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Identification of Tunisian Barley Lines Tolerant to Both Net Botch and Scald in the Adult Stage

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ABSTRACT

Net blotch and scald are the two prevalent foliar diseases of barley (*Hordeum vulgare* L.) in Tunisia, causing significant losses in yield and quality of seeds. Their impact can be reduced with the identification and use of effective sources of resistance. In order to identify genotypes with double resistance to net blotch and scald, 91 inbred barley lines derived from crosses between susceptible cultivars to net blotch were screened at the adult growth stage in the field. Net blotch and scald reactions were evaluated two times using the mass disease index (MDI). The least significant difference (LSD) test showed that 62.1 and 37.8% of the screened total lines had the highest level of resistance to both net blotch and scald for the first and the second assessments, respectively. Furthermore, the evaluated lines were partitioned into four groups according to their simultaneous reaction to both diseases. For the first assessment, 45 lines were considered to be resistant to both diseases with MDIs lower than 7.5 and 1.16% for net blotch and scald, respectively. For the second assessment, 40 lines were resistant to both *Pyrenophora teres* and *Rhynchosporium secalis*, with. MDIs lower than 20.95% for *P. teres* and 3.34% for *R. secalis*. The two methods used in this investigation are efficient to select barely inbred lines tolerant to both diseases. The selected lines could be used in plant breeding programmes for disease resistance after validation of their resistance.

Keywords: double resistance, *Hordeum vulgare*, *Pyrenophora teres*, *Rhynchosporium secalis*
Abbreviations: ESA-Mograne, Ecole Supérieure Agricole de Mograne; **INAT**, Institut National Agronomique de Tunisie; **LSD**, least significant difference; **MDI**, mass disease index

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the second most important cereal in Tunisia after wheat. It covers around 500,000 ha annually representing around 33% of the total cereal area. Barley is used mainly for animal feeding as grain, straw or grazed as forage early in the season, as well as for direct human consumption. However, cultivated varieties are susceptible to several pathogens which are responsible for losses of the yield and the quality of the grain harvested (Yahyaoui 2003; Feriani *et al.* 2007). Net blotch, incited by *Pyrenophora teres* [(Died.) Drechsl.] and scald, caused by *Rhynchosporium secalis* [(Oudem.) J.J. Davis], are the two most important foliar diseases of barley in Tunisia (Cherif *et al.* 1994) that are associated with high severity levels (70-80%) in some regions particularly during favorable weather conditions (pers. obs.).

Among the various strategies to manage crop diseases, disease resistance is of immense practical importance. Use of cultivars with an acceptable level of resistance to major diseases is considered as the best approach, since it can reduce or eliminate the expense and the effects of other chemical, physical, biological, cultural and regulatory control methods. Plant breeders have explored germplasm collections and wild species as sources of favorable alleles for continued crop improvement. Sources of resistance to net blotch (Robinson and Jalli 1997; Afanasenko *et al.* 2004; Silvar *et al.* 2009) and scald (Bjørnstad *et al.* 2002; Genger *et al.* 2005; Silvar *et al.* 2009) are well documented. Most of these germplasms were identified under controlled conditions in the greenhouse or in the field. Little information is available about the existence of double resistance to both diseases, although they are commonly present on barley leaves and are frequently observed on the same plant in a field (Xue *et al.* 1994). In a previous study, Cherif *et al.* (2007) identified some doubled-haploid lines that showed a high level of adult plant resistance to both net blotch and scald diseases in the field. The selected lines could be used in a breeding program to control simultaneously these two foliar diseases. However, the high variability of Tunisian pathotypes of *P. teres* (Harrabi and Kamel 1990) and *R. secalis* (Bouajila *et al*. 2006) require continuous selection of new sources of double disease resistance under diverse epidemic conditions. The goal of this study was to evaluate a collection of inbred barley lines in order to identify genotypes with double resistance to net blotch and scald in the adult growth stage.

MATERIALS AND METHODS

Plant material used in this study consisted of 91 (52 six-rowed and 39 two-rowed) spring inbred barley lines. These lines were obtained from crosses carried out at INAT (Institut National Agronomique de Tunisie) between cultivars chosen on the basis of their yielding ability, good adaptation to the Mediterranean climate and susceptibility to net blotch. The Tunisian commercial cultivars 'Martin', 'Manel', 'Rihane' and 'Roho' were used as susceptible checks.

All barley lines were evaluated for their resistance to net blotch and scald at the research farm of ESA-Mograne (Ecole Supérieure Agricole de Mograne) localized in a particularly hot spot area for net blotch and scald diseases. Barley lines were sown in two rows on 20 November, 2009 using a randomized block design with three replications. Row-lines were 2 m long and spaced 0.3 m apart. The trial was conducted following optimal cultural practices, but without applying fungicides. Artificial inoculations of *P. teres* and *R. secalis* have been achieved at the mid-tillering stage of growth (GS 22-26) (Zadoks *et al.* 1974) using infected barley seeds with a mixture of local isolates of each of the two pathogens prepared according to Onfroy (1997).

