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PREVALENCE OF THE ABILITY TO PRODUCE ABSCISIC ACID IN PHYTOPATHOGENIC FUNGI

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Phytohormone abscisic acid (ABA) plays significant role in many physiological processes and response of plants to abiotic and biotic stresses. Phytopathogenic fungi also produce ABA, but the role of this trait in interactions with host plants is poorly understood. In this work 65 collection strains of phytopathogenic fungi (13 genera, 25 species) were screened for ABA production in batch culture using a modified potato dextrose (MPD) and original chemically defined (OCD) media. Analysis of ABA content was carried out by ultra-performance liquid chromatography. Thirty-four strains belonging total of 13 species produced ABA growing on MPD medium, and among them nineteen strains also produced ABA growing on OCD medium. A maximum ABA concentration was detected in MPD culture fluid of strain *Apiospora montagnei* MF-R13.8 (56.5 ± 0.1 μg L^{-1}), whereas strain MF-S41.5 of the same species was the most active ABA producer (13.4 ± 1.1 μg L^{-1}) growing on OCD medium. For the first time ABA was detected in species *Alternaria tenuissima*, *Apiospora montagnei*, *Bipolaris sorokiniana*, *Fusarium avenaceum*, *F. solani*, *Pythium ultimum*, *Sclerotinia sclerotiorum*, and *Sclerotium varium*. No correlation between the ability to produce ABA and host plant, plant organ of isolation or region of strain origin was found. In agar dish culture three tomato cultivars were inoculated with strains of *Fusarium solani* or *F. oxysporum* differing in ABA production *in vitro* to test relationship between the ability of fungi to produce ABA and to appear negative effects on plants. Generally, ABA production didn't correlate with the effects of fungi of tomato roots, with one exception that ABA production by *F. solani* strains negatively correlated (r = –0.82, P = 0.046, n = 6) with root length of cultivar Ailsa-Craig. The results suggest possibility for the role of fungal ABA as a positive modulator of pathogenesis, but manifestation of this effect depends on plant genotype and fungus species. The selected ABA-producing strains can be used to study mechanisms underlying involvement of fungal ABA in plant-microbe interactions.

Keywords: abscisic acid, fungi, phytohormones, phytopathogens, tomato

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INTRODUCTION

Abscisic acid (ABA) is a phytohormone playing significant role in many physiological processes in plants, including seed and bud dormancy, flowering, root growth, leaf shape and senescence, distribution of assimilates between root and shoot, growth inhibition, seed ripening, as well as in plant responses to abiotic stresses, particularly via regulation of stomata conductance (Davies, Zhang, 1991; Dodd, 2005; Sah, Reddy, 2016). In plants ABA is biosynthesized from carot-

enoids 9-*cis*-violaxanthin or 9-*cis*-neoxanthin via their enzymatic cleavage to xanthoxin followed by subsequent multiplied biochemical steps and finally converting abscisic aldehyde to ABA (Taylor et al., 2000; Oritani, Kiyota, 2003).

At the same time ABA was detected in microorganisms such as bacteria, algae and fungi (reviewed by Hartung, 2010). Many phytopathogenic fungi produce ABA *in vitro* and information about these fungi including taxonomic position, growth medium and conditions used for batch cultures, ABA concentrations in culture fluids and host plants is summarized in Table 1. Up to now total of eight genera and 14 species of phytopathogenic fungi were described as ABA producers. ABA biosynthesis in fungi was overviewed by Oritani and Kiyota (2003). Briefly, fungal species unable to synthesize 9'-*cis*-neoxanthin can form ABA directly via farnesyl-diphosphate and different ionylidene derivates, but when 9'-*cis*-neoxanthin is synthesized its converts to abscisic aldehyde and then to ABA. Another pathway is related to oxidative cleavage of a carotenoid precursor (9*Z*)-γ-carotene to various forms of γ-ionylideneacetic acid and then to ABA (Oritani, Kiyota, 2003).

