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Research Article

A Case Study of RNA-Silencing Resistance against Potato Virus (PVY) in Condition of Climate Change

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Global environmental issues shift crop production to the new conditions. This increases all kinds of biotic and abiotic stresses, beside others also plant diseases. Such changes are also in Kazakhstan where potato production is dramatically increasing, and crop is growing in regions with continental climate. Problems with potato virus type Y (PVY) need cultivars with better resistance. Introduction of silencing small interfering RNA (siRNA) is new technology. *In vitro* experiment was done on potato (*Solanium tuberosum* L.) local cultivar Izolda. Results show significant effect of siRNA in decreasing mortality and occurrence of PVY symptoms and allow expansion of potato cultivation to continental climate conditions, where are faced with new virus problems.

Keywords: potato, *Solanium tuberosum*, potato virus, siRNA

1 Introduction

Population growth and global change of climate impact agriculture in form of higher drought, air temperature, soil salination, occurrence of pests, and disease. Such changed conditions form stress for crop. Population pressure brings a need of production extension out of crop growing optimum (Sahoo et al., 2023).

For example, potato production in Kazakhstan has grown exponentially: while in year 2000 annual potato production was 0.25 million of tones, in 2014 was 1.25 million tones, and in 2022 was 4.0 million tones (Knoema, 2021). This ranks Kazakhstan among major potato world producers (Food and Agriculture Organization of the United Nations (FAO), 2024). Growing potato in the continental climate faces farmers with serious issues in plant protection. In eastern Kazakhstan is it mainly potato virus type Y (PVY) (Loebenstein & Manadilova, 2003). A possible solution can be using not only proper cultivars and breading new ones, but also implementation of new biotechnologies which might enhance crop resistance against different diseases (Afiukwa et al., 2021).

Some promising methods has been currently tested and are introduced in this process. It has been found for example that plants can react on some level of environmental stresses on molecular level mainly specific, so called small ribonucleic acid (sRNAs), which work within the plants in responses to such adverse conditions. sRNAs acts collectively as well as individually to help the plants with their maintenance, homeostasis, and survival under adverse conditions (Guleria et al., 2011). Small RNA has been recognized as a key genetic and epigenic regulator functioning in processes ranging from the modification of DNA to the modulation of the abundance of coding or non-coding RNA in various organisms. In plants, sRNAs are versatile regulators of development growth and response to biotic and abiotic stresses (Bilir et al., 2022). Small- interfering RNAs provides an antiviral immune system that can be applied to plants. RNA silencing is antiviral defence mechanisms in plants. Recent advances in siRNA mediated potato resistance against viruses (incl. PVA) has been reviewed by Jiang et al. (2022).

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Figure 1 Potato production under irrigation in Eastern Kazakhstan

PVY is a virus of the genus *Potyvirus*, one of the most important in potato production. It is easily spread and can reduce production up to 80%. The control is extremely difficult when propagation is done vegetatively, which is common practice, anthropogenic influences are important in virus dynamic (Gray & Power, 2018).

Potato is mostly planted in eastern Kazakstan only under irrigation (Fig. 1). This area is limited by access to water from the main resource which is river Irtish. Beside water, also reasonable quality of soil is limited, because of salinization. A fast increase of area for potato will early come to its limits in this continental climate. High temperatures and humidity are important factors supporting virus PVY (Iftikhar et al., 2020). Therefore changes in irrigation leading to drip technology should be placed as well as biotechnologies for better resistance against virus.

Figure 2 Locality of research

Technology of siRNAs is promising, another important aspect is that legislation does not value it as genetically modified organism production (Mezzetti et al., 2020).

The aim of this study was to test effect of small interfering RNA on resistance against potato virus (PVY). Young plants of potato (*Solanium tuberosum* L.) local cultivar Izolda were used *in vitro* after inoculation of disease potato virus type Y (PVY). Study was done as a practical part of final work of the main author. Laboratory works were done with back-up and under supervision of Sarsen Amanzholov East Kazakhstan University, Ust-Kamenogorsk, Kazakhstan.

2 Material and methods

2.1 Research locality

Study was done in Eastern Kazakhstan (Fig. 2), town Ust-kamenogorsk where research and breading of potato are provided by East Kazakhstan Agricultural Experimental station. For further discussion, conditions around town Pavlodar has been taken under consideration. East Kazakhstan has continental climate with cold winters and hot and dry summers, precipitation occurs mostly in form of snow. Average annual precipitation in Ust- Kamenogorsk is 597 mm, in Pavlodar 348 mm; average annual temperature is 4.2 °C in both sites.

