EVALUATION OF THE NEW EGYPTIAN RICE CULTIVARS TO DISEASES

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Introduction

INTRODUCTION

Rice diseases are one of the limiting factors in rice production in Egypt. The most destructive disease is rice blast caused by Pyricularia oryzae Cavara , which can be epidemic whenever the three factors are present, a susceptible cultivar, virulent isolate(s) and favorable weather conditions. The time factor has been added more recently to these factors to formulate the disease pyramid (Browning et al 1977).

This study aims to clarify the role of some factors, which affect rice crop.

a) The role of the causal organism of rice blast disease (P. oryzae Cav.) and its variability from region to region and from season to season.

b) Evaluation of some promising lines to blast reaction under greenhouse conditions.

c) Antigenic structure of protein in resistant and susceptible rice varieties as well as P. oryzae Cav. by using crossed immunoelectrophoretical technique(CIE) and double diffusion test (DGD) of Ouchterlong as an attempt to test the applicability of (CIE) in detecting the antigenic relationships (common antigens) between rice (Oryza sativa) and the causal agent of rice blast disease (P. oryzae Cav.).

d) Correlation between time of planting as an important factor in blast development and blast disease incidence under two dates of planting for seven rice entries.

d) Blast disease development under different methods and different times of nitrogen fertilizer application.

e) Correlation between both leaf and panicle infections as well as the correlation between each of them and grain yield.

REVIEW OF LITERATURE

Blast is one of the most important disease of rice, occurring in most of the rice growing areas of the world (Rice Path. Newsletter 2/74, International Rice Research Institute, Manila, Philippines). In Egypt, blast disease of rice caused by Pyricularia oryzae Cavara, is the major disease that has economic importance.

I. PHYSIOLOGIC RACES:

Breeding for blast resistance is complicated by the existence of different pathogenic races. Many investigators studied physiologic races of P. oryzae in different rice growing countries (Atkins, 1962; Goto et al, 1964; Ou and Ayad, 1968; Padmanabhan et al, 1970; Marchetti and Abdel-Hak, 1985; and Yamada 1985). Jin and Tao (1984) in China, reported that 7 race groups were recorded in the period from 1980-82 on rice plants. A, B and C (Indica type) mainly infected during early rice season; D, E, F and G (Japonica) were dominant in late season rice fields.

Yaegashi and Yamada (1986) reported that 130 isolates of P. oryzae from 6 countries were tested. The number of races identified from each country of origin were USSR 3,based on the reaction of Japanese differential

Cvs. (3 on the international set); China 7(9); Nepal 2(2); Thailand 3(2); Indonisia 4(4) and Colombia 5(12). The reaction of both sets of differentials did not always correspond. They mentioned that the phenotypic variability in pathogenic reaction may be due to heterogenecity of the differential rice cultivars as well as to pathogenic changes in the fungus.

Bunman et al (1987) examined the pathogenic variation among isolates of P. oryzae with two differential cultivar sets in Korea Republic and the Philippines. The data indicated some variants and support the hypothesis that the fungus is relatively stable pathogenically.

In Egypt, El-Kazzaz (1973) was the first to identify races of P. oryzae from 10 isolates, 5 races were identified as IB-3, IC-3, IE-1, IH-1 and II according to Atkins et al (1967) or IB-47, IC-17, IF-3, IH-1 and II-1 according to Ling and Ou (1969). Kamel (1975) identified 17 races in the period from 1969-1972 . Abdel-Hak (1981) stated that the identification of 38 isolates collected in 1973, resulted in 8 races i.e. IA-5, IA-13, IA-93, IB-29, IB-55, IC-16, IE-1 and IE-4. In 1975, the races identified were IA-13, IB-55 and IC-5. While in 1977 ten races were identified as IA-5, IA-13, IB-6, IB-55, IC-5, ID-5, ID-6, IE-1, IE-2, IF-1 and IG-1.

In 1983-a, Shatla et al, identified six races from ten isolates collected from 5 rice growing governorates . IA-68 and IG-2 were identified from Sakha, IA-22 from Gemmiza, IC-8 from Zarzora, IE-3 from Sherbien and IC-24 from El-Serw. Kamel et al (1985), reported that 31 races out of 121 isolates of P. oryzae were tested against 8 pre-released promising lines, the commercial cvs. Giza 171 and Giza 172 and from IRRI varieties i.e. IR-28, IR-30, IR-50, IR 1626-203 and Giza 180 in addition to Arabi, Giza 159 and Reiho. They stated that IR 28, IR 50 and IR 1626-203 and Giza 180 were resistant to all the tested races, while Giza 159 and Reiho were susceptible to almost all the races,except II . The eight pre-released lines were susceptible to different degrees. El-Refaei et al (1986), identified 6, 8 and 11 international races during 1981, 1982 and 1983 rice growing seasons,respectively.

Sehly et al (1990) , identified 5 race groups i.e. IC, ID, IG, IH and II. Race IG-1 was isolated from Behiera, Kafr El-Sheikh, Dakahlia and Sharkia governorates, with the frequency of 30%. While, race IH-1 was isolated from Behiera, Gharbia, Sharkia and Damietta governorates with the frequency of 25%. Race ID-13 was isolated from Behiera and Dakahlia and race ID-15 was isolated from Behiera and Damietta governorates with the frequency of 10

and 15%, respectively. Both IC-31 and II races were isolated from Behiera and Kafr El-Sheikh governorates with the frequency of 10% for each race.

II. SEROLOGICAL STUDIES:

a) Since the beginning of the twentieth century immunological procedures have become an integral part of microbiology. Aside from the many useful applications in the study of human and animal microbial infections. Serological tests play an important role in the identification and classification of microorganisms.

Such tests surpass in sensitivity practically all chemical and biochemical methods used for this type of work. The general principles governing the serology of microorganisms have been applied to fungi (Seeliger, 1968). The rapid development of mycology early in this decade increased the use of serological techniques by mycologists as an aid in the taxonomy, classification, and identification of fungi (Badami 1960 ; Preece 1971 ; Seeliger 1960 ; Seeliger 1962 ; Seeliger 1968 & Tempel, 1959).

After the second world war, little more was published in the field of serology of phytopathogenic fungi. Tempel (1959), reported that

number of physiologic races of five Puccinia species, a number of physio's of P. graminis f. sp. tritici could be be serologically distinguished; they behaved more as serological groups than as serological units. A better serological differentiation was obtained with physio's of P. graminis f. sp. avenae, especially with application of the complement-binding reaction .

Serology studies on certain fungi has not been used as extensively in research with phytopathogenic fungi (Preece, 1971; Seeliger, 1968).
Kalyanasund and Charudattan (1969), reported that three strains of F. oxysporum f. sp. vasinfectum share major antigens. The nonpathogenic strain showed significant antigenic differences when compared to pathogenic strains. Tempel (1959), found that, using gel immuno-diffusion and immunoelectrophoresis tests, it was difficult to distinguish serologically among the formae specials of Fusarium oxysporum. A gel immunodiffusion test was found to be the most suitable test for differentiating among Fusarium spp. when compared to microconidial agglutination and precipitin tests. Most sera did not differentiate the formae specials specifically. However, antisera to F. oxysporum f. sp. pisi and F. oxysporum f. sp. lupini antigens reacted specifically.

Morton & Duke (1967), demonstrated distinct serological differences between the genera Phytophthora and Pythium by means of immunodiffusion tests, but could not demonstrate differences between Phytophthora parasitica Dast. and P. parasitica var, nicotiana Tucker.

b) **Common antigens between fungal pathogens and their hosts:** Dineen (1963), proposed that the degree of antigenic disparity between the host and the pathogen may be the primary factor in the host-parasite interaction which would lead either to susceptibility or resistance of the host.

The greater the antigenic disparity between the host and the pathogen, the greater will be the resistance of that host to the pathogen. The literature in this area has been extensively reviewed (Carroll et al , 1972; DeVay et al, 1967 and Wimalajeewa & DeVey,1971). It has been proposed that the presence of a common pathogen host antigen in specific plant diseases may be an important factor that prevents triggering of the host defense mechanism, thus allowing the pathogen to parasitize the host (Devay et al, 1967; Doubly et al, 1960; Schnathorst,1969 and Schnathorst & DeVay 1963). In contrast to previous studies on the role of common antigens in plant disease, Carroll et al, (1972)demonstrated the lack of common antigens between

the host and pathogen in bacterial wilt of alfalfa caused by Corynebacterium insidiosum.

Comparison of fungal races and strains by gel-electrophoresis: The possibility of using the electrophoretic patterns of soluble protein and enzyme extracts as an acid in the classification of plant pathogenic fungi has been investigated (Gill and Powell, 1968; Hall , Zentmyer and Erwin-a,1969; Matsuyama and Kosaka, 1971 ; Shipton and Fleischman, 1969; Whitney & Vaughan and Heale, 1968).

One hundred and thirty-two isolates of the rice blast fungus from various sources were tested for their soluble protein and peroxidase enzyme patterns by Matsuyama and Kozaka (1971). Soluble proteins extracted from mycelium and enzymes from culture filtrates were subjected to polyacrylamide disc gel and thin layer electrophoresis. Two isolate groups were distinguishable in the soluble protein patterns and peroxidase zymograms. However, there was no significant correlation between the geographical distribution of the isolates and their pathogenicity of their protein or enzyme patterns.

C) Serological methods for determining disease resistance:

First Trials to compare the serological groups of cereals established by botanists with groupings

based on a number of other properties and thereby found a parallel between serological relationship and susceptibility to Puccinia rubigo-vera f.sp. triticina. This was confirmed by the investigations of Nelson & Birkeland (1929) and those of Edgecombe(1931) with wheat species and races.

Tempel (1959), mentioned that, the Russian investigator extracted protein from seed of different cotton species and allowed these to react with antiserums against Verticillium dahliae, Fusarium buharicum and Bacterium malvacearum , the reactions were positive or negative according to the susceptibility to, or resistance against, these micro-organisms in the field. Antigenic comparisons were made among the Flax varieties and two pairs races of Melampsora lini with the widest different in pathogenicity. The results indicated that a specific antigen was present in each of the rust races and in each of the rust differentiating lines of flax. Races of rust were pathogenic on only those times of flax containing its specific antigen as a minor constituent. (Doubly et al. 1960).

Fawzia Fadel and Abdel Hady (1988), investigated the antigenic strcture of protein in resistant and susceptible wheat varieties and fungal stem rust by using crossed immunoelectrophoretical technique and double diffusion test, beside detecting the antigenic relationships (common antigens)

between wheat (Triticum vulgare) and wheat stem rust (Puccinia graminis f. sp. tritici). Results showed that, the double diffusion test is not able to explain the antigenic relationships between wheat varieties and stem rust races However, the CIE was the most reliable technique for studying antigenic structure and host cultivar-rust race relationships (common antigen).

III. CULTURAL PRACTICES IN RELATION TO RICE BLAST INFECTION:

Rice blast disease caused by Pyricularia oryzae Cava, is the most important disease to rice crop in Egypt as well as in other rice growing countries.

Time of planting has been demonstrated to be an important factor in blast development. Early plantings in Japan usually show less disease than later plantings (Hashioka, 1950, 1950b; Kuribayashi Ichikawa, 1952). Marchetti and Dimond . (1976), found that increased resistance with increase in plant age occurred in greenhouse and field plants grown under flooded or nonflooded conditions. Chandramohan & Palaniswamy (1963), studied the relation between time of planting and blast disease incidence and noted that, severe blast was correlated with low temperature, high humidity and heavy

dews. In Barksdale's work in Florida (1961), the Colusa variety was planted in four fields on three dates space two weeks apart. The typical pattern of disease development showed the greatest rate of increase in the youngest planting, the least in the oldest. Many investigators reported that blast infection gradually decreased with aging of plants in all tested cultivars , indicating that both resistant and susceptible cultivars become increasingly resistance (Kohn & Libby,1958; Atkins, 1974; Koh et al, 1987 and Hwang et al, 1987). Loganathan & Ramaswang (1984), stated that although IR-50 rice cultivar had a disease index score for infection with P. oryzae of 16.9% when planted on September 14, 1983, it had a score of 66.8% when planted on October 13. Gowda and Pandurange Gowda (1982) found that crop sown fortnightly in January-June developed no more than 5% leaf blast and 1% neck blast, but in July disease incidence increased to 20 and 25% respectively, however, they added that maximum incidence of the disease i.e. 55-70% and 40-95% occurred when the crop was sown in Aug-Nov. Whereas, Bhatt and Chaukan (1985), found that the disease was more serious in early May plantings than in the late June planting.
Reddy & Bonman (1987), in their report on epidemics of rice blast in India and Egypt indicated that late sown fields were totally lost.

The evaluation of the commercial cultivars and the promising lines including both indica and japonica types for blast reaction: A considerable variation in the blast disease incidence between the varieties reported by some workers. Marchetti and Abdel-Hak (1985), reported that, when 22 Egyptian rice lines were evaluated against 7 U.S.A. races, most of the local traditional cultivars seemed to possess only the Pi-K^s gene, which afforded protection against only one race. Recombination of Pi-gene from U.S.A. and Egyptian lines through hybridization should multiply disease resistance to many P. oryzae races.

Kamel et al (1986), found that the number of blast lesions on the leaves of Reiho variety was four folds as those appeared on the leaves of Giza 171 and Giza 172 cultivars. Sporulation of the pathogen on Reiho variety was four folds that on Giza 171 cultivar and two folds that of Giza 172 cultivar. Reddy and Bonman (1987), reported that in Egypt, yields from demonstration fields indicated that the average loss in Giza 173(Reiho) was about 50%, whereas locally developed Giza 171 and Giza 172, although susceptible to Egyptian races of P. oryzae, were only mildly affected.

IV. NITROGEN AND BLAST INFECTION:

Timing and methods of nitrogen application are ameneable to improved fertilizer management practices which can be used to optimize nitrogen uptake. Two peak periods of requirement occur, one during the early vegetative growth stage which promotes optimum tillering, expands leaf area and maximizes the number of panicles per unit of area. A second period of high nitrogen requirement occurs as the plant enter the reproductive growth stage (Mikkelson, (1981).

It was reported that heavy nitrogenous fertilization generally predisposes the crop to blast infection in susceptible cultivars, while it has little or no effect on resistant ones (Atkins, 1956 ; Padmanabhan, 1974; Amin & Venkatarao, 1979 and Sehly 1982). Numerous experiments from different countries have indicated that Pyricularia oryzae incidence increased linearly with increasing nitrogen (Abdel-Hak, et al, 1975; Li et al, 1983; Shatla et al, 1983-b; El-Refaei et al, 1985 and Westcott & Guice, 1983). Also Chuke et al (1979), reported that high soil nitrogen increases blast severity regardless of available phosphorus or potassium. Sridhar (1974), found that nitrogen fertilizer generally reduced

phenolic compounds in both susceptible and resistant cultivars. Also Veerraju & Prasad (1975), reported that nitrogen fertilization up to 40 Lb/acre increased synthesis of the inhibitor to P. oryzae, while at higher levels there was a decline. Faria et al (1982), reported that incidence of leaf,neck and panicle blast increased with increasing nitrogen rates. Whereas, Park et al (1980-a) found that different levels of nitrogen fertilizer influenced leaf blast significantly, but had only a slight effect on panicle blast in Tongil cultivar. Abdel-Hak et al (1975), reported that incidence of disease was the lowest on adding $(NH_4)_2SO_4$, 15 or 45 days after transplanting. Also,Amin & Venkatarao (1979), found that maximum leaf blast was found on susceptible variety HR 12 at a high nitrogen level of 150 kg N/ha when applied as a basal dose. They added that, incidence of neck blast was low at a moderate nitrogen level of 75 kg/ha applied in split top dressings, with maximum grain yield. They pointed out that minimum neck blast was noticed when a lower level of 30 kg N/ha was applied in 3 split top dressings, but it reduced grain yield.

On the other hand, Huang et al (1980), reported that split application of nitrogen increased blast damage. Matsuyama and Dimond (1973), found that high nitrogen fertilizer reduced resistance to P. oryzae.

While Efinova & Dyakunchak (1986), reported that application of silicon fertilizers with higher rates of nitrogen resulted in increased thickness of the outer wall of rice epidermal cells and increased resistance to P. oryzae.

Some investigators reported that, when nitrogen application was supplemented with chemical control of the blast pathogen, yield was increased but without fungicide treatment, higher nitrogen increased disease severity and yield losses (Ribevo, 1980 and Montoya, 1985).

