

DIVERSITY ASSESSMENT AMONG DIFFERENT EGYPTIAN RICE BLAST FUNGUS ISOLATES

EI-Wahsh S. M.¹ and M. H. M. Ammar²

1-Rice Pathology Dept., Plant Pathol. Res. Inst., Agric. Res. Center, Sakha , 33717, Kafr El-Sheikh, Egypt.

2- Rice Biotechnology Laboratory, Rice research and Training Center, Sakha 33717, Kafr El-Sheikh, Egypt

Crossponding auther Email: elwahsh7@yahoo.com

ABSTRACT

Twenty-four purified rice blast isolates of *Pyricularia grisea* were isolated at Rice Pathology lab., RRTC, Sakha Kafr El-Seikh Egypt; thirteen from different rice cultivars, and eleven from different weeds. Those isolates were identified to physiological races using the eight international differential varieties under greenhouse conditions. For rice blast isolates, the results show that the isolates are categorized in six race-groups, i.e. five isolates for race group IA, three from group IB, two isolates from ID race group and one isolate conformed each of IC, IG and II race groups. The isolates obtained from weeds were eight isolates conformed of IG race group and three isolates from ID race groups.

A total of 24 rice blast isolates were analysed for their morphological, geographical and molecular diversity. Two Intron Splice Junction (ISJ) and 9 Sequence Tagged Microsatellite (STMS) markers wer used for the molecular diversity assessment of the tested rice blast isolates. Their was a noticable colnearity detected between haplotype and pathotype with few exceptions noticed. All rice specific markers used were successfully amplified with the fungus isolates, indicating a sufficient level of similarity between the rice and the blast fungus in these genetic loci. All weed isolated were clustered in the middle of the dendrogram except one aggressive isolate which clustered with another rice aggressive isolate, the later one is the isolate broke down the resistance of the leading variety Sakha 101. These results demonstrate the usfulness of molecular diversity in assessment and differentiation of pathogenicity and host specificity among the blast fungus isolats. One dworback of the current investigation is the low number of molecular markers used and this may in partial explains the few exceptions recorded.

Keywords: Blast, *Pyricularia grisea*, Races, Molecular diversity, Markers, Weeds, ISJ, STMS

INTRODUCTION

Rice blast disease caused by *Pyricularia grisea* (Cooke) Saco. is a major disease of rice. Up to the present, most breeders concentrate on the development of the highly resistant cultivars to manage the disease (Ou, 1985; Roumen, 1992). Since 1973, many Egyptian investigators (El-Kazzaz 1973 and Sehly *et al* 2000) identified the physiological races of *Pyricularia grisea* using the international system proposed by Atkins *et al* (1967).. However, breakdown of a resistant cultivars may be due to the development of new race(s) initiated from the high variability of the causal blast fungus as well as to the effect of prevalent environmental conditions and increased cultivated areas (Bunman *et al* 1987 and Sehly *et al*, 2002). Sehly *et al* (2002) evaluated ten purified isolates of *Pyricularia grisea* under greenhouse

conditions on eight international differential varieties and categorized the isolates into seven race-groups, i.e. IA, IC, ID, IB, IG, IH and II races.

Ou (1985) reported that *Pyricularia grisea* parasitizes more than 50 species of grasses and sedges, many of which are common weeds in rice fields. Borromeo *et al* (1993) studied the genetic differentiation among isolates for *Pyricularia* infecting rice and weed hosts. They surveyed some hosts of *Pyricularia grisea* like *Digitaria ciliaria*, *Elusine indica* *Cyperus rotundus* and *Echinochloa colona*.

El-Shafey (2002) obtained fifteen *Pyricularia grisea* isolates from different weeds and identified four races as IG-1 (10 isolates), two isolates for each of IB-57 and IB- 61 and only one was identified as ID-13. Gabr (2004) collected 40 isolates from different rice cultivars and weeds, and identified the isolates as 6 races, i.e. one isolate from each of IB-41, IB-45, IB-57, IB-63 and two isolates from IB-61; one isolate from IC-5 and two isolates from IC-31; one isolate from each of ID-9, ID-13 and ID-15, one isolate for each of IF-3 and IF-4; 23 isolates from IG-1(20 rice isolates and 3 weed isolates), and 3 isolates from IH-1.

