VEGETATIVE COMPATIBILITY GROUPS OF PHYSIOLOGICAL RACES OF *PYRICULARIA GRISEA* IN Egypt

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Abstract

One hundred forty *Pyricularia grisea* isolates were collected from the six main rice growing governorates; Kafrelsheikh, Beheira, Sharkia, Gharbia, Dakahlia and Damietta, and from different research stations during 2008, 2009 and 2010 growing seasons. The isolates were identified to their group races as IA (1); IB (12); IC (15); ID (45); IE (1); IF (4); IG (22); IH (32) and II (8) using the international differential varieties under greenhouse conditions. Vegetative compatibility groups (VCGs) among the races were studied through producing nitrate nonutilizing (*nit*) mutants for the races on chlorate minimal medium (CMM). The results revealed that the *nit* mutants were grouped into eight VCGs. VCG 1 included members of the IB race group (70.59 %). VCG 2 included members of IC race group with 87.5% and two isolates were identified as IH and II. The largest group, VCG 3 included 44 isolates, most of these isolates were identified as ID (86.36 %). All isolates in VCG 4 were identified as IF. 95.65% of isolates in VCG 5 represented IG and one belonged to II (4.35%). All isolates in VCG 6 corresponded to IH (96.43 %), and only one as ID. VCG 7 included eight isolates, 5 isolates represented ID and one each of IG, IF and IH. Only one isolate was identified as IE race group in VCG 8. The reaction among different *nit* mutants was examined using scanning electron microscope and revealed that heterokaryon, incompatibility and inhibition zone were observed. This investigation revealed that the correspondence between VCGs and races ranged from 62.5 to 100 %; however, the latter percentage was relevant to very small number of isolates.

Key words: Rice blast, *Pyricularia grisea*, VCGs, Heterokarion and Egypt.

INTRODUCTION

Rice (*Oryza sativa* L.) is the second staple food crop after wheat in Egypt, and is very important for local consumption, and for exportation. Paddy production is about 5 million tons and the national yield productivity is about 4.03 t/feddan (9.6 t/ha) (RRTC, 2011). The rice blast is widespread and damaging disease in most rice growing areas of the world (Ou, 1985). Under Egyptian conditions, rice blast disease may affect annual rice production by about 5% in normal or mildly infected seasons. In epidemic seasons, yield losses may reach as high as 30-50% (Sehly *et al.,* 2002). Vegetative compatibility grouping (VCG) is a useful tool for identifying genetic diversity among fungal isolates (Correll *et al.,* 1987; Leslie, 1993 and Fourie *et al.,*

2009). Many fungi can convert nitrate to ammonium by the action of nitrate reductase (Garraway and Eveans, 1984). Meanwhile, nitrate reductase can convert chlorate to chlorite, a fungitoxic substance. So, wild strains which can assimilate nitrate, are sensitive to chlorate, but nitrate nonutilizing *(nit)* mutants are resistant to chlorate. Many investigators studied the race population using VCGs as a means to measure the pathogen diversity. The multiple-locus base for the VCG subdivisions means that applying one test, the relationship at multiple loci is being assessed and permit to determine identities relatively quickly (Desjardins *et al*., 1992). However, some others reported that VCGs are not useful in determining the degree of relatedness if the two isolates were not identical (Leslie, 1993). Busso *et al*. (2007) studied VCGs among *P. grisea* isolates which were isolated from wheat. Mutants which were unable to use sodium nitrate as nitrogen source, were obtained with potassium chlorate and were divided into two VCGs. Correll *et al*. (2009) characterized isolates of *P. oryzae* using different tests including DNA fingerprinting with MGR 586 and vegetative compatibility test. These isolates were classified into four distinct VCGs with complete correspondence of the four MGR 586 DNA fingerprinting groups. Motallebi *et al*. (2009) studied VCGs in 75 monoconidial isolates of *Magnaporthe grisea* strains complex obtained from rice and some grasses from north Iran. All the isolates and eight standard mating type tester isolates were analyzed with complementation tests using *nit* mutants to determine VCGs and genetic relationship between the two groups of isolates. All rice isolates were grouped into four VCGs, while all grasses isolates belonged to three VCGs. In addition, there was no correlation between field isolates and standard mating type isolates. Leslie (1993) reported that an alternative is to create specific tester strain for each VCG that needs to be detected. The tester strain would have one recessive marker that could be selected against and one dominant marker that could be selected. The use of VCGs may be particularly helpful in characterization and interpretation of diversity within and between rice blast pathogen populations in many rice growing regions of the world where cost and laboratory facilities may limit the number of isolates that can be analyzed by molecular methods. The objectives of this study were to (i) determine the number and distribution of rice blast races in the six Egyptian rice growing governorates using VCG method and determine the relationship between VCGs and physiological races, (ii) investigate the genetic diversity among the populations of *P. grisea* isolates with respect to vegetative compatibility groups.

