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Evaluation of the virulence of *Sclerotium rolfsii* isolates on *Arachis hypogaea* and screening for resistant genotypes in greenhouse conditions

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Summary *Sclerotium rolfsii* is a soil borne pathogen responsible for root and stem rot on a wide range of crops. This study was conducted to identify the virulence of different *S. rolfsii* isolates on a susceptible local peanut germplasm and determine the resistance of 20 peanut genotypes to the most virulent isolate and also the relationship between virulence and mycelial compatibility groups (MCGs). Seventy eight isolates of this fungus from 10 host plants and six known MCGs were used in the experiment. The experiment was done in greenhouse conditions (25±5°C) using a complete randomized block design with three replications. Pots containing sterile soil (pH=6.7) were inoculated with barley seeds colonized by each isolate separately before being seeded with the peanut germplasm. Disease severity was assessed by scoring the wilting, yellowing or death of plants, mycelia or sclerotia production on the soil surface or on plant stem, stem area affected (%) and stem lesion length, at the stage of plant maturity. Also, shoot wet weight and plant height were recorded at this stage. According to the results of the pathogenicity tests, all of the isolates were virulent on the susceptible peanut germplasm and the virulence differed significantly between the isolates ($P \leq 0.01$). There was no relationship between the virulence of the five groups of isolates identified in the present study and the MCGs. The peanut genotype 140, which was better than the others based on seed size, plant height and the canopy size, was also the most resistant one.

Additional keywords: diversity, groundnut, pathogenicity, southern blight, stem rot

Introduction

Sclerotium rolfsii Sacc. (teleomorph: *Athelia rolfsii* (Curzi) Tu & Kimbrough) is one of those soil borne plant pathogenic fungi that are prevalent in warm temperate and subtropical regions of the world (Punja *et al*., 1984). This pathogen has a host range of over 500 plant species mostly of dicotyledonous plants. A wide range of symptoms are produced by this pathogen on its hosts including crown and root rot, stem canker and damping-off and resulting diseases called southern wilt, blight or stem rot (Punja, 1985). The pathogen is of great importance especially when the disease severity is high in the fields. The crop loss may be between 10-25% or even more than 81% in some fields (Mehan *et al*., 1995).

Groundnut or peanut (*Arachis hypogaea* L.) is an annual legume crop cultivated in more than 80 countries in the tropics, subtropics and warm temperate zones (Hammons, 1994). It is a major source of edible oil, vitamins and amino acids and is used extensively for feed and food (Savage and Keenan, 1994). Groundnut is also a main crop in Guilan province of Iran with about 3500 hectares cultivation area.

Southern blight, stem rot or white mould, caused by *S. rolfsii*, is one of the most important diseases of peanut. The disease appears in peanut growing areas and causes great yield losses when climatic conditions, such as soil temperature and humidity, are favorable for fungal development and the dis-

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ease incidence is high (Kolte, 1984; Le, 2004; Nguyen, 2004).

Sclerotium rolfsii overwinters as mycelium or sclerotia in infected plant tissues and soil. Under favorable conditions, hyphae or germinating sclerotia infect the plant and subsequently colonize and invade the root and stem tissue with typical silky white mycelium (Brewster, 2001). Infected plants become yellow and then wilt, the collar root turns brown and rots. In groundnut, *S. rolfsii* also infects the pegs and pods, leading to yield losses.

Sclerotium rolfsii is difficult to control by physical and cultural practices due to its wide host range of over 500 plant species (Aycock, 1966; Punja, 1985) and persistent sclerotia (Lakpale, 2007; Punja, 1985). To successfully implement management practices (e.g., chemical and biological) to control *S. rolfsii*, knowledge of the distribution and diversity especially in pathogenicity and virulence of the pathogen is essential.