The current investigation aimed to differentiate and assess the diversity among 24 different blast fungus isolates differing in their location, host (rice / weeds) and rice variety. The differentiation was carried out using the international differential groups as well as the molecular diversity using a set of 2 Intron Splice Junction (ISJ) and 9 Sequence Tagged Microsatellites (STMS) markers .

MATERIALS AND METHODS

Laboratory and greenhouse studies: -

Thirteen *Pyricularia grisea* isolates were collected and isolated from different rice varieties Giza 159, Giza 171, Reiho, Sakha 101 Sakha 104, IR 10011 and Aichi Asahi grown at different governorates. In addition, eleven isolates were isolated from different weeds; *Echinochloa colona*, *E.crus-galli*, *E.indica* *Elusine indica*, *Cyperus rotundus* and *C. alopecuroides*.

Isolation of rice blast fungus: -

Single conidium isolates were generated by streaking conidia from sporulating lesions on 20% water agar (WA) for 24 hr., then germinated single conidia were picked and transferred to water agar for another 24 hr. The tip of a single hyphae was cut and grown on banana dextrose agar medium (200g banana +15g glucose + 20g agar / 1000 ml water) on a piece of sterile filter paper disc. When the filter papers were completely occupied by the fungal growth, the paper discs were individually transferred into petri dishes. About one week later, the dried filter papers having the fungus isolates were cut into small pieces. Pieces obtained from each isolate were altogether and introduced into a plastic vial and kept at -20 °C for long - term storage (according to the technique of Mekwatanakarn *et al.*, 1999).

Pathogenicity and race identification:

Pathogenicity tests, race identification studies and cross inoculation were carried out for the obtained isolates. Eight international differential

varieties namely: Raminad Str.3, Zenith, NP-125, Usen, Dular, Kanto, CI 8970 S and Caloro (Atkins *et al.*, 1967) were seeded in plastic trays (30 x 20 x15 cm.). Each tray comprised 10 rows representing eight international differential varieties and two susceptible checks (Giza 159 and Giza 171). The trays were kept in the greenhouse at 25-30°C, and fertilized with Urea 46.5%N (5 gm/tray). The plants were inoculated for evaluation under greenhouse condition in each test for each isolate. Seedlings were ready for inoculation at 3-4-leaf stage, about 3-4 weeks after sowing.

For spore production, isolates were grown on banana dextrose agar medium (200g banana, 15g glucose, 15g agar/ 1000 ml water) under florescent light for 10 days at 28 °C for spore production. The spores were harvested at a density of at least 25 spores / microscopic field, examined by 10 x objective. Rice seedlings of 20-day old, in the trays, were inoculated by spraying with water suspension of isolates. Spore suspension(100 ml) was prepared from each isolate and adjusted to 5×10^4 spores/ml. Gelatin was added to the spore suspension at a concentration of 2.5 g/L (Bastiaans, 1993) to enhance the adhesion of spores on leaf surfaces. Each isolate was sprayed using electrical spray gun. The inoculated seedlings were held in a moist chamber with at least 90% R.H. and 25-28 °C for 24 hr, and then moved to the greenhouse. Seven days after inoculation, blast reaction was recorded according to the standard evaluation using 0-9 scale (IRRI 1996) as follows:

- 1-2 = resistant (R)
- 3 = moderately resistant (MR)
- 4-6 = susceptible (S)
- 7-9 = highly susceptible (HS)

DNA extraction:

The twenty four isolates of *P. grisea*, (13 from rice and 11 from weeds) identified by international differential varieties, were inoculated into 50 ml liquid medium of potato dextrose PD (200g P + 15g D) at 25 °C in dark condition with three replications. The mycelia were harvested two weeks after incubation, and stored at -20°C.

Molecular analysis

The twenty-four isolates of *P. grisea* identified by international differential varieties were inoculated into 50 ml. liquid medium of potato dextrose PD (200g P + 15g D) at 25 °C in dark condition with three replications. The mycelia were harvested two weeks after incubation and stored at -20°C. DNA isolation and purification was carried out using CTAB method (Murray and Thompson,1980).

The DNA was quantified using gel assay method and then PCR was performed. A total of eleven pairs of STMS and ISJ primers were used for the screening purpose. The PCR was performed in 10µl PCR volume containing 50 ng of template DNA, 5 pmole of each of forward and reverse primers, 0.1mM dNTP's, 1x PCR buffer (10mM Tris,pH 8.0, 50mM KCl and 50mM ammonium sulphate), 1.8mM MgCl₂, and 0.2 units of Taq DNA polymerase. Initial denaturation at 94oC for 5 minutes was followed by 35 cycles of amplification with template denaturation at 94oC for 1 minute, primer annealing at 55.7oC for 1 min and primer extension at 72oC for 2 min.

