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ELABORATION OF *in vitro* ROOT CULTURE PROTOCOLS TO EFFICIENTLY LIMIT DAPHNE SUDDEN DEATH SYNDROME

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Abstract. In this study *in vitro* root culture protocol was elaborated in order to diversify screening methods and develop quick and reliable assay to test the level of chosen *Daphne* genotype resistance to fungal pathogen *Thielaviopsis basicola*. Plantlets of *Daphne caucasica*, *D. cneorum*, *D. jasminea*, and *D. pontica* were propagated aseptically on medium composed of WPM mineral salts and MS vitamins, supplemented with 1.0 mg dm^{-3} 2iP, 0.1 mg dm^{-3} NAA, 0.5 g dm^{-3} PVP, 0.5 g dm^{-3} MES, 0.65 g dm^{-3} calcium gluconate, and 20 g dm^{-3} sucrose. Root cultures were initiated from adventitious roots regenerated on micropropagated shoots. Proliferative root cultures of examined *Daphne* species were obtained both on solidified and liquid medium, supplemented with various doses of NAA. Cultures of detached roots proved to be a convenient system of testing to *T. basicola* resistance. *Daphne* roots cultured *in vitro* were distinctly contaminated under laboratory conditions. Susceptibility of cultured organs differed between species. This simple method is appropriate to be put into practice for quick selection of resistant/tolerant genotypes to the soil-borne fungal pathogens affecting plants *via* root system.

Key words: biotic stress, fungal pathogen, *in vitro* root culture, *Thielaviopsis basicola*, DSDS

INTRODUCTION

For centuries efforts of breeders were directed at improving characters associated with either plant aesthetics, such as its morphology, tint or fragrance and size of flowers, or yield characteristics. Nowadays, breeding for resistance to environmental stressors, of both biotic and abiotic origin, has become a priority in breeding programs of numerous cultivated plants. The availability of species or cultivars resistant or tolerant to diseases, drought or salinity is of great importance, and in that respect the potential of biotechnology to improve plant productivity is unquestionable [Altman 1999, Halford

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2012]. The insertion of resistance to pathogens, and pests to the plant genome could be perceived as vital factor, indispensable to efficient crop plants production, especially those of economic importance [Nishimura and Dangl 2010, Orsini et al. 2010, Zang et al. 2010, Karban 2011]. Chemical control usually must be applied in production of both crop and ornamental plants, which often are vulnerable to numerous pathogenic agents. Apart from a direct importance of resistance breeding, there is also so called indirect importance, that is a need of yield intensification obtained with maximally reduced pesticide use. Due to the above, resistance breeding approach is currently gaining even greater urgency, accompanied by constantly growing social awareness to achieve and maintain sustainable development. Moreover, when susceptible species or varieties are cultivated, the next crucial issue is related with affordable costs of applying appropriate chemical control to counteract diseases [Tilman et al. 2002, Noshad 2007, Brunelli 2010, Wani et al. 2010].

In natural environment plants belonging to the genus *Daphne* L. (Thymelaeaceae Juss.) are distributed mainly in Europe and Asia, and numerous species are also commercially exploited in pharmacy/herbalism and horticulture [Hanus-Fajerska et al. 2012]. During the nursery production of ornamental shrubs belonging to the genus major looses result from detrimental effect of the infection with root pathogens, with *Thielaviopsis basicola* (Berk. & Br.) Ferraris (syn. *Chalara elegans* Nag Raj et Kendrick) being especially harmful. The contaminated plants suffer from disease defined as Daphne Sudden Death Syndrome (DSDS) [Noshad et al. 2006]. As a consequence of root necrotization, the stem breaks down, and plant collapses. Therefore, to have a commercially workable system, it is not always sufficient to yield rooted plants, and the primary concern is to have at disposal the plant material resistant to infection. For that reason resistant cultivars and genotypes are sought for urgently [Punja 2001, Noshad et al. 2007]. Numerous authors pointed at advantages of using *in vitro* cultures over resistance testing in natural conditions: unfavorable weather and climate conditions are avoided, a small space is exploited for testing a large number of individuals, and mass screening of mutants for resistance is facilitated. It has been also suggested that in experiments involving resistance selection an increase in percentage of resistant regenerants can be expected when a preselection in *in vitro* conditions is applied [van den Bulk 1991, Švabova and Lebeda 2005, Sowik et al. 2008].