Branch and Brenneman (1999) evaluated the resistance of mass-selected populations derived from combinations of crosses among two resistant and two susceptible peanut cultivars. Fery and Dukes sr. (2002) determined the cowpea resistance to *S. rolfsii*. There was significant variability in cowpea germplasm for resistance to southern blight. In another study (Flores-Moctezuma *et al*., 2006), two onion isolates of *S. rolfsii* were inoculated to 51 plant species and disease severity levels were determined. Subsequently, 12 out of 51 plant species were selected for the determination of pathogenic reaction to 20 isolates of *S. rolfsii* from different regions. Onion isolates produced variable levels of disease severity for half of the plants tested. Five plant species were susceptible or highly susceptible to all isolates.

Eleven sugar beet genotypes were evaluated at National Agricultural Research Center, Islamabad, Pakistan, during the year 2009 for their resistance against root rot caused by *S. rolfsii* (Farooq *et al*., 2011). Inoculation of eleven genotypes with *S. rolfsii* exhibited resistant response only in SD-PAK-09/07 and moderate resistance in SD-PAK-07/071.

The results of a recent study showed that *S. rolfsii* isolates originating from groundnut, tomato and taro were all pathogenic on groundnut, but displayed substantial diversity of various genetic and phenotypic traits, including mycelial compatibility, growth rate, and sclerotial characteristics (Le *et al*., 2012).

The aim of this study was to identify the virulence of different *S. rolfsii* isolates on a susceptible local peanut germplasm and determine the resistance of twenty peanut genotypes to the most virulent isolate and also the relationship between virulence and mycelial compatibility groups (MCGs).

Materials and Methods

Isolates virulence determination

Seventy eight isolates of *S. rolfsii* from ten different hosts in Guilan province with known MCGs (Mehri *et al*., 2013) were applied for inoculation of a local susceptible peanut germplasm in greenhouse conditions (Table 1).

Barley seeds were boiled in distilled water for twenty minutes and then 12 gr of seeds were added to each 100 ml Erlenmeyer flask and autoclaved twice at 121°C and 1.5 atmospheres for thirty minutes. For each isolate a 5 mm disk of growing fungus on PDA medium was transferred to the Erlenmeyer flask containing sterilized barley seeds and the cultures were maintained in the growth chamber (27±1°C) (Sennoi *et al*., 2010).

The applied soil (1:1:2 clay, compost, sand, pH=6.7) was autoclaved at 121°C and 1.5 atmospheres for thirty minutes and added to the pots with 500 gr soil capacity. Seeds of a local susceptible peanut germplasm were sterilized with Sodium Hypochlorite 1% solution for three minutes and rinsed with sterilized distilled water three times, then soaked in sterilized distilled water. The peanut seeds were placed in a moist chamber at 25±5°C for 72 h to germinate.

When the mycelium covered all the barley seeds and enough sclerotia were formed, each pot was inoculated with thirty infected barley seeds and the seeds were covered

Table 1. *Sclerotium rolfsii* isolates from different MCGs and host plants.

MCGs	pepper	tomato	squash	bean	sunflower	eggplant	groundnut	*Amaranthus* sp.	*Euphorbia* sp.	cowpea	Total for all hosts
MCG1	13	0	0	13	2	0	5	2	0	1	36
MCG2	1	0	0	1	0	0	0	0	0	0	2
MCG3	3	5	1	3	0	2	15	0	2	1	32
MCG4	0	0	0	3	0	1	0	0	0	0	4
MCG5	2	0	0	1	0	0	0	0	0	0	3
MCG6	0	0	0	1	0	0	0	0	0	0	1
Total for all MCGs	19	5	1	22	2	3	20	2	2	2	78

with a thin soil layer. The experiment was done as a complete randomized block design with three replications. There were two controls for each treatment including pots inoculated with thirty sterile barley seeds. After establishment of the fungus in soil, the germinated peanut seeds were cultured into pots (one seed per pot) (Toribio *et al*., 1992). The pots were maintained in greenhouse conditions at 25±5°C (Erkilic *et al.,* 2006). The pots were irrigated based on seedlings need for prevention of water stress (Flores-Moctezuma *et al*., 2006; Sennoi *et al*., 2010).

