Varietal dependent response of barley to soil-borne *Waitea circinata* infection

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SUMMARY

The disease syndrome caused by Waitea circinata*, a soil-borne pathogen introduced in the past decade into Carpathian basin, visually indistinguishable of those caused by various* Rhizoctonia *strains in diverse host plant. Dicotyledonaceous species in general proved to be more tolerant to this new pathogen than monotyledonaceous ones. This mesophilic fungus can seriously damage cereals. The barley varieties, similarly to other plants, exhibited highly different individual reaction to soil borne infection, Bivoy being the most while Maresi the less tolerant among the 9 tested varieties. Two groups could be separated on the base of their response to* Rhizoctonia*; Jubilant, Bivoy, Pasadena formed one group being moderately tolerant and Anabell, Scarlett, Rex and Omega the other group of more susceptibles. Three significant factors influence on the virulence of* Rhizoctonia *strains comprised 62% of total variation*.

Keywords: *Rhizoctonia*, *Waitea*, barley, susceptibility, soil-borne pathogens

INTRODUCTION

Traditionally, farmers in temperate zone paid little attention to field damage caused by soil-borne *Rhizoctonia* infection in cereals, because either seed-borne or air-borne fungi (rust, mildew, smut etc.) infecting stem, leaves and spikelets had been the main constrains of yield. Due to success in breading and arise new synthetic fungicides, these fungi presently do not cause catastrophic yield losses. However, in the last two decades increasing number of papers was published on yield losses (30% to 70%) caused by *Rhizoctonia* species in main cereal cultivating areas (Dorofeyeva *et al*., 1996; Oros *et al*., 2013). In South Eastern Hungary damage by the *R. cerealis* and *R. solani* has been observed (Simay, 1998; Kövics and Lőrinc, 2001). In August 2002, brown patches were observed on turf grasses in Budapest (Vajna and Oros, 2005). The causative agent associated to *R. solani* was identified as *Rhizoctonia zeae* Voorhees (teleomorph *Waitea circinata* Warcup and P.H.B. Talbot) and seemingly is a complex of diverse physiological groups (Ogoshi *et al*., 2000). This fungus was first found and described on maize in the USA (Vorhees, 1934), than was found in India (Narayanaswamy and Rao, 1984), Japan (Oniki *et al*., 1985), England (Burton *et al*., 1988), Alaska (Leiner and Carling, 1994), New Zealand (Christensen, 1996), Turkey (Demirci and Eken, 1999), Iran (Aghajani, 2000), Australia (Lanoiselet *et al*., 2001) and Brasilia (Poltronieri *et al*., 2002). *R. zeae* was associated to *R. solani* in other samples as well (Sumner and Bell, 1982), By means of comparative studies on more than 150 potential host plants its host range similar to that of *R. solani* (Vajna and Oros, 2005), although the monocotyledonaceous species proved to be less tolerant than dicotyledonaceous ones, contrary *to R. solani* strains.
Both roots and leaves of barley can be infected by *Rhizoctonia* (Murray, 1982) and coexists as an endophytic fungus frequently without symptoms, however, under unfavorable environmental conditions typical disease syndrome evolves (stunting, wilting, lesions). These typically soil-borne pathogens most frequently cause damping off prevalently in moist and cool conditions that are the main stress factors requested to induce disposition to increased susceptibility of potential host plants (Grogan, 1981). In a comparative study involving 19 wheat varieties, the symptomes caused by 26 *R solani* strains and *R. zeae* were indistinguishable with unarmed eye, and all varieties showed highly variable differences in their individual responses to soil-borne infection in both cases (Oros *et al.*, 2013). Demirci (1998) isolated *R. solani* on barley and wheat in near the same frequency, however, the abundance of *R. zeae* was 2.5 times more in barley samples. Few data are available of the barley/*Waitea* interaction (Leiner and Carling, 1994; Demirci, 1998; Paulitz *et al*., 2003; Al-Abdalall, 2010).
Our objectives of this study to make comparative evaluation of responses of germinating barley seeds to *Rhizoctonia* strains of various origin and taxonomic position as well as to reveal factors influence on barley/*Rhizoctonia* interaction with special regard to the new Carpathian basin pathogen, *R. zeae*.

