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Induction of Resistance to Phytophthora in Tubers of Transgenic Potato

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Abstract—Resistance of transgenic cultivars based on the expression of one or more resistance genes is sooner or later broken by pathogens whose race-producing rates are high. Thus, combining transgenesis with elicitor-induced resistance is a promising approach. The elicitor-induced resistance is based on the expression of multiple resistance genes, which can prevent the adaptation of pathogens to transgenic cultivars, maintain the stability of cultivars, and increase their lifespan. In this work, we used transgenic potato cultivars *Temp* and *Superior* transformed with *Bacillus thuringiensis* Δ-endotoxin gene and *Luk'yanovskii* transformed with leukocyte interferon gene. Arachidonic acid (10^{-8} M) and soluble chitosan (5 kDa, 100 μg/ml) were used as elicitors for tuber treatment. Our data showed that pretreatment with elicitors causes a 15–25% increase in both the systemic prolonged resistance of potato tubers to *Phytophthora infestans* and their ability to repair mechanical damage.

Recent molecular-genetic studies enriched the technology with novel approaches to plant protection allowing one to avoid the wide usage of pesticides in agricultural practice. These methods are the development of transgenic forms of resistant plants and induction of resistance with elicitors [1]. Each of them is has its own advantages, but neither is free of drawbacks.

Gene engineers aim to develop genetically resistant plant races that are ideally not at all affected by pathogen(s). Advantages of transgenesis are directed transformation of plants with a desired gene and a relatively rapid development of resistant races that can be further accelerated by improved methods of gene engineering. These methods allow inserting resistance genes into high-yield cultivars, whereas productivity and resistance are often mutually exclusive from the classical selection standpoint.

However, transgenic forms of resistant plants are still based on transformation with a single gene or, at best, with a limited number of genes that might sooner or later be overcome by pathogens due to the high rate of race-forming process. The life of resistant transgenic cultivars can be rather short, because the narrow genetic basis of their resistance strongly limits their ability to resist diseases. In these circumstances, gene engineers, like former selectionists, have to work on a permanent program, that is, to develop quickly a sequence of cultivars in order to outride pathogenic race-forming processes [2].

Transformation of plants by individual resistance genes strongly resembles the vertical resistance of plants, which is most often broken by pathogens [3]. The fate of many cultivars with vertical resistance is known to be lamentable. In many cases, an initial success ended by resounding defeat after a short time.

Alternatively, elicitor-induced resistance is based on induction of multiple genes, thus resembling the horizontal polygenic resistance, which, as a rule, is prolonged and not broken by pathogens. The difference is that the horizontal resistance is genetic, whereas the induced one is phenotypical. Immunostimulation underlying the induced resistance makes it nonspecific and broad against pathogens of various taxonomic groups, which is a great advantage [4].

However, induced plant resistance is only relative and provides only partial protection against diseases. However, in spite of the relativity, this effect is steady.

In light this, integration of transgenesis and induced resistance is the most promising, because it can eliminate faults and consolidate advantages of both methods.

In this study, we attempted to induce disease resistance in transgenic potato forms with biogenic elicitors.

MATERIALS AND METHODS

Potato (*Solanum tuberosum* L.) cultivars Temp (Belarus), Luk'yanovskii (Russia), and Superior (USA) were studied. The first two cultivars bear the *Phytoph-*

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Table 1. *Phytophthora* invasion in transgenic potato tubers of the cultivar *Temp* T-3 and T-7 treated with 10^{-8} M arachidonic acid (AA)

Tubers	Treatment	Race 3.4 (incompatible), necrotic cells		Race 1.3 (compatible), depth of fungus penetration	
		count	% of control	cell rows	% of control
Initial	Water	13.8 ± 1.0	100	20.5 ± 2.3	100
	AA	10.9 ± 0.7	79	15.3 ± 1.1	70
Transgenic T-3	Water	18.8 ± 1.2	100	27.9 ± 2.4	100
	AA	15.2 ± 1.3	81	21.2 ± 2.2	76
Transgenic T-7	Water	17.0 ± 1.3	100	25.5 ± 2.0	100
	AA	12.5 ± 0.9	74	18.3 ± 1.7	72

