TESTING OF MEGAGAMETOPHYTES OF SOME CONIFER SPECIES FOR DEFENSE REACTIONS TO THE FUNGUS *PHAEOLUS SCHWEINITZII*

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ABSTRACT

The megametophytes of *Picea abies*, *Pinus sylvestris*, *Pinus mugo* and *Larix decidua* were cultured on modified solid agar MS medium with 5 mg.l⁻¹ α -naphthaleneacetic acid and 0.1 mg.l⁻¹ 6-benzylaminopurine and exposed to a tester, the basidiomycete *Phaeolus schweinitzii*. The megametophytes of all tested species showed defense reactions manifested by inhibition of mycelial growth. The weakest defense reactions were shown by the megametophytes of *Picea abies* and *Larix decidua*. The strongest defense reactions were found in *Pinus sylvestris* and *Pinus mugo* megametophytes.

KEY WORDS: conifers, megametophytes, defense reactions.

INTRODUCTION

Basidiomycete *Phaeolus schweinitzii* (Fr.) Pat. can be found on roots and butts of living and dead conifers (mainly spruce), exceptionally on deciduous trees and on their stumps. In 145 in Czechoslovak collections of the mentioned hosts, 57 (39%) were found on *Picea abies*, 48 (33%) on pines (*Pinus cembra* 1, *P. nigra* 1, *P. strobus* 10, *P. sylvestris* 35, *P. uncinata* 1) and 22 on *Larix decidua*. There are only a few findings on other woody plants (Kotlaba, 1984).

Phaeolus schweinitzii was successfully used as a tester for in vitro study of defense reactions of embryo and megametophyte of European black pine (*Pinus nigra* Arn.). Very intensive defense reactions of megametophyte were revealed (Hřib and Vooková, 1993; Hřib et al., 1995a, b). However, defense reactions of megametophytes of other conifers have not been studied.

In the present work defense reactions of Scots pine, Norway spruce, European larch and Mugo pine were tested by the fungus *P. schweinitzii*.

MATERIALS AND METHODS

Seeds of *Picea abies* (L.) Karst., *Pinus sylvestris* L., *Pinus mugo* Turra and *Larix decidua* Mill. were sterilized in 70% (v/v) ethanol (1 min) followed by 10-15 min. treatment in 0.1% HgCl₂ (v/v).

The tester, wood-rotting fungus *Phaeolus schweinitzii* (Fr.) Pat., isolate No. 151 was used. This strain was obtained from the Collection of Wood Destroying Fungi, J.E. Purkyně University (now Masaryk University) in Brno. The culture was grown at a temperature 23±1°C in the dark on 3% malt extract and 2% Difco Bacto agar.

In the experiment, growth of *P. schweinitzii* mycelium in presence of megametophyte was studied on modified MS medium (Murashige and Skoog, 1962) with 5mg.l⁻¹ NAA and 0.1 mg.l⁻¹ BAP (Table 1). A 10 x 10 mm inoculum of the fungus, taken from the margin of colonies on malt-agar, was placed at a distance of 2.5 cm from the megametophytes. Dual cultures were maintained at 23±1°C in the dark. The fungal mycelium growth was measured every other day through 20 days. Measurements for each variant were repeated four times. Inhibition of tester mycelium in presence of the megametophytes served as an indicator of intensity of defense reactions.

RESULTS AND DISCUSSION

In all tested species, the presence of megametophytes in dual culture with the tester *P. schweinitzii* caused an inhibition of mycelial growth of the tester. Megametophytes of both pine species and Norway spruce weakly stimulated the growth of mycelium during the first days. This phenomenon was probably caused by volatile substances. After ten days of co-cultivation when tester mycelium closely approached the megametophytes, the inhibition effect was shown.

The most intensive defense reactions were found in *Pinus sylvestris* and *Pinus mugo* megametophytes. Weaker defense reactions of the megametophytes of *Larix decidua* and the weakest defense reaction was observed in *Picea abies* megametophyte.

Many different metabolites, including growth regulators, diffused from the megametophytes into agar and, at in some distance from them, evoked inhibition of mycelial growth.

Seeds contain extra amounts of substances stored as a source of food reserves to support early seedling growth. These

