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Differentiation among *Sclerotium rolfsii* isolates in response to saponins treatment

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Abstract

Genetical and morphological variability among four Egyptian isolates of *Sclerotium rolfsii*, their response to saponin treatment and their ability to control root rot disease in guar plant (*Cyamopsis tetragonoloba*) using saponin seed dressing were investigated. PDA medium amended with saponin reduced significantly mycelial growth and sclerotial formation of *S. rolfsii* isolates. *S. rolfsii* isolates from tomato and/or sesame were more sensitive to saponin than the isolates from guar and/or peanut plants. Saponin application also lowered polygalacturonase activity in cultural filtrate of *S. rolfsii* isolates. In greenhouse experiment, guar seeds pretreated with saponin gave 31.1% to 58.9% protection against root rot disease incidence. RAPD technique was carried out for four isolates of *S. rolfsii* using two random primers. Size of DNA fragments amplified by the two primers ranged from 80 bp to 1116 bp indicating polymorphism among *S. rolfsii* isolates. Genetic similarities ranged from 31% to 71% and from 40% to 92% using primer 5 and primer OPA-3, respectively.

Key words – saponin – RAPD – PCR – polygalacturonase activity

Introduction

Sclerotium rolfsii is a pathogenic soil-borne fungus capable of infecting a wide array of economic plants, e.g., cotton, tomato, potato, soybean, jack bean, eggplant and chickpea and may ruin crops sometimes catastrophically (Garibaldi et al. 2006, Dwivedi & Prasad 2016, Roca et al. 2016, Queiroz et al. 2017). The fungus exists in many soil types, occurs in different geographical locations all over the world and is able to attack plants at various growth stages. Disease symptoms caused by *S. rolfsii* are seedling damping-off, basal rot, dry collar rot, foot rot, stem blight, boll rot and root rot (Parvin et al. 2016, You et al. 2016). Sclerotia formed by the fungus are persistent and can survive for long periods under diverse soil conditions due to their unique structure.

Diversity within *S. rolfsii* isolates based on their morphological characters in terms of mycelium growth habits, sclerotia size, number, colour, formation, dry weight were reviewed by Rasu et al. (2013) and Parvin et al. (2016). Genetic characteristics of *S. rolfsii* isolates using the internal transcribed spacer (ITS), RAPD banding patterns and size of DNA fragments has been reported by many investigators (Ahmed 2010, Pandey et al. 2013, Parvin et al. 2016, You et al.

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2016). Their findings indicated that there was an important degree of genetic differentiation among the tested *S. rolfsii* isolates.

Although integrated disease management of the fungus has achieved some success, Dwivedi & Prasad (2016) found that some bio-agents and heating the soil (solarization), as well as specific plant extracts, gave significant control against *S. rolfsii* pathogen. Moreover, fungicidal application to reduce *S. rolfsii* infection gave limited effects. Among different ways to control plant disease pathogens, saponins have emerged in this respect. Saponins are a constitutive in numerous plants and classified to two groups, steroid or triterpenoid (Fenwick et al. 1992, Hostettmann & Marson 1995). Biological, pharmaceutical, medicinal, and industrial properties of saponins have been extensively reviewed (Levy et al. 1986, Lacai-Dubois 1999). The antifungal properties of saponins against a variety of fungal pathogens has been well demonstrated in previous studies (Leath et al. 1972, Levy et al. 1986, Abdel-Momen et al. 2000, Aly et al. 2000, Omar et al. 2009, Salem et al. 2012). The principal effect of saponin involves reduction in plant disease impact and inhibition in number, weight, or viability of fungal infection units. Moreover, saponins have been implicated in resistance of plants and are considered to be one of the compounds of phytoalexins (Bailey & Mansifield 1982).

The present work aimed to investigate morphological and genetical variability among four *S. rolfsii* isolates collected from tomato, peanut, sesame, and guar infected plants in Egypt. Antifungal activity of saponins as a means of controlling *S. rolfsii* was also examined.

Materials & Methods

Sources of *S. rolfsii* isolates

The tested isolates of *S. rolfsii* were previously isolated from diseased plants of guar (isolate no. 1, *Cyamopsis tetragonoloba*), peanut (isolate no. 2, *Arachis hypogaea*), sesame (isolate no. 3, *Sesamum indicum*) and tomato (isolate no. 4, *Solanum lycopersicum*).