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Research Note

Net blotch and scald symptoms on the foliage were recorded using levels of disease incidence (percentage of plants having at least one lesion) and severity (average percentage of leaf area affected for the whole plant) on 10 randomly selected plants per line. The two diseases appeared at the start of the ear emergence stage; thus assessments were made two times: at the ear emergence (GS 53-58) and at the flowering (GS 61-65) stage. All data were transformed onto mass disease index (MDI) following the method of Ding *et al*. (1993):

MDI = (DI x DS) / 100

where, DI is the disease incidence and DS is the disease severity.

In order to identify resistant lines to both net blotch and scald, the MDI values of all the lines were compared with the respective mean values of the whole population. Genotypes with MDI values superior or inferior to the mean of the population were considered susceptible or resistant respectively. Thus, four groups were obtained – group I: lines were susceptible to both net blotch and scald; group II: lines were resistant to net blotch and susceptible to scald; group III: lines were resistant to both net blotch and scald; and group IV: lines were susceptible to net blotch and resistant to scald. Moreover, to compare lines for the percent case of diseased leaf tissue area infected by net blotch and scald, an analysis of variance was performed for MDI of the sum net blotch + scald using PROC ANOVA (SAS Institute 1988). Lines were separated for the MDI of the sum net blotch + scald using the least significant difference (LSD) test at a probability level of 0.05.

RESULTS AND DISCUSSION

Variation in the distribution of rainfall and temperatures was observed between September and May of the 2009–2010 season. During this cropping season, total rainfall was 362 mm and the temperature varied from 7 to 30°C. Thirty nine percent (39%) of total precipitation was recorded in March and April allowing the expression and the propagation of net blotch and scald on different foliar stages. In fact, the development of net blotch and scald were higher for the second assessment than for the first one. During the first assessment, the average MDIs for net blotch and scald were 7.5 and 1.2%, respectively whereas during the second assessment, they were 20.9 and 3.3%, respectively. In Canada, net blotch severity varied from 2.8 to 4.8 and scald severity varied from 0.9 to 4.0 using a rating scale of 0 to 9 (Xue *et al.* 1994; Orr and Turkington 2005; Xue *et al.* 2005). In Norway, net blotch and scald severities were 13.3 and 7.5%, respectively (Elen 2003) and in Denmark, they reached up to 35 and 20%, respectively (Vollmer *et al.* 2005). In Australia, net blotch was present in 90% of crops with severity ranging from 0 to 42% and scald was found in 23% of crops with severity ranging from 0 to 16% (McLean *et al.* 2009).

Table 1 shows a significant difference between block ($P<0.05$) only for the first evaluation. A significant ($P<0.05$) and a highly significant ($P<0.01$) variation among the 95 barley genotypes were observed for MDI of the sum net blotch + scald calculated for the first and the second assessments, respectively.

Barely lines were compared for the percent diseased leaf tissue area infected by net blotch and scald. **Table 2** shows the most tolerant lines for the sum net blotch + scald using MDI_1 (MDI calculated during the first assessment) and MDI_2 (MDI calculated during the second assessment). For the first evaluation, 62.1% of total lines showed the highest level of resistance for both diseases. These lines revealed a MDI of sum net blotch + scald lower than 10.6%. For the second evaluation, the most tolerant genotypes for percent leaf tissue area infected by net blotch and scald represent only 37.8% of the total screened lines with a MDI of sum net blotch + scald lower than 20%. It seems that the number of tolerant lines depend on the level of attack by the two diseases. In fact, higher number of tolerant genotypes was observed in low epidemic conditions. Cherif *et al*. (2007) identified a greater number of tolerant genotypes in low epidemic conditions than in high epidemic conditions.

Table 1 Mean squares of mass disease index of the sum net blotch + scald for the first and the second assessments.

Source of variation	df	MDI_1[a]	MDI_2[b]
Bloc	2	649.95**	299.53^{ns}
Genotype	94	57.17*	340.35**
Error	188	38.41	102.72
CV (%)		71.45	41.70

[a] MDI_1 : mass disease index of the sum net blotch + scald for the first assessment
[b] MDI_2 : mass disease index of the sum net blotch + scald for the second assessment
**, *, ^{ns} Significant at $P<0.01$ and at $P<0.05$, and not significant at $P<0.05$

Table 2 The most resistant lines for mass disease index of the sum net blotch + scald for the first and the second assessments.

