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CONTENTS

OBSAH

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Horticultural Science

Analysis concerning a rapid spread of apple proliferation phytoplasmas in a selection orchard of apple seedlings

J. Blažek, R. Vávra, J. Kučerová

Determination of yield and yield components in wild thyme (*Thymbra spicata* L. var. *spicata*) as influenced by development stages

O. Tonçer, S. Kizil

Response of three strawberry cultivars (*Fragaria* × *ananassa* Duch.) to different salinity levels in irrigation water

A. Kurunc, C. Cekic

Prospects of classical cross protection technique against *Citrus tristeza closterovirus* in Pakistan

M. Abbas, M. M. Khan, S. M. Mughal, I. A. Khan

Energetic aspects of the productive potential of grapevine in Malé Karpaty vineyard region

F. Špánik, B. Šiška, Š. Hronský

Influence of growth regulators on plant regeneration in tomato

J. Gubiš, Z. Lajchová, L. Klčová, Z. Jureková

Estimates of genetic variability in vegetable amaranth (*A. tricolor*) over different cuttings

S. Shukla, A. Bhargava, A. Chatterjee, A. Srivastava, S. P. Singh

Effect of CO_2 treatment on dormancy duration, sprout growth and sugar content in two potato cultivars

R. Ezekiel, B. Singh

The effect of polypropylene fleece covering on the yield of early potatoes

K. Hamouz, P. Dvořák, J. Čepl, J. Pivec

Adaptation ot twenty peach and nectarine varieties in Kos and their susceptibility to *Plum pox virus* and *Phytophthora citrophthora*

X. Papanikolaou, C. Tsipouridis, T. Thomidis, D. C. Stylianidis

Use of rooted leaves for screening of *Brassica* germplasm response to clubroot (*Plasmodiophora brassicae*) and downy mildew (*Hyaloperonospora parasitica*)

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ABSTRACT: Rooted leaves and cotyledons of various cruciferous crops were used for the screening of *Brassica* germplasm response to two obligatory pathogens: clubroot (*Plasmodiophora brassicae*) and downy mildew (*Hyaloperonospora parasitica*). The development of roots was induced after 20-hour dipping of leaf pedicels in the mixture of growth regulators: indolyl-butyric acid (10 mg/l) and nicotinic acid (5 mg/l). The detached rooted leaves and cotyledons were maintained in 250ml plastic containers with perlite under fluorescent tubes in a growth chamber. With additional foliar fertilizing they remain vital for four months, producing clubroot galls on roots when dip-inoculated with *Plasmodiophora* spores and sporulating mycelia of downy mildew on leaves after drop inoculation with *Hyaloperonospora parasitica*. The possibilities of enhancing the sensitivity of this alternative assay in combination with immunochemical methods are discussed.

Keywords: *Brassica*; clubroot; *Plasmodiophora brassicae*; downy mildew; *Hyaloperonospora parasitica*; rooted leaves

Parallel testing of numerous plant pathogens, pathogen races, and/or multiple screening of host resistance belong to frequent tasks for many phytopathologists, breeders as well as for curators of germplasm collections. Williams (1985) outlined the procedure of multiple pathogen inoculation (MPI) of crucifer seedlings using the various parts of the same juvenile intact plant as targets for 10 pathogenic species. MPI was primarily designed for the routine screening of huge germplasm collections searching for phenomena of multiple resistance. However, it seems to be under serious risk of undesirable contamination if the selections of healthy incompletely resistant host phenotypes are required. The use of detached cotyledons, whole leaves or leaf disks incubated in Petri dishes is a common practice for the laboratory bioassay of pesticides (Urban, Lebeda 2004) and testing of the races of obligatory pathogens as downy mildews (Lebeda 1986). The good correlations of the downy mildew (*Hyaloperonospora parasitica*) resistance of adult crucifer (*Brassica* sp.) plants with that expressed with detached cotyledons (Jensen et al. 1999) open this routine technology for the resistance screening of crucifer genetic resources. However, detached cotyledons do not allow to carry out more than four separate inoculations per one seedling (Williams 1985). Agnola et al. (2003) enhanced the effectivity of this screening strategy using the practically unlimited number of leaf disks, harvested from adult leaves. Seeking for genetic resources of the multiple resistance of crucifer crops to obligatory biotrophic pathogenic fungi (*Plasmodiophora brassicae* – clubroot, colonising the roots, and *Hyaloperonospora parasitica* – downy mildew, growing on leaves) we probed a laboratory assay, based on the use of detached and previously rooted leaves. Details of this procedure and preliminary evaluation of symptom severity are the topics of this report.

