устойчивость представителей рода *Triticum* к обыкновенной злаковой тле *Schizaphis graminum* Rond.

С.Д. Румянцев ✉, С.В. Веселова, Г.Ф. Бурханова, И.В. Максимов

Институт биохимии и генетики – обособленное структурное подразделение Уфимского федерального исследовательского центра Российской академии наук, Уфа, Россия
✉ e-mail: Rumyantsev-Serg@mail.ru

Обыкновенная злаковая тля *Schizaphis graminum* Rond. наносит значительный ущерб посевам зерновых культур, поэтому повышение устойчивости растений к тлям – одна из первостепенных задач. Проблема создания устойчивых сортов к насекомым, питающимся флоэмным соком, стоит достаточно остро, так как механизмы, характеризующие устойчивость растений к насекомым, до конца не изучены. Тем не менее в современной литературе описывают механизмы, связанные с изменением редокс-статуса растений, заселенных тлей. Кроме того, в последнее время все большее внимание уделяется изучению механизмов индуцируемой устойчивости пшеницы к тлям, регулируемой гормональными сигнальными системами. Для обнаружения связи между редокс-статусом, показателями устойчивости растений пшеницы (антибиозом и выносливостью) к вредителю и индукцией жасмонат (ЖАК)- и салицилат (СК)-сигнальных путей у представителей трех видов растений пшеницы, *Triticum aestivum* L., *T. monococcum* L. и *T. timopheevii* Zhuk., заселенных обыкновенной злаковой тлей *S. graminum*, были проведены исследования с помощью физиологических, биохимических и молекулярно-генетических методов. Тесты на антибиоз и выносливость показали, что устойчивыми по отношению к *S. graminum* были образцы *T. timopheevii* – к-58666 и *T. monococcum* – к-39471, последний из которых оказался наиболее выносливым. Устойчивые образцы отличались высоким содержанием перекиси водорода и повышенной активностью пероксидазы. В нашей работе исследована экспрессия генов, кодирующих

PR-белки, маркеры и регуляторы СК- (*TaRboh*, *TaPAL*, *TaPr1*, *TaPrx*) и ЖАК-сигнальных путей (*TaPI*, *TaLOX*, *TaPrx*). При ранних ответных реакциях после заселения тлей у восприимчивого сорта Салават Юлаев (*T. aestivum*) активировалась экспрессия только ЖАК-зависимых генов, что отражало реакцию растений на повреждение. У устойчивого образца *T. timopheevii* к-58666 повышалась транскрипционная активность СК-зависимых генов, при этом тля практически не размножалась. У устойчивого образца *T. monococcum* к-39471, проявившего наибольшую выносливость, увеличивалась экспрессия как СК-, так и ЖАК-зависимых генов. Мы предполагаем, что окислительный взрыв у устойчивых форм пшеницы индуцировался благодаря запуску СК-сигнального пути, что имело решающее значение в дальнейшем каскаде химических реакций, ведущих к развитию устойчивости.

Ключевые слова: *Schizaphis graminum* Rond.; *Triticum aestivum* L.; *T. monococcum* L.; *T. timopheevii* Zhuk.; полимеразная цепная реакция; фитоиммунитет; редокс-метаболизм; гормональные сигнальные системы; экспрессия генов.

Introduction

Greenbug aphids (*Schizaphis graminum*) damage wheat crops significantly (Morkunas et al., 2011; Radchenko, 2012). Today, improvement of the resistance of grain crops to aphids is among the primary concerns for both science and practice. One of the approaches to this problem is the breeding of resistant plant genotypes. However, there are few resistant genotypes of *Triticum aestivum* L., and thus the study of wild wheat species is of great importance. It has been shown that most *T. monococcum* L. and *T. timopheevii* Zhuk. accessions are resistant to a broad spectrum of pests (Radchenko, 2012). However, knowledge of the molecular mechanisms of plant resistance is required to create a resistant variety.

