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Preliminary Genetic Analysis of Resistance to Scald in Tunisian Barley

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ABSTRACT

Leaf scald (*Rhynchosporium secalis*) of barley (*Hordeum vulgare* L.) is a serious disease in many barley-growing areas in Tunisia. A doubled-haploid barley population from a cross between the two-rowed cultivar 'Roho' and the six-rowed line '90' was evaluated for its resistance at the seedling stage to three isolates (Bousalem2, Krib5 and Teboursouk4) of *R. secalis*. The obtained frequency distributions suggest that resistance to Bousalem2 and Teboursouk4 isolates were polygenic whereas resistance to Krib5 isolate seems to be governed by two complementary genes. Heritability of the resistance to all isolates was relatively high (64%), although the genotype × isolate interaction effects were significant. Analysis of variance indicated that scald resistance was not dependent on row type. Three QTLs localised on chromosomes 3H, 4H and 6H were identified by the individual marker analysis with the Marker × Isolate interaction model.

Keywords: doubled-haploid, genetic of resistance, quantitative trait loci, *Rhynchosporium secalis*

Abbreviations: AFLP, Amplified Fragment Length Polymorphism; **DH**, doubled-haploid; **LSD**, least significant difference; **QTL**, quantitative trait loci; **SSR**, Simple Sequence Repeat

INTRODUCTION

In Tunisia, barley (*Hordeum vulgare* L.) is the second cultivated cereal crop after durum wheat. It is primarily used for animal feed, secondly for malt, followed by human consumption. However, this crop is usually subjected to several biotic constraints that affect its cultivation and productivity (Najar *et al*. 2010). One of the most important foliar diseases of barley is scald, also known as leaf blotch, which is caused by *Rhynchosporium secalis* (Oudem.) J.J. Davis (Genger *et al.* 2003; Dizkirici *et al.* 2010). This pathogen is responsible for yield losses and quality of the grain harvested (Yahyaoui 2003; Feriani *et al.* 2007). Yield losses are about 30% and could reach 70% in epidemic years (Bouajila *et al.* 2007). Intensification of barley cultivation and the absence of resistant cultivars are among the most important factors favoring scald incidence. Thus, breeding for disease resistance is an aim for controlling this disease. Many sources of resistance to scald have been identified, especially from the Middle East. In fact, landraces from this region have already proven to be a very fruitful source of genes for scald resistance (Ellis *et al.* 2000). Resistance of barley to *R. secalis* is often controlled by a gene-for-gene interaction (Zhan *et al*. 2007). This race-specific resistance has not proved to be durable because of the high genetic variability of *R. secalis* (Genger *et al*. 2005). Thus, another form of barley resistance to scald was identified which was shown to be polygenically inherited (Cselenyi *et al*. 1998). Studies on the inheritance of resistance to *R. secalis* in barley led to the mapping of several major and minor resistance genes. At least 16 different resistance genes have been reported (Abbott *et al*. 1992, 1995; Garvin *et al*. 1997). Furthermore, the rise of molecular markers has facilitated the identification and localization of loci for quantitative traits (QTLs) (Celeste *et al.* 2005). Several QTLs for scald resistance have been mapped on chromosomes 2H, 3H, 4H, 6H and 7H whose loci often coincided with locations of known scald resistance genes (Bjørnstad *et al*. 2002; Genger *et al*. 2003; Bjørnstad *et al*. 2004; Von Korff *et al.* 2004; Cheong *et al*. 2006; Wagner *et al.* 2008; Li and Zhou 2011).

The objectives of this study were to investigate genetic of scald resistance in barley and to identify QTLs associated with this resistance in the seedling stage using a doubled-haploid (DH) population.

MATERIALS AND METHODS

Plant material

Plant material used in this study consisted of 59 DH barley lines that were produced by the seed coop 'Florimond Desprez Veuve and Fils France', from F_1 plants of the cross 'Roho' x '90'. 'Roho' is a two-rowed Tunisian cultivar introduced by the International Center for Agricultural Research in the Dry Areas (ICARDA) which is susceptible to the most important foliar diseases. '90' is a six-rowed line selected from a Tunisian national breeding program for its resistance to net blotch in the field. The original cross was made at INAT (Institut National Agronomique de Tunisie) to carry out genetic study.