MATERIALS AND METHODS

1- Fungal isolates and race identification

A total of one hundred forty *P. grisea* isolates were collected from19 rice entries, namely, Sakha 101, Sakha 104, Giza 159, Giza 171, Nishihikari, Fukunishiki, BL-1, M 202, M201, Pi.No4, Usen, Reiho, B9C-MD-3-3, B46-1B-PN-3, IR70554-48-1-2, IRTP21662(IRB-LA-A), Hybrid 2, IR-82225-11-3-1and IR-82737-B-182 from six rice growing governorates; Kafrelsheikh, Beheira, Damietta, Gharbia, Dakahlia and Sharkia during 2008, 2009 and 2010 growing seasons. The isolates were identified to races on international differential varieties (IDVs) namely: Raminad str.3, Zenith, NP-125, Usen, Dular, Kanto 51, CI 8970 S and Caloro (Atkins *et al*. 1967) under greenhouse conditions as described by Sehly *et al.* (2009).

2. Nitrate nonutilizing mutant production

The blast isolates were identified and classified on the IDVs under greenhouse conditions to race groups. Three hyphal tips were transferred from culture representing each race group to 9 cm diameter Petri dishes containing chlorate minimal medium (CMM) according to Correll *et al*., (1987) and incubated at 27 ^{o}C for 3 - 4 weeks. Sectors that grew as thin, expansive colonies with little or without aerial mycelium were designated as *nit* mutant. Sectors were transferred to minimal medium (Correll *et al*., 1987) containing sodium nitrate as a nitrogen source and examined after ten to fifteen days.

3. Selection and tester production

Nine testers of the *nit* mutant isolates each represented one of the nine race groups (IA-II) were selected and tested. Three hyphal tips from selected *nit* mutants were transferred to each Petri dish containing minimal medium as described by Puhala and Hummel (1983). The dishes were incubated at 27 ^{o}C for 15 days. The hyphal tip was selected from the best colony and transferred on a piece of sterilized filter paper disc on minimal medium. Filter paper discs were transferred individually into sterilized Petri dishes after the complete growth of the *nit* mutant. About one week later, the dried filter papers, having the *nit* mutant (testers) were cut into small pieces. Pieces obtained from each tester were altogether introduced into a plastic vial and kept at – 20 ^{o}C for long term storage (Metkwatanakarn *et al.,* 1999).

4. Vegetative complementary test

Complementation is evident by dense aerial mycelium where the two thin *nit* mutant colonies contact. Vegetative compatible *nit* mutants may complement one another by forming a heterokaryon on minimal medium i.e. dense aerial growth develops where mycelia of the two *nit* mutant colonies come in contact, anastomose and form a heterokaryon (Correll *et al*., 1987). Complementation tests were conducted on minimal medium in Petri dishes. The selected testers (nine testers) and

the other *nit* mutants of *P. grisea* races were grown on minimal medium for 10-15 days. The *nit* mutant of each tester was placed in the center of a 9-cm diameter Petri dish containing minimal medium. Four *nit* mutants for the races were transferred and distributed equidistantly apart around the tester with three replications at least. These daisy parings were incubated at 27 ^{o}C for 30-35 days. Complementation indicating heterokaryon formation was recognized as a line of dense aerial mycelial growth where two *nit* mutants grew together on minimal medium (Correll *et al.,* 1987). Lack of complementation may mean that the mutants are not complementary, that the *nit* mutant races are in different VCGs, or that the *nit* mutants are unable to form a heterokaryon in which complementation can occur. Complementation score was recorded after 30-35 days as follow:-