MATERIALS AND METHODS

Greenhouse experiment was undertaken to compare the infectivity of *Rhizoctonia zeae* strain with seven *R. solani* strains of various origin. Susceptibility of nine barley varieties and fifteen other monocot species were involved in the tests (*Table 1*). No seed-dressing or any other manners were applied to avoid repression of microbiota in spermosphere. The potting medium was made by mixing forest soil with peat before autoclaving (1.15 atm per 20 mins), at the ratio of 3:1.

Test Plants

Seeds of barley varieties (*Table 1*) were supplied by Dr. A. Tomcsányi (Martonvásár, Hungary), except a local one with unknown genetic background. *Triticum monococcum* L. cv. Alcor, *T. turgidum* L. cv. Hegyes were supplied by Elitmag Ltd. (Martonvásár, Hungary), while *T. aestivum* L. cv. Alcedo was of own propagation. *Zea mays* L. *saccharata* cv. Beregi szürke is a local collected variety (Bereg county, East Hungary), and all other seeds were purchased on the market (HERMES Ltd., Budapest, Hungary).

Test Fungi

The origin of *Rhizoctonia* strains were from different locations and various hosts. *R. zeae* B-405 (mixed grass of *Festuca* and *Lolium*, Hungary). *R. solani* strains were isolated in Hungary: B-321 (*Solanum tuberosum* cv. Ella), B-409 (*Hibiscus rosa-chinensis* L., imported from Tripoli, Lybia), B-410 (*S. tuberosum* cv. Kisvárdai rózsa), B-411 (*S. tuberosum* cv. Desirée), B-412 (*S. tuberosum* cv. Cleopatra), B-413 (*Malus domestica* L.) and B-433 (*Festuca arundinacea* Schreb.). The strains were maintained on potato dextrose agar (Merck, Darmstadt, Germany) amended with 2 g soya peptone L44 (Oxoid, Basingstoke, UK).

Test for Pathogenicity

The sterile potting medium prepared as above was admixed with chickpea seeds previously infested with the pathogen (10 seeds per 250 g pot), than incubated 96 hours at 26-28 ºC to evolve mycelia. The seeds were put on the surface of infested soil (1×1 cm), than covered with 5 mm layer of sterile soil. Sterile distilled water was used to moist the surface (15 mL per pot) and covered with plastic wrap layer to avoid desiccation. The control plants were grown up in *Rhizoctonia*-free soil.

When the coleoptiles of control plants had been fully developed (8 days after emergence of first germling) the pathological status of all seedlings was evaluated following four fold scale to assess the tolerance of test plants at the 8th days: 0 = none of seedlings had no visible symptoms by the naked eye; 1 = most of seedlings were similar to control, but as minimum as one diseased; 2 = the majority of seedlings was dead, but at least one survivor was presented either symptomless or bearing severe symptoms (the coleoptyle and the roots damaged, the root neck scoring); 3 = all seedlings were destroyed. The results of observations were compiled into data matrix ((9 barley varieties + 15 reference plants) × [1+7] *Rhizoctonia* strains). The method was discussed in detail previously (Oros *et al.*, 2013).

Data Analysis

The relationships between host (barley varieties and reference species) and *Rhizoctonia* strains (potential soil-borne pathogens) have been analyzed by multivariate methods: Potency Mapping (PM) technique and Spectral Component Analysis (Lewi, 2005) combined with Principal Component Analysis (PCA), following a previously described scheme (Magyar and Oros, 2012). In the latter case only the components having an eigenvalue greater than one were included in the evaluation of data to demonstrate potential number of factors remarkably influencing on host-parasite system. The similarity in host spectra of strains was evaluated by Canonic Correlation Analysis (CanCor). Box plot analysis was applied to demonstrate selective differences both in tolerance of test plants and and virulence of *Rhizoctonia* strains.

Statistical functions of Microsoft Office Excel 2003 (Microsoft, Redmondton, USA) and Statistica5 program (StatSoft 5.0., Tusla, USA) were used for analysis of data. The graphical presentation of result of data analysis was edited uniformly in MS Office Power Point 2003.