Pathogen isolates and inoculum preparation

Fresh leaves with symptoms of the scald disease were collected from the Tunisian sites: Béja, Bousalem, Kef, Krib and Teboursouk. The fungus was cultivated and monosporal cultures were generated on lima bean agar medium at 17°C in the dark for 15-17 days. Inoculum was prepared by scraping conidia from fungal cultures using a small volume of distilled water. The suspensions of conidia were filtered through double layers of gauze and the concentration was adjusted to 10^6 spores/ml. Tween 20 was added as one drop/100 ml of inoculum suspension.

Disease assessment

Five seeds of each of the two parents 'Roho' and '90' were planted in small pot (7 cm diameter) and they were grown in a growth chamber maintained at 20-22°C and a 16-h photoperiod with a

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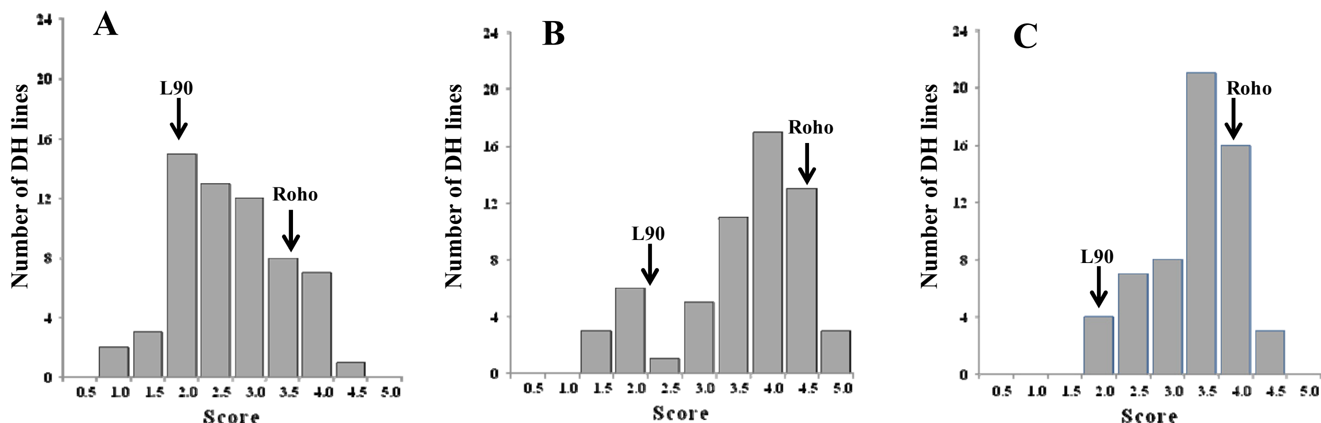

Fig. 1 Frequency distributions of scald reaction among the 59 DH lines derived from the cross 'Roho' x '90' evaluated in the seedling stage for (**A**) Bousalem2 isolate, (**B**) Krib5 isolate and (**C**) Teboursouk4 isolate. Downward point arrows indicate means for the parents 'Roho' and '90'.

photosynthetically active radiation of about 350 μmol/m^2/s. The Tunisian commercial cultivar 'Rihane' was grown as a susceptible check (Bouajila *et al.* 2007). Fifteen days after sowing, seedlings were inoculated separately with fifteen monosporal isolates of *R. secalis*: Béja1, Béja2, Bousalem1, Bousalem2, Kef, Krib1, Krib2, Krib3, Krib4, Krib5, Teboursouk1, Teboursouk2, Teboursouk3, Teboursouk4 and Teboursouk5. Inoculum was applied with an atomizer until the plants were uniformly wet. Inoculated plants were incubated in a mist chamber at 100% RH at 17°C for 48 h and then returned to the growth chamber. The infection responses of barley seedlings to *R. secalis* were scored on the second leaf 14 days after inoculation using the qualitative 0 to 5 scale of Salamati and Tronsmo (1997).

