INTRODUCTION

Potato virus Y (PVY) is one of the major viral pathogens of potato cultures. It can decrease yields by as much as 80%. Protection against this pathogen is based on breeding and cultivation of resistant cultivars (Valkonen, 2007).

Two main types of potato resistance to PVY infection are known. One is field resistance based on polygenes. It is found in several cultivars, which can be classified according to their degree of resistance. The most resistant plants are infected only accidentally. This relative classification remains practically unchanged in various epidemiological conditions; what changes is the number of infected plants (Rohloff, 1979; Weidemann, 1988).

The other type of resistance to PVY, recognized in wild and cultivated varieties of potato (Solanum tuberosum spp.) is based on a single gene. This resistance, controlled by a single dominant gene, comprises two types of reactions: hypersensitive response (HR) and extreme resistance (ER) (Ross, 1986). The hypersensitive response, determined by Ny genes, is often specific for the PVY strain (Celebi-Toprak et al., 2002), while extreme resistance controlled by Ry genes is effective against all strains of this virus (Cookerham, 1970; Jones, 1990). Plants with Ny gene expression are characterized by the development of necrotic damage after mechanical inoculation of the leaves and by necrosis in systemically infected parts of the plants. Extremely resistant plants whose genome contains the Ry gene do not show symptoms after inoculation and are rarely infected (Ross, 1986; Valkonen et al., 1994; Marczewski, 2001). Occasionally, limited necrosis can develop in systemically infected leaves of several genotypes after inoculation through grafting. In extremely resistant plants the virus is undetectable by the ELISA test even after infection (Ross, 1986). Potato cultivation programs have employed resistance determined mainly by two major genes: Ryedium, derived from Solanum tuberosum subspecies andigena (Brigneti et al., 1997), or Ry_sto, originating from Solanum stoloniferum (Ross, 1986; Hamalainen et al., 1997; Brigneti et al., 1997).

Our study compares the cytological reactions of two varieties of potato: the extremely resistant potato cv. Ania, with resistance determined by the Ry_sto gene, and susceptible potato cv. Glada after infection by PVYN Wi, introduced by grafting.
**MATERIALS AND METHODS**

**PLANT MATERIAL**

We used two genera of Solanaceae family plants: tobacco (Nicotiana tabacum L.) cv. Samsun and potato (Solanum tuberosum L.) cvs Ania and Glada. The choice of cultivars was based on their differing levels of resistance to PVY infection. **Ania** is an extremely resistant variety, 9 on the 1-to-9 scale of field resistance. **Glada** is a relatively resistant variety in field conditions, 7.5 on the scale, but after grafting or mechanical inoculation with PVY it can be systemically infected; in our study it was treated as the susceptible variety.

Disease symptoms were observed on potato stems infected with necrotic strain PVYN Wi (Wilga) in the experiment with bridge grafting inoculation. A healthy tobacco cv. Samsun plant was used as rootstock. The potato stem (resistant cv. Ania or susceptible cv. Glada) was used as insert, and tobacco cv. Samsun infected with PVYN Wi served as infection graft (Fig. 1). After 2-3 weeks, new lateral shoots (offshoots) were growing from the insert. The plant material was obtained from M. Chrzanowska (IHAR, Młochów, Poland).

**SAMPLE PREPARATION FOR LIGHT AND TRANSMISSION ELECTRON MICROSCOPY**

Plant tissue samples (~1 mm³) were fixed by a classical method. The tissue samples were placed for 2 h in Karnowsky’s fixing agent (1965), a mixture of 2% glutaraldehyde and 2% paraformaldehyde in sodium cacodylate buffer (pH 7.2), and then rinsed with 0.1 M sodium cacodylate buffer 4 times and additionally fixed with 2% osmium tetroxide for 2 h at 4°C (Watson, 1958). The tissue sections were dehydrated in an ethanol series, then in acetone or propylene oxide, after which they were embedded in Epon 812 (Fluka) and polymerized for 24 h at 60°C.

