Pathological and physiological studies on blast and brown spot diseases of rice in A.R.E

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Blast disease incited by Pyricularia oryzae and brown spot disease caused by Drechslera oryzae (Uelminthosparium oryzae) are considered the most serious diseases which attack rice plant causing heavy losses in the yield,The present investigation was conducted to elucidate some of the main problems of brown spot and blast diseases of rice with emphasis on the causal pathogens including mor-phological aspects as well as some physiological studies on different isolates were also undertaken. The studies in-clude also, components of leaf exudates of different rice varieties. Factors associated with the resistance of rice to D. oryzae and P. oryzae were studied. The relationship between development of both brown spot and blast diseases and chemical components of rice plant i.e. amino acids, sugars, and phenols was studied. Also the relationship bet-ween chemical components of the rice plant i.e. amino acids, sugars and phenols and resistance or susceptibility of rice varieties to brown spot was studied. The toxic effect of the culture filtrate of D. oryzae was studied. Artificial inoculation, with brown spot, of different rice varieties for examining their resistance was also undertaken.Results obtained could be summarized as follows : 1) Reaction of different rice varieties, to brown spotdisease (D. oryzae) differs according to the testedisolateS, In this respect Giza 180, IR36' Sakha 2 and Agami Ml varieties showed complete resistance to most of the tested isolates. The pattern was reversed with IR24, IR8, IR20, Amrecani 22, Giza 14, .Nabatat Asmer, Yabani 15, Yabani M3, Yabani M7, Sabieny, Giza 159 and Araby varieties. Similar results as regards P. oryzae and Giza 180, IR36 and Sakha2 varieties showed complete resistance to all tested isolates. whereas the pattern was reversed with Giza 159, Yabani 15, Yabani 1b17 Sabieny which were susceptible.2)The percentage of spore germination of the tested iso-lates of D. oryzae reached the maximum after 6 hours from incubation on 30°C and onwards in all concentrations also germ-tube lengths increased steadily by prolonging the incubation period till 12 hours in all concentra-tions. As regards the effect of spore concentration re-sults indicated that the increase in spore concentration affected their germination and of the six spore concen-trations used, the most significant inhibition was at the highest concentration of 4 x 105 spore/m1., whereas the le ast inhibition was observed at the lowest concen-tration. of 2 x 104 spore/ml. in all the tested isolates. Similar results were noticed as regards P. oryzae isolates.3)The effect of age of D. oryzae culture on percentageof spore germination and germ-tube lengths was clear and the highest percentage of spore germination was obtained from those collected from 10 to 15-days-old cultures. However in this respect isolate No. (9) gave the maximum rate of spore germination. As regards germ-tube lengths, the longest ones were obtained from spores collected from 10-days-old cultures for isolate No. (6) and (9) and 15-days-old culture for isolate No.(5). Similar results as regards the effect of age of P. oryzae culture. In this regard, the highest percentage of spore germination was obtained from spores collected from 10 to 30-days-old cultures. However, isolate No. (5) gave the maximumrate of spore germination. As regards the length of germ-tubes of P. oryzae, the longest ones were obtained from spores collected from 10-days-old cultures by iso-late No. (2) and (4), and from spores obtained from 15-days--old cultures of isolates No. (1), (3) and (5). 4)D. oryzae and P. oryzae spores geminated on a wide range of temperature i.e. from 10-45°C and the optimum tem-perature for both germination and germ-tube lengths of the tested isolates was at 30°C.5)D. oryzae and P. oryzae spores germinated at a pH range from 5-8, with optimum pH about pH 6.5-7