After the end of the 35th cycle, a final extension at 72oC for 7 min was given followed by storage at 4.0 oC. The PCR products were separated using 1.5% agarose gel stained with Et Br solution (1 mg/l). The banding pattern was then scored and used to prepare the matrix. Employing the computer package NTSYS .pc (Ralf, 1998), Jaccord's similarity coefficients were calculated and used to establish genetic relationship among the genotypes based on unweighted pair group method of arithmetic averages (UPGMA) and sequential agglomerative hierarchical nested (SAHN) clustering.

Table (1): list of ISJ and STMS primers used in the current study.

	Name	Forward Sequence	Reverse Se
1	ISJ 6	ACTTACCTGAGCCAGCGA	
2	ISJ 9	AGGTGACCGACCTGCA	
3	RM 527	GGCTCGATCTAGAAAATCCG	TTGCACAGGTTGCGATAGAG
4	RM 2 64	GAGCTCCATCAGCCATTCAG	CTGAGTGCTGCTGCGACT
5	RM8094	AAGTTTGTACACATCGTATACA	CGCGACCAGTACTACTACTA
6	RM223	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG
7	RM315	GAGGTACTTCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG
8	RM294	TTGGCCTAGTGCCCTCAATC	GAGGGTACAACCTTAGGACGCA
9	RM5699	ATCGTTTCGCATATGTTT	ATCGGTAAAAGATGAGCC
10	RM186	TCCTCCATCTCCTCCGCTCCCG	GGGCGTGGTGGCCTTCTTCGTC
11	RM36	CAACTATGCACCATTGTCCG	GTA CTCCACAAGACCGTACC

RESULTS AND DISCUSSIONS

Twenty-four purified isolates of *Pyricularia grisea* were isolated thirteen from different rice cultivars, and eleven from different weeds. Those isolates were identified to races using the eight international differential varieties. For rice blast isolates, data in Table (2) show that the isolates are categorized in six race-groups, i.e. five isolates for group IA race, three from group IB, two isolates from ID group race and one isolate conformed each of IC, IG and II group races. The isolates obtained from weeds Table (3) were eight isolates conformed of IG race group and three isolates from ID group races. Bidaux (1976) and Notteghem (1981) observed that virulent strains existed for all the identified genes of vertical resistance and most of the strains possessed virulent genes, which were not necessary for their survival. Sehly *et al* (2000) inoculated forty-five isolates of *P. grisea* on eight international differential varieties. The most common races were IH-1 (36.6%), I D-race group (17.8%), I A (13.3%), I G-1 (13.3%) and a virulent race group II (9.0%).

Table (2): Race identification of different single isolates of *Pyricularia grisea* from rice on international differential varieties under greenhouse conditions at seedling stage:

No.	Location	1	2	3	4	5	6	7	8	9	10	11	13	14
		Sakha	Beheira	Abu Kabier	Sakha	Sakha	Dakahlia	Sakha	Dakahlia	Sharkia	Garbia	Gharbia	Shobr-akhet	Gharbia
	Cultivar	Giza 159	Sakha 101	G,171	Giza 159	Sakha 104	Aicch Asahi	IR10011	Reiho	Giza 171	Giza 171	Giza 171	G 171	Giza 159
1	Raminad	S	R	S	R	R	S	S	R	R	MR	R	S	R
2	Zenith	R	R	R	S	S	R	S	R	S	R	R	R	R
3	NP-125	S	R	R	R	MR	R	R	R	S	MR	S	S	MR
4	Usen	S	R	R	MR	S	S	S	R	MR	S	S	S	S
5	Dular	S	R	R	R	R	R	R	R,R	R	S	MR	R	S
6	Kanto 51	R	R	S	R	MR	MR	R	MR	R	MR	MR	MR	S
7	CI 8970 S	S	S	S	S	S	MR	S	R	S	R	S	R	S
8	Caloro	HS	HS	HS	S	HS	S	HS	R	S	S	S	S	S
Race		IA - 69	IG-1	IA-27	IB - 63	IB-45	IA-111	IA - 45	II	IB - 31	ID - 7	IC - 13	IA-79	ID-3

R = Resistant reaction, MR = moderately resistant, S = Susceptible HS = Highly susceptible