2.2 Process of research

In vitro pre-cultivated plants of local potato cultivar Izolda were used. Young plants were divided to 4 groups each of 15 plants.

1. Control group: plants without any treatment.
2. Group siRNA, where siRNA was applied.
3. Group PVY, plants were inoculated by virus PVY.

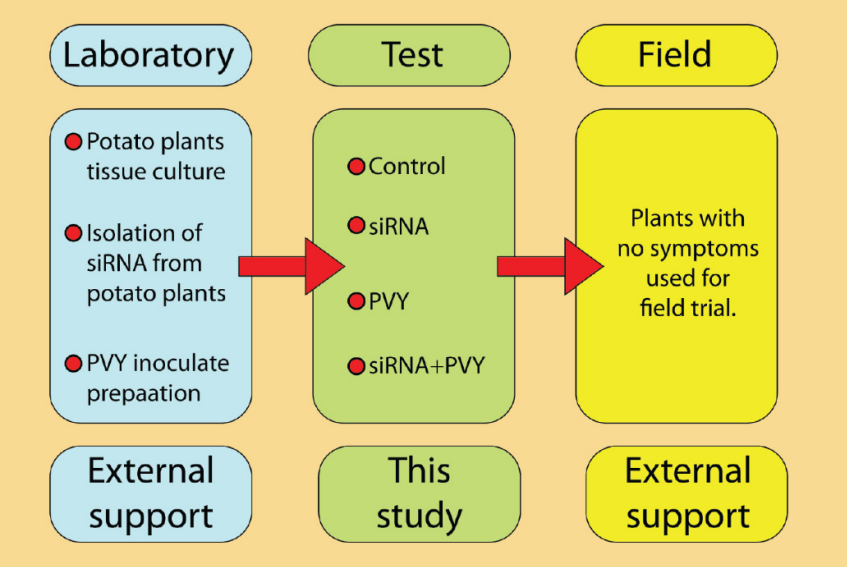

Figure 3 Process of research

4. Group siRNA + PVY, where both, siRNA and PVY, were applied (Fig. 3).

Plants were carefully observed to check the following effects of the treatment. Mortality – plants are dry, no green. Symptoms of PVY – brown or black dots on leaves, mosaic patterns on leaves, leaf drop. For siRNA introduction the mechanical method of application to the leaf surface was used. For Introduction of PVY the liquid form of cultivated virus was injected into leaves. Laboratory support by National Science Shared Laboratory, Sarsen Amanzholov East Kazakhstan University, Ust-Kamenogorsk and East Kazahstan Agriculture Experimental Station, Ust-Kamenogorsk, in detail data about the project see Sutula et al. (2022), Sutula et al. (2023).

3 Results and discussion

All groups show similar mortality, about 20% (group control 20.8%, group siRNA 24.1%, group PVY 30.5%, group siRNA+PVY 21.7%) this can be explained by identical problem with re-planting of young plants which has effected all groups. Relatively higher mortality by group of PVY can be explained as one of the effects of virus. Symptoms were recorded more (26.7%) in group PVY, than in group siRNA+PVY which can be due to functionality of added siRNA. Symptoms in groups Control and siRNA were not recorded.

Data (Fig. 4) show no effect on mortality in group siRNA compared to control. Also show effect of siRNA when introduced into the plant, it decreased occurrence of plants with symptoms from 26.7% to 8.8%.

Other studies show similar results on potatoes. For example, results from transformation experiments, much comparable to our experiment, where 75% of recovery and 25% of mortality after treatment (Sajid et al., 2019). In the next generation of treated plants resistance to PVY is also significant. For example Jahromi et al. (2022) report up to 100% symptoms in virus not treated variants by replanted plants compare to treated in field conditions. In Missiou et al. (2004) the variety siRNA + PVY was in 95% resistant against PVY and variety without siRNA was fully infected (100%). Similarly in Tabassum et al. (2016) transgenic plants PVY knockdown 20%, control plants 100%, and Chung et al. (2013) transgenic potato line 100% resistance, compare to non-transgenic were 8.6%. Many authors expressed results evaluation in ELISA absorbance values, like Valkonen et Rokka (1998), where treated somatic hybrids of potato show ELISA values between 1.12–0.61, when virus-susceptible control were 2.49, non-infected control 0,02. Also in second generations of treated potato plants, reported by Hameed et al. (2017) and other authors (Voloudakis et al., 2022).

