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The posibility of soil micromycetes produced the abscisic acid

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Key words: Abscisic acid, *Aspergillus niger*, *Cladosporium cladosporioides*, TLC, biotest, HPLC, spectroscopy

Abstract

The typical soil micromycetes *Aspergillus niger* and *Cladosporium cladosporioides* from the family moniliaceae were investigated with emphasis on production of ABA into the culture medium. The both fungi were cultivated in a static liquid Czapek - Dox medium and agar Czapek - Dox medium. *Aspergillus niger* and *Cladosporium cladosporioides* showed ability to produce ABA. Analytical detection of ABA from the culture medium was performed by TLC combinated with biotest and HPLC with spectroscopy.

List of abbreviations: Abscisic acid (ABA), Thin layers chromatography (TLC), High performance liquid chromatography (HPLC)

Introduction

When Assante (Assante *et al.* 1977) published, that fungus *Cercospora rosicola* produces ABA, they also suggested that this fact may play a role in growth regulation within ecosystem. Production of ABA by the fungus, was first, described in this work. In the last years literature takes notice of the ability of some saprophytic and parasitic fungi to synthesize ABA into the culture medium (Crocoll *et al.* 1991, Filimonova 1991, Dörffling and Peterson 1984, Okamoto *et al.* 1988, Michniewicz *et al.* 1986, Janitor and Vizárová 1994, Vizárová *et al.* 1997). In this literature are described some fungi from subdivision of *Deuteromycotina* which form terrestrial ecosystem, however they are also parasitical during their lifetime. In the present literature the data is absent about the secretion of mentioned substances by typical soil micromycetes, but the content of ABA in soils was described (Vizárová *et al.* 1998). The present work deals with production of ABA into the culture medium by *Aspergillus niger* and *Cladosporium cladosporioides* (*Deuteromycotina - Hyphomycetes - Moniliales*), which are a typical representantves of micromycetes of eutric fluvisols (Je) (WRB, 1994) in southern Slovak Republic.

Material and methods

The fungus *Aspergillus niger* and *Cladosporium cladosporioides* were isolated from localities Gabčíkovo (Fig. 1, 2). The fungi were cultivated in a Czapek-Dox agar and a liquid medium (Fassa-

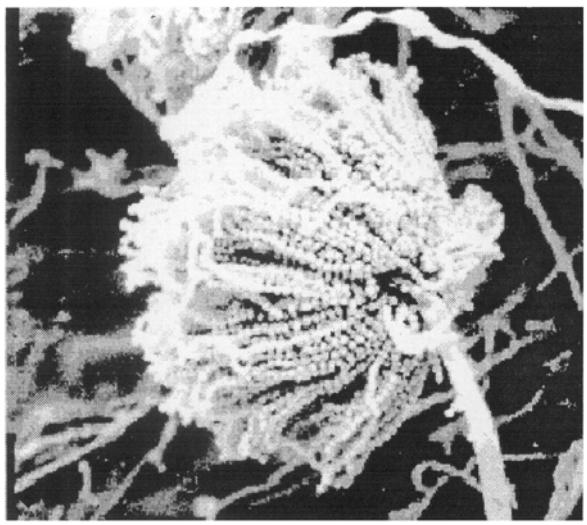

Fig. 1. *Aspergillus niger* - intact conidial head (SEM, x580)

Fig. 2. *Cladosporium cladosporioides* - conidiophore and conidia (SEM, x1100)

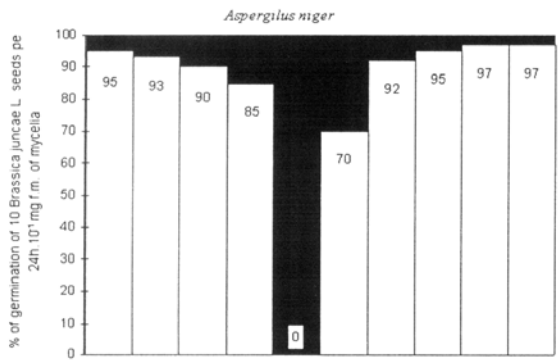

Fig. 3. Determination of ABA production by TLC and by biotest. TLC system (benzene-ethylacetate-acetic acid 100:20:5 (v/v))

