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Response of *Arabidopsis* Clones to Toxic Compounds Released by Various *Rhizoctonia* Species

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ABSTRACT

Response of 3 *Arabidopsis* clones to 41 strains of eight *Rhizoctonia* species was studied in model experiments. The seed germination was decelerated in most of the cases, although the inhibitory effect varied within large limits. The pre-emergence damping off and root neck rot leading to damping off were the most frequent symptoms of disease syndrome caused by toxic metabolites. The clone transformed with cDNA clone overexpressing *gstf*4 gene exhibited significantly improved tolerance as compared to parental one, meanwhile the sensitivity of D-mannose pyrophosphorylase/mannose-1-pyrophosphatase deficient clone dramatically increased. Strains of *R. solani* of AG-2, AG-4 and AG-7 and *Athelia rolfsii* produced the most toxic metabolites, however, no strict relationships were revealed between taxonomic position of *Rhizoctonia* strains and toxicity of their metabolites.

KEYWORDS

Arabidopsis*; *Rhizoctonia*; Toxin; *Athelia*; *Ceratobasidium*; *Ceratorhiza*; *Thanatephorus*; *Waitea

1. Introduction

Damping off caused by various soil-borne pathogenic microbes is a well-known phenomenon in plant cultivation. The traditional control measures (seed dressing, soil fumigation) cannot be applied in large scale due to high risk of irreversible environmental pollution and possible toxic residuals in food and forage. Moreover, the soil-borne fungi (mainly *Fusarium spp*, *Pythium*, *Rhizoctonia*) can attack several hundred cultivated plant species during all vegetation period that makes impossible the long lasting protection of plants with selectively acting synthetic compounds or antibiotic preparations. Recently, none of the marketed fungicides is translocated basipetally, which counteracts their use after development of foliage against root parasiting fungi. Although, *Rhizoctonia* species are not among the top ten pathogens [1], economic importance of their control is increasing from severe to catastrophic yield losses reported from main wheat cultivating areas [2-4]. Changes in agricultural practices of cereals with special regard to minimum or no tillage cultivation [5,6] led to increased importance of *Rhizoctonia* infections [7,8]. The most plausible method is the breeding of tolerant varieties.

The *Rhizoctonia* species are abundant in soils as mutualistic members of microbial consortium associated with plants. Their relationship may change from symbiosis (as in various orchids) to parasitism. The stunted growth can be most frequently observed, which might be related to the effect of *Rhizoctonia* toxins although few evident experimental proof has been demonstrated yet [9]. Presence of parasitic hyphae can also be commonly seen even in symptomless tissues, and in normal conditions does not cause visible disease symptoms either in phyllosphere or in roots. However, when environmental stress factors overwhelm the homeostatic regulation of plant, the disease syndrome may develop at various degrees. Both biotic and abiotic stress manifests as an oxidative stress [10], thus by scavenging free radicals an active antioxidative defense system comprising enzymatic and non-enzymatic antioxidants reduces the level of oxidative

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stress in plant cells and counteracts evolution of disease. Regulation of substrate pools such as glutathione or ascorbic acid is the key element of cell protection [11], and the capacity of the biochemical path sustaining the oxido-reductive potential of cells has crucial importance in elimination of harmful free radicals [12] and confers in resistance to pathogens [13,14]. The high level of ascorbic acid reduces the disease severity [15], while the deficiency increases the sensitivity to environmental stress factors [12]. Glutathione S-transferases play an important role in adaptation of various organisms to adverse factors [16-18], and in detoxification of electrophilic compounds either exogenic or of natural origin [19,20]. The formation of glutathione adducts catalyzed by GST is usually the first step of deterioration [21]. The level of GST increased in okra [22] and ricinus [23] seedlings damaged by soil-borne *Rhizoctonia*, suggesting activation of glutathione conjugation system in defense mechanisms counteracting invasion and eliminating grave consequences of the infection. The deteriorative capacity of plant and associated microbes is related to the tolerance of host plant to the pathogen. Thus, the selection of tolerant plants to these pathogenic fungi is important for quality food production as well.