Disease symptoms were monitored daily from one week after peanut seeding, when the symptoms were observed. At the plants maturity (about 6 weeks after seeding), all the plants were uprooted at the same time and the roots were washed in running tap water to remove soil particles (Yaqub and Shahzad, 2005).

Disease severity was assessed by scoring the plant wilting, yellowing or death, mycelia or sclerotia production on the soil surface or on plant stem, stem area affected (%) and lesion length. Disease severity index was calculated for each treatment using these scores according to the Townsend-Heuberger formula (Erkilic *et al.,* 2006) as bellow:

$$DS(\%) = \frac{\sum(n \times v)}{N \times V} \times 100$$

Where,
n: degree of infection according to the scale (Le *et al*., 2012),
v: number of seedlings per category,
N: total number of seedlings were screened and
V: highest degree of infection.
Also, shoot wet weight and plant height were recorded at this stage. The data were analyzed by the one-way ANOVA followed by Tukey's multiple range test for mean comparison using SAS v. 9.0 software.

Peanut genotypes resistance evaluation

One of the most virulent isolates of *S. rolfsii* on the tested germplasm was used for evaluation of the twenty peanut genotypes resistance in greenhouse conditions as completely randomized design. The germinated peanut seeds and the infected barley seeds were prepared in the same way as mentioned in isolates virulence determination experiment. The germinated peanut seeds were cultured into pots and two weeks after seeding, the seedlings were inoculated with three infected barley seeds (Sennoi *et al*., 2010). Each cultivar was also inoculated by three sterile barley seeds as control treatment. The genotype resistance was evaluated one month after seeding, based on the scale and characteristics mentioned before (Le *et al*., 2012).

Results

S. rolfsii isolates covered the barley seeds 2-3 weeks after inoculation as white mycelia. In the isolates virulence determination experiment, two weeks after seeding of peanut germplasm, disease symptoms were observed on peanut seedling stems as water soaked spots which turned to rot soon (Figure 1). These spots resulted in wilting and death of plants during their maturation. The fungus mycelia extended around the stems and on the soil surface. Sclerotia were also observed on these mycelia.

All of the isolates were virulent on tested peanut germplasm and the virulence was significantly different within the isolates at $p \leq 0.01$ (Figures 2 and 3). There were significant differences between the shoot wet weight and plant height in the treatments ($p \leq 0.01$). The plants which were inoculated with isolates 8 and 73 were the highest (33.5 cm) and those inoculated with isolate 64 were the shortest plants (7.33 cm). There was a significant negative correlation between plant height and disease severity index (DSI). The higher was the DSI, the shorter was the plant height (Table 2).

Stem and root rot resulting from this disease reduced the shoot wet weight. There was a significant negative correlation between shoot wet weight and DSI. The higher was the DSI, the less was the shoot weight.

Variance analysis of the data related to stem lesion length showed significant difference between the isolates ($p \leq 0.01$). Isolate 73 caused the shortest lesion (2.35 mm) and isolate 53 caused the longest one (100.56 mm).

Figure 1. Stem lesion caused by a virulent isolate of *Sclerotium rolfsii* on a susceptible local peanut germplasm. The sclerotia produced on the infected stem and on the soil surface.

Stem area affected (%) shows the proportion of the lesion width to the healthy stem circumference. For the isolates 1, 6, 38, 42, 44, 47, 53, 57, 64, 69 and 70 this proportion was 100%. The least amount was for the isolates 8 (15%).

Disease symptoms occurrence in an infected plant can be compared with a healthy control plant and this criterion can be expressed as percent in an overview. Thus, the symptoms like wilting, yellowing of the entire leaves or only the lower leaves, occurrence of the lesions and crown infection were evaluated. This criterion was 100% for isolates 1, 6, 38, 42, 44, 47, 53, 57, 64, 69 and 70 and 16.66% for isolate 8 (Figure 3).