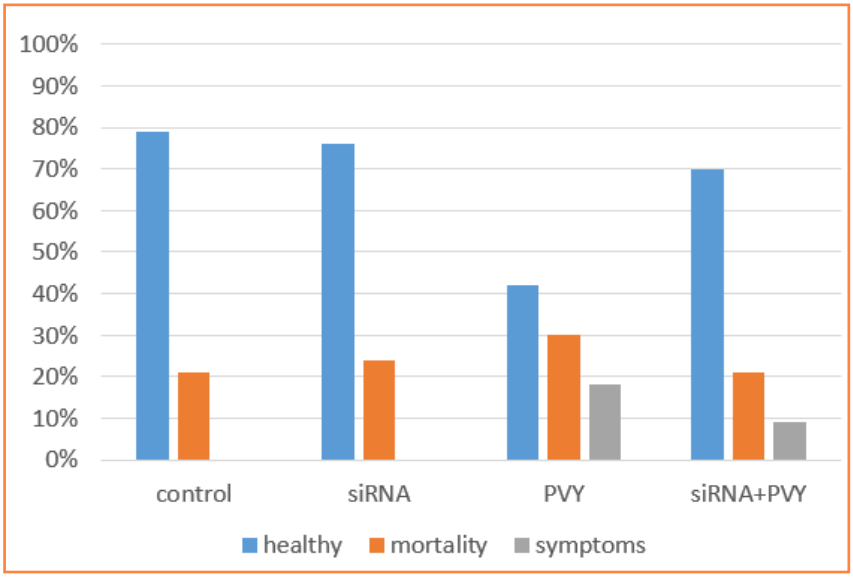

Figure 4 Results

4 Conclusions

In vitro experiment on young potato plants when silencing small interfering RNA (siRNA) was introduced to the plants increase resistance against potato virus Y (PVY) thrice. Compare to control group of plants (with no siRNA and PVY) mortality was similar, but in group where only PVY was introduced, the mortality was much higher as well as occurrence of symptoms. Such results can allow expansion of potato cultivation to continental climate conditions, where they are faced with new virus problems. Further research results in gene regulators including microRNAs should enhance plant tolerance to draught and salinity and together virus resistance can help farmers in changing environment.

References

Afiukwa, C., Azubuike, I., David, O., & Ubi, B. E. (2021). *Biotechnology Role in Climate Change Adaptation and Mitigation for Sustainable Crop Production.* Handbook of Climate Change Management: Research, Leadership, Transformation (pp. 167–1931). https://doi.org/10.1007/978-3-030-22759-3_332-1

Bilir, Ö., Göl, D., Hong, Y., McDowell, J., Tör, M. (2022). Small RNA-based plant protection against diseases. *Frontiers in Plant Science*, 13. https://doi.org/10.3389/fpls.2022.951097

Chung, B., Yoon, J.Y., Palukaitis, P. (2013). Engineered resistance in potato against potato leafroll virus, potato virus A and potato virus Y. *Virus Genes,* 47, 86–92. https://doi.org/10.1007/s11262-013-0904-4

Food and Agriculture Organization of the United Nations (FAO) (2024, January 9). Food and agriculture data. https://www.fao.org/faostat/en/#home

Gray, S. M., & Power, A. G. (2018). Antropogenic influences on emergence of vector-borne plant viruses: the persistent problem of Potato virus Y. *Curent Option in Virology*, 33, 177–183. https://doi.org/10.1016/j.coviro.2018.10.002

Guleria, P., Mahajan, M., Bhardwaj, J., & Yadav, S. (2011). Plant Small RNAs: Biogenesis, Mode of Action and Their Roles in Abiotic Stresses. *Genomics, proteomics & bioinformatics*, 9, 183–199. https://doi.org/10.1016/S1672-0229(11)60022-3

Hameed, A., Tahir, N., Asad, S., Bilal, R., Eck, J., Jander, G., & Mansoor, S. (2017). RNAi-Mediated Simultaneous Resistance Against Three RNA Viruses in Potato. *Molecular Biotechnology,* 59, 73–83. https://doi.org/10.1007/s12033-017-9995-9