In this paper we present the experiments undertaken to evaluate the effect of exposure of isolated and, cultivated *in vitro, Daphn*e roots to infective doses of *T. basicola* chlamydospore suspension. To verify usefulness of such host-pathogen model in selection for disease resistance we have chosen four out of numerous species previously tested to differentiate the intensity of germplasm reaction to the infection with *T. basicola* isolate on the level of detached organ culture. Chosen species were previously described to display different degree of susceptibility to this necrotrophic pathogen [Noshad et al. 2007, Hanus-Fajerska et al. 2008]. Therefore the main objective of this research was to develop rapid, convenient and simple tissue culture system to test the level of species/genotype susceptibility to the soil-borne pathogen affecting plants *via* root system.

MATERIALS AND METHODS

Plant material and tissue culture protocol. The source material were pathogen-free clones of *Daphne caucasica* Pall., *D. cneorum* L., *D. jasminea* Sibth. & Sm., and *D. pontica* L., cultivated *in vitro*. Plant material was collected and maintained at the UBC Botanical Garden and Center for Plant Research, Vancouver, Canada. As highly susceptible control treatment cultures of *D. cneorum* L. were used [Noshad et al. 2007]. This species was treated as a model material in preliminary experiments testing suitability of root culture to *in vitro* pathogen-resistance screening [Hanus-Fajerska et al. 2008]. From stabilized shoot cultures apical shoot fragments 25 mm long were isolated and micropropagated in order to obtain multi-apex cultures of respective species. Propagation medium was composed of woody plant medium – WPM [Lloyd and McCown 1981] mineral salts and vitamin set from MS medium [Murashige and Skoog 1962], 1.0 mg dm^{-3} N6-2-isopentyladenine (2iP), 0.1 mg dm^{-3}, 1-naphtaleneacetic acid (NAA), 0.5 g dm^{-3} polyvinylpyrrolidone (PVP), 0.5 g dm^{-3} 2-N-morpholino-etanosulphonic acid (MES), 0.65 g dm^{-3} calcium gluconate, 20 g dm^{-3} sucrose, 6.0 g dm^{-3} activated charcoal, and solidified with 0.6% (w/v) Difco agar. Medium pH was adjusted to 5.6. Plant material was subcultured every four weeks onto fresh medium of the same composition. The culture environment was maintained at 24 ±2°C, with 16-h photoperiod per day, with light intensity amounted to 48 μmol m^{-2} s^{-1}, coming from cool-white fluorescent lamps. Root induction on obtained shoots was set in aseptic conditions, in perlite moistened with liquid medium consisting of 1/3 WPM mineral salt content supplemented with 3 mg dm^{-3} of indole-3-butyric acid (IBA) for a week. In a control treatment 1/3WPM0 medium without plant growth regulators was used. After 7 days shoots were transferred to perlite moistened with liquid medium consisting of 1/3 WPM mineral salt content. Obtained adventitious roots were excised to start root culture on WPM medium supplemented with 1-naphtaleneacetic acid. Three NAA concentrations, that is 1 mg dm^{-3}, 2 mg dm^{-3}, 3 mg dm^{-3} (and WPM0 – control treatment) were tested to find the optimal one for root tissue proliferation. Experiments were conducted using solid medium with 1.5% Difco agar and 0.5% Phytagel (in 100 mm diameter Petri dishes) and liquid medium (in 100 ml Erlenmeyer flasks capacity). Weighted portions of 50 mg root tissue were fragmented on c.a. 20 mm long pieces. Such root explants were put onto 25 ml of solid medium or into 50 ml of liquid medium. Cultures were maintained in darkness, at 22 ±2°C. Experiments were carried out with at least 50 explants in each treatment, and repeated thrice. Macroscopic observations were conducted every week, biometrical data (number and length of lateral roots, fresh mass, dry mass), and histological examination of tissue samples were taken at the end of second four-week-passage.

