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Comparative study of *Pyrenophora graminea* incidence on *Hordeum vulgare* L. hybrid populations and their parents.

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Abstract

The seed- borne pathogen *Pyrenophora graminea* Ito& Kur. is the causal agent of barley leaf stripe, a destructive disease in most barley growing region in the world. In order to improve the barley species in Algeria against this disease, it is necessary to know their tolerance reaction to it. The genotype that have been tested are five parent genotypes: ACSAD, Tichedrett, Saida, Rihane, Hamra and eight populations hybrid obtained by intraspecific crosses of the parents and then sowing of F1 to F8. We compared the incidence of the isolate of *Pyrenophora graminea* for morphological characters (Stem height) and yield (1000-grain weight, medium yield of plant) of the studied variety. The results obtained showed the existence of variability between our thirteen genotypes of barley which had a different resistance level in the quantitative and qualitative characters.

1. INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the oldest cereals cultivated by humans (7000 years B.C.) (Badr et al., 2000). It is in fourth order after wheat, rice and corn (Lammari et al., 2019). With a global production of 150 Mt for a sown area of 57 million hectares, with an average yield of 27 qx/hectare (FAOSTAT, 2020).Barley cultivation is better adapted to the semi-arid areas where it can replace wheat advantageously and give a good yield (Benbelkacem et al., 2000a, 2020b). In Algeria, cereal products occupy a major place in the food system and in the national economy, and barley *Hordeum vulgare* L. is one of the most cultivated cereals with an area of 1.133.005 ha and 1.647.746 tons produced in 2019 (FAOSTAT, 2020). It is an important composent in animal feed and human food. Unfortunately this production does not cover the high demands of the population (Bessaoud et al., 2019). This decrease in barely production is explained by the regression of the sown surfaces; climate changement, and the use of low yielding local varieties sensitive to diseases . Theforeign varieties recently introduced in Algeria, which are supposed to be resistant to diseases and with high yields, but are not adapted to the edapho-climatic conditions of the country (Bendif, 1994; Sayoud et al., 1996); The absence of phytosanitary measures has led to the appearance of new strains of pathogen. The increasing of barley yields can be done by the control of cultural practices, fertilization and phytosanitary treatments, but especially by the search for genotypes with an interesting agronomic potential, resistant to diseases and adapted to the different growing areas. The most frequent fungal diseases on barley crops are: leaf stripe, rhynchosporiosis, bunt and covered smut and powdery mildew. However, the losses caused by these diseases have never been

evaluated in Algeria (Bendif, 1994). The leaf stripe of barley caused by *Pyrenophora graminea* Ito & Kuribayashi is a very frequent disease on barley crops in Algeria, it causes considerable losses (Lammari et al., 2019). The use of resistant varieties remains the most efficient, the most economic and the least polluting method to control this disease (Benbelkacem et al., 2000a, 2000b).Our study is part of a global research program of resistance to leaf stripe; The aim is to evaluate the incidence of *Pyrenophora graminea* on some characters (Stem height,1000-grain weight, Medium yield of plant), after having made an artificial inoculation that permitted us to demonstrate the best resistant genitors and the interesting hybrid populations against this disease.

2. MATERIALS AND METHODS

2.1 Plant material

We used in our experimentation thirteen genotypes of barley (*Hordeum vulgare* L.) from different origins, of which five genotypes represent the parents: ACSAD, Tichedrett, Saida, Rihane, Hamra. Eight hybrid populations of barley of the F8 generation obtained by intra - specific crosses of the parent genitors (Table 1).

Table 1. Origins of the genitors and different hybrid populations of F8

Origins and crossing of genitors	Hybrid populations
ACSAD (Syria) x Tichedrett (Algeria)	AcTi
Saida (Algeria) x Hamra (Algeria)	SaHa
Saida (Algeria) x ACSAD (Syria)	SaAc
ACSAD (Syria) x Hamra (Algeria)	AcHa
Tichedrett (Algeria) x Rihane (Syria)	Ti Ri
Saida (Algeria) x Tichedrett (Algeria)	SaTi
Rihane (Syria) x Saida (Algeria)	RiSa
ACSAD (Syria) x Rihane (Syria)	AcRi

2.2 Fungal material

We tested a strain of *P. graminea*, which was isolated from a leaf limb of barley with typical symptoms of leaf stripe from our experimental stations at ENSA and ITGC. The isolation of this strain was realized at the mycology laboratories of the INPV.