Table 1

Susceptibility of barley varieties and reference species to soil borne *Rhizoctonia* infection

Test plants	*Rhiz.*	*Rhizoctonia solani*							
	zeae	*Festuc*	*Malus*	*Hibisc*	Potato strains				PS[a]
	B-405	B-433	B-413	B-409	B-151	B-412	B-410	B321	
1. *Hordeum vulgare* L.[b]	0	0	0	2	2	2	0	0	0.9
2. cv. Anabell	2	0	1	2	2	1	1	1	1.1
3. cv. Bivoy	2	0	0	2	2	0	1	1	0.9
4. cv. Jubilant	2	0	0	3	1	0	0	0	0.6
5. cv. Maresi	2	0	0	2	2	0	0	0	0.6
6. cv. Pasadena	2	0	0	2	1	0	1	1	0.7
7. cv. Scarlett	1	0	0	2	1	1	0	0	0.6
8. cv. Rex	2	1	1	2	1	0	0	0	0.7
9. cv. Omega	1	1	0	2	2	0	0	0	0.7
10. *Triticum durum* Desf.	1	1	1	3	2	0	1	0	1.1
11. *T. spelta* L.	0	0	0	1	0	0	0	0	0.1
12. *T. aestivum* L.	2	0	0	3	2	2	0	1	1.1
13. *Festuca arundinacea* Schreb.	2	1	1	1	1	0	1	0	0.7
14. *Festuca rubra* L.	1	0	0	0	1	0	1	0	0.3
15. *Festuca* sp.	0	0	0	3	1	0	0	0	0.6
16. *Zea mays* L. *cornata*	0	0	0	2	1	1	0	0	0.6
17. *Z. mays* L. *everta*	0	0	0	2	1	1	0	0	0.6
18. *Z. mays* L. *saccharata*	1	3	3	3	0	1	0	0	1.4
19. *Allium cepa* L. cv. Owa	3	2	2	3	3	1	3	1	1.1
20. *A. cepa* L. cv. Makói bronz	1	0	1	3	3	1	0	0	2.1
21. *A. cepa* L. cv. Makói bronz	3	3	3	3	2	2	3	3	2.7
22. *Allium sativus* L.	1	1	1	2	2	1	2	2	1.6
23. *Allium schoenoprasum* L.	1	0	0	0	3	0	0	0	0.4
24. *Allium tuberosum* Rottler	3	3	3	3	3	3	3	3	3.0
PV[b] barley	1.6	0.2	0.2	2.1	1.6	0.4	0.3	0.3	0.7
PV *Triticum*	1.0	0.3	0.3	2.3	1.3	0.7	0.3	0.3	0.8
V *Festuca*	1.0	0.3	0.3	1.3	1.0	0.0	0.7	0.0	0.5
PV *Zea*	0.3	1.0	1.0	2.3	0.7	1.0	0.0	0.0	0.9
PV *Allium*	2.0	1.5	1.7	2.3	2.7	1.3	1.8	1.5	1.8

[a]= Potential susceptibility of plant to *R. solani* strains, [b]= local variety, [c]= Potential aggressivity of strains to test plants. Border limits of the scale of evaluation: 0= no damage, 1=as minimum as one plant injured, 2= as minimum as one plant survivied, 3= all plants destroyed.