V. RELATIONSHIP BETWEEN SUSCEPTIBILITY TO LEAF AND NECK BLAST:

It has been reported that certain cultivars, resistant to blast in the leaf stage were observed to be susceptible to neck rot and conversely, cultivars susceptible to leaf blast showed little or no neck blast (Ono and Zuzuki, 1960; Chang et al, 1965; Padmanabhan, 1974 and Hwang et al, 1987).

Similar phenomena were reported by Asaga and Yoshimura (1970); Chung and Koh (1987), which indicates that resistance to panicle blast may be expressed in some genotypes of rice independently of that to leaf blast. They added that the reduction in leaf blast on rice plants at later growth stages may affect

the severity. The highest yield reduction occurred in the cultivar Jinju which was highly susceptible to both leaf and panicle blast. However, higher yield reduction were obtained in the cultivars Jinhewing and Akibara , both highly susceptible to panicle than in cultivar Nakadong, which was only susceptible to leaf blast. This also indicates that yield reduction by panicle blast infection is twice as much as that by leaf blast. However, positive correlation between leaf and panicle infection exists for the more susceptible cultivars was proved by many investigators (Rangaswami and Subramanian, 1957; Ou & Nuquei, 1963; Chin & Amin , 1983 and Ahn (1977).

Willis et al (1968), found a high correlation between seedling and panicle reaction of rice cultivars to different races of P. oryzae.

Park et al (1980-b)indicated that leaf blast lesions were more prevalent from the flag leaf to the third leaf from the top in Tongil lines and on the second leaf from the top of Japonica type rice cultivars. Lesions on the flag leaf and second leaf from the top were the main inoculum source for panicle blast after the booting stage.

MATERIALS AND METHODS

I. LABORATORY AND GREENHOUSE EXPERIMENTS:

(1) Isolation, identification of rice blast fungus:

Diseased rice leaves and panicles showing different types of lesions or discoloration were collected from different localities representing rice growing governorates, i.e. Kafr El-Sheikh , Gharbia, Behiera and Dakahlia, throughout 1984 and 1985 seasons. Isolation trials were carried out from diseased materials after surface sterilization with 3.5% sodium hypochlorite solution for two minutes, then, thoroughly washed in sterile water and carefully dried between two sterilized filter papers. Small pieces of thus surface sterilized plant materials were transferred onto plain agar (WA) medium.

Monoconidial cultures of the isolates were obtained by the use of the single spore method.

The pathogenic propensities of the isolated pathogen during this work were performed using spore suspension prepared from 14 day-old cultures of the isolates,grown on banana media at 28°C to be tested. In seedling stage, 21 day-old plants with 3-4 leaf stage,were sprayed with spore suspension ($5x10^4$ - spores/ml.). Seedlings were kept inside

growth chamber,under favourable environmental condition, until being inoculated. The inoculated plants were put inside inoculation chambers which were made of wooden frames,1.5 x 1 x 0.6 m, and covered from outside with polyethylene layer with cheese-cloth impregnated with water, in addition to a pot full of water was put inside the chamber to maintain high relative humidity required to complete the infection process. The inoculation chambers were removed after 24 hrs. of inoculation and seedlings were kept inside the growth chamber at 28°C until appearance of disease symptoms.

(2) Race identification:

Methods recommended in the United States and Japan (Atkins et al, 1967) and Padmanabhan et al, (1970) in India, for the identification of the pathogenic races of P. oryzae were followed in this work. The eight international differential varieties i.e. Raminad-Str. 3, Zenith, NP 125, Usen , Dular, Kanto 51, Sha-tia-tsao and Caloro, which were obtained from the International Rice Research Institute (IRRI), Philippines, were used in this study. Seven Egyptian cultivars namely, Giza 159, Giza 171, Giza 172, Reiho, IR 28, IR 1626-203(Giza 181) and IR 50 were kindly obtained from the Rice Research and Training Center, Sakha, Kafr El-Sheikh, were

also included. Twenty one-day old seedlings of the tested cultivars were grown in 50x20x7 cm. plastic trays, each tray comprised ten rows with ten plants/row. The outside rows were grown by highly susceptible cultivar Giza 159 as a check. The trays were kept in the greenhouse at 25-30°C, green manure was incorporated with soil (200 g/tray), while Urea 46% (5 g/tray) and Zinc Sulphate (1 g/tray) were added ten days after germination. Inoculations were performed in the evening in order to avoid the retarding effect of light on the spore germination and growth of the germ tubes . The reaction of each of the differential varieties to the tested isolate of P. oryzae was estimated after seven days of inoculation according to IRRI Scale, 1980.

(3) **Serological study on Pyricularia oryzae and the tested rice Cultivars:**

The objective of this study is to determine the level of resistance in three rice cultivars to P. oryzae by the serological tests as a new system of differentiation; (Park et al, 1986). The following materials were used in the present study:

1. Hyphal and conidial growth of P. oryzae.
2. Inoculated and uninoculated seedlings of Reiho cv. as a highly susceptible short grain.

3. Inoculated and uninoculated seedlings of GZ 2175-5-6 as a resistant new short grain promising line.
4. Healthy seedlings of IR 28 as a highly resistant long grain cv.

A. Preparation of antigens: An isolate of P. oryzae was cultured on (PD) liquid medium for two weeks at 28°C. The culture was harvested by filtration, washed several times with saline solution (0.85% NaCl solution), then with sterile distilled water, freezed-dried , ground to fine powder, diluted with saline solution and kept at 4°C for 24 hrs. The resultant mixture was centrifuged at 8000 r.p.m. for 20 min., then the supernatant was collected and the protein content adjusted to 20 mg/ml. before injection into rabbits,this material was used for the immunization.

B. Preparation of rice plants juice: Twenty one days-old seedlings of the tested cultivars were artificially inoculated by a virulent isolate of P. oryzae, ten days later the leaves were removed and homogenized in equal volume of extraction buffer containing 84 mM citric acid, 32 mM Na_2HPO_4, 14 mM mercaptoethanol and 6 mM L-ascorbic acid, pH 2.8 (Antoniw and Pierpoini, 1978). The homogenate was centrifuged at low speed (10.000 g for 20 min.), then the supernatant was collected and the protein content

was adjusted to 20 mg/ml; this material was used for the immunization.

C. **Immunization and production of antibodies:** Antigens of P. oryzae growth and rice seedlings of the three cultivars were used for injection to rabbits. Each rabbit received 10 injections (two injections per week). Incomplete adjuvant was mixed with antigen (ratio 1:1) before the injection, the volume of antigens were 2, 2.5, 3, 3.5, 4, 4.5 and 5 ml for injections nos. 1,2,3,4,5,6 and 7, respectively; 5 ml was used for each of the remaining ones. The injection was first given subcutaneously, then intramuscularly. Rabbits were bled one week after the last injection. Antisera were collected.

D. **Preparation of the glass plates and gels for immunoelectrophoresis:** 80x60x1 mm. glass plates were used, cleaned by washing in detergent followed by repeated washing in distilled water, a final washing was in ethanol 95%, then dried, and the glass plates were coated with a thin film of agarose.

E. **Agar double-diffusion test:** The agar double-diffusion method, was used to compare antigen-antibody reaction following the standard Ouchterlong method. The diameter of the holes was 10 mm and the distance

between the holes from outer to center was 10mm. The agar plates were maintained at 25°C and reaction was observed after 48 hours.

F. Crossed immunoelectrophoresis (CIE) techniques: CIE techniques were performed according to the procedure of Axelsen et al (1973) with some modifications. Agarose (1.0% Bio-Rad agarose powder) dissolved by heating in barbital-sodium barbital buffer pH 8.6, ionic strength (0.03). Electrophoresis in the first dimension was performed on 6x8 cm glass plates. The prewarmed plates (45 °C) were covered with 7.5 ml of agarose gel. After cooling at room temperature, three antigen wells (4 mm in diameter) were punched out 3.5 cm from the cathodic end. Antigen samples centrifuged at 20000g for 1 hr were placed in each well. Bromophenol blue was used in one well an indicator for 5 cm migration. Electrophoresis was performed for one hour in a water cooled electrophoresis chamber at 10 v/cm. After the first dimension electrophoresis, agarose strips (6x1.5 cm) containing antigen were cut and transferred on one side of the glass plate. The remaining space on the plate was then covered with 6 ml agarose containing 0.3 ml purified immunoglobulin preparation. Electrophoresis in the second dimension was performed at

2 V/cm for 16-20 hrs, after immunoelectrophoresis. Precipitation peaks were developed with Coomassie Brilliant Blue G-250 Staining, and the gels were destained with a methanol : acetic acid : water (4:1:5 v/v) mixture.

G. Preparation of the glass plates for the staining: Steps were carried out as follows: **1)** Pressing of the gel according to Laurell (1965), after electrophoresis, the gel was covered with a layer of a wet filter paper (air bubbles avoided) and 2-3cm layer of soft blotting paper. A slight pressure (about 10 g per cm^2) was sustained by means of a thick glass plates or books. After 10-15 minutes the pressure and the blotting paper were removed. Two washings with 0.1M NaCl was performed 15 min. each, a third wash for at least 15 min., in distilled water was done. A new pressing of the gel for 10-15 minutes, filter paper was removed and the gel was dried in cold air.

H. Staining of immunoprecipitates: After the gel was dried completely to a fine film, the staining procedure was carried out as follows: The plates were placed in the staining solution for about 10 minutes , sequency destained three times for 10 min. each then washed in washing solution (Ethyl alcohol + acetic acid glacial) finally dried in cold air.

II. FIELD STUDIES:

1. Effect of time of planting on rice blast infection and grain yield :

The experiment was carried out at Sakha Agricultural Research station during two successive seasons i.e. 1986 and 1987, under the favourable environmental conditions for the disease development. Giza 172 as a commercial cv.; Giza 175 and Giza 181 as new cvs.; GZ 2175-5-6 and GZ 1368-5-4 as promissing lines; Reiho as a highly susceptible cv. and IR-50 as a resistant long grain cultivar under Egyptian environmental condition, were used. A split-plot design with four replicates was followed the main plots were assigned to two dates of planting, while the seven cultivars were allocated in the sub-plots. The period between first date of sowing and the second date was fifteen days during 1986 and 1987 growing seasons. The experimental area was transplanted with 30 day -old seedlings. The plot size was 2.25x5 m, it comprised 11 rows and the hills were 20 cm apart. Giza 159, the highly susceptible cv., was cultivated as borders to increase inoculum density. The nitrogen fertilizer was used in the form of Urea (46.5% N) at the rate of 40 units of nitrogen per feddan. One half of the nitrogen dose was

incorporated to top 15 cm of the dry soil, while the other half was added at panicle initiation. To estimate the leaf blast infection and to determine the disease progress curve for each variety in each planting date, samples were taken four times at 15 days intervals, starting 15 days after transplanting. Estimation of both leaf and neck blast infection was recorded.

2. Effect of methods and time of N-feretilizer application on rice blast disease development:

The effect of nitrogen fertilizer dose and all possibilities of application on rice blast infection and yield of three local cultivars, Reiho, Giza 172 and Giza 175 were used for the present investigation. Thirty days-old seedlings were transplanted in the farm of the Rice Research and Training Center, Sakha Station . The experiment was conducted throughout 1986 and 1987 seasons, it was designed in split plot with four replicates. Varieties were the main treatment , whereas the nitrogen dose and time of their application were sub-treatments. Urea 46% was applied at the rate of 40 units of nitrogen per feddan. The method of N-fertilizer application were as follow:

1) All amount of N was incorporated into soil just before transplanting.

2) Half amount was incorporated into soil before transplanting and the other half was added after 25 days from transplanting.
3) Half amount was added after 25 days from transplanting and the other half after 50 days from transplant-
4) Two third of the fertilizer was incorporated into soil before transplanting and the rest was added after 25 days from transplanting.
5) Two third of the fertilizer was incorborated into soil as the treatment No. 4, while the third was added after 50 days.
6) The amount of fertilizer was divided to three equal doses (1/3), the first dose was added ~~with~~ the soil before transplanting, the second was added after 25 days, and the last third was added after 50 days from transplanting.
7) All amount of fertilizer was added after 25 days from transplanting.
8) Control, without addition any fertilizer.

The experimental area was transplanted with 30 days -old seedlings. The plot size was 2.25x5 m, comprised 11 rows, and the hills were 20 cm apart.

Estimation of leaf blast infection: A sample of 100 leaves was randomly collected from each plot to determine leaf blast infection. Percent of the infected

leaves was calculated, while severity of infection was estimated by counting the total number of type (4) lesions. To determine the disease progress curve for each treatment, samples were taken four times at 15 days intervals, starting 15 days after transplanting.

Estimation of neck blast infection: Samples of one hundred panicles were randomly collected from each treatment to determine the percentage of infected plants with neck rot, while the severity of neck blast infection was calculated by using the following formula adopted by :

$$S = \frac{\text{Sum } (n \times v) \times 100}{10\ N}$$

where, S= Severity of infection; n= number of panicles within infection category, (from one with one infected primary branch of the panicle to 10 for the complete infection in the upper most internode of the panicle which name neck infection); v= numerical values of infection categories; N= Total number of panicles; 10= Constant (highest numerical value).

From the percentage and severity of neck blast infection, degree of neck infection was obtained from the equation:

$$\text{Degree of neck infection} = \frac{\text{\% of neck infection} \times \text{Severity of neck infection}}{10}$$

(Abd El-Hak *et al*, 1982).

Grain yield: Yield of each plot was harvested leaving the two outer rows round each plot. Grain yield at 18 % moisture content was weighted in kg/plot.

EXPERIMENTAL RESULTS

I. Isolation of the causal organism :

Trials were carried out during 1984 and 1985 rice growing seasons to isolate and study the pathogen. Inoculation of 21 days-old rice seedlings, grown under greenhouse condition, showed typical blast lesions similar to those on the naturally infected plants . Samples of diseased rice leaves were collected. Samples were collected from different localities of the rice growing areas i.e. Behiera, Gharbia, Dakahlia and Kafr El-Sheikh Governorates (Table 1). Portions of surface sterilized diseased tissues were placed on potato dextrose agar (PDA) medium in Petri-dishes and were incubated at 28°C. Single spore technique was used for obtaining pure culture. The causal agent was identified as Pyricularia oryzae Cavara. The mycelium of fungus was hyaline to pale olive , 1.5-6.0 in width, septate and branched. Conidia variable in size and shape, terminal, pyriform, base rounded, 2-septate, rarely 1-3 septate, almost hyaline to pale olive, the average length of conidia ranged from 20-26 u and width from 6-11 u.

Table (1): Distribution of physiologic races of Pyricularia oryzae in different localities in Egypt during 1984 and 1985 rice growing seasons.

Season	No. of isolates	Physiologic* races	Geographical distribution of physiologic races			
			Kafr El-Sheikh	Beheira	Gharbia	Dakahlia
1984	1	IA-107	Kalleen			
	1	IB-60			El-Gimmeza	
	3	ID-11	Sakha**, Ibshan			
	1	IF-3			Basioun	
	2	IG-1	Desouk	El-Khazan		
1985	1	IB-38	Sakha			
	2	IB-44		Abo-Homos		Dekernis
	1	IC-17			El-Gimmeza	
	1	ID-1		Abo-Homos		
	1	ID-16	El-Hamrawy			
	1	IE-1	El-Agozeen			

* The standard international race number adopted by Ling & Ou (1969).

** Two isolates were obtained from two locations in Sakha.

II. **Identification of the prevalent races of the blast fungus in Egypt :**

Experiments were designed for this purpose in which the method recommended by Atkins et al (1967) for the identification of the pathogenic races of P. oryzae was followed.