Based on the correlation analysis results, there was a significant correlation within all measured criteria at $p \leq 0.01$ (Table 2). There was a significant positive correlation between percent of symptoms occurrence, DSI, stem lesion length and stem area affected (%), but a negative correlation between these mentioned criteria and plant height and shoot wet weight. The greater was the amount of lesion length or stem area affected, the higher were the symptoms occurrence and DSI, the less the plant height and wet weight and therefore the more virulent the tested isolate. When the plants had been infected by the fungus especially in their seedling stage, the seedlings lost their normal growth and the final plant height decreased compared to uninfected control plants. The isolates which were more virulent also decreased the peanut seedlings emergence rate more.

The 78 tested isolates in this study were significantly different in their virulence on the tested peanut germplasm and were divided to five groups based on their virulence ($P \leq 0.01$). The isolates 38, 6, 1, 42, 44, 47, 53, 57, 64, 69 and 70 were the most virulent and the isolates 8 and 73 were the least virulent ones. Considering the calculated DSI for each isolate, the tested peanut germplasm reaction to these isolates was expressed as

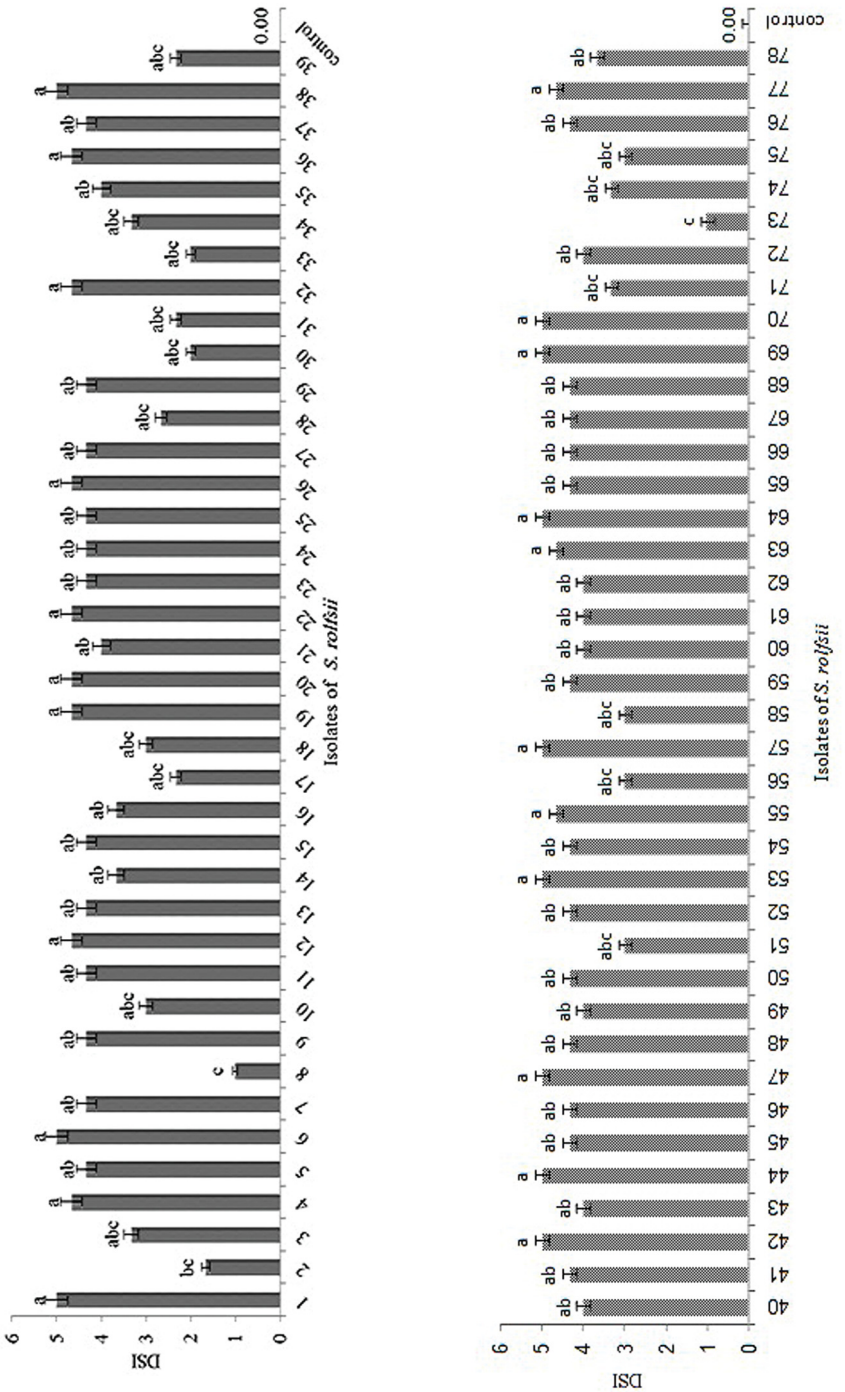

Figure 2. Disease severity index (DSI) for the peanut tested germplasm when inoculated with different isolates of *Sclerotium rolfsii*.