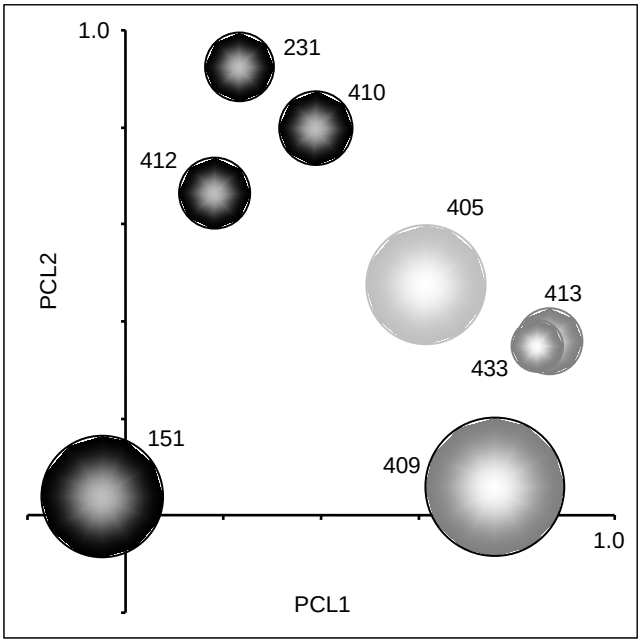
RESULTS

Responses of host/pathogen pairs

The susceptibility of test plants varied within large limits (*Table 1*), and low correlation was revealed between responses to *R. zeae* and *R. solani* strains ($r_{wc,tc}$=0.759, p=0.028) by means of multiple correlation. *Festuca rubra*, *Triticum turgidum* and a local variety of barley tolerated well both *Waitea* and *Thanatephorus* strains, while *Allium tuberosum* proved to be the most susceptible in both host/parasite systems. In general, barley varieties exhibited medium tolerance to *Rhizoctonia*s with marked selectivity to strains. No differences were observed in symptoms caused in various host/parasite pairs, although there were great alterations in individual responses of seedlings. Stunted growth was the most frequent symptom. The leaf spots occurred in the case of each test plant independently on the longitudinal growth of coleoptiles randomly. No wilted plant was found without root neck decay. Most of *A. tuberosum* seeds were destroyed during the germination, and none of them developed coleoptiles longer than 5 mm before dumping off.

Figure 1: **Scatter plot of strains as PC loadings by two major Principal Components of Spectral Map**

Spectral scores		Spectral Components		
No.	Strains	PCL1	PCL2	PCL3
1.	B405	0.603	0.475	0.061
2.	B433	0.838	0.348	-0.208
3.	B413	0.862	0.357	-0.210
4.	B409	0.741	0.057	0.371
5.	B151	-0.060	0.039	0.891
6.	B412	0.176	0.664	0.378
7.	B410	0.383	0.797	-0.158
8.	B231	0.226	0.924	0.008
	Eigenvalues	2.59	2.41	1.19
	Prop. Total (%)	32.4	30.1	14.9



The accession numbers of strains were underlined PC loadings are significantly influencing the component concerned. The distribution of variables on the plot is determined by 62% of total variation. The grey and black spheres are proportional to overall potential virulence and mark clusters *A* and *B* of *R. solani* strains, respectively.

The strains isolated from *Hibiscus* twig and potato tubers (cv. Desirée) pairing with *R. zeae* were significantly more virulent than the other *R. solani* strains (*Table 1*), but their host spectrum showed low similarity ($r_{rz,hib}$=0.34, $r_{rz,des}$=0.14, $r_{des,hib}$=0.09 < r0.1=0.36). Interestingly, only one of *Thanatephorus* strains (B151) harmed *A. schoenoprasum* and *Waitea* caused significant stunting only.

