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Identification of Some Rice Genotypes Resistant to Blast Disease in Egypt

Wafaa M. Haggag[1*] and Medhat Mekhail Tawfik[2]

[1]*Plant Pathology Department, National Research Centre, Egypt.*
[2]*Field Crops Research Department, National Research Centre, Egypt.*

Authors' contributions

This work was carried out in collaboration between both authors. Author WMH designed the study, followed up the practical work and wrote the final version of the manuscript. Author MMT managed the analyses of the study, managed the literature searches. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Blast disease caused by *Pyricularia oryzae*, is the most important disease of rice in Egypt and in the world. Field surveys in main production regions, revealed that the highest disease incidence was recorded in El-Behera Governorate. Seven rice cultivars were screened for resistance to blast disease. Resistance to disease was studied under both artificially and naturally with local pathogen. A bioassay was developed based on (0-9) scale, Area Under the Disease Progress Curve (AUDPC), disease area and sporulation. Differential expression to pathogen was observed between cultivars. Among seven cultivars, Sakha 101 and Sakha 103 were highly resistant and Giza 175 and Giza 178 were highly susceptible to three strains of fungus *P. oryzae*. Meanwhile other lines showed moderate resistance and susceptible. At the same time there were great differences concerning the growth rate and yield between different rice cultivars.

Keywords: Blast disease; Pyricularia oryzae; rice cultivars.

1. INTRODUCTION

The rice blast fungus *Pyricularia oryzae* was named by Cavara in 1891, since that time knowledge about rice diseases increased in a considerable manner especially for

**Corresponding author: Email: wafaa_haggag@yahoo.com; medhatnrc@hotmail.com;*

Magnaporthe grisea (anamorph *Pyricularia oryzae*) [1]. Rice blast causes between 11% and 30% crop losses annually. This represents a loss of 157 million tonnes of rice [2]. Rice is among the most important field crops in Egypt. It occupies about 0.5 million h /year with 7.9 t/ha as a national average [3]. However, rice blast caused by *Pyricularia oryzea* Cav. is considered as the major constraint to Egypt's rice production [4-6].

This disease is related to inappropriate cultural practice, such as excess use of nitrogen, and cultivation of too susceptible varieties. The severity and significance of damage caused by diseases in rice have necessitated the development of strategies to control and manage them to reduce crop loss and to avert an epidemic. *Pyricularia oryzea*, causes losses in different regions, more and more fungicides are used world wide, where several chemical fungicides are authorised. Though Bordeaux mixture, antibiotics, and other copper and mercurial compounds were used in the early 1950s, environmentally safe and stable chemical control agents rendering control at very low concentrations have yet to be developed. Breeding for blast resistance are our concern in varietal improvement program. Since the Egyptian cultivars were showing susceptibility to blast diseases. Breeding for resistance is the most economical way within the integrated disease management strategy [4]. However, the breakdown of major gene resistance of the new cultivars is known to occur as the area of the cultivar increased. Today, the exploitation of host resistance appears to be the reliable method of disease management. Fortunately, modern tools such as transformation (transgenics) for their manifold advantages can be effectively used to complement conventional breeding for the development of built-in resistance in rice cultivars [7-9].

The objectives of this research was to determine disease resistance of local rice cultivars against blast pathogen. Relationships between blast population changes and blast reaction of the cultivars in the greenhouse and field were being examined under laboratory.

2. MATERIALS AND METHODS

2.1 Plant Materials and Rice Blast Inoculation

Three strains of *Pyricularia oryzea* were isolated from the diseased plants grown in different regions of major producing regions of Egypt i.e. experimental farm of National Research Center, El-Kanater El-Khairea (Kaluobia Governorate), Kafr El-shekh (Bohera Governorate) and Senbalaween (Dakahleea Governorate). Fungal isolates were identified according to Valent [10]. For inoculum production, each isolate was grown separately on V-8 juice agar at 20ºC [11]. After 7 days in culture, conidia were washed from the agar surface, filtered through a 330µm strainer and made up into an aqueous suspension containing 10^5 conidia mL^{-1}.

Disease survey in Kaluobia, Bohera and Dakahleea Governorates was performed during the growing period. Mean percentages of the disease incidence at the chosen fields were calculated.