The reports describing ability of phytopathogenic fungi to produce ABA aroused interest in the study the role of this trait in interactions between phytopathogen and host plant. It was shown that ABA increased the susceptibility of rice to *Magnaporthe oryzae* (Matsumoto et al., 1980), soybean to *Phytophthora sojae* (Mohr and Cahill, 2001), tomato to *Botrytis cinerea* (Audenaert, et al., 2002) and *Arabidopsis thaliana* to *Peronospora parasitica* (Mohr and Cahill, 2003). Low temperature condition increased ABA biosynthesis making rice plants susceptible to *Magnaporthe grisea* (Koga et al., 2004). ABA suppressed the activity of phenylalanine ammonia-lyase catalyzing the synthesis of polyphenyl compounds involved in defense mechanisms at the transcriptional level (Ward et al., 1989). Suppression of pathogen defense responses related to jasmonate-ethylene (Anderson et al., 2004) and salicylic acid (Audenaert et al. 2002) signaling pathways by ABA was also reported. It was also shown that ABA stimulated spore germination in *Botrytis cinerea* (Marumo et al., 1982) and mycelium growth of *Ceratocystis fimbriata* ABA (Stopinska, Michniewicz, 1988). On the other hand, it was shown that ABA increased resistance of *Arabidopsis thaliana* to *Alternaria brassicicola* and *Plectosphaerella cucumerina* via stimulation of callose deposition in a border area with infection zone (Ton, Mauch-Mani, 2004). Stomatal closure caused by high ABA concentrations may prevent invasion of phytopathogens into plant tissues (Asselbergh et al., 2008). Review of these contradictory results leaded to a conclusion that the control of disease resistance by ABA is very complex phenomenon varying from positive to negative depending on fungal and plant species, the timing of infection, growth conditions, presence of abiotic stresses and other unknown factors (Asselbergh et al., 2008; Ton et al., 2009).

This work was aimed to screen the collection strains of different phytopathogenic fungi for ABA production to find new species having this trait and to find relationships between ability to produce ABA and their characteristic features.

MATERIALS AND METHODS

Objects of research. Sixty five strains of phytopathogenic fungi were obtained from the Collection of the All-Russian Institute of Plant Protection and the Russian Collection of Agricultural Microorganisms (RCAM, St. Petersburg, Russia, http://www.arriam.ru/kollekciya-kul-tur1/). Species affiliation and origin of the studied fungal strains are shown in Table 2. The stock cultures of fungi were maintained on Czapek-Dox (CD) agar at 4°C. Tomato (*Solanum lycopersicum* synonym *Lycopersicon esculentum* Mill.) cultivar Ailsa-Craig (VIR 1930, England) was obtained from the Moles Seeds (UK, Ltd) and cultivars (cv.) Altai-Ground (VIR 2311, Russia) and Early-Uzbekistan (VIR 4750, Uzbekistan) were obtained from the N.I. Vavilov Institute of Plant Genetic Resources (St. Petersburg, Russia).

Growth media. Two liquid media were used for estimation the ability of fungi to produce ABA. The first medium was a modified potato dextrose (MPD) agar (Okamoto et al, 1988) supplemented after autoclaving with 1 mL L^{-1} of a juice obtained from fresh potato tubers. For this purpose, fresh potato tubers were homogenized using a household mixer and the juice was collected. Then the juice was centrifuged for 10 min (3000 g, 4°C), sterilized using 0.2 μm filters (Corning, Germany) and stored at –20°C until use. This modification aimed on the enrichment of PD medium with some putative heat sensitive components which can induce ABA production by fungi, but probably were inactivated due to autoclaving of a handmade PD medium. The second medium was an original chemically defined (OCD) medium containing (g L^{-1}): glucose – 10, glutamic acid – 0.2, asparagine – 0.2, aspartic acid – 0.2, serine – 0.2, thiamine – 0.001, $MgSO_4$ – 0.2, KCl – 0.5, $CaCl_2$ – 0.1, KH_2PO_4 – 0.8, $FeCl_3$ – 0.05 (pH = 6.0). An autoclaved OCD medium was supplemented with sterile solution of micronutrients (μM): H_3BO_3 – 2, $MnSO_4$ – 1, $ZnSO_4$ – 3, NaCl – 6, Na_2MoO_4 – 0.06; $CoCl_2$ – 0.06, $CuCl_2$ – 0.06, $NiCl_2$ – 0.06. This medium was composed based on the information available in the literature about chemically defined media used for the study of ABA-producing fungi (see Table 1 for references).

Fungi batch culture. Fungal strains were cultivated for 7 days on CD agar at 28°C. Then water suspensions containing spores and mycelium pieces in the amount of 10^7 colony forming units (CFU) per mL were pre-

Table 1. Information on phytopathogenic abscisic acid-producing fungi obtained from the literature