for both spore germination and germ-tube lengths.6)Percentage of spore germination of each of D. oryzaeand P. oryzae was higher on the susceptible varieties than the resistant ones. Also growth of both fungi was less on the resistant varieties as compared with the susceptible ones.7)The interaction between artifical inoculation of rice plants with two isolates of each of D. oryzae and P. ,oryzae (one virulent isolate and the other avirulent one) was conducted. It was found that when inoculation was with a mixture of the two isolates of both fungi or with the avirulent isolate alone, the resulting lesions and severity of infection were lesser than, the case when inoculation was with the virulent isolate alone. On the other hand, when the plants were inoculated first with the avirulent isolate followed-after 24 hours-by the virulent one, lesions and severity were much lesser than in the previous cases.8)Maximum radial growth of all the tested isolates of D. oryzae was obtained when they were kept under continueous darkness for 5 or 6 days, while continueous light for five or six days resulted in relatively poor growth. Maximum sporulation of all the tested isolates was ob-tained when they were incubated under darkness for five or six days or under alternative darkness for two days and light for other two days then 2 days darkness. The high percentage of spore germination of all the testedisolates was obtained when they were exposed to contin-ueous darkness for six days. whereas, under diffused light, the percentage of conidial germination was reduced to about half that under darkness. 9)Similarly the maximum radial growth of all the tested isolates of D. oryzae was obtained when they were in-cubated under darkness or red light. However the maxi-mum sporulation of all the tested isolates was obtained when they were kept under darkness or green colour. On the other hand, Blue light inhibited conidial produc-tion of all tested isolates. Darkness and red light were effective on spore germination on contrast with white colour. 10)Recognized differences were found between different iso-lates of D. oryzae in morphological characteristics and the colour of culture growths differed from white to dark olivaceous. Also the nature of cultural growth differed among different isolates, some isolates were characterized by superficial growths, while others were with aerial cottony growths. Also the different iso-lates differed in secreting stains colouring the media due to their growth, from white to dark. Conidial measurements varied among different isolates from 74 x 11) to 117 x 16)1, also the number of septa per conidium differed among different isolates from 8 to12 septa. The different isolates differed also in their ability to spore production as regards the nature of thecolony.11)Amino acids content in mycelium of different isolates of D. oryzae were.determined. Most of the tested isolates, contained higher amounts of glycine and (fatty amino acid) serine and small amounts of lysine and histidirw. Noticable amounts of acidic amino acids aspartic and glutamic were detected in most tested isolates. In this respect, isolate No. (5) (virulent) contained higher amounts of total amino acids whereas, isolate No. (25) (avirulent) contains the lowest level of total amino acids. Similar trent of results was noticed as regards sugar contents of different isolates.12)Amino acids produced by D. oryzae isolates in culture media varied among isolates. Generally large amounts of glycine and fatty amino acids i.e. serine, valine and alanine acids were produced in culture ,Iedium. However in certain isolates large amounts of leucine, isoleucine, therionine and acidic amino acids i• e. aspartic and glu-tamic acids were noticed.As for Heterocyclic amino acids i.e. proline and histidine, aromatic amino acids such as phenylalanine and tyrosine, and thioamino acids such as cyStine were produced relatively in small amounts. This indicatesthat metabolism varies considerably among different iso-lates, however, isolate No. (9) (virulent) produced higher amounts of total amino acids in culture broths, while isolate No. (25) (avirulent) produced the least amounts in this respect. Ale results revealed that sugars content varied considerably among different iso-lates.13)Leaf exudates taken from susceptible rice varieties to both D. oryzae and P. oryzae, inhanced the linear growth of both fungi compared with exudates of the resistant ones which caused slight inhibition in mycelial growth. As regards spore germination, exudates of susceptible varieties caused more enhancement in this respect than resistant ones,14)Leaf exudates of the susceptible varieties (Araby, Giza 159, Sabieny and Yabani M7) -30 days old-showed clear increase in glutamic, aspartic, therionine. glycine and serine amino acids. whereas, phenylalanine and tyrosine were higher in the resistant varieties Giza 180 and 1R36. However, total amino acids was higher in leaf exudates of susceptible varieties when compared with resistant ones. In this respect, exudates of 45-days-old plants of the susceptible varieties contained higher amounts of glutamic, aspartic and therionine amino acids, as

