J. Agric. Sci. Mansoura Univ., 32 (5): 3791 - 3802, 2007

DIVERSITY ASSESSMENT AMONG DIFFERENT EGYPTIAN RICE BLAST FUNGUS ISOLATES

El-Wahsh S. M.[1] and M. H. M. Ammar[2]

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 colenearity detected between haplotype and pathotype with few exceptions noticed. All rice specific markers used were successfully amplified with the fungus isolates, indicating a sufficent level of similarity between the rice and the blast fungus in these genetic loci. All weed isolated were clusterd in the middele of the dendrogram except one agressive isolate which clustered with another rice agressive isolate, the later one is the isolate broke down the resistance of the leading variety Sakha 101. These results demonstrate the usfelness 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. alopcuroides*.

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 ^{o}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^{o}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 ^{o}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 x 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 ^{o}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 ^{0}C in dark condition with three replications. The mycelia were harvested two weeks after incubation, and stored at -20^{0}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 ^{0}C in dark condition with three replications. The mycelia were harvested two weeks after incubation and stored at -20^{0}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	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG
8	RM294	TTGGCCTAGTGCCTCCAATC	GAGGGTACAACTTAGGACGCA
9	RM5699	ATCGTTTCGCATATGTTT	ATCGGTAAAAGATGAGCC
10	RM186	TCCTCCATCTCCTCCGCTCCCG	GGGCGTGGTGGCCTTCTTCGTC
11	RM36	CAACTATGCACCATTGTCGC	GTACTCCACAAGACCGTACC

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%).

T2-3

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 condstructed using Jaccord`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 agressive races that attached the leading rice variety sakha 101.