2.3 Fungal isolation

Leaves showing symptoms of leaf stripe were selected and cut into small fragments of 2 cm in length at the level of the symptoms. These fragments were disinfected, rinsed, dried and then placed on a Petri dish containing V8 medium with the following composition:

Sterile distilled water.....800ml
Juice8.....200ml
Agar.....15g
Caco3.....2g

After isolation, the Petri dishes were incubated at 20°C for 10 days (Dickson, 1956); In order to obtain a pure culture, we proceeded to several mycelial transplants on V8 medium to confirm first the presence of conidia typical of *P. graminea* and then several transplants on PDA medium which allow a favorable development of its mycelium.

2.3.1 Fungal inoculation

We opted for the Sandwich method described by Houston et Oswald (1948) and Hammouda (1986), based on the principle that the fungus is seed-transmitted. This method consists of placing barley seeds between two layers of *P. graminea* mycelium to obtain the inoculated specimens. The steps of the method are

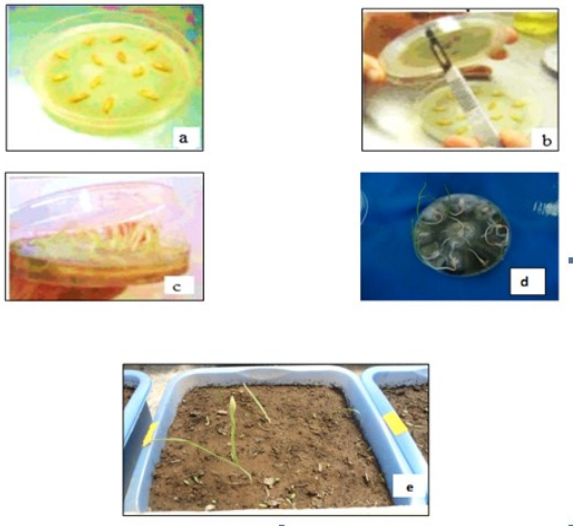

Fig. 1. Inoculation of barley seeds with the mycelium of *Pyrenophora graminea* on PDA medium.
(a) Barley seeds deposited on a PDA medium colonized by the mycelium of *Pyrenophora graminea*. (b) Disposition of barley seeds between the two layers of PDA medium colonized by*Pyrenophora graminea* mycelium. (c) and (d) Germination of barley seeds through the two layers of PDA medium. (e) Terrine in plastic showing the barley plants germinated from inoculated seeds.

described in (Fig. 1.). The dishes containing the Sandwich seeds are closed and then incubated for 72h (3 days) at a temperature of 4C°, According to Nilsson (1976), the inoculation methods used at low incubation temperatures give better results. We prepared the uninoculated controls of the hybrid populations and their genitors by disinfecting the seeds and then putting them between two layers of PDA medium under the incubation conditions.

2.4 Plant cultivation

2.4.1 Preparation of the substrate

The substrate is a mixture of (2/3 soil for 1/3 potting soil). This was previously sterilized in the oven for 24 hours at a temperature of 120°C.

2.4.2 Experimental device

Our experiment was realized in a glass greenhouse on the experimental station of the ENSA. The experimental device adopted is a complete randomized block, this device includes, two factors with three repetitions: genotypes factor and treatment factor which includes two tests, inoculated and not inoculated (Fig. 2.). Each genotype was represented by 5 barley plants.

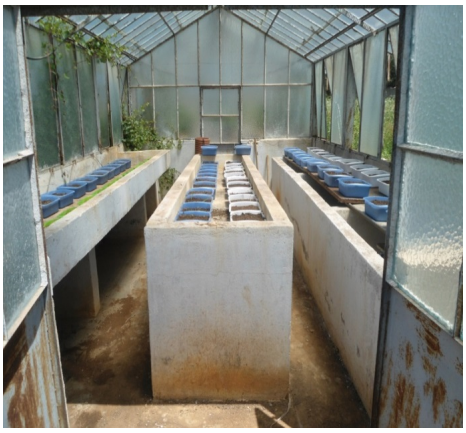

Fig. 2. Experimental device under glass in greenhouse. (From right to left: Block 1; Block 2; Block 3).