2.2 Greenhouse Test

Seven lines i.e. Sakha 101, Sakha 102, Sakha 103, Sakha 104, Giza 175, Giza 177 and Giza 178 were evaluated under artificial infested conditions. Seedlings of rice were sown in 30 cm- diameter plastic pots (2 seedlings per pot) under a 16h photoperiod and 22 to 25ºC.

Leaf blast inoculations were preformed on seedlings at 4-5 leaf stage. Spores were suspended in a light mineral oil and inoculated using a concentration of 10^5 spores mL^{-1} with 0.01% surfactant, by using a hand held atomizer (about 0.5mL per leaf). Inoculated seedlings were planted for 18-24h at greenhouse temperatures in a dark room. Each treatment was replicated three times. Plants were grown in a greenhouse 24 to 30ºC with 16 h light for 2 to 4 wk until plants were at the 4-leaf stage for disease reaction testing and scale (0-9) were tested using IRRI [12].

2.3 Natural Infection in the Field

Field screening using naturally occurring inoculum was used to identify resistance in a range of cultivars (Sakha 101, Sakha 102, Sakha 103, Sakha 104, Giza 175, Giza 177 and Giza 178) to blast disease. Field experiments were conducted in National Research Center Experimental Farm at Kanater El-Khairea. Each cultivars was sown in 5x5 m plots. A randomized complete block design with five replicates for each treatment was used. Irrigation was carried out as recommended. Diseases incidence of leaves was assessed monthly using the (0-9) scale of IRRI [12] as follows:

0) No lesions observed Highly resistant; 1) Small brown specks of pinpoint size or larger brown specks without sporulating center, Resistant; 2) Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin, Moderately resistant; 3) Lesion type is the same as in scale 2, but a significant number of lesions are on the upper leaves; Moderately resistant; 4) Typical susceptible blast lesions 3mm or longer, infecting less than 4% of the leaf area, Moderately susceptible; 5) Typical blast lesions infecting 4-10% of the leaf area , Moderately susceptible; 6) Typical blast lesions infection 11-25% of the leaf area, Susceptible; 7) Typical blast lesions infection 26-50% of the leaf area; Susceptible; 8) Typical blast lesions infection 51-75% of the leaf area and many leaves are dead; Highly susceptible; 9) More than 75% leaf area affected ; Highly susceptible

Mean number of spores/cm^{-2} were counted during growth periods using hemocytometer. Resistant reaction was based on no visible infection and no conidia produced from affected tissue. Susceptible reaction was based on a lesion size greater than 3 cm in length, visible infection, and conidia evident in affected tissue [10]. The intensity of blast infection was measured for each plot by calculation of the Area under the Disease Progress Curve (AUDPC) using numerical integration**.** The largest length (L) and width (W) of each lesion were measured and used to calculate the lesion area (A); ($A=0.25\pi LW$). AUDPC is expressed as units of ‘percentage months’. Data were recorded per 10 plants, 20 days intervals on randomly selected lines per replicate**.** At harvest stage, plant height and grain dry weight were measured.

2.4 Statistical Analysis

The percentages of disease severity were transformed before analysis of variance to improve homogeneity of variance. Analysis of variance was performed to analyze the transformed data. The completely randomized method Snedecor and Cochran [13], was used for analysis of variance and LSD (0.05).

3. RESULTS

3.1 Disease Survey

Table 1 indicated that the highest infection at Dakahleea Governorate (34.8% 0. Kaluobia Governorate had lower infection. Rice blast is a leaf spot disease which is spread by splash dispersal. Under conditions of heavy dew all aerial parts of the plant can be affected; leaf surfaces become speckled with oval lesions, plants are liable to lodging if stems are infected and if the panicle is infected then a severe yield loss results (Fig. 1).

3.2 Artificial Infection in the Greenhouse

The reaction of the seven cultivars plants to the *P. oryza* pathogen was studied with three different races: RI, RII, and RIII (Table 2). All strains exhibited distinct differential virulence responses on several cultivars. Isolates of *P. oryza* RI exhibited high infection on all cultivars (Fig. 2). Among seven cultivars, Sakha 101 and Sakha 103 were highly resistant and Giza 175 and Giza 178 were highly susceptible to all strains. Meanwhile other lines showed moderate resistance and susceptibility. Diseases score of rice cultivars infected with *P. oryza* in the greenhouse are presented in Table 2 and (Fig. 2). The same result was also, obtained in disease score, which Sakha 101 and Sakha 103 were low scored of disease incidence. Meanwhile, Giza 175 and Giza 178 were highly scored in all stranis.