Effect of saponin on linear growth, sclerotial formation and sclerotial germination of *S. rolfsii* isolates

Synthetic saponin was kindly provided by ICN Company USA. Saponin substance was added to potato dextrose agar (PDA) medium to give final concentrations of 1, 3 and 5 g/l, and autoclaved at 121°C for 15 min. The amended PDA was poured into 9 cm diameter sterilized Petri dishes, using three replicates for each concentration of saponin. Plates containing only PDA medium were used as control. All plates were then inoculated with discs (5 mm diameter) of 6-days-old culture of the tested *S. rolfsii* isolates individually and incubated at 25°C for 6 days. Diameter of fungal growth was measured daily and number of sclerotia was counted 8 and 15 days after inoculation.

The effect of different concentrations of saponin (1, 3, 5 g/l in sterile water) on sclerotial germination of *S. rolfsii* isolates was also tested. Sclerotia of the tested isolates were immersed individually in 200 ml beakers containing the different saponin concentrations for 2 h. The sclerotia were then dried between layers of filter papers and then transferred to Petri dishes containing PDA medium and incubated at 25°C for 24 h. The numbers of germinated sclerotia in each treatment were counted. Three replicates were used for each saponin concentration.

Effect of saponin on polygalacturonase activity of *S. rolfsii* isolates

Polygalacturanse produced by necrotrophic pathogens during plant infection causing rapid and degradation of cell wall and cell death. The effect of saponin on polygalacturonase activity (PG) of *S. rolfsii* isolates was studied using Czapek´s liquid medium. The carbon source was replaced by 1.2% pectin, and saponin was added to the medium at the rate of 5 g/l before autoclaving. Flasks (250 ml) containing 100 ml of the modified Czapek's medium were individually inoculated with a 5 mm diameter agar culture disc of the tested isolates. The flasks were incubated at 25°C for 10 days. Three replicates were used for each isolate. Cultures were then filtrated and the filtrates centrifuged at 3000 rpm for 20 minutes. The clear supernatants were

utilized as crude enzyme preparations to estimate the enzyme activity according to Talboys & Bush (1970) as follows.

The relative activity of polygalacturonase was determined by measuring the reduction in viscosity and by determining the reducing groups liberated by enzyme action of the substrate in Ostwaled viscometer. The substrate used for measuring PG was 1.2% pectin in phosphate buffer solution at pH 5.6. Five ml of the filtrate was added to 10 ml of buffered substrate, and incubated at 30°C. The activity of PG was measured after 0 and 30 min. Viscosity was measured and the percentage of loss in viscosity was recorded using the following formula:

$$\frac{T_0 - T_t}{T_0 - T_w} \times 100 \quad \text{where:}$$

T_0 = the time of flow in second of the reacted mixture at zero time.
T_t = the time of flow at a given time interval.
T_w = the time of flow of distilled water.

Effect of guar seed pretreated with saponin on root rot disease incidence caused by *S. rolfsii* under greenhouse conditions

A greenhouse experiment was carried out to determine the effect of guar seed pretreated with saponin on root rot disease incidence caused by *S. rolfsii*. Glass bottles (500 ml) each containing corn meal-sand medium (3:1 w/w) were autoclaved at 121°C for 15 min. The sterilized bottles were then inoculated individually with 5 mm diameter discs of a 6-days-old culture of the tested *S. rolfsii* isolates and incubated at 25°C for 15 days.

The fungal inoculum of each isolate was then individually mixed with sterilized potted soil at the rate of 2.5% (w/w). The infested soils were watered for 7 days to enhance growth and distribution of the fungal inoculum. Pots (30 cm diam.) containing infested soil of each isolate were sown with guar seeds (Local cv.) that had been pre-treated with saponin at the rate of 5 g/l. Three replicates were used for each isolate and five seeds were sown in each pot. Untreated guar seeds were also sown and used as a control. The growing plants were examined periodically and root-rot disease incidence was recorded 55 days after sowing.

Electrophoretic studies

DNA extraction and RAPD technique

S. rolfsii isolates were grown in potato dextrose broth medium for 7 days. The mycelium of each isolate was harvested and subsequently dried on sterilized blotter papers. The mycelium was then wrapped in aluminum foil and kept in a freezer at -20°C until further use. The CTAB extraction procedure was followed as described by Passone et al. (2010) with some modifications. About 100 mg of mycelium was ground in liquid nitrogen until a powder was produced. This was then transferred into a sterile 1.5 ml tube containing 500 µl of extraction buffer (100 mM tris HCl pH8, 2% CTAB, 20 mM EDTA, 1.4 M NaCl, 20 mg polyvinylpyrrolidone, 2.5 µl *β*-mercaptoethanol), followed by vortex for 2 min. After incubation at 65°C for 60 min, 500 µl of chloroform: isoamyl alcohol (24:1, v/v) was added to the sample, mixing well and centrifuged for 5 min at 14,000 rpm. Four hundred microliters of the aqueous phase was then transferred into a new 1.5 ml tube containing 32 µl of 7.5 M NaOAc and 233 µl of isopropanol. The samples were homogenized and centrifuged again for 5 min at 14,000 rpm. The aqueous phase was discarded and 500 µl of 70 % ethanol was added. The samples were then centrifuged for 5 min at 14,000 rpm. The aqueous phase was discarded and the DNA pellet was dried at room temperature. The dried pellet was suspended in 20 µl autoclaved distilled H_2O.