Plant protection against phloem-feeding insect is provided by mechanisms of both specific gene-for-gene resistance and nonspecific resistance associated with plant hormonal signaling systems (Morkunas et al., 2011; Radchenko, 2012). Presently, increasing attention is focused on mechanisms of induced (termed active by N.I. Vavilov) resistance of wheat to aphids. The induced nonspecific resistance of plants developed against phytophagous insects is regulated by hormonal signaling pathways, including those depending on jasmonate (JA), ethylene, and salicylate (SA), which affect gene expression, synthesis of protective proteins and various enzymes, redox metabolism of plants, activity of peroxidases in the apoplast, plant cell wall strength, etc. (War et al., 2012). Hormonal signaling pathways do not act independently of each other but form a complex network of interactions playing an important role in the fine-tuning of plant protective reactions (Morkunas et al., 2011). It has been shown that infestation by aphids induces both JA- and SA-dependent protective responses in plants (Morkunas et al., 2011; Kerchev et al., 2012). Unfortunately, the mechanisms underlying the cross-talk of these signaling pathways in the response of plants to aphid damage are not fully understood. The activation of the JA-dependent protective pathway was observed in both susceptible and resistant plants, whereas the induction of the SA-dependent way was faster and stronger only in resistant genotypes (Morkunas et al., 2011).

However, it has been demonstrated that both the JA and SA signaling pathways cause the accumulation of reactive oxygen species (ROS), in particular, hydrogen peroxide (H_2O_2), in plants infested by aphids (Morkunas et al., 2011). ROS are known as the second messenger in a broad spectrum of plant reactions to environmental stress, and the redox state of plants infested by aphids is an important indicator of resistance in a variety (Morkunas et al., 2011; Koch et al., 2016). Oxidative burst during aphid colonization is considered to be a typical reaction implicated in plant resistance to the pest (Koch et al., 2016). The involvement of ROS in the early signal response and in the regulation of the protective response along with both the JA- and SA-dependent signaling pathways has been shown, but the molecular mechanism underlying these processes is not known yet (Kerchev et al., 2012).

In this regard, the aim of this work was to investigate links among the redox state, indicators of resistance of wheat plants (antibiosis and endurance) to the pest, and the induction of JA and SA signaling pathways in three wheat species: *Triticum aestivum*, *T. monococcum* and *T. timopheevii*, infested with greenbug aphids (*S. graminum* Rond.).

Materials and methods

Objects of research. The greenbug aphid *Schizaphis graminum* Rond. was cultivated on young seedlings of soft spring wheat, *Triticum aestivum* L., cultivar Salavat Yulaev (SY) (bred at the Bashkir State Agrarian University, Russia) grown in isolated flasks filled with soil preheated at 180 °C and placed into a KS-200 SPU climatic chamber (Russia) at the 16 : 8 h light/dark schedule, 20/24 °C (night/day) and light intensity 146 W/m^2 (lamps Osram L 36W/77, Germany). The tested accessions included the susceptible soft spring wheat (*T. aestivum*) variety Salavat Yulaev (SY), *T. monococcum* accession k-39471, and *T. timopheevii* accession k-58666, all obtained from the N.I. Vavilov All-Russia Institute of Plant Genetic Resources (St. Petersburg).

Biochemical parameters. Three-day-old wheat seedlings hydroponically grown in isolated plastic vessels (10 % Hoagland–Arnon solution) under the conditions described above were settled with aphids. For this, equal numbers of aphids of different ages were flipped into each vessel with plants so that the load be 10 aphids per plant (Radchenko, 2008). The vessels were covered with plastic insulators tightened with porous nonwoven fabric. The concentrations of hydrogen peroxide (H_2O_2) and the activities of peroxidase (PO) were estimated on days 1, 3, and 10 after the infestation as described in (Veselova et al., 2016).

Antibiosis test. First instar nymphs were placed under the insulators on three-day-old seedlings, one per plant. The numbers of dead and living aphids, imagoes and larvae, were counted after 14 days (Radchenko, 2008). Mortality was expressed as percentage of the total number of aphids. The propagation coefficient was calculated by the formula: K = average fertility of females during the experiment/duration of the experiment, days (Radchenko, 2008).

Test of plant endurance. The length of a seedling was measured from the level of the raft to the tip of the leaf at the age of three days, and then each plant was settled with 20 wingless females and insulated. The constant number of aphids was maintained by removing hatching larvae at 48-h intervals for two weeks. At the end of the experiment, the heights of the first and second leaves of plants uninhabited and infested by aphids were measured again, and the results were compared with the original measurement (Radchenko, 2008). Endurance was expressed as percentage of leaf growth inhibition compared to the intact control.