Fifteen isolates of P. oryzae, which were isolated from lesions of diseased rice leaves collected from different rice growing governorates, were tested in these experiments. Isolation trials were made on PDA medium. Cultures were purified by dilution method and single-spore isolates were cultivated on PDA medium at 25°C. Stock cultures were maintained on straw decoction agar medium at 25°C. Spore suspensions were prepared from 10 day-old cultures grown on Banana medium at 28°C, having at least 25 spores/microscopic field under 10X objective. Twenty one day-old seedlings of the eight differential varieties namely, Raminad st. 3, Zenith, NP-125, Usen, Dullar, Kanto-51, Sha-tia-tsao and Caloro, and two Egyptian cultivars namely Giza 159 and Giza 172 which were used as supplementary local vars., were sprayed with the inoculation suspensions to insure uniform covering of the leaves. Inoculation was carried out in the evening, and the inoculated plants

were held at 25-30°C for 24 hrs. under the inoculation chambers to maintain high relative humidity, then transferred to the greenhouse for 7-9 days. Plant reactions to each isolate were estimated after seven days from inoculation. Special stress was given to the reaction of leaves which were expanded at the time of inoculation. The reaction of each of the differential varieties to the isolates tested was scored according to the reaction types previously described in the materials and methods. Only two reactions, resistant = (R) and susceptible = (S), were used for race identification.

The obtained data are shown in Table (1). From the presented data, the following findings could be concluded. Fifteen isolates of P. oryzae were tested and identified in both 1984 and 1985 seasons according to Ling & Ou (1969). Eight isolates out of fifteen were tested in 1984. They were grouped in five races i.e. IA-107 , IB-60, ID-11, IF-3 and IG-1 which were prevalent in different locations ,representing Kafr El-Sheikh, Behiera and Gharbia governorates. However, races ID-11 and IG-1 were the most frequently common in Kafr El-Sheikh and Behiera Governorates, while IB-60 and IF-3 were found in Gharbia Governorate .

In 1985 rice growing season, seven isolates of the fungus were grouped to six races, i.e. IB-38, IB-44 ,

IC-17, ID-1, ID-16 and IE-1 which were distributed in different locations representing Kafr El-Sheikh, Behiera, Gharbia and Dakahlia Governorates. The two Egyptian cultivars namely Giza 159 and Giza 172 were susceptible to most of the tested isolates.

III. Evaluation of some rice cultivars and promising lines to blast disease infection under greenhouse conditions :

In 1984 rice growing season, 8 isolates representing races IA-107, IB-60, ID-11, IF-3 and IG-1 showed different reaction types on 10 rice cultivars and lines namely Giza 159, Giza 171, Giza 172 and Reiho as Japonica type (local cultivars); IR 28, IR 1626-203 and IR 50 as Indica type, and GZ 882, GZ 991 and GZ 1394-10-1 as promising lines.

Data in Table (2) indicated that Reiho and GZ 991 were susceptible to all tested races. Giza 159 and GZ 882 were susceptible to all tested races, except race IB-60 which gave MR and R, respectively. On the other hand, IR 28, IR 50 and GZ 1394-10-1 were completely resistant to all tested isolates . IR 1626-203 was resistant to all races, except race IB-60 which gave susceptible reaction. Giza 171 and Giza 172 were moderately resistant. Since they

Table (2): Reaction of ten rice cultivars and entries to eight isolates representing 5 races of Pyricularia oryzae under greenhouse conditions in 1984.

No.	Entries and cultivars	Reaction of races and sub-races of P. oryzae						
		IA 107	IB 60	ID-11 a	ID-11 b	ID-11 c	IF-3	IG-1
1	Giza 159	S	MR	S	S	S	S	S
2	Giza 171	R	MR	MR	S	S	R	R
3	Giza 172	R	MR	MR	S	R	R	S
4	Reiho	S	S	S	S	S	S	S
5	IR 28	R	R	R	R	R	R	R
6	IR 1626-203 (Giza 181)	R	S	R	R	R	R	R
7	IR 50	R	-	S	S	S	S	S
8	GZ 882	S	R	S	S	S	S	S
9	GZ 991	S	S	S	S	S	S	S
10	GZ 1394-10-1 (Giza 175)	R	R	-	R	R	R	R

R = Resistant
MR = Moderately Resistant
S = Susceptible

showed different reaction types toward infection with the tested isolates or races. However, three isolates of race ID-11 showed different reactions on those two cultivars. Thus the three isolates could be differentiated into three sub-races i.e. ID-11a, ID-11b, and ID-11c. Since they gave different reaction types on either Giza 171 or Giza 172 .

In 1985 season, twelve rice entries, lines and commercial cultivars were inoculated with six races of blast fungus i.e. IB-38, IB-44, IC-17, ID-1, ID-16 and IE-1, Data are presented in Table (3). The data showed that IR 28 and IR 50 were resistant to all tested races , IR 1626-203 showed susceptible reaction (type-4 lesions) to races IB 38, IB 44a and IC 17, while it was resistant to the rest of the tested races.

Promising lines GZ 1368-5-2 & GZ 1368-5-4 and GZ 1394-10-1 were resistant to all tested races, while GZ 1108 was resistant to races IB-38, ID-1 and IE-1. However, GZ 1443-8-2 was susceptible to races IB-44a and ID-16, and susceptible to rest of the tested races.

On the other hand, Giza 159, Giza 171, Giza 172 and Reiho were susceptible to most of the tested races and showed resistant or moderately resistant to one or another of the tested races.

Table (3): Reaction of 12 rice entries to seven isolates representing seven races of Pyricularia oryzae under greenhouse conditions in 1985.

No.	Entries and cultivars	Reaction of races of P. oryzae					
		IB-38	IB-44 a	IB-44 b	IC-17	ID-1	ID-16	IE-1
1	Giza 159	S	S	S	R	R	S	S
2	Giza 171	S	S	R	S	S	S	S
3	Giza 172	S	S	R	R	S	S	S
4	Reiho	S	S	R	S	S	S	MR
5	IR 28	R	R	MR	R	R	R	R
6	IR 1626-203 (Giza 181)	S	S	R	S	MR	R	R
7	IR 50	R	R	R	MR	R	MR	MR
8	GZ 1108	R	S	S	S	MR	S	R
9	GZ 1368-5-2	-	R	R	R	MR	-	R
10	GZ 1368-5-4	-	R	R	R	R	-	R
11	GZ 1394-10-1 (Giza 175)	-	R	MR	-	MR	R	R
12	GZ 1443-8-2	-	S	R	-	R	S	R

R = Resistant
MR = Moderately Resistant
S = Susceptible

IV. Serological studies on the antigenic relationships between Pyricularia oryzae and some susceptible and resistant rice cultivars:

A) Serological differences among rice cultivars inoculated or not inoculated with P. oryzae:

Three rice cultivars, Reiho as susceptible cv., IR 28 as resistant cv. and GZ 2175-5-6 as promising lines, inoculated or uninoculated with P. oryzae, were compared serologically by using double gel diffusion (DGD) and crossed immuno-electrophoresis (CIE) techniques. The DGD test showed that one antigen was not detected in inoculated Reiho plants, that was detected in the uninoculated plants, i.e. five and six antigens were detected, respectively, in the homologous reactions (Table 1; Fig. 1). In the cross heterologous reactions between antibodies against inoculated and uninoculated Reiho, and antigens of uninoculated and inoculated plants, the same antigens were detected.

No differences in the antigenic structures of uninoculated and inoculated plants of IR 28 cv. were observed when antigens of its uninoculated and inoculated plants were reacted with antibodies against inoculated and uninoculated plants of all the rice cultivars.

Three antigens were found in common between IR 28 cv. and Reiho cv., while six antigens were common between IR 28 and GZ 2175-5-6. On the other hand, four antigens were found in common between inoculated Reiho and inoculated GZ 2175-5-6, while five antigens were common between uninoculated plants of Reiho and GZ 2175-5-6.
One antigen was not detected in the inoculated plants of GZ 2175-5-6, when compared to the uninoculated plants, as six and seven antigens were detected, respectively, in the homologous reactions (Table 4).

The present results clearly showed that antigenic differences occurred between uninoculated and inoculated plants of the rice cultivars, Reiho and GZ 2175-5-6, while no differences were found between uninoculated and inoculated plants of IR 28. Also, the results revealed that GZ 2175-5-6 was serologically related to both Reiho and IR 28 cvs., since four and six antigens were found in common, respectively, while three antigens were found in common between Reiho and IR 28 cvs. (Fig. 2b).

In the CIE technique, the numbers of precipitin peaks detected in all the reactions were almost twice those detected in the DGD test. CIE showed that three antigens were undetectable in inoculated Reiho cv. by

Table (4): Serological reactions among uninoculated and inoculated rice cultivars and P. oryzae using the double gel diffusion test.

Antibodies \ Antigens		Number of precipitin lines detected						
		Reiho 73		IR 28		Giza 2175		P. oryzae
		-	+	-	+	-	+	
Reiho	-	5+S	5	3	3	4+S	4	1+S
	+	5	5	3	3	4	4	1
IR 28	-	3	3	6	6	6	6	1
	+	3	3	6	6	6	6	1
Giza 2175	-	4+S	4	6	6	6+S	6	1+S
	+	4	4	6	6	6	6	1
P. oryzae		1+S	1	1	1	1+5	1	6+5

- : Uninoculated plants
+ : Inoculated
S : Antigens associated to susceptibility

comparison with uninoculated plants. Since eleven and fourteen antigens were detected, respectively, in the homologous reactions (Table 5, Fig. 4 a,b), while eleven antigens were detected in the heterologous reactions (Fig. 5).
Furthermore, CIE with an intermediate gel showed these three antigens with the uninoculated Reiho by comparison with the inoculated plants (Fig. 6).

Antibodies against uninoculated Reiho differentiated between uninoculated and inoculated GZ 2175-5-6 , since one antigen was not detected in the inoculated plants, while thirteen antigens were found common among both inoculated and uninoculated plants (Table 5).

On the other hand, no antigenic differences were found between uninoculated and inoculated plants of all the tested cultivars when their antigens were tested with antibodies against inoculated and uninoculated IR 28, i.e. fourteen antigens were detected in the homologous reactions of uninoculated and inoculated IR 28 (Fig. 7 a,b). On the other hand fourteen and seven antigens were detected between antibodies against IR 28 (inoculated and uninoculated) and antigens of GZ 2175-5-6 and Reiho,respectively (Fig 8 a,b). One antigen was not detected from inoculated GZ 2175-5-6 by comparison

Table (5): Serological reactions among uninoculated and inoculated rice cultivars and Pyricularia oryzae using CIE technique.

Antigen/Antibody	Precipitin peaks detected																	No. of precipitin peaks
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	20	21	22	
(-) (-) Reiho X Reiho	1	1	1	1	1	1	1	1	1	1	1	1	1	1	–	–	–	14
(+) (+) Reiho X Reiho	1	1	1	1	1	1	1	–	1	1	1	–	1	–	–	–	–	11
(+) (-) Reiho X Reiho	1	1	1	1	1	1	1	–	1	1	1	–	1	–	–	–	–	11
(-) (-) IR 28 X IR 28	1	1	1	1	1	1	1	1	1	1	1	–	1	–	1	1	–	14
(+) (-) IR 28 X IR 28	1	1	1	1	1	1	1	1	1	1	1	–	1	–	1	1	–	14
(-) (-) GZ 2175 X GZ 2175	1	1	1	1	1	1	1	1	1	1	1	–	1	–	1	1	–	14
(+) (+) GZ 2175 X GZ 2175	1	1	1	1	1	1	1	1	–	1	1	–	1	–	1	1	–	13
(-) (-) Reiho X IR 28	–	–	–	1	1	1	1	–	1	–	1	–	–	1	–	–	–	7
(-) (+) Reiho X GZ 2175	1	1	1	1	1	1	–	1	–	–	1	–	–	–	–	1	–	9
(-) (-) GZ 2175 X IR 28	1	1	1	1	1	1	1	1	–	1	1	–	1	–	1	1	1	14
(-) GZ 2175 X Fungus	1	1	1	–	1	1	–	1	–	–	1	–	–	–	–	1	–	8
Fungus X Fungus	1	1	1	1	1	1	1	1	1	1	1	–	–	–	1	1	–	13

- = Uninoculated + = Inoculated

with uninoculated, since thirteen and fourteen antigens were detected, respectively (Table 5). Thriteen antigens were detected between antibodies against GZ 2175-5-6 (inoculated) and antigens of inoculated GZ 2175-5-6 (Fig. 9 a,b).

Generally CIE technique showed that the serological relationship between GZ 2175-5-6 and IR 28 was more than those between Reiho and any of these cultivars . Also, the results revealed that three and one antigens were not detected in inoculated cultivars of Reiho and GZ 2175-5-6 respectively, when compared with uninoculated plants . No changes in the antigenic structures were found between inoculated and uninoculated IR 28 (Fig. 7 a,b).

B) Common antigens between Pyricularia oryzae and rice cvs.:

Both the DGD and CIE techniques showed that there were common antigens between P. oryzae and all tested rice cultivars. The common antigens between P. oryzae and the uninoculated plants were more than those detected with the inoculated plants, except, for IR 28 , whereas no differences were found. In the DGD tests (Table 4) two antigens were found common between P. oryzae and the uninoculated Reiho, while one antigen

Fig (1a) : Precipitin bands detected when antiserum of uninoculated plants of Reiho cv. reacted with its homologous, ,heterologous (3^-,3^+) and GZ 2175(5^-,5^+) antigens .

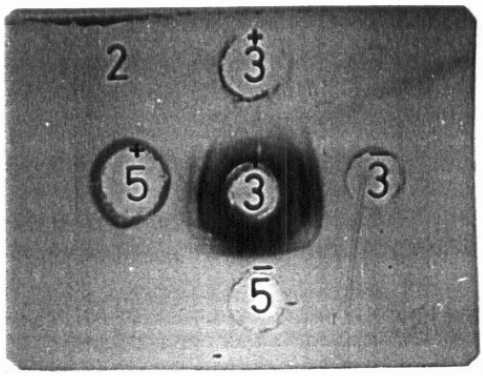


Fig (1b) : Precipitin bands detected when antiserum of inoculated plants of Reiho cv. reacted with its homologous,heterologous (3^+,3^-) and GZ 2175 (5^+,5^-) antigens .

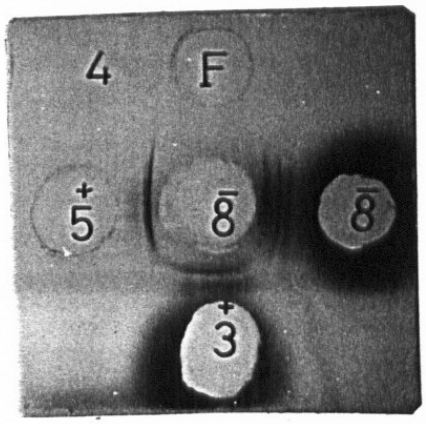


Fig (2a) : Precipitin bands detected when antiserum of uninoculated plants of IR28 cv. reacted with its homologous and inoculated Reiho cv. (3^+) , inoculated GZ 2175 (5^+) , Poryzue, antigens .

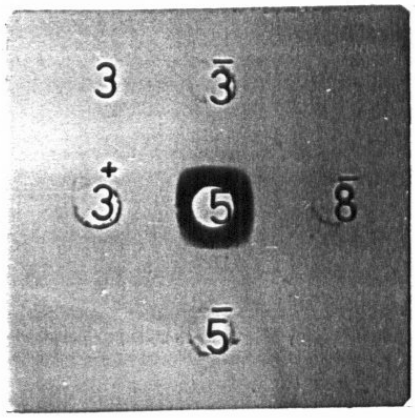


Fig (2b) : Precipitin bands detected when antiserum of inoculated plants of GZ 2175 cv. reacted with, GZ 2175 (5^-) , IR28 (8^-) and Reiho (3^-,3^+) antigens .

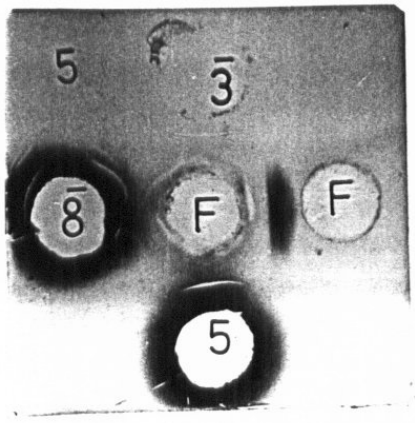
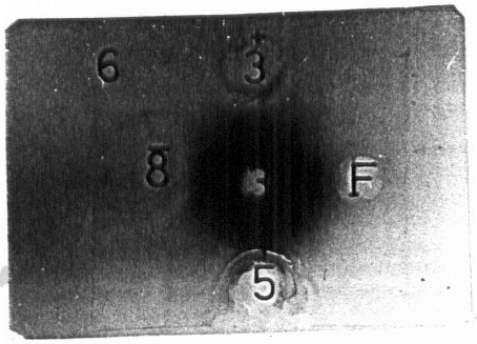


Fig (3a) : Precipitin bands detected when antiserum of P.oryzue (Pathogen) reacted with its homologous and uninoculated plants Reiho (3^-) , IR28 (8^-) , GZ 2175 (5^-) antigens .



