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LABORATORY SEARCH OF RESISTANT SOURCE TO *Ralstonia solanacearum*

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Summary. This paper described the technique of nightshades cultivars susceptibility study to *Ralstonia solanacearum* in controlled laboratory terms with using plants, growing on artificial substrate. There were no determined any enough resistant potato and tomato samples resistant for the following usage in breeding work.

Keywords*: Ralstonia solanacearum, potato, tomatos,variety resistance.*

Introduction. Bacterium *Ralstonia solanacearum* (Smith) Yabuuchi et al.is important pathogen for nightshades cultivars in terms of temperate climate. It's low temperature race causes the following diseases potato's brown rot and bacterial wilt of tobacco and vegetables. This pathogen spread in many European and Mediterranean countries during the second half of XXth century. Its sources determine in area of following countries: Bulgaria, Great Britain, Greece, Spain, Italy, Cyprus, Portugal, Poland, Romania, Egypt, Libya, Lebanon, Marocco, Tunis, Turkey. The disease determines high harmfulness through the yield loss, tubers deterioration during its' save and impossibility to use potato from defeated fields as a seedling material. The quarantine measures following needs additional costs. [1-3].

The work for breeding resistant potato varieties and other nightshades to brown rot causative agents takes important place in strategy for decrease it's spread and decrease loses caused by this disease. That is why main attention pays for the search among wild kinds and forms of wild potato forms. The potato varieties especially bred with usage of potato wild forms S*olanum demissum, S. phureja* та *S. commersonii* [4-7]. The same work conducted with vegetable cultivars e..g.. tomato, pepper, egg-plant. [8-10].

The main attention of breeding paid on resistance to fungi diseases in Ukraine before nowadays. The dangerous for penetration brown rot causative

agent and area of bacterial diseases increase every day in Ukraine in the same days through the global climatic changes. The register of potato varieties and other nightshades cultivars and hybrids increased every year, their resistance to *Ralstonia solanacearum* unknown. That is why the main purpose of the present work was to improve methods for determining nightshades resistance to brown rot in laboratory terms for determining varieties and hybrids with high resistance to this pathogen. It may be used as a resistance source in breeding work.

Materials and methods. The potato samples and hybrids used for researches. They were given by Institute for Potato study, Ukrainian Institute for Plant Varieties Examination. The tomatoes of national and foreign breeding used too. The researched plants grew in control laboratory terms on artificial substrate (medium fraction pearlite) with nutrition Knopps solution.

Bacterium suspension for plants infecting produced from two-days culture *Ralstonia solanacearum*. It was grown on potato agar or King's medium (strains Rs9080 and Rs9081, which were presented by Institute of Microbiology and Virology). The bacterium pathogeny testing provided on black nightshade plant before their usage. It was grown too on artificial substrate.



The researches plants growing from potato separated shots on artificial substrate

The researches put on in three respectabilities on 10 samples. The intensity of defeating development during pathogen artificial inoculation conducted as per following scale:

0 — defeating is absent

1 — damaged to 10% of planting material

2 — damaged 11-25%

3 — damaged 25-50%

4 — damaged more than 50%

All investigations conducted as per requirements and rules for the work with quarantine objects. The used substrate and plant remnants neutralized through the autoclave sterilization. [11].

Results and discussions. It is necessary to follow some terms for receiving truly results during the resistance evaluation from phytopathogen bacterium for varieties and hybrids. Among them are: to use the healthy quality researched material, to check bacterium pathogenicity before trial put up, to follow the necessary temperature mode, lightning, nutrient and humidity during the researched plants growing, and also to follow necessary terms for pathogenic process development during experiment (temperature, humidity etc). So the laboratory technique for determining susceptibility to *Ralstonia solanacearum* at artificial defeating in controlled terms was developed by laboratory of quarantine pests and diseases UkrSRPQS IPP.

The visually healthy tubers choose from received samples. Then they washed from soils remnants, disinfected by 1% sodium hypochlorite solution at temperature 18^0C (in the terms of natural lighting) till the well-defined sprouts.

The well-developed sprouts checked on the absence of defeating Brown patch, late blight, black leg and etc. These sprouts separated from tubers and set out in containers with artificial substrate. Then they put in separate vessels (by piece) after the rooting and the third leaf unfolding. The pearlite usage as a substrate for researched plants caused the following factors as comfortability and velocity for work providing, following standard terms during some trials, repeatability, following terms of phytosanitary measures, decrease the remnants quantity, which needs disinfecting.

The healthy well-developed potato plants selected after fifth leaf unfolding for the next researches. They were inoculated by bacterium suspension. Then they kept in separate (isolated) premises at temperature 24^oC. The observation conducted aboveground part development. The seedlings of different varieties used for determining tomato plants resistance. They grew in the same way in pearlite. They were inoculated by bacterium suspension after $5\text{-}6^{th}$ leaves unfolding. You may also add the bacterium suspension to substrate for plant defeating. It imitates the pathogen spread with irrigation water in field conditions.