Table 2. *Phytophthora* invasion in transgenic potato tubers of the cultivar *Temp* T-3 and T-7 treated with chitosan (100 μg/ml)

Tubers	Treatment	Race 3.4 (incompatible), necrotic cells		Race 1.3 (compatible), depth of fungus penetration	
		count	% of control	cell rows	% of control
Initial	Water	11.5 ± 0.7	100	21.9 ± 1.3	100
	Chitosan	9.1 ± 0.6	79	15.3 ± 1.1	70
Transgenic T-3	Water	13.1 ± 1.1	100	24.8 ± 1.7	100
	Chitosan	9.8 ± 0.6	75	17.6 ± 1.5	71
Transgenic T-7	Water	14.5 ± 1.2	100	28.7 ± 2.4	100
	Chitosan	10.0 ± 0.7	69	18.6 ± 1.2	65

thora resistance gene R_1, that is, possess vertical monogenic resistance against the race 3.4 and susceptibility to race 1.3 of *Phytophthora* used in this study. Cultivar *Superior* was found to be sensitive to both races of the fungus.

Transgenic variants T-3 and T-4 bearing *Bacillus thuringiensis* v. *tenebrionis* (Bt) Δ-endotoxin gene that protects plants against beetles, including the Colorado potato beetle [5], were selected from the potato cultivar Temp by the Bioengineriya Center, RAS. Transgenic potato variants bearing Δ-endotoxin of this pathogen and resistant to the Colorado potato beetle were also created by Monsanto (USA) from the cultivar *Superior.* A variant transformed with leukocyte α-interferon gene (a construct with this gene was designed by the Department of Virology, Moscow State University) was created from the cultivar Luk'yanovskii at the Institute of Potato Growing. This potato variant was supposed to possess an elevated antiviral resistance.

Arachidonic acid (AA) (Sigma, USA; 10^{-8} M) and water-soluble low-molecular-weight chitosan (5 kDa, deacetylation extent 85%; 100 μg/ml) purified in the Bioinzheneriya Center from crab shells using the enzymatic complex of *Streptomyces kurssanovi* [6] were used as biogenic elicitors. The elicitors were optimal for induction, which was determined in preliminary tests [7].

The causative agent of potato late blight (*Phytophthora infestans* (Mont) de Bary) was grown for 11 days on oat agar medium; zoospores were isolated from zoosporangia washed from the medium surface with sterile distilled water.

Both the systemic resistance of potato tubers to *Phytophthora* and their ability to repair mechanical damage were determined. Systemic resistance is known to involve the whole plant or its organ tissues independently of the area of pathogen invasion or elicitor application.

Potato tubers matched in size were treated with elicitors (20 ml per ten tubers). Control tubers were treated with water. One week after the treatment, cylinders oriented from the apical to stolon part were plugged out of the tubers. The cylinders were dissected into disks of 5 mm in height and 16 mm in diameter; middle disk samples were infected with a suspension of *Phytophthora* zoospores (5×10^4 spores per ml). The results of infection were examined after 72 h.

Distribution of the incompatible *Phytophthora* race 3.4 spreading along the disk tissues was evaluated from the number of necrotic cells penetrated by the pathogen hyphae. The greater the number of necrotic cells, the larger the pathogen distribution area. The extent of disk lesion caused by the compatible fungus race 1.3 was evaluated from the depth of its penetration into the disk tissues estimated by the number of infected cell layers from the surface infected.

Wounded periderm formed on the surface of noninfected disks treated with various elicitor doses was evaluated from the count of newly formed layers.