Fungal species	Growth medium	Growth conditions*	Concentration of abscisic acid	Host plant	Reference
Alternaria alternata	GAMS	20–40 days, 23°C, CL	84 mg g^{-1} DM	NS	Crocol et al. (1991)
A. brassicae	PD	20 days, at 25°C, CL	NS	Canola	Dahiya et al. (1988)
Botrytis cinerea	PD	7 days, 27°C, dark or light	2 (dark) or 14 (light) mg L^{-1}	NS	Marumo et al. (1982)
B. cinerea	GAMS	5 days	2.8 μg L^{-1}	Lettuce	Dorffling, Petersen (1984)
B. cinerea	PD	7 days, CL	3.5 mg L^{-1}	Geranium	Hirai et al. (1986)
B. cinerea	PD	15 days, 28°C	39.2 mg L^{-1}	Grape	Wu, Shi (1998)
B. cinerea	PD	20 days, statically, 23°C, dark	1.4 mg L^{-1}	Geranium	Inomata et al. (2004)
B. cinerea	CD	7 days, shaking, 20°C	0.8 mg L^{-1}	NS	Siewers et al. (2004)
Ceratocystis coerulescens	GAMS	5 days	1.6 μg L^{-1}	Pine	Dorffling, Petersen (1984)
C. fimbriata	GAMS	5 days	2.4 μg L^{-1}	Aspen	Dorffling, Petersen (1984)
Cercospora cruenta	PD	9 days, shaking, 28°C, CL	10 mg L^{-1}	NS	Oritani et al. (1982)
C. fici	PD	30 days, statically, 25°C, 12 h FL/12 h dark	10 μg L^{-1}	Pine	Okamoto et al. (1988)
C. pinidensiflorae	PD or CzD	30 days, statically, 25°C, 12 h FL/12 h dark	110 (PDM) or 380 (CD) μg L^{-1}	Pine	Okamoto et al. (1988)
C. pinidensiflorae	PD	17 days, 23°C, 12 h FL/12 h dark	NS	Pine	Hirai-et al. (2000)
C. rosicola	CD	7–21 days, shaking, 24–26°C, CL	1–10 mg g^{-1} DM	Rose	Norman et al. (1981)
C. rosicola	MSGM	7 days, shaking, 23–24°C, CL	0.2–13.6 mg L^{-1}	Rose	Bennet et al. (1981)
C. theae	PD	30 days, statically, 25°C, 12 h light/12 h dark	10 μg L^{-1}	Pine	Okamoto et al. (1988)
Fusariam culmorum	CzD	14 days	0.05 ng g^{-1} DM	Tomato	Michniewicz (1989)
F. oxysporum	GAMS	5 days	3.7 μg L^{-1}	Tomato	Dorffling, Petersen (1984)
Fusarium sp.	PD	15 days, 28°C	3.1 mg L^{-1}	Grape	Wu, Shi (1998)
Rhizoctonia solani	GAMS	5 days	4.6 μg L^{-1}	Tomato	Dorffling, Petersen (1984)
Rhizopus nigricans	GAMS	20–40 days, 23°C, CL	202 mg g^{-1} DM	NS	Crocol et al. (1991)
Rhizopus sp.	PD	15 days, 28°C	7.2 mg L^{-1}	Strawberry	Wu, Shi (1998)
Verticillum dahliae	PD	30 days, statically, 25°C, 12 h light/12 h dark	10 μg L^{-1}	Pine	Okamoto et al. (1988)

Note. *Growth conditions mean cultivation period, shaking, temperature and lighting, if available in the corresponding report. Abbreviations: GAMS – glucose-asparagine-mineral salt medium; PD – potato dextrose medium; MSG – mineral salts with glucose medium; CzD – Czapek-Dox medium; CD – various chemically defined media containing mineral salts with glucose or lactose, various amino acids and thiamine (for more details see corresponding references); CL – continuous lighting; NS – not shown; DM – dry mycelium.

Table 2. Characteristics of the studied fungal strains and their ability to produce abscisic acid in batch culture