There is an urgent need in sustainable management strategy to combat damages induced by soil habiting *Rhizoctonia* complex. No major resistance genes to this pathogen have been identified so far in spite of increasing efforts in studies of physiology and genetics of *Rhizoctonia*/host interaction [8]. Currently, the genetic engineering is widely used in breeding technologies providing genetically modified (GM) plants with a very high resistance against abiotic and biotic factors. Some plants have been objected for increasing tolerance to unfavorable environmental conditions [24] and remediative capacity [25] by gene engineering that can be a determinative factor for producing healthy food [26].

The genomes and physiology of *Arabidopsis thaliana* are broadly studied, and numerous mutans or transgenic clones are described in literature. The *Arabidopsis* model system greatly help us to understand specific genetic and biochemical processes. The aim of this work was to get information on the effect of toxic metabolites of *Rhizoctonia* species complex on seed germination of *Arabidopsis*.

The scoring of disease incidence and severity meet difficulties in the case of *Rhizoctonia*s as some symptoms of disease syndrome can be induced by toxic metabolites of *Rhizoctonia* alone [9]. In agar cultures, the above two effects can be separated during first days of germination, however, later the damage caused by parasiting hyphae secondary infections of germlings caused by various microbes of spermosphere can mask the visual effect of *Rhizoctonia* toxins even in agar cultures. To avoid the altering effects, the observations were limited up to five days, as the aim of this work was the evaluation of sensitivity of *Arabidopsis* to toxic metabolites of *Rhizoctonia* released to the medium.

2. Materials and Methods

Response of three *A. thaliana* clones to metabolites of 41 *Rhizoctonia* strains five genera (*Athelia*, *Ceratobasidium*, *Ceratorhiza*, *Thanatephorus* and *Waitea*) was compared in model experiment applying vertical agar diffusion technique. The seeds were surface sterilized for 2 min in 70% alcohol followed by 5 min in commercial bleach (0.5% hypochlorite) then washed five times in sterile water and diluted in sterile water slightly solidified with 0.1% agar before stratification for 48 hours at 4˚C in the dark.

2.1. Plant Material

Seeds of *Arabidopsis thaliana* wild type (*Col*-5; #195), the vitamin C deficient mutant *vtc*1-1 [12,27] and a transgenic clone overexpressing glutathione S-transferase [28] (gst f; #193) were used for all exepriments.

***Col*-5** [wild type] and *vtc*1-1 seeds were obtained from *Arabidopsis* stock Center (www.arabidopsis.info).

A-193—For gstf, ecotype: Col-5 was transformed with cDNA clone overexpressing *gstf*4 gene (*Zea mays*, EMBL: U12679/X79515; Uniprot: P46420) driven by cauliflower mosaic virus 35S promoter. cDNA were introduced by using the floral dip transformation method [29] using the hygromycin phosphotransferase (*hpt*) gene as selectable marker. The pCAMBIA1301 binary vector was used for transformation.

***vtc*1** ecotype is deficient in the function of GDP-mannose pyrophosphorylase/mannose-1-pyrophosphatase. This enzyme provides GDP-mannose, which is used for cell wall carbohydrate biosynthesis, protein glycosylation and for ascorbate (vitamin C) biosynthesis. Total acorbic acid content in *vtc*1 clone is about 40% compared to wild type. Earlier work revealed that *vtc*1 mutant has increased resistance against *Pseudomonas syringae* or *Peronospora parasitica* [30] but more sensitive to *Alternaria brassicicola* [15].

2.2. Test Fungi

Rhizoctonia strains were originated of different locations and various hosts:

Rhizoctonia solani Kühn strains of CBS collection: B-415 (AG-1, *Pinus sylvestris* L., Canada, CBS 522.96), B-432 (AG-2, *Daucus carota* L., Netherlands, CBS 326.84), B-446 (AG-3, *Solanum tuberosum* L., Spain, CBS 117248), B-417 (AG-4, *Citrus sp.*, Argentina, CBS 341.35), B-430 (AG-4, *Phaseolus sp.*, England, CBS 340.51), B-418 (AG-5, *Zea mays* L., Netherlands, CBS 339.84), B-419 (AG-6, *Erigeron canadensis* (L.) Cronquist, CBS 137.82, USA), B-420 (AG-7, soil, Japan, CBS 214.84), B-421 (AG-8, *Triticum aestivum* L., Australia, CBS 101782), B-422 (AG-9, *S. tuberosum*, USA, CBS 970.96), B-423 (AG-10, *T. aestivum*, USA, CBS 971.96), B-424 (AG-11, *Lupinus angustifolius* L., Australia, CBS 974.96), B-434 (AG-E, *Malus sp.*, Netherlands, CBS 340.84).