2.5. Calculate parameters and data analysis

In order to discuss the results concerning the impact of leaf stripe caused by *P. graminea* on morphological traits (mean Stem Height, SH), and yield components (Thousand Grain Weight, TGW, and average Yield per Plant, Y/P) for all hybrid populations and their genitors. We performed an analysis of variance with the statistical software "STATICF" using the NEWMAN KEULS test at probability level of 5%, the Student-Newman-Keuls means comparison test.

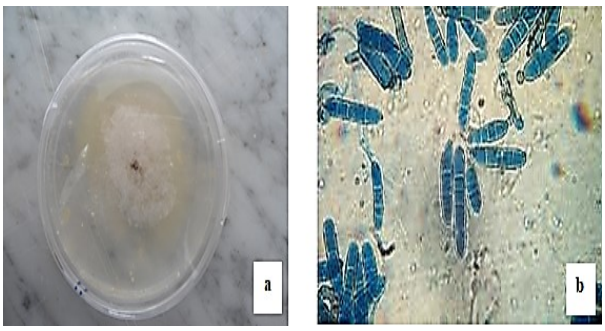

Fig. 3. (a) Aspects of mycelia of the *Pyrenophora graminea* strain on PDA medium. and (b) Conidia morphology (40X).

3. RESULTS AND DISCUSSION

Our strain of *Pyrenophora graminea* exhibited a cottony aspect of colony and a white mycelia on PDA medium.

3.1 Disease incidence on barley varieties

The results of the artificial inoculation of the hybrid populations and their genitors by *P. graminea*, expressed a ratio of infection varied from one genotype to another. The results obtained are represented in (Fig. 4.).

The hybrid populations AcHa and AcRi are classified as resistant compared to other hybrid populations, and the The ACSAD genitor is classified as resistant in comparison to the studied parents genitors ; The AcTi and SaHa hybrid populations are classified as intermediate resistant genotypes; The hybrid populations SaAc, RiSa, TiRi and SaTi are sensitive to the pathogen attack.

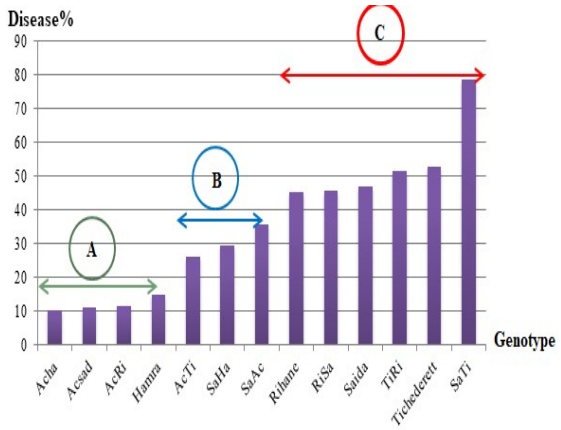

Fig. 4. Classification of different barley genotypes according to the infection ratio caused by *Pyrenophora graminea.*

Table 2. Summary table of the impact of leaf stripe on the set of traits studied

Character	Stem height (cm)	1000-grain weight (g)	Medium yield of plant (g/plant)
Genotype			
ACSAD			
Inoculated	42.56	41.94	2.21
Control	46.71	47.32	2.901
Incidence*	04.15	05.38	0.69
	8.88%	**11.36%**	**23.78%**
Hamra			
Inoculated	44.33	43.75	2.26
Control	47.79	48.53	2.83
Incidence	03.46	04.78	0.57
	7.24%	**9.84%**	**20.14%**
Saida			
Inoculated	38.22	37.33	1.72
Control	45.30	45.58	2.35
Incidence	07.08	08.25	0.63
	15.62%	**18.1%**	**26.80%**
Rihane			
Inoculated	40.36	39.69	1.96
Control	45.70	46.39	2.67
Incidence	05.34	06.70	0.71
	11.68%	**14.44%**	**26.59%**
Tichedrett			
Inoculated	36.10	35.40	2.15
Control	43.96	44.49	3.73
Incidence	07.86	09.09	1.58
	17.87%	**20.43%**	**42.35%**
SaAc			
Inoculated	40.50	40.82	1.93
Control	45.90	46.89	2.56
Incidence	05.40	06.07	0.63
	11.76%	**12.94%**	**24.6%**
SaTi			
Inoculated	38.02	37.54	1.8
ControlContr	45.03	45.90	2.49
ol	07.01	08.36	0.69
Incidence	**15.56%**	**18.21%**	**27.71%**
SaHa			
Inoculated	41.70	41.07	1.90
Control	46.45	47.05	2.58
Incidence	04.75	05.98	0.68
	10.22%	**12.7%**	**26.35%**
AcRi			
Inoculated	42.92	42.43	2.34
Control	47	47.71	2.90
Incidence	04.08	05.28	0.56
	8.68%	**11.06%**	**19.31%**
RiSa			
Inoculated	40.65	40	1.95
Control	46.13	46.71	2.60
Incidence	05.48	06.71	0.65
	11.87%	**14.36%**	**25%**
AcTi			
Inoculated	43.03	42.31	1.77
Control	47.08	47.62	2.46
Incidence	04.05	05.31	0.69
	8.6%	**11.15%**	**28.04%**
TiRi			
Inoculated	40.36	39.82	1.67
Control	46.30	46.47	2.33
Incidence	05.94	06.65	0.66
	12.82%	**14.31%**	**28.32%**
AcHa			
Inoculated	47.22	46.65	1.8
Control	49.34	49.99	2.31
Incidence	02.12	03.34	0.51
	4.29%	**6.68%**	**22.07%**

