Ribonuclease activity as a new prospective disease resistance marker in potato

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Disease resistance is an important characteristic for each variety of potato, and the search for pathogen resistance markers is one of the primary tasks of plant breeding. Higher plants possess a wide spectrum of enzymes catalyzing the hydrolysis of nucleic acids; it is believed that protection against pathogens is the most probable function of the enzymes. RNases are actively involved in several immune systems of higher plants, for example, systemic acquired resistance (SAR) and genetic silencing, hence RNase activity in plant leaves, as a relatively easily measured parameter, can serve as a good marker for the selection of pathogen resistant varieties. We have analyzed sixteen varieties of potatoes permitted for use on the territory of the Russian Federation and tested the correlation of the level of variety-specific ribonuclease (RNase) activity with such economically valuable traits as maturity and resistance to viruses, late blight and common scab. In general, the level of RNase activity was variety-specific, which was confirmed by very small values of average squared error for the majority of tested varieties. We have detected a statistically significant positive correlation of RNase activity in potato leaves with increased resistance of varieties to phytopathogenic viruses, a negative correlation with resistance to scab and an absence of a significant connection with maturity and resistance to late blight, regardless of the organ affected by the oomycete. Thus, the level of RNase activity in potato leaves can be used as a selective marker for resistance to viruses, while varieties with increased RNase activity should be avoided when selecting resistance to scab.

Key words: *Solanum tuberosum*; phytopathogenic viruses; late blight; common scab; RNase activity.

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Рибонуклеазная активность как потенциальный новый маркер устойчивости к фитопатогенам у картофеля

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Устойчивость к фитопатогенам является важной характеристикой для каждого сорта картофеля, а поиск маркеров устойчивости к патогенам – одна из приоритетных задач селекции растений. Высшие растения обладают широким спектром ферментов с нуклеазной активностью. Основной наиболее вероятной функцией этих ферментов считается защита растений от патогенов. РНКазы активно участвуют в иммунных системах растений, например таких, как системная приобретенная устойчивость и генетический сайленсинг, следовательно, РНКазная активность в листьях растений, как относительно легко измеряемый параметр, может служить хорошим маркером для отбора устойчивых к патогенам сортов. В настоящей работе проанализированы шестнадцать сортов картофеля, включенных в Государственный реестр селекционных достижений, допущенных к использованию на территории Российской Федерации. Проверена корреляция уровня рибонуклеазной (РНКазной) активности сортов с такими хозяйственно ценными признаками, как сроки созревания и устойчивость к вирусам, фитофторе и парше обыкновенной. В целом уровень РНКазной активности оказался сортоспецифичным параметром, что было подтверждено очень малыми значениями средней квадратичной ошибки для большинства тестируемых сортов. Выявлена статистически значимая позитивная корреляция РНКазной активности в листьях картофеля с повышенной устойчивостью сортов к фитопатогенным вирусам, негативная корреляция с устойчивостью к парше обыкновенной и отсутствие значимой связи с устойчивостью к фитофторозу вне зависимости от органа, поражаемого оомицетом. Таким образом, уровень РНКазной активности в листьях картофеля может быть использован как селективный маркер устойчивости к вирусам, в то время как при селекции устойчивости к парше сортов с повышенной РНКазной активностью следует избегать.

Ключевые слова: *Solanum tuberosum*; рибонуклеазная активность; фитопатогенные вирусы; фитофтороз; парша обыкновенная.

Potatoes are the third most important crop and the first of non-cereals, annually more than 300 million tons of potatoes are produced in the world, and about 1 billion people consider potatoes to be the basis of their diet (Barrell et al., 2013). Currently, more than 3000 varieties of potatoes are grown in 60 countries of the world with different resistance to pathogens and unfavorable climatic conditions, with different taste and maturity. Resistance to late blight, viral infections and other phytopathogens is an important characteristic for each variety of potato, and the search for pathogen resistance markers is one of the primary tasks of plant breeding and genetics. It is known that higher plants have a large spectrum of enzymes with nuclease activity, 16 ribonucleases (RNase) with a molecular weight from 9 to 41 kDa in *Arabidopsis thaliana* (Yen, Green, 1991), 15 RNase in wheat and 14 RNase in barley with molecular weights from 16.3 to 40.1 kDa (Yen, Baenziger, 1993). Several RNase were detected in wild potato *Solanum chacoense* (Qin et al., 2005) in connection with studies of the mechanisms of gametophytic self-incompatibility in Solanaceae, but a systematic study of RNase activity in potatoes has not yet been carried out.