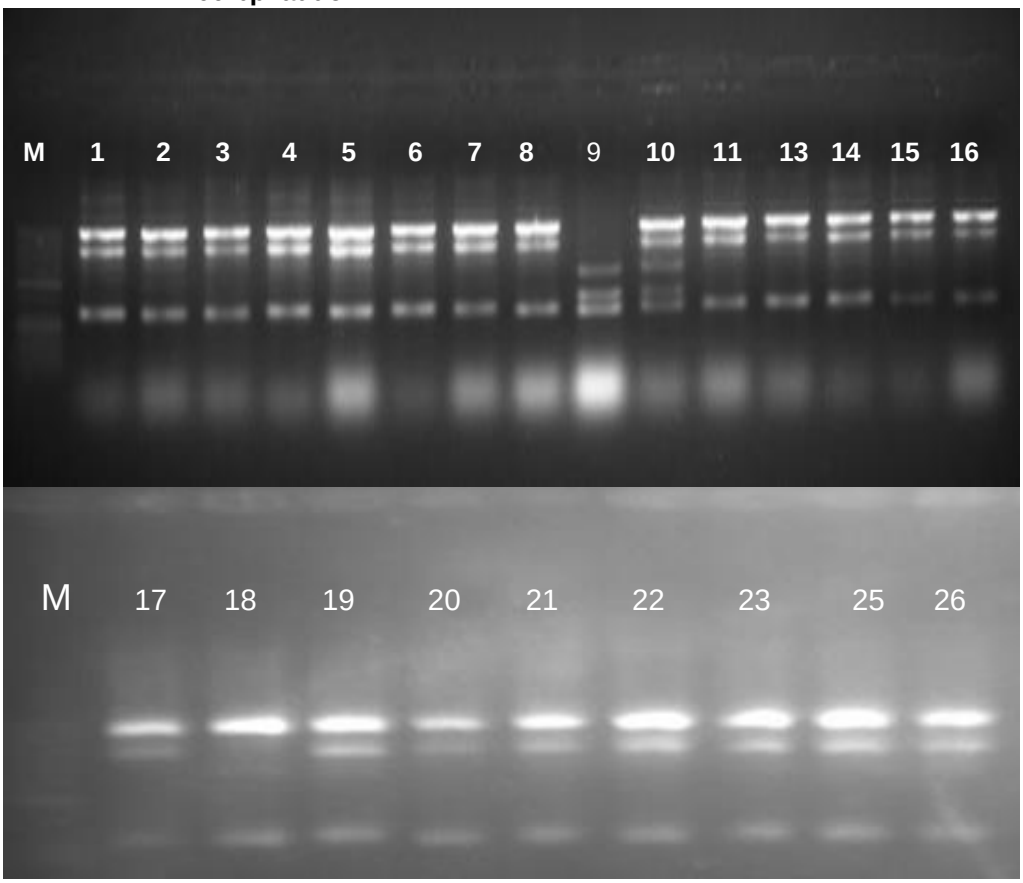
T4

The second lineage containd the rest of 22 isolatsand 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 verieties Giza 159 and Sakha 104,respectively. The two isolates belonged to IB group of which IB-45 considered an agressive race againest the newly released rice variety Sakha 104. The fourth lineage contained only one isolate (14) that is agressive againest giza 159 at Gharbia governrate.The fifth lineage consisted of two isolates (9 and 10) that attacks rice varietiey Giza 171 at Sharkia and Gharbia governrates, 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 governrates and belonged to ID-3 and ID-1, respectively . The clustering of the tested isolates showed incostenscey 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 worthnoting that these two isolates have a common origin and diversed from each other . Both of them were collected from the same location, and even had the same legion 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, Their was a noticeable colenearity detected between haplotype and pathotype with few exceptions noticed. All rice specific markers used were successfully amplified with the fungus isolates, indicating a sufficent 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 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 thetested 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.