3.3 Natural Infection in the Field

There was a wide range of resistance among the rice cultivars grown under natural infested conditions (Table 3) and (Fig. 3). Based on the AUDPC, lesion number and score observed on rice cultivars in the field, a wide range of rice cultivars had good resistance to *P. oryza* isolates. Sakha 101 and Sakha 103 exhibited high resistance to *P. oryza.* Meanwhile, Giza 175 and Giza 178 were highly susceptible. Anther rice cultivars identified as moderate resistance to *P. oryza*.

In order to study the interaction of rice cultivars with pathogen, we counted spores on leaves during growth periods and compared in all rice cultivars. Significant difference in spores counts were found between the rice cultivars. Spores count on resistance cultivars (Sakha 101 and Sakha 103) were not appeared than those of the moderate resistance (Sakha 104 and Giza 177). Susceptible rice cultivars Giza 175 and Giza 178 had the highest apparent spores numbers, and these values were significantly higher**.**

Disease development of rice cultivars was tested during growth periods, indicating adequate distribution of inoculums (Fig. 3). Disease reactions were determined 120 d after transplanting. Disease reaction was based on base on spot area(s). Of the seven cultivars, Sakha 103 was successful resistant cultivar. Meanwhile, Giza 175 and Giza 178 were highly susceptible.

2.4 Growth Parameters and Yield

Growth rate (mean plant height and mean plant dry weight) and yield (grain dry weight of 1000 ears) of rice cultivars were recorded at harvest stage (Table 4). There were significant differences concerning the growth rate and yield between different rice cultivars. It is evident that Sakha 103 recorded the highest growth rates and grain dry weight followed by

Sakha 104. The moderate growth rate and grain dry weight were obtained for Giza 177 and Giza 175.