Table 3. The number of wound periderm layers in transgenic potato tubers of the cultivar *Temp* T-3 and T-7 treated with 10^{-8} M arachidonic acid and chitosan (100 μg/ml)

Tubers	Treatment	Wound periderm	
		number of layers	% of control
Initial	Water	2.98 ± 0.01	100
	AA	3.66 ± 0.07	123
	Chitosan	3.61 ± 0.2	121
Transgenic T-3	Water	3.16 ± 0.05	100
	AA	3.86 ± 0.01	122
	Chitosan	3.72 ± 0.08	118
Transgenic T-7	Water	3.24 ± 0.01	100
	AA	3.82 ± 0.01	118
	Chitosan	3.95 ± 0.02	122

Table 4. Phytophthora invasion of novel transgenic potato tubers of the cultivar *Luk'yanovskii* transformed with leukocyte α-interferon gene (potato underwent a preplant treatment with 10^{-8} M arachidonic acid)

Tubers	Treatment	Race 3.4 (incompatible), necrotic cells	
		count	% of control
Initial	Water	12.9 ± 0.9	100
	AA	10.7 ± 0.6	83
Transgenic int	Water	8.5 ± 0.2	100
	AA	7.3 ± 0.4	86

Statistical analysis was performed at the confidence level of 0.95.

RESULTS AND DISCUSSION

Transgenic potato forms T-3 and T-7 are remarkably less resistant to *Phytophthora* than the parent cultivar Temp (Tables 1 and 2). This was observed in respect to both incompatible pathogen race whose spreading along the tuber tissues is more prominent than in the parent potato form and the compatible race penetrating deeper into the disk tissues than in the control. However, since the numbers of periderm layers formed on wounded disk surface even exceeded the initial form (compare 3.16, 3.24, and 2.98), both transgenic forms did not lose their capability to repair mechanical damage (Table 3).

The use of AA and chitosan makes it possible to increase the resistance to *Phytophthora* in both transgenic forms, resulting in an about 1/4 or even 1/3 decrease in the amount of tissue infected by the incompatible or compatible *Phytophthora* races (Tables 1 and 2). Chitosan and AA equally induced the potato resistance to *Phytophthora*.

Treatment with elicitors can enhance the capability of transgenic tubers (even compared to the initial form) to form wounded periderm in abrasion areas (Table 3). This means that transgenic tubers treated with elicitors can have better storage characteristics due to active formation of wounded periderm that hampers the spread of infection through abrasion sites unavoidably formed during mechanized tuber harvesting.

Of prime importance is the fact that not only superficial but also deep tissues gained resistance after treatment of intact tuber surface with AA or chitosan. Thus, elicitors can induce systemic resistance in transgenic forms, which is a particularly valuable feature in agriculture.

Not only systemic resistance, but also prolonged resistance to *Phytophthora* was further demonstrated to be induced by elicitors in transgenic potato tubers. This was evident from experiments in which tubers of the transgenic cultivar Luk'yanovskii transformed with α-interferon (Table 4) and tubers of the cultivar Superior bearing the *B. thuringiensis* toxin gene (Table 5) substantially increased their resistance to *Phytophthora* as a result of treatment with AA before seeding. Thus, systemic resistance induced by elicitors was maintained for at least four months from seeding to harvesting. Previous studies showed that the proper usage of elicitors can prolong induced systemic resistance against plant diseases (particularly in potato) up to one or more years [1].

Results of studies of three transgenic types in the system potato–*Phytophthora* showed that systemic and prolonged resistance can be induced in transgenic plants by treatment with elicitors. A combination of transgenesis with induction of plant resistance by elicitors can elevate not only the resistance in transgenic forms, but also endow resistance with an integrated character, and, what is more important, it can prevent

Table 5. *Phytophthora* invasion of novel transgenic potato tubers of the cultivar *Superior* bearing the *B. thuringiensis* Δ-endotoxin gene Bt (potato underwent a preplant treatment with 10^{-8} M arachidonic acid)

Tubers	Treatment	Race 3.4		Race 1.3	
		Depth of fungus penetration			
		cell rows	% of control	cell rows	% of control
Initial	Water	40.4 ± 2.57	100	42.6 ± 3.47	100
	AA	34.4 ± 3.0	85	37.0 ± 1.57	87
Transgenic	Water	50.6 ± 4.0	100	50.4 ± 3.25	100
	AA	40.8 ± 3.8	81	40.6 ± 2.35	81

the pathogens from adapting to the transformed gene for the period of induced resistance, thereby prolonging the life of the transgenic form. After treatment with an elicitor, additional expression of transformed gene may occur, thereby elevating the protective response even more.

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