Species	Strain number	Host plant		Region of origin	Abscisic acid production, $\mu g\ L^{-1}$	
		Species	Organ		MPD medium	OCD medium
Alternaria radicina	MF-P190-031	*Daucus sativus*	leaf	Minsk	ND	ND
A. solani	MF-P043-021	*Solanum tuberosum*	leaf	Primorsk	ND	ND
A. solani	MF-P043-041	*Solanum tuberosum*	leaf	Primorsk	1.2 ± 0.1	ND
A. tenuissima	MF-P480-011	*Triticum aestivum*	seed	Primorsk	0.7 ± 0.1	0.1 ± 0.01
Alternariaster helianthi	MF-P16-011	*Helianthus annuus*	stem	Krasnodar	ND	ND
Apiospora montagnei	MF-S41.4	*Elytrigia repens*	leaf	St. Petersburg	0.2 ± 0.1	0.2 ± 0.01
A. montagnei	MF-S41.5	*Heracleum sibiricum*	leaf	Novgorod	6.8 ± 0.6	13.4 ± 1.1
Arthrinium arundinis	MF-R13.7	*Brassica napus*	seed	St. Petersburg	0.9 ± 0.07	3.9 ± 0.3
A. arundinis	MF-R13.8	*Brassica napus*	seed	St. Petersburg	56.5 ± 0.1	19.8 ± 1.7
A. arundinis	MF-R41.5	*Heracleum sibiricum*	leaf	Novgorod	47.8 ± 0.1	ND
Bipolaris sorokiniana	MF-M17.1	*Papaver rhoeas*	leaf	Stavropol	ND	ND
B. sorokiniana	MF-R16.6	*Brassica napus*	seed	St. Petersburg	0.1 ± 0.1	ND
Botrytis cinerea	MF-R33.7	*Crambe abyssinica*	stem	St. Petersburg	0.7 ± 0.2	0.1 ± 0.01
Fusarium avenaceum	MF-W496	*Secale sereale*	seed	St. Petersburg	0.7 ± 0.1	0.1 ± 0.01
F. avenaceum	MF-W509	*Helianthus annuus*	stem	St. Petersburg	0.6 ± 0.1	ND
F. culmorum	MF-W993	*Triticum aestivum*	seed	Gomel	ND	ND
F. culmorum	MF-W30	*Hordeum vulgare*	root	St. Petersburg	0.2 ± 0.1	1.5 ± 0.1
F. equiseti	MF-W1081	*Hordeum vulgare*	seed	Novgorod	ND	ND
F. equiseti	MF-W1090	*Triticum aestivum*	seed	Gomel	ND	ND
F. graminearum	MF-W218	*Triticum aestivum*	seed	North Ossetia	0.7 ± 0.2	ND
F. oxysporum	MF-W1111	*Ocimum basilicum*	seed	St. Petersburg	ND	ND
F. oxysporum	MF-W1115	*Cucumis sativus*	stem	St. Petersburg	ND	ND
F. oxysporum	MF-G58284	*Solanum tuberosum*	tuber	St. Petersburg	4.3 ± 0.4	1.9 ± 0.2
F. oxysporum	MF-G58767	*Cucumis sativus*	stem	St. Petersburg	ND	ND
F. oxysporum	MF-G59014	*Solanum lycopersicum*	stem	Krasnodar	0.5 ± 0.04	ND
F. oxysporum	MF-G59120	*Gossypium sp.*	root	Kazahstan	0.9 ± 0.1	ND
F. oxysporum	MF-G59124	*Beta vulgaris*	root	Kazahstan	ND	ND
F. oxysporum	MF-G93656	*Capsicum annuum*	root	Kiev	ND	ND
F. solani	MF-W1109	*Ocimum basilicum*	seed	St. Petersburg	0.5 ± 0.3	2.1 ± 1.1
F. solani	MF-W1110	*Cola sp.*	stem	St. Petersburg	ND	ND
F. solani	MF-W83	*Secale cereale*	seed	Riga	5.4 ± 0.5	0.5 ± 0.3
F. solani	MF-W87	*Triticum aestivum*	seed	Irkutsk	1.1 ± 0.2	ND
F. solani	MF-W436	*Avena sativa*	seed	Pskov	10.6 ± 1.0	ND

Table 2. (Contd.)