Transcriptional gene activity. Total RNA was isolated from both control and experimental wheat plants fixed in liquid nitrogen with Trizol reagent (Sigma, Germany) on days 3 and 10 after infestation by aphids in accordance with manufacturer's recommendations. The nucleic acid concentration was assessed by the A_{260}/A_{280} ratio with a Spec Plus spectrophotometer (Bio-Rad, United States). cDNA was obtained by reverse transcription with M-MuLV reverse transcriptase (Sintol, Russia). With the cDNA as the template, PCR was performed with primers flanking conservative gene sites of the studied PR proteins in a TP4-PCR-01-Tertsik thermocycler (DNA-Technology, Russia). We used primers to genes encoding PR1 protein (*TaPr1*, AF384143) (Veselova et al., 2016), proteinase inhibitors (*TaPI*, EU293132.1), anionic peroxidase (*TaPrx*, TC151917), NADPH oxidase (*TaRboh*, HE674332) (Veselova et al., 2019), phenylalanine ammonia lyase (*TaPAL*, X99725) (Ding et al., 2011), and lipoxygenase (*TaLOX*, BJ223744) (Diallo et al., 2014). To calculate the normalized levels of genes expression, primers to the gene for constitutively expressed protein RNase inhibitor were used (RNase L inhibitor-like) (*TaRLI*, AY059462) (Veselova et al., 2016). Primers to these genes were matched to *T. aestivum* L. Homologous genes were obtained from *T. monococcum* and *T. timopheevii* in pilot experiments with these primers. It is known that carriers of the primary (for example, *T. monococcum*, *Ae. tauschii* Coss., *T. spelta* L., *T. compactum* Host., *T. durum* Desf., *T. dicoccum* (Schrank.) Schuebl.) and secondary (*T. timopheevii*, *T. araraticum* Jakubz., *Ae. speltoides* Tausch., *Ae. sharonensis* Eig, *Ae. longissima* Schw. et Musch.) gene pools contain genomes with high or partial homology to soft wheat *T. aestivum* genomes A, B, and D (Chaudhary et al., 2014).

Statistical analysis. All experiments were carried out in three biological and analytical replications and were repeated three times ($n = 9$ in total), except for the tests for antibiosis and endurance, which included at least ten biological replications ($n = 30$). The Figures 1–4 and Tables 1–2 present the arithmetic mean values and their confidence intervals calculated from standard errors. The reliability of the differences between the variants of the experiment was assessed by Student's t test at the confidence level $p \leq 0.05$.

Results

Indicators of resistance of a plant accession. Two types of plant resistance to aphids were studied: antibiosis and endurance. Aphids fed on SY showed the lowest mortality and the highest propagation rate in the antibiosis test (see Fig. 1, *a*, *b*); i. e., this variety was susceptible to greenbug aphid. The mortality of aphids on *T. timopheevii* k-58666 was about 3–4 times higher and the propagation rate 4–5 times lower than on the susceptible variety (see Fig. 1, *a*, *b*). However, *T. monococcum* k-39471 had the highest effect on aphid viability, as the aphids colonizing it had the highest mortality and the lowest propagation rates (see Fig. 1, *a*, *b*). *T. monococcum* k-39471 was the most resistant among the studied accessions.

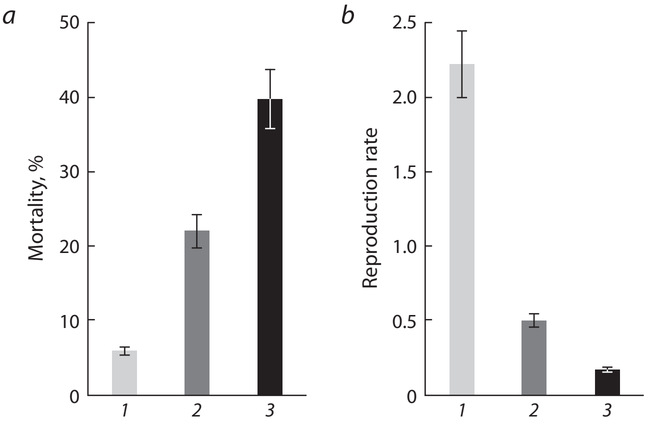

Fig. 1. Mortality (*a*) and propagation (*b*) of greenbug aphid *S. graminum* settled on different species of wheat *Triticum* spp.