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MATERIALS AND METHODS

Plant material

Three sets of *Brassica* genotypes were selected for experiments described here (Table 1). The first, ECD set, is identical with the one recommended as European Clubroot Differential (Crute et al. 1983) having been previously used for the evaluation of *Plasmodiophora* races in the Czech Republic (Rod 1994). The second group consisted of experimental cauliflower (*Brassica oleracea* var. *botrytis* L.) lines, experimental clones of asymmetric somatic hybrid *Armobrassica* (horseradish + cauliflower) and their both parents (horseradish Morava and cauliflower breeding line OLH 66) as reported by Navrátilová et al. (1997). The third set comprised selected accessions of primitive cabbage landraces (*Brassica oleracea* var. *capitata* L.) from the Czech-Slovak border, collected during two collection missions in Carpathian area and maintained in Vegetable Genebank of the Research Institute of Crop Production Prague-Ruzyně, Olomouc workplace.

Before sowing all seeds were disinfected with 2.5% solution of chloramine for 20 min and thoroughly rinsed with tap water as prevention of contamination by *Alternaria* or *Phoma*. Seedlings were raised in perlite (Agroperlit, 2.5 mm grain fraction) in a growth chamber (see the following paragraph).

Procedure of rooting leaves

Fully developed leaves from the middle part of 20–30 days old plants were cut off tightly to the stem and their whole petioles were immediately immersed into a solution of 10 mg/l of indolyl-butyric acid (IBA) and 5 mg/l of nicotinic acid (NOA) for 20 hours. Then the leaves were thoroughly rinsed under tap water and transplanted into moist perlite in 250ml plastic beakers closed for the following week by another transparent plastic cover. Perlite was moistened with tap water but the leaf blades were weekly sprayed with diluted foliar fertiliser 0.3 g/l (Kristalon Start, Agri Rotterdam, Netherlands). Beakers were incubated in a growth chamber (22°C/18°C day/night, 16-hour day length) under batteries of fluorescent tubes, generating 140 μmol/m^2 of irradiance at the plant level. When rooting young cotyledons, the same procedure was used except the cotyledons with petioles were harvested from 10 to 11 days old seedlings.

Pathogens and inoculations

Plasmodiophora brassicae Wor., clubroot:

Isolate OL24 (ECD index 16/31/31), caught and propagated on Chinese cabbage plants (*Brassica campestris* var. *pekinensis* (Metzg.) Sinsk. cv. Granaat, ECD05) from soil samples in Olomouc region was used in this study. Freshly harvested fully developed galls were cleaned under tap water and were crushed in a kitchen mincer. The homogenate was squeezed through two layers of cheesecloth and diluted with tap water to 10^7 spores per 1 ml. Roots of plantlets or rooted leaves and cotyledons were inoculated by means of short dipping in the inoculum. Inoculated plants or plant parts were transplanted