Species	Strain number	Host plant		Region of origin	Abscisic acid production, μg L^{-1}	
		Species	Organ		MPD medium	OCD medium
F. solani	MF-W448	*Zea mays*	stem	Krasnodar	1.2 ± 0.7	0.3 ± 0.1
F. solani	MF-W483	*Solanum lycopersicum*	stem	Volgograd	2.3 ± 1.4	0.2 ± 0.04
F. solani	MF-W632	*Triticum aestivum*	seed	Krasnodar	1.4 ± 0.2	0.6 ± 0.1
F. solani	MF-W723	*Zea mays*	stem	Stavropol	0.9 ± 0.2	0.2 ± 0.02
F. solani	MF-W725	*Triticum aestivum*	stem	Altay	8.9 ± 3.3	1.7 ± 0.5
F. solani	MF-W728	*Triticum aestivum*	seed	Primorsk	2.3 ± 0.3	ND
F. solani	MF-W841	*Triticum aestivum*	stem	Stavropol	ND	ND
F. solani	MF-W869	*Cucumis sativus*	stem	St. Petersburg	ND	1.3 ± 0.8
F. solani	MF-W894	*Cannabis sativa*	stem	St. Petersburg	0.6 ± 0.1	ND
F. solani	MF-W898	*Cannabis sativa*	stem	St. Petersburg	0.4 ± 0.1	ND
F. solani	MF-W934	*Papaver rhoeas*	stem	St. Petersburg	3.4 ± 0.4	ND
F. solani	MF-W1014	*Pisum sativum*	stem	Voronezh	7.1 ± 4.2	ND
F. solani	MF-W1099	*Solanum lycopersicum*	stem	St. Petersburg	ND	ND
F. solani	MF-W1100	*Solanum lycopersicum*	stem	St. Petersburg	ND	0.8 ± 0.4
Macrophomina phaseolina	MF-16-001	*Helianthus annuus*	stem	Krasnodar	ND	ND
Neocamarosporium betae	MF-Ch16-001	*Beta vulgaris*	leaf	Primorsk	ND	ND
Phomopsis sojicola	MF-15-002	*Glycine max*	seed	Krasnodar	ND	ND
Piricularia grisea	MF-S72.1	*Cynodon dactylon*	leaf	Krasnodar	ND	ND
Plenodomus biglobosus	MF-Br16-001	*Barbarea sp.*	stem	Krasnodar	ND	ND
P. lindquistii	MF-Ha16-001	*Helianthus annuus*	stem	Krasnodar	ND	ND
Pythium ultimum	MF-R26.1	*Brassica napus*	stem	St. Petersburg	0.6 ± 0.1	ND
Rhizoctonia solani	MF-R15.2	*Brassica napus*	root	St. Petersburg	ND	ND
R. solani	MF-R15.6	*Brassica napus*	stem	St. Petersburg	ND	ND
R. solani	MF-P915-010	NR	NR	Germany	0.4 ± 0.1	ND
Sclerotinia sclerotiorum	MF-R22.14	*Brassica campestris*	stem	St. Petersburg	2.2 ± 0.2	ND
S. sclerotiorum	MF-R22.16	*Thlaspi arvense*	stem	St. Petersburg	ND	ND
S. sclerotiorum	MF-R22.16	*Thlaspi arvense*	stem	St. Petersburg	ND	ND
Sclerotium rhizodes	MF-S-60.2	*Agrostis tenuis*	leaf	St. Petersburg	ND	ND
S. rhizodes	MF-S-60.3	*Calamagrostis epigeios*	leaf	St. Petersburg	ND	ND
S. varium	MF-P992-010	*Raphanus raphanistrum*	leaf	St. Petersburg	3.7 ± 0.3	1.4 ± 0.1
Verticillium albo-atrum	MF-M18.5	*Papaver somniferum*	stem	Kiev	ND	ND

Note. MPD – Modified potato dextrose medium; OCD – original chemically defined medium; ND – not detected; NR – not registered. The data are means ± standard error ($n = 4$).

pared via flushing from the agar surface and used as inoculum. One mL of the inoculum was added to the flasks containing 50 mL liquid MPD or OCD media in two replicates for each strain. The uninoculated flasks were used as control treatments and for monitoring the presence of ABA in MPD medium. The flasks were incubated for 20 days at 23°C and lighting of 20 μmol m^{-2} s^{-1} with a 12 h photoperiod. The experiments were conducted twice for the strains showing ABA production.

ABA determination. The culture fluids were separated from mycelium using disposable cotton filters, acidified with 1 N HCl to pH = 3.0 and extracted with equal volumes of ethyl acetate. The extracts were evaporated to dryness at 35°C under vacuum on rotary evaporator Heidolph Hei-VAP Advantage (Heidolph, Germany) and the dry residues were dissolved in 0.2 ml of 18% acetonitrile, followed by filtration through 0.2 μm nylon membrane filters (Corning, USA). Analysis of ABA content was carried out by ultra-performance liquid chromatography using a Waters Acquity UPLC H-class system (Waters, USA) on a Waters Acquity UPLC BEH Shield RP18 (Waters, USA) column. Chromatographic separation was carried out for 6 minutes in isocratic mode in 18% aqueous acetonitrile containing 0.1% acetic acid followed by a 3 min flush of the chromatography column with mixture of 80% acetonitrile, 20% water and 0.1% acetic acid. The flow rate of the chromatographic mixture was 0.25 mL min^{-1}. ABA was determined using a Waters PDA diode-matrix UV detector at 265 nm by comparison the retention time and UV spectra (210–400 nm) of ABA peaks in the standard solution of chemically pure ABA (0.1 mg mL^{-1}, and Sigma-Aldrich, USA) with the corresponding peaks in samples. In the end of experiments the uninoculated MPD medium contained some amount of ABA varying from 0.9 to 4.7 μg L^{-1} depending on the experiment. The source of ABA in MPD medium was potato tubers used for medium preparation. The values of ABA concentration in the uninoculated MPD medium were always subtracted from those detected in the fungal cultures.