Thirty ng of the extracted DNA and ten picomole of the primer were used for amplification reaction using Ready Mix PCR Reaction (Sigma). PCR amplification was performed in a

thermocycler (Biometra Thermocycler T-Gradient Thermo Block) using the following conditions: 30 s at 94°C following of 40 amplification cycles at 94°C for 1 min, 52°C for 1 min and 72°C for 2 min, followed by final extension at 72°C for 7 min. Two random primers were used in this study; primer 5 (5′- AACGCGCAAC-3′) and primer OPA-3 (5′- AGTCAGCCAC-3′).

Fifteen microliters of the amplified DNA were electrophoresed using electrophoresis unit (WIDE mini-sub-cell GT Bio-RAD) on 1% agarose containing 5% ethedium bromide (0.5 µg/ml), at 75 constant volt, and determined with UV transilluminator. DNA agarose gel was scanned for band Rf using gel documentation system (AAB Advanced American Biotechnology 1166 E. Valencia Dr. Unit 6 C, Fullerton CA 92631). The different M.W. of bands were determined against PCR marker promega G 317A. The banding patterns were analyzed by using the PC-Windows software AAB package. This program calculated the genetic similarities (GS) and differences between each DNA profile, with the Pearson's product moment correlation coefficient (r) between samples to construct a matrix. The samples were then clustered using the UN weighted pair group method of arithmetic average (UPGMA) which resulted in a dendrogram based on their overall similarities.

Results

Effect of saponin on linear growth and sclerotial formation of *S. rolfsii* isolates

Saponin treatments affected significantly ($P \leq 0.5$) mycelium linear growth of *S. rolfsii* isolates compared to the control (Table 1). A positive correlation between saponin concentration and reduction of fungal liner growth was detected. Response of fungal isolates to saponin application was varied. *S. rolfsii* isolate no. 4 (tomato) was the most sensitive to saponin even at low level, followed by isolate no. 3 (sesame), no. 1 (guar) and no. 2 (peanut).

Table 1 Effect of different saponin concentrations on linear growth (cm) of *Sclerotium rolfsii* isolates.

Isolates No.	Saponin concentrations (g/l)			
	0 (Control)	1	3	5
1 (From guar)	(9.00) [a]	(6.30) [a] (30%) [b]	(5.35) [a] (40.5%) [b]	(4.10) [a] (54.4%) [b]
2 (From peanut)	(9.00) [a]	(7.35) [a] (18.3%) [b]	(5.70) [a] (36.6%) [b]	(4.75) [a] (47.2%) [b]
3 (From sesame)	(9.00) [a]	(6.10) [a] (32.2%) [b]	(4.53) [a] (49.6%) [b]	(3.17) [a] (64.7%) [b]
4 (From tomato)	(9.00) [a]	(5.55) [a] (38.3%) [b]	(3.75) [a] (58.3%) [b]	(2.70) [a] (70.0%) [b]

* L.S.D 5%: Isolate (I): 0.439, Concentration (C): 0.415, Interaction (I) x (C): 0.878, [a] mean of linear growth (cm) of four replicates, [b] Reduction percentage of fungal growth compared to the control.

Saponin application reduced significantly ($P \leq 0.5$) the number of sclerotia formed by *S. rolfsii* isolates. A negative correlation between saponin concentration and sclerotia number was detected after 8 days incubation (Fig. 1A). This result was observed in each of the four examined *S. rolfsii* isolates. However, after 15 days incubation, the number of sclerotia increased about 2–3 fold more than that formed after 8 days incubation in all treatments with or without saponin (Fig. 1B). Isolate no. 1 (from guar) was greatly affected by saponin and produced fewer sclerotia.

Effect of saponin on sclerotial germination of *S. rolfsii* isolates

Germination of *S. rolfsii* sclerotia was greatly affected as a result of saponin treatment (Fig. 2). Saponin at low concentration (1 g/l) inhibited significantly sclerotia germination compared

with the check (saponin-free). The reduction percentage, however, was more pronounced in the isolates from guar and sesame. Use of high saponin concentrations (3 and 5 g/l) did not affect sclerotia germination at all. This finding was confirmed in the four tested fungal isolates.