Table (3): Race identification of different single isolates of *Pyricularia grisea* from weeds on international differential varieties under greenhouse conditions at seedling stage:

No.	Location	15	16	17	18	19	20	21	22	23	25	26
		Gharbia	Gharbia	Sakha	Gemmiza	Beheira	Beheira	Beheira	Sakha	Gemmiza	Sarkia	Beheira
	Cultivar	<i>Echinochloa indica</i>	<i>Echinochloa colona</i>	<i>Echinochloa crus-galli</i>	<i>Echinochloa colona</i>	<i>Elusine indica</i>	<i>Echinochloa colona</i>	<i>Elusine indica</i>	<i>Elusine indica</i>	<i>Cyperus rotundus</i>	<i>Elusine indica</i>	<i>Elusine indica</i>
1	Raminad	R	R	R	R	R	R	R	R	R	R	R
2	Zenith	R	R	R	R	R	R	R	R	R	R	R
3	NP-125	R	R	R	R	S	R	S	R	R	R	R
4	Usen	R	R	R	R	S	R	R	S	R	R	S
5	Dular	R	R	MR	R	R	R	R	R	R	R	R
6	Kanto 51	R	MR	R	R	R	R	R	R	R	R	R
7	CI 8970 S	S	S	R	S	S	S	S	S	S	S	S
8	Caloro	S	S	S	HS	S	S	S	HS	S	S	HS
Race		IG-1	IG-1	IG-1	IG-1	IG-1	IG-1	ID - 1	ID - 3	IG - 1	IG-1	ID-13

R = Resistant reaction, MR = moderately resistant, S = Susceptible HS = Highly susceptible

El-Shafey (2002) collected eight rice blast isolates from rice cultivars and identified them by the international differential varieties. He identified the isolates as six races, i.e. two isolates from each IB-45 and IG-1, and one isolate for each of IB-13, IC-1, IC-5 and ID-1. In addition, he isolated fifteen isolates of *P. grisea* from different weed species, the isolates were divided into four race groups, the common race was IG-1 (10 isolates), two isolates from each of IB-57 and IB-61 and one identified as ID-13. Gabr (2004) collected 37 blast isolates from rice and three from weeds, from different locations. He identified those isolates as six race groups, IB, IC, IF, IG and IH from rice, while one race group from weeds as IG. The most common races obtained from rice was race group IG (57.5% of the total races), IB race group represented 15%, IG, ID and IH race groups represented 7.5% for each, while IF race group represented 5%. Mackill and Bonman (1986) surveyed several isolates attacking rice and grass weeds. Some grasses were not susceptible to *P. grisea* isolated from rice although isolates of grasses readily infected their original hosts.

The molecular analysis data proved that their was significant amount of polymorphism among the tested isolates . Fig. 1 shows the banding pattern for the 24 isolates using ISJ 9. Genetic similarity index between different pairs of isolates are presented in Table 4. based on this similarity index, a dendrogram of the genetic relationship among the tested isolates was constructed using Jaccard's coefficient (Fig. 2). The level of polymorphism detected using ISJ primers were quite higher than that of STMS. This was due to the fact that ISJ gives a higher number of bands / marker than that of STMS due to the higher probability of ISJ to occur in multilocus fashion . ISJ 6 showed 8 different bands and ISJ 9 showed 6 bands (Fig. 1). Meanwhile the STMS markers considered a good tool for diversity assessment due to their high level of polymorphism (McCouch *et al*, 1988, 2002) STMS possessed a good level of polymorphism in the current investigation with number of alleles ranging from 1 with RM527 (Rice Microsatellite) to four alleles with RM264. The results showed clearly that their is quite high similarity between the rice and blast sequenes allowing the rice markers to be utilized for the fungus molecular analysis. The isolates number 1 (IA-69), 3 (IA-27) and 7 (IA-45) were identical as far as the molecular similarity is concerned with similarity percentage of 100% (Table 4). The three isolates infects the old traditional rice varieties Giza 159, Giza 171 and IR 10011. The same case holded also true with isolates 6 (IA-111) and 13 (IA -79) with 100 % similarity.

The isolates 19 (IG-1) and 25 (IG-1) were also identical, both of these isolates are isolated from weeds. The similarity percentage ranged from 54% to 100 %. The fingerprint patterns for The 24 tested isolates thus revealed the persence of 20 haplotypes. At 54% similarity, two main lineages were observed, the first one contains only two isolates, 2 and 20 , both are belonging to IG-1group and isolated from one location, Behira governrate. Isolate 2 isolated from rice variety Sakha 101 and isolate 20 from weeds (*E. colona*) at the same location . the two isolates have79% similarity and considered one of the most aggressive races that attached the leading rice variety sakha 101.