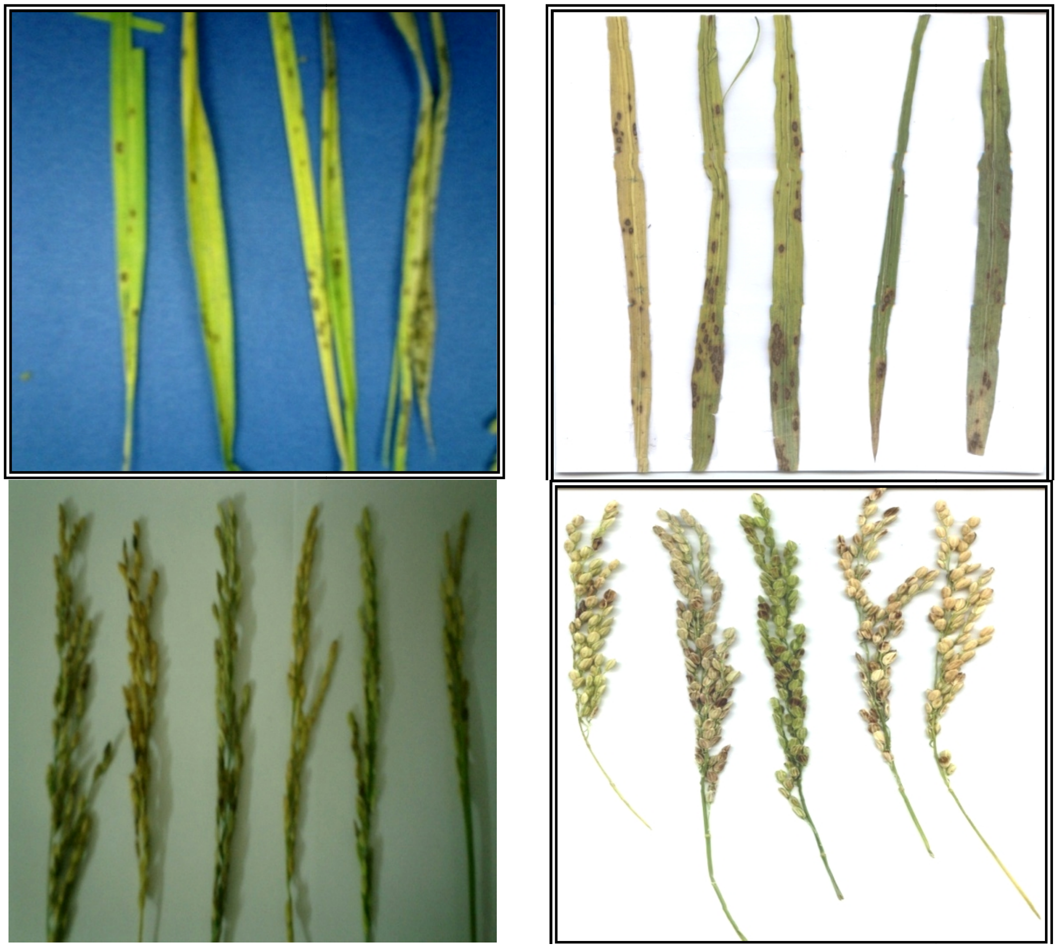

Fig. 1. Severely infected leaves and panicle

Table 1. Screening of blast fungus *P. oryza* in the main regions of rice production in Egypt

Region	Disease incidence %	Strain
Kafr El-shekh (Bohera governorate)	31.9	RI
Senbalaween (Dakahleea governorate)	34.8	RII
El-Kanater El-khairea (Kaluobia governorate)	22.7	RIII

Table 2. Observed infection reaction for different rice cultivars after exposure to Egyptian strains of *Pyricularia oryzae* under artificial infested conditions

Cultivar	Isolates			Diseased score (0-9)		
	RI	RII	RIII	RI	RII	RIII
Sakha 101	R	R	R	1.65	1.76	1.9
Sakha 102	R	MR	MR	2.54	3.88	2.87
Sakha 103	R	R	HR	0.64	0.88	0.56
Sakha 104	S	M	M	5.87	4.76	5.65
Giza 175	S	S	S	8.34	7.54	7.23
Giza 177	M	R	MR	5.76	4.87	4.53
Giza 178	S	S	S	8.56	8.45	7.51
LSD	--	--	--	0.57	0.87	0.77

Diseases incidence of leaves was assessed monthly using the (0-9) scale of IRRI (1980) as follows: 0) No lesions observed Highly resistant; 1) Small brown specks of pinpoint size or larger brown specks without sporulating center, Resistant; 2) Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin, Moderately resistant; 3) Lesion type is the same as in scale 2, but a significant number of lesions are on the upper leaves; Moderately resistant; 4) Typical susceptible blast lesions 3 mm or longer, infecting less than 4% of the leaf area, Moderately susceptible; 5) Typical blast lesions infecting 4-10% of the leaf area , Moderately susceptible; 6) Typical blast lesions infection 11-25% of the leaf area , Susceptible; 7) Typical blast lesions infection 26-50% of the leaf area ; Susceptible; 8) Typical blast lesions infection 51-75% of the leaf area and many leaves are dead; Highly susceptible; 9) More than 75% leaf area affected ; Highly susceptible

