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ИНДУЦИРОВАННАЯ УСТОЙЧИВОСТЬ РАСТЕНИЙ КАК АЛЬТЕРНАТИВА ХИМИЧЕСКИМ СРЕДСТВАМ ЗАЩИТЫ РАСТЕНИЙ

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Реферат

На системах картофеля — *Globodera rostochiensis* и томаты — *Meloidogyne incognita* показано, что обработка семян томатов и клубней картофеля перед посадкой биогенными индукторами (хитозаном, жасмоновой и салициловой кислотами по отдельности и в сочетании с хитозаном) индуцирует системную устойчивость (СИУ) восприимчивых растений и популяция нематод на растениях, обработанных этими препаратами в определенных концентрациях, проявляла признаки морфо-физиологического угнетения. Показано, что механизм действия СИУ связан с изменением тех же защитных механизмов, которые отмечены при естественном иммунитете растений к нематодам. Применение для повышения устойчивости растений исследованных биогенных индукторов удовлетворяет требованиям нового поколения препаратов для защиты растений и их использование может быть перспективным.

Ключевые слова: нематоды, системная индуцированная устойчивость, хитозан, салициловой кислота, жасмоновая кислота.

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INDUCTION OF PLANT RESISTANCE TO NEMATODES SEDENTARY BIOGENIC ELICITORS

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Abstract

Objective of research: to study the mechanisms of induced tomato plant resistance to root-knot nematode *Meloidogyne incognita* and potato to cyst nematode *Globodera rostochiensis*.

Materials and methods: The biogenic elicitors — chitosan and signal molecules — SA, JA for the modulation of immune plant responses were used. In experiment 1, tubers of potato cultivars Istrinskii (PCN-susceptibility) and Krinitsa (PCN-resistant), were treated with aqueous solutions of the immunomodulators at the specified concentrations. A low molecular weight soluble chitosan and acetylation degree of 15% and signal molecule — SA was used as an elicitor. In experiment 2, system tomato *M. incognia* was studied. Water solutions of chitosan, signal molecules: SA, JA were used for treatment of tomato seeds for 2 h and then the seeds were planted in sterile soil. The control plants were treated with water. Cultivation of plants was carried out by the standard technique. Plants were maintained in a greenhouse long enough for the nematodes to complete their life cycle. Development of nematodes in the processed plants estimated on morphophysiological and population characteristics. Biochemical indicators of roots and leaves of tomatoes estimated for 14 days after infection of plants. Previously identified major biochemical indicators of the plants in the genome that contain genes that determine the resistance of plants. The effects of biogenic elicitors on plant resistance were also evaluated by some metabolic changes related to natural plant resistance to tomato and potato to plant nematodes. These indicators were studied in clarifying mechanisms of induced resistance.

Results and discussion: Biogenic elicitors induce systemic resistance of plants to plant parasitic nematodes — *Meloidogyne incognita* and *Globodera rostochiensis* (decrease in the parasitic invasion of the roots; an inhibition of the vital activity of the parasite; a decrease in fertility and the amount of agents sources (larvae and eggs) capable of infecting the plants). The addition of signal molecules (salicylic and jasmonic acid) to elicitors increased their activity as immunomodulators. In present investigation, the mechanisms of induced plant resistance nematode were studied. The data obtained suggest that the mechanisms natural and induced by biogenic elicitors tomato resistance to the nematode have the same origin. These features meet all requirements of the new generation of methods of plant protection and the use of biogenic elicitors to raise plant resistance to parasitic nematodes may be promising.

Keywords: nematodes, systemic acquired resistance, elicitors, signal molecules, chitosan, salicylic acid, jasmonic acid.

Introduced

Plants are often exposed to biotic stresses derived from viruses, bacteria, fungi, nematodes, and insects that can endanger crop yields and cause relevant economic losses.