Although the specific functions of each plant RNase are only clarified, it is believed that protection from pathogens is the most likely function of these enzymes in higher plants (Kao, McCubbin, 1996). It was reported that RNase activity was higher in diseased plants (Green, 1994; Lusso, Kuc, 1995; Galiana et al., 1997; Hugot et al., 2002). Wounding, senescence, and phosphate starvation also cause induction of RNase expression (Galiana et al., 1997; Lers et al., 1998; Kurata et al., 2002). The direct involvement of phosphate starvation-inducible RNase in the acquired resistance to *Phytophthora parasitica* was shown in *N. tabacum* (Galiana et al., 1997; Hugot et al., 2002). Previously, we have demonstrated that the transgenic plants with an increased level of RNase activity were more resistant to at least two different unrelated viruses (Trifonova et al., 2007b; Sugawara et al., 2016). For buckwheat varieties with different resistance to the buckwheat burn virus (BBV), a positive correlation between resistance to virus and RNase activity was shown, the authors have analyzed two varieties, Roksolana and Kara-Dag (Sindarovska et al., 2014).

In the present work, we have analyzed sixteen varieties of potatoes presented in the State Register of Selection Achievements Admitted to Use in the Russian Federation and tested the correlation of ribonuclease activity level of varieties with such economically valuable features as maturity and resistance to viruses, late blight and common scab.

Materials and methods

Plants material. Thirteen potato varieties were used from the *in vitro* collection of the Genetic engineering laboratory of the Institute of Cytology and Genetics of the SB RAS (Novosibirsk) and three potato varieties of the Siberian selection (Tuleevskiy, Kemerovchanin and Safo) from the *in vitro* variety collection of Bio Collection Center "GenAgro". The original plants free from viral and viroid infections were cultivated in sterile conditions *in vitro*, at a temperature of 20±2 °C in the daytime and 18±2 °C at night, with an illumination of 8.000 lux. To prepare the material, apical shoots measuring 2.5–3 cm in length were placed in test tubes 200 × 23 mm and cultured on Murashige and Skoog medium (Murashige, Skoog, 1962). To the main medium of Murashige and Skoog, 20 g/L of sucrose and 6 g/L of agar were added and cultivated for three to four weeks, until the plants were formed. The tubes were covered with cotton-gauze stoppers. For each variety, no less than 5 test plants were grown in each experiment. All procedures for the cultivation of the starting material were carried out according to generally accepted procedures (Pershina, 2005).

Ribonuclease assay. For one RNase activity measurement, leaves from the entire 4–6 weeks old potato plant grown *in vitro* under standard conditions were used. The level of RNase activity in crude leaf extracts of plants was evaluated by the change in the amount of acid-soluble matter in total yeast RNA (Trifonova et al., 2007b). Leaf tissue 1 g, was ground in liquid nitrogen, suspended in 1 ml of 50 mM Tris–HCl (pH 7.0) and centrifuged for 10 min (12.000 g, 4 °C). The total protein was assayed in the supernatant according to Bradford (Bradford, 1976). The extracts, containing 25 g of total protein, were added to the reaction mixture containing 0.4 % total yeast RNA, 0.1 % bovine serum albumin, and 0.1 M Tris–HCl (pH 7.0). The total volume of the reaction mixture was 300 µl. The reaction was stopped by adding 1 ml of 3.4 % $HClO_4$. The test tubes were cooled at 4 °C for 10 min and centrifuged at 12.000 g and 4 °C for 5 min. The optical densities of the supernatants were measured at 260 nm relative to the control (reaction mixture without leaf extract).