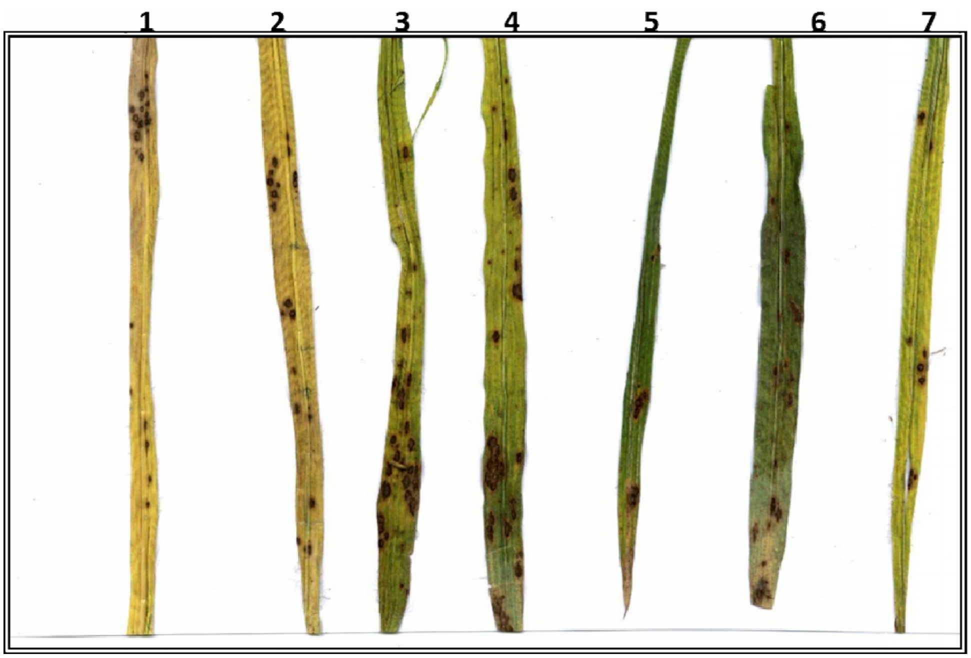

Fig. 2. Phenotypic reaction of rice cultivars against rice blast isolate RI 1) Sakha 104; 2) Sakha 102; 3) Sakha 101; 4) Sakha 103; 5) Giza 177; 6)Giza 175 and 7) Giza 178

Table 3. Area under disease progress curves (AUDPC), lesion number and disease score of 7 rice cultivars with leaf blast under natural field conditions after 90 days of growth

Cultivar	AUDPC	Lesion number/leaf	Diseased score (0-9)*	Sporulation leaf/cm
Sakha 101	1.9	6.83	2.35	16.9
Sakha 102	4.8	7.81	4.24	23.9
Sakha 103	1.5	5.80	1.12	12.9
Sakha 104	5.9	11.9	5.97	69.0
Giza 175	3.8	5.76	7.34	41.9
Giza 177	2.9	4.87	5.16	27.9
Giza 178	6.8	6.98	7.26	38.9
LSD	0.76	0.98	0.76	8.86

Analysis of variance was performed to analyze the transformed data. The completely randomized method Snedecor and Cochran (1972), was used for analysis of variance and LSD (0.05)

Diseases incidence of leaves was assessed monthly using the (0-9) scale of IRRI

0; No lesions observed highly resistant;
1; Small brown specks of pinpoint size or larger brown specks without sporulating center; Resistant
2; Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin; moderately resistant
3; Lesion type is the same as in scale 2, but a significant number of lesions are on the upper leaves; moderately resistant
4; Typical susceptible blast lesions 3 mm or longer, infecting less than 4% of the leaf area; moderately susceptible
5; Typical blast lesions infecting 4-10% of the leaf area; moderately susceptible
6; Typical blast lesions infection 11-25% of the leaf area; Susceptible
7; Typical blast lesions infection 26-50% of the leaf area; Susceptible
8; Typical blast lesions infection 51-75% of the leaf area and many leaves are dead; Highly susceptible
9; More than 75% leaf area affected; highly susceptible.

Table 4. Growth rate and yield of tested rice cultivars grown under natural field conditions after 90 days of growth

Cultivars	Plant length (cm)	Plant weight (g)	Yield/plant (g/1000 grain)
Sakha 101	37.9	15.8	31.9
Sakha 102	44.8	13.7	29.7
Sakha 103	71.7	18.9	36.9
Sakha 104	53.9	10.7	24.8
Giza 175	51.8	16.8	37.7
Giza 177	50.8	14.9	29.8
Giza 178	39.9	11.9	24.8
LSD	1.98	1.21	1.54

Analysis of variance was performed to analyze the transformed data. The completely randomized method Snedecor and Cochran (1972), was used for analysis of variance and LSD (0.05)