The gradual wilting of plants happened through the disease development. It caused the destroy of water move by pathogen defeated vascular. The leaves of diseased plants enrolled and gradually died. The stem became brown color through vascular bundles. It spread gradually from inoculation place. There were tested 117 potato samples of different origin on resistance to brown rot causative agent according to this technique in laboratory terms. These varieties and also hybrids were provided by Institute for potato study NAAS. There were any identified resistant varieties. The majority of them reached defeating level on 3-4 points. The most defeated varieties were: Agatha, Vedruska, Vira, Volodarka, Gorlytsya, Dyvo, Kondor, Obriy, Soncedar, Spadschyna and Fantasia. The lowest defeating received the following varieties: Glazurna, Zabava, Knyazha, Kobza, Levada, Letana, Lugivska, Poliske Dzherelo, Prolisok, Serpanok, Slauta, Strumok and Favor. Among 26 researched tomato varieties: De Barao, Izumrudne yabluko, Iskorka, Lemon giant, Lyana, Persey, Peto-86, Sayvo, Flandria and Yana appeared relatively resistant. Their defeating level did not increase 15% (1-2points). Some variety was in the scope 40-45% (Gyna, Kibits, Lagidnyi, Perlyna) to full plant`s dye (variety Druzhba).

Conclusions:

There were any identified samples with resistance to brown rot causative agent among researched potato and tomato varieties. It is necessary to account the variety origin and to determine resistance (susceptibility) of parents` forms from their received crossing.

The proposed way for receiving the researched plants have some advantages in comparison with growing on traditional soil mixes and it may be use for determining agricultural crops resistance to bacterium pathogens in controlled laboratory terms.

References

1. Quarantine pests of Europe. Data sheets on quarantine pests for the European Union and for the European and Mediterranean Plant Protection Organization // CAB International. 1997. pp. 1071—1081.
2. *Jance J.* Potato Brown rot in western Europe — history, present occurrence and some remarks on possible origin, epidemiology and control strategies. 1996. № 26. P. 679—695.

3. *Elphinstone J. G*. The current bacterial wilt situation: A global overview. Bacterial Wilt: The Disease and the *Ralstonia solanacearum* Species Complex. C. Allen, P. Prior, and A. C. Hayward, eds. American Phytopathological Society, St. Paul, MN. 2005. P. 9—28.
4. *Carputo D., Aversano R., Barone A. and all*. Resistance to *Ralstonia solanacearum* of sexual hybrids between *Solanum commersonii* and *Solanum tuberosum*. *American Journal of Potato Research*. 2009. Vol. 86, № 3. P. 196—202.
5. *González M., Galván G., Siri M. I., Borges A., Vilaró F.* Resistencia a la marchitez bacteriana de la papa en *Solanum commersonii*. *Agrociencia Uruguay*. 2013. 7, p. 45–54.
6. *Fock I., Collonnier C., Purwito A. et al.* Resistance to bacterial wilt in somatic hybrids between *Solanum tuberosum* and *Solanum phureja*. *Plant Science*. 2000. 160. P. 165–176.
7. *Patil V.U., Gopa J. & Singh B.P.* Improvement for Bacterial Wilt Resistance in Potato By Conventional and Biotechnological Approaches. *Agricultural Research*. 2012. 1, p. 299–316.
8. *Ano G., Hebert Y., Prior P., Messiaen C.M.* A new source of resistance to bacterial wilt of eggplant obtained from a cross: *S. aethiopicum* L. x *Solanum melongena* L. *Agronomie*. 11 (1991). 11, p. 555 – 560.
9. *Prior P., Bart S., Leclercq S., Darrasse A., Anais G.* Resistance to bacterial wilt in tomato as discerned by spread of *Pseudomonas (Burholderia) solanacearum* in the stem tissues. *Plant Pathology*. 1996. 45, p. 720 – 726.
10. *Lebeau A., Daunay M.C., Frary A., Palloix A., Wang J.F., Dintinger J. et al*. Bacterial wilt resistance in tomato, pepper, and eggplant: genetic resources respond to diverse strains in the *Ralstonia solanacearum* species complex. *Phytopathology*. 2011. 101, p. 154–165.
11. *Krym I.V., Zelia A.H.* Metodyka vyznachennia stiikosti kartopli do zbudnyka buroi bakterialnoi hnyli kartopli *Ralstonia solanacearum* (Smith) v laboratornykh umovakh. [Technique for evaluation of potato resistance to potato brown rot *Ralstonia solanacearum* (Smith) in laboratory conditions]. Chernivtsi: Misto, 2016. 16 s. (in Ukrainian)