0 = No reaction 1=Little or no aerial mycelium

2= Abundant aerial mycelium 3= Very abundant aerial mycelium

In addition, inhibition zone (IZ) reaction was also considered

5- Scanning Electron Microscope (SEM)

The contact area between the pairings on Petri dishes was examined using scanning electron microscope at Electron Microscope Unit (EMU), Faculty of medicine, Tanta University, Egypt.

RESULTS AND DISCUSSION

1- Physiological races

The distribution of the 140 isolates of *P. grisea* identified as race groups during in three seasons (2008-2010) was presented in Table (1).

Table 1. Number and percentage of *Pyricularia grisea* race groups collected from different rice growing governorates during 2008- 2010 seasons.

Race group	Season						Total	Percentage of common race
	2008		2009		2010			
	Number	%	Number	%	Number	%		
IA	0	0	1	1.49	0	0	1	0.69
IB	3	6.52	3	4.48	6	19.35	12	8.6
IC	2	4.2	8	11.94	4	12.9	14	10.0
ID	1	2.17	25	37.31	19	61.2	45	32.1
IE	1	2.17	0	0	0	0	1	0.69
IF	4	8.70	0	0	0	0	4	2.78
IG	7	15.22	14	20.9	1	3.23	22	15.7
IH	19	41.30	14	20.9	0	0	33	23.6
II	8	17.39	0	0	0	0	8	5.7
Total	45		65		30		140	

2- Complementation test reaction

One hundred forty blast isolates were grown to produce nit mutants, on chlorate minimal medium. The chlorate resistant sectors were transferred on to minimal medium free from potassium chlorate (KClO3). Out of the selected sectors, one was selected for complementary test. One out of five reactions (o, 1, 2, 3 and inhibition zone) was obtained through complementary test among different *nit* mutants (one for each 140 *nit* mutants for *P. grisea* races) and its testers. The *nit* mutant producing dense aerial wild type mycelia when two *nit* mutants were paired together and mycelia growth of their colonies were met and formed heterokaryons giving reaction after four to five weeks.

Data in Table (2) show that the *nit* mutant races were grouped into eight VCGs. Group 1 (VCG 1) recovered all IB blast races (12 races represented 70.59 % from the total of VCG 1 races); two races identified IH group (11.76 %) and one race for each II, IA and ID as 5.88 % for each (Table 2). Most of races in Group 2 represented IC race group with (87.5%). The biggest group VCG 3 included 44 isolates; most of these isolates were identified as ID race group (86.36 %). All races in VCG 4 belonged to IF race group. Twenty two out of 23 isolates of VCG 5 belonged to IG group race as (95.65%) and one represented II group race. For VCG 6, most of these isolates in this group were in IH race group as (96.43 %). The seventh group as (VCG 7) including eight isolates, 5 represented ID race group and one for each of IG, IF and IH group races. Only one isolate was identified as IE-7 race, in VCG 8.

The complementation among different tested isolates in the same VCG ranged from 70.59 in group 1 to 100 % in group 4 and group 8 except group 7 which showed complementation at 62.5 %. Members of the IB group race were found in four governorates (Dakahlia, Beheira, Kafrelshiekh and Gharbia). While, IC group race was distributed to three governorates (Beheira, Kafrelsheikh and Gharbia). The biggest group race ID, in addition to IG and IH group races were distributed in five rice governorates. VCG4 and VCG 8 which included IF and IE group race, respectively, came from Kafrelsheikh only (Table2). Similar studies were carried out by Correll *et al.* (2000), Mosa-Nejad *et al*. (2005), Javan-Nikkhah *et al.* (2007), Amir-Dehi *et al.* (2008) and Motallebi *et al.* (2009) on *M. grisea* isolates and different VCGs were identified.

