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Biological and Molecular Variability of Rice Sheath Rot Pathogen *Sarocladium oryzae* using SCAR and SRAP Markers

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Rice sheath rot disease caused by *Sarocladium oryzae* is a seed borne disease and is a major production constraint in some rice growing countries. This study aimed to evaluate some of our local rice entries to sheath rot infection with different isolates, elucidate their phytotoxicity effect and the molecular variability among some selected isolates derived from different locations. Data revealed that Sakha 101 was the most resistant cultivar against sheath rot. The Toxin of *S. oryzae* completely inhibited differentiation of rice roots and negatively affected seed germination of rice and weeds. *S. oryzae* isolates showed morphological variability and polymorphism in DNA, high levels of genetic variability among isolates were identified. Using MR specific primers revealed that the two bands (1500 and 1400bp) were found only in low virulent isolates. Using SRAP dendogram supported the similarity of data. Moreover, using SCAR and SRAP primers could be able to differentiate among *S. oryzae* isolates collected from different locations or varieties and differed in their virulence and classified in two main clusters as high and low virulent. Thus, the molecular variability of *S. oryzae* isolates may be an important consideration in breeding programs to develop resistance for sheath rot disease.

Keywords: Breeding programs, molecular variability, rice entries, *Sarocladium oryzae* and sheath rot.

Rice (*Oryzae sativa* L.) is one of the most important cereal food crops in Egypt. The major rice diseases common in Egypt are blast, brown spot and false smut. Recently, sheath rot disease has been detected by El-Gremi and Saleh (2013). Rice sheath rot disease is a seed borne and is a major production constraint in rice growing countries. It has recently become a serious disease of rice during flag sheath development when climatic conditions are unfavorable (Lalan *et al.,* 2013), causing severe yield losses (Saranya and Sowndaram, 2014), reached up to 85% (OU, 1985; Webster and Gunnell, 1992). This disease is characterized by grayish brown lesions on the flag leaf sheath which encloses panicle. Lesions start small on flag leaf sheath, then enlarge, coalesce and cover most of the sheath. Panicles may emerge partially or do not emerge, rotted resulting in spikelet sterility. Rice sheath rot disease was first described in Taiwan by Sawada (1922), the causal fungus was named as *Acrocylindrium oryzae* Sawada, synonym *Sarocladium oryzae* Sawada (Gams and Hawksworth, 1975). This fungus secretes certain toxic substances in the

culture which may play a role in the pathogen's virulence (Vasantha and Gnanamanickam, 1993) and inhibit seed germination or vigorous seedling with reducing shoot and root length, (Velazhahan, 1991). It is difficult to improve host resistance due to lack of knowledge on the genetic variability and virulence of this pathogen. Using fungicides, insignificantly affect the fungus; therefore, fungicide treatments have been unsuccessful in management of this pathogen and expensive as well as toxic to the environment (Sakthivel, 2001). Therefore, the development of resistant cultivars deserves attention. Successful breeding and effective deployment of durable plant resistance require an understanding of pathogen diversity and of the way in which virulence evolves in the pathogen populations (Manzanares *et al.,* 2001). Thus, molecular techniques are used for the assessment of genetic diversity in microbe complex communities (Saad *et al.,* 2004; Gonzalez and Saiz-Jimenez 2005). Sequence related amplified polymorphism (SRAP) is a PCR based marker system as described by Li and Quiros (2001). The SRAPs is a simple and efficient marker system that can be adapted for a variety of purposes in different crops. It is simple, discloses numerous co-dominant markers, targets open reading frames (ORFs), and allows easy isolation of bands for sequencing. However, SRAP markers were used for many crops; it is still scare for fungi, recently, Yuejin *et al.* (2011) used it for filamentous fungi. Furthermore, sequence characterized amplified regions (SCAR), MR primer based on Ayyadurai *et al.* (2005) was used to differentiate among *S. oryzae* isolates collected from different locations or varieties, they found high level of polymorphism within isolates using MR primer. Similarly, Southwood *et al*. (2012) used SCAR to differentiate among *Fusarium* sp*.,* isolates. Also, Zhang *et al*. (2012) developed a diagnostic SCAR marker specific towards *Tilletia foetida*, the causal agent of wheat bunt disease. This SCAR marker could distinguish related pathogenic fungi efficiently and could be used for the early diagnosis of the common bunt of wheat in the field and provide an efficient way for disease surveillance and disease forecasting in cereal crops.

According to the available literature, there is no report concerning the molecular variability of *S. oryzae* under Egyptian conditions. In the present investigation we aimed to study biological and molecular characterization among *S. oryzae* isolates derived from different locations in Egypt. The study also included the pathogenicity, phytotoxicity effect of the fungus on some rice genotypes and weeds. Study was also expanded to elucidate DNA polymorphism among some selected isolates based on SCAR and SRAP markers.