1 – *Triticum aestivum* variety Salavat Yulaev; *2* – *T. timopheevii* accession k-58666; *3* – *T. monococcum* accession k-39471.

In our experiments, SY exhibited poor endurance to *S. graminum*, manifesting itself as strong inhibition of the growth of the 1st and 2nd leaves (Table 1). The k-58666 accession of *T. timopheevii* showed intermediate endurance to greenbug aphid (see Table 1). *T. monococcum* k-39471 was the most endurant accession, as the infestation by aphids did not inhibit the growth of the 1st leaf, and the inhibition of the growth of the 2nd leaf was the least (see Table 1).

The redox state of plants infested by aphids. Colonization of the susceptible variety SY with aphids led to a decrease in the content of H_2O_2 at the initial stages of feeding: on days 1 and 3 (Table 2). In contrast, resistant plants of *T. timopheevii* k-58666 showed elevated H_2O_2 levels at the early stages of response to infestation by aphids (see Table 2). Accession *T. monococcum* k-39471, resistant to *S. graminum*, showed drastic H_2O_2 accumulation on the 1st day after settling of aphids and reduction of H_2O_2 generation on the 3rd day of feeding (see Table 2). In SY and *T. timopheevii* k-58666, the contents of H_2O_2 remained at the level of control plants on day 10 after the settling with aphids but greatly decreased in the resistant *T. monococcum* accession k-39471 (see Table 2).

Triticum timopheevii k-58666 and *T. monococcum* k-39471, resistant to *S. graminum*, showed elevated PO activities and H_2O_2 generation at early response stages (days 1–3) after settling with aphids (see Table 2). In resistant genotypes, the increase in PO activity was found to continue on day 10

Table 1. Resistance of different *Triticum* spp. species settled with greenbug aphid *S. graminum*

Wheat species	Growth inhibition, %	
	first leaf	second leaf
Triticum aestivum – SY	25.8 ± 6.1	45.0 ± 3.7
T. timopheevii – k-58666	12.3 ± 6.6	22.6 ± 4.2
T. monococcum – k-39471	0.1 ± 2.4	7.6 ± 2.8

Table 2. The effect of greenbug aphid *S. graminum* on H_2O_2 content and peroxidase activity in three wheat *Triticum* spp. accessions at different stages of feeding

Variant	Time after settling, days		
	1	3	10
H_2O_2 content, µmol/g fr wt			
SY	25.2 ± 5.6	26.8 ± 1.7	26.3 ± 2.7
SY + *S. graminum*	12.6 ± 1.3	19.0 ± 2.4	27.0 ± 1.1
k-58666	27.8 ± 5.3	28.9 ± 2.8	22.9 ± 1.7
k-58666 + *S. graminum*	58.7 ± 5.5	48.6 ± 4.5	28.0 ± 3.3
k-39471	28.2 ± 3.6	28.7 ± 3.1	28.8 ± 1.1
k-39471 + *S. graminum*	53.7 ± 5.3	20.4 ± 3.0	12.0 ± 1.2
Peroxidase activity, OD/mg protein min			
SY	30.4 ± 0.7	29.0 ± 0.4	30.3 ± 1.8
SY + *S. graminum*	26.1 ± 1.0	25.8 ± 0.6	28.8 ± 1.2
k-58666	29.6 ± 0.4	31.4 ± 0.8	28.7 ± 1.7
k-58666 + *S. graminum*	35.7 ± 1.2	36.0 ± 0.3	57.1 ± 2.2
k-39471	23.6 ± 0.5	32.1 ± 1.1	30.2 ± 1.2
k-39471 + *S. graminum*	43.2 ± 1.4	60.5 ± 2.6	90.7 ± 3.4

after aphid settling, especially in *T. monococcum* k-39471 (see Table 2). On the contrary, increased activity of PO and low concentration of H_2O_2 were found in the susceptible wheat variety SY both on the first days and on day 10 of aphid feeding (see Table 2).

Transcriptional activity of genes associated with redox metabolism. The contents of mRNA of the *Rboh* gene increased in the resistant accession *T. timopheevii* k-58666 and in *T. monococcum* k-39471 during the early response, on day 3 after aphid settling, but no increase was detected in the susceptible variety SY (Fig. 2, *a*). On the contrary, the content of *Rboh* transcripts increased in the susceptible variety SY at a later response stage, on day 10, whereas it decreased by about one-half in the resistant accessions (see Fig. 2, *a*). The content of *Prx* mRNA increased slightly in the susceptible variety SY. In *T. timopheevii* k-58666, this parameter decreased throughout the experiment (see Fig. 2, *b*). A significant (eightfold) increase in *Prx* gene mRNA content was found in *T. monococcum* k-39471 on day 3 after aphid infestation, and a threefold increase was detected on day 10 (see Fig. 2, *b*).