R. solani strains isolated in Hungary: B-151 (*S. tuberosum* cv Desirée), B-245 (*Allium cepa* L., China, Henan), B-246 (*S. tuberosum cv* Gül Baba), B-399 (*Sesamum indicum* L.), B-403 and B-404 (*S. tuberosum cv* Ella), B-409 (*Hibiscus rosa-chinensis* L., Lybia, Tripoli), B-410 (*S. tuberosum cv* Kisvárdai rózsa), B-411 (*S. tuberosum* cv Desirée), B-412 (*S. tuberosum cv* Cleopatra), B-413 (*Malus sp.* L.), B-433 (*Festuca arundinacea* Schreb.), B-444 (*Viola* × *wittrockiana* Gams.), B-446 (*S. tuberosum cv* Százszorszép), B-521 (*Impatiens balsamina* L.), B-522 (*Oxalis triangularis* A.St.-Hil., Oxalidales, Oxalidaceae), B-573 (*T. aestivum*)., B-548 (*Phragmipedium schlimii* (Lind. & Reich.f.) Rolfe, Orchidaceae), B-553 (*Phalenopsis* Orchidaceae), B-557 (*Dendrobium* × *Phalenopsis* hybrid, Orchidaceae), B-560 (*Doritis pulcherissima* Lindley, Orchidaceae).

R. fragariae S. Husain & W. E. McKeen 1963 (teleomorph: *Ceratorhiza fragariae* (S. S. Husain & W. E. McKeen) R. T. Moore 1987), B-438 (*Fragaria* × *ananassa* Duchense, Canada, CBS 335.62).

R. cerealis E. P. Hoeven 1977 (teleomorph: *Ceratobasidium cereale* D. I. Murray & Burpee 1984 Basidiomycota, Cantharellales), B-447 (*T. aestivum*, Germany, CBS 559.77).

R. stahlii Burgeff 1936 (teleomorph: *Mycelium radicis Platantherae chloranthae* (Custer) Rchb.), *Thanatephorus* Donk *sp.*, Basidiomycota, Cantharellales, Germany), B-441 (*P. chlorantha* (Custer) Rchb.), Asparagales, Orchidaceae, Germany, CBS 119.92).

R. ramicola W. A. Weber & D. A. Roberts (teleomorph: *Ceratorhiza ramicola* (W. A. Weber & D. A. Roberts) R. T. Moore 1987, Basidiomycota, Cantharellales), B-427 (*Pittosporum tobira* (Thunb.) W. T. Aiton, Asparagales, Orchidaceae, Florida, USA, CBS 400.51).

R. carotae Rader 1948 (teleomorph: *Athelia arachnoidea* (Berk.) Jülich 1972, Basidiomycota, *Atheliales*), B-440 (*D. carota*, USA, CBS 464.48).

Athelia rolfsii (Curzi) C. C. Tu & Kimbr. 1978 (Basiiomycota, *Athelia*les), B-442 (*S. tuberosum* L., Italy, CBS 464.48).

R. zeae Voorhees 1938 (teleomorph: *Waitea circinata* Warcup and P. H. B. Talbot 1962), B-405 (*F. arundinacea*, Hungary).

The strains were maintained on potato dextrose agar (Merck, Darmstadt, Germany) amended with 2 g soya peptone L44, 0.5 g Trypton T L43 (Oxoid, Basingstoke, sUK) and 1 g yeast extract L21 (Oxoid).