Five seeds of each DH lines were planted in a plastic trays (60 × 40 × 4 cm) using a completely randomized design with three replications. The trays were placed in a growth chamber with the same conditions as the parents. Fifteen days after sowing, seedlings were inoculated separately with three isolates of *R secalis* which showed differential responses on parents. The DH lines were evaluated under the same conditions of inoculation and scoring of scald disease as the parents.

Statistical analysis

The frequency distributions were generated using Excel 2007.

The data were primarily submitted to an analysis of variance by isolate using PROC ANOVA (SAS Institute 1988) to highlight the differences between the DH. The least significant difference (LSD 0.05) was used to compare DH lines reaction with parents reaction and then to identify transgressive lines. Likewise, a two-way ANOVA was carried out to estimate the isolate, the genotype and the isolate × genotype interaction effects.

Broad sense heritability (H^2) for scald resistance to all isolates was estimated from variance components using the following formula:

$$H^2 = \sigma^2 g / (\sigma^2 g + \sigma^2 gi/I + \sigma^2 \varepsilon/IR)$$

where σ^2g, σ^2gi, and $\sigma^2\varepsilon$ are the genotypic variance, the genotype × isolate interaction variance and the residual variance, respectively. I and R are number of isolates and replicates, respectively.

The effect of the spike type on scald resistance was studied by comparing the reaction against *R. secalis* of the two classes of barley (two rows and six rows). For this purpose, an analysis of variance was carried out using PROC GLM (SAS Institute 1988) with the LSMEANS option to compare the between-row type against the within-row type mean squares (Choo 1983).

For QTL detection, we used the segregation data of 34 SSR markers of the same DH population (Cherif 2009). An individual marker analysis using the combined Marker × Isolate model was conducted for all isolates of *R. secalis*. A significant marker effect ($P < 0.005$) indicates that a QTL closely linked to that marker influences the scald resistance. First, the proportion of phenotypic variation explained by the QTL was estimated by the value of the coefficient of determination R^2. Then, main additive effects and additive effect × isolate which are the mean effects of substituting one allele of 'Roho' by an allele of the line '90 ' were determined.

Table 1 Scald reaction of the barley parents 'Roho' and '90' and the check cultivar 'Rihane'scored on the second leaf 14 days after inoculation for 15 isolates of *R secalis* on the qualitative 0 to 5 scale.

Isolate	Parent and check cultivar	Infection response
Béja1	'Roho'	1.50
	'90'	1.70
	'Rihane'	2.44
Béja2	'Roho'	1.62
	'90'	1.40
	'Rihane'	1.83
Bousalem1	'Roho'	4.10
	'90'	3.07
	'Rihane'	3.57
Bousalem2	'Roho'	3.73
	'90'	1.03
	'Rihane'	3.71
Kef	'Roho'	3.06
	'90'	1.70
	'Rihane'	3.33
Krib1	'Roho'	3.15
	'90'	1.70
	'Rihane'	3.80
Krib2	'Roho'	2.80
	'90'	1.55
	'Rihane'	2.80
Krib3	'Roho'	2.81
	'90'	1.12
	'Rihane'	2.00
Krib4	'Roho'	3.06
	'90'	1.67
	'Rihane'	3.71
Krib5	'Roho'	4.30
	'90'	1.20
	'Rihane'	4.43
Teboursouk1	'Roho'	3.41
	'90'	1.82
	'Rihane'	3.43
Teboursouk2	'Roho'	2.92
	'90'	1.16
	'Rihane'	2.6
Teboursouk3	'Roho'	2.52
	'90'	2.00
	'Rihane'	2.4
Teboursouk4	'Roho'	3.80
	'90'	1.65
	'Rihane'	3.2
Teboursouk5	'Roho'	3.30
	'90'	1.92
	'Rihane'	2.8

Table 2 The most resistant and the most sensitive doubled haploid lines compared with parents for their reaction to three isolates of scald using the LSD test.