Transcriptional activity of genes regulated by the JA-dependent signaling cascade. In the susceptible variety SY, elevated levels of transcripts of the *LOX* and *PI* genes, which are regulated by the JA signaling cascade, were observed on days 3 and 10 after aphid settling (Fig. 3, *a*, *b*). In the resistant *T. timopheevii* accession k-58666 the transcriptional activity of the genes in question was close to that in control plants and even decreased by day 10 (see Fig. 3, *a*, *b*). The resistant *T. monococcum* accession k-39471 showed a significant increase in the mRNA contents of the *LOX* and *PI* genes on day 3 after aphid settling, by factors of 2.5 and 6.4, respectively (see Fig. 3, *a*, *b*). On day 10 of feeding, this accession showed a significant accumulation of transcripts only for the LOX gene, by a factor of 3.7 times compared to the intact plants (see Fig. 3, *a*).

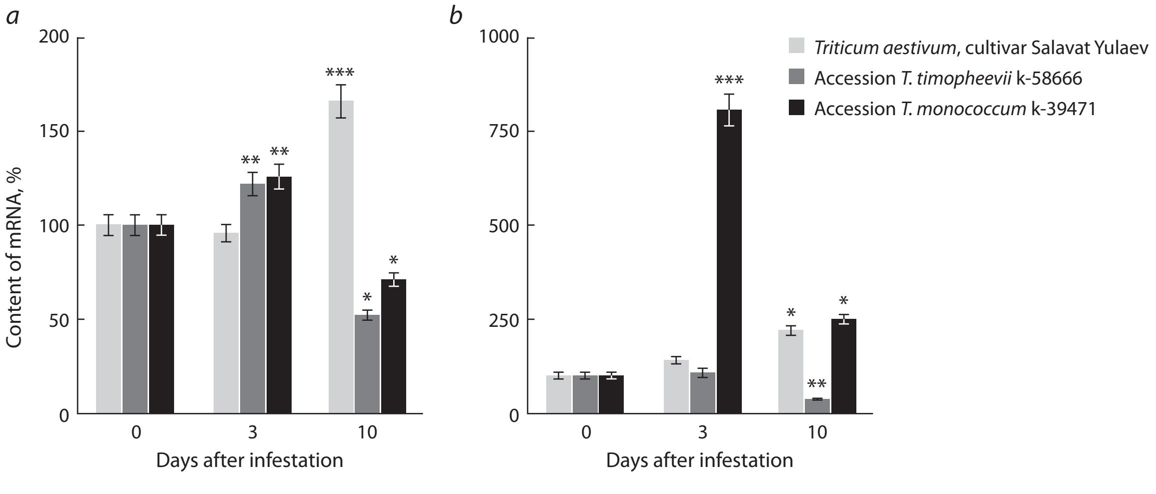

Fig. 2. Effect of greenbug aphid *S. graminum* on the contents mRNAs of *Rboh* (*a*) and *Prx* (*b*) genes in three *Triticum* spp. species at different stages of feeding.

Asterisks show statistically significant differences from the control group, and different numbers of asterisks mean differences between the variants significant at $p \le 0.05$.

