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ORIGINAL ARTICLE

Marker-assisted selection for scald (*Rhynchosporium commune* L.) resistance gene(s) in barley breeding for dry areas

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Abstract

Barley scald, caused by *Rhynchosporium commune* is one of the most prevalent diseases in barley (*Hordeum vulgare* L.) worldwide. The primary loss from scald is reduced yield, which can exceed 25% in dry areas. In our earlier studies, we developed a low-resolution linkage map for recombinant inbred lines of the cross Tadmor/WI2291. Quantitative trait loci (QTLs) for scald were localized on chromosomes 2H and 3H flanked by Simple Sequence Repeat (SSR) markers HVM54 and Bmac0093b on 2H and HVLTPP8, HVM62 and Bmag0006 on 3H. These chromosome 3H markers were found to be located close to the *Rrs1* – *R. commune* resistance gene(s) on chromosome 3H. In this study, 10 homozygous resistant and 10 homozygous susceptible plants each from the F7 population of Tadmor/Sel160, a panel of 23 barley varieties used routinely in the International Centre for Agricultural Research in the Dry Areas (ICARDA) breeding program and three populations were used for scald resistance screening using 25 DNA markers that are located very close to scald resistance gene(s) on barley chromosomes. Only five of those markers clearly discriminated co-dominantly between resistant and susceptible plants. These markers, Ebmac0871-SSR, HVS3-SCAR, Bmag0006-SSR, reside on different arms of barley chromosome 3H. Ebmac871 is localized on the short arm of 3H and HVS3 and Bmag0006 are localized on the long arm of 3H. This result indicates that the scald resistance genes which they tag are probably close to the centromeric region of this chromosome. Scald resistance from several sources map to the proximal region of the long arm of chromosome 3H, forming the complex *Rrs1* locus. The availability of highly polymorphic markers for the discrimination of breeding material would be extremely useful for barley breeders to select for the trait at the DNA level rather than relying on phenotypic expression and infection reaction.

Keywords: barley, Marker-Assisted Selection (MAS), resistant gene, *Rhynchosporium commune*, scald

Introduction

Scald or leaf blotch, caused by *Rhynchosporium commune* (*R. secalis*, Zaffarano *et al.* 2011) is a serious foliar disease that affects barley (*Hordeum vulgare* L.) in many of the major production regions of the crop around the world. The primary loss from scald is reduced yield, which can exceed 25% (James *et al.* 1968; Khan 1986; Vivar *et al.* 1987; Abbott *et al.* 1991). The losses caused by scald are greater than those caused by powdery mildew in dry areas. One approach to reducing the severity of scald in the field is through the use of fungicides. An alternative approach to scald control is by using naturally occurring scald-resistance genes. Resistance of barley to the pathogen is often controlled by a gene-for-gene interaction, a gene for resistance in the plant corresponding to a gene for avirulence in the pathogen. Genetic analysis of resistance to *R. commune* led to the definition of several major and minor resistance genes.

Several resistance genes for resistance to leaf scald have been found, 14 of those have already been named and nine of those have been localized on chromosomes. It has been found that many of the known genes for scald resistance are alleles at a complex locus on the long arm of chromosome 3H, *Rrs*1 (Dyck and Schaller 1961a, b; Habgood and Hayes 1971; Bockelman *et al.* 1977; Barua *et al.* 1993; Graner and Tekauz 1996). Others have been mapped to the short arm of chromosome 7H (*Rrs*2; Dyck and Schaller 1961a; Schweizer *et al.* 1995) and to chromosome 4H (*Rrs*3; Bockelman *et al.* 1977).