Materials and Methods

Sampling:

Mature rice plants showing typical symptoms of sheath rot disease, *i.e*. grayish brown lesions on the flag leaf sheath beside rotted panicles, were collected from farms located at Kafer El-Sheikh, Behira and Gharbia governorates.

Isolation and identification of the associated microorganisms:

Isolation trails from naturally infected rice plants collected from the three governorates were carried out following the method described by Saleh (2012). The emerged fungi were picked up, purified and identified according to keys given by

Riker and Riker (1968), Amin *et al*. (1974) and Ou (1985). Pure cultures were maintained on potato dextrose agar (PDA) slants as stock cultures. Percentage of frequency of each fungus was also determined.

Pathogenicity test:

Pathogenicity test using the most frequently isolated fungus, *i.e*. *S. oryzae* was carried out under greenhouse conditions. Thirty isolates of *S. oryzae* (5 days old cultures on PDA medium) were grown on PD broth medium in conical flasks (250ml) at 28-30°C for 7 days using shaking incubator (200 rpm/ minute). These isolates were artificially inoculated into rice Hybrid 2. The hybrid was planted in the greenhouse in rows of 1.5m length; each row had 7 hills. At booting stage, tillers were sprayed with spore suspension (10^8 spore/ml of *S. oryzae*) of any isolate tested. Inoculated plants were checked for sheath rot incidence after 14 days and disease assessment was recorded after 21 days of inoculation, till full disease symptoms. Infection percentage was calculated according to Thapak *et al*. (2003) as follows:

$$\text{Infection (\%)} = \frac{\text{No. of infected plants}}{\text{No. of total tested plants}} \times 100$$

Evaluation of nine rice entries to infection by eight isolates of S. oryzae:

Nine rice entries, *i.e.* Hybrid1, Hybrid 2, Sakha 101, Giza 178, Giza 181, Giza 182, IR58025A line, IR69625A line and IR70368A line were checked for their resistance to eight isolates of *S. oryzae* (1, 8, 16, 17, 19, 27, 29 and 30) under greenhouse conditions. Rows of 1.5 m length with 7 hills/ row were cultivated with any of the entries tested with three replicates. At booting stage, three rows were sprayed individually with spore suspension (10^8 spore/ml) of the eight isolates. Untreated rows were used as control and disease incidence was recorded as mentioned before.

Phytotoxicity of S. oryzae isolates

Effects of culture filtrates of *S. oryzae* isolates on seedling growth (shoot and root length) of Hybrid 1 and Sakha 101 rice cultivars and on seed germination of two weeds, *i.e*. *Echinocloa crus-galli* and *Echinocloa colonum* were tested. Three isolates (8, 16 and 29) were selected for this test according to their virulence. The three isolates were grown on two liquid media (potato dextrose broth medium and liquid medium, ($CaCO_3$ medium), containing 6% glucose, 0.5% peptone, 0.30% NaCl and 0.30% $CaCO_3$; according to Ayyadurai *et al.* (2005). Flasks containing 100 ml of sterilized medium were inoculated with PDA-mycelial disks (0.5 cm diameter) of 7-day old culture of any of *S. oryzae* isolates tested (one disk/flask). The flasks were incubated for 10, 20 and 30 days at 27°C in darkness. Then, the mycelium mats were removed through three cheese cloth layers followed by Whatman No.1 filter paper and the filtrates were subjected to centrifuge (3000rpm). Then, the filtrates were sterilized using millipore filter (0.22mμ). Seeds of rice cultivars and the two kinds of weeds were impregnated with culture filtrate (10 ml) in 9 cm Petri dishes, each containing 25 seeds and three replicates were used for each treatment. Seeds impregnated in water were served as a check treatment. Shoot and root length were measured after 14 days for rice and weeds seeds, in addition to seed germination percentage of weeds seeds.

DNA extraction and purification:

As a result of variation in pathological, cultural and morphological characters of the isolates tested, the genetic basis of variation was tested. Eight isolates (1, 8, 16, 17, 19, 27, 29 and 30) varied in source of isolation, aggressiveness were chosen to be tested using SRAP and SCAR- markers. The selected isolates were grown on PD broth medium at 28°C under continuous shaking for a period of 7 days according to Ayyadurai *et al*. (2005). Mycelia after centrifugation at 3,000 g for 10 min at 4°C were used for DNA extraction using cetyltrimethyl ammonium bromide (CTAB) based on Doyle and Doyle (1990). Mycelia were suspended in CTAB extraction buffer incubated at 65°C for 45 min and centrifuged. The supernatant was removed and mixed with an equal volume of phenol : chloroform (1:1, vol/vol) and centrifuged. The top layer was removed and mixed with choloroform:iso-amyl alcohol (24:1). After centrifugation, DNA was precipitated using ice-cold ethanol. Purity and the concentration of DNA were estimated using Nano drop at 260 and 280 nm.