Table 1. List and origin of used accessions

1. ECD (European Clubroot Differential) set
   Brassica campestris var. *pekinensis* (ECD 01, aaBBCC; ECD 02, AabbCC; ECD 03, AABBcc; ECD 04, AABBCC; ECD 05, cv. Granaat)
   Brassica napus (ECD 06, cv. Nevin; ECD 07, cv. Giant Rape; ECD 08, line Dc126; ECD 09, cv. Clubroot Resistant; ECD 10, cv. Wilhelmsburger)
   Brassica oleracea var. *capitata* (ECD 11, cv. Badgershipper; ECD 12, cv. Sachsener; ECD 13, cv. Jersey Queen; ECD 14, cv. Septa)
   Brassica oleracea var. *fimbriata* (ECD 15, cv. Verheul)
   Original seeds were obtained from former Vegetable Research Institute Olomouc (Ing. J. Rod, Ph.D.), afterwards they were maintained and multiplied at Vegetable Genebank at Olomouc Workplace, Research Institute of Crop Production Praha-Ruzyně
2. Armobrassica set (see Navrátilová et al. 1997)
   cauliflower *Brassica oleracea* var. *botrytis,* cv. Fastman (Bejo Zaden, Warmenhuizen, The Netherlands); cauliflower *Brassica oleracea* var. *botrytis,* experimental line OLH 66 (now cv. Arktur); horseradish *Armoracia rusticana*, experimental clone Morava derived from seed-bearing wild plants; somatic hybrid Armobrassica, clone 28A
3. cabbage landraces (Research Institute of Crop Production, Praha-Ruzyně, Vegetable Genebank Olomouc):
   H1800142 Landrace (Lutiše 46); H1800143 Landrace (Lutiše); H1800144 Landrace (Zázrivá 367); H1800145 Landrace (Zázrivá); H1800146 Landrace (Zákamenné); H1800147 Landrace (Breza); H1800148 Landrace (Babín); H1800149 Landrace (Veličná); H1800150 Landrace (Veličná 1); H1800151 Landrace (Parnica); H1800152 Landrace (Kralovany); H 1800005 Kodaňské tržní rané; H1800014 Křimické; H1800015 Vysocké krajové; H1800079 Moskovskaja pozdnaja 9; H1800080 Losinoostrovskaja; H1800083 Křimické; H1800277 Turnovské; H1800283 Šumavské-Böhmerwaldkohl; H1800287 Vysocké (Frýdštejn); H1800319 Vysocké (Jenišovice)

into new perlite beakers and were incubated in a growth chamber (for conditions see above). Symptoms of disease were indexed after 40–42 days according to Rod (1987).

Hyaloperonospora parasitica Constant. (previously *Peronospora parasitica* Gäumann), downy mildew:

Isolate OL1 from kohlrabi (*Brassica oleracea* var. *gongylodes* L.) was propagated on young detached cotyledons of cabbage cv. Kodaňské on moist filter paper in plastic Petri dishes under fluorescent tubes at 16°C. After 7–10 days the cotyledons covered with sporulating mycelia were frozen and maintained at –80°C. Water suspensions of conidia from this stock were used for the preparation of inocula diluted to 5.10^{-4}–10^{-5} spores per 1 ml. Surfaces of leaf disks, rooted leaves or cotyledons were drop-inoculated (7 µl droplets). Closed plastic containers with plant organs growing in perlite were incubated for the next two days in dark at 10°C and thereafter under fluorescent tubes at 16°C/10°C (day/night, day length 16 hours).

The disease was assessed by the six-point scale according to Williams (1985).

RESULTS

Response of explants to induced rooting

Three to six root primordia on leaf petioles of all *Brassica* species were visible within a week after IBA + NOA treatment and roots reached 3 cm in length in the next week. Rooted leaves remain vital without symptoms of chlorophyll breakdown for more than four months when fortified with foliar nutrition. On untreated controls the spontaneous rooting was rather irregular and unrooted leaf explants rapidly senesced and died during the first month of cultivation. Rooting response was very similar in all tested *Brassica* species but we noticed the unexpectedly slow and protracted root development with horseradish (*Armoracia rusticana* L.) leaves. Nevertheless, once rooted, horseradish leaves remain vital for the period comparable with the other crucifers. With OLH66, an experimental line of cauliflower, we noticed the development of secondary shoots together with roots on treated petioles (Fig. 1).