***Incidence = ((value of the control – Value of the inoculated)/value of the control)*100**

3.2 Incidence of leaf stripe on barley morphological characteristics and yields components.

The objective of this analysis is to evaluate the potential production of the different hybrid barley populations and their parents, as well as their resistance to *P. graminea* . The measures and weights obtained for all the characteristics for each genotype are shown in (Table 2). Stem height) and yield (1000-grain weight, Medium yield of plant).

3.2.1 Incidence of leaf stripe on medium stem height (SH)

The results of the analysis of variance for the two inoculated and uninoculated (control) treatments are shown in the tables below:

Table 3. Analysis variance. of stem height in *Pyrenophora graminea* inoculated plants of barley cultivars

	S.S.D.	DF	MS	F-test	PROBA	S.D C.V.
TOTAL VAR.	438.91	38	11.55			
FACTOR1 VAR.	304.62	12	25.38	4.95	0.0005	
BLOCK VAR.	11.33	2	5.67	1.11	0.3482	
RESIDUAL 1 VAR.	122.96	24	5.12	2.26	5.5%	
INTERACTION	SSD. TUKEY 's test = 57.68			PROBA = 0.0002		

Table 4. Analysis variance of Stem height in control plants of barley cultivars

	S.S.D.	DF	MS	F-test	PROBA	S.D C.V.
TOTAL VAR.	175.46	38	4.62			
FACTOR1 VAR.	64.13	12	5.34	1.46	0.2072	
BLOCK VAR.	23.47	2	11.74	3.21	0.0572	
RESIDUAL 1 VAR.	87.86	24	3.66		1.91	4.1%
INTERACTION	SSD. TUKEY 's test =		0.06	PROBA =	0.8938	

Based on the analysis variance about the effect of leaf stripe on the Stem height trait; We can say that there is a very highly significant difference for the inoculated treatment (Table 3), while it revealed no significant difference for the control (uninoculated) treatment (Table 4).

According to the summary in Table 2, the genitors Hamra and ACSAD presented moderate

values of stem height and their hybrid population AcHa inoculated with *P. graminea* expressed the highest medium stem height in the order of 47.22 cm, the uninoculated treatment of the same hybrid population gave a value of 49,34cm, the leaf stripe had a low incidence on the stem height character of the genotype AcHa in the order of 4,29%. While the Tichederett progenitor expressed the highest incidence of leaf stripe with a value of 17.87% (Fig. 5.). The Newman and Keuls test was used to classify the barley genotypes into 3 homogeneous groups.

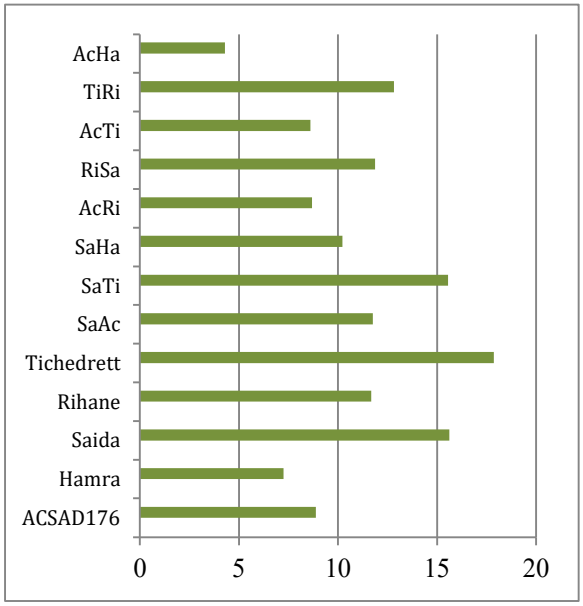

Fig. 5. Classification of genotypes according to the incidence (%) of leaf stripe on the stem height (SH) character.