Data convenient for statistical estimation according to Compton [1994] were subjected to one-way Statistica 9.1 ANOVA (StatSoft Inc., Tulsa, OK, USA), and a posteriori Fisher's test was used to determine the significance of differences between studied objects, with the significance level $\alpha = 0.05$.

Culture of pathogen and in vitro screen. Working culture of highly virulent isolate of *Thielaviopsis basicola* (Berk. & Br.) Ferraris, was maintained on 5% carrot agar (50 ml of canned carrot extract per liter of deionized water supplemented with 18 g dm^{-3} agar) and was incubated in the darkness at 24 ±2°C. Pathogen inoculum was prepared

according to the procedure elaborated by Noshad et al. [2007]. It was a suspension of endoconidia deriving from three week old colonies of *T. basicola* in a concentration 1×10^6 ml^{-1}. *Daphne* cultures were inoculated by dropping 1 ml of inoculum onto roots cultured *in vitro*. The course of symptom expression was monitored weekly, and eight weeks after inoculation cultures were rated for disease severity (DS) using scale 0–5, where 0 stands for no symptoms and 5 for root necrotization – according to Hanus-Fajerska et al. [2008]. Plant disease index (PDI) was calculated for each treatment 56 days after inoculation using following formula: PDI = DS/L ×DI/N ×100, where DS – disease severity, L – number of roots, DI –number of infected roots, N – sample quantity (25), in three replicates for each combination.

RESULTS

Proliferative shoot cultures of *Daphne caucasica*, *D. cneorum*, *D. jasminea*, and *D. pontica* were obtained from pathogen-free *in vitro* material. Applied technique of shoot apical fragment culture enabled efficient propagation on solidified medium composed of WPM mineral salts, MS vitamins, and additionally supplemented with 1.0 mg dm^{-3} 2iP, 0.1 mg dm^{-3} 1-NAA, 0.5 g dm^{-3} PVP, 0.5 g dm^{-3} MES, 0.65 g dm^{-3} calcium gluconate, 20 g dm^{-3} sucrose, and 6.0 g dm^3 charcoal.

The next step in protocol optimization was an induction and regeneration of adventitious roots on the micropropagated shoots. Overall, 65% of shoot explants developed roots during twenty eight days of culture in aseptic conditions on perlite moistened with liquid rooting medium. The efficiency of rooting, expressed as the percentage of rooted shoots, was proved to be relatively high especially in *D. jasminea* (83%) and *D. caucasica* (56%). Fragments of adventitious roots were then used to start aseptic root culture, in order to establish a convenient system of testing to *T. basicola* resistance. Proliferative root cultures of examined *Daphne* species were obtained both on solidified and liquid medium (fig. 1). The general appearance of roots as well as their growth habit in both culture systems were equally satisfactory on media supplemented with 2 and 3 mg dm^{-3} NAA. Both the number and the length of regenerated lateral roots were significantly higher than in control treatment. However, on media supplemented with 3 mg dm^{-3} NAA callusing of explants was observed. The growth of *D. pontica* roots was the slowest among tested genotypes, since the mean root length reached about 6 mm in twenty eight days on medium supplemented with 2 mg dm^{-3} NAA (tab. 1). As far as the number of lateral roots is concerned, no univocal reaction of cultures was displayed during the course of the experiment, irrespective of applied treatment and genotype. It was found that in liquid media roots exhibited higher uniformity in relative water content (expressed as relative water content coefficient, RWC), in comparison with roots cultured on solid media, where the RWC values fluctuated from 10.5 to 52.6 (tab. 1). The best proliferation rate of *D. cneorum* roots was achieved when root tissue was cultured on a medium containing 2 mg dm^{-3} NAA. For *D. caucasica*, *D. pontica* and *D. jasminea* NAA concentrations of either 2 or 3 mg dm^{-3} were equally beneficial (fig. 1). The roots of examined genotypes exhibited proper appearance when cultured on auxin-rich medium, and displayed rather stable proliferation rate in long-term culture.