The Epon blocks with plant material were cut in the following ways: (a) for LM, with a Supercut 2065 microtome (Leica/Reichert-Jung) into 0.3 mm sections and stained with methylene blue in 2% borax and 1% Azure B solution; (b) for TEM, with an Ultracut E microtome (Reichert) into ultrathin sections ~90 nm thick, which were collected on Formvar-coated slot-grids and contrasted with 1% lead citrate and 2% uranyl acetate (Venable and Coggeshall, 1965).

Semithin sections were examined and documented with the use of an Axioscope (Zeiss) light microscope with a Contax 167 MT camera, and ultrathin sections with the use of JEM 100C or JOEL 1220 transmission electron microscopes in the Electron Microscopy Laboratory of the Warsaw University of Life Sciences – SGGW.

**RESULTS AND DISCUSSION**

Potato virus Y particles and viral protein inclusions (CI, AI) were identified only in the cells of susceptible cv. Glada; in the resistant cv. Ania no pathogenic agent structures were found. Since both the PVY-resistant and the susceptible cultivars were inoculated, it can be assumed that the presence/absence of the virus in the observed cells differentiates the cultivars according to their resistance to that pathogen. However, regardless of the degree of resistance of the investigated plants, necrosis symptoms can develop in tissues in response to the infecting agent. In the insert stem of susceptible potato cv. Glada infected with strain PVYN Wi, virions and cytoplasmic inclu-
sions (CI) were observed in parenchymatous cells of the cortex (Fig. 2). Cytoplasmic inclusions were also found in vascular tissue. The phloem cells underwent irreversible structural changes (Fig. 3). Similarly, in the stem of the offshoots in potato cv. Glada there were PVY particles (Fig. 2b) and viral protein inclusions, both cytoplasmic (CI) (Fig. 4a) and amorphous (AI) (Fig. 4b). In the stem of the insert in potato cv. Anita infected with PVYN Wi, cortex collenchyma cells with thin cell walls became split (Fig. 5a). Widespread necrosis was observed in vascular tissue (Fig. 5b). Coagulated protoplasts and split cell walls were observed in offshoot potato cv. Anita (Fig. 6a, b). Anatomical observations (Fig. 7) showed large internal necroses in the cortex and axial cylinder in the stem of offshoot potato cv. Anita.

Hinrichs-Berger et al. (1999) noted two phenotypes of necrotic reactions on the cellular level. Early in the necrotization process, protoplast plasmolysis, cell wall thickening and deformation are seen. Later the protoplasts remain in the form of a highly osmophilic mass of membranous structures in which the cell organelles are completely degraded.

For an understanding of the scope of resistance it is important to characterize the distribution of necrotic reactions in plants.

In mechanically inoculated highly resistant potato cultivars, PVY did not spread systemically and the necrotic reaction was restricted to leaf epidermis, mesophyll and collenchyma cells. To explain the lack of necrosis in the vascular system we note that the resistance reaction is effective at the stage of infection.
when the virus is still outside the vascular tissue (Hinrichs et al., 1998). In mechanically inoculated potato, Hinrichs-Berger et al. (1999) found necrotic damage in leaves of both the resistant potato cv. *Pirola* and susceptible cv. *Quarta*, but the virus induced systemic infection only in the susceptible variety.

Ultrastructural studies showed necrosis on the inserts and the offshoots of the two Polish potato varieties, resistant cv. *Ania* and susceptible cv. *Glada*. Necroses were within the bicollateral vascular bundle. In the susceptible variety the pathogen also occurred outside necroses (Fig. 3a,b). In the resistant variety there was only necrosis (Fig. 5b) involving individual cells as well as large ensembles of tissue.

The presence of internal necrosis in the vascular tissue of resistant potato cv. *Ania* (Fig. 5b) confirms the effectiveness of the resistance mechanism at the stage of systemic infection. Possibly such localization of the necroses is due to the grafting method of inoculation, in which transport of the virus is largely conditioned by the vascular tissue.