Agar dish culture with tomato. Tomato seeds were surface sterilized by 1% Na-hypochlorite for 15 min and germinated on a sterile wet filter paper with tap water for 7 days at 22°C. Germinated seeds were transferred to Petri dishes (3 seedlings per dish) with 25 mL of 1.5% agar nutrient solution (μM): $MgSO_4 \cdot 7H_2O$ – 500, $CaCl_2 \cdot 2H_2O$ – 500; K_2HPO_4 – 500, KH_2PO_4 – 210, KNO_3 – 100, NaFeEDTA – 10, HBO_3 – 1; $MnSO_4$ –1; $ZnSO_4$ – 1; Na_2MoO_4 – 0.03; $CuSO_4$ – 0.8; pH 6.3. Each seedling was inoculated with 30 μL of the above mentioned inoculum. After 10 days incubation in growth chamber at day/night cycle of 16/8 h and temperature of 23°C for 7 days all dishes were inspected and scanned. The obtained images were used to count the number of lateral roots and to measure total root length and using a curvimeter LX-3 (Sprinter, Ukraine).

Statistical analysis of the data was performed using the software Statistica version 10 (StatSoft Inc., USA).

RESULTS AND DISCUSSION

Thirty-four strains of 13 species produced ABA growing on MPD medium (Table 2). The maximum ABA concentrations were found in the culture liquids of both strains MF-R13.8 and MF-R41.5 belonging to *Arthrinium arundinis*. Nineteen strains produced ABA growing on OCD medium and the maximum ABA concentrations were in culture liquids of strains *Apiospora montagnei* MF-S-41.5 and *A. arundinis* MF-R13.8. All the strains producing ABA on MPD medium also did it on OCD medium as well, but among them only 17 strains produced ABA growing on both MPD and OCD media. The results suggest that the ability to produce ABA is a wide spread trait among phytopathogenic fungi of different taxonomic groups isolated from various host plants and environments. Some fungal species were among previously described ABA-producers (Table 1), namely *Botrytis cinerea* (e.g. Dorffling, Petersen 1984; Inomata-et al., 2004), *Fusarium culmorum* (Michniewicz, 1989) and *Rhizoctonia solani* (Dorffling, Petersen, 1984). However, for the first time we detected ABA production in nine species such as *Alternaria tenuissima*, *Apiospora montagnei*, *Bipolaris sorokiniana*, *Fusarium avenaceum*, *F. solani*, *Pythium ultimum*, *Sclerotinia sclerotiorum*, and *Sclerotium varium*. Ability to produce ABA highly varied among strains of the same species. For example, only three of eight *Fusarium oxysporum* strains and 16 of 19 *F. solani* strains produced ABA growing on the used media. No correlation between the ability to produce ABA and host plant ($R = -0.06$, $P = 0.63$, $n = 65$), plant organ of isolation ($R = -0.01$, $P = 0, 94$, $n = 65$) or region of strain origin ($R = 0.06$, $P = 0.66$, $n = 65$) was found. This suggests that fungal ABA production is not closely associated with these features. Otherwise, it should be mentioned that the presence and concentration of ABA produced by the same fungal strain may significantly vary depending on growth conditions and medium composition (Norman et al., 1981; Okamoto et al., 1988; Oritani, Kiyota, 2003) resulting in the disguise of such correlations. Indeed, the absence of correlations between the *in vitro* studied traits and the natural properties of biological objects is a well-known fact. The use of knock-out fungal mutants unable to produce ABA or the study of expression of genes related to fungal ABA production in patho-systems may shed light on this problem.

The agar dish culture was applied with three tomato cultivars inoculated with six strains of *F. solani* and four strains of *F. oxysporum* differing in ABA production to test relationship between the ability of fungi to produce ABA and to exert negative effects on plants. Similar