Eig (3b) : Precipitin bands detected whenantiserum of inoculated plants of Reiho cv. , reacted with homologous (3^+) , IR28(8^-) , GZ 2175 (5^+) and P.oryzue , antigens .

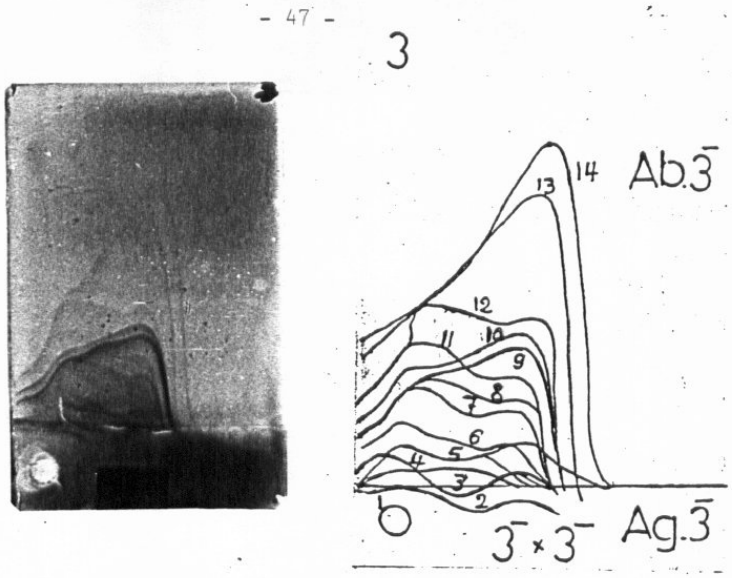


Fig. (4a): Crossed immunoelectrophoresis of uninoculated plants of Reiho cv., antigen-antibody system. Antigen (Ag-$\bar{3}$) was electrophoresed, reference gel contained the homologous antibody (Ab.$\bar{3}$). Anodes at the right and the top.

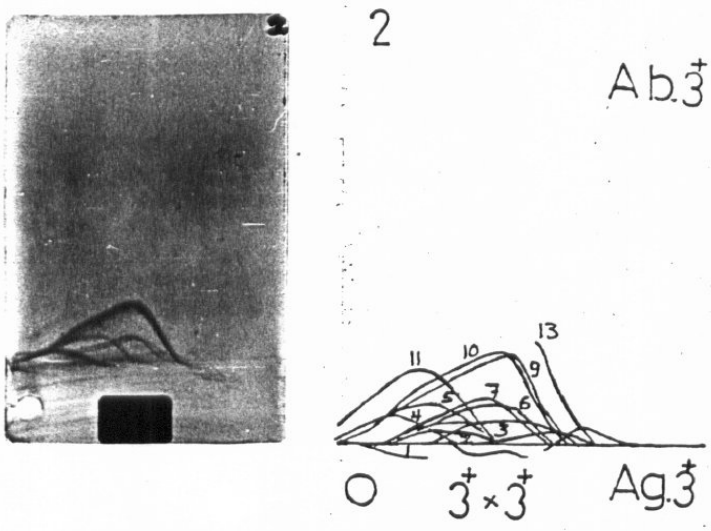


Fig. (4b): CIE of inoculated plants of Reiho cv., antigen-antibody system. Antigen (Ag.3^+) was electrophoresed, reference gel contained the homologous antibody (Ab.3^+). Anodes at the right and the top.

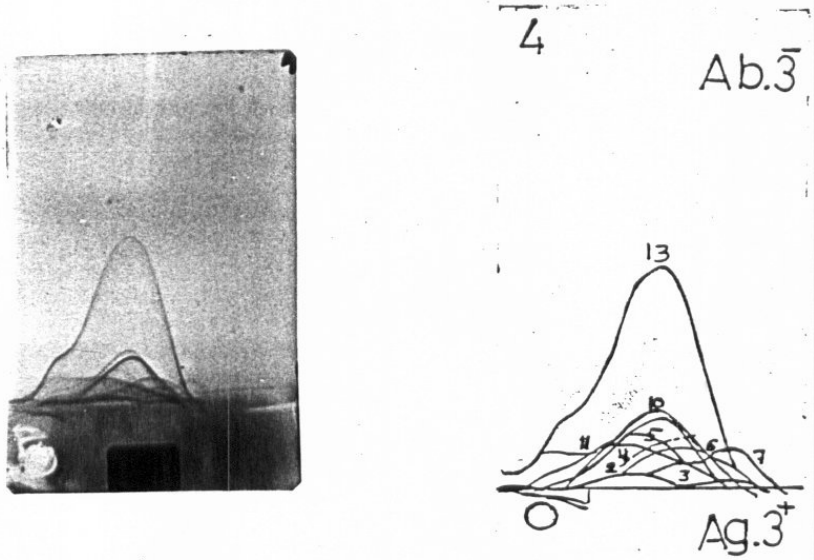
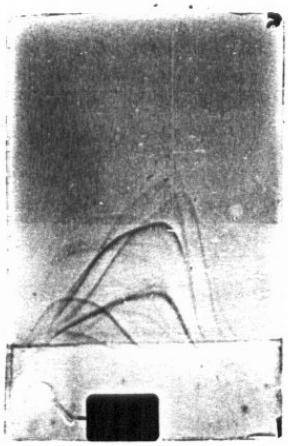


Fig. (5): Crossed immunoelectrophoresis of inoculated plants of Reiho cv., uninoculated plants of Reiho cv., antigen-antibody system. Antigen ($Ag.3^{+}$) was electrophoresed reference gel contained the antibody ($Ab.\bar{3}$). Anodes at the right and the top.



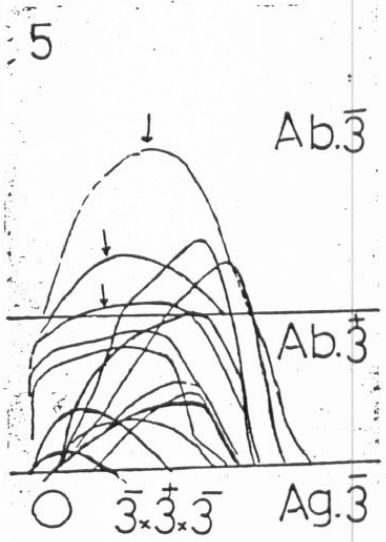


Fig. (6): CIE with an intermediate gel of reference $\bar{3}$ and anti $\bar{3}$, antibody system. reference gel contained $\bar{3}$, intermediate gel contained (Ab.3^{+}), ($\bar{3}$) antigen was electrophoresed . Anodes to the right and the top. Arrows refers to the specific peak for $\bar{3}$.

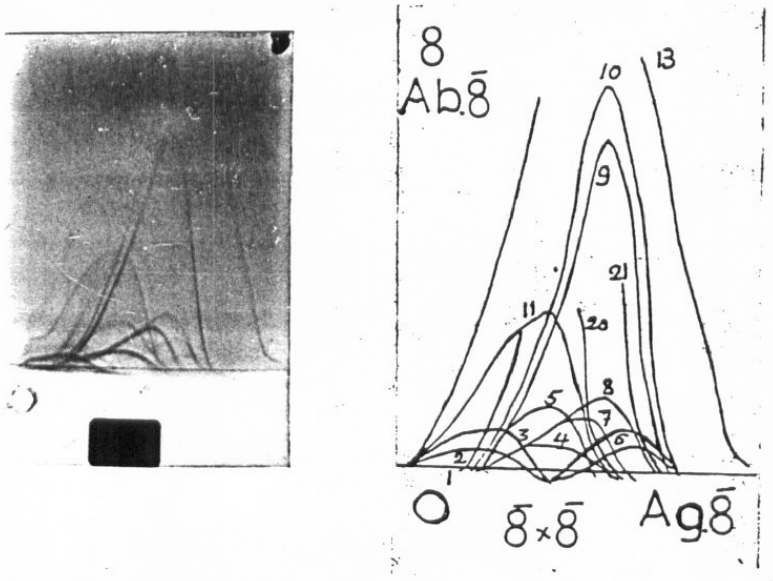


Fig. (7a): Crossed immunoelectrophoresis of uninoculated plants of IR 28 cv. antigen-antibody system. Antigen (Ag.$\bar{8}$) was electrophoresed, reference gel contained the homologous antibody (Ab.$\bar{8}$). Anodes at the right and the top.

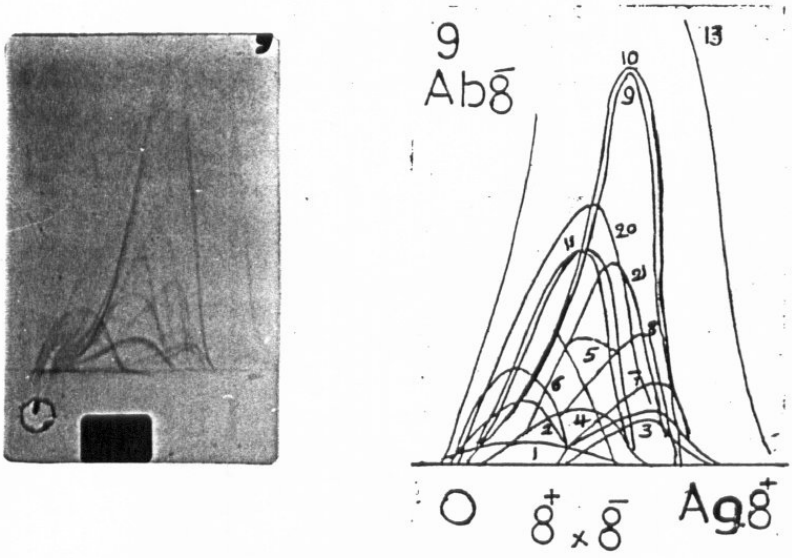


Fig. (7b): CIE of inoculated plants of IR 28 cv. antigen-antibody system. Antigen (Ag.8^+) was electrophoresed, reference gel contained the antibody (Ab.$\bar{8}$). Anodes at the right and the top.

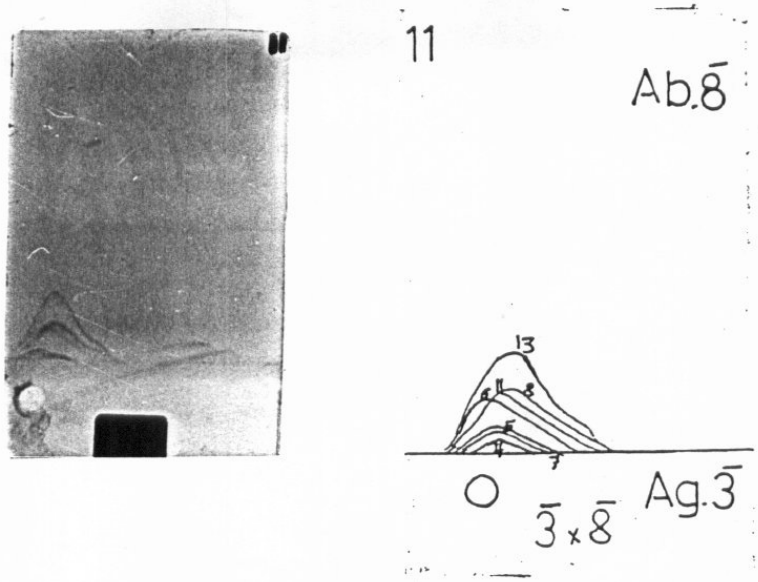


Fig. (8a): Crossed immunoelectrophoresis of uninoculated plants of Reiho, IR 28 cvs. antigen-antibody system Antigen (Ag.3̄) was electrophoresed, refrence gel contained antibody (Ab.8̄). Anodes at the right and the top.

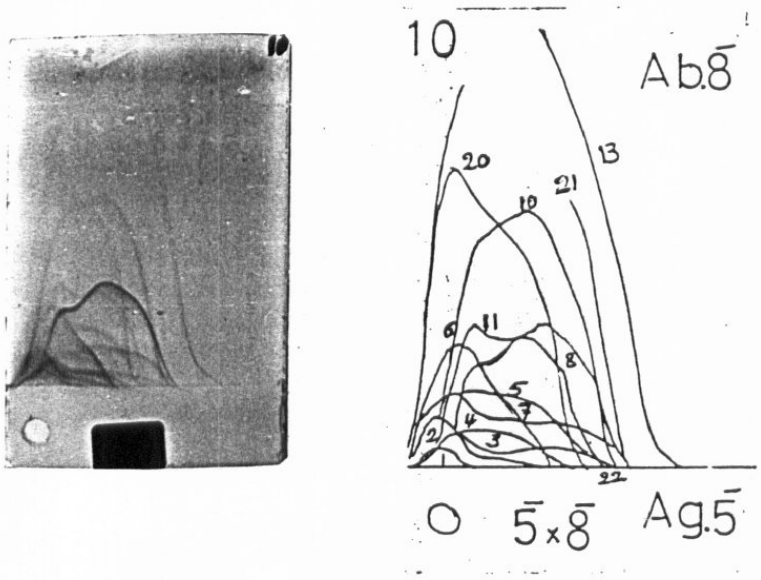
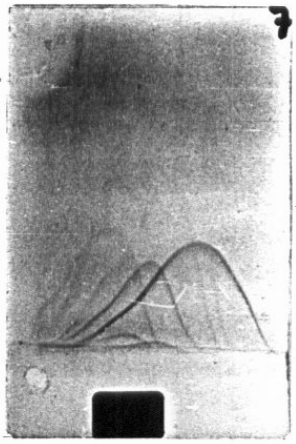
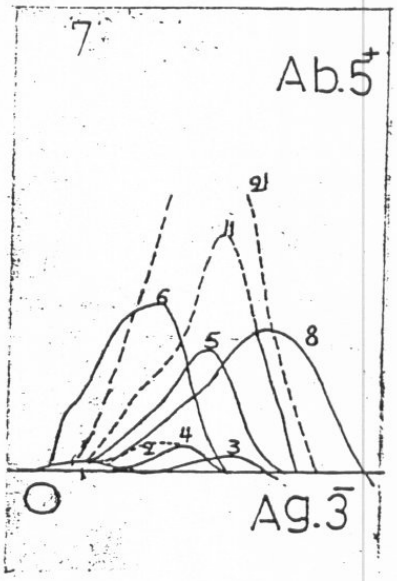


Fig. (8b): CIE, of uninoculated plants of GZ 2175-5-6, IR 28 cvs. antigen-antibody system. Antigen (Ag.5̄) was electrophoresis, reference gel contained antibody (Ab.8̄). Anodes at the right and the top.

was common with the inoculated plants. Also, the same results was found between P. oryzae and GZ 2175-5-6. Two antigens were found in common between the pathogen and the uninoculated plants, while one antigen was found with the inoculated plants. No differences in the common antigens between P. oryzae and both of the inoculated and uninoculated plants of IR 28 were found, since one antigen was common. Seven precipitin lines (antigens) were detected in the homologous reaction of P. oryzae .

CIE showed the same results, except that the number of common antigens detected by CIE were more than those detected with DGD test. For example, five and two antigens were found common between P. oryzae and the uninoculated and inoculated Reiho, respectively (Table 5), while were two and one antigens were detected by DGD test (Table 4). Also, three and two antigens were found common between P. oryzae and the uninoculated and inoculated GZ 2175-5-6, respectively, by using CIE, while two and one antigens were found with the DGD test. Thirteen antigens were detected in the homologous reactions of the pathogen by CIE, while seven antigens were detected using DGD test. (Figs. 11a, 3a).





Fig(10): Crossed immunoelectrophoresis of uninoculated plants of Reiho cv., inoculated GZ 2175, antigen-antibody system. Antigen (ag.3̄) was electrophoresed, reference gel contained the antibody (Ab.5^{+}). Anodes at the right and the top.

