Abscisic Acid in Phytopathogenic Fungi of the Genera *Botrytis, Ceratocystis, Fusarium,* and *Rhizoctonia*

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Z. Naturforsch. **39 c,** 683–684 (1984); received March 14, 1983

Ceratocystis fimbriata, Fusarium oxysporum, Rhizoctonia solani, Phytopathogenic Fungi, Abscisic Acid

Strains of the phytopathogenic species *Botrytis cinerea, Ceratocystis coerulescens, C. fimbriata, Fusarium oxysporum,* and *Rhizoctonia solani* were cultivated on a defined synthetic culture medium. The plant hormone abscisic acid (ABA) was identified by gas chromatography/mass spectrometry and gas chromatography data, and the contents determined in mycelia (15.9–31.9 ng/g mycel) and culture media (1.0–4.6 ng/ml) by scintillation counting.

Introduction

Abscisic acid (ABA) is an important plant hormone which occurs in higher plants, in ferns, and in some mosses. It is involved in many physiological processes, especially in stress phenomena [1]. So far, ABA has been described as a fungal metabolite only from *Cercospora* species and from *Botrytis cinerea* [2–4]. We report here on the occurrence of ABA in five species from four genera of phytopathogenic ascomycetes.

Results and Discussion

Botrytis cinerea Pers. (strain Bc 1.Sa), *Ceratocystis coerulescens* Bakshi (Münch) (RWD 390 and RWD 705), *C. fimbriata* Ellis & Halst. (RWD 835 C and RWD 856), *Fusarium oxysporum* f. sp. *lycopersici* Snyder & Hansen (CBS 758.68), and *Rhizoctonia solani* Kühn (Rs 4. IU To) were cultivated on a synthetic culture medium containing glucose as carbon source, asparagine as nitrogen source, and mineral salts [5]. In all strains investigated, ABA could be unequivocally identified. The gas chromatograms, HPLC data and mass spectra provided complete coincidence with authentic ABA. Except for *Botrytis cinerea,* the occurrence of ABA has not so far been reported for these genera. The amounts of ABA found in mycelia and culture media (Table I) are distinctly lower than those found by other authors in *Cercospora rosicola* [2, 6] and *Botrytis cinerea* [4]. These differences could be due to the cultivation method, since the biosynthesis of ABA is strongly influenced by the composition of the culture medium, the age of the cultures, and also by environmental factors [4, 7].

Recently, we reported on a strain-dependent formation of volatile terpenes in *Ceratocystis* species [5, 8]. Regardless of this ability, ABA could be traced in all screened *Ceratocystis* strains. Since ABA was found as a metabolite of all fungal strains investigated, we suppose a widespread distribution of this plant hormone at least among phytopathogenic ascomycetes. Distribution, kinetic studies, metabolism, and the possible role of this substance in phytopathogenic processes are under investigation.

Materials and Methods

The fungal strains listed in Table I were cultivated on a glucose-asparagine-mineral salt medium as described in [5]. In each case, five cultures were harvested during the stationary phase. Mycelia and culture media were separated carefully and frozen at −20 °C before screening of ABA. Mycelia were extracted three times with 80% methanol at pH 8. The extract was shaken against hexane, then acidified and extracted with ether. Culture media were directly shaken against hexane and then extracted at pH 2.8 with ether. The ether phases were further purified by means of C-18-Sep-Pak cartridges after a modified technique of [9] and afterwards by thin-layer chromatography using hexane–ethylacetate (2:1, v/v) as solvent system. The purified ABA eluates were methylated with diazomethane and subjected to gas chromatography on packed glass columns (QF 1, SE 30) or fused silica capillary columns (CPSil 5 CB) with nitrogen as carrier gas and ^{63}Ni or ^{3}H electron capture detection [10].

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0341-0382/84/0600-0683 $ 01.30/0

Table I. Contents of abscisic acid (ABA) in mycelia and culture media of different phytopathogenic fungi cultivated on a synthetic glucose-asparagine-mineral salt medium.

Species	Strain	Host	Culture age	ABA content	
			[days]	mycelium [ng/g fresh weight]	culture medium [ng/ml]
Botrytis cinerea Pers.	Bc 1. Sa	garden lettuce	30	23.7	2.8
Rhizoctonia solani Kühn	Rs 4. IU To	tomato	23	16.9	4.6
Fusarium oxysporum f. sp. *lycopersici* Snyder & Hansen	CBS 758.68	tomato	23	31.9	3.7
Ceratocystis coerulescens Bakshi (Münch)	RWD 390	pine	27	31.0	1.6
	RWD 705	Douglas fir	35	n.d.	1.0
C. fimbriata Ellis & Halst	RWD 835 C	aspen	29	15.9	2.4
	RWD 856	sycamore	29	n.d.	1.3

n.d.: not determined.

For exact quantitative determination, 22 ng [^{14}C]ABA (Amersham, spec. activity 3.6 MBq/mg) was added to each extract as an internal standard. Recovery was determined in the purified extract by scintillation counting [10]. No such internal standard was used when greater amounts of mycelia or culture media were further analyzed by combined gas chromatography/mass spectrometry. This procedure involved an additional purification step by reversed phase high pressure liquid chromatography (HPLC) using an analytical C-18 Bondapak column and a methanol gradient from 20 to 60%. Purity was determined gaschromatographically (packed 2 m steel column 3% SE 30, Volaspher 100–120 mesh, isothermic 230 °C, FID).

Mass spectra were recorded on a Varian MAT 112 mass spectrometer at 70 eV combined with a Perkin Elmer PE F 22 gas chromatograph using a 30 m glass capillary column SE 54 (i.D. 0.32 mm) and helium as carrier gas. After injection, the split was shut for 2 min at 70 °C. Thereafter, a temperature programme (from 70 to 180 °C at 30 °C/min, and from 180 to 270 °C at 2 °C/min) was started. For identification, authentic methyl-ABA (Fluka) was compared under identical conditions. In each case, the mass spectra displayed characteristic major ions at 190 (base peak), 162, 134, and 125.

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