The greatest challenge to breeding for scald resistance is the rapid 'breakdown' of single gene resistance in the field. Although *R. commune* has no known sexual stage, the pathogen is highly variable pathogenically and has a range of other genetically controlled characters (Ali *et al.* 1976; Jackson and Webster 1976; Tekauz 1991; Goodwin *et al.* 1992; Zhang *et al.* 1992; Burdon *et al.* 1994; Salamati and Tronsmo 1997; McDonald *et al.* 1999; Williams *et al.* 2003; Wallwork and Grcic 2011; Hofmann *et al.* 2013). This high variability allows rapid selection and accumulation of pathotypes able to overcome newly released resistance genes (Salamati and Tronsmo 1997; McDonald *et al.* 1999; Genger *et al.* 2003). Cultivars with pyramided scald resistance genes are likely to show more durable resistance. Wallwork and Grcic (2011) identified at least two distinct patterns of reaction to scald caused by the gene(s) located close to the centromere on 3H, which are distinguishable using panels of differential isolates and cultivars.

The fact that a molecular marker tags each resistance means that screening for multiple resistances in segregating families of crosses between lines is simplified. Although microsatellites or Simple Sequence Repeats (SSR) and Sequence Characterised Amplified Region (SCARs) markers have been used for mapping genes of interest or map-based cloning, their application in Marker-Assisted Selection (MAS) has been limited in the past.

Another requisite to improve breeding for disease resistance is the identification of additional resistance sources in *H. vulgare*. A collection of landraces represents valuable resources containing broad genetic variability for numerous agronomically important traits. One of these collections is hosted in the International Centre for Agricultural Research in Dry Areas (ICARDA), which was used in this study.

The main objective of this study was to test a set of 25 DNA markers which are closely linked to scald resistance genes to identify the presence of known resistance genes in a few populations from the barley breeding program at ICARDA by using molecular markers as marker-assisted selection.

Materials and Methods

Plant material

Genetic linkage mapping studies used three populations (Arta/*H. spontaneum*, Tadmor/Sel160 and Tadmor/WI2291). The populations were derived from a single selfed plant (Arta/*H. spontaneum*) developed by doubled haploid technique (DH, Tadesse *et al.* 2012) (Tadmor/Sel160) or by single seed descent (Tadmor/WI2291), respectively. The populations segregate for scald resistance. One hundred eighty-four lines of the population Arta/*H. spontaneum*, 243 lines of the population T/Sel160 and 71 lines of the population T/WI2291 developed by ICARDA, had been screened previously with low-resolution mapping. The parental lines were screened to isolate polymorphic markers, which are closely linked to the scald resistance gene(s). *Hordeum spontaneum*, Sel160 and WI2291 parents contain the resistance gene(s) for scald while Arta and Tadmor are susceptible to scald. A panel of 21 barley varieties was also tested for scald resistance and compared with two scald susceptible cultivars Clipper and Rihane-2 as controls.

Seedling inoculation

Scald isolates were continuously collected from different parts of Syria to sample the full pathogenic

Table 1. Origin of isolates of *Rhynchosporium commune* used in seedling inoculation

R. commune isolate	Country source	Host	Location	Collection year
RS1	Syria	Barley cultivar	Aleppo	2005
RS2	Syria	Landrace (A. Aswad)	Daraa	2005
RS3	Syria	Barley grass	Aleppo	2004
RS4	Syria	Barley cultivar	Hama	2004
RS5	Syria	Barley breeding line	Aleppo	2004
RS6	Syria	Barley cultivar (Rihane)	Aleppo	2006
RS7	Syria	Barley cultivar (Rihane)	Aleppo	2006

variability occurring in nature (Table 1). Seeds were pre-germinated for 48 h at 23°C on moist filter paper in order to produce uniform seedlings of all cultivars.