Iftikhar, Y.,Mubeen, M., Raza, W., Shakeel, Q., Abbas, W., Iqbal, S., & Sajid, A. (2020). Effect of Environmental Factors on Potato Leaf Roll Virus (PLRV) Infecting Potato Varieties and Myzus persicae (Sulzer). *Pakistan Journal of Agricultural Research*, 33(3), 473–479. https://doi.org/10.17582/journal.pjar/2020/33.3.473.479

Jahromi, M., Rahnama, H., Mousavi, A., & Safarnejad, R. (2022). Comparative evaluation of resistance to potato virus Y (PVY) in three different RNAi-based transgenic potato plants. *Transgenic Research*, 31, 313–323. https://doi.org/10.1007/s11248-022-00302-0

Jiang, L., Du, Z., Zhang, G., Wang, T., & Jin, G. (2022). Advances in RNA-Silencing-Related Resistance against Viruses in Potato, Genes (Basel). *Genes*. 13, 731. https://doi.org/10.3390/genes13050731

Knoema (2021). Production statistics. https://knoema.com/FAOPRDSC2020/production-statistics-crops-crops-processed?tsId=1182770

Loebenstein, G., & Manadilova, A. (2003). *Potatoes in the Central Asian Republics.* https://doi.org/10.1007/978-94-007-0791-7_8

Mezzetti, B., Smagghe, G., Arpaia, S., Christiaens, O., Dietz-Pfeilstetter, A., Jones, H., Kaloyan V. K, Sabbadini, S., Opsahl-Sorteberg, H. G., Ventura, V., Nji Tizi Taning, C., & Sweet, J. (2020). RNAi: What is its position in agriculture. *Journal of Pest Science*, 93, 112593, 1125–1130. https://doi.org/10.1007/s10340-020-01238-2

Missiou, A., Kalantidis, K., Boutla, A., Tzortzakaki, S., Tabler, M., & Tsagris, E. M. (2004). Generation of transgenic potato plants highly resistant to potato virus Y (PVY) through RNA silencing. *Molecular Breeding*, 14, 185–197. https://doi.org/10.1023/B:MOLB.0000038006.32812.52

Sahoo, G., Mishra, P., Wani, A.M., Mishra, I., & Behera, M. (2023). *Impact of Climate Change on Livelihood Security and Biodiversity* – Issues and Mitigation Strategies. Springer Climate (pp. 1–27). https://doi.org/10.1007/978-3-031-19059-9_1

Sajid, I., Tabassum, B., Yousaf, I., Khan, A., Adeyinka, O., Shahid, N., Nasir, I., & Husnain, T. (2019). *In Vivo* Gene Silencing of Potato Virus X by Small Interference RNAs in Transgenic Potato. *Potato Res*., 63, 143–155. https://doi.org/10.1007/s11540-019-09433-0

Sutula, M. Y., Kabataeva, Z.K, & Komekova, G. K. (2023). Isolation of CP-PVY-Specific siRNA from PVY-Infected Plants of *Solanum tuberosum*. *Russian Journal of Plant Physiology*, 70. https://doi.org/10.1134/s1021443722700108

Sutula, M., Khosnutdinova, T., Zhakmanova, E., & Akhmadiyeva, A. (2022). The prevalence of recombinant strains of potato virus Y in the East Kazakhstan region. *Plant disease,* 107(1). https://doi.org/10.1094/PDIS-05-22-1027-PDN

Tabassum, B., Nasir, I., Khan, A., Aslam, U., Tariq, R., Shahid, N., & Husnain, T. (2016). Short hairpin RNA engineering: In planta gene silencing of potato virus Y. *Crop Protection*, 86, 1–8. https://doi.org/10.1016/j.cropro.2016.04.005

Valkonen, J.P. T., & Rokka, V. M. (1998). Combination and expression of two virus resistance mechanisms in interspecific somatic hybrids of potato. *Plant Science*, 131(1), 85–94. https://doi.org/10.1016/S0168-9452(97)00238-0

Voloudakis, A., Kaldis, A., & Patil, B. (2022). RNA-Based Vaccination of Plants for Control of Viruses. *Annual Review of Virology*, 9, 521–548. https://doi.org/10.1146/annurev-virology-091919-073708