Bousalem2 Isolate				Krib5 Isolate				Teboursouk4 Isolate			
The most resistant DH lines / the resistant parent	**Reaction**	**The most sensitive DH lines / the sensitive parent**	**Reaction**	**The most resistant DH lines / the resistant parent**	**Reaction**	**The most sensitive DH lines / the sensitive parent**	**Reaction**	**The most resistant DH lines / the resistant parent**	**Reaction**	**The most sensitive DH lines / the sensitive parent**	**Reaction**
18	0.72a	**'Roho'**	**3.14e**	16	1.02a	**'Roho'**	**4.26f**	18	1.66a	**'Roho'**	**3.52k**
42	0.81a	26	3.33e	18	1.27a	51	4.33f	**'90'**	**1.68a**	35	3.53k
40	1.25a	58	3.48e	17	1.33a	59	4.40f			43	3.56k
34	1.36a	33	3.50e	22	1.55a	5	4.40f			49	3.58k
28	1.45a	24	3.50e	4	1.63a	26	4.43f			23	3.60k
52	1.54a	11	3.55e	45	1.66a	50	4.45f			27	3.64k
12	1.55a	46	3.59e	**'90'**	**1.93a**	47	4.45f			33	3.75k
17	1.58a	19	3.62e			8	4.60f			24	3.75k
36	1.64a	21	3.66e			37	4.66f			34	3.75k
32	1.66a	31	3.67e			60	4.80f			46	3.80k
39	1.66a	59	3.91e							59	3.81k
61	1.69a	50	4.14e							58	3.81k
'90'	**1.73a**									16	3.84k
										61	3.84k
										52	3.87k
										6	3.99k
										60	3.99k
										31	4.02k
										11	4.16k
										8	4.44k

Values followed by the same letter for each isolate are not significantly different at $LSD_{0.05}$

RESULTS AND DISCUSSION

Scald development progressed slowly and symptoms appeared on the parents and the check cultivar 10-12 days after inoculation. Therefore, scald reactions were recorded 14 days after inoculation. **Table 1** shows the infection responses of the parents 'Roho' and '90' for the fifteen isolates of *R secalis*. Indeed the parent 'Roho' showed susceptibility reactions towards most isolates while '90' showed resistance reactions. Accordingly, '90' was the resistant parent and 'Roho' was the susceptible one. The check cultivar 'Rihane' behaved as expected and showed susceptibility to all isolates (**Table 1**). According to the obtained results, we selected isolates of Bousalem2, Krib5 and Teboursouk4 which expressed the strongest differential responses on parents for the inoculation of the DH lines. Reaction to scald of the DH progeny ranged from 0.72 to 4.14; from 1.02 to 4.80 and from 1.66 to 4.44 for Bousalem2, Krib5 and Teboursouk4 isolates, respectively (**Fig. 1**). Frequency distributions of the DH lines varied depending on the isolate. The DH genotypes showed continuous distributions for the isolates of Bousalem2 and Teboursouk4 (**Fig. 1A, 1C**). The continuity of distribution of the scald ratings among the DH lines is probably due to its quantitative inheritance. Concerning the isolate of Krib5, the frequency distributions of the progeny was discontinuous (**Fig. 1B**). The χ^2 test was applied with the consideration that genotypes having 0, 1 or 2 scores are resistant and those having 3, 4 or 5 scores are susceptible. Consequently, we obtained a highly significant phenotypic ratio of 1:3 ($\chi^2 = 0.005$, $P = 0.01$), corresponding to the segregation of two complementary genes. In a previous study, Habgood and Hayes (1971) found the same ratio in an F_3 population from the cross 'Osiris' x 'Proctor' whereas, a ratio of 13:1 was recorded by Spaner *et al.* (1998), which fits to the segregation of three genes.

Fig. 1A, 1B and **1C** show transgressive lines for resistance and for susceptibility. However, no DH line was significantly more resistant than the parent '90' or more susceptible than the parent 'Roho' for the three isolates of scald ($LSD_{0.05}$ = 1.02, 1.12 and 0.98 for isolates of Bousalem2, Krib5 and Teboursouk4, respectively) (**Table 2**). This could be attributed to the experimental error that is sometimes high due to the subjectivity of the evaluation. Transgressive segregation is due to the presence of minor genes for resistance to scald in the parents. During hybridization, there was addition of these minor genes that were expressed in DH lines. Indeed, previous studies have attributed the genetic basis of scald resistance to additive and epistasic effects (Sorkhilalehloo *et al.* 2002).