2.3. Assay for Toxin Sensitivity

The culture medium consisted of agar No. 1. (Oxoid) (11 gl^{-1}), soya peptone (5 gl^{-1}), yeast extract (3 gl^{-1}), starch (5 gl^{-1}), glucose (5 gl^{-1}) glycerol (2 gl^{-1}), Na-b-glycerophosphate × $6H_2O$ (0.5 gl^{-1}), Tween 80 (0.25 gl^{-1}), KH_2PO_4 (0.5 gl^{-1}), Na_2HPO_4 × $7H_2O$ (0.5 gl^{-1}), KCl (0.25 gl^{-1}), $MgSO_4$ (0.15 gl^{-1}), $CaCl_2$ (0.15 gl^{-1}), $FeSO_4$ × $5H_2O$ (0.025 gl^{-1}), $CuSO_4$ × $5H_2O$ (5 mgl^{-1}), $MnSO_4$ × H_2O (5 mgl^{-1}), $Ni(NO_3)_2$ × $6H_2O$ (1 mgl^{-1}) and $CoCl_2$ × $6H_2O$ (1 mgl^{-1}) and vitamins pyridoxine×HCl (1 mgl^{-1}), thiamine × HCl (10 mgl^{-1}), riboflavine (1 mgl^{-1}) and nicotinamide (20 mgl^{-1}). The agar plates (5 ml medium in 50 mm diameter) were centrally inoculated, and incubated at ambiental conditions for 21 days. The thickness of medium reduced up to 1 mm, and the mycelium completely covered the surface. These cultures were covered with 5 ml agar solution (10 gl^{-1}), and after 8 hours stratified seeds were dropped over the surface (25 - 30 seeds in 200 microl). The plates were incubated in ambiental conditions, and the germination was followed up to 10 days. Number of germinated seeds was counted in each morning, moreover, the germlings were studied under dissecting microscope to check their response to metabolites of *Rizoctonia* strains released into the medium. The half time of germination and ratio of survivors were calculated.

2.4. Data Analysis

Fisher's test was applied to evaluate significance of differences between variant at $p = 0.05$ level. The results of counting of germinated/dormant and healthy/diseased individuals were calculated as percentages. The half time of germination (hours requested for germination of the 50% of seeds) was determined by linear regression analysis, where the percent values were transformed into probits. Similarity of *Arabidopsis* clones and relationships between parameters of assessment were investigated by Canonic Correlation Analysis (CCA). Statistical functions of Microsoft Office Excel 2003 (Microsoft, Redmond, USA) and Statistica5 program (StatSoft 5.0., Tulsa, USA) were used for analysis of data. The graphical presentation of result of data analysis was edited uniformly in MS Office Power Point 2003.

3. Results

3.1. Germination of Seeds

The number of dropped seeds varied between 25 ± 6 and they disseminated in a spot with diameter between 10 - 14 mm on agar plate. On control plates 96% - 100% of them have germinated indicating the good quality of seed material. Seeds of each clone germinated synchronously within 48 hours. The half time of germination approached by linear regression varied within −12 and 18 hours, where the 48 hours of stratification should be taken into consideration for negative value. Predominant majority of *Rhizoctonia* strains did not break through the agar layer in first five days of incubation, thus most of seeds in each variant could germinate and develop cotyledons until meeting with hyphae.

The hyphae of strains started to colonize the surface after six days of incubation, but their attack cannot be accurately evaluated due to airborne contaminations and bacteria arising of spermosphere. The first visual symptom of toxicose was the formation of brown spot on root-neck of the germlings even before full opening of cotyledons that was followed with damping off within 24 hours. The killed seeds became dark brown. Seedlings that survived the effect of toxic metabolites were subsequently destroyed by fungal attack rapidly in the case of most aggressive strains (B-151, B-245, B-420, B-427 and B-557), while in the case of other strains the variation in number of survivors within series was too high for correct assessment of pathogenicity, thus the data analysis was limited to 5 days of incubation. In some cases germination started after 24 - 72 hours lag phase (**Table 1**).

Table 1. Toxic effect of *Rhizoctonia* metabolites to germinating *Arabidopsis* seeds.