Effect of saponin on polygalacturonase activity of *S. rolfsii* isolates

Polygalacturonase activity of cultural filtrate of *S. rolfsii* isolates treated and untreated with saponin is illustrated in (Fig. 3). Presence of saponin in the fungal medium reduced poly-galacturonase activity by about half that of the control (saponin-free). This result was seen in all examined *S. rolfsii* isolates with different degrees.

Effect of guar seed pretreated with saponin on root rot disease incidence caused by *S. rolfsii* under greenhouse conditions

Under greenhouse conditions, guar seeds pre-treated with saponin at the rate of (5 g/kg seeds) reduced significantly root rot disease incidence compared to untreated seeds (Table 2). Reduction percentage of root rot in infected plants ranged from (31.1 to 58.9%). The highest reduction in disease incidence was detected in the isolates from guar plants.

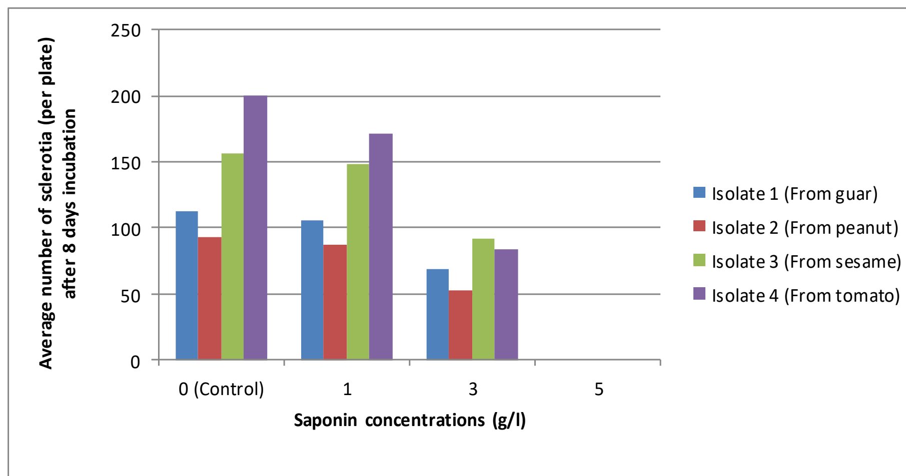

Fig. 1A – Effect of different saponin concentrations (g/l) on sclerotial formation by *Sclerotium rolfsii* isolates after 8 days incubation; {L.S.D 5%: Isolate (I) = 32.05, Conc. (C) = 35.04, Inter. (I)x(C) = N.S.},

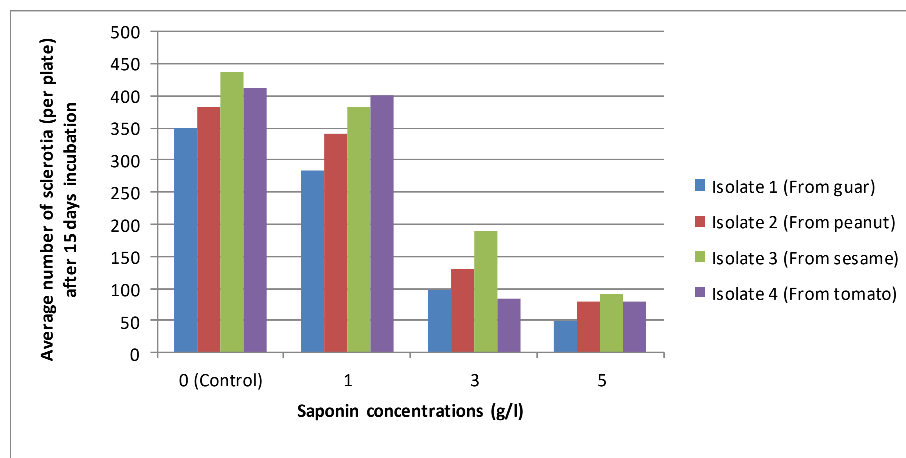

Fig. 1B – Effect of different saponin concentrations (g/l) on sclerotial formation by *Sclerotium rolfsii* isolates after 15 days incubation; {L.S.D 5%: Isolate (I) = 23.53, Conc. (C) = 20.38, Inter. (I)x(C) = N.S.}.