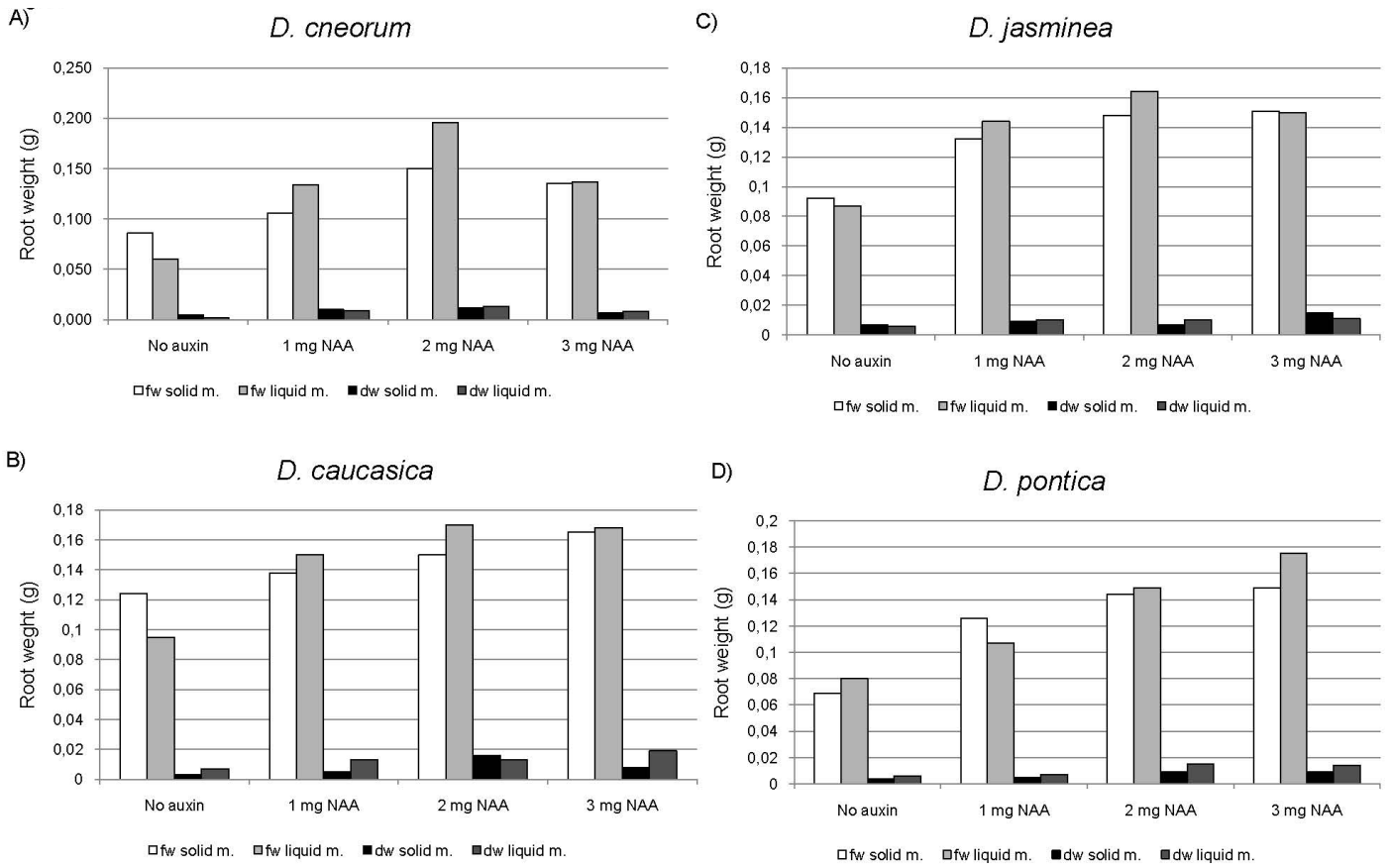

Fig. 1. The effect of NAA concentration on proliferation of roots in solid and liquid medium (m.) culture system of Daphne spp.; fw – fresh weight, dw – dry weight

Table 1. The effect of NAA dose on lateral roots development and water content of Daphne root cultures