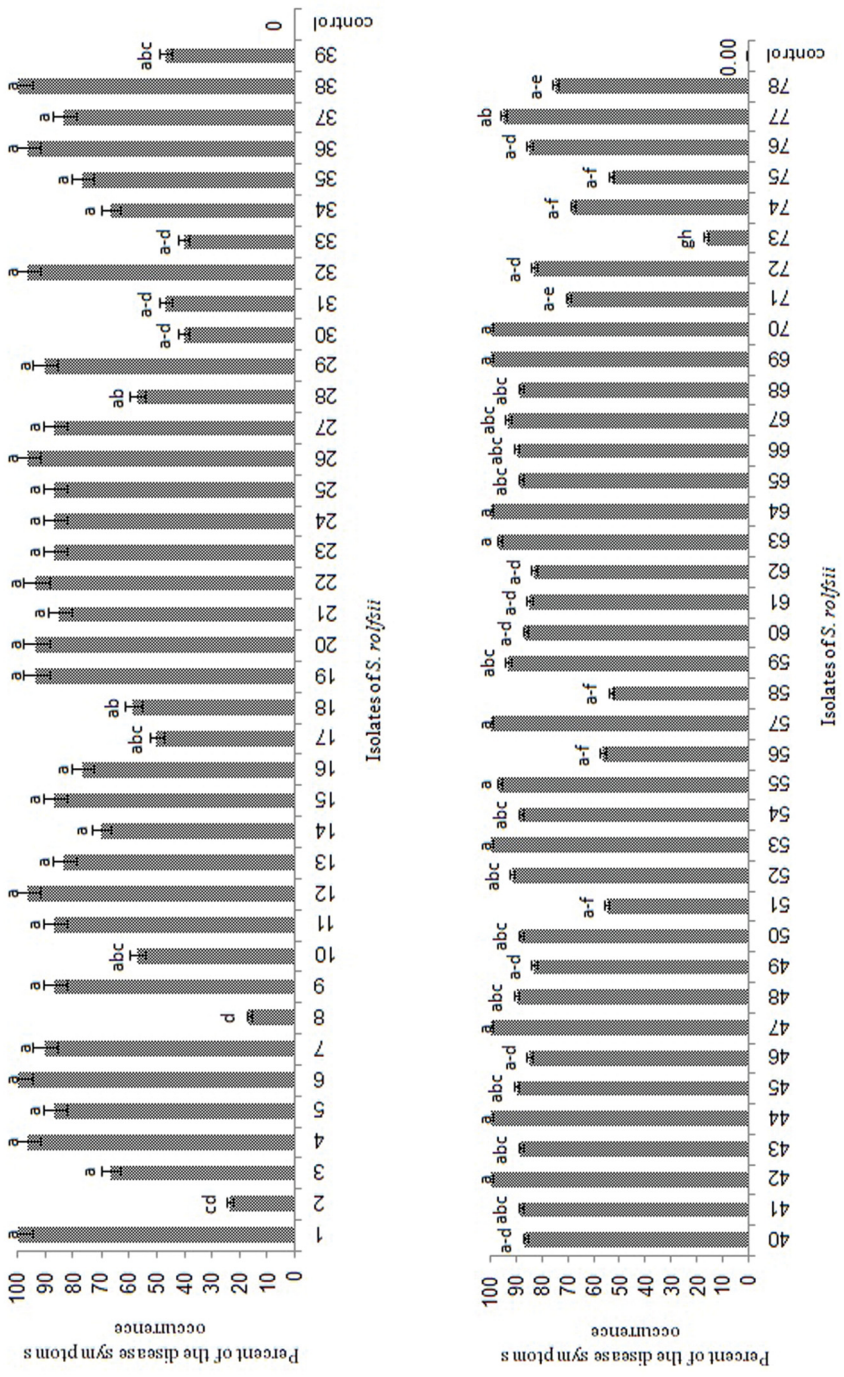

Figure 3. Percent of the disease symptoms occurrence in the peanut tested germplasm compared to the control plant when inoculated with different isolates of *Sclerotium rolfsii*.

Table 2. Correlation between the measured criteria in the *Sclerotium rolfsii* isolates virulence evaluation test.

		Lesion length	Stem area affected (%)	Symptom occurrence (%)	DSI	Plant height	Shoot wet weight
Lesion length	Pearson Correlation	1					
	N	78					
Stem area affected (%)	Pearson Correlation	0.810**	1				
	N	78	78				
Symptom occurrence (%)	Pearson Correlation	0.815**	0.981**	1			
	N	78	78	78			
DSI	Pearson Correlation	0.831**	0.989**	0.990**	1		
	N	78	78	78	78		
Plant height	Pearson Correlation	-0.730**	-0.896**	-0.894**	-0.906**	1	
	N	78	78	78	78	78	
Shoot wet weight	Pearson Correlation	-0.299**	-0.404**	-0.371**	-0.396**	0.349**	1
	N	78	78	78	78	78	78

R= resistant with scores of 0-2, MR= moderately resistant with scores 2.1-3, S= susceptible with scores 3.1-4.99 or HS= highly susceptible with score 5 (Farooq *et al*., 2011; Flores-Moctezuma *et al*., 2006). The tested peanut germplasm was resistant, moderately resistant, susceptible and highly susceptible to 6.41, 12.82, 66.67 and 14.1 percent of tested isolates, respectively.