Rhizoctonia strains[1]			Anastomosis	Destroyed (%)			Lag phase[3] (days)		
No.	Code	Source	groups	A-195[2]	A-193	*vtc* 1	A-193	A-195	*vtc* 1
1	B-415	Scots pine	AG-1	75.0	15.4	76.2	0	0	0
2	B-432	Carott	AG-2	100.0	100.0	100.0	6	6	6
3	B-446	Potato	AG-3	47.8	0.0	20.0	1	1	1
4	B-417	*Citrus sp.*	AG-4	66.7	56.7	0.0	2	1	2
5	B-430	*Phaseolus sp.*	AG-4	87.0	44.4	90.6	6	0	1
6	B-418	Maize	AG-5	96.7	67.9	100.0	3	1	6
7	B-419	Horseweed	AG-6	15.4	20.8	0.0	0	0	0
8	B-420	Soil	AG-7	100.0	95.0	100.0	6	3	6
9	B-421	Wheat	AG-8	16.7	14.3	81.0	1	1	1
10	B-422	Potato	AG-9	96.4	75.0	100.0	2	1	6
11	B-423	Wheat	AG-10	58.3	8.7	28.6	1	0	1
12	B-424	Lupin	AG-11	4.0	0.0	5.0	0	0	0
13	B-434	Apple tree	AG-E	0.0	4.5	21.4	0	0	0
14	B-151	Potato cv Desirée		78.8	92.1	85.7	0	0	1
15	B-412	Potato cv Cleopatra		100.0	20.0	40.7	6	0	0
16	B-246	Potato cv Gül Baba		57.1	33.3	71.4	0	0	0
17	B-410	Potato cv Kisvárdai rózsa		100.0	100.0	100.0	6	6	6
18	B-411	Potato cv Desirée		39.1	20.0	25.0	0	0	0
19	B-446	Potato cv Százszorszép		22.6	25.7	15.6	0	0	0
20	B-403	Potato cv Ella		3.7	0.0	22.2	0	0	0
21	B-404	Potato cv Ella		4.0	6.7	21.1	0	0	0
22	B-522	Purple shamrock		100.0	100.0	100.0	6	6	6
23	B-409	Rose mallow		100.0	100.0	100.0	6	6	6
24	B-521	Touch-me-not		100.0	100.0	100.0	6	4	3[5]
25	B-399	Sesame		50.0	73.7	58.1	0	0	1
26	B-413	Apple tree		44.4	50.0	68.2	0	0	0
27	B-444	Pansy		14.8	20.0	25.0	0	0	0
28	B-245	Onion		90.3	88.2	92.3	1	1	1
29	B-433	Tall fescue		100.0	100.0	100	6	6	1[5]
30	B-573	Wheat		4.3	11.1	5.0	0	0	0
31	B-553	*Phalenopsis*[4]		100.0	100.0	100.0	6	6	6
32	B-557	*Dendrobium Blue Violetta*[4]		50.0	37.5	74.1	1	0	0
33	B-441	*Plantathera*[4]	*R. stahlii*	20.0	29.2	19.2	1	0	1
34	B-560	*Doritis*[4]		5.4	0.0	30.8	0	0	0
35	B-548	*Phragmipedium*[4]		0.0	0.0	14.3	0	0	0
36	B-405	Tall fescue	*R. zeae*	0.0	3.8	0.0	0	0	0
37	B-447	Wheat	*R. cerealis*	14.3	18.2	21.7	0	0	0
38	B-438	Strawberry	*R. fragariae*	61.8	27.3	38.9	1	0	1
39	B-427	*Pittosporum*[4]	*R. ramicola*	52.2	7.1	81.3	0	0	1
40	B-440	Carott	*R. carotae*	15.6	40.9	67.9	0	0	0
41	B-442	Potato	*A. rolfsii*	100.0	100.0	100.0	6	6	6
		$SD_{0.05}$		4.7	6.9	12.2			

[1]Accession numbers of the Mycological Collection of PPI HAS (WDCM824). Strains 1-35 are *Thanatephorus* anamorph: 1-13 reference strains of CBS, 14-21 isolated of potato, 22-27 isolates of dicot plants, 28-80 isolates of gramineas, 31-35 isolates of orchids; strains 36 *Waitea*, 37 *Ceratobasidium*, 38-39 *Ceratorhiza*, 40-41 anamorphs; [2]Parental clone (A-195); [3]Time requested for the emergence of first seedling; [4]Orchid species, host plants of *Rhizoctonias*; [5]All seeds killed after 4th day.

3.2. Toxic Effect of Metabolites

Response of germinating seeds was evaluated in two aspect. First the lethal effect, *i.e.*, the ratio of non germinated seeds was calculated of recorded data at 5th days of incubation for each strain (**Table 1**). The reaction to metabolites of *Rhizoctonia*s varied within large limit and strain dependents manner. The toxicity of AG-3 (B-446 and B-433) and AG-4 (B-417 and B-430) strain pairs proved to be significantly different. Similarly, great differences were revealed between strains isolated of the same host, the toxicity of potato originated ones varied between 4% and 100%. The genetic manipulation altered significantly the response *Arabidospsis* clones to *Rhizoctonia* metabolites (**Table 2**). The clone A-193 with improved capacity of glutathione conjugation system (GCS) tolerated at higher level the toxins than both parental (A-195) and vitamin C deficient mutant (*vtc*1), while the latter slightly got behind of A-195.