PCR amplification and gel electrophoresis:

Polymerase Chain Reaction (PCR) was carried out in presence of 1X *Taq* DNA polymerase buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 200 µM dNTPs, 5 picomole for each primer, 25 ng template DNA, 0.5 unit of *Taq* DNA polymerase in a total volume of 20 µl. Two types of primers were used, SRAP (Table 1) and SCAR (primer MR, 5`GAGGGTGGCGGTTCT3`). PCR amplification was performed as follow, 95°C for 4 min followed by 35 cycles of 1 min for denaturation at 94°C, 30 sec for annealing at 40°C and 1.30 min for polymerization at 72°C, followed by a final extension step at 72°C for 7 min for SCAR. For SRAP, initial denaturation at 94°C for 4min, followed by five cycles comprising 1-min denaturation at 94°C, 1-min annealing at 35°C, and 30s of elongation at 72°C. the following 30 cycles, denaturation at 94 °C for 1 min, annealing at 50°C for 1 min, and elongation at 72 C for 30s were carried out, ending with an elongation step for 10 min at 72°C. The amplified products were stored at 4°C. Electrophoresis was done using 1X TAE buffer in agarose gel 1% to detect banding pattern.

Table 1. Primers name and sequences of SRAP*

No.	Primer name	Primer sequences 5`→3□`
1	me1	TGAGTCCAAACCGGATA
2	me2	TGAGTCCAAACCGGAGC
3	me3	TGAGTCCAAACCGGAAT
4	me4	TGAGTCCAAACCGGACC
5	me5	GAGTCCAAACCGGAAG
6	em1	GACTGCGTACGAATTAAT
7	em2	GACTGCGTACGAATTTGC
8	em3	GACTGCGTACGAATTGAC

* all possible 15 combinations (me1+em1, me1+em2, me1+em3, me2+em1, me2+em2, me2+em3, me3+em1, me3+em2, me3+em3, me4+em1, me4+em2, me4+em3, me5+em1, me5+em2 and me5+em3).

Data analysis:

Data were subjected to analysis of variance using Genstat Computer Program. Means were compared using least significant difference method (LSD) at 5% (Steel and Torrie, 1980). Amplification profiles for the used eight isolates as a result of SRAP were compared with each other and DNA fragments were scored as a binary data, where (1) means presence and (0) means absence. The data were used to estimate genetic similarity on the basis of number of shared amplification products (Nei and Li, 1979). The distance coefficients were calculated by the following statistical equation: $F= 2N_{xy}/ (N_x + N_y)$

Whereas: F is the distance coefficient in which N_x and N_y are the numbers of fragments in genotypes x and y, respectively, and N_{xy} is the number of fragments differed by the two genotypes (Lynch, 1990).

The electrophoresis patterns of the reproducible banding patterns of each primer produced by SRAP were chosen for analysis. Each band was scored as present (1) or absent (0), and pairwise comparisons between individuals were made to calculate the Jukes-Cantor coefficient using PAST program adapted by Hammer *et al.* (2001). Cluster analysis was performed to produce a denderogram using unweighed pair-group method with arithmetical average (UPGMA).

Results and Discussion

Isolation and identification of the associated microorganisms:

Isolation trails from naturally infected rice plants collected from three Egyptian governorates resulted in the presence of three different fungal genera, *i.e. Fusarium*, *Helminthosporium* and *Sarocladium*. These fungi were identified as *Fusarium* sp. *Helminthosporium oryzae* and *Sarocladium oryzae*. *S. oryzae* was the most dominant fungus followed by *Fusarium* sp. and *H. oryzae* (Table 2). It is worthy to note that isolation trails resulted in thirty different isolates of *S. oryzae*.

Table 2. Incidence (%) of fungi isolated from rice sheath showing rot symptoms

No.	Detected fungi	Incidence (%)
1	*Fusarium* sp.	30
2	*Helminthosporium oryzae*	10
3	*Sarocladium oryzae*	60

Pathogenicity test:

Data presented in Table (3) and Fig. (1) clearly show that three weeks after inoculation of rice hybrid 2, sheath rot symptoms were developed on the uppermost flag leaf enclosing the panicle. The Virulence which represented as infection percentage ranged from 0 to 69%. the highest percentage of infection (69%) occurred when plants were artificially inoculated with *S. oryzae* isolate No.16. Meanwhile, inoculation with *S. oryzae* isolate no.8 failed to cause any infection to the tested rice hybrid. Isolation trials from three locations (*i.e.* Kafr El-Sheikh, Beheira and Gharbia Governorates) produced 30 isolates which were identified as *S. oryzae*

Table 3. Infection percentage of thirty *Sarocladium oryzae* isolates on rice hybrid 2 cultivar under greenhouse conditions