The five identified groups in the tested isolates based on the virulence, overlapped to some extent with six MCGs identified for these isolates before (Mehri *et al*., 2013). For example, MCG4 only included 4 isolates and the tested peanut germplasm was susceptible to all of them. The MCG5 only had three members which caused susceptible or highly susceptible reaction on the tested germplasm. This overlap has not been observed for all MCGs and some of them included the isolates with different virulence.

Different peanut genotypes resistance to one of the most virulent isolates of *S. rolfsii* (isolate 53) was determined based on the scores and criteria mentioned above for isolates virulence evaluation. Variance analysis of the data showed that the genotypes were significantly different in their resistance to *S. rolfsii*. Considering the calculated DSI for each genotype (Figure 4), its reaction to the tested isolate was expressed as R= resistant with scores of 0-2, MR= moderately resistant with scores 2.1-3, S= susceptible with scores 3.1-4.99 or HS= highly susceptible with score 5 (Farooq *et al*., 2011; Flores-Moctezuma *et al*., 2006).

The genotypes 140 and 183 were resistant to the tested isolate. The genotype 129 was moderately resistant and 137, 138, 193 and 208 were highly susceptible. All the other genotypes showed susceptible reaction. The genotypes 140 and 183 had the least amount of stem lesion length, stem area affection and disease symptom occurrence