Fig. 1. Rooted leaves of cauliflower (*Brassica oleracea* var. *botrytis*) breeding line OLH66 with clubroot (*Plasmodiophora brassicae*) galls. On the right, a rooted leaf with secondary shoot

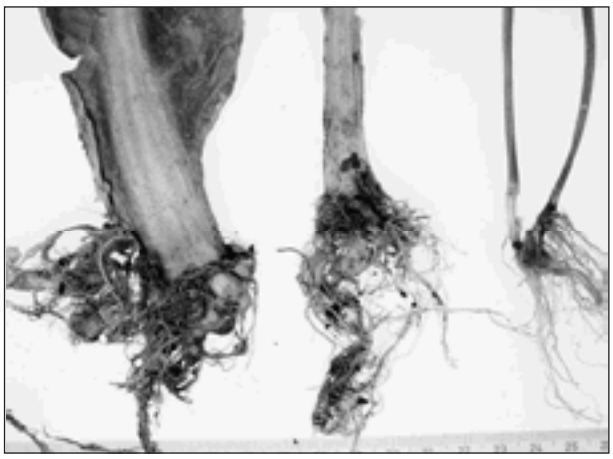

Fig. 2. Response of the rooted leaves of cauliflower (*Brassica oleracea* var. *botrytis*) breeding line OLH66, *Armobrassica* somatic hybrid 28A, and horseradish *Armoracia rusticana* Morava (from right to left) to *Plasmodiophora brassicae*. Note the absence of clubroot symptoms on the horseradish leaf

Young detached cotyledons responded to the rooting treatment with similar longevity as true leaves but they developed only one to three root primordia per explant.

Response of rooted explants to *Plasmodiophora brassicae*

Four weeks after dip inoculation the root galls occurred on rooted explants but they reached only a half-size in comparison with intact seedlings (Fig. 2). As a consequence of small club size on rooted explants, the indexing of resistance of ECD testers to clubroot based only on gall size seems to be unreliable. Nevertheless, no symptoms of clubroot development were found with rooted horseradish leaf explants and only negligible clubs were noticed with somatic *Armobrassica* hybrid clone 28A (Fig. 2).

Response of rooted explants to *Hyaloperonospora parasitica*

Three days after drop inoculation the symptoms of downy mildew were visible on rooted explants regardless of their age (one to four months) or origin (leaf or cotyledons). Next three days later heavy sporulation occurred there. No symptoms of downy mildew occurred on rooted leaves of horseradish and only hypersensitive reaction without consequential sporulation was noticed with somatic *Armobrassica* hybrid clone 28A.

DISCUSSION

Rooted leaves and cotyledons of *Brassica* crops extend the arsenal of model objects (Williams 1985) for the study of plant-pathogen interactions especially in combination with obligatory pathogens with different demands for the target tissue – roots versus leaves. It may be useful for crucifer breeders in situations of parallel testing of the same plant individual with numerous races of *Plasmodiophora* and *Hyaloperonospora*, substantially diminishing the risk of accidental contamination. However, the smaller size of *Plasmodiophora* galls in induced roots hampers the indexing of the disease severity and calls for more objective methods of evaluation such as histochemical staining (Morgner, Sacristán 1995). Experiments with immunochemical staining of *Plasmodiophora*-specific antigens are in progress in our team (Kudlíková, Slováček 2002).

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References

AGNOLA B., BOURY S., MONOT C., QUILLÉVÉRÉ A., HERVÉ Y., SILUÉ D., 2003. Evidence that a leaf-disk test allows assessment of isolate-specific resistance in *Brassica oleracea* crops against downy mildew (*Peronospora parasitica*). European Journal of Plant Pathology, *109*: 471–478.

CRUTE I.R., PHELPS K., BARNES A., BUCZACKI S.T., CRISP P., 1983. The relationship between genotypes of three *Brassica* species and collections of *Plasmodiophora brassicae*. Plant Pathology, *32*: 405–420.