Table 2. Distribution of vegetative compatibility groups of *Pyricularia* grisea races and its pathotypes collected from different locations during 2008 – 2010 seasons.

No . of VCG	Isolate No.	Location	Race	Year
VCG 1	3	Beheira	IB-61	2008
	25	Dakahlia	IB-61	2008
	29	Dakahlia	IB-63	2008
	54	Gharbia	IB-63	2009
	86	Ghabia	IB-61	2009
	87	Gharbia	IB-29	2009
	115	Gharbia	IB-47	2010
	118	Kafrelsheikh	IB-41	2010
	121	Beheira	IB-41	2010
	125	Kafrelsheikh	IB-33	2010
	133	Kafrelsheikh	IB-33	2010
	136	Kafrelsheikh	IB-41	2010
	1	Beheira	IH-1	2008
	22	Dakahlia	IH-1	2008
	28	Sharkia	II-1	2008
	76	Kafrelsheikh	IA-41	2009
	91	Kafrelsheikh	ID-5	2009
VCG 2	2	Beheira	IC-31	2008
	39	Kafrelsheikh	IC-32	2008
	47	Kafrelsheikh	IC-27	2009
	56	Kafrelsheikh	IC-13	2009
	92	Gharbia	IC-13	2009
	97	Kafrelsheikh	IC-9	2009
	98	Kafrelsheikh	IC-12	2009
	99	Beheira	IC-13	2009
	110	Kafrelsheikh	IC-13	2009
	111	Kafrelsheikh	IC-13	2009
	137	Kafrelsheikh	IC-13	2010
	138	Kafrelsheikh	IC-29	2010
	141	Kafrelsheikh	IC-13	2010
	142	Kafrelsheikh	IC-9	2010
	4	Kafrelsheikh	IH-1	2008
	13	Kafrelsheikh	II-1	2008
VCG 3	35	Sharkia	ID-7	2008
	49	Kafrelsheikh	ID-15	2009
	53	Gharbia	ID-9	2009
	61	Gharbia	ID-5	2009
	64	Gharbia	ID-13	2009
	79	Kafrelsheikh	ID-11	2009
	80	Kafrelsheikh	ID-13	2009
	81	Beheira	ID-13	2009
	89	Gharbia	ID-13	2009
	93	Beheira	ID-10	2009
	95	Kafrelsheikh	ID-13	2009
	96	Kafrelsheikh	ID-15	2009
	101	Gharbia	ID-9	2009
	104	Gharbia	ID-13	2009
	106	Beheira	ID-13	2009
	107	Kafrelsheikh	ID-13	2009
	108	Beheira	ID-13	2009
	109	Kafrelsheikh	ID-13	2009
	112	Beheira	ID-13	2009
	113	Beheira	ID-13	2009
	114	Sharkia	ID-13	2010
	116	Kafrelsheikh	ID-5	2010
	117	Kafrelsheikh	ID-13	2010
	119	Beheira	ID-13	2010
	120	Beheira	ID-13	2010
	122	Dakahlia	ID-13	2010