Table (4).Genetic similarity index between pairs of the tested blast fungus isolates.

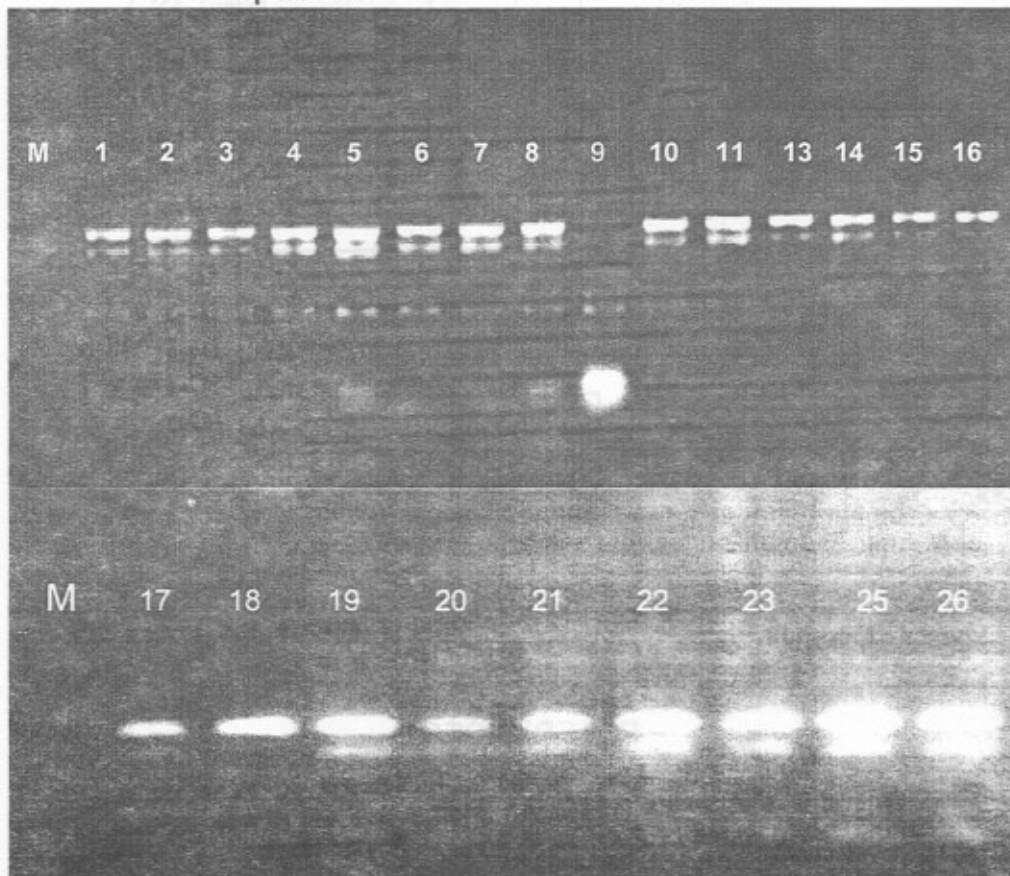
	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8	Isolate 9	Isolate 10	Isolate 11	Isolate 13	Isolate 14	Isolate 15	Isolate 16	Isolate 17	Isolate 18	Isolate 19	Isolate 20	Isolate 21	Isolate 22	Isolate 23	Isolate 25	Isolate 26
Isolate 1	1.0000																							
Isolate 2	0.6316	1.0000																						
Isolate 3	1.0000	0.6316	1.0000																					
Isolate 4	0.8421	0.5263	0.8421	1.0000																				
Isolate 5	0.8421	0.5263	0.8421	0.8947	1.0000																			
Isolate 6	0.9474	0.6316	0.9474	0.8947	0.7895	1.0000																		
Isolate 7	1.0000	0.6316	1.0000	0.8421	0.8421	0.9474	1.0000																	
Isolate 8	0.9474	0.6316	0.9474	0.7895	0.8947	0.9474	1.0000																	
Isolate 9	0.6842	0.3684	0.6842	0.6316	0.5263	0.7368	0.8642	0.6316	1.0000															
Isolate 10	0.7895	0.5263	0.7895	0.7368	0.6316	0.8421	0.7895	0.7368	0.7895	1.0000														
Isolate 11	0.8947	0.6316	0.8947	0.8421	0.7368	0.9474	0.8947	0.8421	0.6842	0.8947	1.0000													
Isolate 13	0.9474	0.6316	0.9474	0.8947	0.7895	1.0000	0.9474	0.8947	0.7368	0.8421	0.9474	1.0000												
Isolate 14	0.7895	0.6316	0.7895	0.7368	0.6316	0.8421	0.7895	0.7368	0.5789	0.7894	0.8947	0.8421	1.0000											
Isolate 15	0.8421	0.5789	0.8421	0.7895	0.6842	0.8947	0.8421	0.7895	0.6316	0.8421	0.9475	0.8947	0.8421	1.0000										