Source of isolate			Infection (%) on Hybrid 2
Isolate No.	Location	Entry	
1	Kafer El-Sheikh	Giza 171	34.65
2		CMS line 3	21.90
3		A line 9	10.51
4		A line 10	11.12
5		A line 11	12.10
6		A line 1	12.10
7		A line 12	20.67
8		A line 5	0.00
9		A line 70	18.63
10		A line 70	8.18
11		Giza 171	7.20
12		Sakha 104	9.80
13		A line 4	12.75
14		PG-B/37	19.61
15		A line 7	25.49
16		A line 8	69.00
17		A line 3	56.00
18		BNE/65	11.76
19		Sakha103/1	2.13
20		BNS/66	13.08
21		BNS/69	17
22		BNS/73	15.69
23		BNS/50	22.88
24		CMS line 3	14.06
25	Behira	Hybrid 1	22.55
26		Hybrid 2	10.78
27		Giza 177	36
28		Giza 171	19.94
29		Hybrid 2	2.29
30	Gharbia	Sakha 101	6.21
LSD			18.64

based on their morphological characters. These isolates differed in their virulence. Isolates derived from Kafr El-Sheikh Governorate were the most virulent and this might be due to the number of collected samples and the wide range of genotypes from which they were isolated.

Fig. 1. Rice sheath rot disease symptoms on panicles of hybrid 2 inoculated with *Sarocladium oryzae*

Evaluation of nine rice entries to infection by eight isolates of S. oryzae:

Rice entries were differently responded to infection by *S. oryzae* isolates tested (Table 4). Among the entries tested, Sakha 101 appeared the less susceptible one to infection by the 8 isolates of *S. oryzae* tested, as the corresponding mean value of infection was 0.6%. On the other hand, IR70368A was the highly susceptible entry tested. The corresponding mean value of infection was 13.1%. The highest percentage of infection caused by fungal isolates on all tested rice entries was obtained from the isolate No.1, being 6.1% on the average. Meanwhile, the highly virulent isolate in this respect was the isolate No.1 (6.1%) followed by the isolate No. 16 (5.4%). Data (Table 4) also show that among the 8 isolates tested isolates No.8 and No.29 gave the lowest percentages of infection, being 1.9 and 2.0%, respectively. The previously mentioned results were similar to those found by Sakthivel and Gnanamanickam (1987) who pointed out that the pathogen attacks both semi dwarf and traditional tall cultivars with variation in their response to infection with *S. oryzae*. Amin *et al.* (1974) observed sheath rot on dwarf and local tall varieties, the dwarf varieties appeared to be more prone to sheath rot because of their shortened internodes and poor exertion of the panicle from the flag leaf sheath. We observed that complete and fast exerted panicles which are completely free from sheaths and surrounded by dry air had very low or free from the disease.

Phytotoxicity of S. oryzae isolates:

The effect of culture filtrates of the three *S. oryzae* isolates, *i.e.* 8, 16 and 29 grown on two growth media on the rice seedling growth of Sakha101 and Hybrid1 is illustrated in Fig. (2). There was a complete inhibition of rice seedling growth of both cultivars in case of isolates grown in $Caco_3$ broth medium. Culture filtrate of *S. oryzae* isolate 8 in PD broth medium caused a great reduction in shoot and root length of rice seedlings comparing with the untreated control. The toxic effect of metabolites secreted by the isolates tested in liquid media was greater after 10 and 20 days of incubation than after 30 days, (Figs. 3 and 4). Also, culture filtrates of *S. oryzae* isolates grown in $Caco_3$ broth medium reflected the highest inhibitory effect on germination of weed seed than those grown in PD broth medium under the three growth intervals (Fig.5).

Table 4. Infection percentage of nine rice entries artificially inoculated with eight *Sarocladium oryzae* isolates under greenhouse conditions

Isolates No.	Entries									
	Hybrid 1	Hybrid 2	Sakha 101	Giza178	Giza181	Giza182	IR58025A	IR69625A	IR70368A	Mean
1	0.30	2.66	0.00	0.86	0.00	1.30	16.05	4.76	29.36	6.13
8	0.00	0.56	0.00	2.16	0.91	2.38	1.23	1.59	7.14	1.93
16	4.32	1.40	1.06	0.86	0.00	0.00	19.75	3.88	17.06	5.37
17	2.08	0.70	0.88	0.00	5.67	5.41	8.82	6.35	4.36	3.81
19	1.79	2.52	0.53	0.21	0.23	0.21	13.23	10.76	0.00	3.28
27	0.45	0.70	0.88	1.08	1.81	1.30	3.70	4.06	20.63	3.85
29	0.60	0.70	1.41	6.49	0.00	0.00	0.53	5.64	3.77	1.97
30	3.42	1.54	0.35	0.65	2.50	2.38	4.94	8.29	22.82	5.21
Mean	1.61	1.35	0.64	1.54	1.39	1.62	8.53	5.67	13.14	
LSD at 5% for:		Entries(E) 0.25			Isolates(I) 0.24			E*I 0.72		