In every multicellular organism, death signals, when released, trigger the active process of programmed cell death (PCD) (Gilchrist, 1998; Lam et al., 1999; Baehrecke, 2002). In animal cells the characteristic form of this process is apoptosis, involving fragmentation and absorption of decaying cells by neighboring ones. In plant cells the trigger of programmed cell death is the hypersensitive response of cells affected by metabolically active necrotization (Heath, 2000; Shirasu and Schulze-Lefert, 2000; Goldbach et al., 2003). The mechanism of cell death is regulated via the expression of...
genes encoding proteins that activate (Bax) or silence (BCL-2, BCL-XL) (Doukhanina et al., 2006; Hofius, 2007) this process with agents released from mitochondria, such as cytochrome c and reactive oxygen species (ROS) (Wojtaszek, 1997; Lam, 1999). ROS production during pathogen recognition can also be conditioned by membrane-associated NADP(H) oxidase or by peroxidase in the apoplast. Severe oxidative stress associated with a high local concentration of ROS can participate directly in killing the pathogen affecting the plant cell, due to cell death in the hypersensitive response (Hofius et al., 2007).

In potato plants extremely resistant to PVY, after mechanical inoculation the rapid and localized hypersensitive response is activated. Cell death occurred at sites of pathogen attack and the virus was blocked and restricted to necrotic damaged areas. (Hinrichs-Berger et al., 1999). In PVY-susceptible potato plants, virus replication could proceed unrestricted for 4–5 days after inoculation. After that period, necroses began to develop. Late activation of this reaction enables the virus to spread outside the cells reacting with necrosis and caused by PVY systemic transport (Hinrichs-Berger et al., 1999).

CONCLUSIONS

1. Both the PVY-resistant and susceptible potato cultivars responded to infection with necrotic reactions. Necroses could develop in all plant tissue types.
2. The development of necroses in vascular tissues in upper shoots of the observed potato plants differing in their degree resistance to PVY may have been due to the method of inoculation by grafting. In highly resistant potato plants (Ania)
systemically infected by grafting, the development of internal necroses in the vascular tissue of upper shoots blocked the spread of potato virus Y outside the necrotic regions. These necroses developed in the highly resistant cultivar as a result of the resistance reaction. In the plants with lower field resistance (Glada), development of necroses in the vascular tissue did not prevent the virus from spreading outside the necrotic regions.

3. High resistance to PVY in potato plants determined by the Ryst0 gene was manifest in the lack of viral particles or viral protein inclusions in infected plant cells.

ACKNOWLEDGEMENTS

We thank Professor Mirosława Chrzanowska (IHAR, Młochów, Poland) for making available the plant material, and Ewa Znojek for expert technical assistance.

Conflict of interest status: The authors declare that they have no conflict of interest.

REFERENCES

BAEHRECKE EH. 2002. How death shapes life during development. Nature Reviews Molecular Cell Biology 3: 779–787.

BRIGNETI G, GARCIA-MAS J, and BAULCOMBE DC. 1997. Molecular mapping of the potato virus Y resistance gene RYst0 in potato. Theoretical and Applied Genetics 94: 198–203.

CELEBI-TOPRAK F, SLACK SA, and JAHN MM. 2002. A new gene, Nprev, for hypersensitivity to Potato virus Y from Solanum tuberosum Maps to Chromosome IV. Theoretical and Applied Genetics 104: 669–674.

COOCKERHAM G. 1970. Genetical studies on resistance to viruses in cultivated and wild potato species (Solanum spp.). Theoretical and Applied Genetics 94: 198–203.

DOUKHANINA EV, CHEN S, VAN DER ZALM E, GODZIK A, REED J, and DICKMAN MB. 2006. Identification and functional characterization of the BAG protein family in Arabidopsis thaliana. Journal of Biological Chemistry 13: 84–95.