Factors influence on virulence of *Rhizoctonia*s and plant responses

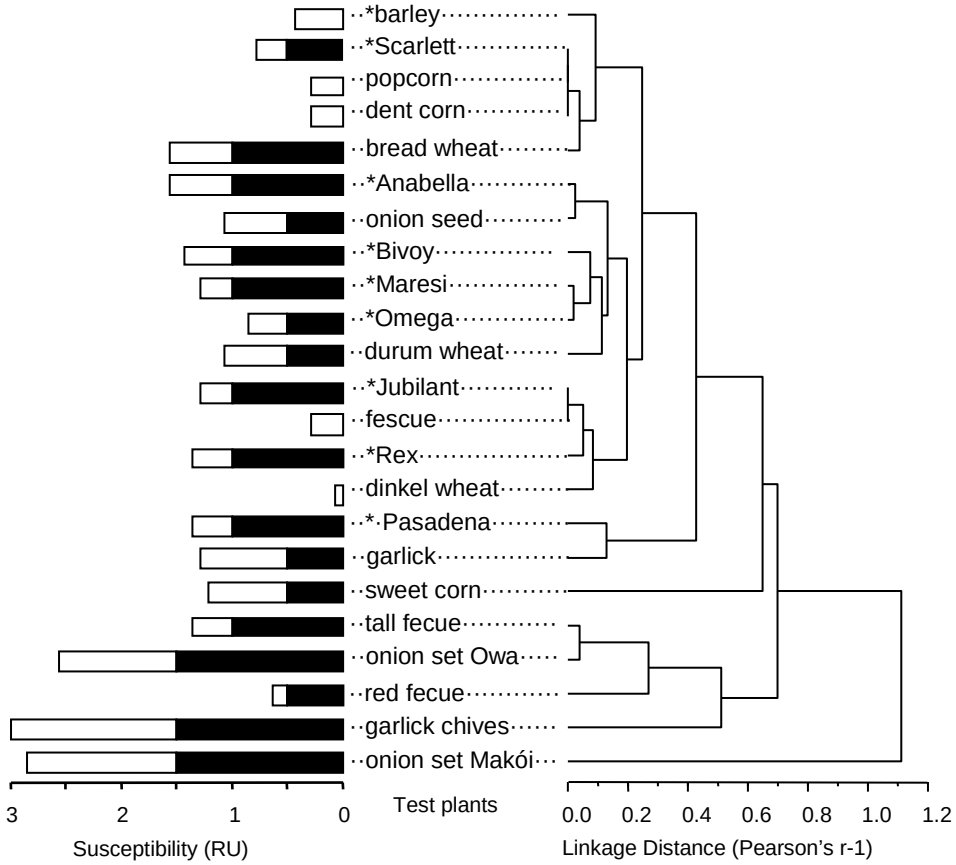
The strength of virulence of *Rhizoctonia* strains was separated by Potency Mapping and their host selectivity analyzed by multivariate techniques (PCA and CanCor). Neither the overall infective potential (Table 1) nor the host range of *Rhizoctonia* strains were related to their origin (*Figure 1*). Three components comprised 77% of total variance of the Spectral Map, where the potato strain (B151) significantly deviated of others that strains clustered into two groups (*A* and *B*) as it was demonstrated on scatterplot (*Figure 2*). The similarity of host spectrum of *R. zeae* to these groups was significantly different being $R_{405,A}$=0.54 > $R_{405,B}$=0.70. The origin of strains seemingly did not take role in their host spectrum that was for example, similar ($r_{433,413}$=0.94) of strains originated of *Festuca* roots and apple cambium.

Factors influencing on plant responses to soil-borne *Rhizoctonia* infection

The potential susceptibility of test plant was separated by Potency Mapping. The strength of response to *Rhizoctonia*s varied within large limits (*Figure 2*), and there was not revealed relationship between taxonomic position and overall potential susceptibility. Although some plants (1, 11, 15, 16, 17) proved to be tolerant to *R. zeae*, all others but garlic and sweet corn exhibited higher potential susceptibility to this new pathogen than to *R. solani* strains (*Figure 2*).

The relationship between test plants and their host spectra was evaluated applying Cluster Analysis (unweighted pair group averages) based on Pearson's correlation matrix of Spectral Map (*Figure 2*). The plants formed five groups with two outliers (18, 20), where barley varieties distributed within four clusters. Seemingly, both response of host and virulence of pathogens are influenced by complex interaction of diverse factors.

Figure 2: **Potential susceptibility of monocotyledonaceous species to soil borne *Rhizoctonia* infection and their relationships in host specificity**