Isolate 16	0.8947	0.5789	0.8947	0.8421	0.7368	0.9474	0.8947	0.8421	0.6842	0.7895	0.8947	0.9474	0.7895	0.9474	1.0000									
Isolate 17	0.8947	0.5789	0.8947	0.8421	0.7368	0.9474	0.8947	0.8421	0.7895	0.7895	0.8947	0.9474	0.7895	0.8421	0.8947	1.0000								
Isolate 18	0.8421	0.5263	0.8421	0.7895	0.6842	0.8947	0.8421	0.7895	0.8421	0.7368	0.8421	0.8947	0.7368	0.7865	0.8421	0.9474	1.0000							
Isolate 19	0.8421	0.5263	0.8421	0.8421	0.7368	0.8947	0.8421	0.7895	0.7368	0.7368	0.8421	0.8947	0.7368	0.7895	0.8421	0.9474	0.8947	1.0000						
Isolate 20	0.5263	0.7895	0.5263	0.4737	0.4737	0.5263	0.5263	0.5263	0.3684	0.4211	0.5263	0.5263	0.4737	0.4737	0.4737	0.5789	0.5263	0.6316	1.0000					
Isolate 21	0.7368	0.5263	0.7368	0.5789	0.6842	0.6842	0.7368	0.7895	0.5263	0.5263	0.6316	0.6842	0.6316	0.5789	0.6316	0.7368	0.6842	0.6842	0.5789	1.0000				
Isolate 22	0.7368	0.4737	0.7368	0.6316	0.7368	0.6842	0.7368	0.7895	0.5263	0.6316	0.7368	0.6842	0.6316	0.6842	0.6316	0.7368	0.6842	0.7895	0.5789	0.6842	1.0000			
Isolate 23	0.8947	0.5263	0.8947	0.7895	0.7895	0.8421	0.8947	0.8421	0.6842	0.6842	0.7895	0.8421	0.6842	0.7368	0.7895	0.8947	0.8421	0.9473	0.6316	0.7368	0.8421	1.0000		
Isolate 25	0.8421	0.5263	0.8421	0.8421	0.7368	0.8947	0.8421	0.7895	0.7368	0.7368	0.8421	0.8947	0.7368	0.7895	0.8421	0.9474	0.8947	1.0000	0.6316	0.6842	0.7895	0.9474	1.0000	
Isolate 26	0.7894	0.4737	0.7895	0.7895	0.6842	0.8421	0.7895	0.7368	0.6842	0.6842	0.7895	0.8421	0.6842	0.7368	0.7895	0.8947	0.8421	0.9474	0.5789	0.6316	0.7368	0.8947	0.9474	1.0000