Cultures were grown at 16°C with a 12-h photoperiod for 7 days to produce plates covered with spores. A spore solution was produced as described above and diluted to 10^6 spores · ml^{-1} in sterile reverse osmosis (RO) water. One droplet of Tween 20 was added to 100 ml of spore solution which was then sprayed onto five seedlings at the three-leaf stage that had been grown in 10-cm plastic pots (Fig. 1). Following inoculation, the seedlings were placed in the dark with 100% humidity in a controlled-atmosphere growth room at 13°C for 24 h and then transferred to a 10/14 h photoperiod with similar humidity and temperature. The first signs of infection were observed 10 days later and assessments made 18 days after inoculation. The number of lesions on each leaf was recorded and the area of the leaf was measured. Since symptoms are ill-defined and preclude more precise ratings individual seedlings were rated using a simple 5-category scale (R = no symptoms, LS = symptoms only on leaf sheaths, MR = very minor lesions on leaves, often only on leaf margins, MS = a few leaf blotches, often only on the margins of leaves and S = many large lesions on leaves or death of whole leaves). These ratings were converted to a numerical scale in which R = 1, LS = 2, MR = 3, MS = 5 and S = 7, and mean scores were computed for five seedlings. This numerical scale was designed to align with a 1–9 numbering system used in National Variety Trial disease ratings for cereals in Australia (Wallwork *et al.* 2014).

Genotyping

Ten homozygous resistant and 10 homozygous susceptible plants from the recombinant inbred line (RIL) population of Tadmor/Sel160 were screened with all the available markers close to scald resistance genes (*Rrs1*-3H: HVS3, HVS9, Bmac209 markers; Genger *et al.* 2003), (*Rrs1*-3H: Ebmac0871, Bmac209; Bmac67, Bmag6, Bmag603; Karakousis *et al.* 2003), scald quantitative trait locus (QTL) marker (Bmag0006, HVM62, Bbmac0093b, HVM54, HVLTPP8; Sayed *et al.* 2004), (*Rrs13*-6H: CXP3EX5,7; Baulcombe *et al.* 1987), (*Rrs13*-6H: ABG378; Abbott *et al.* 1995), (*Rrs2*-7H: CDO545-1; Heun *et al.* 1991, Schweizer *et al.* 1995) (*Rrs2*-7H: CDO545-2; Grain Genes Database) (*Rrs2*-7H: MWG2018; Schweizer *et al.* 1995), scald QTL markers (CDO1174; Heun *et al.* 1991, Barua *et al.* 1993), (agtc17; Grønnerød *et al.* 1996), (SM1000, Falc666; Penner *et al.* 1996), (YLM; Paltrige *et al.* 1998), (*Rhy*-3H: OPA-D8, OPA-R3; Barua *et al.* 1993) (Table 1) were tested with the set of 10 resistant and 10 susceptible lines.

Microsatellite assays were carried out according to Becker and Heun (1995), Liu *et al.* (1996) and Ramsay *et al.* (2000). Polymerase chain reaction (PCR) was performed in a volume of 20 µl. The reaction mixture consisted of 5 µM of each dNTP, 10 pM of each primer, 50 ng template DNA, 0.2 units *Taq* DNA polymerase, 10 mM Tris-HCl, 50 mM KCl and 2 mM $MgCl_2$. Gel electrophoresis was carried out on standard sequencing gel systems (GIBCO/BRL, Life Technologies),