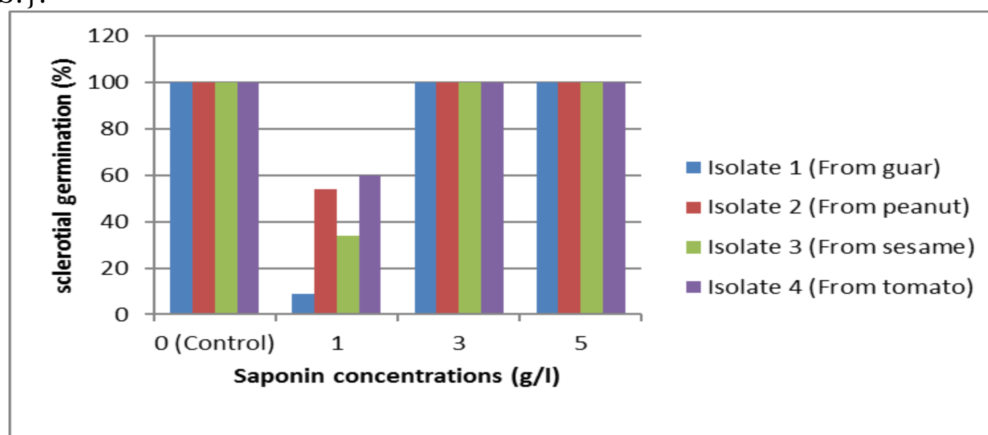

Fig. 2 – Effect of different saponin concentrations (g/l) on sclerotial germination of *Sclerotium rolfsii* isolates.

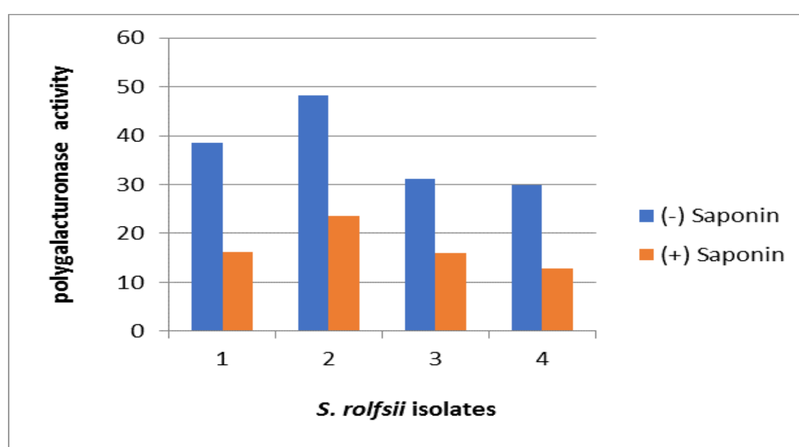

Fig. 3 – Influence of saponin on polygalacturonase activity of *Sclerotium rolfsii* isolates.

Table 2 Effect of saponin treated guar seeds on disease incidence of root rot caused by *Sclerotium rolfsii* under greenhouse conditions**.**

Isolates No.	Disease incidence (%)		Reduction (%)
	(-) Saponin Control	(+) Saponin	
1 (From guar)	56.7	23.3	58.9
2 (From peanut)	63.3	40	36.8
3 (From sesame)	53.3	36.7	31.1
4 (From tomato)	46.7	26.7	42.8
L.S.D. 5%	10.75		-

RAPD analysis

RAPD analysis of *Sclerotium rolfsii* isolates was performed using primer 5 (5′- AACGCGCAAC-3′) (Fig. 4A) and primer OPA-3 (5′- AGTCAGCCAC-3′) (Fig. 4B). DNA

molecular weights of the four *Sclerotium rolfsii* isolates using primer 5 ranged from 138 bp to 1116 bp, while it ranged from 80 bp to 1040 bp using primer OPA-3. Data illustrated in Fig. 5A shows the cluster analysis of RAPD of the four isolates using primer 5, two main groups were observed in the dendrogram, similarities ranged from 31% to 71%. The first group included isolate no.1 (guar) and no. 2 (peanut). The second group included sample no. 3 (sesame) and no. 4 (tomato), which showed the highest genetic similarity of 71%. Fig. 5B shows the cluster analysis of RAPD of the four isolates using primer OPA-3. The highest genetic similarity (92%) was observed between sample no. 2 (peanut) and no. 3 (sesame). Sample no. 4 (tomato) showed the lowest similarity (40%) compared with the other samples.

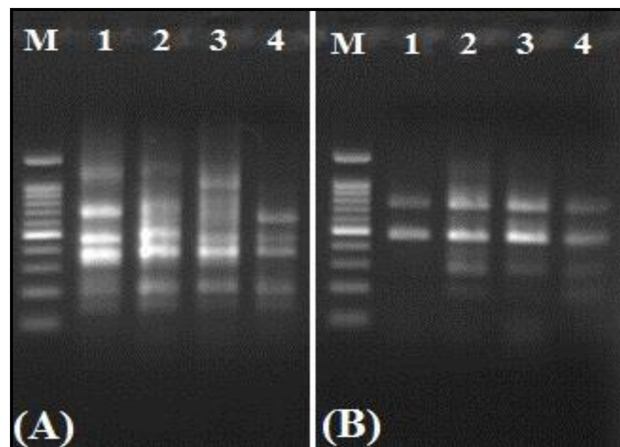

Fig. 4 – (**A**) RAPD profiles of *Sclerotium rolfsii* isolates using primer 5. (**B**) RAPD profiles of *Sclerotium rolfsii* isolates using primer OPA-3. M: 100 bp DNA marker, lane 1: guar, lane 2: peanut, lane 3: sesame, lane 4: tomato.