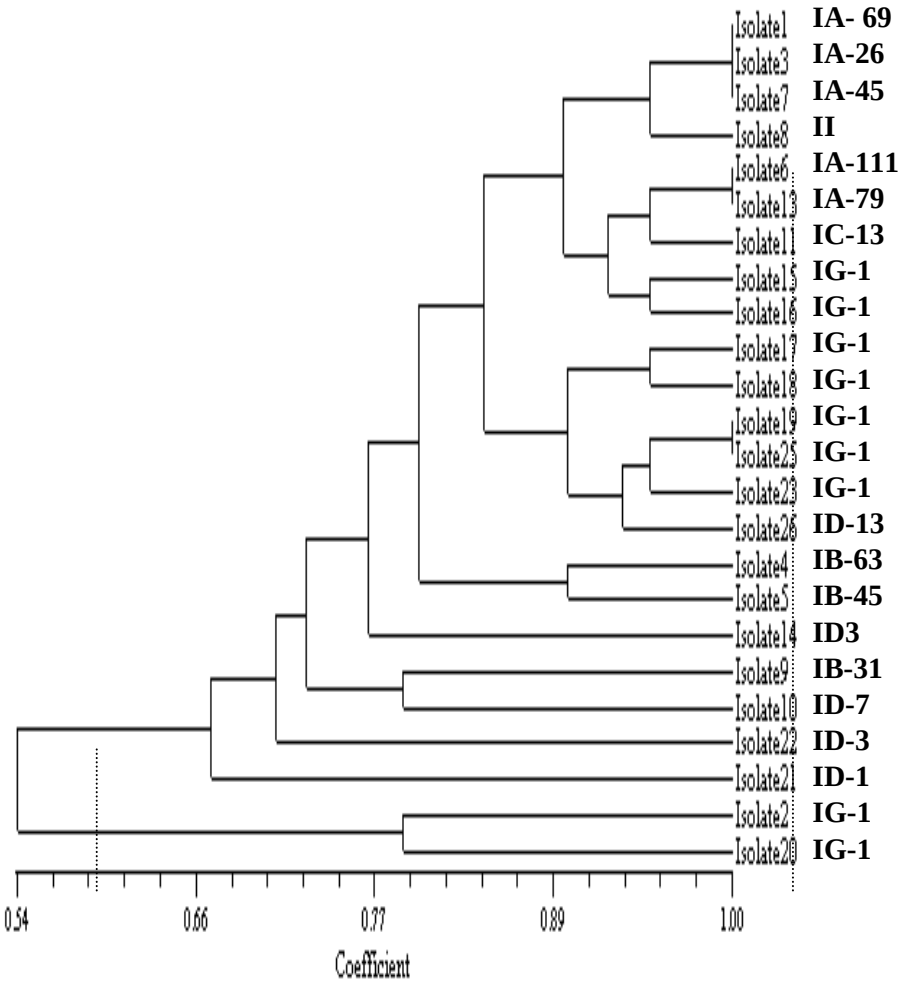


Figure (2). Dendrogram of genetic relationship among the tested isolates using Jaccord`s coefficient.

REFERENCES

Atkins, J.G.; A.L. Rebert; C.R. Adsir; K. Goto; T. Kozako; R. Yanagida; Y. Yamada and S. Matsumoto (1967). An international set of rice varieties for differentiating races of *Pyricularia oryzae*. Phytopathology, 57: 298-301.

Bastiaans, L. (1993). Effects of leaf blast on photosynthesis of rice.1- Leaf photosynthesis. Netherlands Journal of Plant Pathology 99: 197-203.

Bidaux J.M. (1976). Screening for horizontal resistance to rice blast *Pyricularia oryzae* in Africa. Academic Press, London, 159-172.

Borromeo, E.S.; R.J. Nelson; J.B. Bonman and H. Leung (1993). Genetic differentiation among isolates of *Pyricularia oryzae* infecting rice and weed hosts. 83(4): 393-399.

Bunman, T.M.; T.I. Vergel De Dios; J.M. Bandong and E.I. Lee (1987). Pathogenic variability of monoconidial isolates of *Pyricularia oryzae* in Korea and in the Philippines. Plant Disease, 71 (2): 127-130.

Chen Q. H.; Y. C. Wang and X. B. Zheng (2006) Genetic Diversity of Magnaporthe grisea in China as Revealed by DNA Fingerprint Haplotypes and Pathotypes. J. Phytopathology 154: 361 –369

El-Kazzaz, M.K. (1973). Studies on diseases affecting rice crop in Eygpt. Ph.D. Thesis, Fac. Agric., Alexandria Univ

El-Shafey, R.A. (2002). Studies on *Pyricularia oryzae* the causal organism of rice blast disease and its control. M.Sc. Thesis, Fac. Agric. Monufeia University.

Gabr, W.E. (2004). Studies on rice blast disease in Egypt. MSc. Thesis. Fac. of Agric., Tanta University.

IRRI (1996). Standard evaluation system for rice . 3rd ed. IRRI. Los Banos, Philippines.

Mackill, A.O. and J.M. Bonman (1986). New hosts of *Pyricularia oryzae*. Plant Disease., 70: 125-127.

McCouch S.R. , G. Kochert, Z.Y. Yu and G.S. Khush (1988). Molecular mappingof rice chromosomes. Theor. Appl.Genet. 76:815-829.

McCouch S.R., L. Teytelman, Y. Xu, K.B. Lobos, K. Clare, M. Walton, B. Fu, R. Maghirang, Z. Li, Y. Xing, Q. Zhang, I. Kono, M. Yano, R. Fjellstorm, G. DeClerck, D. Schneider, S. Cartinhour, D. Ware and L. Stein (2002). Development and mapping of 2240 new SSR markers for rice (Oryza sativa L.). DNA Research 9: 199-207.

Mekwatanakarn, P.; W. Kositratana; T. Phromraksa and R.S. Zeigler (1999). Sexually feriole *Magnaporthe grisea* rice pathogens in Thailand. Plant Disease. 83: 939-943.

Mishra, D.;U.D. Singh; A. B. Dash; J. N. Reddy and R. Sridhar (2006). Analysis of Pyricularia grisea populations from three different blast epidemics. IRRN, 31(1): 22-24.

Murray M.G. and W.F. Thompson (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research, 8: 4321-3425.