GILCHRIST DG. 1998. Programmed cell death in plant disease: the purpose and promise of cellular suicide. Annual Review of Phytopathology 36: 393–414.

GOLDRACI R, BUCHER E, and PRINS M. 2003. Resistance mechanisms to plant viruses: an overview. Virus Research 92: 201–212.

HAMALAINE JP, WATANABE KN, VALKONEN JPT. 1994. Natural genes and mechanisms for resistance to viruses in cultivated and wild potato species (Solanum spp.). Plant Breeding 112: 1–16.

HINRICHIS-J, BERGER S, and SHAW JG. 1998. A hypersensitive response-like mechanism is involved in resistance of potato plants bearing the Ry(st0) gene to the Potyviruses potato virus Y and tobacco etch virus. Journal of General Virology 79: 167–176.

HINRICHIS-BERGER J, HAROLD M, BERGER S, and BUCHENAUER H. 1999. Cytological responses of susceptible and extremely resistant potato plants to inoculation with potato virus Y. Physiological and Molecular Plant Pathology 55: 143–150.

HEATH MC. 2000. Hypersensitive response – related death. Plant Molecular Biology 44: 321–334.

HOFUS D, TSITSIPIANNIS DI, JONES JD, and MUNDY J. 2007. Inducible cell death in plant immunity. Seminars in Cancer Biology 17: 166–187.

JONES RAC. 1990. Strain group specific and virus specific hypersensitive reactions to infection with Potyviruses in potato cultivars. Annals of Applied Biology 117: 93–105.

KARNOWSKY MJ. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. Journal of Cell Biology 25: 137A.

LAM E, DEL POZO O, and PORTIER D. 1999. BAXing in the hypersensitive response. Trends in Plant Science 4: 419–421.

MARKCZESKI W, FLIS B, SYLLER J, SCHAFFER-FREGEL R, and GEBHARDT C. 2001. A major quantitative trait locus for resistance to Potato leafroll virus is located in a resistance hotspot on potato chromosome XI and is tightly linked to N-gene-like markers. Molecular Plant Microbe Interaction 14: 1420–1425.

ROHLOFF H. 1979. Beitrag zur Analyse der Kartoffeln-Y-Virus-Epidemie in 1976. Gesunde Pflanzen 31: 296–299.

ROSS H. 1986. Potato breeding-problems and perspectives. Journal of Plant Breeding, Suppl. 13.

SHIRASU K, and SCHULZE-LEFERT P. 2000. Regulators of cell death in disease resistance. Plant Molecular Biology 44: 371–385.

VALKONEN JPT. 1994. Natural genes and mechanisms for resistance to viruses in cultivated and wild potato species (Solanum spp.). Plant Breeding 112: 1–16.

VALKONEN JPT, SLACK SA, PLAISTED RL, and WATANABE KN. 1994. Extreme resistance is epistatic to hypersensitive resistance to potato virus Y0 in Solanum tuberosum ssp. andigena – derived potato genotype. Plant Disease 78: 1177–1180.

VALKONEN JPT. 2007. Viruses: economical losses and biotechnological potential. In: Vreugdenhil D, Bradshaw J, Gebhardt C, Govers F, Taylor M, MacKerron D, Ross H [eds.], Potato Biology and Biotechnology: Advances and Perspectives, 619–641. Elsevier, Amsterdam.

VENABLE JH, and COGGESHALL R. 1965. Simplified lead citrate stain for use in electron microscopy. Journal of Cell Biology 25: 407.

WATSON ML. 1958. Staining of tissue sections for electron microscopy. Journal of Cell Biology 25: 407.

WATSON ML. 1958. Staining of tissue sections for electron microscopy with heavy metals. Journal of Biophysical and Biochemical Cytology 4: 475.

WATERSJESZ P. 1997. Oxidative burst: an early plant response to pathogen infection. Biochemical Journal 322: 681–692.

WEIDEMANN HL. 1998. Importance and control of potato virus Y (PVYN) in seed potato production. Potato Research 31: 85–94.