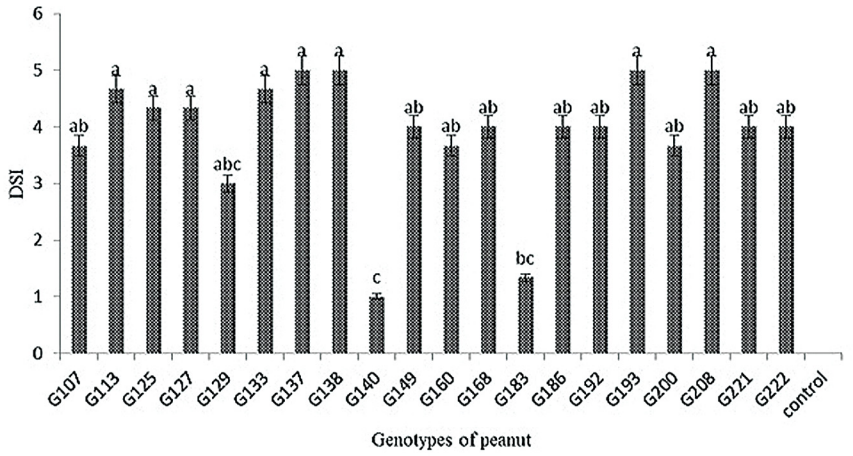

Figure 4. Disease severity index (DSI) for different peanut genotypes when inoculated with one of the most virulent isolates of *Sclerotium rolfsii*.

and the highest amount of height or shoot wet weight. No systemic infection symptoms such as yellowing or wilting were observed in these two genotypes.

Discussion

Based on our research results, the shoot wet weight and plant height decreased in different treatments related to the isolates virulence. Also, the isolates which were more virulent decreased the peanut seedlings emergence rate more. These results on isolates virulence evaluation were compatible with those achieved by Yaqub and Shahzad (2005) who evaluated the *S. rolfsii* isolates virulence on different host plants. Based on their study results, soil infestation with *S. rolfsii* caused a significant reduction in germination of sunflower, mungbean and sugar beet seeds as compared to control. Germination of tomato, sweet pumpkin, cabbage and cauliflower seeds were slightly reduced. The highest reduction in plant length, weight and shoot weight as compared to control was observed in sunflower and mungbean followed by sugar beet, tomato, sweet pumpkin and cabbage. Cauliflower plants showed no effect of *S. rolfsii* infection on plant growth. *S. rolfsii* proved to be highly pathogenic on sunflower, mungbean and sugar beet, mildly pathogenic on tomato, lentil, sweet pumpkin and cabbage, and non-pathogenic on cauliflower plants in pot experiments in their study.

Our results related to isolates virulence and genotypes resistance differences are comparable with those in the study by Flores-Moctezuma *et al*. (2006), in which two onion isolates of *S. rolfsii* from the states of Morelos and Guanajuato, Mexico were inoculated to 51 plant species and disease severity levels were determined. Subsequently, 12 out of 51 plant species were selected for the determination of pathogenic reaction to 20 isolates of *S. rolfsii* from different regions of Mexico. Onion isolates from Morelos and Guanajuato produced variable levels of disease severity for half of the plants tested. Five plant species were susceptible or highly susceptible to all isolates. The remaining plants tested showed differential reactions to individual isolates, ranging from highly resistant to highly susceptible.

As already mentioned, the five identified groups in the tested isolates based on the virulence, overlapped to some extent

with six MCGs identified for these isolates by Mehri *et al*. (2013). Harlton *et al*. (1995) found 49 MCG and 12 RFLP-ITS groups in a worldwide collection of *S. rolfsii* isolates and they did not find a correlation between MCG groups and pathogenicity. In our case, there was also no significant correlation between the isolates virulence and their geographical or host plant origin. In a random selection, not all the identified high virulent isolates had been isolated from peanut and some, which were isolated from peanut, were not virulent on the tested peanut local germplasm. These results were compatible with the results of research conducted by Flores-Moctezuma *et al*. (2006) and Le *et al*. (2012).

Regarding the peanut genotypes resistance evaluation, most of the genotypes were susceptible to the selected most virulent isolate and the resistant reaction was observed only in few genotypes which showed no systemic infection symptoms such as yellowing or wilting. The results were similar to the results reported in other investigations (Farooq *et al*., 2011; Flores-Moctezuma *et al*., 2006; Yaqub and Shahzad, 2005).