DNA analysis

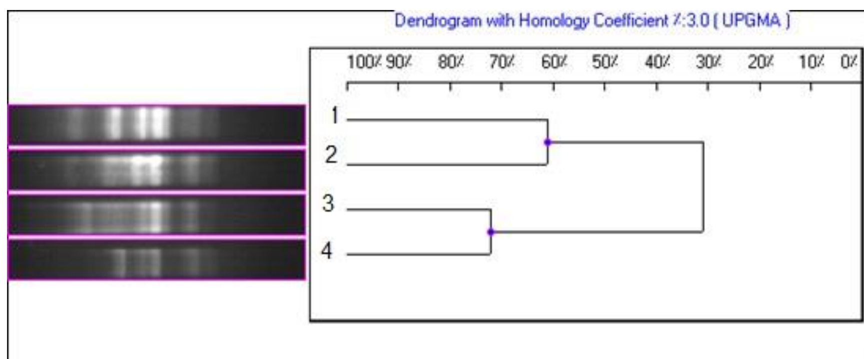

Fig. 5A – Similarities between the DNA samples using primer 5 (5′- AACGCGCAAC-3′).

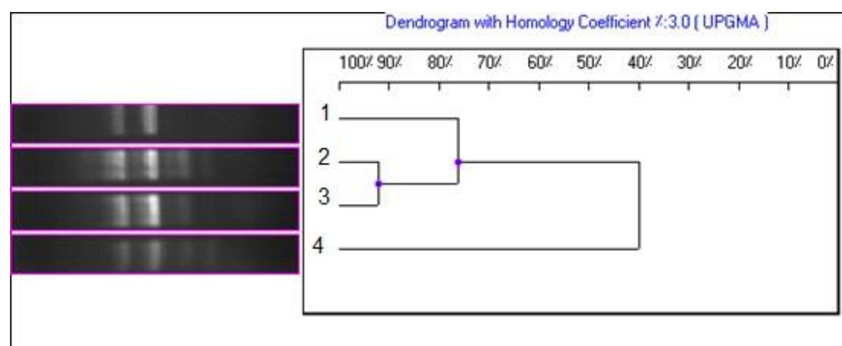

Fig. 5B – Similarities between the DNA samples using primer OPA-3 (5′- AGTCAGCCAC -3′).

Discussion

To achieve a comprehensive and efficient control of any pathogenic fungi, knowledge of variations within fungal isolates is greatly needed. The genetic variability and morphological characteristic of the fungal isolates might respond differently to the application of plant disease control measure(s). The current investigation revealed diversity in both morphological and genetical characters among the tested *S. rolfsii* isolates derived from four different host plants. These results confirmed the previous finding by Ahmed (2010) and You et al. (2016).

The properties of saponin as antifungal are well known. Previous investigators (Leath et al. 1972, Horber et al. 1974, Levy et al. 1986, Bowyer et al. 1995, Wubber et al. 1996, Omar et al. 2009, Salem et al. 2012) determined the biological influence of saponin derivatives against various microorganisms and highly specific reaction of individual fungi to saponin. They found that most of the tested fungi were inhibited, although some were not affected and a few, however, were stimulated. These findings depend mainly on saponin sources, saponin levels and species of fungi tested. The mechanism(s) of saponin to diminish fungal growth is assumed to be due to their membrane lytic action causing the formation of pores and subsequent loss of membrane integrity (Hostettman & Marston 1995). Moreover, (Gestetner et al. 1971) revealed that the biological activity of alfalfa saponins on *S. rolfsii* was due primarily to the sapogenin medicagenic acid, which is essentially responsible for the antifungal properties of alfalfa saponin.

The importance of polygalacturonase produced by several necrotrophic fungi in plant disease resistance is well known. Increasing pectin-degrading enzymes causing subsequently severe degradation of cell wall and cell death (Alghisi & Favaron 1995). The present research work has shown that saponin application reduced polygalacturonase levels in cultural filtrate of *S. rolfsii* isolates. This finding might explain also the protection effects of saponin against *S. rolfsii* infection. Similarly, Mall & Suresh (1988) indicated that inhibition of endopolygalacturonase (endo-PG) ultimately restricts lesion expansion of *Rhizoctonia solani* in potato plants. The results showed that saponin amended in PDA medium inhibited mycelial growth and sclerotia formation of *S. rolfsii* isolates. Such inhibitory effects were increased proportionally with increasing saponin concentrations.