The other aspect of evaluation of toxic effects was the delay of germination. Toxins of ten strains, except *A. rolfsii* (B-442) all of them *Thanatephorus anamorphs*, killed the seeds before seed coat rupture. The introduction of *gstf*4 gene (A-193) counteracted to this effect in three cases (**Table 2**), that means the overexpression of GST increased the vitality of *Arabidopsis*. In some cases the germination started after 24 - 72 hours of lag phase (**Table 1**). In this case, the overexpression of GST unlocked the inhibitory effect as well. The lag phase of germination poorly correlated with lethal effect (Canonic $R^2 = 0.389$, $\chi^2 = 13.75$, $p = 0.13$), that means the retardation of emergence is not strictly connected to response of seedlings to toxic effect in subsequent stages of germination (**Table 3**). The half time of germination (**Table 4**) varied within large limits (13 - 150 hr), nevertheless, this parameter could be calculated surprisingly high precision (the regression could be fitted in $p < 0.05$ in all cases) that underlines again the good quality of seed material as well as indicates the reproducibility of toxin production following the method applied in our experiments.

The presence *gstf*4 gene (A-193) accelerated the germination at about 20 hr as related to parental and vitamin C deficient clones, although, this protective effect varied in *Rhizoctonia* strain dependent manner.

The CCA revealed that the effect on half time of germination correlated slightly better to lethal effect on seedlings than to lag phase (**Table 3**). Plotting the strains as canonic scores (plots are not shown) no grouping was revealed in the case of HT vs LE and HT vs LP. However, two groups was formed on plot LE vs LP (**Table 4**). The major one (Group A, 22 strains) did not show any correlation, but in the other one (Group B, 19 strains) the correlation between lag phase and lethal effect was significant ($R^2 = 0.75$). The difference between this two groups is in the selective effect of their toxins to *Arabidopsis* clones. The parental clone (A-195) exhibited slightly higher sensitivity to B group than to A (57% and 50%, resp.), while the genetically altered A-193 and *vtc*1 clones responded exhibited higher sensitivity to A group (48% and 59%, resp.) than to B group (41% and 54%, resp.).

No significant relationships is between selective toxicity of taxonomic position, source and origin of strains could be revealed.

The seedlings of A-193 clone differed at higher extent than *vtc*1 one of parental A-195 in tolerance to *Rhizoctonia* toxins, but the response of germinating seeds of *vtc*1 clone altered at higher extent of parental A-195 that those of A-193 (**Table 5**). The Person's coefficient between responses of A-193 and *vtc*1 as evaluated by half time of germination was lower than in scoring of lethal effect (0.553 < 0.873) that indicates changes in sensitivity spectrum of clones during the seed germination.

Table 2. Response of germinating seeds of *Arabidopsis* to toxic metabolites of *Rhizoctonia* strains.

Type of Response	*Arabidopsis* clones		
	A-195	A-193	*vtc*1
Lag Phase[1]			
No	20	26	19
24 hr	7	6	11
>24 hr	14	9	11
Tolerated[2]	3	5	2
Lethal[3]	10	8	11
Inhibition[4]			
Mean	57.7	50.2	59.0
±s	36.9	36.7	36.5

[1]Time requested to outcrop the first seed; [2]Control like germination; [3]All seeds became dark brown up to 6th day of incubation = 100 percent of inhibition; [4]Average inhibition of germination recorded at 6th day.

Table 3. Similarity of response as evaluated by various parameters of germination.

Matrix B (R^2)	Matrix A (χ^2)		
	Half time	Lethal effect	Lag phase
Half time (HT)		32.30	26.89
Lethal effect (LE)	0.670		13.75
Lag phase (LP)	0.610	0.389	

The sub-matrix up to diagonal (A) shows χ^2 values, while the sub-matrix down the diagonal (B) Canonic R^2 of the first root.