Notteghem J.L. (1981). Analyse resultats dinoculation de 67 varieties de riz par 15 souches de *Pyricularia oryzae*. In Proceeding of the syposium on rice resistance to blast, IRAT, Montpellier, pp:301-318.

Ou, S.H. (1985). Rice disease. Commonwealth Agric. Bureau. 380 pp. U.K.

Ralf F.J. (1998). *NTSYS-PC Version 2.0*, Exeter software, Setauket, New York, USA

Roumen, E.C. (1992). Effect of leaf age on components of partial resistance in rice to blast. Euphytica 63: 271-279.

Sehly, M.R.; Z. H. Osman and E.A. Salem (2002). Rice diseases In: T. Castello, pp 198-247. Rice in Egypt. Rice Research and Training Center, Sakha, Kafr El-Sheikh, Egypt.

Sehly. M. R.; Osman, Z. H.; Salem E. A., Badr, E. A. S. and S. M. El- Wahsh (2000). Proceedings of the fourth. Nrational Rice Research and Development Program Workshop, 12-13 Feb.

تقدير الإختلافات الوراثية بين عزلات مختلفة لفطر اللفحة تحت الظروف المصرية

صلاح محمود الوحش [١] و مجاهد حلمي عمار [٢]

١- قسم بحوث أمراض الارز- معهد بحوث أمراض النباتات مركز البحوث الزراعية - جيزة

٢- معمل الارزللتكنولوجيا الحيوية – مركز البحوث والتدريب في الارز - 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 كفرالشيخ - مصر. من بين هذه العزلات ثلاث عشر عزلة تم عزلهم من أصناف أرز مختلفة. واحدي عشر عزلة تم عزلهم من أصناف حشائش مختلفة. تم تعريف السلالات الفسيولوجية لهذه العزلات علي الأصناف العلمية المفرقة 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 الإختلافات المظهرية ,الجغرافية والجزيئية بإستخدام ٢ بادئ جزيئى ISJ, ٩ بادئ جزيئى STMS وقد وجد أن هناك درجة ملحوظة من التوافق بين القدرة المرضية ودرجة القرابة الوراثية للعزلات .

وكل البوادئ المستخدمة هى بوادئ متخصصة للأرز ونجاح PCR يعنى وجود درجة من التشابه الوراثى بين الفطر والأرز يسمح بذلك . وقد تم عمل شجرة للقرابة الوراثية وأظهرت نتائجها تجمع عزلات الحشائش فى وسط الشجرة بإستثناء عزلة واحدة تجمعت مع عزلة أرز وكلاهما شديد القدرة لإصابة الأرز والحشائش وتلك العزلة للأرز هى المسئولة عن كسر المقاومة للصنف سخا ١٠١ , وأظهرت هذه الدراسة أهمية إستخدام درجة التباين على المستوى الجزيئى لتقسيم وتوصيف درجة الشدة المرضية لعزلات الفطر المختلفة .

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	M R	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	M R	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	M R	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	*Echinochloa indica*	*Echinochloa colona*	*Echinochloa crus-galli*	*Echinochloa colona*	*Elusine indica*	*Echinochloa colona*	*Elusine indica*	*Elusine indica*	*Cyperus rotundus*	*Elusine indica*	*Elusine indica*
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	M R	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

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

	Isol-ate 1	Isol-ate 2	Isol-ate 3	Isol-ate 4	Isol-ate 5	Isol-ate 6	Isol-ate 7	Isol-ate 8	Isol-ate 9	Isol-ate 10	Isol-ate 11	Isol-ate 13	Isol-ate 14	Isol-ate 15	Isol-ate 16	Isol-ate 17	Isol-ate 18	Isolate 19	Isol-ate 20	Isol-ate 21	Isol-ate 22	Iso-late 23	Isol-ate 25	Isol-ate 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	0.8947	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

3791 3792 3793 3794 3795 3796 3797 3798 3799 3800 3801 3802