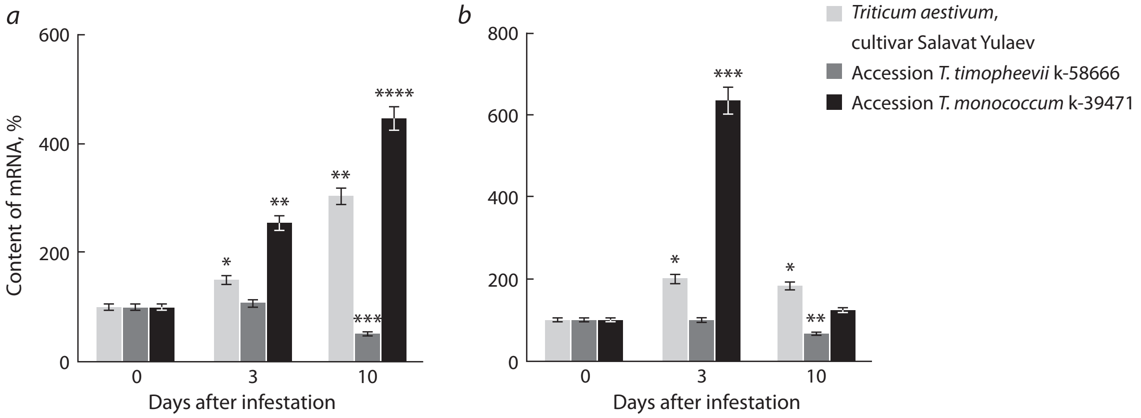

Fig. 3. Effect of greenbug aphid *S. graminum* on the mRNA contents of the *LOX* (*a*) and *PI* (*b*) genes of three *Triticum* spp. accessions at different stages of feeding.

Designations follow Fig. 2.

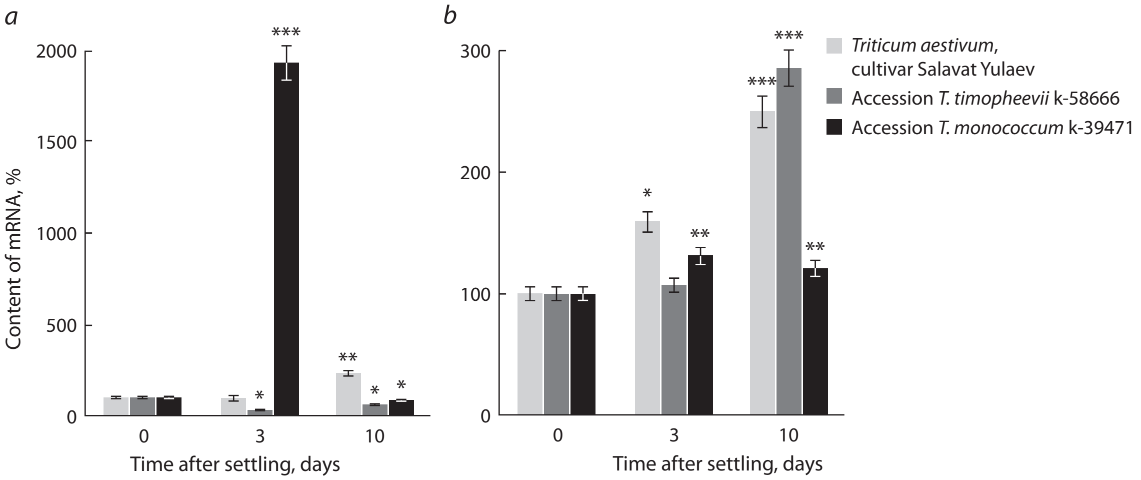

Fig. 4. Effect of greenbug aphid *S. graminum* on the mRNA contents of the *Pr1* (*a*) and *PAL* (*b*) genes of three *Triticum* spp. accessions at different stages of feeding.

Designations follow Fig. 2.

Transcriptional activity of genes regulated by SA-dependent signaling cascade. In susceptible SY, twofold accumulation of transcripts of the *Pr1* gene was detected only on day 10 after aphid settling (Fig. 4, *a*). Resistant *T. timopheevii* k-58666 showed a slightly lowered transcription level of the main gene of the SA signaling pathway throughout the experiment (see Fig. 4, *a*). In contrast, a significant 20-fold increase in the mRNA of the *Pr1* gene was discovered in the resistant *T. monococcum* accession k-39471, which showed the greatest endurance, on day 3 after aphid settling (see Fig. 4, *a*). The pattern of changes in the content of *PAL* gene transcripts was similar in susceptible SY and resistant *T. timopheevii* k-58666: the mRNA level increased by 20–50 % on day 3 after aphid settling and almost threefold on day 10 (see Fig. 4, *b*). The resistant *T. monococcum* accession k-39471 showed a slight increase in the transcriptional activity of the studied gene, by 20–30 % compared to the intact control throughout the experiment (see Fig. 4, *b*).