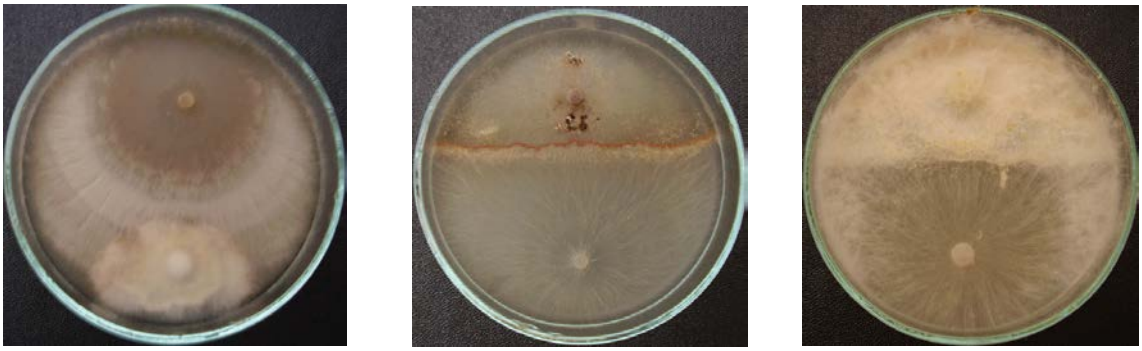
The bold and opened prism on the left graph are proportional to potential susceptibility to soil-borne *Waitea circinata* and *Thanatephorus cucumeris* strains. The clusterogram on the right side was calculated of Spectral Map derived of *Table 1* according to Lewi (1976). The asterisk (*) labelled plants are barley varieties.

DISCUSSION

The gene center of barley was most probably in Levant (Gyulai, 2004), and nowadays the most diverse group of *Rhizoctonia*s was found in this area: strains of five anastomosis groups of *Thanatephorus cucumeris* and two pathotypes of *W. circinata* were isolated of barley in East Turkey (Demirci, 1998). These two fungi occurred frequently together, and different types are infecting in consortium (Roget *et al*., 1996; Yamauchi *et al*., 2002). Nevertheless, their ecological requirements seem to be different, as *R. zeae* was not affected tillage methods contrary to *R. solani* (Schroeder and Paulitz, 2008). The virulence of new for Carpathian basin soil-borne pathogen, *W. circinata* was demonstrated in this study to be almost the most virulent *R. solani* strains tested on barley varieties. Similar results were found with set of strains used in this study with okra (Bittsánszky *et al*., 2012), ricinus (Bittsánszky *et al*., 2015) and wheat varieties (Oros *et al*., 2013). *R. solani* strains divided into two groups having different host range within monocot plants, and the host range of *R. zeae* strain studied showed significantly similar pattern to one of them.

The elucidation of physiological background of host/*Rhizoctonia* needs further studies as well as use of experimental models mimicking the field conditions. More attention should be also paid to interaction among associated *Rhizoctonia* strains residing in the field (Yamauchi *et al.,* 2002). The *R. zeae* (B405) strain antagonized the associated *R. solani* strain (B433) in brown patches of *Festuca*/*Lolium* turf (*Figure 3*), and it was found in microscopic studies to parasite some other strains as well. However, this parasitic action was strain specific, and there was not revealed relationship between virulence of *R. solani* strains and their susceptibility to *R. zeae*. The strain specific toxin production may take place in determination of virulence and antagonism (Oros *et al*., 2014), where the acceptor's reaction might be specific as well.

Figure 3: **Strain specific interaction between *R. zeae* and *R. solani***



R. zeae vs B151 | *R. zeae vs* B433 | *R. zeae vs* B409

Five days old cultures are shown on Potato Dextrose Agar

Some efforts have been done to utilize the mycoparasitic property of *W. circinata* in control of several common root diseases (Webb *et al*., 2015). This initiative underlines the importance of the use of more complex experimental models, as testing only one cultivar of plant to be protected does not result supportive data for application of any preparation in large scale, due to highly varietal selective response of cultivated plants to *W. circinata* infection as well as strain-selective interaction between fungi, thus there is no surety to positive outcome of the application of *W. circinata* based mycopreparate.

CONCLUSIONS

No relationship was found between taxonomic position and origin of *Rhizoctonia* strains, indicating that traits used for their classification are not closely related to the expression of their pathogenicity against barley cultivars and other test plants.

Three factors were revealed that significantly affect the host range and virulence of strains in barley/*Rhizoctonia* system, and only limited overlapping was revealed between *R. zeae* and *R. solani* strains.

We have got empirical evidence from plant/pathogen system on the possibility of selection; the barley phenotype resistant to *R. zeae* and one pathotype of *R. solani*.

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