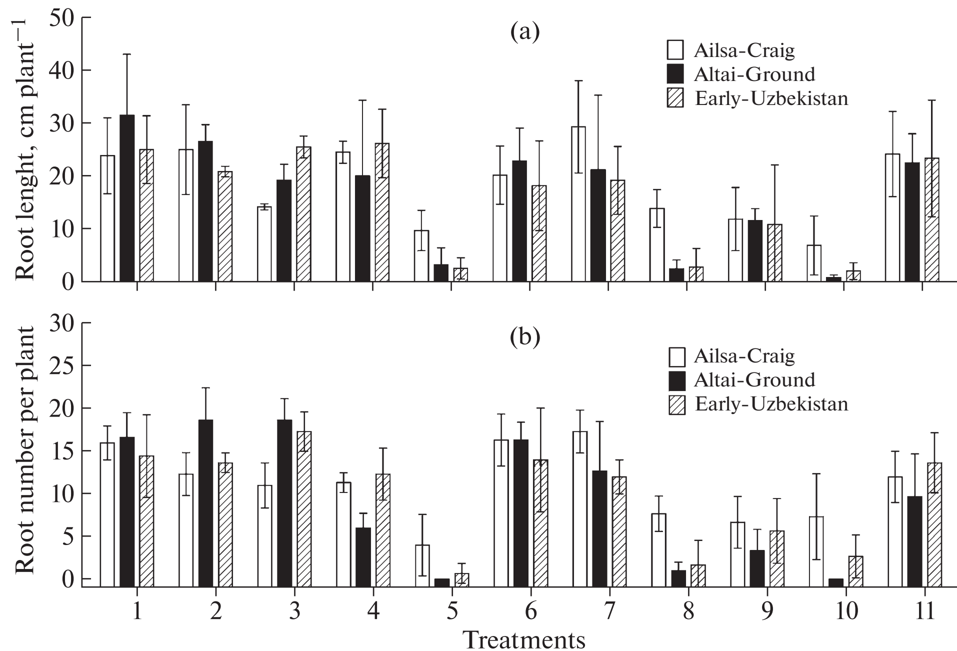

Fig. 1. Effect of *Fusarium* strains on root length (a) and number of lateral roots (b) of tomato seedlings in agar dish culture. Tomato cultivars: Ailsa-Craig (VIR 1930) (white fill), Altai-Ground (VIR 2311) (black fill) and Early-Uzbekistan (VIR 4750) (speckled fill). Treatments: 1 – uninoculated control, 2 – *Fusarium solani* MF-W483, 3 – *F. solani* MF-W725, 4 – *F. solani* MF-W869, 5 – *F. solani* MF-W1014, 6 – *F. solani* MF-W1100, 7 – *F. solani* MF-W1109, 8 – *F. oxysporum* MF-G58284, 9 – *F. oxysporum* MF-G58767, 10 – *F. oxysporum* MF-G59120, 11 – *F. oxysporum* MF-G93656. Bars show standard deviations (n = 3).

agar culture we previously applied to investigate effects of ABA-utilizing rhizobacteria on growth and tissue ABA concentrations of tomato cultivar Ailsa-Craig (Belimov et al., 2014). In this study the strains *F. solani* MF-W1014 and *F. oxysporum* MF-G58284, MF-G58767 and MF-G59120 significantly inhibited root elongation (Fig. 1a) and root branching (Fig. 1b) of all three tomato cultivars. These treatments also resulted in yellowing of roots and significant reduction of shoot growth (visual observations, data not shown). Root elongation and root branching was also inhibited after inoculation of cultivar Ailsa-Craig by *F. solani* 725 (Fig. 1). However, the negative effects of strains *F. solani* MF-W1014 and *F. oxysporum* MF-G58284 and MF-G59120 on cultivars Altai-Ground and Early-Uzbekistan were more pronounced as compared with Ailsa-Craig (Fig. 1). In general, the root growth inhibiting effects of fungi were similar for all cultivars, since correlations between cultivars for root length ($r > 0.75$; $P < 0.012$; $n = 10$) and root number ($r > 0.76$; $P < 0.011$; $n = 10$) were significant. This suggests similarity in mechanisms of growth inhibiting effects of the studied strains. It is known that *F. solani* and *F. oxysporum* cause disease of various crops, including tomato (Imazaki, Kadota, 2015; Akbar et al., 2018). Most probably the root growth inhibition observed in our study on the inoculated tomato seedlings was due to fungal phytotoxins, particularly fusaric acid which causes negative effect on plants (Bohni et al., 2016; Lopez-Diaz et al., 2018).

Generally, ABA concentration in culture fluids did not correlate with the effects of fungi on tomato roots,

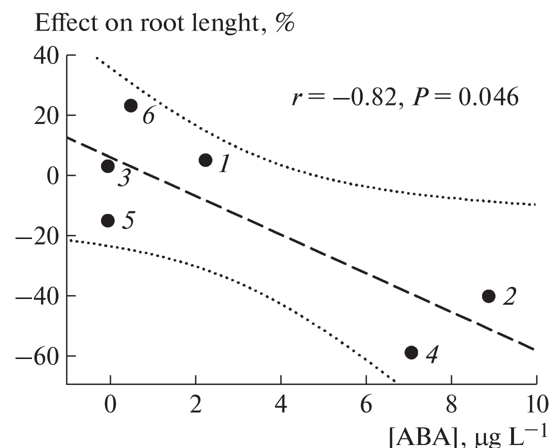

Fig. 2. Linear regression curve (dash line) showing correlation between abscisic acid production *in vitro* by *Fusarium solani* strains and their effect on root elongation of tomato cultivar Ailsa-Craig (VIR 1930) in agar dish culture. Strains: *1* – *F. solani* MF-W483, *2* – *F. solani* MF-W725, *3* – *F. solani* MF-W869, *4* – *F. solani* MF-W1014, *5* – *F. solani* MF-W1100, *6* – *F. solani* MF-W1109. Dotted lines show regression confidence area at $P = 0.05$.