Table 2. continued

No . of VCG	Isolate No.	Location	Race	Year
	123	Dakahlia	ID-13	2010
	124	Kafrelsheikh	ID-13	2010
	126	Sharkia	ID-13	2010
	128	Sharkia	ID-13	2010
	129	Sharkia	ID-13	2010
	130	Kafrelsheikh	ID-13	2010
	131	Gharbia	ID-13	2010
	132	Kafrelsheikh	ID-1	2010
	134	Kafrelsheikh	ID-13	2010
	135	Kafrelsheikh	ID-13	2010
	139	Kafrelsheikh	ID-13	2010
	140	Kafrelsheikh	ID-13	2010
	15	Kafrelsheikh	II-1	2008
	21	Dakahlia	II-1	2008
	23	Dakahlia	II-1	2008
	24	Dakahlia	II-1	2008
	26	Dammieta	II-1	2008
	77	Dakahlia	IH-1	2009
VCG 4	5	Kafrelsheikh	IF-3	2008
	16	Kafrelsheikh	IF-1	2008
	31	Kafrelsheikh	IF-3	2008
VCG5	8	Kafrelsheikh	IG-1	2008
	12	Kafrelsheikh	IG-2	2008
	18	Beheira	IG-2	2008
	19	Beheira	IG-1	2008
	37	Sharkia	IG-1	2008
	43	Kafrelsheikh	IG-1	2008
	46	Kafrelsheikh	IG-1	2008
	48	Kafrelsheikh	IG-2	2009
	51	Gharbia	IG-1	2009
	52	Gharbia	IG-1	2009
	58	Kafrelsheikh	IG-1	2009
	62	Gharbia	IG-1	2009
	65	Sharkia	IG-1	2009
	66	Sharkia	IG-1	2009
	68	Sharkia	IG-1	2009
	71	Sharkia	IG-1	2009
	73	Kafrelsheikh	IG-1	2009
	74	Kafrelsheikh	IG-1	2009
	78	Dakahlia	IG-1	2009
	83	Kafrelsheikh	IG-1	2009
	103	Sharkia	IG-1	2009
	127	Sharkia	IG-1	2010
	14	Kafrelsheikh	II-1	2008
VCG 6	7	Kafrelsheikh	IH-1	2008
	9	Kafrelsheikh	IH-1	2008
	10	Kafrelsheikh	IH-1	2008
	11	Kafrelsheikh	IH-1	2008
	20	Beheira	IH-1	2008
	27	Dakahlia	IH-1	2008
	30	Sharkia	IH-1	2008
	32	Kafrelsheikh	IH-1	2008
	33	Kafrelsheikh	IH-1	2008
	34	Kafrelsheikh	IH-1	2008
	36	Sharkia	IH-1	2008
	40	Kafrelsheikh	IH-1	2008
	41	Kafrelsheikh	IH-1	2008
	44	Kafrelsheikh	IH-1	2008
	45	Kafrelsheikh	IH-1	2008

Table 2. continued

No . of VCG	Isolate No.	Location	Race	Year
VCG 6	50	Gharbia	IH-1	2009
	55	Kafrelsheikh	IH-1	2009
	57	Kafrelsheikh	IH-1	2009
	59	Kafrelsheikh	IH-1	2009
	60	Kafrelsheikh	IH-1	2009
	63	Gharbia	IH-1	009
	67	Sharkia	IH-1	2009
	69	Sharkia	IH-1	2009
	70	Sharkia	IH-1	2009
	72	Sharkia	IH-1	2009
	85	Beheira	IH-1	2009
	102	Sharkia	IH-1	2009
	84	Beheira	ID-15	2009
VCG 7	6	Kafrelsheikh	IH-1	2008
	38	Kafrelsheikh	IF-3	2008
	42	Kafrelsheikh	IC-29	2008
	90	Beheira	ID-13	2009
	94	Beheira	ID-15	2009
	100	Beheira	ID-15	2009
	105	Gharbia	ID-13	2009
	144	Kafrelsheikh	ID-9	2010
VCG 8	17	Kafrelsheikh	IE-7	2008