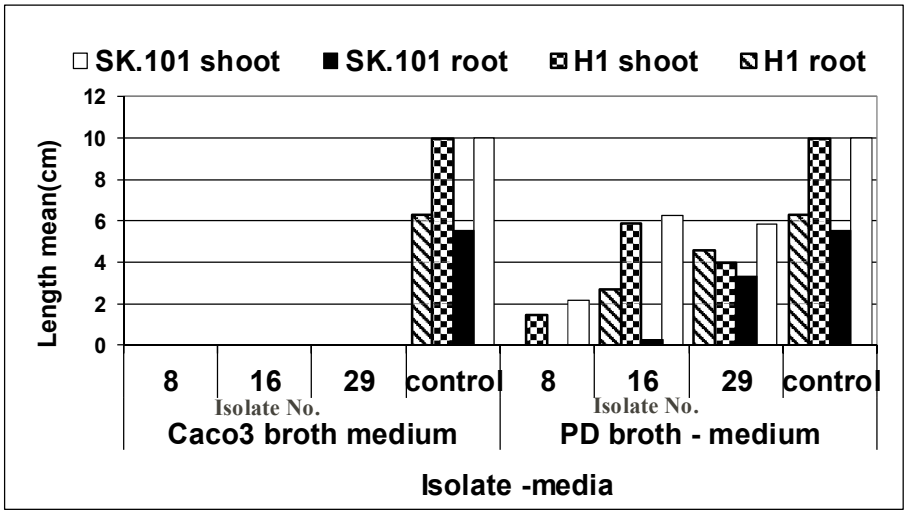

Fig. 2. Effect of culture filtrates of three isolates of *Sarocladium oryzae* on shoot length and root length of rice seedlings of Sakha101 and Hybrid 1.

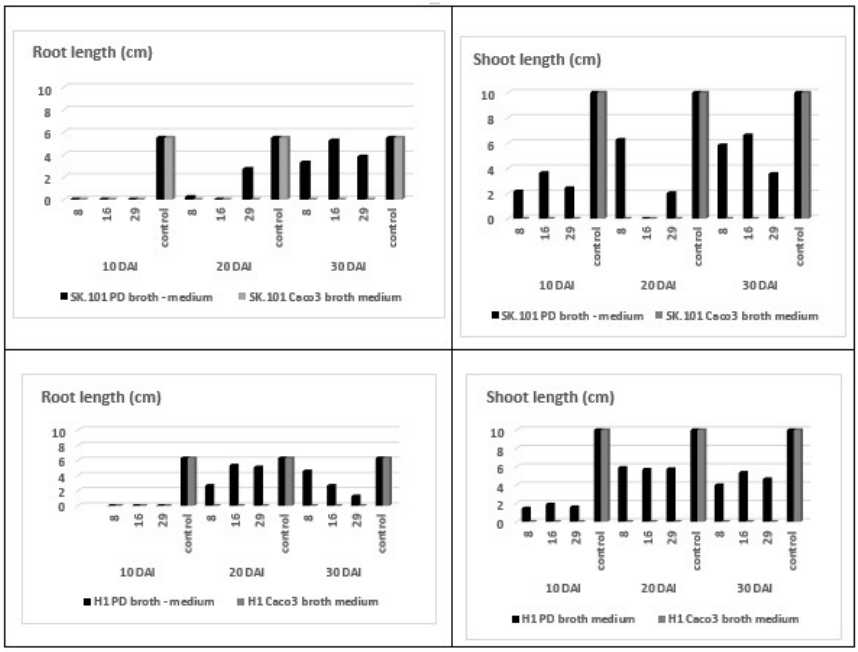

Fig. 3. Effect of culture filtrates of three isolates of *Sarocladium oryzae* grown in two different liquid media for 10, 20 and 30 days after inoculation (DAI) on length of shoot and root of seedlings of two different rice entries (Sakha 101 and Hybrid 1).

Fig. 4. Sakha 101 and Hybrid 1 seedlings grown under culture filtrate of *Sarocladium oryzae* isolate No. 8 compared with untreated seedlings.