Table 5. Analysis variance of thousand grain weight in *Pyrenophora graminea* inoculated plants of barley cultivars.

	S.S.D.	DF	MS	F-test	PROBA	S.D	C.V.
TOTAL VAR.	443.55	38	11.67				
FACTOR1 VAR.	310.77	12	25.90	4.86	0.0005		
BLOCK VAR.	4.96	2	2.48	0.47	0.6358		
RESIDUAL 1 VAR.	127.82	24	5.3			2.31	5.7%
INTERACTION	SSD. TUKEY 's test =		0.87	PROBA =		0.6965	

Table 6. Analysis variance of thousand grain weight in control plants of barley cultivars.

	S.S.D.	DF	MS	F-test	PROBA	S.D	C.V.
TOTAL VAR.	173.93	38	4.58				
FACTOR1 VAR.	67.73	12	5.64	1.40	0.2324		
BLOCK VAR.	9.43	2	4.72	1.17	0.3281		
RESIDUAL 1 VAR.	96.76	24	4.03			2.01	4.3%
INTERACTION	SSD. TUKEY 's test =		1.52	PROBA =		0.5570	

3.2.2 Incidence of leaf stripe on thousand grain weight (TGW)

The results of the analysis of variance for the two inoculated and uninoculated (control) treatments are shown in the tables below:
Analysis of variance showed a significant difference about the effect of leaf stripe on Thousand Grains Weight (TGW) between inoculated (Table 5) and not inoculated (control) treatment (Table 6).

As shown in the summary of Table 2, the Hamra progenitor has a high value in the order of 43.75g for the inoculated treatment and 48.53g for the uninoculated treatment and the very low incidence of 9.84%. The hybrid population AcHa presents a higher TGW of 46.65g for the inoculated treatment and 49.99g for the control treatment with a very low incidence of 6.68%. The hybrid population SaTi and the variety Tichederett present a very low value for the control treatment of 45.9g and 44.49g respectively, with a very high incidence of 18.1% and 20.43% respectively. The Newman and Keuls test was used to classify the inoculated treatment barley genotypes into 5 homogeneous groups.

3.2.3 Incidence of leaf stripe on medium of Yield per Plant (Y/P)

The results of the analysis variance for the two inoculated and uninoculated (control) treatments are shown in the tables below:

Table 7. Analysis variance of yield per plant in *Pyrenophora graminea* inoculated plants of barley cultivars

	S.S.D.	DF	MS	F-test	PROBA	S.D	C.V.
TOTAL VAR.	13.17	38	0.35				
FACTOR1 VAR.	1.65	12	0.14	0.35	0.9684		
BLOCK VAR.	2.13	2	1.07	2.72	0.0844		
RESIDUAL 1 VAR.	9.39	24	0.39			0.63	31.8%
INTERACTION	SSD. TUKEY 's test =		0.06	PROBA =		0.7086	

Table 8. Analysis variance of yield per plant in control plants of barley cultivars.

	S.S.D.	DF	MS	F-test	PROBA	S.D	C.V.
TOTAL VAR.	21.61	38	0.57				
FACTOR1 VAR.	4.97	12	0.41	0.68	0.7500		
BLOCK VAR.	2.12	2	1.06	1.75	0.1937		
RESIDUAL 1 VAR.	14.52	24	0.60			0.78	29.2%
INTERACTION	SSD. TUKEY 's test =		0.42	PROBA =		0.4227	

The analysis variance of the leaf stripe on medium yield per plant showed a significant difference between inoculated (Table 7) and uninoculated (control) treatment (Table 8). According to the summary Table 2, the AcRi hybrid population presents the highest value about 2.9 g/p for the uninoculated (control) treatment and 2.34 g/p for the inoculated treatment. The incidence observed on this