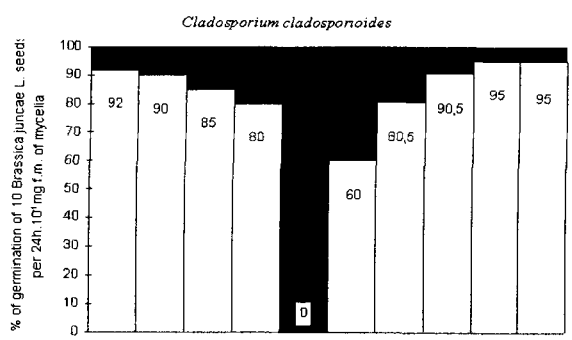

Fig. 4. Determination of ABA production by TLC and by biotest. TLC system (benzene-ethylacetate-acetic acid 100:20:5 (v/v))

tiová 1979) containing the following compounds: 3 g $NaNO_3$, 1 g KH_2PO_4, 0.5 g KCl, 0.5 g $MgSO_4 \cdot 7 H_2O$, 0.1 g $FeSO_4 \cdot 7 H_2O$, 30 g glucose, agar, water 1,000 cm^3 (pH before inoculation was 5.0). The cultures were inoculated with a spore suspension which is prepared by washing of 14 day old culture on Czapek-Dox with sterile water and kept in an incubator at 25 °C. After 20 days of cultivation the analyses were performed.

Extraction and purification of ABA from Czapek - Dox agar medium

Each culture medium (16 ml) was extracted with 76% methanol three times at the temperature +5 °C. The extract was filtered through filter paper. The filtrate was evaporated under vacuum at the temperature 35 °C to evaporate of methanol fraction. The water fraction was filtered on Dowex 50 column in H+ cycle (20x3 cm). The filtrate which had pH 3.5 was extracted 3 times with ethylacetate. The mixed ethylacetate extracts were evaporated in vacuum to dryness at 35 °C. The residue were disolved in methanol. The solution was filtered on DEAE cellulose column (3x5 cm) in methanol using vacuum filtration. The methanol extract was dried and disolved in 1 ml of MeOH for spectroscopy. One part of this extract was used for HPLC determination, and the second part was used for biological determination using biotest according Nikolaeva and Daleckaja (1963).

Extraction and purification of ABA from static liquid Czapek - Dox medium

The method of column thickening on Dowex 50 (in H^+ cycle) was applied. The filtrate before the separation was of pH 5.2. The filtrate was further purified on Dowex column (20 cm long, 3 cm diameter). Resulting purified filtrate showed pH of 3-3.2. This lower pH indicated separation of low basic substances. The filtrate after column thickening on Dowex 50 (in H^+ cycle) was three times extracted with ethylacetate. Mixed ethylacetate extracts were evaporated in vacuum to dry at 35C. The residue were disolved in methanol. Obtained solution was filtered on DEAE celulose column (3x5 cm) by vacuum filtration. The extract was dried and again disolved in methanol for spectroscopy and used for TLC, biotest and HPLC.

TLC on silica gel

As a developing mixture the benzene - ethylacetate - acetic acid (100/20/5; v/v) was used. For rechromatography mixture the benzene - ethylacetate - acetic acid (70/30/1; v/v) (Rypak and Kamenicka 1986). The R_F position responsible for ABA was determined with UV lamp at 254 nm. This position was further used for ABA detection by a biotest according to Nikolaeva and Daleckaja (1963). The method is based on inhibition of seed germination of mustard seeds.

High performance liquid chromatography (HPLC)

The HPLC system for analyses consisted of a column Separon SGxRPS 7m (150x3 mm) eluated with mobile phase water - methanol - acetic acid 55:44:1 (v/v/v), flow rate 0.4 ml·min^{-1}, detection UV at 245 nm. Retention time for ABA standard (MERCK) DL - cis, trans - abscisic acid (99.5 %) ranged from 8 to 9 min.

HPLC determination of ABA with spectroscopy

For this determination we used liquid chromatograph with two dosimeters f. LABORAT model 3 s, programator GP 2 with spectrometry detector for UV and determination program f. DATA APEX.