Plant-parasitic nematodes are pests of a wide range of economically important crops, causing severe losses to agriculture, incurring estimated economic losses in excess of €100 billion/year in worldwide. Sedentary cyst and root knot nematodes are obligatory parasites of a wide range of crops. Sedentary nematodes which cause characteristic formations on roots, galls or root-knots, and cyst-forming ones which females transform into cysts at the last stage of their life cycle, have been of great economic importance and a subject for intent studies of nematologists and phytopathologists worldwide. These sedentary endoparasites start a lasting relationship with their host plant; the nematodes force the plant to create an exclusive feeding site located in the plant root. For growth and development, the nematode fully depends on this food source.

Potato cyst nematode (PCN) *Globodera rostochiensis* is classified with the most dangerous and economically significant pathogens of the family Solanaceae. This nematode decreases the yield of potato (to 60%), worsens the quality and marketable condition of tubers, as well as enables potato infection with other diseases. PCN is a sedentary endoparasite of potato roots, fighting with which is hampered due to the good adaptation of the parasite to environmental conditions, long (10 to 15 years) lifespan of cysts in the absence of the host plant, and the threat of occurrence of aggressive pathotypes in the case of reduction of nematode-resistant potato varieties in a monoculture.

Root-knot nematodes (*Meloidogyne* spp.) are one of the most important plant parasitic nematodes of great economic importance which reduce the quantity and the quality of the yields of many cultivated and wild plants everywhere (in tropical, subtropical and temperate regions).



Root-knot nematodes *Meloidogyne incognita* — parasitize the root systems of a wide variety of crops, including cultivated tomato (*Lycopersicon esculentum* Mill).

High level of adaptation to the parasitism of the nematode, complicates the control of these parasites. In many crops including potato, sugar beet and a range of vegetables, this small parasite is so successful that populations need to be controlled. Their adaptation to parasitism is so perfect that in many cases control strategies have been unsuccessful.

Currently, three main options are available for sedentary nematode control: i) crop rotation, ii) chemical control, and iii) the use of resistant host plant varieties. Genetically based resistance may be a simple and efficient solution to protect plants against pathogens and pests.

R-mediated resistance to nematodes correlates with hypersensitivity reactions, which is characterized by the rapid local death of plant cells at sites of penetration of nematodes and is accompanied by the accumulation of toxic products in the necrotic dead cells. The invading pathogen dies together with cells.

The new ecologically safe direction in plant protection based on the induction of plant resistance using the corresponding natural mechanism — i. e., systemic induced resistance (SAR) — is developed successfully during the last 10–20 years [3]. Using resistance induction in plants, it is planned to substitute or at least decrease the adverse effect of pesticides, which are now used in large volumes for treating agricultural crops. Various compounds (oligosaccharides, glycoproteins, fatty acids, and other substances) induce resistance in plants; they were named elicitors. SAR activated under the influence of metabolites of plant pathogens and various biotic and abiotic factors and reflecting a certain adaptive potential of the organism.

As known chitosan and its derivatives are elicitors or signal-transducing molecules involved in the regulation of expression of a broad array defence genes [7]. Numerous attempts have been made to use chitosan and other oligomers as elicitors of plant protection from various diseases including against nematodes [8, 11]. It is known that several chito-oligomers are able to bind to the receptor site on the membrane of plant cells [9]. SAR in plants activated the same defense mechanisms that operate in genetically determined resistance, but unlike this degree of protection, usually, does not exceed 20%.

One of the possibilities to elevate the efficiency of elicitor-induced defense is to supplement the elicitor with systemic signal molecules, such as jasmonic acid (JA), jasmonate methyl ester, salicylic acid (SA), ethylene, systemin, oligosaccharins, or some other compounds. It is known that the process of recognition of elicitors is mediated by signalling systems that determine the response of cells to various chemical and physical effects. For example SA and JA are important mediators in the transmission of stress signals in the genome of plant cells [2]. It was shown that application of JA and SA to tomato foliage induces systemic effects that suppress root-knot nematode infestation [18]. It is considered that the concentration of these substances increases at the site of infection by an incompatible pathogen or treatment with elicitor with subsequent transport of these substances or their derivatives via the phloem to various parts of the plant (or its organ) where they induce the defensive effects.