In this investigation, disease severity was evaluated using several scoring methods like the plant wilting, yellowing or death, mycelia or sclerotia production on the soil surface or on plant stem, stem area affected (%) and lesion length, shoot wet weight and plant height and also percent of the disease symptom occurrence. We finally concluded that stem area affection is a very useful criterion for the evaluation of isolates virulence or genotype resistance and stem lesion length is of second importance. The two resistant peanut genotypes to *S. rolfsii* identified in our study will be useful for the control of the white rot disease in the peanut fields and the reduction of the losses through the introduction of the genotypes in Guilan province, especially because the genotype 140 is better than the others based on seed size, plant height and the canopy.

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Αξιολόγηση της μολυσματικότητας απομονώσεων του *Sclerotium rolfsii* στην αραχίδα (*Arachis hypogaea*) και διερεύνηση της ανθεκτικότητας γονοτύπων αραχίδας σε συνθήκες θερμοκηπίου

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Ο μύκητας *Sclerotium rolfsii* είναι εδαφογενές παθογόνο που προκαλεί σήψη ριζών και στελεχών σε ένα μεγάλο εύρος καλλιεργειών. Η παρούσα μελέτη πραγματοποιήθηκε με σκοπό τον προσδιορισμό της μολυσματικότητας διάφορων απομονώσεων του *S. rolfsii* σε ευπαθές τοπικό γενετικό υλικό αραχίδας, τον καθορισμό του βαθμού ανθεκτικότητας 20 γονοτύπων αραχίδας στην πιο μολυσματική απομόνωση του παθογόνου καθώς και τη διερεύνηση της σχέσης μεταξύ μολυσματικότητας και ομάδων μυκηλιακής συμβατότητας (Mycelial Compatibility Groups, MCGs). Χρησιμοποιήθηκαν 78 απομονώσεις του μύκητα που προέρχονταν από δέκα διαφορετικά φυτά-ξενιστές και ανήκαν σε έξι γνωστές MCGs. Το πείραμα πραγματοποιήθηκε σε συνθήκες θερμοκηπίου (25 ± 5°C) εφαρμόζοντας σχέδιο τυχαιοποιημένων πλήρων ομάδων με τρεις επαναλήψεις. Γλάστρες που περιείχαν αποστειρωμένο έδαφος (pH=6,7) εμβολιάστηκαν με σπόρους κριθής εποικισμένους με καθεμία από τις απομονώσεις του παθογόνου ξεχωριστά πριν από τη σπορά του τοπικού γενετικού υλικού αραχίδας. Η εκτίμηση της έντασης της ασθένειας έγινε βαθμολογώντας τη μάρανση, χλώρωση ή νέκρωση των φυτών, την ανάπτυξη μυκηλίου ή το σχηματισμό σκληρωτίων στην επιφάνεια του εδάφους ή στο στέλεχος των φυτών, το ποσοστό (%) της προσβεβλημένης επιφάνειας του στελέχους και το μήκος της κηλίδας στη βάση του στελέχους στο στάδιο της ωρίμανσης των φυτών. Επίσης έγιναν μετρήσεις του νωπού βάρους του στελέχους και του ύψους των φυτών. Με βάση τα αποτελέσματα των δοκιμών παθογένειας, όλες οι απομονώσεις ήταν μο-

λυσματικές στο τοπικό γενετικό υλικό αραχίδας και υπήρχαν στατιστικά σημαντικές διαφορές (P≤0,01) μεταξύ των απομονώσεων ως προς το βαθμό μολυσματικότητας. Δε διαπιστώθηκε συσχέτιση μεταξύ των πέντε ομάδων μολυσματικών απομονώσεων του μύκητα που προσδιορίστηκαν στην παρούσα μελέτη και των MCGs. Ο γονότυπος αραχίδας 140, ο οποίος ήταν ο καλύτερος σε σχέση με τους υπόλοιπους με βάση το μέγεθος του σπόρου, το ύψος των φυτών, και το μέγεθος της κόμης ήταν και ο πιο ανθεκτικός στις μολύνσεις του παθογόνου.

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