4. Discussion

Rhizoctonia species are well known soil borne pathogens, which are habiting mainly in rhizosphere, however, they may survive as saprobionts in the upper layer of the soil

Table 4. **Influence of *Rhizoctonia* metabolites on germination of *Arabidopsis* seeds.**

Rhizoctonia			*Arabidopsis* clones					
	Strains[1]		A-195[2]		A-193		*vtc*1	
No.	Code	Type[3]	Half time ± st[4]	(r)	Half time ± st	(r)	Half time ± st	(r)
1	B-415	A	22.06 ± 4.21	(0.992)	79.87 ± 2.46	(0.992)	32.93 ± 9.74	(0.924)
2	B-432	A	n.g.		n.g.		n.g.[5]	
3	B-446	B	65.00 ± 9.28	(0.878)	61.14 ± 3.79	(0.980)	58.94 ± 5.39	(0.959)
4	B-417	B	92.06 ± 14.54	(0.957)	61.70 ± 12.26	(0.873)	83.49 ± 13.62	(0.947)
5	B-430	B	150.63 ± 20.04	0.971)	105.29 ± 13.37	(0.918)	133.95 ± 21.99	0.971)
6	B-418	B	138.19 ± 25.56	(0.872)	96.94 ± 12.13	(0.964)	n.g.	
7	B-419	B	66.97 ± 4.84	(0.984)	49.11 ± 8.84	(0.893)	55.16 ± 5.62	(0.963)
8	B-420	B	n.g.		142.93 ± 28.81	(0.866)	n.g.	
9	B-421	B	81.15 ± 4.47	(0.983)	71.55 ± 5.91	(0.959)	104.75 ± 14.96	(0.924)
10	B-422	B	105.73 ± 9.24	(0.973)	68.75 ± 15.48	(0.866)	n.g.	
11	B-423	B	80.23 ± 11.97	(0.878)	49.85 ± 10.67	(0.898)	77.15 ± 9.07	(0.924)
12	B-424	A	13.44 ± 17.19	(0.915)	1.64 ± 30.47	(0.832)	26.89 ± 11.63	(0.922)
13	B-434	A	44.10 ± 6.65	(0.943)	28.19 ± 4.01	(0.992)	67.18 ± 11.78	(0.912)
14	B-151	B	114.56 ± 38.93	(0.870)	125.39 ± 49.22	(0.876)	78.36 ± 14.83	(0.922)
15	B-412	B	n.g.		66.28 ± 5.58	(0.960)	90.41 ± 16.41	(0.928)
16	B-246	B	87.87 ± 19.40	(0.914)	20.14 ± 2.09	(0.998)	90.61 ± 11.27	(0.972)
17	B-410	A	n.g.		n.g.		n.g.	
18	B-411	A	85.83 ± 13.55	(0.934)	42.69 ± 9.31	(0.936)	73.83 ± 3.40	(0.994)
19	B-446	A	57.12 ± 5.97	(0.950)	60.95 ± 4.05	(0.987)	52.70 ± 6.86	(0.938)
20	B-403	A	38.59 ± 11.42	(0.958)	8.92 ± 10.37	(0.972)	47.88 ± 16.41	(0.884)
21	B-404	A	25.77 ± 6.77	(0.974)	18.64 ± 21.81	(0.833)	58.16 ± 2.50	(0.994)
22	B-522	B	n.g.		n.g.		n.g.	
23	B-409	A	n.g.		n.g.		n.g.	
24	B-521	A	n.g.		156.89 ± 14.99	(0.974)	138.76 ± 15.06	(0.943)
25	B-399	B	54.80 ± 10.56	(0.866)	76.11 ± 1.63	(0.999)	65.43 ± 11.32	(0.911)
26	B-413	A	35.14 ± 12.29	(0.866)	34.23 ± 2.66	(0.996)	78.02 ± 16.16	(0.907)
27	B-444	A	55.21 ± 3.60	(0.985)	35.08 ± 8.09	(0.964)	47.75 ± 2.09	(0.994)
28	B-245	B	61.99 ± 3.86	(0.992)	112.37 ± 26.92	(0.945)	60.28 ± 0.41	(0.999)
29	B-433	A	n.g.		n.g.		n.g.	
30	B-573	A	38.82 ± 11.12	(0.866)	35.06 ± 1.91	(0.998)	38.53 ± 8.96	(0.915)
31	B-553	A	n.g.		n.g.		n.g.	
32	B-557	B	86.33 ± 7.61	(0.992)	56.80 ± 7.02	(0.960)	86.69 ± 12.10	(0.924)
33	B-441	B	70.95 ± 8.45	(0.914)	62.15 ± 3.33	(0.992)	13.03 ± 3.09	(0.948)
34	B-560	A	70.62 ± 15.30	(0.921)	5.72 ± 5.49	(0.992)	57.85 ± 8.59	(0.923)
35	B-548	A	35.79 ± 7.80	(0.943)	24.70 ± 17.05	(0.871)	38.47 ± 5.60	(0.967)
36	B-405	A	33.26 ± 6.55	(0.965)	22.78 ± 5.78	(0.986)	37.08 ± 2.88	(0.992)
37	B-447	A	25.26 ± 12.28	(0.960)	8.35 ± 1.38	(0.999)	32.73 ± 16.78	(0.905)
38	B-438	B	81.04 ± 6.10	(0.968)	64.97 ± 1.10	(0.999)	81.75 ± 8.51	(0.941)
39	B-427	B	91.18 ± 13.63	(0.958)	42.25 ± 5.01	(0.982)	97.36 ± 27.98	(0.868)
40	B-440	A	46.91 ± 0.77	(0.999)	57.93 ± 4.20	(0.993)	94.96 ± 4.19	(0.997)
41	B-442	A	n.g.		n.g.		n.g.	