It is now recognized that the regulation of the number of phytopathogens by specific elicitors may be an addition or alternative to chemical methods of protection, since, unlike the latter this method does not cause damage to the environment. SAR continuously protects plants against broad spectrum of pathogens, including viruses, bacteria, fungi, and oomycetes.

Materials and methods

In present investigation the mechanisms of induced tomato plant resistance to root-knot nematode *M. incognita* (Kofoid et White, 1919) Chitwood, 1949 and potato to cyst nematode *G. rostochiensis* (Wollenweber, 1923) Behrens, 1975 were studied.

The biogenic elicitors — chitosan and signal molecules — SA, JA for the modulation of immune plant responses were used.

In experiment 1, tubers of potato cultivars Istrinskii (PCN-susceptibility) and *Krinitza* (PCN-resistant), were treated with aqueous solutions of the immunomodulators at the specified concentrations. A low molecular weight soluble chitosan with an average molecular weight of 5 kDa and acetylation degree of 15% and signal molecule — SA (7×10^{-7} – 7×10^{-8} M) [5] was used as an elicitor. In experiment 2, system tomato *M. incognita* was studied. In this work we

used tomato resistant hybrids Shagane (resistance index (RI)- 100%) and susceptible Gamayun (RI — 30%).

Water solutions of chitosan, signal molecules: SA, JA ($10^{-7}M$ — $10^{-4}M$) were used for treatment of tomato seeds for 2 h and then the seeds were planted in sterile soil. The control plants were treated with water. Cultivation of plants was carried out by the standard technique [14]. Plants were maintained in a greenhouse ($\sim 24-27$ °C; 16 : 8 L : D photoperiod) long enough for the nematodes to complete their life cycle (6,5 wk). Development of nematodes in the processed plants estimated on morphophysiological and population characteristics. Biochemical indicators of roots and leaves of tomatoes estimated for 14 days after infection of plants.

Previously identified major biochemical indicators of the plants in the genome that contain genes that determine the resistance of plants (R-genes) [10, 16]. The resistance of plants to phytopathogens is known to be related to a multicomponent defense response. The effects of biogenic elicitors on plant resistance were also evaluated by some metabolic changes related to natural plant resistance to tomato and potato to plant nematodes. These indicators were studied in clarifying mechanisms of induced resistance.

The activities of enzymes were measured as described in [11]. Colloidal chitin (10 mg/ml) and laminarin were used as substrates for chitinase (EC 3.2.1.14) and β -1,3-glucanase (EC 3.2.1.39) activities. The specific activities of these enzymes were expressed in micromoles of N-acetylglucosamine and glucose formed per mg protein in 1 min, respectively. Lipoxygenase (LOX, EC 1.13.1.13) activity was spectrophotometrically using linoleic acid as a substrate [6]. The activity of phenylalanine ammonium lyase (PAL EC 4.3.1.5) the method described earlier in our modification [5] was determined spectrophotometrically (290 nm) by the formation of trans-cinnamic acid ($\mu M/g$ protein for hour) (phenylalanine was as substrate). Proteinase inhibitors (PIs) were assayed by the inhibition of trypsin activity. The PI activity in leaves and roots was estimated at the level of suppression of the amidase activity of trypsin using benzoylarginine paranitroanilide (BAPA) in accordance with Erlanger's method [4]. Sterols were isolated from tomato roots as described [15]. Fractions of free sterols were identified by high-performance liquid chromatography. The determination of phytoalexin — rishitin was performed as determined as described before [1].

Results and discussion

On the most reliable parameters characterizing the degree of potato resistance to *G. rostochiensis* is the number and size of cysts of this nematode in soil at the end of experiment, as well as the number of eggs in cysts (Table 1).