Table 3 Two-way ANOVA of 59 doubled haploid barley reactions inoculated with three isolates of scald in the seedling stage.

Sources of Variation	df	MS
Genotype	58	3.06**
Isolate	2	44.23**
Genotype x Isolate	116	1.19**
Residual	372	0.43

df: degrees of freedom. MS: mean of squares. **: significant at 0.01 probability

Moreover, pooled analysis of variance across isolates showed highly significant differences between genotypes, isolates, and genotype × isolate interaction (**Table 3**). The variation among isolates can be attributed to their different geographical origins. The genetic variability of *R. secalis* was previously reported by MacDonald *et al.* (1999) who collected 265 isolates of *R. secalis* from Australian barley fields and proved that they differed significantly both in allele frequencies and genotype diversities. In addition, Arabi *et al.* (2008) identified 18 unique haplotypes among sixty-three isolates of *R. secalis* of diverse geographical origin for pathogenicity and variability of genomic DNA using random amplified polymorphic DNA markers. Besides, the significant interaction observed between the isolates and the DH lines indicated that the resistance level of a given genotype is influenced by the virulence of the isolate and that resistance genes in the DH lines varied with the tested isolate. Genotype × isolate interactions for scald resistance were also reported by Arabi et *al.* (2010) who tested the reaction of barley differential cultivars to 49 single spore scald isolates from Syria.

The broad-sense heritability analyzes the relative contribution of genetic factors to the total phenotypic variance. In our study, the broad-sense heritability for scald reaction to all isolates reached 0.64. Thus, the genotypic variation explained 64% of the total variation, suggesting that resistance to scald in the seedling stage is a relatively high inherited

Table 4 Mean squares of scald reaction in relation to spike type of barley doubled-haploid lines derived from the cross 'Roho' x '90' evaluated with three isolates in the seedling stage.

Isolates	Sources of variation	df	MS
Bousalem2	TR	1	0.30^{ns}
	DH(TR)	59	1.89^{ns}
Krib5	TR	1	3.25^{ns}
	DH(TR)	59	2.63^{ns}
Teboursouk4	TR	1	3.14^{ns}
	DH(TR)	59	1.16^{ns}

TR: row type (intertype). DH(TR): row type within DH lines. df: degree of freedom. MS: mean of squares. ^{ns}: not significant at 0.01 probability

genetic trait. In their study, Sorkhilalehloo *et al.* (2002) obtained a higher (72%) heritability for resistance to scald. Although the highly significance of genotype × isolate interaction, the estimated heritability in our study suggest that breeder could select tolerant lines to scald in the seedling stage independently of the isolate.

Mean squares of the between-row type and the within-row type were not significant (**Table 4**). The two-rowed DH barley lines behaved as the six-rowed lines towards resistance to *R. secalis*. Therefore, genes coding for resistance to scald in DH lines do not appear to be linked with the *vrs1* gene which codes for row type in barley. Our result was not in agreement with that of Mert and Karakaya (2004), who showed that resistance to scald was linked to row type in a Turkish barley population, and that the six-rowed genotypes were more resistant than the two-rowed ones.