Minor differences in the vegetative growth response of the four examined *S. rolfsii* isolates were noted. The reasons for such variations in mycelial growth and sclerotia formation of *S. rolfsii* isolates to saponin is probably due to the host plant from which the fungal isolates came from. Tomato isolate was ranked as more sensitive to saponin followed by sesame isolate, while peanut isolate was classified as less sensitive to saponin treatment. Accordingly, it can be suggested that response of *S. rolfsii* isolates to saponin is host dependent. Moreover, geographical locations of *S. rolfsii* populations might also affect cultural characteristic (mycelial growth and sclerotia formation) in their reaction to saponin application. Although the vegetative growth response of different fungal species to saponin was well documented, to our knowledge, there are few attempts to investigate influence of saponin on growth reaction of different isolates within one fungus. In whole plant test under artificial inoculation with *S. rolfsii*, saponin treated guar seeds reduced significantly disease incidence. This finding confirmed the efficacy of saponin in controlling *S. rolfsii* fungus under pure cultural in the laboratory and under greenhouse conditions. These results are in agreement with Rasu et al. (2013) who found variations in cultural morphology of 17 *S. rolfsii* isolates derived from different plants at various geographic sites in India.

RAPD banding patterns was carried out for the four isolates of *S. rolfsii* using two random primers. The results obtained by analysis of DNA profiles of the isolates revealed that genetic variability existed among the tested *S. rolfsii* isolates. These results are in agreement with the findings of Pandey et al. (2013) who found variations among 14 Indian *Sclerotium* isolates based on protein profile generated by polyacrylamide gel–electrophoresis (SDS-PAGE). The authors

reported that all fungal isolates showed a significant variation among themselves with respect to number and intensity of major and minor bands.

Kumar et al. (2010) studied genetic diversity among 15 isolates of *S. oryzae* collected from major rice growing areas of Haryana in India based on DNA polymorphism using 22 RAPD primers. Their findings proved the genetic variability among the tested *S. oryzae*, since they divided into different sub-clusters. Parvin et al. (2016) used RAPD polymorphism as a tool to determine genetic variation among isolates of *S. rolfsii.* They mentioned that DNA fingerprinting by RAPD prompted the grouping of eight *S. rolfsii* isolates. Rasu et al. (2013) established RAPD banding patterns for ten *S. rolfsii* isolates using five random primers. Their findings revealed that all the isolates have about 54% similarity coefficient indicating that they were genetically varied by their unique banding patterns. This indicated genetic variability existing among *S. rolfsii* isolates.

In short, genetical and morphological diversity of four isolates of *S. rolfsii* were detected. Saponin treatment affected greatly both growth and development of the fungus in pure culture and greenhouse experiments. Accordingly, saponin application is more likely to be used as a disease control measure against the fungus.

References

Abdel-Momen SM, Omar SA, Hanafi AA, Abdel-Rahman TM. 2000 – Different sources of saponin affecting white rot disease in onion (*Allium cepa* L.) Bulletin Faculty of Agriculture, Cairo University, Egypt 51, 365–378.

Ahmed HZ. 2010 – Diversity of *Sclerotium rolfsii* isolates infecting some oil crops. M.Sc. Thesis. Faculty of Agriculture, Ain-Shams University, Egypt.

Alghisi P, Favaron F. 1995 – Pectin-degrading enzymes and plant-parasite interactions. European Journal of Plant Pathology 101, 365–375.

Aly AA, Omar SA, Zayed SME, Mansour MTM. 2000 – Use of saponin-containing *Atriplex nummularia* to suppress damping-off of cotton seedlings. Journal of Agricultural Science Mansoura University 25, 7621–7631.

Bailey JA, Manfsield JM. 1982 – Phytoalexins. Black Pub. Glasgow and London.

Bowyer P, Clark PR, Lunness P, Daniels MJ, Osbourn AE. 1995 – Host range of a plant pathogenic fungus determined by a saponin detoxifying enzyme. Science 267, 371–374.

Dwivedi SK, Prasad G. 2016 – Integrated management of *Sclerotium rolfsii*: an overview. European Journal of Biomedical and Pharmaceutical Science 3, 137–146.

Fenwick GR, Price KR, Tsukamota C, Okubo K. 1992 – Saponins. pp. 285–327. In: Crop plants, D'Mello JP, Duffus CM, Duffus JH (eds). The Royal Society of Chemistry, Cambridge.