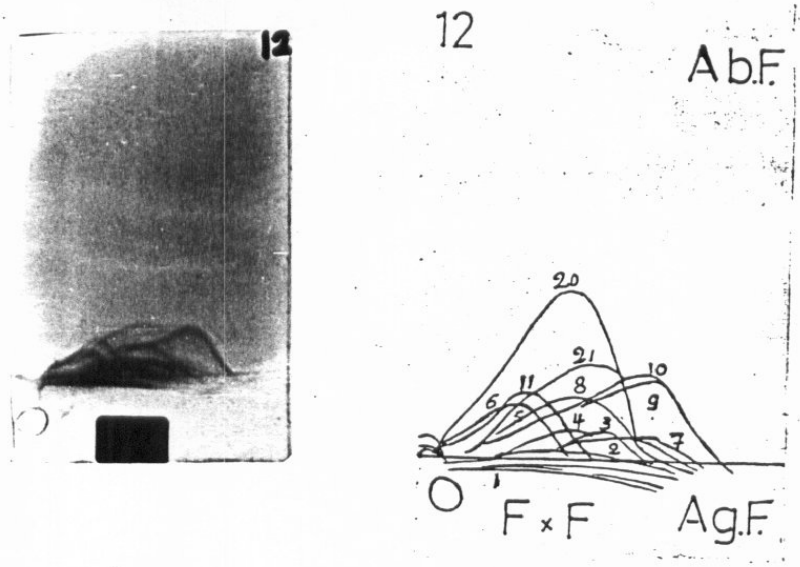


Fig.(11a): Crossed immunoelectrophoresis of Pyricularia oryzae antigen-antibody system. Antigen (Ag.F) was electrophoresed, reference gel contained the homologous antibody (Ab.F). Anodes at the right and the top.

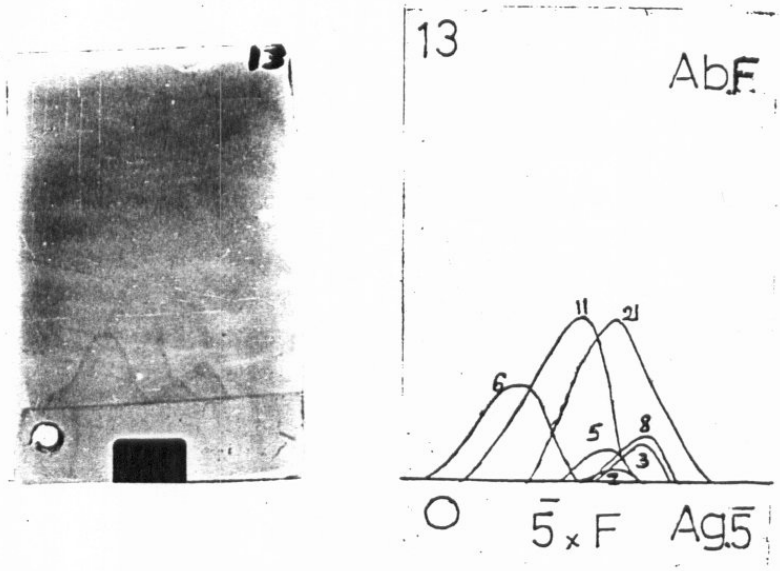


Fig.(11b): CIE of uninoculated GZ 2175-5-6 and Pyricularia oryzae, antigen-antibody system. Antigen (Ag.5)was electrophoresed reference gel contained the antibody (Ab.F). Anodes at the right and the top.

Generally, both of DGD and CIE revealed that more common antigens were detected between P. oryzae and the rice cultivars from the uninoculated plants than from inoculated plants.

C) Reciprocal analysis procedure by using CIE:

Antibodies against inoculated and uninoculated plants of rice cultivars Reiho, IR 28 and GZ 2175-5-6 and P. oryzae were observed by their antigens in homologous and heterologous reaction. The absorbed antibodies were used to test all the plant and fungal antigens separately. The numbers of precipitin peaks (antigens) detected were recorded in (Table 6) and showed the following:

1. Three antigens were not detectable from the inoculated plants of Reiho by comparison with the uninoculated ones. Absorbed antibodies of the uninoculated Reiho (by antigens of inoculated Reiho and GZ 2175-5-6 plants) differentiated between inoculated and uninoculated plants of these cultivars, while the absorbed antibodies of inoculated Reiho did not.
2. No antigenic differences were found between uninoculated and inoculated plants of IR 28. The absorbed antibodies of IR 28 (by antigens of any inoculated

Table 6: Reciprocal analysis procedure of P. oryzae and some rice cultivars by using crossed immunoelectrophoresis (CIE).

Antibodies	Antigens used for absorption	Number of precipitin peaks detected						
		Reiho		IR 28		GZ 2175		P. oryzae
		-	+	-	+	-	+	
Reiho -	Reiho -	-	-	-	-	-	-	-
	Reiho +	3S	-	-	-	S	-	3S
	IR 28 -	4+3S	4	-	-	2+S	2	3S
	IR 28 +	4+3S	4	-	-	2+S	2	3S
	GZ 2175 -	2+2S	2	-	-	-	-	2S
	GZ 2175 +	2+3S	1	-	-	S	-	3S
	P. oryzae	9	9	-	-	8	8	-
Reiho +	Reiho -	-	-	-	-	-	-	-
	Reiho +	-	-	-	-	-	-	-
	IR 28 -	4	4	-	-	2	2	-
	IR 28 +	4	4	-	-	2	2	-
	GZ 2175 -	2	2	-	-	-	-	-
	GZ 2175 +	2	2	-	-	-	-	-
	P. oryzae	9	9	-	-	8	8	-
IR 28 -	Reiho -	-	-	7	7	7	7	-
	Reiho +	-	-	7	7	7	7	-
	IR 28 -	-	-	-	-	-	-	-
	IR 28 +	-	-	-	-	-	-	-
	GZ 2175 -	-	-	-	-	-	-	-
	GZ 2175 +	-	-	-	-	-	-	-
	P. oryzae	9	9	-	-	8	8	-
IR 28 +	Reiho -	-	-	7	7	7	7	-
	Reiho +	-	-	7	7	7	7	-
	IR 28 -	-	-	-	-	-	-	-
	IR 28 +	-	-	-	-	-	-	-
	GZ 2175 -	-	-	-	-	-	-	-
	GZ 2175 +	-	-	-	-	-	-	-
	P. oryzae	9	9	-	-	8	8	-
GZ 2175 -	Reiho -	-	-	4	4	4	4	-
	Reiho +	2	-	4	4	4+S	4	S
	IR 28 -	S	-	-	-	-	S	S
	IR 28 +	S	-	-	-	S	-	S
	GZ 2175 -	-	-	-	-	-	-	-
	GZ 2175 +	S	-	-	-	S	-	S
	P. oryzae	6	6	6	6	6	6	-
GZ 2175 +	Reiho -	-	-	4	4	4	4	-
	Reiho +	-	-	4	4	4	4	-
	IR 28 -	-	-	-	-	-	-	-
	IR 28 +	-	-	-	-	-	-	-
	GZ 2175 -	-	-	-	-	-	-	-
	GZ 2175 +	-	-	-	-	-	-	-
	P. oryzae	6	6	6	6	6	6	-
P. oryzae	Reiho -	-	-	-	-	-	-	11
	Reiho +	3S	-	-	-	S	-	11+3S
	IR 28 -	-	-	-	-	S	-	11+3S
	IR 28 +	-	-	-	-	S	-	11+3S
	GZ 2175 -	2S	-	-	-	-	-	11+2S
	GZ 2175 +	3S	-	-	-	S	-	11+3S
	P. oryzae	-	-	-	-	-	-	-

- : Uninoculated
+ : Inoculated
S : Antigens associated to susceptibility

and uninoculated cultivar) did not differentiate between inoculated and uninoculated plants of any tested cultivar.

3. One antigen was not detectable from the inoculated plants of GZ 2175-5-6 when compared with the uninoculated plants. Absorbed antibodies of only the uninoculated GZ 2175-5-6 (by antigens of inoculated Reiho, GZ 2175-5-6 and IR 28 plants) differentiated between inoculated and uninoculated plants of Reiho and GZ 2175-5-6.

4. The different antigens between inoculated and uninoculated plants of Reiho and GZ 2175-5-6 were common with P. oryzae indicating the association of these antigens with susceptibility.

5. The serological relationship "expressed as number of common antigens" between GZ 2175-5-6 and IR 28 was more than that between GZ 2175-5-6 and Reiho.

V. Relationship between time of planting and blast disease incidence:

This experiment was carried out for two seasons , i.e. 1986 and 1987, under Egyptian environmental conditions for disease development. A split plot design with four replicates was used, the main plots were assigned to two dates of planting, while the 7 cultivars

namely Giza 172, Giza 175, Giza 181, GZ 2175-5-6, GZ 1368-5-4, Reiho and IR 50 were allocated in the sub-plots . The period between first date of sowing and the second date was fifteen days during both growing seasons. To determine leaf blast infection a sample of 100 leaves was randomly collected from each plot. Samples were taken four times at 15 days intervals, starting 15 days after transplanting to determine the disease progress curve for every variety in every planting date.

A sample of 100 panicles was collected at random from each plot to determine the percentage and severity of neck blast infection.

Data in Tables (7) & (8) show that in general infected rice leaves with blast was lower in 1986 compared to 1987 growing season. Giza 172 and Reiho cultivars showed heavy infection in both seasons, however, disease severity was higher in 1987 than 1986 growing season. Giza 175 cv. on the other hand, showed minor infection since only few type-4 lesions were observed in 1987 season under late transplanting (July 10) Table (8) and illustrated in Figs. (12, 13).

The present data in both 1986 and 1987 growing seasons indicated that Reiho cv. was the most susceptible one, among all the tested rice cvs. However, early transplanting was better for avoiding heavy infection with blast disease in both 1986 and 1987 seasons. Other

Table (7): Development of leaf blast infection on three rice cultivars at two different dates of transplanting at Sakha Experimental Research Station during 1986 rice growing season.

No.	Varieties*	Disease index throughout													
		Early transplanting (June 25)								Late transplanting (July 10)					
		1st Score (July 10)		2nd Score (July 25)		3rd Score (Aug. 10)		4th Score (Aug. 25)		1st Score (July 25)		2nd Score (Aug. 10)		3rd Score (Aug. 25)		4rh Score (Sept. 10)	
		%	S	%	S	%	S	%	S	%	S	%	S	%	S	%	S
1	Giza 172	13.86	32.80	20.42	139.30	29.09	88.00	19.71	50.50	15.10	90.80	27.20	168.50	30.90	233.50	27.10	121.50
2	Reiho	19.61	109.80	30.63	410.50	35.55	313.50	31.15	115.00	25.10	120.50	38.60	438.80	41.20	544.50	43.00	252.80
3	Giza 175	2.87	0.25	2.87	0.25	2.87	0.25	2.87	0.25	2.87	0.25	2.87	0.25	2.87	0.25	2.87	0.25
L.S.D. 0.05		2.40	30.60	4.00	53.50	3.30	65.10	3.30	28.70	2.40	30.60	4.00	53.60	3.30	65.10	3.30	28.70

* Rest of the tested varieties i.e. Giza 181, IR 50, GZ 2175-5-6 and GZ 1368-5-4 were completely resistant.

Table (8): Development of leaf blast infection on three rice cultivars at two different dates of transplanting at Sakha Experimental Research Station during 1987 rice growing season.

No.	Varieties*	Disease index throughout											
		Early transplanting (June 25)								Late transplanting (July 10)			
		1st Score (July 10)		2nd Score (July 25)		3rd Score (Aug. 10)		4th Score (Aug. 25)		1st Score (July 25)		2nd Score (Aug. 10)		3rd Score (Aug. 25)		4th Score (Sept. 10)	
		%	S	%	S	%	S	%	S	%	S	%	S	%	S	%	S
1	Giza 172	38.60	66.80	52.00	250.30	39.70	68.30	39.70	64.50	52.10	126.30	60.90	486.80	49.90	155.30	46.10	86.30
2	Reiho	57.50	397.50	60.90	791.50	53.20	155.30	47.80	106.80	47.20	113.30	67.10	625.30	55.10	218.30	50.80	130.80
3	Giza 175	2.87	0.25	2.87	0.50	2.87	0.25	2.87	0.25	6.30	2.25	9.90	5.25	9.90	5.25	7.80	2.75
L.S.D. 0.05		10.20	33.70	6.70	59.50	8.20	19.60	6.70	29.30	10.20	33.70	6.70	59.50	8.20	19.60	6.70	29.30

* Rest of the tested varieties i.e. Giza 181, IR 50, GZ 2175-5-6 and GZ 1368-5-4 were completely resistant.

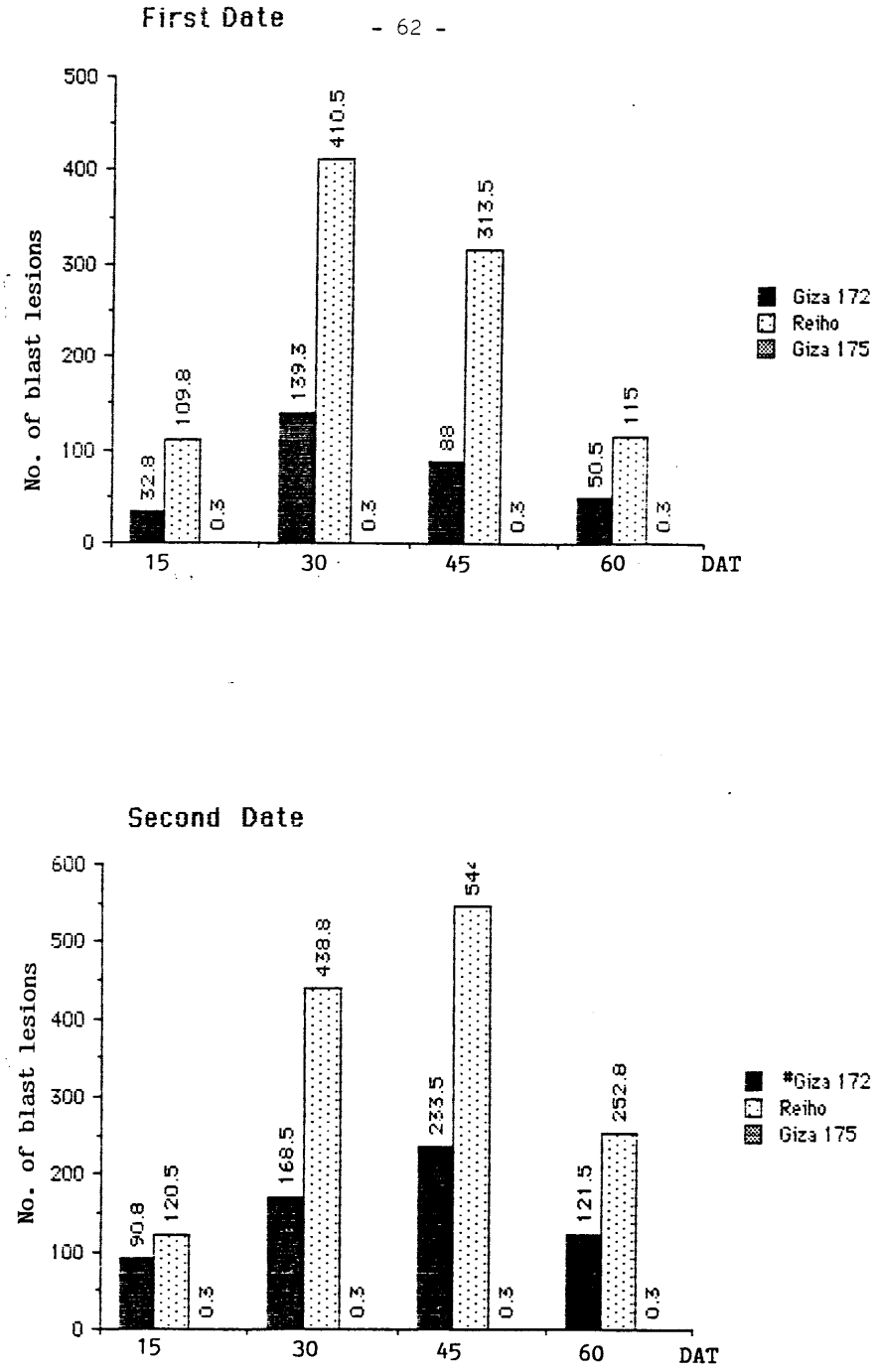


Fig.(12): Development of leaf blast infection on three rice cvs. at two dates of transplanting in 1986.