VCGs = Vegetative Compatibility Groups

3- Scanning electron microscope and appearance on media

The reaction among different *nit* mutants visually differed. Figure 1 A illustrates that *nit* mutant for blast isolates (number 39) was highly compatible with its tester (*nit* mutant. 76) on minimal medium forming a clear and dense aerial mycelium in the contact area and robust heterokaryon formation was observed. Under scanning electron microscope (SEM) in Fig. 1 B the aerial mycelium appeared with hyphal anastomosis formed between distinct mycelia in contacted *nit* mutants. Vegetative compatibility systems generally act to restrict the transfer of nuclear and cytoplasmic elements during growth. Most studies of vegetative compatibility have focused on the fusion (anastomosis) of hyphae rather than on the fusion of protoplast. The work done with fused protoplast is quite interesting, however, since the heterokaryons formed following protoplast fusion appear to be significantly different from those formed following hyphal anastomosis (Stasz *et al.*, 1989). Anastomosis formation and the cytological events involved have been studied extensively in ascomycetes and basidiomycetes, (Leslie, 1993). Chen and Wu (1977) mentioned that hyphal anastomosis in *P. oryzae* occurs naturally in the lower epidermal cells and in the vascular bundles of young lesions on rice. In those cells, the invading blast fungus is active. A fusion aperture of 0.2 – 0.6µm in diameter is formed allowing the migration

or exchange of nuclei and cytoplasm between two anastamosing hyphal cells. Also, Correll *et al*. (2000) reported that anastomosis state, the nucleus of a strain migrated into the cell of another strain during plasmogamy to produce a heterokaryon mycelium or heterokaryon cell and occasionally to become diploid after fusing, so anastomosis is considered as a source of recombination or variability.

