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DISEASE RESPONSES OF BARLEY GENOTYPES WITH *Puccinia hordei* Otth. in IRAQ

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ABSTRACT

Disease responses of barley genotypes and leaf rust pathogen *Puccinia hordei* Otth. through pustule density (Uredinia/cm ^{2} of flag leaf, uredinia/flag leaf and tiller) and latent period (LP_{50}) were investigated under growth room and field conditions near Baghdad. Barley genotypes used included 9 cultivars, 13 induced mutants, 8 introduced accessions and 300 M2 selected variants from Numar cultivar following gamma irradiation. Pustule density per cm ^{2} area of flag leaf under heavy artificially epiphytotic produced 15 in the mutant SA/12 to 127 pustules in cultivar Golden Melon and the naked barley strain Aamer. Disease responses of all tested genotypes could be classified into four groups: Highly susceptible such as the cultivars Golden Melon, Aamer, Arivat, Beacher and Weah and mutants C/50, D/21, D/24 D/30 and D/32 and accessions 480 and 557; Susceptible such as Prior cultivar, mutants NA/20, C/63 and D/34, mildew resistance source H-421 and the accession 552; Moderate susceptible as in cultivar Jazera 2, mutants VB/7 and TB/15, the accessions 102,576,577 and 657; Moderate resistance included Numar with all its variants, cultivars Jazera 1, the mutants OA/15, VB/6, and SA/12. The latent period (LP_{50}) of *P. hordei* on detached leaves at 20±2°C was 132 hr. in the first group, 132- 156 hr. in the second and third while it was 168 hr. on genotypes of the fourth group. Barley genotypes of the fourth group might have moderate resistant reaction to *P.hordei* in Iraq. Thus a convenient screening method to aid breeding for disease resistance and variety recommendation was established

INTRODUCTION

Barley (*Hordeum vulgare* L.) represents one of the most important four cereal crops around the world and it's the second crop in Iraq. Barley is annually cultivated in all Iraqi provinces for animal feed mainly and for domestic malting. Like all cereal crops, barley annually suffers from numerous biotic (phytopathogenic and insects) and abiotic (drought and salinity) agents that limit yield quantity and quality. Barley leaf rust, *Puccinia hordei* Otth. is one of the most destructive and widely distributed diseases in most barley growing regions. The causal agent is an obligate parasite in which its signs may be range from small chlorotic flecks to large pustules containing urediniospores. The pustules, or uredinia, of barley leaf rust are small, circular, light orange-brown usually formed on leaves and producing a mass of orange-brown powdery spores. The pustules are predominantly on the upper leaf surfaces and might develop on leaf sheath. The pustules turn dark (telitia with telitiospores) during host maturity and produce black spores embedded in host tissues. Since the disease is one of the multiple cycle diseases, the urediniospores of *P. hordei* are wind borne and repeat or recycle the infections during the season as long as both the environmental conditions and the host leaf tissues are suitable for disease development.

This disease has caused significant losses in many regions and continues to cause a serious threat due to its epidemic forms since the epidemic of such disease frequently reduce yield and seed quality. Yield loss due to barley leaf rust could be near 30 % (Arnst *et al*. 1979). Yield losses have been reported in Australia 31 %(Dill-Macky *et al*. 1989), Europe 17-31 %(Clifford 1985; Cotterill *et al*. 1992) and about 32 % in USA on susceptible barley cultivars under epiphytotic conditions (Griffey *et al*. 1994; Mammadov *et al*. 2003). Barley leaf rust can be controlled by fungicides, but because of the environmental and health risks, and the need to reduce production costs, there is a strong tendency to reduce the use of fungicides in favor of genetic resistance or by the use of resistant cultivars (Shtaya *et al*. 2006) even the sources of leaf rust resistance in cultivated barley are limited (Jin and Steffenson 1994; Jin *et al*. 1995). In recent years, the economic importance of barley leaf rust has increased in Europe, especially in Central and Northwestern areas due to fitness increase in pathogen populations to landrace cultivars (Czembor and Bladenopoulos 2007).

