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Barley Leaf Stripe Disease in Algeria: Evaluation of Virulent *Pyrenophora graminea* Isolates and Identification of Resistant Algerian Barley Genotypes

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ABSTRACT

Nine isolates of *Pyrenophora graminea*, barley leaf stripe disease causal agent, collected from several regions in Algeria were evaluated under greenhouse conditions for their virulence to a collection of barley cultivars including three most cultivated Algerian varieties. Virulence levels were observed among the set of isolates and a mean disease rate ranging from 3.33% to 75.83% was found. Pathogenic variability of *P. graminea* and resistant gene effects in barley cultivars were revealed. Isolate OS was the most virulent among *P. graminea* isolates making it a suitable virulent isolate in future breeding programs. A set of 8 barley genotypes composed of common Algerian cultivars and local developed lines were tested for their reaction to *P. graminea* and yield response. Barley cultivar Minnesota 23 and line 18/17/7L2 were the most resistant of the collection with high grain number/ear and thousand grains weight even when diseased. These genotypes could be useful to integrate as candidate genitor plants into barley breeding programs to develop resistant cultivars to leaf stripe disease.

Keywords: Barley stripe disease; Aggressiveness; Barley; Resistance; Breeding; Algeria

1. Introduction

Barley (*Hordeum vulgare* L.) is one of the largest sown crops in the world. In Algeria, barley is a strategic crop and occupies an important place after durum and bread wheat. Thirty five to 40% of crop areas were reserved for cereals in Algeria (Benmohammed 2004; Rahal-Bouziane 2015; Zairi et al 2016) and a production around 1400 to 2000 kg ha^{-1} occurs (ONFAA 2015). Barley is heavily devastated by barley leaf stripe disease, a widely disseminated seed-born disease caused by the fungal pathogen *Pyrenophora graminea* (Ito & Kuribayashi) (anamorph *Drechslera graminea* ((Rabenh ex. Schlech) Shoemaker). This disease decreases barley yield and quality and induces important economic losses in many countries (Porta-Puglia et al 1986; Arabi et al 2004; Karakaya

et al 2016). It is the most important barley disease in Algeria. Benbelkacem et al (2000a) evaluated the mean incidence of barley leaf stripe averaging 27.94%, which corresponds to one_third of the Algerian potential barley production and estimated an overall yield reduction of 29 kg per hectare for each 1% of the disease incidence. Fungicides are effective for reducing the severity of this disease, but the most practical and environmentally sound means of control is through the use of resistant cultivars (Arabi & Jawhar 2005). Developing resistant cultivars is the best method environmentally and effective one for disease control. However, to breed for resistance to disease, a solid knowledge of the virulence variation information related to pathogen is necessary (Arabi & Jawhar 2012).

The objective of the present study was to investigate the most aggressive isolate of the *P. graminea* among a collection from different regions of Algeria and to evaluate the reaction and yield response of most cultivated Algerian varieties and local selected lines, under greenhouse conditions to *P. graminea*. The study was also carried out in attempt to develop a breeding program for improving barley local cultivars to leaf stripe disease.

2. Materials and Methods

2.1. Fungal isolates

Barley leaves showing *P. graminea* stripes, collected from different regions of Algeria, were used to obtain the fungus isolates (Table 1). Infected leaves were cut into pieces and sterilized using 5% sodium hypochlorite solution (NaOCl) for 5 min and then washed three times with sterile distilled water. Under sterile conditions and using a binocular loupe, a single spore was removed with a handle and put into Potato Dextrose Agar (PDA) medium. The Petri dishes were left for 7 days of incubation at 21±1 °C in the dark. All isolates were derived from single spores cultures.