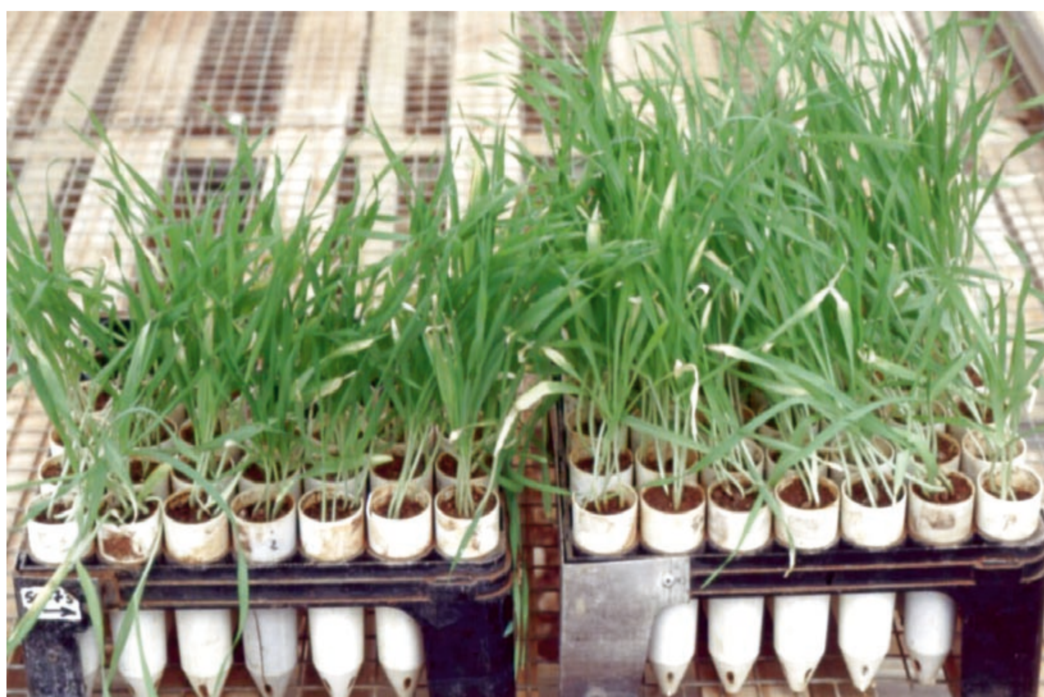

Fig. 1. Seedling infection for genotyping under controlled conditions. Infection by spraying the pathogen. The reading is taken after 18 days

where detection was performed with the silver staining procedure (Bassam *et al.* 1991) or on agarose gels.

Statistical analysis

For each of the three populations, Chi-square values (χ^2) and probability levels at p = 0.05 and p = 0.01 were calculated according to Mather (1957) using the following formula:

$$\chi^2 = [(|O_1 - E_1| - 0.5)^2/E_1] + [(|O_2 - E_2| - 0.5)^2/E_2],$$

where: | | – absolute value; O_1 and O_2 – observed values; E_1 and E_2 – expected values.

Allele frequency (F) for each locus was calculated using the following formula:

$$F_{A,B} = (A, B)/n,$$

where: $F_{A,B}$ – the estimate of the allele frequency either A or B allele; (A, B) – the presence of allele A or B at one locus; n – the population size for each locus. All loci were tested by (χ^2) analysis against an expected 1 : 1 ratio (Sayed *et al.* 2002).

Results and Discussion

Infections with the seven scald isolates (RS1–RS7) using different mixtures of different isolates were repeated seven times over a period of a year on the 71 DH progeny of the Tadmor/WI2291 cross (Table 2).

Two sets of extremely resistant and susceptible lines were selected from the population Tadmor/Sel160 to be tested with all available QTL markers for resistance genes. Twenty-five DNA markers closely linked to scald resistance genes on different chromosomes were tested with the parents Tadmor, WI2291, Sel160 and a set of 10 homozygous resistant and 10 homozygous susceptible plants from the population of Tadmor/Sel160 (Table 3).

Table 2. Analysis of resistance/susceptibility to scald (*Rhynchosporium commune*) in the 71 lines of the DH population of Tadmor/WI2291, 18 days after inoculation

R. commune isolate	Number of plants infected/not infected*					Total
	R (1)	LS (2)	MR (3)	MS (4)	S (5)	
RS1	21	10	14	16	10	71
RS2	50	8	10	3	0	71
RS3	6	8	10	14	13	51
RS4	15	10	10	24	12	71
RS5	26	13	12	11	9	71
RS6	3	4	5	23	31	66
RS7	7	6	10	20	23	66

*scale for scoring (1–5): 1 = no infection (resistant, R), 2 = less susceptible (LS), 3 = moderate resistant (MR), 4 = moderate susceptible (MS), 5 = leaves almost 100% covered with lesions (susceptible, S)

A 10-cM window bracketing the *Rrs1* was defined via the low-resolution interval-mapping population to compare the 10 DNAs that were homogeneous within the window for *Rrs1* resistance specific. Out of those, only five markers (Bmac209, HVS3, Bmac67, Ebmac871 and Bmag0006*), which are localized on chromosome 3H, clearly discriminated co-dominantly between resistant and susceptible plants. For Bmac209 the marker which is probably linked to a resistant allele expressing resistance was 179 bp and the marker which is probably linked to a susceptible allele expressing susceptibility was 192 bp; for HVS3-SCAR 250 bp and 575 bp, for Bmac67 the resistant allele was 175 bp and the susceptible allele 220 bp; for Ebmac871-SSR the resistant allele was 182 bp and the susceptible allele 199 bp; and for Bmag0006-SSR 178 bp and 210 bp, respectively (Fig. 2).