Table 1

Effect of biogenic elicitors on morphophysiological parameters of *G. rostochiensis*

Treatment	Krinitsa (R)			Istrinskii (S)		
	Cyst size, mm	Number of eggs per female	Number of cysts per 100 g soil	Cyst size, mm	Number of eggs per female	Number of cysts per 100 g soil
Control	0,129	0	33	0,164	71	180
Chitosan	0,115	0	0	0,144	59	81
SA	0,130	0	25	0,188	51	85
Chitosan +SA	—	0	0	0,129	61	60
LSD at $P \leq 0,05$	0,010			0,015	10	15

As evident from this table, the highest resistance was exhibited by cultivar *Krinitsa*, on roots of which *G. rostochiensis* formed immature eggs containing no cysts. On the roots of *Istrinskii* in the control found a large quantity of cysts. Biogenic elicitors (5 kDa chitosan and SA) induced protective effects. The low molecular weight water-soluble chitosan + SA displayed the maximum protective activity. This order of arrangement of potato plants with respect to their resistance

Table 3

Catalase and PAL activity in eaves of infected potato plants

Treatment	Catalase activity, ($\mu\text{M H}_2\text{O}_2 / (\text{mg protein} \cdot \text{min})$)	PAL activity, mM cinnamic acid/(mg protein h)
Water (control)	0,310	1,8
Chitosan	0,137	2,7
LSD at $P \geq 0,05$	0,046	0,51

Table 4

PAL and LOX iactivity in leaves of tomatoes infested by the root-knot nematode

Treatment	PAL activity in leaves μM cinnamic acid/ (mg protein h)	LOX activity, $\Delta\text{D}_{234} \text{min}^{-1}$
Control (water)	1,6	0,38
SA	3,3	–
JA	1,7	0,58
Chitosan	4,2	0,52
Chitosan + SA	–	0,78
Chitosan + JA	2,0	0,62
LSD at $P \geq 0,05$	0,98	0,14

Another important defense reaction is the formation of PR proteins in tissues of resistant plants infected by fungi, bacteria, and viruses or treated with elicitors. Chitinase and β -1,3-glucanases are the most extensively studied. These enzymes probably destroy cell walls in phytopathogens and are involved in the formation of oligosaccharides that regulate plant immune reactions.

It is known that higher plants do not have, or contain only minor amounts of, chitin and chitosan. However, enzymes cleaving these substances are abundant in higher plants. Under normal conditions, plants contain only minor quantities of chitinase and chitosanase. The content of these enzymes sharply increases under the effects of biotic and abiotic stress factors. After treatment of elicitors increased β -1,3-glucanase and chitinase activities in plants (Table 5).

Table 5

Activities of β -glucanase and chitinase in tissues of potato treated with chitosans (500 $\mu\text{g/ml}$) and water (control)

Treatment	Activity, $\mu\text{M}/(\text{min mg protein})$	
	β -1,3-glucanase	chitinase
Chitosan, 5 kDa	0,067	0,053
Control	0,029	0,052

Chitosan with a molecular weight of 5 kDa increased β -1,3-glucanase and chitinase activities in potato tubers.

Earlier studies have shown that activity of chitinase and β -1,3-glucanase in leaves of tomato plants, seeds of which were treated by chitosan, increased on 312% and 34% in comparison control, according [17].

The roles of proteinases and their inhibitors in the formation of protective reactions of plants to parasitic invasions is actively discussed interactions in modern scientific literature. Proteins inactivating the enzymes of pathogens are involved in plant protection from microorganisms. The protective function of PIs has attracted much attention in recent research.