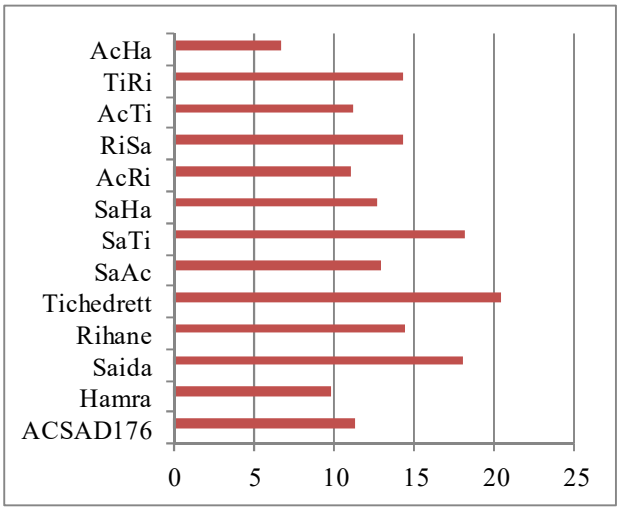

Fig. 6. Classification of genotypes according to the incidence (%) of leaf stripe on the thousand grain weight (TGW) character.

Fig. 7. Classification of genotypes according to the incidence (%) of leaf stripe on yield per plant (Y/P) character.

genotype is very low at 19.31% while the Tichederett genotype has the highest incidence with a value of 42.35% for the Y/P trait. The Newman and Keuls test carried out at the 5% probability threshold on the basis of the number of grains per plant made it possible to divide the barley genotypes into 1 homogeneous group (A).

The study of *Pyrenophora graminea* impact's on the quantitative and qualitative characteristics for the different barley genotypes, shows a variability within cultivars and between cultivars, as well as a homogeneity and stability in some genotypes, the variability is due to the influence of the genetic potentials on the expression of the characteristics during the test period. The stability of the characters is in general quite clear in the qualitative characters of the parent genotypes towards the hybrids (Rahal–Bouziane, 2006). The development of leaf stripe is very well remarkable on sensitive barley varieties, this showing the efficacy of the inoculation method that was used, and the interaction "variety-isolate" confirmed the hypothesis of physiological specialization in *Pyrenophora graminea* reported by many authors (Benbelkacem et al., 2000a, 2000b; Benkorteby-Lyazid, 2019), and the levels of incidence on some varieties explain the sensibility of Algerian barley cultivars contrary to imported cultivars and prove the resistance of this pathogen on the local hosts. The large variations observed between replications can be explained by the fact that some seeds were highly infested by large amounts of inoculum and some lost their seed coat during the tempering process, this result confirms the observation made by Mohammad and Mahmood (1974).

In the same way, the incidence of the leaf stripe disease on the Algerian varieties: Saida and Tichedrett revealed an excessive sensitivity compared to that of the systematic investigation made during campaign 1994/1995 on 226 fields through the main cereal zones with an average incidence of 27.97% of this disease; These data allowed to estimate losses about 1/3 of the potential production of barley in Algeria (Benbelkacem et al., 2000a, 2000b). Losses result from a reduction in tillering and yield per plant where the grains of affected plants are empty or rarely fill (Mathur et al., 1964). 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 cultivars : ACSAD, Hamra, and hybrides : AcHa, AcRi , showed a high yield/plant and a high thousand grain weight. Arabi et al. (2001) showed that *P. graminea* had a direct impact on proteins in grain content susceptible cultivars, where no effects were detected in the resistant ones. In our study, the resistance of Syrian cultivars are mostly the same to those reported by Arabi et al.

(2001). Therefore, the cultivars ACSAD and Hamra should be integrated in breeding studies for introducing leaf stripe disease resistance into most cultivated and high yielding varieties in Algeria.

4. CONCLUSION

In the aim of improving barley resistance against the leaf stripe disease, it is important to study the reaction of barley genotypes to *Pyrenophora graminea* infection. This study showed that ACSAD and Hamra progenitors are characterized by a high genetic potential for resistance against this disease. Their hybrid populations in turn have a good genetic potential. On the other hand, the Saida and Tichedrett progenitors are characterized by a low genetic potential which makes them susceptible to leaf stripe. The hybrid populations AcHa, SaHa and AcRi are the most interesting for the characteristics of stem height (SH), the weight of thousand grain (TGW) and yield per plant (Y/P).

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