In an attempt to confirm the results, a panel of 23 barley varieties which is used routinely in the ICARDA breeding program was used for assessing the presence of a major scald resistance gene *Rrs1* on chromosome 3H for marker-assisted selection (MAS) using the five markers (Bmac209, HVS3, Bmac67, Ebmac871 and Bmag0006) and to identify the resistance genes in the varieties (Figs 3 and 4). In this study, we were able to verify the usefulness of these DNA markers for MAS for *Rrs1* scald resistance gene on chromosome 3H. The SCAR marker HVS3 (Genger *et al.* 2003) was tested for scald resistance screening of *Rrs1* gene, the one major gene on chromosome 3H. This marker can clearly discriminate between resistant and susceptible plants. The 250 bp and 575 bp bands revealed resistance and susceptible plants, respectively (Fig. 3).

The results showed (Table 4) that the breeding material *H. spontaneum* 41-1, Hml(Union/C103576//Coho), WI2291, SLB45-40/*H. spontaneum* 41-5, *H. spontaneum* 41-5, Moroc9-75/Arabi Aswad are resistant to scald. Also, we verified that varieties Zanbaka and Sara which were selected from the landrace Arabi Aswad, Harmal-2 and Sel160 were also resistant to scald in semi-dry and dry areas in Syria while the landraces Tadmor, Arta, Arabi Abiad and Rihane-2 were susceptible to scald.

The mapping populations (Tadmor/WI2291: DHL, 71 lines), (Tadmor/Sel160: RIL, 242 lines) and (Arta/*H. spontaneum*: RIL, 184 lines) were phenotyped for scald resistance with the *R. commune* isolate 7 (Sayed *et al.* 2004). Infection scores for the parental lines averaged 0.0 for WI2291, Sel160 and *H. spontaneum*, and 5.0 for Tadmor and Arta. The three populations were also genotyped with the Bmac0006 marker and the results were 31(R): 40 (S) for Tadmor/WI2291 population, 111 (R): 131 (S) for Tadmor/Sel160 population, and 86 (R): 98 (S) for Arta/*H. spontaneum* population. Allele frequencies were 0.6 (R): 0.6 (S) for Tadmor/

Table 3. Summary of the linked DNA markers to scald resistance genes, chromosomal location, marker type, resistance linked genes, references and polymorphic target (+) polymorphism, (–) either monomorphic or not amplified