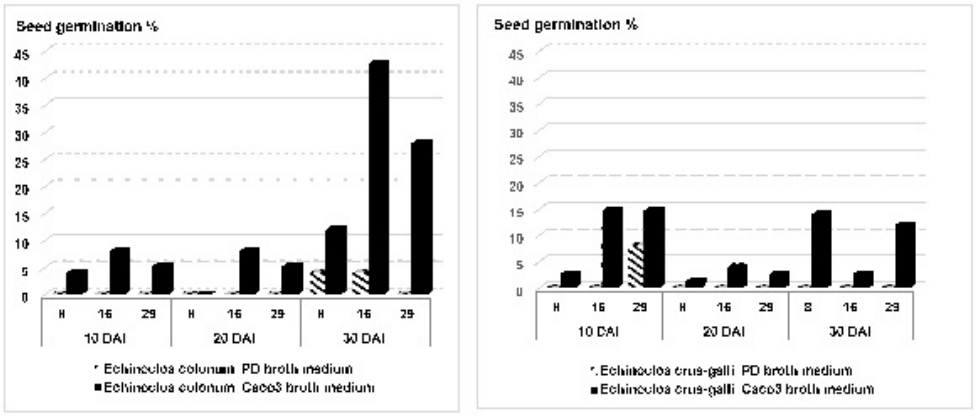

Fig 5. Effect of culture filtrates of three isolates of *Sarocladium oryzae* grown in two different liquid media for 10, 20 and 30 days on *Echinocloa colonum* and *E. crus-galli* seed germination.

Concerning with the toxic effect of culture filtrates of *S. oryzae* isolates tested, the present study indicated that the culture filtrates of the three tested isolates affected seed germination and seedling vigor. The effect of culture media, $CaCO_3$ broth medium was more inducible for toxin production than PD broth medium. In the case of $CaCO_3$ broth medium, there was no growth for the two rice cultivars seeds in comparison with PD broth medium where rice cultivars gave short shoot and root compared with the check which had normal growth for both cultivars. These results are in agreement with the findings of Bills *et al.* (2004) and Ayyaduari *et al.* (2005). The results contradict for the toxin production and its involvement in pathogenesis with the findings of many investigators (Ghosh *et al.,* 2002; Garcia *et al.,* 2003 and Nandakumar *et al.,* 2007). The tested isolates filtrate of 10 days period were the most effective, completely inhibited shoots and roots of the seedlings, especially those grown in $CaCO_3$ broth medium, followed by 20 and 30 days filtrate period, respectively. Similar trend was observed in case of weed seed germination. This might be due to the four phases of fungal growth: lag, log, stationary and death, where the toxins increased to a maximum during the lag phase, and subsequently decreased with fungus aging (Gottlieb and Etten, 1964). Negi and Das (2003), Hafiz *et al.* (2009), Bodalkar and Awadhiya, (2014) proved pathogenicity by inoculating rice plants of various stages with different *S. oryzae* isolates and proved their pathogenic potential to initiate grain discoloration.

Molecular analysis:

Sequence Characterized Amplified Regions (SCAR) analysis was a useful tool for characterizing the genetic variability among the eight tested isolates of *S. oryzae*. Using SCAR (MR primer), the results (Fig.6) indicate that the tested isolates were highly diverged. PCR amplification with MR specific primers revealed that the two bands (1500 and 1400bp) were found only in low virulence isolates (8, 19, 29 and 30) in the same manner, the band with size 650 bp was found only in the aforementioned isolates; indicating that those isolates were similar and these bands

could be used for identification of pathogenicity of *S. oryzae*. However, all the isolates participated in the monomorphic band with 800bp size, as shown in Fig. (6).

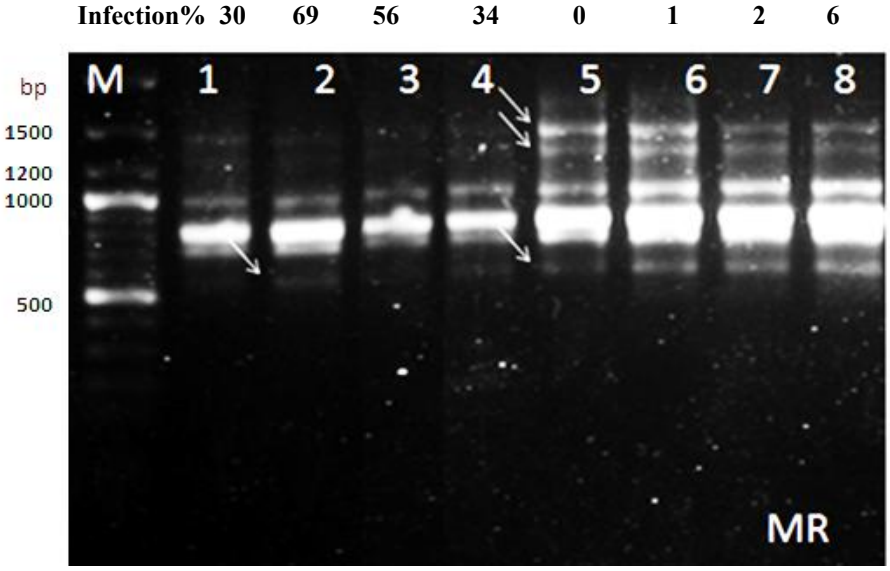

Fig. 6. SCAR patterns generated following PCR amplification from eight isolates of *Sarocladium oryzae* with primer MR, M: ladder marker 100bp, 1-8: isolates 1, 16, 17, 27, 8, 19, 29 and 30, respectively.