[1]The codes are accession numbers of the Mycological Collection of Plant Protection Institute HAS (WDCM824). Strains 1-35 are *Thanatephorus* anamorph: 1 -13 reference strains of CBS, 14-21 isolated of potato, 22-27 isolates of dicot plants, 28-80 isolates of gramineas, 31-35 isolates of orchids; strains 36 *Waitea*, 37 *Ceratobasidium*, 38-39 *Ceratorhiza*, 40-41 *Athelia*; [2]Parental clone; [3]Toxogenic groups; [4]Hours requested for germination of 50% of seeds; [5]No one seed germinated (n.g.) during 10 days of incubation.

Table 5. **Similarity of response of *Arabidopsis* clones to *Rhizoctonia* toxins.**

Matrix B	Matrix A (Susceptibility)		
	A-195	A-193	*vtc*1
A-195	1	0.838	0.850
A-193	0.700	1	0.873
*vtc*1	0.635	0.553	1

The sub-matrix up to diagonal (A) shows similarity (Pearson's coeffients) of clones as evaluated on the base of incidence of diseased individuals (Table 1), while the sub-matrix down the diagonal (B) on the base of delay of germination (Table 3).

forming a mycelial web, thus the undisturbed soil enhance the risk of the infection of young roots. The disease syndrome may evolve rapidly to a fatal consequence in formerly symptomless host (damping off and wilting). Our data support the assumption of crucial role of mycotoxins in evolution of disease syndrome [9,22]. Like to series of host plants [8,10,31] the strain dependent response of *A. thaliana* clones to *Rhizoctonia* was demonstrated in our experiments as well. The strains examined highly diverged in toxic properties. The poor correlation between reaction of *Arabidopsis* seedlings in subsequent steps indicates the qualitative differences in composition of toxic substances released into the medium. Further research requested for both analysis of this complex and elucidation of toxic properties of each component.

The fact of increased tolerance of transgenic clone bearing overexpressed alien GST and increased sensitivity of ascorbic acid deficient clone evidently support the importance of elimination of free radicals in response of plants to pathogen attack.

5. Conclusions

Rhizoctonia strains of various taxonomic position release medium metabolites toxic to *Arabidopsis thaliana*. Neither taxonomic position nor origin of strains was related to inhibitory effect of their toxins.

The genetic manipulation of *A. thaliana* significantly altered the response of germinating seeds to *Rhizoctonia* toxins. Contrary to ascorbic acid deficiency the overexpression of alien glutathione-S-transferase significantly improved the tolerance of seeds.

The improvement of tolerance of transgenic plant to wild range of soil-borne *Rhizoctonia* strains was demonstrated here, illustrating the usefulness of *A. thaliana* as a model for research of biochemical background of resistance to soil-borne pathogens. In our opinion, this approach can significantly accelerate the progress in breeding of wheat tolerant to brown patch disease

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