In Iraq, unfortunately, the breeders and plant pathologists have ignored barley leaf rust, although the environmental conditions in our region are quite suitable for its development every year. The first attempt to improve disease resistance was reported in 2004, when a promising resistance source for mildew and leaf rust was identified (AL-Hamdany *et al*. 2004). This source, line 7020, was successfully selected from H-421 (AL-Hamdany *et al*. 1993).

Thus, this investigation was initiated to establish or measure the range of disease responses among cultivars and breeding lines in the field and growth room through pustule density and latent period.

MATERIALS AND METHODS

Inoculum units of *Puccinia hordei:* Rust-infected barley leaves were collected from barley fields at the Tuwaitha Experiment Station and from farmer's fields in the Tuwaitha region 30 km south of Baghdad. Urediniospores were air dried and stored at -20°C. The stored urediniospores were reproduced on seedlings of susceptible cultivar Golden Melon under growth room conditions (20±2°C with 12hr/day light along with high humidity). Following inoculation, the potted seedlings were placed in darkened boxes to maintain moisture for 18 hr. The pots then were transferred to the above-mentioned conditions.

Host plants: Three hundred and thirty barley genotypes were used in this study. The genotypes were: 9 cultivars, 13 induced mutants (AL-Khalissii 1980), 7 introduced accessions, and 1 mildew resistance source (H-421), 227 M3 variants from Numar cultivar following 250 Gy of gamma rays, and 73 M3 variants from the same cultivar but following 550 Gy of gamma rays (Table 1).

Latent period of *Puccinia hordei:* The first leaf of 10 - day old seedlings of all barley genotypes used in this study (Table 1) was detached. The detached leaves were floated on 50 ppm of benzimidazole solution inside Petri dishes (15 ml/plate) (Browder 1964) with three replicates for each genotype. The dishes were covered and placed in the dark for 18 hr immediately after inoculation by atomizing the leaf segments with spore suspension of *P. hordei* (100 urediniospores/ cm ^{2}). Inoculum density was measured on 1-cm ^{2} water agar slides distributed among the dishes during inoculation.
The number of visible pustules on leaf segments of each genotype was counted every day until no more pustules appeared. The latent period (the time (hrs) between inoculation and the appearance of 50 % of the pustules was recorded. The relative latent period on detached barley leaves (RLP_{50}) was calculated based on the latent period of Golden Melon, where $Lp_{50\ of}$ Golden Melon =100 (Parlevliet 1975). Data of latent period were analyzed (Snedecor and Cochran 1976).

Pustule density of *Puccinia hordei* under field conditions: A randomized complete block design with three replicates was applied in the Tuwaitha Experimental Station using 7 X 15m field plots. The plot borders were seeded with Golden Melon as a spreader. The barley genotypes were sown during the third week of December in 1-m rows 30 cm apart. Fresh uredinial pustules of *P. hordei* were established on potted Golden Melon seedlings in the greenhouse two weeks before sowing. An epidemic in the field plot was initiated by placing seedlings of Golden Melon with heavy rust between the spreader and tested genotypes during the third week of February. All the plants were subjected to urediniospores suspension during the season. Pustule density during dough stage was recorded on 10 tillers per genotype per replicate. The number of pustules on the upper three leaves of each tiller and pustules in 1-cm ^{2} areas of flag leaf/tiller was also counted (Parlevliet 1976). Data of pustule density in 1-cm ^{2} of flag leaves were analyzed (Snedecor and Cochran 1976).