2.2. Aggressiveness test

Nine isolates of *P. graminea* were assessed for their aggressivity on four barley varieties, known for their reaction to *P. graminea* which ranged from susceptible to resistant (Table 1): Rihane 03 (ICARDA), Saïda and Tichedrett (Algeria) and Minnesota 23 (USA). Rihane 03, Saïda and Tichedrett are frequently cultivated in Algeria whereas Minnesota 23 is experimentally used. The inoculum was prepared according to Hammouda (1986) modified sandwich method. Under aseptic conditions, barley seeds were disinfected, following leaf surface sterilization protocol previously described, and put onto a half part of an 8 days old mycelia culture plates, and the other half of the fungal colony was flipped over seeds. Each prepared plate was sealed. For control treatment, seeds were placed between two half of PDA medium without *P. graminea*. The seeds were incubated at 6 °C for 14 days in the dark. Inoculated and uninoculated seeds were carefully planted into 25 cm diameter plastic

Table 1- *P. graminea* isolates and barley genotypes used in this study

Fungal isolates	*Region*	*Algerian geographic area*	*Barley genotypes*	*Origin*
SST	Sidi taleb/Setif	East	Minnesota 23	USA
BBN	Biskra	South-East	Rihane 03	ICARDA
IP48	Algiers	North	Saïda	Algeria
STF	Setif	East	Tichedrett	Algeria
OS	Algiers	North	P11L4 and	Line developed from Algerian
MSK	Mascara	North-West	P48/L2	Populations 48 and 11 (*)
SFOU	Fouara/Setif	East	18/17/7L2	Doubled haploid line (*)
AD	Aind Defla	South-West	18/3/2BL2	Genealogical line (*)
MBS	Medea	South-West		

(*), developed by Professor Mekliche L.

pots, at rate of 10 seeds per pots and placed under greenhouse conditions in a randomized complete block design, with three replicates. Infected and uninfected plants were scored at heading stage (GS 50) (Zadoks et al 1974). To estimate infection level, percentage of infected plants was calculated, according to Delogu et al (1989) scale, described as follows: highly resistant (*HR*) (0-5% of infected plants), resistant (*R*) (6-11%), moderately resistant (*MR*) (12-26%), susceptible (*S*) (27-78%) and highly susceptible (*HS*) (79-100% of infected plants).

2.3. Assessment of barley cultivars' reaction to leaf stripe and grain production components

Eight barley genotypes were used to evaluate their reaction to the most virulent isolate of the nine *P. graminea* isolates collection. These eight genotypes are composed of the four varieties described above and four lines (Table 1) developed by Professor Mekliche L. at our Plant Production Department. Inoculated and control seeds were treated as previously described and then carefully removed from Petri dishes and planted, in greenhouse, directly to cement vats previously filled with soil. In this experiment, randomized complete block design with three replicates was used. Infected and healthy plants were counted at heading stage and infection level was assessed according to Delogu et al (1989) scale as described above. At maturity, plants of each plot were harvested to evaluate grain number per ear (NG/E) and thousand grain weight (TGW).

2.4. Computation and data analysis

Statistical ANOVA and homogeneous groups (Newman-Keuls tests) analyzes were performed to evaluate differences between experienced different factor values.

3. Results and Discussion

3.1. Evaluation of isolates' aggressiveness

Highly significant virulence levels were observed among five separated homogenous groups (Tables 2 and 3). The mean disease rating was ranged from 3.33% to 75.83%. Çetin et al (1995) observed infection ranging from 15.4% to 96.3%. Isolate OS was the most virulent on four cultivars. Therefore, this isolate may be considered as a suitable virulent isolate in the future plant-breeding programs. MBS isolate was found as the least virulent (Table 3). Reaction of cultivars to *P. graminea* isolates showed highly significant differences (Tables 2 and 3). The cultivar Minnesota 23 was shown to be resistant to all pathogen isolates with <5% of disease reaction. Rihane 03 was resistant to 7 isolates. However, the varieties Saïda and Tichedrett were the most susceptible ones and were resistant to only three isolates. The cultivars Minnesota 23, Rihane 03, Saïda and Tichedrett were suitable for defining virulence because they all provided clear response to the pathogen isolates investigated, whether in aggressiveness test or in barley reactions. They revealed a high level of variability in their reactions. Similar reactions were found by other researchers. Bayraktar & Akan (2012) also found resistant cultivars with <20% of disease reaction. In this study, Minnesota 23 genotype was the most resistant, Rihane 03 was intermediate, Tichedrett and Saïda were susceptible. These results are in agreement with those obtained by Benbelkacem et al (2000b). Analysis of variance showed also highly significant interaction of isolates and cultivars (Table 2) suggesting that cultivars reacted differently to the isolates. Based on those differential reactions between different cultivars and several isolates collected from diverse regions, specific resistance gene effects may exist in the host barley cultivars. Consequently, this indicates the presence of pathogenic variability of *P. graminea* isolates. Variation in pathogenicity of *P. graminea* isolates was mentioned by many researchers (Zriba & Harrabi 1995; Delogu et al 1995; Benbelkacem et al 2000b; Aminnejad et al 2009; Bayraktar & Akan 2012; Çelik et al 2016; Karakaya et al 2017). However, groups clustering showed that isolates OS, IP48, MSK and MBS constituted by themselves as statistically different individual groups (Table 3), suggesting that those isolates can be different distinct physiologic races. On the other hand, Algerian East and