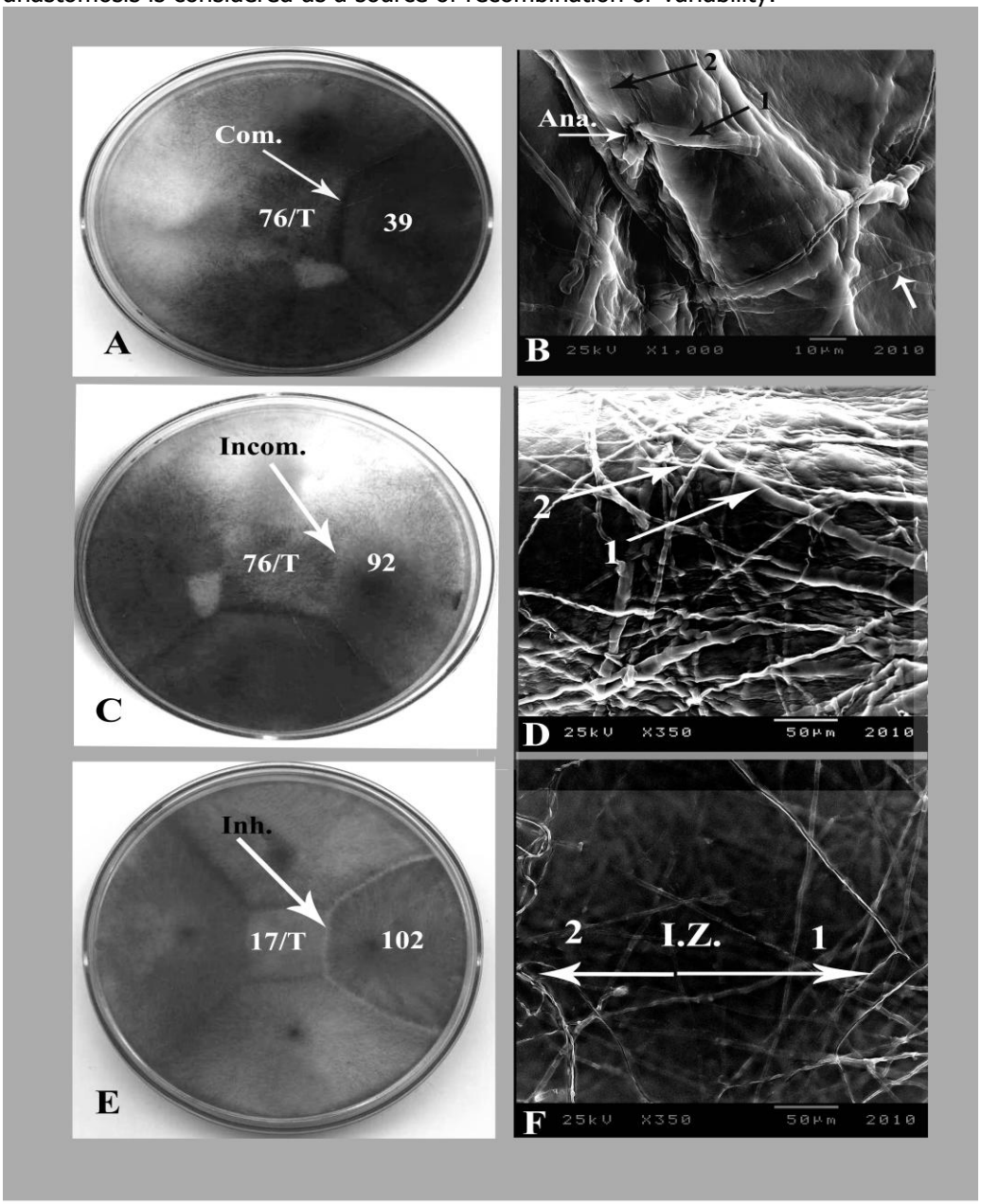

Fig. 1. Photos of vegetative compatibility groups test for *nit* mutants of some isolates of *Pyricularia grisea* on Petri dishes and scanning electron microscope, examined different reactions among them as A, B, C, D, E and F.

Com. = Compatibility

Incom.= Incompatibility

Ana. = Anastomosis

Inh. = Inhibition zone

In Figure (1, C) illustrates that the same previous tester was incompatible with another *nit* mutant (92); the mycelia did not interact with the *nit* mutant mycelia and developed without the formation of aerial mycelium. In Figure (1, D), the contact area, the two distinct overlapped without anastomosis, indicating an incompatible reaction. In Figure (1, E) exhibited an incompatible reaction between *nit* mutants 17 and 102 with an inhibition zone area. This inhibition zone was free from growing mycelia or contacted mycelia (Fig 1, F). The inhibition zone formed between some isolates on minimal medim, under SEM lethal mycelia exhibited some fusions as a result of some chemicals or allelo-chemicals produced by some blast races and helped to prevent developing mycelia. Also, similar results were obtained by Javan-Nikkhah (2002). Some investigators explained the phenomenon of incompatibility and inhibition zone. Some biochemical features were found to be correlated with the incompatibility reaction including a decrease in RNA production, appearance of new proteins (Boucherie *et al.* 1981), and increase in proteolytic and other enzymatic activities such as phenoloxidases, malate/NADH dehydrogenase, proteases, and amino acid oxidase. Vegetative incompatibility systems in fungi normally impair genetic exchanges among strains and are under strict control of heterokaryon incompatibility and vegetative incompatibility loci. Natural populations of fungi are generally polymorphic for vegetative incompatibility systems (Leslie, 1993). The barrage zone phenomenon is conceptually the opposite of a prototrophic vegetative compatibility heterokaryon. Barrages occur between vegetatively incompatible strains in many fungi, including *Podospora*, *Neurospora* and *Cryphonectria*.

Table 3. Relationship between vegetative compatibility groups for 140 *Pyricularia grisea* races and its group races and percentage of compatibility.

VCG group	No. of isolates	Common group race	Compatibility percentage for the common races
VCG1	17	IB	70.59
VCG 2	16	IC	87.5
VCG 3	44	ID	86.36
VCG 4	3	IF	100
VCG 5	23	IG	95.65
VCG 6	28	IH	96.43
VCG 7	8	ID	62.5
VCG 8	1	IE	100