No.	Marker	Chromosome	Marker type	Gene	Polymorphism	Reference
1	Bmac0093	2H	SSR	*Rh*	–	Sayed *et al*. 2004, Sayed *et al*. 2006
2	HVM54	2H	SSR	*Rh*	–	Sayed *et al*. 2004, Sayed *et al*. 2006
3	Bmac209	3HL	SSR	*Rrs1*	+	Genger *et al*. 2003, Ramsay *et al*. 2000
4	HVS3	3HL	SCAR	*Rrs1*	+	Genger *et al*. 2003, Sayed *et al*. 2006
5	HVS9	3HL	SCAR	*Rrs1*	–	Genger *et al*. 2003
6	Bmag603	3HS	SSR	*Rrs1*	–	Karakousis *et al*. 2003
7	Bmac6	3HS	SSR	*Rrs1*	–	Karakousis *et al*. 2003
8	Bmac67	3HS	SSR	*Rrs1*	+	Karakousis *et al*. 2003
9	Ebmac871	3HS	SSR	*Rrs1*	+	Karakousis *et al*. 2003
10	CDO1174	3HL	SSR	*Rh*	–	Heun *et al*.1991, Barua *et al*. 1993
11	agtc17	3HL	SSR	*Rh*	–	Grønnerød *et al*. 2002
12	SM1000	3HL	SSR	*Rh*	–	Penner *et al*. 1996
13	Falc666	3HL	SSR	*Rh*	–	Penner *et al*. 1996
14	YLM	3HL	SSR	*Rh*	–	Paltrige *et al*. 1998
15	cMWG680	3HL	RFLP-PCR	*Rh*	–	Graner *et al*. 1991, Graner & Tekauze 1996, Smilde *et al*. 2000
16	Bmag0006*	3H	SSR	*Rh*	+	Sayed *et al*. 2004, Sayed *et al*. 2006
17	HVM62	3H	SSR	*Rh*	–	Sayed *et al*. 2004, Sayed *et al*. 2006
18	HVLTPP8	3H	SSR	*Rh*	–	Sayed *et al*. 2004, Sayed *et al*. 2006
19	OPA-D8	3H	RAPD	*Rhy*	–	Barua *et al*. 1993
20	OPA-R3	3H	RAPD	*Rhy*	–	Barua *et al*. 1993
21	ABG378	6HS	RFLP-PCR	*Rrs13*	–	Abbott *et al*. 1995
22	CXP3	6HS	RFLP-PCR	*Rrs13*	–	Baulcombe *et al*. 1987, Abbott *et al*. 1995
23	CDO545.1	7HS	RFLP-PCR	*Rh2*	–	Heun *et al*. 1991, Schweizer *et al*. 1995
24	CDO545.2	7HS	STS	*Rh2*	–	Grain Genes Database
25	MWG2018	7HS	SSR	*Rh2*	–	Schweizer *et al*. 1995

*named Bmag0005B on the map of Tadmor/WI2291 according to Sayed *et al*. (2004)
SSR – Simple Sequence Repeats; SCAR – Sequence Characterised Amplified Region; RFLP-PCR – Restriction Fragment Length Polymorphism-Polymerase Chain Reaction; Random Amplified Polymorphic DNA; STS – Sequence-Tagged Site

WI2291 population, 0.5 (R): 0.5 (S) for Tadmor/Sel160 population, and 0.5 (R): 0.5 (S) for Arta/*H. spontaneum* population. The three populations showed significant segregation for the marker Bmag0006. Chi-square values were ($\chi^2 = 0.7$, $\chi^2 = 0.9$ and $\chi^2 = 1.5$), respectively (Table 5). The result of scald infection for the same populations showed a 98% of the same results as in the markers screening.

The markers Ebmac871-SSR, HVS3-SCAR, Bmag 0006-SSR reside on different arms of barley chromosome 3H. Ebmac871 is localized on the short arm of 3H and HVS3-SCAR and Bmag0006 are localized on the long arm. This result indicates that the scald resistance genes/alleles they tag are likely to be in the centromeric region of chromosome 3H. Scald resistance from several sources map to the proximal region of the long arm of chromosome 3H, forming the complex *Rrs1* locus. This locus may consist of several tightly linked scald resistance genes, or it may contain a single gene with multiple alleles.

There is still much confusion about allele/locus designation. The *Rh-Rh3-Rh4* (old names for scald genes) locus complex on chromosome 3H (Dyck and Schaller 1961a; Wells and Skoropad 1963) was regarded as a single gene with several alleles (Habgood and Hayes 1971). Others suggest that at least two different, but closely linked genes exist (Dyck and Schaller 1961b). Hofmann *et al.* (2013) suggested that although the QTL for Rrs1/3H region is close to the centromere, showing low recombination, the future identification of a candidate gene through the development of a large population to search for recombinants in this region with