Discussion

The study of the mechanisms of plant resistance to phloem-feeding insects requires distinguishing early responses found in plants during the first five days after colonization by aphids and long-term responses observed after 10 days of ingestion by the pest (Koch et al., 2016). Early responses of resistant plants are characterized by increased generation of ROS and activation of basal immunity (Morkunas et al., 2011). The protective role of oxidative burst against aphid feeding consists of their signaling function in the development of systemic resistance and the direct damaging effect of high H_2O_2 concentrations on aphids, leading to the suppression of pest survival (Morkunas et al., 2011; War et al., 2012; Koch et al., 2016). Long-term responses are ROS detoxication and growth recovery, which should be induced by early responses of plants (Koch et al., 2016).

Our results showed that *T. monococcum* k-39471 was the most resistant to greenbug aphid *S. graminum* among the accessions studied. This observation coincides with the literature data on the resistance of numerous *T. monococcum* accessions to different types of aphids (Radchenko, 2012). *Triticum timopheevii* k-58666 also showed medium resistance to the pest in our experiments. In the literature, there is inconsistent information about the resistance of *T. timopheevii* to different types of cereal aphids; however, this wheat species generally shows resistance to the pest and is poorly populated by aphids (Radchenko, 2012).

In the resistant accessions *T. timopheevii* k-58666 and *T. monococcum* k-39471, an increase in H_2O_2 generation during early responses after pest colonization and decrease at the later stage were observed (see Table 2). Thus, it led to high mortality of aphids (see Fig. 1) and a sufficient increase in plant endurance (see Table 1). In susceptible plants of wheat variety SY, suppression of the oxidative burst during early responses after the infestation by pests was observed. The content of H_2O_2 during long-term responses after aphid settling did not differ from control plants (see Table 2), which led to high fertility of the insect (see Fig. 1) and poor plant endurance (see Table 1). An oxidative burst in barley plants populated by the barley aphid *Diuraphis noxia* and *Arabidopsis* plants populated by the peach aphid *Myzus persicae* was detected only in resistant but not in susceptible forms of plants (Lei, Zhu-Salzman, 2015).

In our experiments, the high H_2O_2 generation in resistant wheat forms was probably due to the elevated transcriptional activity of the *Rboh* and *Prx* genes (see Fig. 2). The same was observed earlier by the example of the wheat–*Staganospora nodorum* pathosystem (Veselova et al., 2018). In addition, experiments with mutant *Arabidopsis* plants with the silenced *RbohD* gene showed that NADPH oxidase played an important role in the generation of ROS in plants attacked by aphids (Kerchev et al., 2012).

The oxidative burst induces an immune response in plants, but excessive amounts of ROS inhibit photosynthesis and growth and can cause host cell damage. Therefore, resistant plants have mechanisms of ROS detoxication, which involves antioxidant enzymes such as PO, catalase, and others (Koch et al., 2016). In our experiments, PO activity increased in the resistant accessions *T. timopheevii* k-58666 and *T. monococcum* k-39471 during early responses after aphid settling. The PO activation was accompanied by an H_2O_2 content increase. The PO activity during long-term responses was even more significant than at the early stage (see Table 2). These results suggest the involvement of H_2O_2 and PO in the synthesis of toxic phenolic compounds and the reorganization of plant cell walls via lignification, which may be the cause of reduced pest viability (Morkunas et al., 2011; War et al., 2012; Koch et al., 2016). In our experiments, this kind of response was detected in the resistant accessions (see Fig. 1). In susceptible SY, enhanced activity of PO during early and long-term responses was not detected after aphid settling (see Table 2).

Thus, the activation of peroxidases may play a crucial role in the development of plant resistance to aphids via detoxifying large amounts of ROS (War et al., 2012; Koch et al., 2016). In addition, the H_2O_2 molecule has a signaling function in protection of plants from aphids. It acts as a second messenger in hormonal signaling pathways in the development of systemic resistance (Kerchev et al., 2012). The primary factor inducing the protective response of plants to infestation by aphids is considered to be mechanical injury, which induces JA-dependent activation of proteinase inhibitors and lipoxygenases (Morkunas et al., 2011). Next, the plant responds to the chemical determinants present in aphid saliva and induces a protective response similar to that of plants to biotrophic pathogens, which triggers the SA-dependent signaling cascade (Morkunas et al., 2011). Genes encoding protective proteins *Pr1* and *PAL* are SA-dependent (Van Loon et al., 2006). It is worth noting that the *Rboh* gene is also regulated by the SA-dependent signaling cascade (Kerchev et al., 2012). The *PI* and *LOX* genes are regulated by JA (Van Loon et al., 2006). The *Prx* gene, encoding anionic PO, is induced during JA- and SA-dependent defense reactions (Van Loon et al., 2006).