Results and discussion

The results which were obtained from the analysis of culture medium of *Aspergillus niger* and *Cladosporum cladosporioides*, after 20 days of cultivation showed the ability of those fungi to produce ABA into the culture medium in *in vitro* conditions. The method of purification and identification applied in our experiments demonstrated a considerable production of ABA. These results are highly significant when statistically evaluated (P=0.01). Production of ABA by bouth fungi verified the results which were obtained by HPLC and HPLC with spectroscopy (Fig. 5, 6, 7). HPLC with spectroscopy demonstrated higher quality ABA purification from culture medium.

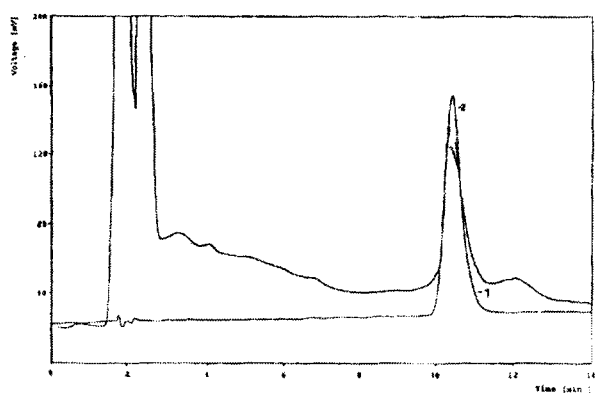

Fig. 5. Determination of ABA in *Aspergillus niger* by HPLC
1 - ABA from *Aspergillus niger*
2 - ABA standard

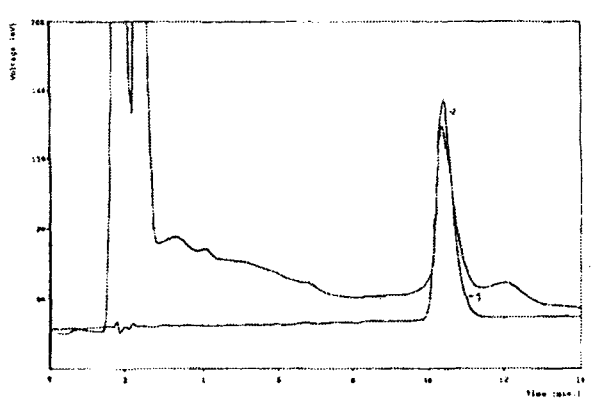

Fig. 6. Determination of ABA in *Cladosporium cladosporioides* by HPLC
1 - ABA from *Cladosporium cladosporioides*
2 - ABA standard