To assess the correlation of the level of RNase activity in varieties with economically valuable traits, we adopted the following notation: 1 – the cultivar is resistant to late blight or viruses (resistance index 6–9 at http://www.kartofel.org/cultivars/main_cult/sorta.htm) or the "+" sign in the World Catalogue of Potato Varieties (Hamester, Hils, 2004) for one or more viruses, 0 – the variety is susceptible (resistance index 1–5 or "–" in the above catalogue). We considered in our study only potato viruses with ssRNA genomes, such as PVX, PVY, PVM, and PVS. For the maturity period, we took the "1" for late and the medium maturity, and "0" for early. Parameters of varieties and RNase activity in leaf extracts are shown in Table 1. The evaluation of the direction and tightness of the relationship of the parameters was made using the Pearson's parametric correlation criterion.

Results and discussion

Potato varieties differ among themselves for a large number of characteristics, starting from the shape of the tuber and taste qualities and ending with resistance to phytopathogens. In our previous research of the expression of heterologous nucleases in transgenic plants, we noticed that different potato varieties have different intrinsic levels of RNase activity (Trifonova et al., 2004). In this study, we first assessed this level for 16 varieties listed in the Federal State Register of Breeding Achievements Admitted to Use in the Russian Federation (2017) and checked whether the level of RNase activity is related to some important agricultural characteristics such as maturity, potato resistance to viral diseases, late blight and common scab.

The results of RNase activity measurements in leaf extracts of different potato varieties are shown in Table 1. Note that, in general, this feature is variety-specific, which is confirmed by very small values of average squared error for the majority of tested varieties. The highest average squared error was

Table 1. RNase activity in leaves and agriculturally valuable parameters of potato varieties

ID	RNase activity	Maturity	Late blight tubers	Late blight leaves	Viruses	Common scab
Arkhideya N	0.318 ± 0.004	0	0	0	1	–
Beloyarskiy ranniy	0.459 ± 0.033	0	0	0	1	–
Vytok	0.291 ± 0.000	1	1	1	1	1
Golubizna	0.271 ± 0.002	1	0	0	1	1
Desiree	0.462 ± 0.005	1	0	0	1	0
Zhukovskiy ranniy N	0.210 ± 0.001	0	0	0	0	1
Ilinskiy	0.443 ± 0.000	0	0	0	1	1
Kemerovchanin	0.258 ± 0.004	0	0	0	0	1
Kolorit	0.472 ± 0.059	1	–	–	1	–
Lugowskoy	0.498 ± 0.014	1	1	1	1	1
Odissey	0.440 ± 0.009	0	1	0	1	0
Sante	0.401 ± 0.000	0	0	0	1	1
Safo	0.233 ± 0.021	0	0	0	0	–
Skoroplodniy	0.286 ± 0.000	0	0	0	0	–
Tuleevskiy	0.466 ± 0.069	0	0	0	1	0
Kapris	0.236 ± 0.007	0	0	0	0	–

Notes: RNase activity is presented as optical density at 260 nm.

Table 2. Interrelation of RNase activity with pathogen resistance in potato (Pearson correlation)

Pearson's correlation parameter	Maturity	Late blight tubers	Late blight leaves	Viruses	Common scab
r	0.1758	0.2923	0.1695	0.7590	–0.5388
p	0.531	0.290	0.546	0.001	0.106

recorded in the Tuleevskiy variety, which also proved to be the most variable in the number of shoots formed per explant, which, apparently, is due to the genetic characteristics of the variety (Ibragimova et al., 2018).