Based on MR primer data which divided these eight tested isolates into two groups, the first included isolates 1, 16, 17 and 27 which had high virulence (34.65% - 69% infection), while the second group (isolates 8, 19, 29 and 30) showed low virulence (0.00% - 6.21% infection), Table (3) and Fig. (8).

Moreover, by using SRAP as a new method with eight primers (15 possible combinations) as shown in Table (1) and Fig. (7), SRAP maker is more frequently used in the genetic study for many crops and rarely in the study of fungi (Sun *et al*., 2006). Among all molecular markers, SRAP was firstly applied in *S. oryzae* isolates in this study. It is more reliable and repeatable than RAPD, and are less-labor-intensive and time-consuming to produce than AFLP and SSR (Ren *et al*., 2010; Cai *et al*., 2011).

The results of SRAP primers combinations gave different banding patterns and there were some specific bands in case of using primer me1+em2, there was band with size ~950 bp found only on all low virulence isolates and absent from others. However, using me3+em2 the band with size ~800bp was found in three of low virulence isolates as shown in Fig (7). Other primers gave some bands specific for each group but not in all isolates. The dendogrms could classify the studied isolates into groups according to their severity. The most aggressive isolates (1, 16, 17 and 27) which have high virulence were included in a cluster with similarity 0.81 between isolates 16 and 17, another cluster included the remained isolates that recorded low severity with similarity 0.86 between isolates 19 and 8 as shown in Table (5).

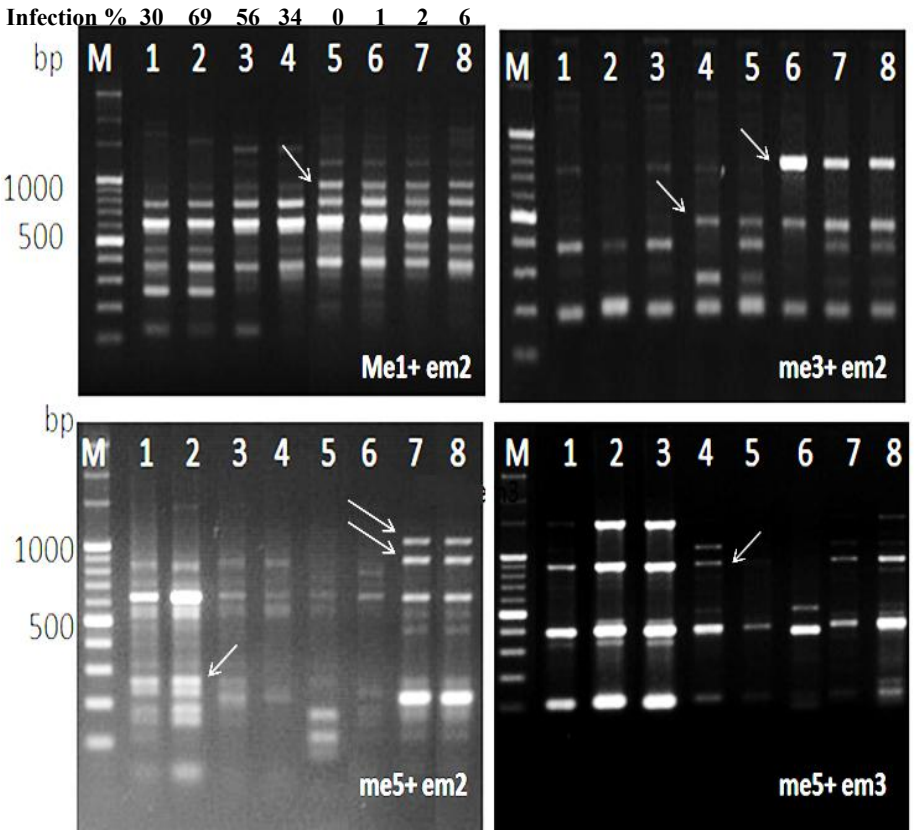

Fig. 7. Fingerprint patterns for eight isolates of *Sarocladium oryzae* generated by SRAP, M: ladder marker 100bp, 1-8: isolates 1, 16, 17, 27, 8, 19, 29 and 30, respectively

Table 5. Similarity index based on Jaccared among the studied eight isolates of *Sarocladium oryzae*

Isolate 16	Isolate 17	Isolate 1	Isolate 27	Isolate 30	Isolate 29	Isolate 19	0
0.26	0.26	0.33	0.41	0.42	0.45	0.86	Isolate 8
0.27	0.27	0.35	0.43	0.50	0.55	1.00	Isolate 19
0.29	0.29	0.41	0.44	0.53	1.00		Isolate 29
0.27	0.32	0.37	0.39	1.00			Isolate 30
0.36	0.36	0.53	1.00				Isolate 27
0.55	0.55	1.00					Isolate 1
0.81	1.00						Isolate 17