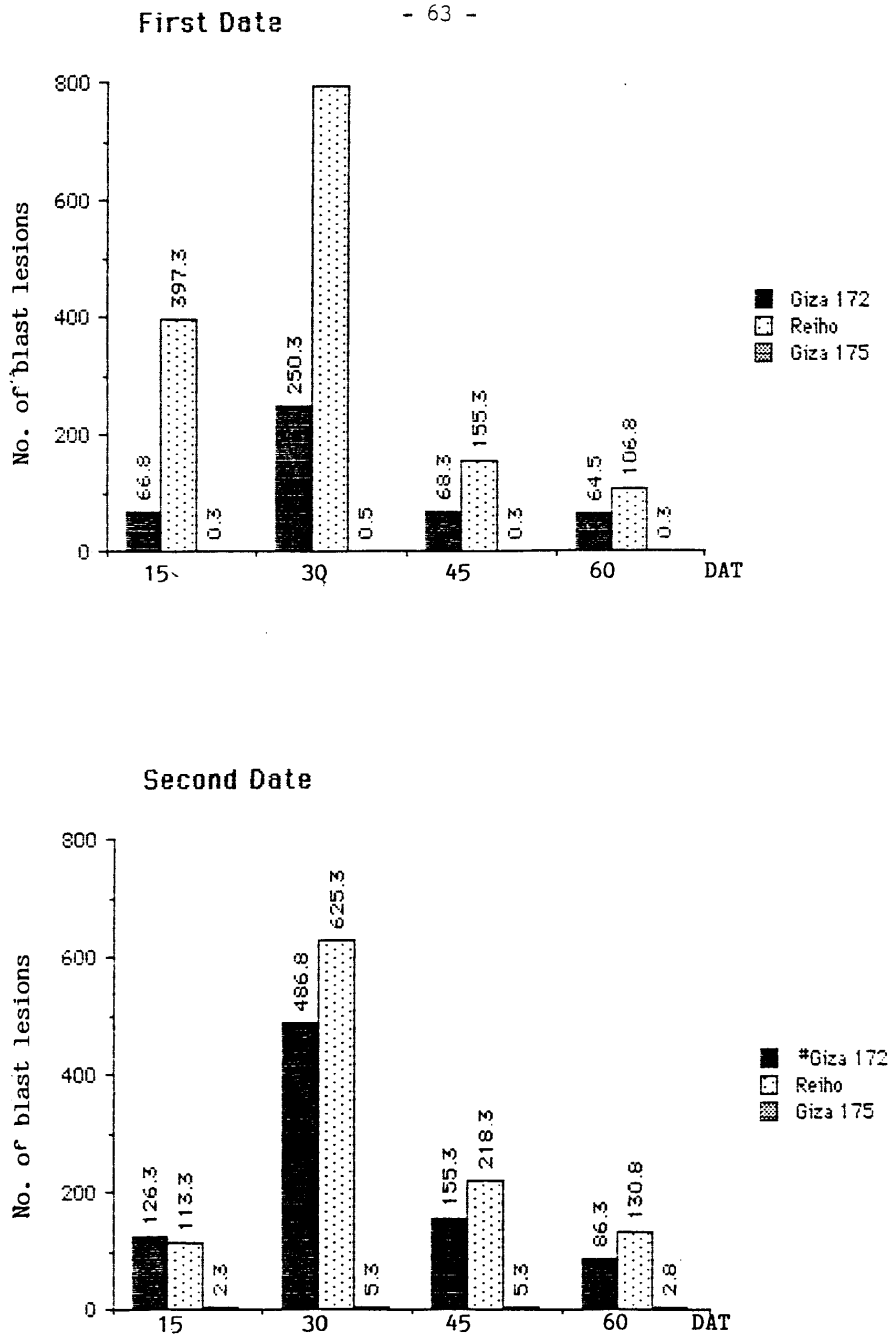


Fig. (13): Development of leaf blast infection on three rice cvs. at two dates of transplanting in 1987.

other tested varieties or lines i.e. Giza 181, IR 50, GZ 2175-5-6 and GZ 1368-5-4 appeared resistant in both seasons under the two dates of transplanting.

oncerning neck infection, it is evident from data presented in Table (9) & (10) that the same trend of leaf infection was similar to those of panicle infection, otherwise late transplanting clearly increased infection of both leaves and panicle with blast in both growing seasons, 1986 and 1987.

In 1986 season, severity of leaf infection on Giza 172 cv. increased from 77.6 lesions per plant at early transplanting to 153.6 lesions at late date of transplanting. Degree of panicle infection of such cultivar increased from 10.1% to 19% and consequently grain yield decreased from 2,72 to 2.45 T/fed. for early and late transplanting respectively (Table 9). As far as the highly susceptible Reiho cv. is concerned leaf infection increased from 237.2 lesions to 339.1 ones, and degree of panicle infection increased from 14.3% to 24.1%, consequently grain yield decreased from 2.48 to 1.9 T/fed. (Table 9). Similar results were obtained in 1987 season with some differences in leaf infection and grain yield (Table 10).

VI. **Effect of methods and time of N-fertilizer application on rice blast disease development:**

Giza 172, Reiho and Giza 175 rice cultivars were tested to determine the relation between N-fertilizer application and blast disease development

Table (9): Influence of transplanting date on leaf and panicle infection with blast disease of three rice cultivars in 1986 growing season.

No.	Varieties*	Disease index											
		First date of transplanting						Second date of transplanting					
		Leaf infection mean		Panicle infection			Yield Ton/ Fed.	Leaf infection mean		Panicle infection			Yield Ton/ Fed.
		%	S	%	S	Degree		%	S	%	S	Degree	
1	Giza 172	20.8	77.6	29.0	34.8	10.1	2.72	25.1	153.6	40.0	46.7	19.0	2.45
2	Reiho	29.2	237.2	37.0	38.8	14.2	2.48	37.0	339.1	52.6	46.0	24.1	1.90
3	Giza 175	2.87	0.25	2.87	2.87	0.08	2.38	2.87	0.25	2.87	2.87	0.08	1.85
L.S.D. 0.05		6.56	134.76	3.42	4.56	2.36	0.33	6.56	34.76	3.42	4.56	2.36	0.33

* Rest of the tested varieties i.e. Giza 181, IR 50, GZ 2175-5-6 and GZ 1368-5-4 were completely resistant.

Table (10): Influence of transplanting date on leaf and panicle infection with blast disease of three rice cultivars in 1987 growing season.

No.	Varieties *	Disease index											
		Firs date of transplanting						Second date of transplanting					
		Leaf infection mean %	S	Panicle infection %	S	Degree	Yield Ton/ Fed.	Leaf infection mean %	S	Panicle infection %	S	Degree	Yield Ton/ Fed.
1	Giza 172	42.50	112.94	34.10	17.2	9.10	2.62	52.30	213.60	41.20	24.00	13.30	2.62
2	Reiho	54.80	362.60	59.50	49.30	30.10	2.81	55.10	271.90	68.90	70.70	42.30	2.43
3	Giza 175	2.87	0.31	2.87	0.25	0.08	2.51	8.46	3.88	2.87	0.25	0.08	2.55
L.S.D. 0.05		6.46	220.40	5.21	7.44	4.64		6.46	220.40	5.21	7.44	4.64	

* Rest of the tested varieties i.e. Giza 181, IR 50, GZ 2175-5-6 and GZ 1368-5-4 were completely resistant.

in the present experiment during 1986 and 1987 rice growing seasons. Urea (46%) was applied at the rate of 40 units of Nitrogen/Feddan. The methods of N-fertilizer application were presented in the materials and methods.

Symptoms estimated starting from 15 days after transplanting (DAT), till 60 DAT with 15 days intervals Data of the present study are presented in Tables (11 & 12) and illustrated in Figs (14, 15 & 16).

The obtained data could be summarized as follow : Both leaf and panicle infections decreased when the amount of N-fertilizer was splitted to three equal doses; all amount was applied 25 DAT or the amount was splitted to two equal doses i.e. one at 25 DAT and the other at 50 DAT Treatments No. 3, 6 and 7 . On the other hand, the rest of the treatments which includes,all; half or two third of N-fertilizer amount was incorporated into soil gave high leaf and panicle infections with maximum leaf infection at 30-45 DAT in both 1986 and 1987 rice growing seasons Treatments No. 1,2,4 and 5 .

Giza 172 and Reiho were susceptible with different degrees of infection depending on the methods and time of N-fertilizer applications in both seasons.

Giza 175 cultivar was free from either leaf or panicle infection throughout 1986. In 1987 growing

Table (11): Effect of time of N-fertilizer application on both leaf and panicle infection with blast of three rice cultivars in 1986 season.

Time of N application (Kg/Fed.)			Leaf and panicle blast infection of the tested rice cultivars															
			Giza 172					Reiho					Giza 175					
Soil incorporation	25 DAT**	50	Mean of leaf infection %	S*	Panicle infection %	S	Degree	Yield Ton/ Fed.	Mean of leaf infection %	S	Panicle infection %	S	Degree	Yield Ton/ Fed.	Mean of leaf infection %	S	Panicle infection %	S	Degree	Yield Ton/ Fed.
40	--	--	9.01	76.94	17.55	27.18	4.77	2.49	11.74	81.88	18.33	39.62	7.26	2.45	2.87	0.25	2.87	2.87	0.08	2.76
20	20	--	8.47	81.06	14.90	29.23	4.36	2.67	10.95	79.50	17.57	36.73	6.45	2.55	2.87	0.25	2.87	2.87	0.08	3.18
--	20	20	7.08	49.13	14.08	25.47	3.59	2.56	8.84	49.38	19.15	29.47	5.64	2.72	2.87	0.25	2.87	2.87	0.08	3.12
27	13	--	9.40	75.06	16.40	31.08	5.10	2.70	12.84	78.38	20.66	37.65	7.78	2.45	2.87	0.25	2.87	2.87	0.08	3.30
27	--	13	7.93	70.00	15.20	32.95	5.01	2.74	10.64	68.56	19.02	37.84	7.20	2.50	2.87	0.25	2.87	2.87	0.08	3.29
13	13	13	7.13	53.25	16.73	27.08	4.53	2.98	9.67	46.31	20.30	34.72	7.05	2.85	2.87	0.25	2.87	2.87	0.08	3.20
--	40	--	5.33	34.63	12.77	21.02	2.69	2.80	7.37	36.94	16.94	28.51	4.83	2.53	2.87	0.25	2.87	2.87	0.08	3.33
--	--	--	3.53	20.06	9.45	18.26	1.73	2.02	4.51	16.94	12.28	21.72	2.67	1.50	2.87	0.25	2.87	2.87	0.08	2.46
L.S.D. 0.05			5.18	21.34	3.19	5.08	1.25	0.31	5.18	21.34	3.19	5.08	1.25	0.31	5.18	21.34	3.19	5.08	1.25	0.31

* Severity of infection.

** Days after transplanting (DAT).

Table (12): Effect of time of N-fertilizer application on both leaf and panicle infection with blast of three rice cultivars in 1987 season.

Time of N application (Kg/Fed.)			Leaf and panicle blast infection of the tested rice cultivars														
			Giza 172					Reiho					Giza 175				
	DAS**		Mean of leaf infection		Panicle infection			Yield Ton/ fed.	Mean of leaf infection		Panicle infection			Yield Ton/ fed.	Mean of leaf infection		Panicle infection			Yield Ton/ Fed.
Soil incorporation	25	50	%	S*	%	S	Degree		%	S	%	S	Degree		%	S	%	S	Degree	
40	--	--	39.45	114.56	42.41	32.41	13.75	2.600	38.33	103.13	59.40	49.12	29.18	2.600	3.14	1.56	2.87	2.87	0.08	2.832
20	20	--	41.95	119.38	42.79	33.98	14.54	3.000	41.04	83.75	57.28	47.37	27.13	2.700	4.19	0.81	2.87	2.87	0.08	3.400
--	20	20	33.90	60.06	43.66	34.85	15.22	2.960	32.44	51.19	53.75	43.57	23.42	2.900	2.87	0.31	2.87	2.87	0.08	3.320
27	13	--	39.92	111.50	44.24	35.93	15.90	2.920	39.35	84.00	57.86	45.89	26.55	2.600	3.68	0.56	2.87	2.87	0.08	3.600
27	--	13	41.45	134.31	45.87	36.81	16.89	2.920	37.24	73.69	58.95	46.80	27.59	2.652	2.87	0.31	2.87	2.87	0.08	3.552
13	13	13	36.02	83.50	43.50	34.99	15.22	3.200	32.56	54.56	56.72	46.11	26.15	3.052	2.87	0.31	2.87	2.87	0.08	3.400
--	40	--	33.22	53.13	38.24	29.46	11.27	3.160	30.37	40.00	48.49	38.04	18.45	2.700	2.87	0.25	2.87	2.87	0.08	3.652
--	--	--	23.27	30.00	35.06	27.04	9.48	1.720	23.83	18.13	40.30	28.85	11.63	1.552	2.87	0.25	2.87	2.87	0.08	2.752
L.S.D. 005			3.96	15.30	6.02	5.14	4.72	0.57	3.96	15.30	6.02	5.14	4.72	0.57	3.96	15.30	6.02	5.14	4.72	0.57

* Severity of infection.
** Days after transplanting (DAT).

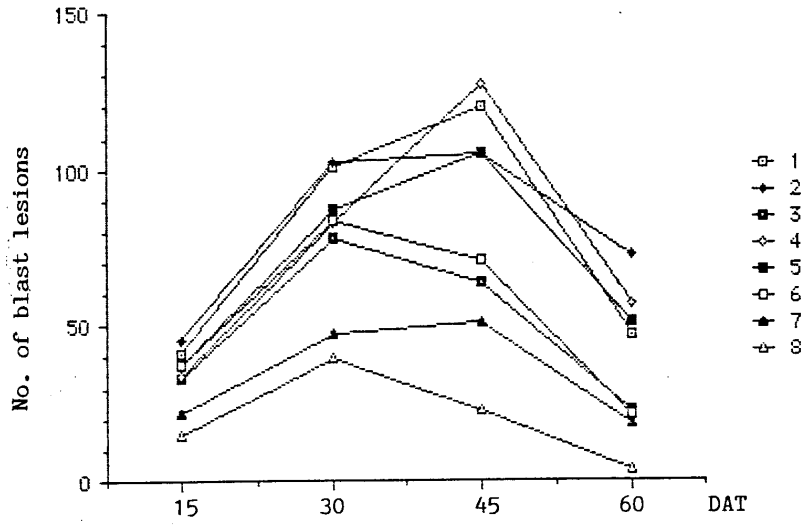


Fig. (14a): Effect of methods and time of nitrogen fertilizer application on the blast disease incidence, on Giza 172, 1986.

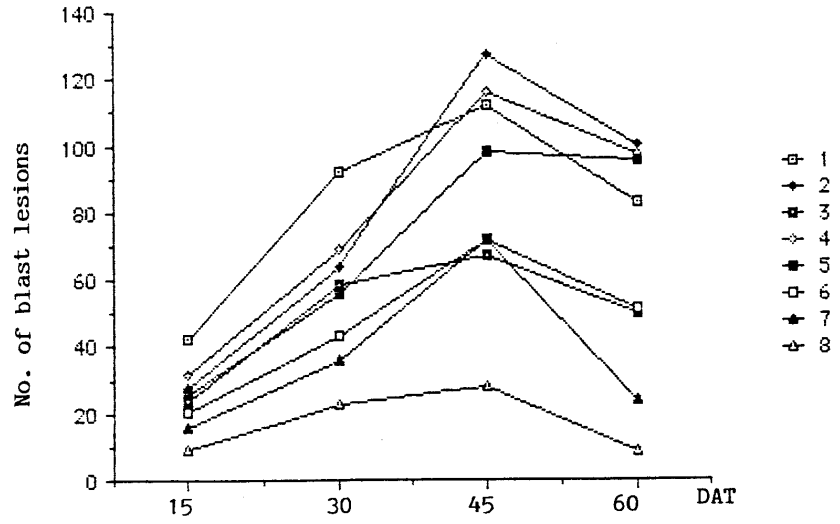


Fig. (14b): Effect of methods and time of nitrogen fertilizer application on the blast disease incidence, on Reiho, 1986.