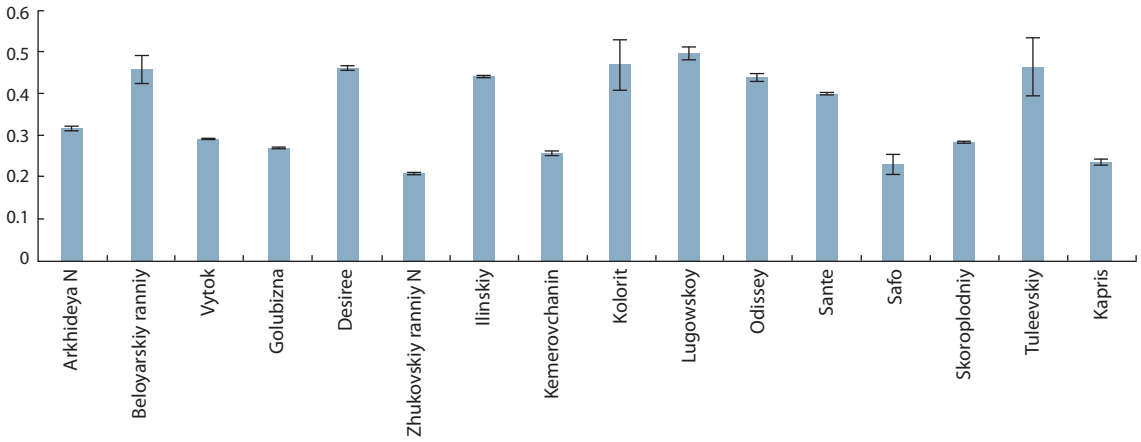
RNases are actively involved in several immune systems of higher plants, for example, systemic acquired resistance (SAR) and genetic silencing (Trifonova et al., 2007a), hence RNase activity in plant leaves, as a relatively easily measured parameter, can serve as a good marker for the selection of pathogen resistant varieties. Most of all, we were interested in the correlation of the level of RNase activity with resistance to the late blight of tubers and leaves, the general increased resistance to viruses and common scab. The results of the correlation analysis are presented in Table 2.

We found a statistically significant positive correlation of RNase activity in potato leaves with increased resistance of varieties to phytopathogenic viruses ($r = 0.7590$, $p = 0.001$).

It was hypothesized that RNase activity may play a protective role in plants (Green, 1994; Galiana et al., 1997; Trifonova et al., 2015), RNase activity increases in diseased plants (Green, 1994; Lusso, Kuc, 1995; Galiana et al., 1997; Hugot et al., 2002; Šindelářová et al., 2002), and injury causes an increase in this activity in tobacco (Kurata et al., 2002) and tomato (Lers et al., 1998). Among the 17 identified proteins associated with pathogenesis (PR proteins), some of the PR-4 and PR-10 proteins are nuclease (Van Loon et al., 2006).

Previously, we have demonstrated that elevated extracellular RNase activity in the transgenic tobacco modulates the resistance of these plants to phytopathogenic viruses, with an increase in resistance proportional to the level of RNase activity (Trifonova et al., 2015; Sugawara et al., 2016). The molecular mechanisms of RNase antiviral effects are still poorly understood. It may be hypothesized that higher level of nuclease activity provides for plant DNA fragmentation during the hypersensitive response or direct pathogen DNA/RNA degradation (Kim et al., 2011), while lower level of nuclease activity or binding without hydrolytic activity explores alternative mechanisms, such as binding DNA/RNA of pathogens and interfering their life cycles (Zhang et al., 2001). However, the level of resistance provided by the second method is lower, as we showed earlier (Trifonova et al., 2015) and most likely does not protect varieties with low RNase activity in the leaves from viruses in the field.

We did not find a statistically significant correlation of the RNase activity in leaves with resistance to late blight of tubers and leaves, as well as the maturity of various potato varieties. RNases involvement in resistance to *Phytophthora parasitica* var. *nicotianae* was proved by Galiana et al. (1997) who demonstrated that induction of SAR with elicitin is accompanied by a rapid induction of RNase activity and by the increase in the activity of at least two different extracellular RNases. Moreover, exogenous application of RNase activity



RNase activity in leaves of potato varieties.

RNase activity is presented as optical density at 260 nm.

in the extracellular space of leaves led to a reduction of the fungus development by up to 90 %, independently of any elicitin treatment and in the absence of apparent necrosis. We assume that the lack of a link between the RNase activity level and the resistance to late blight in potato is due to the insufficiently high level of the activity in the analyzed varieties. This assumption is supported by the fact that Lugowskoy with the highest level of all the varieties studied is characterized by increased resistance to both viruses and late blight of tubers and leaves (Figure).