compared with resistant ones as well as phenolic aminoacids and phenylalanine.15)Total sugars content in leaf exudates markedly increased in susceptible varieties than resistant ones. Amounts of total and reducing sugars increased in exudates of both resistant and susceptible varieties with the in-crease in age from 30 days to 60 days. Also, the per-centage of reducing sugars was higher in susceptible varieties, compared with the resistant ones.16)No clear differences were noticed in the level of total and free phenols in exudates of different resistant and susceptible rice varieties at 30 days old. However, after 45 days, the amounts of total phenols increased in exudates of different varieties, also free phenols in-creased in exudates of different varieties except the susceptible varieties Araby and Giza 159. However the percentage of free phenols was higher in the resistant varieties, Giza 180 and IR36 than the susceptible ones.17)The length of germ tubes produced by D. oryzae spores germinating in sap of inoculated resistant varieties Giza 180 and IR36 were significantly shorter than those from uninoculated leaves of the same varieties. Similar trend was noticed as regards susceptible varieties, Araby and Giza 159. Also the length of germ-tubes of spores germinated in sap from inoculated resistant varieties was lower than those from inoculated suscep.tible ones. Similar results were obtained as regards. P. oryzae.18)The length of germ-tubes of D. oryzae spores produced in the diffusates from leaves of inoculated resistant varieties, Giza 180 and IR36' were shorter than those of uninoculated ones, and also inoculated and uninoculated susceptible varieties Araby and Giza 159. Similar re-sults were obtained with P. oryzae as regards the effect of diffusate of resistant and susceptible rice varieties.19)Leaves of Giza 180 resistant variety (for both 2,- oryzae and P. oryzae) contained 201.5 mg/100 g. fresh weight, of free amino acids before inoculation. Free amino acids content increased as a result of infection of Giza 180 resistant variety after 3 and 5 days of inoculation, and a great reduction followed after 7 and 9 days of in-oculation (with both fungi), except aromatic amino acids. As regards susceptible varieties (Araby for D. oryzae and Sab ienz for P. orTzae) the le ayes contained higher amounts of total free amino acids than the resistant one , leaves of Araby , susceptible, variety contain 285 mg/100 g. fresh weight, before inoculation. This amount increased after 3 and 5 days of inoculation then decrea-sed after 7 and 9 days. whereas Sabieny variety plants contain 270.5 mg/100 fresh weight, this amount increa-sed after 3, 5 and 7 days of inoculation.20)Uninoculated leaves of Giza 180,resistant variety, (for both fungi) contained 16.93 mg/100 g. fresh weight, of total sugars. As regards uninoculated leaves of sus-ceptible varieties (Araby and Sabieny) contained higher amounts of total sugars than the resistant one. Total sugars content in Araby leaves was 20.03 mg/100 g. fresh weight, whereas Sabieny leaves contained 23.16 mg/100 g. fresh weight. Sugar content increased with disease development in all the tested varieties, and this in-crease was higher in the susceptible varieties than the resistant one. Same trend of results was found as re-gards reducing sugars content which was higher in sus-ceptible varieties and increased with disease develop-ment in all the tested varieties, although it was higher in the inoculated and uninoculated susceptible leaves than the resistant ones.21)As regards total and free phenols, uninoculated leaves of Giza 180 resistant (to D. oryzae and P. oryzae) variety contain 91.03 and 72.39 mg/100 g. fresh weight total and free phenols respectively. whereas, Araby and Sabieny susceptible varieties contain lower quanti-ties of total and free phenols as compared with the re-sistant one, which were 58.21 and 83.91 mg. /100 g. fresh weight for total phenols and 36.69 and 56.48 mg./ 100 g. fresh weight for free phenols respectively. Total and free phenols content increased with diseasedevelopment in all the tested varieties.22)Uninoculated plants of Giza 180,resista.nt variety (for both D. oryzae and P. oryzae) contain higher amounts of 0-diphenols than that of Araby and Sabieny susceptible ones. However, 0-diphenols in both uninoculated and in-oculated plants Giza 180(R) variety increased after 3, 5, 7 and 9 days after inoculateion, while 0-diphenols