In our work, the contents of transcripts of the SA-dependent *Rboh* and *PAL* genes increased in plants of the resistant accession *T. timopheevii* k-58666 at both the early and late stages of feeding (see Fig. 2, *a*, 3, *b*). The increase was accompanied by enhanced H_2O_2 generation during early responses and high PO activity (see Table 2). These results suggest that phenylalanine ammonia lyase (encoded by the *PAL* gene), the main enzyme of the phenylpropanoid pathway, is involved in the synthesis of lignin and phenolic compounds, including SA. It is worth noting that lignification of plant cell walls occurs only when apoplastic peroxidases were activated together with elevated H_2O_2 content (Herrero et al., 2013). In addition, the induction of the SA-signaling pathway is likely to be a joint mechanism for antibiosis and aphid repulsion in resistant plant forms (Morkunas et al., 2011).

The resistant accession *T. monococcum* k-39471 showed significantly elevated concentrations of mRNAs of all genes under study, which were regulated by both the SA-dependent and JA-dependent signaling cascades, during early responses after the plants were attacked by aphids (see Fig. 2–4). The transcript contents of some genes decreased during long-term responses after aphid infestation, but the concentration of *LOX* gene mRNA continued to increase (see Fig. 2–4). The most important function of lipoxygenases is the oxidation of linolenic acid as the first step of the JA synthesis pathway and further activation of the JA-dependent signaling system protecting plants from pests. This leads to the synthesis of protease inhibitors and enzymes that participate in the production of lignin, suberin, and cutin and to the generation of volatile organic compounds acting as insect repellents (Wasternack, Strnad, 2018). Proteinase inhibitors bind to digestive enzymes in the insect intestine and inhibit their activity, thereby aggravating protein digestion, which, in turn, causes amino acid deficiency, slow development, and insect hunger (War et al., 2018). However, just the induction of SA-dependent genes (*Pr1*, *PAL*, *Rboh*, and *Prx*) induced the oxidative burst during early responses after the infestation by aphids and triggered the cascade of subsequent protective responses of plants against aphids. Probably, this was a result of the inhibition of catalase activity due to the direct binding of SA to catalase (Mohase, van der Westhuizen, 2002). It is known that some aphid species increase the activity of this enzyme to reduce the oxidative burst and secure favorable conditions for their life (Lei, Zhu-Salzman, 2015). We also showed earlier that the decreased reduced activity of catalase was among the factors ensuring high H_2O_2 levels in the resistant accessions *T. timopheevii* k-58666 and *T. monococcum* k-39471 (Rumyantsev et al., 2018).

In the susceptible variety SY, no significant increase in the contents of transcripts of genes regulated by SA-dependent signaling cascades during early responses after the aphid invasion was detected except for a small increase in the content of the *PAL* mRNA (see Fig. 2, 3), whereas the content of H_2O_2 was reduced. However, an increase in the content of transcripts of genes (*LOX* and *PI*) regulated by the JA-signaling cascade (see Fig. 4) was observed, which most likely reflected the plant's response to the damage (Morkunas et al., 2011). In the susceptible variety, both JA- and SA-sensitive genes were activated during long-term responses after the aphid invasion; however, the lack of oxidative burst appears to have prevented the start of the cascade of protective reactions.

Conclusions

To sum up, the oxidative burst is induced in resistant wheat varieties due to the triggering of the SA-signaling pathway, which is crucial in the subsequent cascade of chemical reactions leading to the development of resistance. Time is another factor important in the development of protective reactions and in the crosstalk between the SA- and JA-dependent signaling systems in plants. Induction of the JA-signaling pathway alone did not lead to the development of resistance, whereas the SA-signaling pathway, either by itself or along with JA-signaling cascade, induced resistance in *T. timopheevii* k-58666 and *T. monococcum* k-39471, respectively.

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ORCID ID

S.D. Rumyantsev orcid.org/0000-0002-5331-448X
S.V. Veselova orcid.org/0000-0002-1219-2383
G.F. Burkhanova orcid.org/ 0000-0003-2346-3502
I.V. Maksimov orcid.org/0000-0002-5707-3265

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