Data in Table (3) summarized the results relevant to the correspondence between VCGs and race groups as identified by differential varieties and VCGs. VCG 4 and VCG8 each had only one race group. This could probably be due to the small number of isolates included in each. The least correspondence percentage was observed in VCG 7 as 62.5 % with reference to ID. This VCG included three other race groups. There are cases where a single group race may belong to several different VCGs. VCGs 3 and 7 showed majorities of race group ID which raises a question about the possible relationship between the two VCGs. This reduces the degree of dependence on VCG as indicative of race groeping. Generally, the degree of correspondence between VCGs and physiological blast races ranged from 62.5 % to 100 %. A similar degree of complexity of race and VCG occur within *Fusarium oxysporum f. sp lycopresici*; in one case, the three known races were found within a single VCG and others where a single race lies in different VCGs (Elias and Schneider, 1991). With the exception of VCGs 1 and 7, a high degree of correspondence was found between race identity and VCGs. So, it is possible to use vegetative compatibility, with some limitations, as a method for primarily identifying and differentiating races of *P. grisea.* However, greater numbers of isolates need to be included in such studies to reach definite conclusion in this respect

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مجموعات التوافق الخضرى لسلالات اللفحة الفسيولوجية للفطر *Pyricularia grisea* فى مصر

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المستخلص

تم تجميع 140 عزلة من الفطر *Pyricularia grisea* المسبب لمرض اللفحة فى الارزوذلك من محافظات زراعة الارز الاساسية وهى كفر الشيخ والبحيره والشرقيه والغربيه والدقهليه و دمياط وكذلك المزارع البحثيه المختلفه خلال ثلاثة مواسم متتاليه 2008، 2009و 2010 . تم تعريف السلالات الفسيولوجية لهذه العزلات على الاصناف المفرقه العالميه تحت ظروف العدوى الصناعية بالصوبة حيث تم تقسيمها الى 9 مجموعات كالاتى

IA (1 race); IB (12); IC (15); ID (45); IE (1); IF (4); IG (22); IH (32) and II (8).

وتم استخدام طريقة التوافق الخضرى فى التفريق بين هذه السلالات الفسيولوجية وذلك من خلال انتاج المطفرات *nit* mutants على بيئة الكلورات بمعمل قسم بحوث امراض الارز بمحطة بحوث سخا– كفرالشيخ– مركز البحوث الزراعية. وقد اوضحت نتائج الدراسة انه تم تقسيم السلالات الفسيولوجية للفطرالى ثمانى مجموعات (VCG 1- VCG8) بناءا على التوافق الخضرى فيما بينها . واشتملت المجموعة الاولى على 14 سلالة فسيولوجية منها 12 سلالة ،(70.59) % تتبع المحموعة IB group race بينما المجموعة الثانية تضمنت 16 سلالة ، 87.5 % منها تتبع IC group race. وتعتبر المجموعة الثالثة من اكبر المجموعات حيث اشتملت على 44 سلالة فسيولوجية ،86.36 % منها تتبع ID group race واشتملت المجموعة الرابعة على 3 سلالات جميعها تتبع المجموعة IF group race. واما المجموعة الخامسة فقد تضمنت 23 سلالة فسيولوجية منها 95.65 % تتبع IG group race بينما المجموعة السادسة اشتملت على 28 سلالة منها 96.43% تتبع IH group race. وقد اشتملت المجموعة السابعة على سلالات مختلفة فسيولوجيا وكانت السيادة فيها ل ID group race بنسبة 62.5 % بينما المجموعة الثامنة فتعتبر اقل المجموعات عددا حيث اشتملت على سلالة واحدة تتبع IE group race. وبدراسة العلاقة بين المطفرات بفحص المناطق الفاصلة بين السلالات الفسيولوجية باستخدام الميكروسكوب الالكترونى الماسح ظهرت حالات التوافق وعدم التوافق بين المطفرات محل الدراسة وكذلك ظهور المناطق الفاصلة بينها. وبربط النتائج المتحصل عليها من التعريف بالاصناف المفرقة العالمية وطريقة التوافق الخضرى اتضح ان هناك توافق (تطابق) بنسبة تراوحت من 62.5 الى 100 %.