Treatment/ NAA level	Species	Mean lateral root length (mm)	Mean number of lateral roots	RWC* on solid media	RWC on liquid media
No auxin added – control treatment	*D. cneorum*	4.2 ±0.6 a**	2.3 ±0.3 ab	13.7 ±1.7 a	18.8 ±5.6 bcd
	D. caucasica	6.2 ±2.0 ab	1.6 ±0.5 a	52.6 ±4.0 c	11.6 ±1.2 ab
	D. jasminea	5.5 ±0.9 ab	2.6 ±0.8 abc	12.4 ±1.9 a	14.1 ±2.1 ab
	D. pontica	3.6 ±1.4 a	1.4 ±0.1 a	10.5 ±3.2 a	7.9 ±2.2 a
1 mg dm^{-3}	*D. cneorum*	7.9 ±1.6 abc	3.2 ±0.2 cde	17.6 ±5.5 ab	18.8 ±3.0 abc
	D. caucasica	10.6 ±4.3 bc	5.1 ±0.7 defg	48.7 ±8.5 c	16.9 ±2.8 abc
	D. jasminea	9.5 ±1.4 abc	4.2 ±0.4 cdef	20.1 ±3.8 ab	20.3 ±3.7 bcd
	D. pontica	5.2 ±0.2 ab	3.4 ±0.6 cde	33.5 ±6.7 bc	29.9 ±4.7 d
2 mg dm^{-3}	*D. cneorum*	7.2 ±0.2 abc	4.6 ±0.6 defg	16.3 ±4.5 ab	27.5 ±3.6 cd
	D. caucasica	10.5 ±1.2 abc	4.5 ±2.1 defg	42.4 ±12.4 c	21.0 ±4.6 bcd
	D. jasminea	12.1 ±1.6 c	5.5 ±0.4 fg	28.3 ±4.7 bc	24.5 ±0.5 bcd
	D. pontica	6.1 ±1.1 ab	2.1 ±0.4 ab	20.1 ±1.4 ab	15.4 ±2.1 abc
3 mg dm^{-3}	*D. cneorum*	14.1 ±2.1 c	5.3 ±0.2 efg	10.0 ±3.0 a	24.1 ±4.5 bcd
	D. caucasica	11.4 ±2.3 bc	6.0 ±1.1 g	20.6 ±2.5 ab	14.8 ±3.7 abc
	D. jasminea	13.1 ±3.6 c	6.6 ±0.9 g	14.6 ±1.3 a	18.9 ±1.2 bcd
	D. pontica	5.3 ±1.0 ab	2.8 ±0.5 bcd	22.6 ±1.5 ab	23.5 ±5.9 bcd

* RWC – relative water content coefficient

** values denoted with the same letter in columns do not differ significantly at $\alpha = 0.05$, and a posteriori Fisher's test was used to determine the significance of differences between studied objects

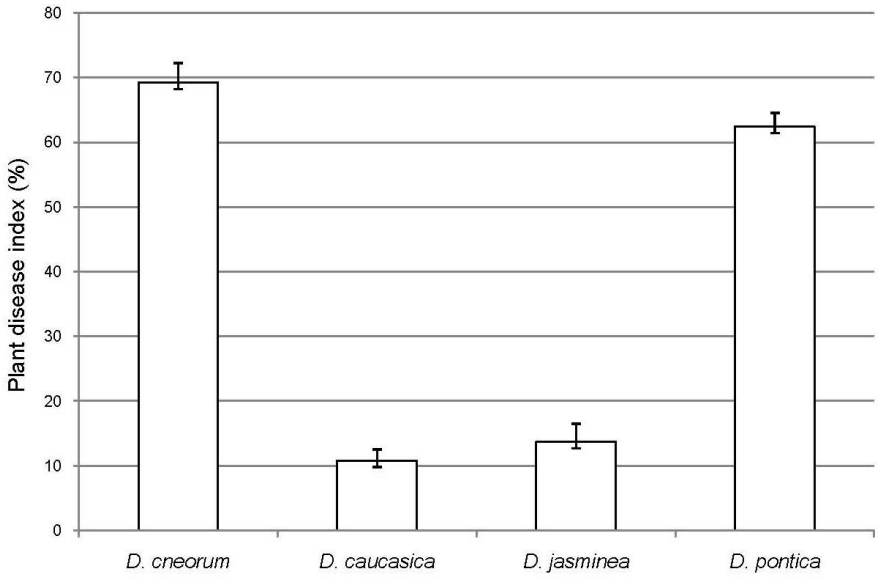

Fig. 2. Mean plant disease index (PDI) value of *Daphne* root cultures inoculated with *Thielaviopsis basicola*