South-West isolates BBN, SFOU, STF, SST, AD (Table1) were gathered into one group (Table 3), with no differential virulence, suggesting that they might be related to the same pathotype. This can be explained by the fact that *P. graminea* is exclusively seed-borne and that Eastern Algerian farmers often supply their needs of barley seeds from the south-western regions and vice versa. The same case happens to South-Western Algerian farmers. Such results were also reported by Benbelkacem et al (2000b).

3.2. Evaluation of barley cultivars' reaction

Differences were observed in the reaction of barley cultivars (Table 2) indicating a high level of variability among barley collection for leaf stripe severity. The line 18/3/2BL2 was highly susceptible barley genotype with disease incidence of 90% followed by Tichedrett (80%). The genotypes P11L4, P48/L2 and the varieties Saïda and Rihane 03 were susceptible with disease incidence ranging from 50 to 77%. The line 18/17/7L2 was moderately resistant (23%). The cultivar Minnesota 23 was highly resistant with 3% disease incidence. Cultivar Minnesota 23 proved to be the most resistant one all through the aggressiveness and the reaction variability tests.

Differences among the reactions of the barley cultivars and lines to the isolates of the barley leaf stripe fungus were also reported by different authors (Ulus & Karakaya 2007; Bayraktar & Akan 2012; Çelik et al 2016; Karakaya et al 2017; Çelik Oğuz et al 2017).

Table 2- Mean squares (MS), degrees of freedom (df) and coefficients of variation (CV) from analysis of variance for the studied traits

Studied traits	*Disease rating aggressivity (%)*		*Incidence (OS isolate inoculation) (%)*		*Number of grain/ear*		*1000-grain weight (g)*	
Sources	*df*	*MS*	*df*	*MS*	*df*	*MS*	*df*	*MS*
Cultivars	3	2.538***	7	0.235***	7	140.952***	7	103.356***
Isolates	9	1.386***	1	8.626***	1	833.333***	1	1631.934***
Varieties isolates	27	0.173***	7	0.235***	7	27.048***	7	166.009***
Error	54	0.006	14	0.023	14	2.610	14	0.698
CV(%)		1.639		5.494		8.073		1.741

***, significant at P<0.001

Table 3- Mean disease aggressiveness rating of *P. graminea* isolates

Isolates	*Mean disease rating (%)*	*Mean disease rating on barley cultivars (%)*			
		Minnesota23	*Rihane03*	*Saida*	*Tichedrett*
OS	75.83 a[y]	10.00 R	96.67 HS	96.67 HS	100.00 HS
IP48	49.17 b	0.00 HR	56.67 S	83.33 HS	56.67 S
MSK	30.83 c	0.00 HR	23.33 MR	66.67 S	33.33 S
BBN	16.67 d	3.33 HR	0.00 HR	23.33 MR	40.00 S
SFOU	14.17 d	0.00 HR	0.00 HR	26.67 S	30.00 S
SST	14.17 d	0.00 HR	0.00 HR	36.67 S	20.00 MR
STF	14.17 d	0.00 HR	0.00 HR	26.67 S	30.00 S
AD	10.83 d	0.00 HR	0.00 HR	23.33 MR	20.00 MR
MBS	3.33 e	0.00 HR	0.00 HR	13.33 MR	0.00 HR