The PI activity in leaves and roots of tomato resistant and susceptible cultivars was determined at 14th day after invasion [10]. The invasion of plants treated with JA caused an increase in the PI activity in leaves of both cultivars and the greatest change was observed in plants of the susceptible species (80%). In leaves of resistant tomatoes treated with JA, the invasion caused an increase in the PI activity by 29%. In roots of both Gamayun (S) and Shagane (R) cultivars treated with JA, during the invasion, there was a considerable increase in the PI activity (by factors of more than 2,5 and 2,2, respectively in susceptible and resistant cultivars) in comparison with non-invaded plants treated with JA. The study of plants invaded by gall nematodes showed that the treatment with JA decreased the invasion of tomato roots susceptible to the root-knot nematode (the number of galls in roots decreased by 31%), but the weight of the aboveground part was considerably higher compared to untreated plants (Table 2). These data agree with the data on the role of proteinases in the life of root-knot nematodes: it has been shown that proteinases of *M. incognita* participate in different processes during the whole life of the nematode, such as feeding, reproduction, and embryogenesis. Our data demonstrate that PIs are components of plant protection against root-knot nematodes.

One of the most important defenses of plant tissues against phytopathogens is their ability to produce low molecular weight antibiotics, phytoalexins, which are *de novo* synthesized in response to incompatible pathogens or races of pathogens and elicitors. Phytoalexins at toxic concentrations inhibit the growth and development of phytopathogens. It was shown that the 5 kDa chitosan, which was the most potent in protecting potato from plant parasitic nematode *Ditylenchus destructor* [13], induced the formation of the greatest amount of the phytoalexin — rishitin (Table 6).

Table 6

Induction of rishitin in potato tubers treated with chitosans 5 kDa

Chitosan concentration, mg/ml	0,1	1,0	3,0
Rishitin, mg/ml	0	8	40

No rishitin production was detected in control roots of tomato (Table7). It is interesting that its level and appearance (over 40 µg/g within five days after invasion in case of pretreatment with chitosan differed from those in resistant cultivars [13].

Table 7

Induction of rishitin in potato tubers treated with chitosans 5 kDa

Chitosan concentration, mg/ml	0,1	1,0	3,0
Rishitin, mg/ml	0	8	40

Sterols as known play a special role in interactions of plants with parasitic nematodes, which are auxotrophs in relation to sterols, and replenish their deficit by sterols taken from the host tissues. The reproduction of the parasite is especially sterol-dependent. The sterol-dependence of nematodes determines the resistance of tomato plants that deprive the parasite of sterols.

Considerable differences between control and chitosan-treated plants were found in the contents and composition of free sterols. The total level of free sterols in roots of treated tomato plants decreased nearly twofold in comparison to control plants. A study of individual sterol fractions showed that campesterol and sitosterol accounted for this effect; their levels in the roots of treated plants decreased more than in control plants. The level of fucosterol in immunized plants increased considerably. The shares of other sterols in the total fraction changed to a small extent [15].

Reduction of sterols in plant roots is associated with accumulation of sesquiterpene rishitine, that have a common biosynthetic pathway with sterols. It is suggested that terpene biogenesis has switched from a healthy plant sterol formation pathway, to the formation of highly toxic phytoalexins. As a result, the parasite loses necessary nutrients for its development and simultaneously the nematode is affected by plant phytoalexins [12].

Thus, the treatment of potato tubers and tomato seeds by biogenic elicitors and composition of elicitors with signal molecules induced SAR in plants to sedentary nematodes simultaneously produced the following effects: a decrease in the parasitic invasion of the roots; an inhibition of



the vital activity of the parasite; a decrease in fertility and the amount of agents sources (larvae and eggs) capable of infecting the plants. The data obtained suggest that the mechanisms natural and induced by biogenic elicitors tomato resistance to the nematode have the same origin. These features meet all requirements of the new generation of methods of plant protection and the use of biogenic elicitors to raise plant resistance to parasitic nematodes may be promising. More knowledge about the resistance induction mechanism involved in inducer treatments of tomato seeds to acquire systemic resistance to plant parasitic nematodes could enhance the development of a new biologically and environmentally safe method for the sustainable management of these important plant pathogens.

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