KJENSEN B.D., HOCKENHULL J., MUNK L., 1999. Seedling and adult plant resistance to downy mildew (*Peronospora parasitica*) in cauliflower (*Brassica oleracea* convar. *botrytis* var. *botrytis*). Plant Pathology, *48*: 604–612.

KUDLÍKOVÁ I., SLOVÁČEK J. 2002. Preliminary report of immunochemical methods to detect resting spores of *Plasmodiophora brassicae* in soil. Book of Abstracts of 6th Conference of European Foundation for Plant Pathology, Czech Republic, 8–14th September 2002: 71.

LEBEDA A., 1986. *Pseudoperonospora cubensis*. In: LEBEDA A. (ed.), Methods of testing vegetable crops for resistance to plant pathogens. Olomouc, VHJ Sempra, Research Institute of Vegetable Crops: 81–85.

MORGNER M., SACRISTÁN M.D., 1995. Quantitative determination of colonization by *Plasmodiophora brassicae* (Wor.) in *Brassica napus* and *Brassica oleracea*. Cruciferae Newsletter, *17*: 80–81.

NAVRÁTILOVÁ B., BŮŽEK J., ŠIROKÝ J., HAVRÁNEK P., 1997. Construction of intergeneric somatic hybrids between *Brassica oleracea* and *Armoracia rusticana*. Biologia Plantarum, *39*: 531–541.

ROD J., 1987. Vliv pěstebních substrátů na intenzitu výskytu nádorovitosti (*Plasmodiophora brassicae*). Olomouc, Bulletin Výzkumný ústav zelinářský, *31*: 11–17.

ROD J., 1994. The occurrence and distribution of pathotypes of *Plasmodiophora brassicae* WOR. in the Czech Republic and Slovakia. Ochrana rostlin, *30*: 171–182.

URBAN J., LEBEDA A., 2004. Differential sensitivity of fungicides in Czech populations of *Pseudoperonospora cubensis*. In: LEBEDA A., PARIS H.S. (eds.), Progress in Cucurbit Genetics and Breeding Research. Proc. of Cucurbitaceae 2004, the 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding. Olomouc, Palacký University: 275–280.

WILLIAMS P.H., 1985. Resource Book of Crucifer Genetic Cooperative (608-262-6496), DSTT 06-17-87 WILLPAU, Workbook of Madison College of Agricultural and Life Sciences, University of Wisconsin.

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Použití zakořeněných listů k průzkumu genofondu rodu *Brassica* na reakci vůči boulovitosti košťálovin (*Plasmodiophora brassicae*) a plísni zelné (*Hyaloperonospora parasitica*)

ABSTRAKT: Zakořeněné listy a dělohy různých brukvovitých rostlin byla použity k průzkumu reakce položek genofondové kolekce košťálovin vůči dvěma častým patogenům: původci boulovitosti – plasmodiofoře (*Plasmodiophora brassicae*)

a plísni zelné (*Hyaloperonospora parasitica*). Vývoj kořenů na řapících byl navozen dvacetihodinovým namočením báze listů ve směsi růstových regulátorů kyseliny indolylmáselné (10 mg/l) a kyseliny nikotinové (5 mg/l). Oddělené zakořeněné listy byly pěstovány v 250ml plastových kontejnerech s perlitem v kultivační místnosti pod zářivkami. S pomocí listového hnojiva, aplikovaného postřikem, zůstaly zakořeněné listy vitální až čtyři měsíce. Namáčením kořenů do suspenze spor *Plasmodiophora* byla navozena tvorba kořenových nádorků a po kapkové inokulaci suspenzí konidií *Hyaloperonospora parasitica* rovněž tvorba sporulujího mycelia plísně zelné. Navrhujeme další zvýšení vypovídací schopnosti tohoto alternativního testu pomocí histochemických postupů.

Klíčová slova: *Brassica*; boulovitost; *Plasmodiophora brassicae*; plíseň zelná; *Hyaloperonospora parasitica*; zakořeněné listy

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