The final step of the protocol elaboration was the inoculation experiment and *in vitro* screen. A comparison of plant disease index (PDI) was conducted using root cultures on solidified media, as Petri plates were more convenient for disease severity estimation. After inoculation root cultures became infected by *T. basicola*, and susceptibility of *D. caucasica*, *D. cneorum*, *D. jasminea* and *D. pontica* detached roots was diversified (fig. 2). Resistance trait of examined *Daphne* species was maintained in root cultures tested under laboratory conditions. In all examined plant species, sensitivity of roots occurred to be irrespective of culture system (liquid/solid) and of auxin concentration in culture medium. For this reason, in figure 2 are presented the mean values obtained in all tested treatments performed for a respective species. Experiment revealed that *D. caucasica* and *D. jasminea* were relatively resistant to *T. basicola,* as their mean plant disease index (PDI) was 10.8 and 13.7, respectively. *D. cneorum*, with PDI = 69.2, was the most susceptible to infection with suspension of *T. basicola* endoconidia. For *D. pontica* PDI value amounted to 62.4. Under laboratory conditions cultured roots were distinctly affected by examined biotic stressor, and vulnerability varied among tested species.

DISCUSSION

Daphne Sudden Death Syndrome evoked by fungus *Thielaviopsis basicola* was identified for the first time as a disease of *Daphne cneorum* [Noshad et al. 2006]. It was

found that *D. cneorum* is one of the most susceptible species to DSDS within the genus, but both numerous species living in the wild, and commercial cultivars are endangered of being infected. Currently, the only known method of controlling the disease, once it has been recognized, is elimination of all infected plants. It is an expensive procedure, particularly in nursery production, and extremely ineffective manner of plant protection. Therefore, there is an urgent need to develop an simple assay, which will enable quick and reliable diagnosis of germplasm resistance to this root system pathogen.

In chosen experimental conditions the levels of *D. caucasica*, *D. cneorum*, *D. jasminea* and *D. pontica* susceptibility to the pathogen were quite parallel to those described in the literature [Noshad et al. 2007], although the described approach is more simplified in comparison with those applied before. *In vivo* screen of 21 container-grown *Daphne* species growing in the natural environment, and subsequent *in vitro* screen of *in vitro* grown rooted microshoots of *D. cneorum*, *D. caucasica*, *D. giraldii*, *D. jasminea*, *D. laureola*, *D. retusa* and *D. tangutica* revealed that in the genus *in vitro* approach can be equivalent to the classical method. Further simplification would be the use of detached roots as a material for diagnostic trial. Noshad et al. [2007], assessing disease symptoms at the same period of time after infection, as we did obtained plant disease index 72.2 for *D. cneorum* rooted microshoots, for *D. jasminea* PDI was under twenty, for *D. caucasica* c.a. eleven. Our results obtained for *D. cnorum, D. jasminea* and *D. caucasica* are quite similar to those above cited. Therefore we can assume that, irrespectively of examined species, the culture of detached roots in aseptic conditions can be regarded as quick and reliable assay to test the level of *Daphne* germplasm resistance to *Thielaviopsis basicola*. The merit of the elaborated approach currently is to shorten time necessary for selection procedures in comparison with other available testing systems.