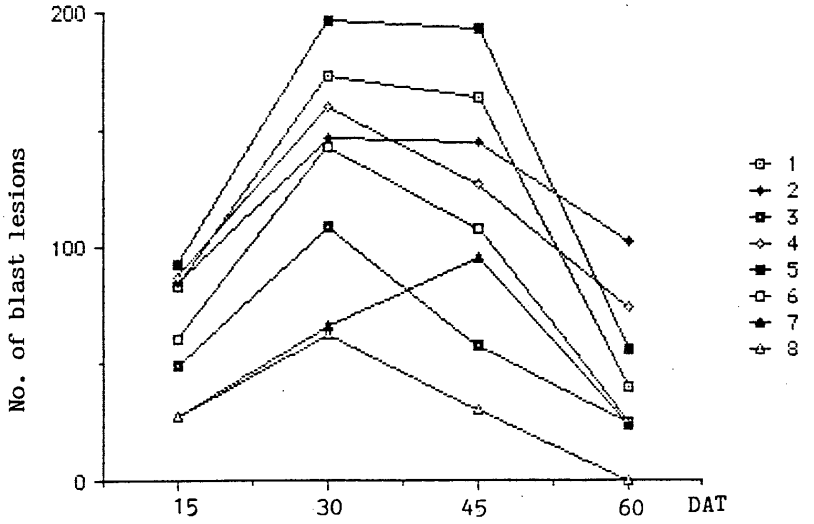


Fig. (15a) : Effect of methods and time of nitrogen fertilizer application on the blast disease incidence, on Giza 172, 1987.

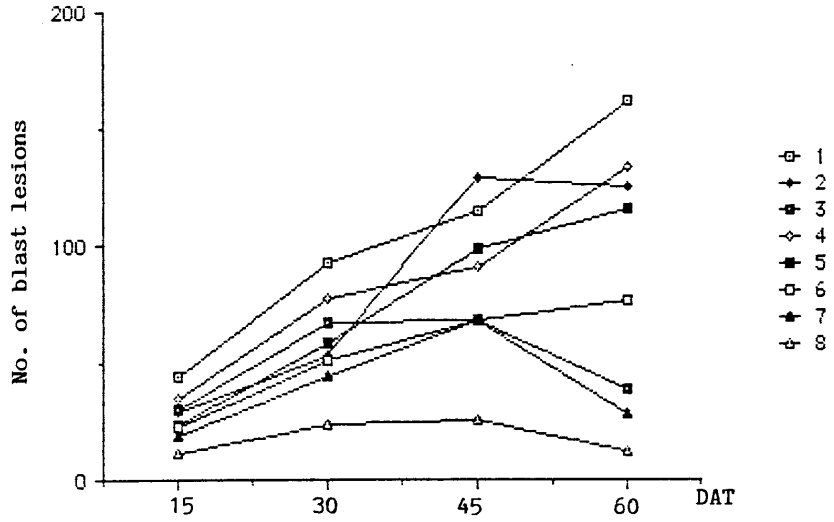


Fig. (15b): Effect of methods and time of nitrogen fertilizer application on the blast disease incidence, on Reiho, 1987.

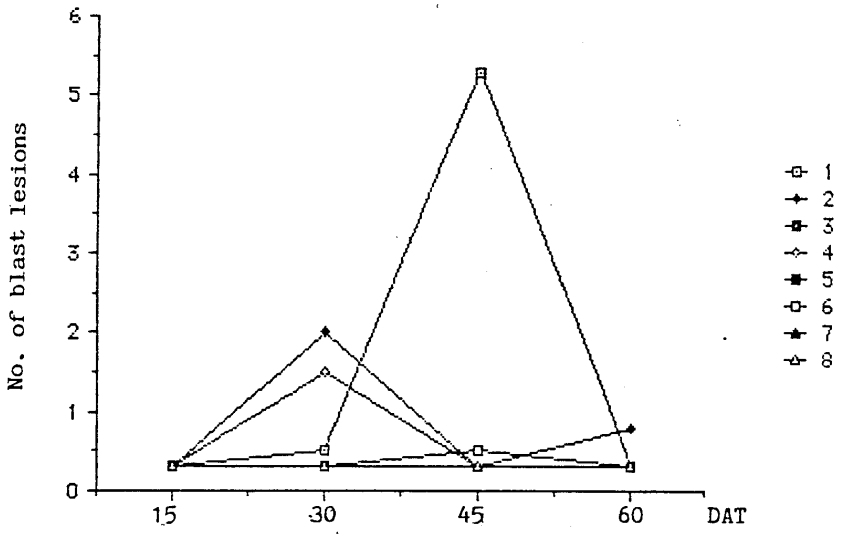


Fig. (16): Effect of methods and time of nitrogen fertilizer application on the blast disease incidence, on Giza 175, 1987.

No	Soil incorporation	25 DAT	50 DAT
1	40	--	--
2	20	20	--
3	--	20	20
4	27	13	--
5	27	--	13
6	13	13	13
7	--	40	--
8	--	--	--

season only few numbers of typical blast lesions (Type 4 lesion) were detected on leaves 30 DAT, when all or partially amounts of N-fertilizer were incorporated into soil pre-transplanting (Treatments No. 1,2,4, and 5).

As concerning yield, the highest yield was obtained from the plots in which N-fertilizer was splitted to three equal doses i.e. one third was incorporated into soil; the second third was added 25 DAT, and the rest of amount was added 50 DAT. Almost the results were obtained when all amounts of N-fertilizer were applied 25 DAT in all tested cultivars during 1986 and 1987 rice growing seasons.

VII. Correlation between leaf and panicle infection: Leaf and panicle infection was studied on the two susceptible rice cvs. i.e. Giza 172 and Reiho during 1987 rice growing season. Data of the present work are presented in Table (13).

The obtained data revealed that, there is a distinct correlation between leaf and panicle infections on both Reiho and Giza 172 cvs. However, the correlation was more obvious in case of Reiho cv. since all scores gave significant differences and yield was affected significantly.

Table (13): Correlation between leaf and panicle blast infection and grain yield of two cultivars in 1987.

Cultivars / Date of score	Giza 172		Reiho	
	Neck infection	Grain yield	Neck infection	Grain yield
Leaf infection (Severity) 22/7/1987	0.74*	0.30	0.72*	0.44
" 6/8/1987	0.77*	0.32	0.76*	0.49
" 21/8/1987	0.57	0.41	0.79*	0.50
" 6/9/1987	0.56	0.40	0.81*	0.33
Leaf infection, Mean	0.71*	0.40	0.84*	0.44
Flag leaf infection (Severity)	0.90*	0.36	0.63	0.28
Neck infection	--	0.60	--	0.79*

* Significant

DISCUSSION

On the basis of the results obtained from the isolation and identification of Pyricularia oryzae Cav. , the causal fungus of rice blast disease, fifteen isolates of the organism collected from various localities, revealed the occurrence of eleven races of P. oryzae , distributed in Kafr El-Sheikh, Beheira, Ghrabia and Dakahlia Governorates, throughout 1984 and 1985 growing seasons.

The races which were identified on basis of their reaction to the eight international differential varieties as well as two Egyptian cultivars (Giza 159 and Giza 172) were IA 107, IB 60, ID 11, IF 3 and IG 1 in 1984, and IB 38, IB 44, IC 17, ID 1, ID 16 and IE 1 in 1985 according to the methods of identification recommended by (Atkins et al 1967 & Ling and Ou, in 1969).

In 1984, races ID 11 and IG 1 were most prevalent in Kafr El-Sheikh and Behiera Govs., while IB 60 and IF 3 were prevalent in Gharbia. In 1984 it was found that IB, ID and IE race groups were isolated from Kafr El-Sheikh.

As far as race group IB is concerned, it was also isolated from Behiera and Dakahlia Govs., while IC group was isolated only from Gharbia Gov. It is obvious from the obtained data that race groups IB and ID are common in the northern governorates (Behiera & Kafr El-Sheikh). However, race groups IA, IB, IC, ID and IG

which comprise different races are predominant in Egypt since many races belong to these groups were frequently isolated in most years by other workers (El-Helaly et al, 1974; Abdel-Hak et al, 1981; Kamel et al, 1986).

The tested Egyptian cultivars, i.e. Giza 159 and Giza 172 cvs. were susceptible to most of the tested isolates. Results obtained indicate that prevalence of P. oryzae races showed a seasonal pattern . Some races were dominant in 1984 season, while they were not detected in 1985 season and vice versa. This may be attributed to the potential variability of the fungus which is capable of producing numerous races. The prevailing races in an area, however, depend upon the rice cultivars growing in the area, as well as the fluctuation in the environmental conditions to another. This is in agreement with the finding of other workers (Ou,1985; Kamel et al, 1985 and Abdel-Hak,1981).

Introduction of new rice cultivars is always directed toward high yielding varieties with blast resistance. However, the resistant genes in the host are correspondingly matched by the virulent genes in the pathogen (Kiyosowa , 1967c).The longevity of any new cultivar to remain resistant under field conditions is controlled by many factors including the type of resistance, the

area of the cultivar, the ability of the pathogen to produce new virulent races (Kiyosawa 1967 c).

The evaluation of some promising lines to the isolates identified in 1984 indicated that, Reiho and GZ-991 were susceptible to all identified races in such season. Giza 159 and GZ-882 were susceptible to all races except one (IB 60 from Gemmiza). This may indicate the existance of specific gene(s) in common between such varieties. However, IR 28, IR 50 and Giza 175 were resistant to all tested races. This may be due to the absence of the virulent gene(s) in the pathogen for the corresponding gene(s) of resistance in this group of Indica and Indica/Japonica type cvs. On the other hand Giza 181 was resistant to all races, except IB 60, while the reaction of Giza 171 and Giza 172 to this race was resistant indicating that, the virulence of this race is resembling its effects on Reiho and GZ-991.

In 1985, among the 12 evaluated entries to the 6 races identified, IR 28, IR 50, GZ 1368-5-2, GZ 1368-5-4 and Giza 175 remain resistant to all tested races, this may due to accumulation of many genes responsible for resistance in those cultivars. Therefore, these resistant cultivars which may have broad spectrum of

resistance for many years in different locations can be used as a best source of resistance in breeding programs. On the other hand, Giza 159, Giza 171, and Reiho were susceptible to most of the tested races throughout the 1984 and 1985 growing seasons. However, Giza 171 and Giza 172 were inconsistent in their reaction, thus they showed resistance to some races and susceptibility to others.

These cultivars may possess partial resistance due to existence of minor genes, consequently this may interpret why those cultivars are still under cultivation in Egypt for more than 15 years. This is in line with the finding of other workers (Kamel et al, 1985 & 1986).

Regarding to serological studies, fungus serology has not been used as extensively in research with phytopathogenic fungi (Preece, 1971; Seeliger, 1968). This work could be the first attempt in this respect in Egypt from the available literature.

Serological studies on the antigenic relationships between P. oryzae and some rice cvs. were conducted by using double gel diffusion (DGD) and crossed immunoelectrophoresis (CIE) techniques. These sensitivity

technique proved to be valuable analytical tools to analyse the broad spectrum of antigens in the micro-organism (Axelsen et al, 1973; Hornok, 1980 and El-Kady et al, 1986).

Results of the present work indicated that, three antigens were lost in the susceptible Reiho cv., following infection by a compatible race of P. oryzae, while only one antigen was lost in GZ 2175-5-6 by inoculation. No differences were found between healthy and inoculated plants of the resistant IR 28 cv.

However, GZ 2175-5-6 was serologically related to both Reiho and IR 28 cvs. as Japonica and Indica types. Therefore, GZ 2175-5-6 may acquire its resistant reaction to most tested races in the present results from its Indica type parents (Calrose 76 and Nagina cvs.) which posses resistant genes to blast fungus so far. Since 11 and 8 antigens were detected in common between GZ 2175-5-6 & IR 28 and GZ 2175-5-6 & Reiho, respectively, while 6 antigens detected between Reiho and IR 28 .

In the CIE method, the number of precipitin peaks detected in all reactions were almost twice those detected in the DGD test which is lesser sensitive technique than the former. As far as antigens of P. oryzae are concerned the obtained results revealed the presence of

15 and seven antigens in the homologous reaction of the pathogen by CIE and DGD, respectively.

There were common antigens among the pathogen and the tested rice cultivars. Since the common antigens between P. oryzae and the healthy plants were more than those detected with the inoculated ones, except for IR 28, no difference were found.

These results are in line with Carroll et al, 1972 ; DeVay et al, 1967 and Wimalajeewa & DeVay, 1971), they mentioned that the greater the antigenic disparity between the host and the pathogen, the greater will be the resistance of that host to the pathogen.

It could be concluded from serological tests that such new technique in this field of study is beneficial in differentiating between resistant and susceptible cultivars. This finding could save breeders time to select varieties or promising lines directly resistant to blast according to such technique. However more work is needed in this point of study in future to be applied widely in breeding programs.

As far as date of transplanting is concerned late planting is one of the important factors affecting rice blast infection. The present results indicated that, when plants were transplanted late at tenth of July, they were more severly

infected with blast than those transplanted early at June 25. This may be due to the exposure of younger plants to the pathogen population in the late transplanted plots, which are more susceptible to infection than older plants as well as peak of the air-bore conidia of blast fungus is generally found in July-August in Egypt. These results are in line with findings of Kohn and Libby, 1958; Atkins, 1974; Koh et al, 1987 ; Hwang et al, 1987 .

The newly evaluated promising lines showed consistence resistant in both growing seasons. However, Giza 175 which was completely resistant in 1986 season under both early and late transplanting dates, showed few susceptible type lesions under late transplanting date in 1987 season. This may be due to minor races which may developed and were able to attack younger plants of Giza 175 in the late transplanted plots, in addition to high inoculum build up in this season which was detected in the high infection severity of the two susceptible cultivars,Giza 172 and Reiho.

Chin-Chung Chin, 1976 stated that susceptibility of rice plants to blast may differ from year to year even with the same variety at the same location. He added that this phenomenon is relatively clear for susceptible

varieties and he believed to be related to environmental condition, type and density of the physiologic races.

In 1986 and 1987 seasons the maximum leaf infection was obtained 30-45 DAT, with higher infection severity in 1987 than 1986 season, especially on Reiho cv. This may be explained by earlier findings of Kamel et al(1986), who found that the number of lesions on Reiho was four folds than that found on Giza 171 and Giza 172. Also sporulation of lesions from Reiho was two times than that from Giza 172. El-Kazzaz et al (1989), found that the peak of leaf blast infection of the susceptible cultivars Giza 159, Giza 171, Giza 172 and Reiho occurred 49-63 DAT, depending upon cultivar sensetivity and environmental conditions.

In this study, adult plant resistance was clearly observed in both Giza 172 and Reiho, 60 days after transplanting. This may be very helpfull to minimize or even neglect chemical application for blast control after this stage depending upon the cultivar and other factors. Koh et al (1987), reported that blast infection gradually decreased with aging of plants in 8 cultivars tested , so lower leaves of rice plants were more severly infected than upper leaves.

The amount of N-fertilizer which is usually applied to the soil for plant nutrition has a great effect on blast disease incidence. The intensity of the effect of nitrogen on the diseases varies with methods and time of N-fertilizer application (Ou, 1985).

The results of the present study indicated that , leaf and neck infection with blast disease decreased when N-fertilizer was applied after transplanting at various doses. On the other hand, when N-fertilizer was incorporated to soil before transplanting at various doses, the disease incidence was obviously higher than the previous one. Consequently affected the yield on susceptible cultivars as compared with the rest of the treatments. The high disease incidence as a result of incorporation of N-fertilizer into soil could be attributed to the high availability of nitrogen at seedling stage which predisposed plants at various growth stages to heavy infection with the disease, since leaves became more succulent and succumb the disease. These infected leaves could be considered as a source of neck infection, consequently was affected yield. Splitting the amounts gave plants their requirements without an access minimize the disease severity and gave a considerable higher yield. These results

are in line with the findings of other workers (Mikkelson 1981 and Amin & Venkatarao, 1979). Since OU (1985), mentioned that split applications usually of blast disease. The results also coincide with the findings of Hamissa et al (1987), who reported that, the best timing and method of N-application was to incorporate 1/3 of the fertilizer in dry soil + 1/3 top dressing 30 days after sowing and 1/3 at panicle initiation, such treatment gave the highest yield. While, incorporation all the nitrogen dose in dry soil before planting gave the lowest yield. On the other hand, the present results contradict with the findings of Huang et al (1980), who found that split application of nitrogen increased blast damage.