are principally carbohydrates, fats and oils, and proteins. For example, the pine megagametophyte contains 35% of proteins, 48% of fats and 6% of carbohydrates (mainly starch). Seeds contain other minor, but nevertheless important reserves (e.g. phytin); of these, a number are recognized as being nutritionally undesirable or even toxic (e.g. alkaloids, lectins, proteinase inhibitors, phytin and raffinose oligosaccharides) (Bewley and Black, 1985). Salmia and Mikola (1980) reported that resting pine seeds contained proteinase inhibitors of high activity. Similar, although smaller, inhibitor activity was present in fresh extracts of "germinating" endosperm. The fresh extracts had a major proteinase peak (mol wt. 42.000 kDa) of high activity at pH 3.7 and moderate activities at pH 5.4 and 7.0 (pine proteinase I) and a smaller peak (mol wt. 30.000 kDa) of high activity at pH 5.4 and 7.0 and smaller activity at pH 3.7 (pine proteinase II) (Salmia, 1981). Seeds also contained auxins and IAA conjugates. Almost all the knowledge of conjugate hydrolysis leading to the formation of free IAA has been derived from the study of seeds and young seedlings (Reinecke and Bandurski, 1987). As early as in 1937 Skoog described that the growth of the coleoptile in intact oat seedlings is inhibited by removal of the endosperm. The *Avena* curvature test demonstrated that endosperm removal decreased the amounts of auxin secreted into agar blocks. In a previous work (Hřib et al., 1993) it was found that tester mycelium responded on auxin and higher level of glutamine by inhibition of growth of mycelium.

In close proximity of European black pine megagametophyte, cultured *in vitro* for 20 days Hřib et al. (1995a, b) revealed 10 released proteins (13 to 64 kDa). Among them protein with mol mass 17.4 kDa. Kurosaki et al. (1988) described extracellular chitinase (mol mass 17.5 kDa) obtained from carrot cell culture. It may be supposed that rich spectrum of extracellular proteins and growth regulators can be found around the megagametophytes of conifer species tested in the present work. We suppose that defense reactions of megagametophytes could be evoked by auxins, L-glutamine and by defense-related proteins. An excellent review of defense-related proteins was written by Bowles (1990). All tested pine species megagametophytes in dual culture had an inhibition effect on mycelial growth. It was inhibited in close proximity of the megagametophytes. We assume that the basidiomycete *P. schweinitzii* can be used as tester of general (non-specific) defense reactions of plant tissues.

Fig. 1. Mycelial growth of fungus *Phaeolus schweinitzii* in presence of conifer megagametophytes.

a – mycelium,
b – mycelium and megagametophytes of Norway spruce,
c – mycelium and megagametophytes of European larch,
d – mycelium fungus and megagametophytes of Mugo pine,
e – mycelium of fungus and megagametophytes of Scots pine,
(●—●— mycelial growth in direction to the megagametophytes, —○—○— mycelial growth in opposite direction).

Fig. 2. Photographs of Petri dishes with dual cultures.
a – mycelium of *P. schweinitzii* with two megagametophytes of Norway spruce,
b – mycelium *P. schweinitzii* with two megaga metophytes of European larch,
c – mycelium with two megagametophytes of *Mugho pine*,
d – mycelium with two megagametophytes of *Scots pine*; after 20 days of cultivation.

TABLE 1. Composition of medium used for co-cultivation of megagametophytes with the tester.

Inorganic nutrients mg.l ⁻¹		Organic supplements mg.l ⁻¹	
NH ₄ NO ₃	1,650	Nicotinic acid	0.5
KNO ₃	1,900	Pyridoxine.HCl	0.1
CaCl ₂ .2 H ₂ O	440	Thiamine.HCl	0.1
MgSO ₄ .7 H ₂ O	370	myo-Inositol	100
KH ₂ PO ₄	170	Asparagine	100
H ₃ BO ₃	6.2	α-naphtaleneacetic acid (NAA)	5
MnSO ₄ .4 H ₂ O	16.9	6-benzylaminopurine (BAP)	0.1
ZnSO ₄ .7 H ₂ O	10.6	Sucrose	30,000 (3%)
KI	0.83	Bacto agar	10,000 (1%)
Na ₂ MoO ₄ . 2 H ₂ O	0.25		
CuSO ₄ .5 H ₂ O	0.025		
CoCl ₂ .6 H ₂ O	0.025		
Fe /as Fe EDTA/	5.6 ^{1/}		

^{1/} 5 ml.l⁻¹ of a stock solution containing 5.57 g FeSO₄.7 H₂O and 7.45 g Na₂EDTA per litre of H₂O.

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TESTOWANIE REAKCJI OBRONNYCH MEGAGAMETOFITÓW
NIEKTÓRYCH GATUNKÓW ROŚLIN IGLASTYCH
NA GRZYB *PHAEOLUS SCHWEINITZII*

STRESZCZENIE

Megagametofity *Picea abies*, *Pinus sylvestris*, *Pinus mugo* i *Larix decidua* hodowano na zmodyfikowanym agarowym podłożu MS z dodatkiem 5 mg.l⁻¹ kwasu α-naftalenooctowego i 0.1 mg.l⁻¹ 6-benzylaminopuryny. Obok gametofitów umieszczono grzybnie podstawczaka *Phaeolus schweinitzii*. Gametofity wszystkich gatunków formowały wzrost strzępek grzybni. Najsilniejszą obronną reakcję wykazywały gametofity *Pinus sylvestris* i *Pinus mugo*, reakcja pozostałych była słaba.

SŁOWA KLUCZOWE: rośliny szpilkowe, megagametofity, reakcje obronne.