Suitability of a simple *in vitro* culture-based resistance screen was demonstrated recently in grapevine, where detached leaves were spot-infected with biotrophic ascomycete *Erysiphe necator* [Miazzi et al. 2010]. On the other hand, our report presents simplified protocol for germplasm screening using cultures of easily proliferating organs. It is postulated that elaborated pathosystem could be used to facilitate studies focused on elucidation of possible virulence factors [D'Ovidio et al. 2010, Orsini et al. 2010]. Grasser et al. [1988a] used tobacco callus cultures as simple model system to study resistance mechanism to *T. basicola*. *Nicotiana tabacum* is known to be susceptible to this necrogenic soil pathogen for a long time [Allison 1938], and Grasser et al. [1988a, b] found that resistance of tobacco cultivars was maintained in callus cultures. They revealed that during an infection by *T. basicola* the concentration of scopoline increased in cultures of resistant tissues. Thus the scopoline level was proposed to be considered as biochemical marker of resistance in tobacco. Afterwards, Hood and Shew [1997] exploited transmission electron microscopy technique to focus on initial cellular interactions between root hairs of tobacco seedlings and *T. basicola*. Hence, the protocol developed here may also be exploited in studies on cellular response to pathogen attack *via* root system.

Root cultures with distinct reaction to the infection would be useful material in such studies, taking into account that tissue and organ cultures are widely exploited in an induction of resistance to fungal pathogens [Švabova and Lebeda 2005]. For that pur-

pose the use of convenient and rapid system of individual genotype testing to *T. basicola* resistance could be taken into consideration both in breeding programs and more general studies regarding plant-pathogen interaction. Protocol elaborated here provides suitable groundwork for further investigations in genus *Daphne* and related plant families.

CONCLUSIONS

1. Application of 1-naphtaleneacetic acid in doses between 1 and 3 mg dm^{-3} resulted in rhisogenesis, and allow to maintain root culture in studied *Daphne* species.
2. *In vitro* root culture method is the most advantageous for quick selection of genotypes resistant or tolerant to the soil-borne fungal pathogens.

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REFERENCES

Allison C.C., 1938. Physiologic specialization of *Thielaviopsis basicola* on tobacco. Phytopathology 28, 1.

Altman A., 1999. Plant biotechnology in the 21st century: the challenges ahead. Elect. J. Biotech. www.ejb.org/content/vol2/issue2/full/1.

Brunelli A., 2010. A new background in the protection of plants from biotic pathogens. J. Plant Pathol. 92, suppl. 4, 447.

Compton M.E., 1994. Statistical methods suitable for the analysis of plant tissue culture data. Plant Cell Tiss. Organ Cult. 37, 217–242.

D'Ovidio R., Janni M., Volpi C., Rochchi V., 2010. Cell wall reinforcement for wheat improvement against fungal pathogen. J. Plant Pathol. 92, suppl. 4, 449.

Grasser R., Kern H., Defago G., 1988a. Resistance of tobacco to *Thielaviopsis basicola* under tissue culture conditions and increased susceptibility after transformation with *Agrobacterium tumefaciens* T-DNA. J. Phytopathol. 123, 105–114.

Grasser R., Kern H., Defago G., 1988b. Scopolin, a biochemical marker for resistance to *Thielaviopsis basicola* in callus and crown-gall tissue cultures of tobacco. J. Phytopathol. 123, 115–123.

Halford N.G., 2012. Toward two decades of plant biotechnology: successes, failures, and prospects. Food Energy Sec. 1(1), 9–28.

Hanus-Fajerska E., Farfán L., Riseman A., 2008. Podatność wawrzynka główkowego na *Thielaviopsis basicola* w testach *in vitro*. Zesz. Probl. Post. Nauk Roln. 531, 57–62.

Hanus-Fajerska E., Wiszniewska A., Czaicki P., 2012. Effectiveness of *Daphne* L. (Thymelaeaceae) *in vitro* propagation, rooting of microshoots and acclimatization of plants. Acta Agrobot. 65(1), 21–28.

Hood M.E., Shew H.D., 1997. Initial cellular interactions between *Thielaviopsis basicola* and tobacco root hairs. Phytopathology 87, 228–235

Karban R., 2011. The ecology and evolution of induced resistance against herbivores. Funct. Ecol. 25, 339–347.

Lloyd G., McCown B., 1981. Commercially feasible micropropagation of mountain laurel (*Kalmia latifolia*) by use of shoot tip culture. Int. Plant Prop. Soc. Proc. 30, 421–427.

Miazzi M.M., Hajjeh H.R., Faretra F., 2010. An in vitro method to evaluate grapevine cultivars for *Erysiphe necator* susceptibility. *In Vitro* Cell Dev. Biol. Plant 46(4), 363–367.