The obtained results also showed only few numbers of typical blast lesions on leaves of Giza 175 cv. when all or partially amount of N-fertilizer were incorporated into soil. These data are in agreement with the findings of Kawai (1952) and Amin & Venkatarao (1979), who found that high blast incidence occurred when the entire N-fertilizer (150 kg N/ha) was applied into the soil in one single basal dose and consequently disease incidence was low whenever moderate N-fertilizer was applied in split top dressing.

Concerning the correlation between leaf and panicle infection on the susceptible cv. Reiho, the obtained results indicated that there was a significant positive correlation between leaf and panicle infection, consequently leading to yield losses. That means close relationship between panicle and leaf infections.

These results are in line with the finding of (Rangaswami & Subramanian, 1957; Ou & Nuquei, 1963 ; Padmanabhan et al, 1974; Chin & Amin, 1983 and Willis et al, 1968 and Ahn , 1977).

SUMMARY

LABORATORY AND GREENHOUSE STUDIES:

Rice blast disease, caused by Pyricularia oryzae Cav., is one of the most important diseases in Egypt as well as in most of the rice growing countries. This study was conducted to clarify some important problems concerning this disease, the obtained results could be summarized as follows:

1. Fifteen isolates of P. oryzae obtained from different locations were collected in 1984 and 1985 seasons. According to the reaction on the international differential varieties, the identified races were IA 107, IB 60, ID 11, IF 3 and IG 1 in 1984 , while in 1985 they were IB 38, IB 44, IC 17, ID , ID 16 and IE 1 races.
2. The promising lines evaluated against these identified races showed that:
   a) Reiho, GZ 991 were susceptible to all tested races. However, Giza 159 and GZ 882 showed susceptible to all tested races, except they were resistant to race IB 60.
   b) IR 28, IR 50, Giża 175 Cvs., were resistant to all tested races. Lines GZ 1368-5-2 and GZ 1368-5-4 showed resistant to races collected in 1985 season.
   c) Giza 171 and Giza 172 were resistant to some races, but susceptible to others.

d) Giza 181 showed resistance most of the identified races. However, races IB 60, IB 38, IB 44 and IC 17, were compatible with this cv. On the other hand, the line GZ 1108 was susceptible to all races except IB 38, ID 1 and IE 1, while GZ 1443-8-2 was resistant to all races, except IB 44a and ID 16.

3. Serological studies on Reiho, GZ 2175-5-6 and IR 28, using DGD and CIE techniques, to study the antigenic changes before and after inoculation with P. oryzae and also to determine the common antigens among the pathogen and the inoculated or uninoculated rice cvs. The results of these experiments were as follows:

a) The healthy plants of Reiho, IR 28 and GZ 2175-5-6 cvs. had 14 detectable antigens in each, by using the CIE method.

b) The inoculated plants of the same cvs., had 11, 14 and 13 detectable antigens respectively.

c) Three antigens were lost in the susceptible cv., Reiho following infection by a compatible race of P. oryzae, while it was one antigen in case of GZ 2175-5-6 .

d) No antigenic differences were observed when antigens from inoculated and healthy plants of the resistant cv. IR 28 were compared.

e) The serological relationship expressed as common antigens between IR 28 and GZ 2175-5-6 was closer than the relationship between Reiho and GZ 2175-5-6. Since 12 common antigens between the first two cvs., however, they were eight between the last two cvs. The expression of common antigens was almost between the resistant cv..IR 28 and the susceptible one Reiho , since only seven antigens were common between them.

f) Several antigens occurred in both P. oryzae and the rice cvs., it is varied according to whether the plants were inoculated or uninoculated with P. oryzae. The healthy plants of Reiho and GZ 2175-5-6 cvs. had more common antigens with P. oryzae, since 5 and 3 antigens detected,respectively, while the inoculated plants showed only two antigens in each.

g) Both of DGD and CIE techniques showed the same antigenic interaction among cultivars and blast fungus. However,CIE was more sensitive than the DGD test, as the number of detected precipitin bands.

FIELD EXPERIEMNTS:

4. Seven rice entries were transplanted at two planting dates i.e. June 25th and July 10th in two seasons 1986 and 1987 at Sakha, in order to study the relationship

between date of transplanting and blast incidence. The obtained results revealed that :

a) Late transplanting (July 10th) showed higher blast incidence on leaves and panicles of the susceptible cvs. Reiho and Giza 172.

b) Reiho cultivar was more susceptible with higher number of lesions and higher degree of panicle infection than that Giza 172 cv.

c) Giza 175 cultivar was completely resistant under both early and late transplanting in 1986 season, while, in 1987 season showed few number of typical blast lesions at late transplanting only.

d) The other tested cvs. IR 28, Giza 181, IR 50, GZ 2175-5-6 and GZ 1368-5-4 were found resistant in both seasons.

5. Concerning the relationship between methods and time of N-fertilizer applications on the rice blast development. Three rice cvs. were used i.e. Giza 172, Reiho and Giza 175. The recommended N-fertilizer level 40 kg N/fed. were applied in seven different ways. The obtained results indicated that :

a) The best results were obtained when the 40 kg N/fed. were added in three equal doses i.e. 1/3 incorporated

into soil, 1/3 at 25 DAT and 1/3 at 50 DAT. Also, when all amount was added 25 DAT or splitted two times 1/2 at 25 DAT and 1/2 at 50 DAT.

b) The application of all amount of N-fertilizer ; half or two third as a basal dose incorporated into soil increased both leaf and panicle infections subsequently affected the yield of susceptible cultivars, Reiho and Giza 172.

c) The obtained results showed only few numbers of typical blast lesions on leaves of Giza 175 cv., when all or partially amount of N-fertilizer was incorporated into soil.

6. The highest number of lesions scored at 15 days intervals was obtained on both Giza 172 and Reiho 30-45 DAT, in the two seasons.

7. The correlation between leaf and panicle infection on the two susceptible cvs. i.e. Giza 172 and Reiho during 1987 season, was studied. The obtained data revealed that, there is distinct correlation between leaf and panicle infections on both Reiho and Giza 172 cvs. However, the correlation was more obvious in case of Reiho cv., since all scores gave significant differences and yield was affected significantly as a result of panicle infection.

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الملخص العربـــى

الدراســة فــى المعمــل والصوبـــــة :

يعتبر مرض اللفحة فى الأرز الذى يسببه فطر بيركيولاريا أوريزا من أهم أمراض الأرز سواء فى مصر أو فى مناطق زراعته فى العالـــــم ٠

أجريت هذه الدراسة لتوضيح بعض النقاط المتعلقة بهذا المرض ـ وكانت النتائج المتحصل عليها كالآتـــــى :ـ

١ـ استخدمـت ١٥ عزلة من الفطر ـ تم الحصول عليها من مناطق مختلفة فى موسمـــى ١٩٨٤ ، ١٩٨٥ ـ وباجراء العدوى الصناعية لهذه العزلات على الأصناف العالميـــة المفرقة تم تعريف سلالات الفطر الآتيـــــة :ـ

فى موسم ١٩٨٤ : IA-107, IB-60, ID-11, IF-3, IG-1

وفى موسم ١٩٨٥ كانت IB-38, IB-44, IC-17, ID-1, ID-16, IE-1

٢ـ نتيجة تقييم بعض الأصناف وسلالات الأرز المبشرة لهذه العزلات من الفطر أظهـرت النتائج الآتـــــى :ـ

أ) أظهر كل من الريهو، سلالـة ٩٩١ ، جيزة ١٥٩ ، سلالة ٨٨٢ قابلية للاصابـة بجميع سلالات الفطر المختبرة ـ بينما استثنى من ذلك السلالة IB-60 التـــى لم تصيب جيزة ١٥٩ ، سلالة ٨٨٢ ٠

ب) كل من الأصناف آى٠آر ٢٨ ، آى٠آر٥٠٠، سلالة ١٣٩٤ـ١٠ـ١ (جيزة ١٧٥) كانـت مقاومة لجميع سلالات الفطر المختبرة ـ أما سلالة ١٣٦٨ـ٥ـ٢ ، سلالة ١٣٦٨ـ٥ـ٤ فكانت مقاومة لسلالات الفطر المعرفة فى موسم ١٩٨٥ ٠

جـ) كل من الصنفين جيزة ١٧١ ، جيزة ١٧٢ كانت مقاومة لبعض السلالات وقابلـة للاصابة للبعض الآخـــــــر ٠

د) جيزة ١٨١ ثبتت مقاومته لمعظم السلالات المعرفة باستثناء السلالات IB-60 ، IB-38 ، IB-44 ، IC-17 خلال فترة الدراسة ٠ أما سلالة ١١٠٨ فكان قابل للاصابة بكل سلالات الفطر المختبرة ما عدا IB-38 ، ID-1 ، IE-1 فى حين كان سلالة ١٤٤٣ـ٨ـ٢ مقاوم لكل السلالات باستثناء IB-44a ، ID-16

٣ـ من الدراسة السيرولوجية على الأصناف ريهو ، آى٠آر ٢٨ ، سلالـة ٢١٧٥ـ ٥ـ ٦ باستخدام طريقتى DGD ، CIE لمعرفة التغيرات الانتيجينية قبل وبعـــــد العدوى بالفطر ، وأيضا لتحديد الانتيجينات المشتركة بين كل من الفطر وأصنـــاف الأرز المعدية وغير المعدية ـ أظهرت نتائج الدراسة الآتـــى :ـ

أ) تم تمييز ١٤ أنتيجين فى النباتات السليمة للأصناف الثلاثة المختبرة باستخـدام طريقـــــة CIE ٠

ب) أما النباتات المعدية فتم تمييز ١١ أنتيجين فى الريهو ، ١٣ أنتيجين فى سلالة ٢١٧٥-٥-٦ ، ١٤ أنتيجين فى آى٠آر ٢٨ ٠

جـ) كانت نتيجة العدوى بسلالة متوافقة من الفطر غياب ثلاثة أنتيجينات فى الصنف ريهو ، بينما غاب أنتيجين واحد فقط فى حالة سلالة ٢١٧٥-٥-٦ ٠

د) لم يلاحظ أى تغيير فى عدد أنتيجينات الصنف آى٠آر ٢٨ بين النباتات المعدية وغير المعدية بالفطر ٠

هـ) من الدراسة السيرولوجية أيضا اتضح وجود قرابه بين كل من سلالة ٢١٧٥-٥-٦ والصنف آى٠آر ٢٨ أكثر من الموجودة بين سلالة ٢١٧٥-٥-٦ والريهو ، بناء على عدد الأنتيجينات المشتركة بينهم ، حيث وجد ١٢ أنتيجين بين سلالة ٢١٧٥ - ٥ - ٦ ، آى٠آر ٢٨ مع ٨ أنتيجينات بين سلالة ٢١٧٥-٥-٦ والريهو ، ٧ أنتيجينات بين آى٠آر والريهو ٠

و) وجدت أنتيجينات عديدة بين الفطر وأصناف الأرز المختبرة ، واختلفت هذه الأنتيجينات بين النباتات المعدية وغير المعدية بالفطر ، حيث وجدت ٥ أنتيجينات بين النباتات السليمة للصنف ريهو والفطر ، ٣ أنتيجينات بين نباتات سلالة ٢١٧٥ والفطر - بينما النباتات المعدية فى كلا الصنفين لوحظ فيها ٢ أنتيجين فقط ٠

ز) أثبتت كل من طريقتى الدراسة السيرولوجية DGD ، CIE وجود تفاعل أنتيجينى بين أصناف الأرز والفطر - بينما كانت طريقة CIE أكثر حساسية عن الأخرى متمثلة فى عدد الخطوط الترسيبية الناتجة ٠

تجارب الحقل :

٤- بزراعة ٧ أصناف وسلالات من الأرز فى ميعادين الأول فى ٢٥ يونيه والثانى فى ١٠ يوليو فى موسمى ١٩٨٦ ، ١٩٨٧ أظهرت النتائج المتحصل عليها الآتى :-

أ) التأخير فى ميعاد الزراعة (الشتل ١٠ يوليو) شجع الاصابة بالمرض حيث اشتدت الاصابة على كل من الأوراق والسنابل فى كلا الصنفين القابلين للاصابة ريهو ، جيزة ١٧٢ ٠

ب) الصنف ريهو كان أكثر قابلية للاصابة - حيث كان عدد البقع على الأوراق ، وشدة اصابة السنابل أعلى من تلك التى على الصنف جيزة ١٧٢ ٠

جـ) بخصوص الصنف جيزة ١٧٥ فكان مقاوم تماما للاصابة فى ميعادى الزراعة فى موسم ١٩٨٦ بينما فى موسم ١٩٨٧ ومع التأخير فى الميعاد الثانى ظهر عدد قليل من بقع اللفحة النموذجية على الأوراق ٠

د) كانت بقية الأصناف المختبرة : جيزة ١٨١ ، آى٠آر ٥٠ ، سلالة ٢١٧٥-٥-٦ ، سلالة ١٣٦٨-٥-٤ مقاومة للاصابة فى كلا الموسمين ١٩٨٦ ، ١٩٨٧ وتحت ظروف ميعادى الزراعة ٠

٥- فيما يتعلق بدراسة العلاقة بين طرق وميعاد اضافة السماد النيتروجينى ومرض اللفحة فى الأرز باستخدام ٣ أصناف (جيزة ١٧٢ ، ريهو ، وجيزة ١٧٥) وباستخدام ٤٠ كيلوجرام / للفدان نيتروجين مضافة بسبعة طرق مختلفة - كانت النتائج المتحصل عليها كالآتى :-

أ) كانت أفضل الطرق من حيث تقليل الاصابة بالمرض واعطاء محصول أعلى - هى تقسيم كمية السماد على ثلاث جرعات : الثلث الأول خلطا بالتربة قبل الشتل ، والثلث الثانى بعد ٢٥ يوم من الشتل أما الثالث فبعد ٥٠ يوم من الشتل مع خلطها بالتربة جيدا بالأقدام ٠
أيضا المعاملة التى يضاف فيها كل كمية السماد بعد ٢٥ يوم من الشتل ، والمعاملة الثالثة والتى يوزع فيها السماد على دفعتين - النصف الأول بعد ٢٥ يوم من الشتل والنصف الآخر بعد ٥٠ يوم من الشتل ٠

ب) وجد أن خلط كل كمية النيتروجين أو نصفها أو ثلثيها مع التربة قبل الشتل شجعت كل من اصابة الأوراق والسنابل وبالتالى أثرت على المحصول فى الأصناف القابلة للاصابة (ريهو ، جيزة ١٧٢) ٠

جـ) كان من نتيجة اضافة كل أو جزء من كمية النيتروجين خلطا بالتربة قبل الزراعة ظهور عدد قليل من البقع المرضية على أوراق الصنف جيزة ١٧٥ فى موسم١٩٨٧٠

٦- من القراءات وبفاصل ١٥ يوما بين كل قراءة والأخرى وجد أن أعلى شدة اصابة على الأوراق فى كلا الصنفين جيزة ١٧٢ ، ريهو فى كلا موسمى الزراعة ١٩٨٦ ، ١٩٨٧ ، كانت فى الفترة من ٣٠ - ٤٥ يوم بعد الشتل ٠

٧- بخصوص العلاقة بين اصابة الأوراق واصابة السنابل على الصنفين جيزة ١٧٢ والريهو خلال موسم ١٩٨٧ أظهرت النتائج الآتى :-
وجود علاقة واضحة بين اصابة كل من الأوراق والسنابل فى كلا الصنفين بينما كانت هذه العلاقة أكثر وضوحا على الصنف ريهو - حيث أعطت كل قراءات الأوراق ارتباط معنوى مع اصابة السنابل - كما نقص المحصول نتيجة اصابة السنابل لوجود ارتباط معنوى أيضا بينهما ٠

تقييم أصناف الأرز المصرية الجديدة بالنسبة للأمراض النباتية

رسالة مقدمة من

عيسى أحمد على سالم

بكالوريوس فى العلوم الزراعية (أمراض النبات ١٩٧١)
كلية الزراعة ـ جامعة الاسكندرية

ماجستير فى العلوم الزراعية (أمراض النبات ١٩٨٢)
كلية الزراعة ـ كفر الشيخ ـ جامعة طنطا

للحصول على درجة

دكتوراه الفلسفة فى العلوم الزراعية

(أمراض النبات)

قسم النبات الزراعى
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١٩٩٠