3.3. Evaluation of grain number per ear and thousand grains weight components

Number per ear and thousand grains weight of inoculated barley cultivars was significantly reduced compared to their associated controls and this decrease varied largely between barley genotypes (Table 4). The disease reduced the NG/E of the resistant cultivars Minnesota 23 and 18/17/7L2 by 29% and 18%. For susceptible cultivars, reduction of NG/E ranged between 19% to 87%. On the other hand, the disease did not affect TGW of the resistant cultivar Minnesota 23 and line 18/17/7L2 while for susceptible genotypes Rihane 03, P11/L4, Saïda, Tichedrett, P48/L2 and 18/3/2BL2, disease impact reduced TGW of 9%, 12%, 54%, 56%, 80% and up to 100% respectively. This study showed that inoculation with *P. graminea* affected grain number per ear and thousand grain weights of diseased cultivars considerably. Arabi et al (2004) reported that the thousand grain weight was affected negatively when barley plants were inoculated with *P. graminea.* However, in our study, the resistant cultivar Minnesota 23 and line 18/17/7L2 showed high number/ear and thousand grain weight while the susceptible ones scored reduced values of these traits. Arabi et al (2001) showed that *P. graminea* had a direct impact on element storage (as proteins) of susceptible cultivars, whereas no effects were detected in the resistant ones. In our study, the resistance demonstrated in Minnesota 23 and 18/17/7L2 approached closely to those reported in Arabi et al (2001) study. Therefore, it is suggested that cultivar Minnesota 23 and line 18/17/7L2 should be integrated in breeding studies for introducing leaf stripe disease resistance into most cultivated and high yielding varieties.

4. Conclusions

The present study showed pathogenic variation among Algerian *P. graminea* isolates and provided insights about reactions of Algerian barley cultivars and resistance sources to barley leaf stripe disease. The agressiveness of the *P. graminea* isolate OS could make it suitable as the virulent isolate in future plant breeding programs. The barley cultivar Minnesota 23 and the line 18/17/7L2 were good sources of resistance and may be useful in developing resistant cutivars. Although our sample size was limited, these prelimenary results indicated presence of good resistance gene effects in Algerian barley cultivars and varieties and diversity in the *P. graminea* pathogen populations of Algeria. For more solid protocols to assist breeding programs, a larger set of isolates collected from different localities should be used and more barley cultivars should be investigated and screened under field conditions.

Table 4- Incidence of *P. graminea* (inoculated with OS isolate) in 8 barley genotypes and its effect on the grain number per ear and 1000-grain weight (g)

Cultivars	*Barley genotypes reactions*		*NGE*			*1000-grain weight*		
	Incidence (%)	*Reaction type*	*Mean*		*Reduction rate (%)*	*Mean*		*Reduction rate (%)*
			Uni.	*Ino.*		*Uni.*	*Ino.*	
18/3/2BL2	90 a[y]	HS	39.33 b	21.00 e	87	52.67 a	23.00 h	129
Tichedrett	80 a	HS	29.67 e	23.00 ge	29	44.67 ef	28.67 f	56
P48/L2	77 a	S	31.67 de	26.33 efg	20	45.67 de	25.33 g	80
Saïda	63 ab	S	32.67 de	25.00 fg	31	47.67 bc	31.04 e	54
Rihane 03	53 ab	S	37.67 bc	31.67 bcd	19	44.67 ef	41.00 c	9
P11L4	50 ab	S	34.67 cd	28.33 def	22	39.67 g	35.33 d	12
18/17/7L2	23 b	MR	43.66 a	37.00 a	18	48.67 b	46.67 a	4
Minnesota23	3 c	HR	42.67 a	33.00 b	29	43.67 f	43.00b	2

[y], different letter, means significantly different at P<0.05 (Newman-Keuls test)

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