RESULT AND DISCUSION

Latent period of *Puccinia hordei* on barley genotypes: Statistical analyses of latent period revealed significant differences among barley genotypes used. Many barley genotypes showed a considerable increment in LP_{50} ranged from 24 to 36 hr. compared to that observed on susceptible cultivar Golden Melon. These groups included 3 cultivars (Numar, Jazera 1 and 2) 6 mutants (D/34, VB/6, OA/15/ SA/12, VB/7 and TB/15) H-421, 4 accessions and all Numar variants. The latent period of the cultivars Golden Melon Aamer, Beacher, Weah, Arivat, Prior, mutants D/21, D/24, D/30, D/32, C50, C63 and NA/20, accessions 480, 525 and 557 was 132 hr which was the lowest (Table 2). Numar cultivar with all its variants regardless the gamma dose used along with three induced mutants from it (OA/15, VB/6 and SA/12) and one cultivar Jazera 1 showed 36 hr increments in LP_{50} over the Golden Melon and the first group. Although the increment in LP_{50} was not as expected, it might reflect a good indication for a partial resistance in many genotypes (Table 2). This is true, since long latent period is one of partial resistance components (Parlevliet 1979. Parlevliet and Ommeren 1975; Statler and Parlevliet 1987). The partial resistance in barley to *P. hordei*, has been characterized by a reduced rate of epidemic development, long latent period and short sporulation or infectious period in spite of susceptible infection types (Parlevliet 1975). The latent period which represents the most reproducible and easiest component to measure its correlation with partial resistance, had been well investigated in all rust diseases (Luke *et al*. 1984; Parlevliet 1979; 1977; 1975; Parlevliet and Ommeren 1984). Necrotic areas surrounded the pustule of the last group were observed. Few leaves of H-421 either in LP test or in the field showed few or no pustules.

Pustule density of *Puccinia hordei* under field conditions: Pustule density of *P. hordei* on the tested barley genotypes was significantly varied

among barley genotypes used due primarily to the host reaction. It was lower for the genotypes Numar cultivar and its M3 Gama induced variants, cultivar Jazera 1, mutants VB/6, OA/15 and SA/12. However, out of these genotypes, Numar mutant SA/12 showed the lowest disease severity (15 pustules/cm ^{2}), which covered 2-3 % of leaf area and 11.8 % of pustules number counted on Golden Melon. (Table 2). The number of pustules per 1 cm ^{2} of the flag leaf of these above mentioned genotypes ranged from 15 to 24. Based on the leaf area of flag leaf of each genotype, the pustule density on flag leaves were ranged between 204 in the mutant VB/6 to 275 pustules in Jazera 1. Using Parlevliet scale and percent of infected leaf area (Parlevliet and Ommeren 1984), pustule per tiller was ranged from 1089 in the mutant SA/12 to1615 pustules in the mutant OA/15, which indicated that the total leaf area covered by uredinial pustules in these genotypes was 2-5 % compare to 18 % in Golden Melon, Beacher, Weah, Aamer, Arivat, mutants D/21, D/30 and D/32, and in accession 480.

Pustule density, either per 1 cm ^{2} or per flag leaf or per tiller, raised a good question on the best way to evaluate the infection density in barley: *P. hordei* relationship. Our study recommend that plant pathologists working with barley leaf rust to use pustule per 1cm^2 of the flag leaf only rather than the pustule per flag leaf or per tiller since the leaf area is always varied among barley genotypes.

Since pustule density in rust diseases reflects the amount of urediniospores released from each cultivar, the clear reduction in their numbers and the long latent period could be together represents good indicators of having partial (general) resistance in our materials as previously reported elsewhere (Parlevliet 1979; 1977; 1976; 1975; Parlevliet and Ommeren 1984; 1975). Out of all genotypes tested, the chlorotic tissues around the pustules were always there on the flag leaves of SA/12, VB/6, OA/15 and Numar and its variants .Statler and Parlevliet 1987 reported that germinated urediniospores failed to penetrate the host tissue, early and late-aborted colonies were higher in barley cultivars with partial resistance than the susceptible one .Thus, the lower numbers of pustules in certain genotypes that might take longer to develop in the field under an epiphytotic form agree with a solid conclusion that partial resistance of any cultivar is always reflected slower development of fewer sporulated colonies.