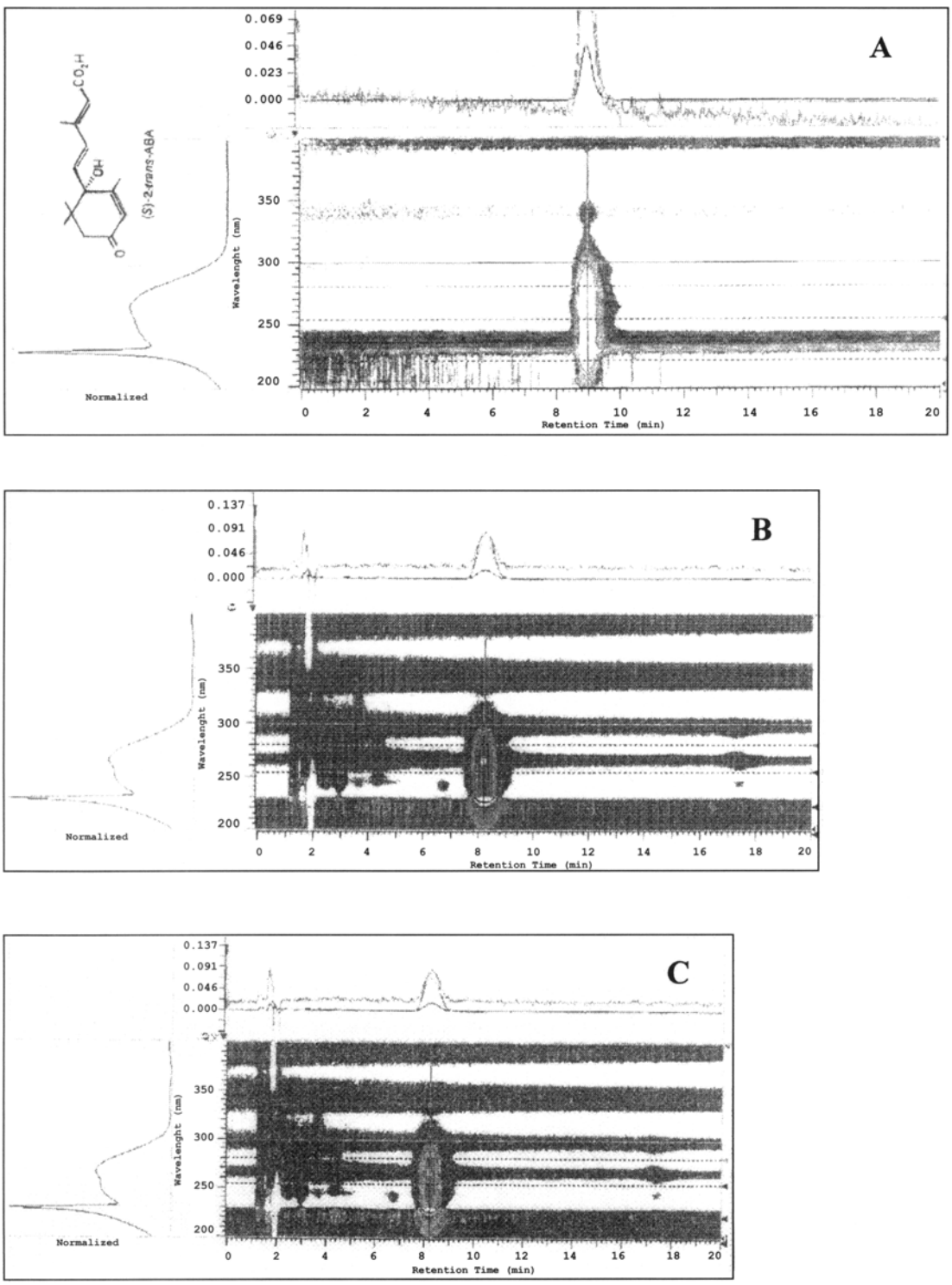

Fig. 7. Determination of ABA produced by *Aspergilus niger* and *Cladosporium cladosporioides* into cultivation medium by HPLC with spectroscopy. A - spectroscopy of ABA standard; B - spectroscopy of ABA from *Aspergillus niger;* C - spectroscopy of ABA from *Cladosporium cladosporioides*

Our results correspond to the literature data that the following fungi produced ABA into the culture medium: *Agrocybe praecox, Alternaria alternata, Coprinus domesticus, Cunninghamella echinulata, Mucor spinosus, Polyporus brumalis, Rhizopus arrhizus, Rhizopus nigricans, Trametes versicolor* (Crocoll *et al.* 1991), *Cercospora rosicola* (Assante et al. 1977), *Cercospora pini-densiflorae, Cercospora theate, Cercospora fici, Verticillium dahlie* (Okamoto *et al.* 1988), *Fusarium culmorum* (Michniewicz *et al.* 1984), *Botrytis* sp. (Dörffling *et al.* 1984), *Schizophyllum commune* (Janitor and Vizárová, 1984). *Monilia fructigena, Monilia laxa* Sacc. and *Cytospora cincta* Sacc. (Vizárová *et al.* 1997). Our results are very interesting as up to date data describe production of ABA, only by phytopathogenic fungi. The fact that soil micromycetes secreted ABA in culture medium is new and correlates with data (Vizárová *et al.* 1998) which described ABA content in soils. At this time abscisic acid is for plants a phytohormone with inhibitory effects characterised at present as a "stress phytohormone". On the other hand, from the literature it is known that ABA stimulated the growth of some fungi *e.g. Cylindrocarpon destructans* (Zins.) Scholl. (Michniewicz *et al.* 1986) and *Fusarium culmorum* (Michniewicz *et al.* 1984) and *Ceratocystis fimbriata* (Stopiska and Michniewicz 1988) and had no inhibitory effect on the growth and development *of Bolumeria graminis* (DC) Speer (Vizárová 1993) and *Septoria tritici* (Vasjuk and Musatenko 1998). Our results support the supposition of Assante *et al.* (1977) that production of ABA by soil micromycetes may play a role in growth regulation in the ecosystem. The results from our study of ABA production by typical soil micromycetes allow us to express assumption that fungi from *Deuteromycotina* have the abbility to produce ABA during their growth and development as a "vital part". Enzymes whose admittance into the processes of ABA biosynthesis are not known at present time and are not described in literature. We hope that our results will contribute to the extension of the knowledge about the important role of ABA production by micromycetes in growth regulation within the ecosystem.