Murashige T., Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473–497.

Nishimura M.T., Dangl J.L., 2010. *Arabidopsis* and the plant immune system. Plant J. 61, 1053–1066.

Noshad D., Punja Z.K., Riseman A., 2006. First report of *Thielaviopsis basicola* on *Daphne cneorum*. Can. J. Plant Pathol. 28, 310–312.

Noshad D., Punja Z.K., Riseman A., 2007. Evaluation of *Daphne* germplasm for resistance of Daphne Sudden Death Syndrome caused by the soil-borne pathogen *Thielaviopsis basicola*. HortSci. 42, 1639–1643.

Noshad D., 2007. Daphne Sudden Death Syndrome (DSDS). Pathogen identification, characterization and screening for disease resistance. Vancouver, Canada, University of British Columbia, PhD Thesis.

Orsini F., Cascone P., De Pascale S., Barbieri G., Corrado G., Rao R., Maggio A., 2010. Systemin-dependent salinity tolerance in tomato: evidence of specific convergence of abiotic and biotic stress responses. Physiol. Plant. 138, 10–21.

Punja Z.K., 2001. Genetic engineering of plants to enhance resistance to fungal pathogens, a review of progress and future prospects. Can. J. Plant Pathol. 23, 216–235.

Sowik I., Michalczuk L., Wójcik D., 2008. A method for *in vitro* testing strawberry susceptibility to *Verticillum* wilt. J. Fruit Ornam. Plant Res. 16, 111–121.

Švabova L., Lebeda A., 2005. *In vitro* selection for improved plant resistance to toxin-producing pathogens. J. Phytopathol. 153, 52–64.

van den Bulk R.W., 1991. Application of cell and tissue culture and *in vitro* selection for disease resistance breeding – a review. Euphytica 56, 269–285.

Tilman D., Cassman K.G., Matson P.A., Naylor R., Polasky S., 2002. Agricultural sustainability and intensive production practices. Nature 418, 671–677.

Wani S.H., Sanghera G.S., Singh N.B., 2010. Biotechnology and plant disease control – role of RNA interference. Am. J. Plant Sci. 1, 55–68.

Zang Y., Shi J.Y., Zang J.D., Guo X.Q., 2010. Identification of novel NPR1-like gene from *Nicotiana glutinosa* and its role in resistance to fungal, bacterial and viral pathogens. Plant Biol. 12, 23–34.

OPRACOWANIE PROTOKOŁU KULTUR *in vitro* KORZENI W CELU OGRANICZANIA PORAŻENIA ZESPOŁEM NAGŁEJ ŚMIERCI WAWRZYNKA

Streszczenie. Opracowano protokół prowadzenia kultur korzeni wybranych genotypów *Daphne* z zamiarem stworzenia możliwości testowania ich podatności na *Thielaviopsis basicola* w warunkach *in vitro*. Mikropędy *Daphne caucasica*, *D. cneorum*, *D. jasminea* i *D. pontica* rosły na pożywce mineralnej WPM wzbogaconej o zestaw witamin i aminokwasów z pożywki MS oraz 1,0 mg dm^{-3} 2iP, 0,1 mg dm^{-3} 1-NAA, 0,5 g dm^{-3} PVP, 0,5 g dm^{-3} MES, 0,65 g dm^{-3} glukonianu wapnia. Kultury korzeni inicjowano z korzeni przybyszowych regenerowanych w bazalnej strefie mikropędów. Uzyskano silnie proliferujące kultury korzeni zarówno na pożywkach płynnych, jak i zestalonych, wzbogaconych NAA na zróżnicowanym poziomie stężeń. Kultury te okazały się przydatne do prowadzenia testów przesiewowych w warunkach laboratoryjnych, przy czym podatność poszczególnych genotypów była zróżnicowana. Ta stosunkowo prosta metoda może być stosowana w celu szybkiej selekcji genotypów odpornych bądź tolerancyjnych na porażenia patogenami grzybowymi infekującymi organizm roślinny poprzez system korzeniowy.

Słowa kluczowe: stres biotyczny, patogen grzybowy, kultura korzeni *in vitro*, *Thielaviopsis basicola*, DSDS

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