Table 1 Barley genotypes used in growth room and field trails

Barley genotypes	
Cultivars	
Golden Melon	
Weah	
Beacher	
Prior	
Aamer	
Numar	
Arivat	
Jazera 1	
Jazera 2	
Mutants[a]	
D/21, D24, D/30,D/32,D/34 ,C/50 and C63	Induced from Arivat cultivar by Sodium azide
VB/6	Induced from Numar cultivar by Sodium azide
OA/15,SA/12,VB/7, TB/15 and NA/20	Induced from Numar cultivar by 200 Gy gamma rays
H-421	Mildew resistance source (*Mla*13)
Introduced accessions	
102, 480, 525, 557, 576, 577, 657	
Numar Variants	
227 Variants[c]	250 Gy of Gamma rays (M2)
73 Variants[c]	550 Gy of Gamma rays (M2)

a. Barley Induced mutants (AL-Khalissii 1980)

b. Introduced barley accessions from International Institutes

c. Following gamma irradiation on Numar seeds, the variants were selected from M2 progenies of both doses.

Table 2 Latent period (LP_{50})[a] and pustule density of barley: *Puccinia hordei* interaction following artificial inoculation in growth room and field conditions

Barley genotypes	LP 50 Hrs.	RLP % [b]	No. Pustules (Uredinia) per			Scale values[c]	Sporulation [d]area in %
			Cm ^{2}/flag leaf	Flag leaf	Tiller		
Golden Melon	132	100	127	1160	7500	16	18
Beacher	132	100	96	672	5526	16	18
Weah	132	100	78	436	5813	16	18
Prior	132	100	61	405	2962	15	10
Aamer	132	100	127	710	5279	16	18
Numar	168	127	20	268	1610	14	5
Arivat	132	100	75	603	5007	16	18
Jazera 1	168	127	24	275	1393	14	5
Jazera 2	156	118	31	210	2631	15	10
Mutant D/21	132	100	98	744	5299	16	18
Mutant D/24	132	100	66	412	4046	15	10
Mutant D/30	132	100	89	725	5790	16	18
Mutant D/32	132	100	75	671	5299	16	18
Mutant D/34	156	118	54	567	2832	15	10
Mutant C/50	132	100	66	545	4007	15	10
Mutant C/63	132	100	61	473	4450	15	10
Mut. NA/20	132	100	62	478	3391	15	10
Mut. VB/6	168	127	17	204	1359	14	5
Mut. OA/15	168	127	18	222	1615	14	5
Mut. SA/12	168	127	15	216	1089	13	2-3
Mut. VB/7	156	118	31	339	2096	14	5
Mut. TB/15	156	118	32	252	2565	15	10
Line H-421	156	118	53	392	2263	14	5
Acc. 102	156	118	33	298	2024	14	5
Acc. 480	132	100	68	534	5637	16	18
Acc.525	132	100	61	691	4160	15	10
Acc.557	132	100	70	557	4548	15	10
Acc.576	156	118	41	336	2697	15	10
Acc.577	156	118	34	362	2823	15	10
Acc.657	156	118	37	386	2443	15	10
Numar Varients[e]	168	127	18	245	1350	14	5
LSD P=0.05	15.77		9.82				

a. LP_{50} = period from inoculation to appearance of 50 % of the pustule on detached leaves floated on 50 ppm of Benzimidazole.
b. Relative percentages to the LD50 on susceptible Golden Melon.
c. Scale value (0-19) represents the amount of leaf rust on one tiller (Parlevliet and Ommeren 1984).
d. Estimated leaf area covered by pustules where scale value 12 =300 pustules per tiller = 1 % of leaf area produced urediniospores (Parlevliet and Ommeren 1984)
e. M3 variants from Numar following 250 and 550 Gy of gamma rays.

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