MDI_1[a]				MDI_2[b]	
Genotype	**Mean**	**Genotype**	**Mean**	**Genotype**	**Mean**
'Roho'	0.76	58	6.95	'Roho'	3.16
91	2.12	38	6.99	78	8.08
36	2.34	94	6.99	65	8.16
78	2.36	90	7.06	55	10.18
'Rihane'	2.73	17	7.20	47	10.26
64	2.79	39	7.26	79	10.66
'Manel'	3.18	63	7.37	43	10.93
11	3.33	'Martin'	7.40	34	10.96
41	3.57	83	7.54	69	11.16
34	3.67	62	7.55	60	11.50
55	3.68	68	7.59	12	11.90
43	3.83	77	7.63	84	12.02
69	4.26	22	7.74	'Manel'	12.49
1	4.29	29	8.20	'Rihane'	12.50
19	4.40	56	8.32	19	12.57
49	4.71	2	8.41	1	13.05
95	4.82	7	8.63	50	13.16
13	4.85	81	8.69	49	14.16
65	4.88	87	8.7	95	14.48
33	4.93	30	8.96	64	14.73
5	4.99	72	9.14	63	14.91
35	5.14	46	9.52	77	15.19
12	5.20	48	9.99	36	15.50
47	5.25	26	10.02	'Martin'	15.71
88	5.37	59	10.09	94	16.21
60	5.42	9	10.16	91	16.44
61	5.42	21	10.19	58	16.48
6	5.55	8	10.28	83	16.49
51	5.92	32	10.31	2	17.17
92	6.05	53	10.35	22	17.24
4	6.32	57	10.42	70	18.16
42	6.33	76	10.54	57	18.42
79	6.36			16	19.13
50	6.42			33	19.16
16	6.75			29	19.23
84	6.76			61	19.45
23	6.81			72	19.48
LSD = 9.98				LSD= 16.32	

[a] MDI_1 : mass disease index of the sum net blotch + scald for the first assessment
[b] MDI_2 : mass disease index of the sum net blotch + scald for the second assessment

The four Tunisian commercial cultivars 'Martin', 'Manel', 'Rihane' and 'Roho' were classified as resistant for the two evaluations using the sum of the two diseases at the adult growth stage. Among these cultivars, 'Roho' was the most resistant to the sum of the two diseases with the smallest attack of foliar area (0.76 and 3.16% for the first and the second assessments, respectively). The resistance observed for the susceptible checks could be explained by the low disease level which did not exceed 10% for the first assessment and 20% for the second, which is associated with a high experimental error especially edging effect. These cultivars were classified as susceptible genotypes by Cherif *et al.* (2007) during two cropping seasons.

Furthermore, the 95 lines were partitioned into four groups by comparing their MDI values for both diseases with the respective mean values of the whole population Resistant lines to both diseases represent group III for