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References

Assante G., Merlini G., Nasini G. 1977. +/- Abscisic acid a metabolite of the fungus *Cercospora rosicola*. Experientia 33: 1551 -1557.

Crocoll C., Kettner J., Dörfling K. 1991. Abscisic acid in saprophytic and parasitic species of fungi. Phytochemistry 30: 1059 - 1060.

Dörfling K., Peterson W. 1984. Abscisic acid in phytopathogenic fungi of the *genera Botrytis, Ceratocystis, Fusarium, Rhizoctonia*. Z. Naturforsch 39: 683 - 686.

Fassatiová O. 1979. Mouds and filamentous fungi in technical microbiology. (In Czech) SNTL, Praha, pp. 211.

Filimonova M. V. 1991. Methods of high press liquid chromatography and thin-layer chromatograpfy for analyse of phytohormone in culture medium of some phytopathogenic fungi. In: Obligate parasitism - cytophysiology aspects (Eds: Andreev L. N., Gorlenko M. N.) Nauka, Moscow, 125 - 127.

Janitor A., Vizárová G. 1994. Production of abscisic acid and cytokinins in static liquid culture by *Schizophyllum commune*. Czech. Mycol. 47: 293 - 302.

Michniewicz M., Michalski L., Czerwińska E. 1984. Control of growth and development of isolates of *Fusarium culmorum* of different pathogeniciti to wheat seedlings by plant growth regulators IV. abscisic acid. Acta Physiol. Plant. 6: 55 - 64.

Michniewicz M., Czerwińska E., Lamparska K. 1986. Stimulatory effect of abscisic acid on the growth and development of *Cylindrocarpon destructans* (Zins.) Scholl. Bull. Polish Acad. Sci. Biol. Sci. 34: 55 - 59.

Nikolaeva M. G., Daleckaja T. V. 1963. Growth of mustard seeds for estimation of biological activity of abscisic acid. Trudy Bot. Inst. AM ZSSR ser. 4: 16 - 19.

Okamoto M., Hirai N., Koshimizu B. 1988. Biosynthesis of abscisic acid. Phytochemistry 27: 1099 - 2103.

Rypák M., Kamenicka A. 1986: Growth regulators vest and germination of seeds. Bratislava, Veda, 150 pp.

Stopinska J., Michniewicz M. 1988. Control of growth and development of *Ceratocystis fimbriata* Ell. et Halst. by plant growth regulators. II. Abscisic acid. Bull. Polish Acad. Sci. Biol. Sci. 36: 260 - 264.

Vasjuk V. A., Musatenko L. I. 1998. The hormonal regulation of growth and developmnet of phytopathogenic fungi *Septoria tritici* Roberge ex Desm. In: International conference of plant onkogenesis in natural and transformed evironments, ed. by O. I.Tepek: 106.

Vizárová G., Chalanyová M., Janitor A., Ďugová O., Bacigalová K., Takáč L. 1997. Secretion of abscisic acid by hemibiotrophic fungi Biologia 52: 807 - 809.

Vizárová G. 1993. Participation of phytohormones in defensive reaction to biotrophic fungi (Overview). Ponohospodárstvo (Agriculture) 39: 545 - 552.

Vizárová G., Ďugová O., Chalányová M., 1999. Biological active substancies in soil loaded with heavy metals. In: Live in soil, ed. by O. Ďugová, G.Vizárová, KARTPRINT, Bratislava: 47-50.

WRB - 1994. World Reference Base for Soil Resources Draft. ISSS - ISRIS - FAO. Wageningen/Rome.

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