Using the Marker × Isolate model, the ANOVA revealed significant associations between the dependent variable: resistance to scald and three of the SSR markers (**Table 5**). The second QTL was the most important since it explained 2.34% of the total phenotypic variance and it showed a main additive effect of 0.16 based on the rating scale 0-5 of Salamati and Tronsmo (1997). This QTL was localized on chromosome 3H near the molecular marker HVM33. For this QTL, the resistant allele was from the susceptible parent 'Roho'. However, the resistance alleles of the two others QTLs (1 and 3) were from the resistant parent '90'. These two QTLs were localized on chromosomes 4H and 6H near the SSR markers HVM68 and EBmac0806 and they explained 1.97 and 1.24% of total phenotypic variance, respectively. QTLs associated with HVM68 and EBmac0806 contributed to scald resistance by about 0.15 and 0.12 (0-5 rating scale), respectively. The obtained results show that the two parents carried alleles for scald resistance, which explained the observed transgressive segregants. However, **Table 5** shows that no QTL had a significant additive effect × isolate interaction. This result indicates that the identified QTLs had no effect on specific resistance to Bousalem2, Krib5 and Teboursouk4 isolates, but they had a general effect on resistance to scald. In order to improve these results, the marker effect should be tested for each isolate.

By the use of the combined Marker × Isolate model, we identified three QTLs for scald resistance in the seedling stage that are linked to HVM33, HVM68 and EBmac0806 SSR markers. According to Varshney *et al.* (2007), these QTLs are eventually located on chromosomes 3H, 4H and 6H. QTLs for scald resistance have previously been identified on these chromosomes by Jensen *et al.* (2002), Genger *et al.* (2003) and Shtaya *et al.* (2006). Jensen *et al.* (2002) identified a QTL on chromosome 6H that is co-located with the Bmac0316 SSR marker at 7.2 cM. This QTL may not be linked to that mapped in our study which is associated to the EBmac0806 SSR marker located at 75.5 cM. Genger *et al.* (2003) detected a QTL on chromosome 3H that is closely linked to Bmag0603 SSR marker at 54.6 cM. This QTL seems to be the same as that identified in our study which is associated to the HVM33 SSR marker located at 65.5 cM. QTL mapped on chromosome 4H may correspond to the QTL for scald resistance in the adult stage detected in the 'Vada' ×'L94' cross with the resistance allele contributed by 'L94' (Shtaya *et al.* 2006).

Moreover, QTLs cited in the literature explained more phenotypic variation and their additive effects were more important than QTLs identified in our study. This could be explained by the limited number of the used SSR markers as well as the low size of the evaluated population. Indeed, in the study of Shtaya and Martínez (2011), a barley population composed of 103 recombinant inbred lines was evaluated at the seedling stage to scald resistance using a dense markers map (709 AFLP and 139 SSR markers). The large size of their population and the great number of molecular markers used allowed the identification of two QTLs mapped on chromosomes 3H and 7H which explained 57.4% of the total phenotypic variation.

For a reliable study, field trials should be carried out in several environments to study the resistance to scald in the adult stage and identify the most stable QTLs in the DH progeny.

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Table 5 Data of QTLs associated with resistance to each of the three isolates of *R. secalis* (Bousalem2, Krib5 and Teboursouk4) in the seedling stage using a doubled haploid population from the cross 'Roho' x '90'.

QTL	Marker	Chromosome (Position cM)	MS (Marker)	R^2 (%) (marker)	Main additive effect	Additive effect x isolate		
						Bousalem2	Krib5	Teboursouk4
1	HVM68	4H (62.5)	11.64^{*}	1.97	-0.15^{*}	-0.07^{ns}	-0.34^{ns}	-0.05^{ns}
2	HVM33	3H (65.5)	13.82^{*}	2.34	$+0.16^{*}$	$+0.11^{ns}$	$+0.19^{ns}$	$+0.17^{ns}$
3	EBmac0806	6H (75.5)	7.34^{*}	1.24	-0.12^{*}	-0.07^{ns}	-0.23^{ns}	-0.08^{ns}

Position (cM): Position in cM of the QTL relative to the first flanking marker. MS: mean of squares. R^2(%): Coefficient of determination, percentage of the phenotypic variance which is explained by the putative QTL. Main additive effects and additive effect x isolate are the mean effects of substituting one allele of 'Roho' by an allele of the line '90'. *: The presence of a QTL or main additive effects are significant at $P < 0.05$. A negative sign reflects that QTL alleles is contributed by line '90' which reduce the scald score. a positive sign reflects that QTL alleles is contributed by 'Roho' which reduce the scald score ^{ns}: The presence of an additive effect by isolate is not significant at $P < 0.005$

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