decreased in inoculated susceptible varieties as a re-sult of infection after (5, 7 and 9 days) for Araby and after (3, 5 and 7 days) for Sabiony.23)Noticeable increase was found. in the amounts of diffe-rent amino acids in response to infection with D. oryzae in the different rice varieties i.e. Agami M1(R), Giza 172 (MR) and Araby(S), however the rate of increase was higher in the susceptible variety. In this respect, amino acids content increased by increasing the age of plant of the different varieties.24)Total and reducing sugars content was higher in the sus-ceptible variety (Araby) than the other two varieties, Giza 172 (MR) and Agami Ml (R). However, total and reducing sugars content tended to increase in all the

tested varieties as a result of infection with D. oryzae, also total and reducing sugars content increased by in-creasing the age of plant of different rice varieties.25)Uninoculated resistant (Agami M1) and moderate resis-tart (Giza 172) varieties contain higher amounts of total and free phenols than the uninoculated susceptible variety (Araby) , however total and free phenols content increased in all the tested varieties due to infection with D. oryzae. Free phenols were higher in the inocu-lated resistant and moderately resistant varieties thanthe susceptible ones. Total and free phenols content in-creased by increasing the age of plants of the different rice varieties except Giza 172 (45 days old). Also, 0-diphenols compounds increased in the resistant andmoderate resistant varieties, whereas the reverse was noticed in case of the susceptible one. 0-diphenols in-creased by increasing the plant age.26)Amino acids content in Araby plants as affected ley in-fection varied among different isolates of D. oryzae. For example the amounts of argenine ranged from 21 to 29 mg./100 g. fresh weight in different isolates. Also different isolates induced different amounts of total and reducing sugars in Araby(S) variety. Total and reducing sugars content increased as a result of infec-tion among different isolates. As regards total and free phenols, these compounds in le ayes of Araby sus-ceptible variety varied also among different isolates of the fungus.27)The crude culture filtrate of two isolates of D.oryzae(one virulent isolate and the other avirulent one)in-duced inhibitory effect on the percentage of seed ger-mination and root density on all the tested varieties i.e. Giza 180, IR36, Araby and Giza 159. The high con-centration of crude culture filtrate was the most effec-tive in this respect in all the tested varieties. How-ever the inhibitory effect of the crude culture filtrate decreased proportionally with decreasing the concentra-tion. The crude culture filtrate which was isolatedfrom 30 days-old-cultures, was the most effective filtrate on inhibiting seed germination, and root density than those collected from 10 or 20-days-old cultures. Inthis respect, filtrates of isolate No. (9), which was the most virulent, induced the highest percentage of in-hibition to seed germination when compared with filtra-tes of the least virulent isolate No. (1).28) One toxic fraction was isolated in crystaline form(Needle crystals), according to the method adopted by Orsenigo (1957) who identified these crystals as(Ophiobolin) and Turner (1971) identified ophiobolins A, B, C as phytotoxins produced by D. oryzae. The virulent isolate No. (9) produced 5.21 g./liter of culture medium of toxin, while the less virulent iso-late No. (1) produced 4.67 g./liter of culture medium, Spraying leaves of Araby susceptible rice variety with-170 - the isolated toxin resulted in yollow pale spots.29)Isolated toxin induced inhibitory effect on seed germi-nation, coleoptile elongation and density of roots. The inhibitory effect of the toxin decreased proportionally by decreasing its concentration.30)Giza 180, 1R36, Sakha2 and Agami 1V11 rice varieties showed complete resistance to most of the tested isolates of D. oryzae,, except Giza 180 for isolate No. (19), 1R36 for isolate No. (13), Sakha2 for isolate No. (19) and Agami M1 for isolates No. 9,13 and 19 which showed moderate resistant reaction respectively. However varieties Giza 172 and Sakha 1 were moderately resistant,and varieties Giza 170 and Giza 171 were moderately susceptible, 13 varieties were susceptible to all the tested isolates namely, IR24, IR8, 1R20, Amrecani 22, Nanda, Giza 14, Nabatat Asmer, Yabani 15, Yabani M3, Yabani M7, Sabieny, Giza 159 and Araby, The remaining 14 varieties showed different reactions to the different isolates.