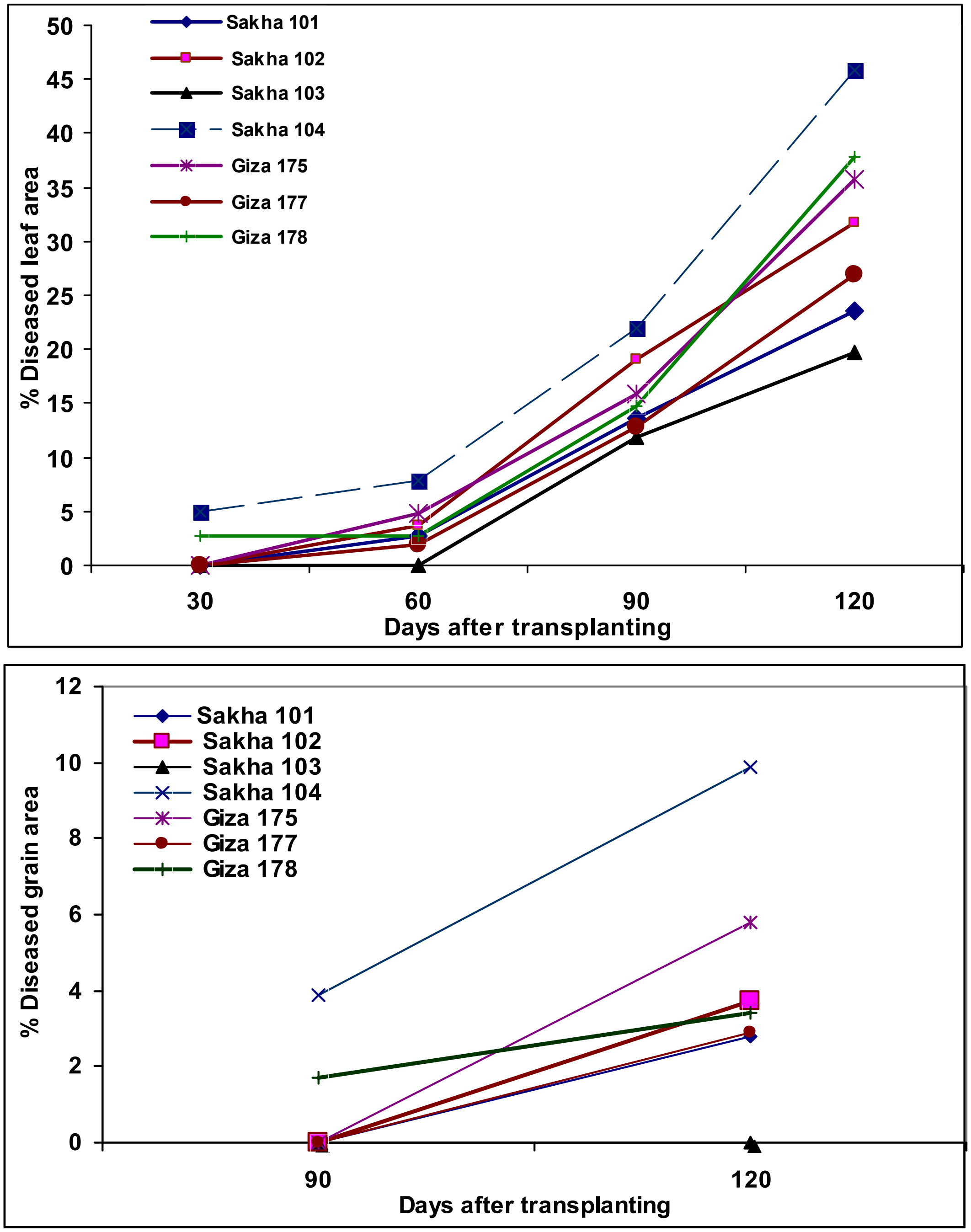

Fig. 3. Blast disease infection (%) of rice cultivars during growth periods in natural field after 90 days of growth

3. DISCUSSION

The blast disease caused by *Pyricularia oryzae* was occasionally present in production regions in Egypt. The main blast manifestations were leaf, node and neck blast [4,5,6]. Many authors reported that the most severe rice disease is blast caused by *Pyricularia oryzae* [4,5,1].

Quantitative resistance is a practical term for describing a type of resistance that can be assessed by estimating the percentage of tissue affected by the pathogens (area and scale type). The data presented clearly indicate that infection response of rice to blast disease in the greenhouse and in field showed quantitative variation. Among 7 collected rice cultivars, tested under both natural and artificial infested conditions, two cultivars include Sakha 101 and Sakha 103 were highly resistant and Giza 175 and Giza 178 were highly susceptible to all strains. Meanwhile other lines showed moderate resistance and susceptible. This indicated that the evaluation will, in general, be useful for selecting cultivars with resistance in plants in breeding application [4]. This study confirmed findings in other investigations that rice cultivars possess diseases resistance genes different from genes present in cultivated varieties that may result from multiple gene control [7-9]. The main criteria for selection was stable performance and high yield.

4. CONCLUSION

In Egypt, rice blast causes between 11% and 30% crop losses annually. Resistance to disease was studied under both artificially and naturally with local pathogen. Among seven cultivars, Sakha 101 and Sakha 103 were highly resistant. The main criteria for selection was stable performance and high yield.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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