One of the unexpected results of our study was a statistically insignificant negative correlation of the level of RNase activity in leaves with resistance to common scab. The main causative agent of this disease is the soil bacterium *Streptomyces scabies*, which affects only tubers (Lerat et al., 2009), therefore, the level of RNase activity in leaves could not impact significantly the infection process. A negative correlation may also be due to the fact that plants with a higher RNase activity are less affected by other types of pathogens and *S. scabies* appears in more favorable competitive conditions. However, for a final conclusion on the effect of RNase activity on the resistance to common scab in potato, more data on resistant varieties is needed, since this type of resistance is very rarely reflected in potato catalogues, despite meaningful crop losses due to infection with the pathogen. Thus, of the 16 varieties studied by us, only 10 of them, we were able to find information on scab susceptibility.

Thus, the level of RNase activity in potato leaves can be used as a prospective selective marker for resistance to viruses, but a systematic study of pathogenesis-related RNase activities in potatoes is needed to find out the specific genes responsible for the function. To create late blight resistant varieties, in our opinion, a greater increase in RNase activity is required than classical methods of selection can provide, and perhaps the best results will be achieved through the methods of transgenesis or editing of the potato genome (Korotkova et al., 2017).

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Conflict of interest

The authors declare no conflict of interest.

References

Barrell P.J., Meiyalaghan S., Jacobs J.M., Conner A.J. Applications of biotechnology and genomics in potato improvement. Plant Biotechnol. J. 2013;11:907-920. DOI 10.1111/pbi.12099.

Bradford M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 1976;72:248-254.

Federal State Budgetary Institution "The State Commission of the Russian Federation for Testing and Protection of Selection Achievements". Available at: http://reestr.gossort.com

Galiana E., Bonnet P., Conrod S., Keller H., Panabieres F., Ponchet M., Poupet A., Ricci P. RNase activity prevents the growth of a fungal pathogen in tobacco leaves and increases upon induction of systemic acquired resistance with elicitin. Plant Physiol. 1997;115:1557-1567.

Green P.J. The ribonucleases of higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 1994;45:421-445.

Hamester W., Hils U. (Eds.) World Catalogue of Potato Varieties. Hamburg: Agrimedia, 2003.

Hugot K., Ponchet M., Marais A., Ricci P., Galiana E. A tobacco S-like RNase inhibits hyphal elongation of plant pathogens. Mol. Plant Microbe Interact. 2002;15:243-250. DOI 10.1094/MPMI.2002.15.3.243.

Ibragimova S.M., Romanova A.V., Myzgina G.Kh., Kochetov A.V. The morphogenic potential of Siberian potato cultivars in tissue cultures. Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding. 2018;22(3):316-320. DOI 10.18699/VJ18.366. (in Russian)

Kao T.H., McCubbin A.G. How flowering plants discriminate between self and non-self pollen to prevent inbreeding. Proc. Natl. Acad. Sci. USA. 1996;93:12059-12065.

Kim S.G., Kim S.T., Wang Y., Yu S., Choi I.S., Kim Y.C., Kim W.T., Agrawal G.K., Rakwal R., Kang K.Y. The RNase activity of rice probenazole-induced protein1 (PBZ1) plays a key role in cell death in plants. Mol. Cells. 2011;31:25-31. DOI 10.1007/s10059-011-0004-z.

Korotkova A.M., Gerasimova S.V., Shumny V.K., Khlestkina E.K. Crop genes modified using the CRISPR/Cas system. Russ. J. Genet.: Appl. Res. 2017;7(8):822-832. DOI 10.1134/S2079059717050124.

Kurata N., Kariu T., Kawano S., Kimura M. Molecular cloning of cDNAs encoding ribonuclease-related proteins in *Nicotiana glutinosa* leaves, as induced in response to wounding or to TMV-infection. Biosci. Biotechnol. Biochem. 2002;66:391-397. DOI 10.1271/bbb.66.391.

Lerat S., Simao-Beaunoir A.M., Beaulieu C. Genetic and physiological determinants of *Streptomyces scabies* pathogenicity. Mol. Plant Pathol. 2009;10:579-585. DOI 10.1111/j.1364-3703.2009.00561.x.