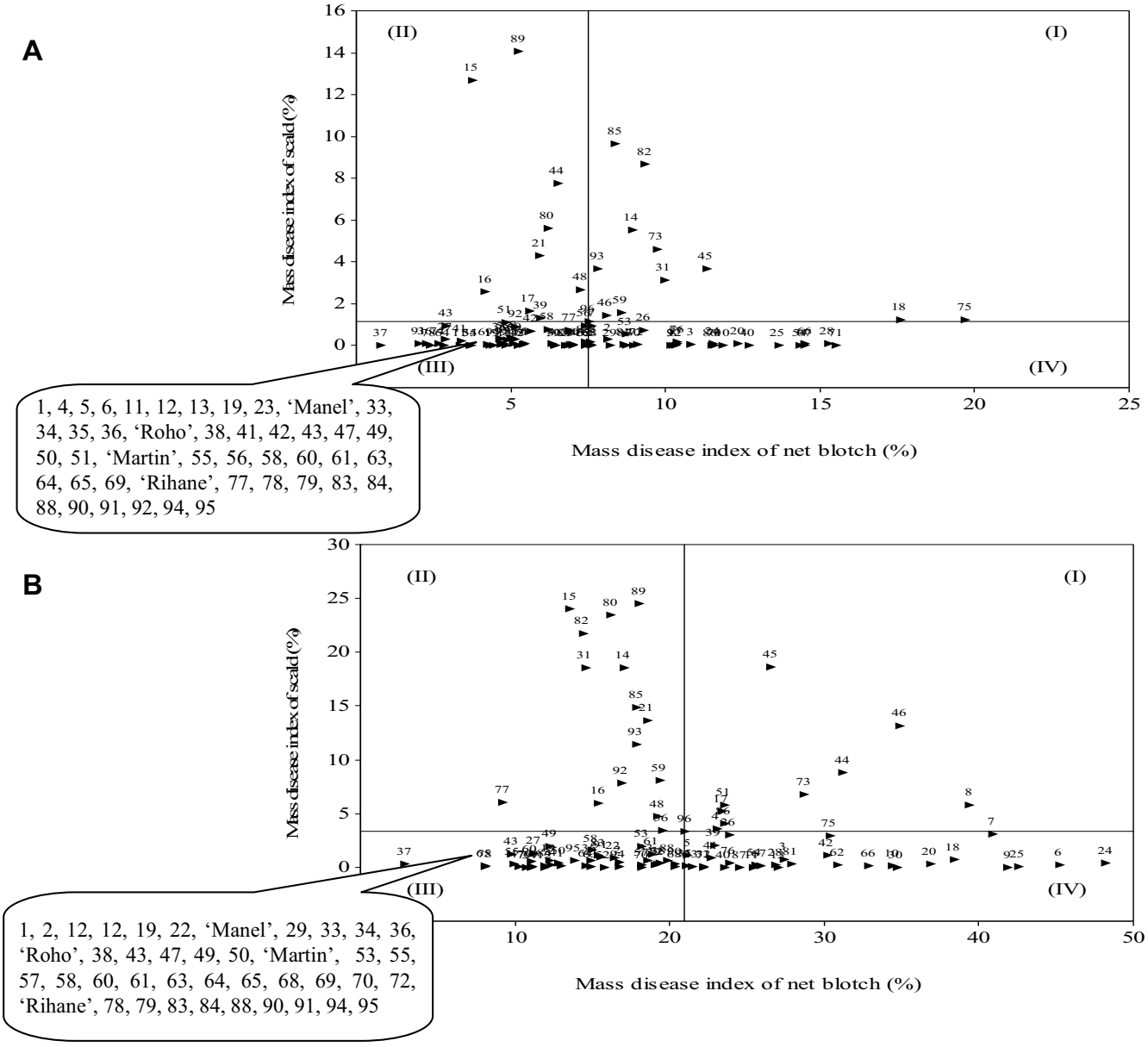

Fig. 1 Association between mass disease index of net blotch and scald evaluated at the level of the whole plant under field conditions. (**A**) Association between net blotch and scald mass disease index evaluated at the ear emergence growth stage. (**B**) Association between net blotch and scald mass disease index evaluated at the flowering growth stage. (I) Set of lines that are susceptible to both net blotch and scald. (II) Set of lines that are resistant to net blotch and susceptible to scald. (III) Set of lines that are resistant to both net blotch and scald. (IV) Set of lines that are susceptible to net blotch and resistant to scald.

MDI_1 and MDI_2 (**Fig. 1**). For the first evaluation, 45 lines (1, 4, 5, 6, 11, 12, 13, 19, 23, 'Manel', 33, 34, 35, 36, 'Roho', 38, 41, 42, 43, 47, 49, 50, 51, 'Martin', 55, 56, 58, 60, 61, 63, 64, 65, 69, 'Rihane', 77, 78, 79, 83, 84, 88, 90, 91, 92, 94, 95) were considered resistant to both net blotch and scald. All these genotypes exhibited MDIs lower than 7.5 and 1.2% for net blotch and scald, respectively. Discrimination between these lines is difficult as their reactions to diseases were very close. For the second assessment, 40 lines (1, 2, 12, 19, 22, 'Manel', 29, 33, 34, 36, 'Roho', 38, 43, 47,49, 50, 'Martin', 53, 55, 57, 58, 60, 61, 63, 64, 65, 68, 69, 70, 72, 'Rihane', 78, 79, 83, 84, 88, 90, 91, 94, 95) were resistant to both *P. teres* and *R. secalis*. All these genotypes had MDIs lower than 20.9% for *P. teres*, and lower than 3.3% for *R. secalis*. On the basis of these results, lines 1, 12, 19, 'Manel', 33, 34, 36, 'Roho', 38, 43, 47, 49, 50, 'Martin', 55, 58, 60, 61, 63, 64, 65, 69, 'Rihane', 78, 79, 83, 84, 88, 90, 91, 94 and 95 showed the highest level of adult plant resistance to both diseases and for the two evaluations.

The two methods used in this investigation are efficient to select barely inbred lines tolerant to both net blotch and scald diseases. Results obtained by the two methods were usually in agreement. However, some genotypes were significantly the most resistant on the basis of the LSD test (as lines 16, 76, 57 for the first evaluation and 16, 77 for the second one) were not classified into group III. Furthermore, other genotypes (as lines 38, 43, 49 for the second evaluation) classified in group III were not the most resistant on the basis of the LSD test.

The present study indicates that several inbred lines exhibited better level of net blotch and scald resistance than 'Rihane' which is the most cultivated variety in field crops. The identified lines could be used in plant breeding programmes for disease resistance. The double resistance obtained should be validated in different geographic areas and in controlled conditions using highly virulent *P. teres* and *R. secalis* isolates.

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