with one exception that ABA production by *F. solani* strains negatively correlated (r = –0.82, $P = 0.046$, n = = 6) with root length of cultivar Ailsa-Craig (Fig. 2). The latter observation is in line with previous reports showing the important negative role of fungal ABA in plant disease resistance (Asselbergh et al., 2008; Ton et al., 2009). Whether ABA production is associated with production of some toxins by such fungi needs more detailed study.

CONCLUSION

In conclusion, we have detected ABA in culture fluids of nine fungal species, for which this property was not previously described, and expanded the species list of ABA-producing phytopathogens. A high variation in the ability to produce ABA was present on both strain and species levels. Production of ABA in a not defined MPD medium, containing extract of potato tubers, was found in more strains and it was generally higher compared with a defined OCD medium. However, the presence of ABA in the uninoculated MPD medium should be taken into account when assessing the ability of fungi to produce ABA. The absence of significant correlations between ABA production and the studied characteristics of strains suggest high complexity of this phenomenon. However, the negative correlation observed here between ABA production by *F. solani* strains and root length of Ailsa-Craig gave new original information about the role of fungal ABA as a positive modulator of pathogenesis. The selected ABA-producing strains can be used to study mechanisms underlying involvement of fungal ABA in plant-microbe interactions.

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СПОСОБНОСТЬ НЕКОТОРЫХ ВИДОВ ФИТОПАТОГЕННЫХ ГРИБОВ ПРОДУЦИРОВАТЬ АБСЦИЗОВУЮ КИСЛОТУ

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Фитогормон абсцизовая кислота (АБК) играет значительную роль во многих физиологических процессах и реакции растений на абиотические и биотические стрессоры. Фитопатогенные грибы также продуцируют АБК, но роль этого признака во взаимодействиях с растениями-хозяевами плохо изучена. В этой работе был проведен скрининг 65 коллекционных штаммов фитопатогенных грибов различных таксонов (13 родов, 26 видов) по способности продуцировать АБК в периодической культуре с использованием модифицированной картофельной (МК) и оригинальной химически определенной (ХО) сред. Анализ содержания АБК проводили с помощью ультраэффективной жидкостной хроматографии. Тридцать четыре штамма, принадлежащие в общей сложности к 13 видам, продуцировали АБК на среде МК, и среди них девятнадцать штаммов также продуцировали АБК на среде ХО. Максимальная концентрация АБК была обнаружена в культуральной жидкости штамма *Apiospora montagnei* MF-R13.8 (56.5 ± 0.1 мкг/л), а штамм MF-S41.5 того же вида был самым активным продуцентом на среде ХО (13.4 ± 1.1 мкг/л). Впервые АБК была обнаружена у видов *Alternaria tenuissima*, *Apiospora montagnei*, *Bipo-*

laris sorokiniana, *Fusarium avenaceum*, *F. solani*, *Pythium ultimum*, *Sclerotinia sclerotiorum* и *Sclerotium varium*. Не обнаружено корреляции между способностью продуцировать АБК и растением-хозяином, органом растения, из которого был изолирован гриб или регионом происхождения штамма. В условиях агаровой культуры три сорта томатов инокулировали штаммами *Fusarium solani* или *F. oxysporum*, отличающимися продукцией АБК *in vitro*, для проверки взаимосвязи между способностью грибов вырабатывать АБК и оказывать негативное воздействие на растения. Как правило, продуцирование АБК не коррелировало с эффектами грибов на корни томатов, за исключением негативной корреляции продукции АБК штаммами *F. solani* с длиной корней сорта Ailsa-Craig ($r = -0.82$, $P = 0.046$, $n = 6$). Полученные результаты свидетельствуют о возможной роли грибной АБК в качестве положительного модулятора патогенеза, но проявление этого эффекта зависит от генотипа растения и вида гриба. Отобранные продуцирующие АБК штаммы могут быть использованы для изучения механизмов, лежащих в основе участия этого свойства грибов во взаимодействиях растений и микробов.

Ключевые слова: абсцизовая кислота, грибы, томаты, фитогормоны, фитопатогены