The second lineage contained the rest of 22 isolates and further subdivided into 7 more lineages at 90 % similarity. The first lineage (from top to bottom , Fig. 2) contained 9 isolates, representing 6 haplotypes . these 9 isolates belonged to IA (5 isolates), II(one isolate) , IC (one isolate) and two isolates of IG type . all isolates except IG were isolated from rice while IG isolates were isolated from weeds (isolates 15 and 16) The second lineage had five haplotypes and six isolates and all this lineage were isolated from weeds from different locations, not from rice . The third lineage had 2 isolates (4 and 5) that attack rice varieties Giza 159 and Sakha 104, respectively. The two isolates belonged to IB group of which IB-45 considered an aggressive race against the newly released rice variety Sakha 104. The fourth lineage contained only one isolate (14) that is aggressive against giza 159 at Gharbia governorate. The fifth lineage consisted of two isolates (9 and 10) that attacks rice variety Giza 171 at Sharkia and Gharbia governorates, respectively. The two isolates had 79% similarity percentage and represent IB-31 and ID-7 (Table 1). The sixth and seventh lineages represented by one isolate each (Isolates 22 and 21) , both the isolates are weeds isolates from Sakha, Kafr El-Sheikh and Behira governorates and belonged to ID-3 and ID-1, respectively . The clustering of the tested isolates showed inconsistency with their locations in most cases. Thus, some isolates are presented in more than one location and more than isolate were found in one location .For example, isolates no. 1, 17, 4, 5, and 22 are from Sakha though their differences and presence in different groups. This clearly indicate the inability of molecular diversity assessment to reflect the geographical diversity of the blast fungus isolates. On the other hands, the clustering was in agreement with race types as we can see from the dendrogram that most of IA isolates has clustered together(i.e, isolates 1, IA-69; 3, IA-27; 7, IA-45;6, IA-111; 13, IA-79) are all derived for the same lineage at 90% similarity with few exceptions, viz, isolate 8, II and both isolate 15 and 16 that were emerged as a new lineage at >90% similarity level. The same observation may hold true for IG group isolates as we can notice from Fig.2, isolates number 15, 16, 17, 18, 25, 23, are all IG group and they laid near by each other, only isolates 2 and 20 are the exceptions . ID group (has clustered together, with a very minor exceptions detected. The other clear point from the data was that the differentiation between isolates based on their host, as we can see that most of weed isolates has gathered together in specific clusters. One exception was noticed with isolate 20 from weeds that was clustered with isolate 2, the aggressive isolate that attacks the leading variety Sakha 101 . It is worth noting that these two isolates have a common origin and diversified from each other . Both of them were collected from the same location, and even had the same lesion morphology and both are quite aggressive on their specific hosts . This may explain their presence in one separate group and their high level of diversity from all other haplotypes.