Garibaldi A, Gilardi G, Gullino ML. 2006 – First report of southern blight incited by *Sclerotium rolfsii* on potato (*Solanum tuberosum*) in northern Italy. Plant Disease 90, 1114.

Gestetner B, Assa Y, Henis Y, Birk Y, Bondi A. 1971 – Lucerne saponins. IV. Relationship between their chemical constitution and haemolytic and antifungal activities. Journal of the Science of Food and Agriculture 22, 168–172.

Horber E, Leath KT, Berrang B, MarcarianV, Hanson CH. 1974 – Biological activities of saponin components from Dupuits and Lahontan alfalfa. Entomologia Experimentalis et Applicata 17, 410–424.

Hostettmann KA, Marson A. 1995 – Saponins. pp. 1–548. In: Chemistry and pharmacology of natural products. Cambridge University Press, Cambridge, U.K.

Kumar A, Singh R, Boora KS, Jalali BL. 2010 – Genetic diversity in *Sclerotium oryzae* population of rice based on random amplified polymorphic DNA markers. Indian Phytopathology 63, 418–420.

Lacai-Dubois MA. 1999 – Biological and pharmacological active saponin from plant: recent advances. Cited in Saponin in Food, Feedstuffs, and Medicinal Plants, Book of abstracts Pulawy, Poland 6–8 Sep. 1999.

Leath KT, Davis JKH, Wall ME, Hanson CH. 1972 – Vegetative growth responses of alfalfa pathogens to saponin and other extracts from alfalfa (*Medicago sativa* L.). Crop Science 12, 851–856.

Levy M, Zehavi U, Naim M, Polacheck I. 1986 – An improved procedure for the isolation of medicagenic acid 3-O-B-D- glucopyranoside from alfalfa roots and its antifungal activity on plant pathogens. Journal of Agricultural and Food Chemistry 34, 960–963.

Mall S, Suresh K. 1988 – Susceptibility of potato plants of different ages to *Rhizoctonia solani* in relation to production of pectolytic and cellulolytic enzymes in pathogenesis. Indian Phytopathology 41, 64–68.

Omar MR, Osman EAM, Asran AA, Omar SA. 2009 – Factors affecting efficiency of saponin in controlling damping-off. Journal of Agricultural Science Mansoura University 34, 5107–5119.

Pandey R, Pandey MK, Singh UP. 2013 – Variability in Indian isolates of *Sclerotium rolfsii* by SDS-PAGE. Archives of Phytopathology and Plant Protection 46, 19–28.

Parvin N, Bilkiss M, Nahar J, Siddiqua MK, Meah MB. 2016 – RAPD analysis of *Sclerotium rolfsii* isolates causing collar rot of eggplant and tomato. International Journal of Agricultural Research, Innovation and Technology 6, 47–57.

Passone MA, Rosso LC, Ciancio A, Etcheverry M. 2010 – Detection and quantification of *Aspergillus* section *Flavi* spp. in stored peanuts by real-time PCR of nor-1 gene, and effects of storage conditions on aflatoxin production. International Journal of Food Microbiology 138, 276–281.

Queiroz JVJ, Inokuti EM, Tsuji SS, Camara MPS, Michereff SJ. 2017 – First report of collar rot on jack bean (*Canavalia ensiformis*) caused by *Sclerotium rolfsii* in Brazil. Plant Disease 101, 388.

Rasu T, Sevugapperumai N, Thiruvengadam R, Ramasamy S. 2013 – Morphological and genomic variability among *Sclerotium rolfsii* populations. An International Quarterly Journal of Life Sciences 8, 1425–1430.

Roca LF, Raya MC, Luque F, Agusti-Brisach C, Romero J, Trapero A. 2016 – First report of *Sclerotium rolfsii* causing soft rot of potato tubers in Spain. Plant Disease 100, 2535.

Salem MF, Rizk NM, Omar SA, Abdel-Hamed AS. 2012 – The potential utilization of alfalfa root saponin in controlling chocolate spot disease in faba bean. Journal of Biological Chemistry and Environmental Sciences 7, 159–171.

Talboys PW, Bush LV. 1970 – Pectic enzymes produced by *Verticillium* spp. Transactions of the British Mycological Society 55, 367–381.

Wubber JP, Price KR, Daniels MJ, Osbourn AE. 1996 – Detoxification of leaf saponins by *Septoria avenae*. Phytopathology 86, 986–992.

You JM, Liu H, Huang BJ. 2016 – First report of southern blight caused by *Sclerotium rolfsii* on *Macleaya cordata* in China. Plant Disease 100, 530.