Contrary, the lower similarity was found among isolates from different groups; isolates, 16 and 17 with isolate 8 the similarity was 0.26. Fig (8) and Table (5), using SRAP dendogram supports the similarity data. Other isolates 29 and 30 from Behira

and Gharbia were found in one subcluster with similarity 0.53. These results are in agreement with Ayyadurai *et al*. (2005) who reported that the primers (GF and MR) were able to differentiate between *S. oryzae* isolates collected from different locations or varieties and were different in their virulence. The isolates showed a high variability in pathogenicity, and DNA variation, as evidenced in other plant pathogenic fungi (Manzanares *et al.,* 2001). Results revealed that isolates were independently grouped into two major clusters and further subdivided into different genotypes based on their virulence. All the isolates that originated as aggressive virulence were clustered in one group with high similarity, while other low virulence were clustered as a separate group. Here, this study is considered as a first report on the biological and molecular variability of *S. oryzae* on rice in Egypt to help in breeding programs to develop resistance to avoid the environment fungicides harm or inconsistency of antagonists under field conditions.

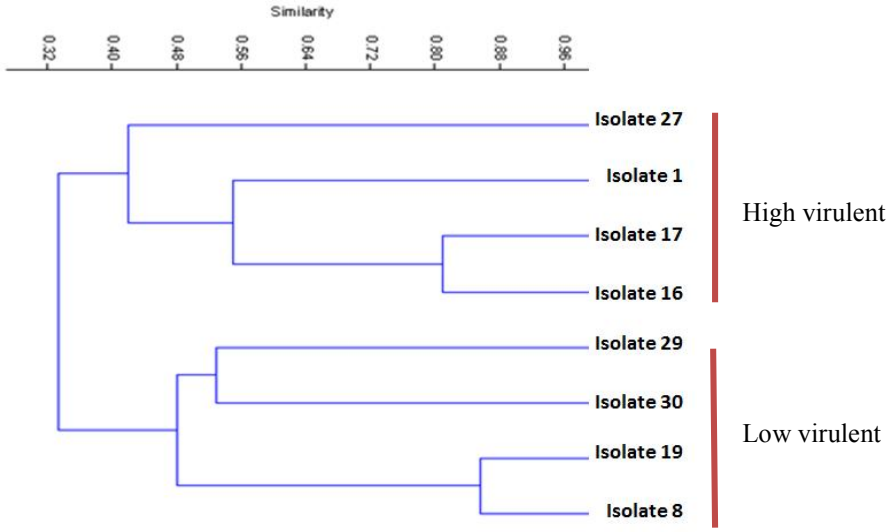

Fig. 8. UPGMA cluster analysis based on Jacarrd using SRAP primers for eight *Sarocladium oryzae* isolates.

A c k n o w l e d g e m e n t s

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R e f e r e n c e s

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الإختلافات البيولوجية والجزيئية لمسبب عفن غمد الأرز ساروكلاديم أوريزا بإستخدام البادئات SCAR و SRAP

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يعتبر مرض عفن الغمد فى الأرز المتسبب عن الفطر *Sarocladium oryzae* من الأمراض المحمولة بالبذرة وهو من الأمراض المحددة لأنتاج الأرز فى بعض مناطق زراعته. ويهدف هذا البحث إلى تقييم بعض التراكيب الوراثية من الارز لمقاومة الأصابة بعفن الغمد بعزلات مختلفة من الفطر وأثر السمية النباتية لها مع توضيح التنوع الوراثى على المستوى الجزيئى وكذا التنوع المورفولوجى و البيولوجى لبعض عزلات الفطر المعزولة من مناطق مختلفة.

ولقد أظهرت النتائج أن صنف سخا 101 أكثر الأصناف مقاومة. ثبط التوكسين تكشف الجذور وأثر سلبيا على انبات حبوب الارز والحشائش إضافة إلى وجود تنوع مورفولوجي ووراثى كبير بين العزلات المختبرة كما أثبت البادئ المتخصص MR باستخدام تكنيك ال SCAR أن هناك حزمتين (1500 bp ، 1400) وجدت فقط فى العزلات ضعيفة المرضية .

و قد يكون استخدام البادئات SCAR و SRAP قادرا على التفرقة بين عزلات الساروكلاديم أوريزا المجمعة من أماكن أو أصناف مختلفة ومختلفة فى قدرتها المرضية حيث صنفت العزلات حسب تركيبها الوراثي وشدتها المرضية الي مجموعتين مجموعة شديده القدرة المرضية واخري ضعيفة ومن ثم قد يكون التنوع الجزيئى للعزلات مهم فى برامج التربية لتطوير وتنمية المقاومة لمرض عفن الغمد.