In conclusion, There was a noticeable colinearity detected between haplotype and pathotype with few exceptions noticed. All rice specific markers used were successfully amplified with the fungus isolates, indicating a sufficient level of similarity between the rice and the blast fungus in these genetic loci. All weed isolates were clustered in the middle of the dendrogram

except one aggressive isolate which clustered with another rice aggressive isolate, the later one is the isolate broke down the resistance of the leading variety Sakha 101. These results demonstrate the usefulness of molecular diversity in assessment and differentiation of pathogenicity and host specificity among the blast fungus isolates. The results obtained here were in coherence with that of Mishra *et al.* (2006). Who found that rice blast isolates populations clustered differently based on their host varieties. Also, Chen *et al.* (2006) studied that *M. grisea* populations cannot be delineated into region-specific groups which is the same as our results reveal.

Fig.(1): Banding pattern of the tested rice blast isolates using Intron Splice Junction (ISJ 9) bands were separated using 1.5% agarose gel, The numbers are the isolate numbers and M is 100 bp ladder.



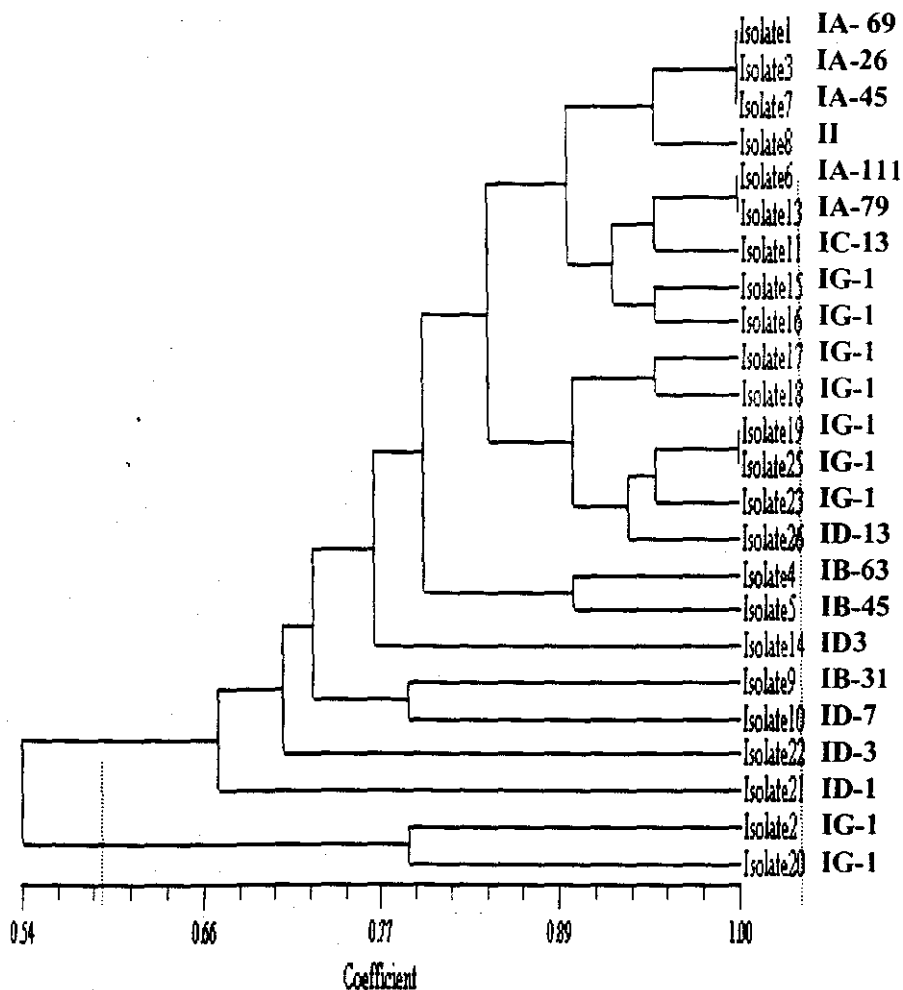


Figure (2). Dendrogram of genetic relationship among the tested isolates using Jaccard's coefficient.

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تقدير الاختلافات الوراثية بين عزلات مختلفة لفطر اللفحة تحت الظروف المصرية
صلاح محمود الوحش¹ و مجاهد حلمي عمار²
١- قسم بحوث أمراض الأرز- معهد بحوث أمراض النباتات مركز البحوث الزراعية - جيزة
٢- معمل الأرز للتكنولوجيا الحيوية - مركز البحوث والتدريب في الأرز - معهد بحوث
المحاصيل الحقلية - مركز البحوث الزراعية - جيزة

تم عزل أربعة وعشرون عزلة لفطر *Pyricularia grisea* المسبب لمرض اللفحة في الأرز، بمعمل قسم بحوث أمراض الأرز مركز البحوث والتدريب في الأرز - سخا كفر الشيخ - مصر. من بين هذه العزلات ثلاث عشر عزلة تم عزلهم من أصناف أرز مختلفة. واحد عشر عزلة تم عزلهم من أصناف حشائش مختلفة. تم تعريف السلالات الفسيولوجية لهذه العزلات على الأصناف العلمية المفترقة تحت ظروف العدوي الصناعية بالصوبة. وقد أظهرت النتائج المتحصل عليها إلي: انقسمت العزلات المنعزلة من نباتات الأرز إلي تحت ستة سلالات فسيولوجية (race-groups)، خمسة تقع تحت (IA race-group)، ثلاثة تحت (IB race group)، واثنين تحت (ID race group) وواحدة تحت كل من (IC, IG and II race groups) أما بالنسبة للعزلات المعزولة من الحشائش فقد أظهرت النتائج أن ثمانية عزلات تقع تحت (IG race group) وثلاثة تحت (ID race group).

تم تحليل ٢٤ عزلة لفطر اللفحة من ناحية الاختلافات المظهرية، الجغرافية والجزيئية باستخدام ٢ بادئ جزيئي ISJ، ٩ بادئ جزيئي STMS وقد وجد أن هناك درجة ملحوظة من التوافق بين القدرة المرضية ودرجة القرابة الوراثية للعزلات.

وكل البوادئ المستخدمة هي بوادئ متخصصة للأرز ونجاح PCR يعنى وجود درجة من التشابه الوراثي بين الفطر والأرز يسمح بذلك. وقد تم عمل شجرة القرابة الوراثية وأظهرت نتائجها تجمع عزلات الحشائش في وسط الشجرة باستثناء عزلة واحدة تجمعت مع عزلة أرز وكلاهما شديد القدرة لإصابة الأرز والحشائش وتلك العزلة للأرز هي المسنولة عن كسر المقاومة للصنف سخا ١٠١، وأظهرت هذه الدراسة أهمية استخدام درجة التباين على المستوى الجزيئي لتقسيم وتوصيف درجة الشدة المرضية لعزلات الفطر المختلفة