Lers A., Khalchitski A., Lomaniec E., Burd S., Green P.J. Senescence-induced RNases in tomato. Plant Mol. Biol. 1998;36:439-449.

Lusso M., Kuc J. Evidence for transcriptional regulation of beta-1,3-glucanase as it relates to induced systemic resistance of tobacco to blue mold. Mol. Plant Microbe Interact. 1995;8:473-475.

Murashige T., Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 1962;15:473-497.

Pershina L.A. Basic Methods of *in vitro* Cultivation in Plant Biotechnology. Novosibirsk: Novosibirsk State University, 2005. (in Russian)

Qin X., Soulard J., Laublin G., Morse D., Cappadocia M. Molecular analysis of the conserved C4 region of the S_{11}-RNase of *Solanum chacoense*. Planta. 2005;221:531-537. DOI 10.1007/s00425-004-1470-8.

Sindarovska Y.R., Guzyk O.I., Yuzvenko L.V., Demchenko O.A., Didenko L.F., Grynevych O.I., Spivak M.Y. Ribonuclease activity of buckwheat plant (*Fagopyrum esculentum*) cultivars with different sensitivities to buckwheat burn virus. Ukr. Biochem. J. 2014;86:33-40.

Šindelářová M., Šindelář L., Burketová L. Glucose-6-phosphate dehydrogenase, ribonucleases and esterases upon tobacco mosaic virus infection and benzothiodiazole treatment in tobacco. Biol. Plant. 2002;45:423-432.

Sugawara T., Trifonova E.A., Kochetov A.V., Kanayama Y. Expression of an extracellular ribonuclease gene increases resistance to *Cucumber mosaic virus* in tobacco. BMC Plant Biol. 2016;16(3):147-152. DOI 10.1186/s12870-016-0928-8.

Trifonova E.A., Komarova M.L., Leonova N.S., Shcherban A.B., Kochetov A.V., Malinovskii V.I., Shumnyi V.K. Transgenic potato (*Solanum tuberosum* L.) plants expressing the gene of secretory nuclease from *Serratia marcescens*. Dokl. Biochem. Biophys. 2004;394:39-41.

Trifonova E.A., Kochetov A.V., Shumny V.K. Molecular mechanisms of system resistance of plants to infections and strategy of raising virus resistance through transgenesis. Biol. Bulletin Reviews. 2007a;127:13-24 (in Russian)

Trifonova E.A., Sapotsky M.V., Komarova M.L., Scherban A.B., Shumny V.K., Polyakova A.M., Lapshina L.A., Kochetov A.V., Malinovsky V.I. Protection of transgenic tobacco plants expressing bovine pancreatic ribonuclease against tobacco mosaic virus. Plant Cell Rep. 2007b;26:1121-1126. DOI 10.1007/s00299-006-0298-z.

Trifonova E.A., Savelyeva A.V., Romanova A.V., Filipenko E.A., Sapotsky M.V., Malinovsky V.I., Kochetov A.V. Transgenic expression of *Serratia marcescens* native and mutant nucleases modulate tobacco mosaic virus resistance in *Nicotiana tabacum* SR1. Russ. J. Genet. 2015;51:715-719.

Van Loon L.C., Rep M., Pieterse C.M. Significance of inducible defense related proteins in infected plants. Annu. Rev. Phytopathol. 2006;44:135-162. DOI 10.1146/annurev.phyto.44.070505.143425.

Yen Y., Baenziger P.S. Identification, characterization, and comparison of RNA-degrading enzymes of wheat and barley. Biochem. Genet. 1993;31:133-145.

Yen Y., Green P.J. Identification and properties of the major ribonucleases of *Arabidopsis thaliana*. Plant Physiol. 1991;97:1487-1493.

Zhang L., French R., Langenberg W.G., Mitra A. Accumulation of barley stripe mosaic virus is significantly reduced in transgenic plants expressing a bacterial ribonuclease. Transgenic. Res. 2001;10:13-19.

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