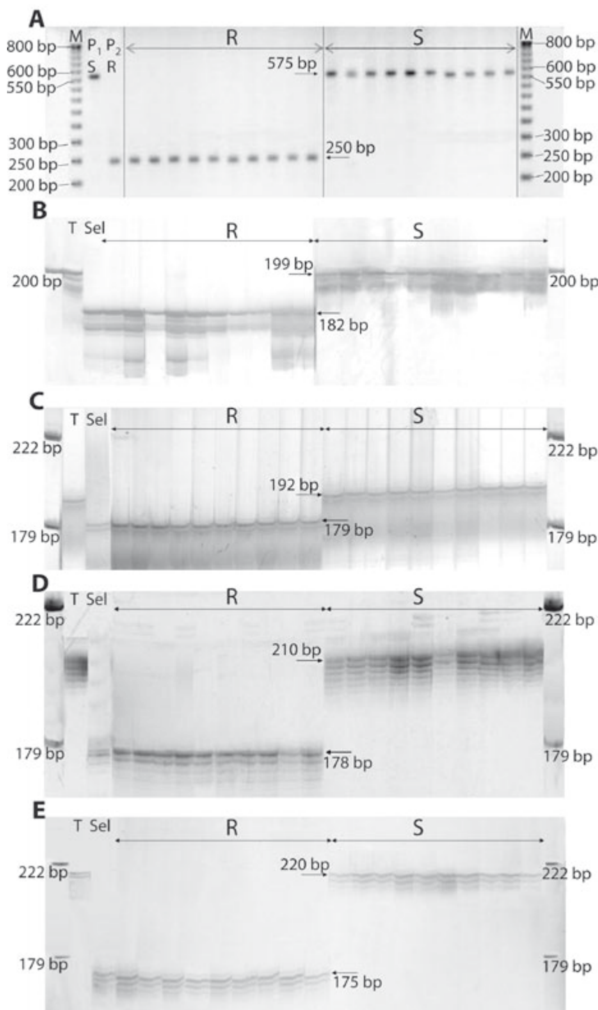

Fig. 2. Screening of 10 homozygous resistant and 10 homozygous susceptible plants from the Tadmor/Sel160 population, with five markers (HVS3, Ebmac871, Bmac209, Bmag0006, and Bmac67) for *Rrs1* scald resistance gene on chromosome 3H. From left to right, M = standard ladder, P_1 = parent 1 – Tadmor (T), P_2 = parent 2 – Sel160 (Sel), 10 resistant lines (R), 10 susceptible lines (S), followed by standard ladder. (A) SCAR marker HVS3 patterns with 20 plants of the progeny Tadmor/Sel160 cross. Upper bands (575 bp) susceptible lines, lower bands (250 bp) resistant lines; (B) Ebmac871 marker, upper bands (199 bp) susceptible lines, lower bands (182 bp) resistant lines; (C) Bmac209 marker, upper bands (192 bp) susceptible lines, lower bands (179 bp) resistant lines; (D) Bmag0006 marker, upper bands (210 bp) susceptible lines, lower bands (179 bp) resistant lines and (E) Bmac67 marker, upper bands (220 bp) susceptible lines, lower bands (175 bp) resistant lines

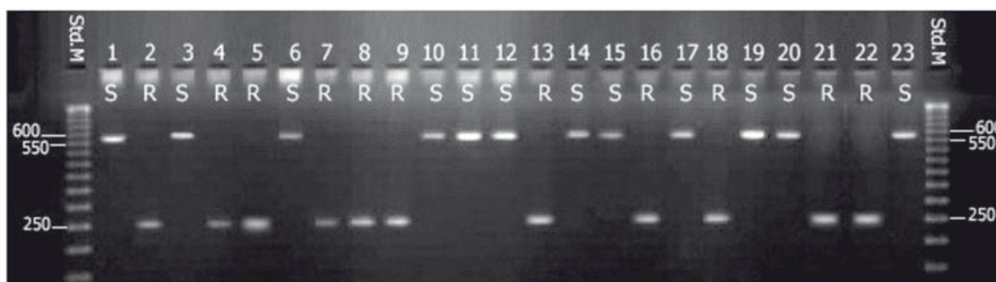

Fig. 3. SCAR marker HVS3 patterns of 23 barley varietes. Upper band (575 bp) susceptible lines, lower band (250 bp) resistant lines. From left to right, standard ladder (50 bp), 1 = Tadmor, 2 = *H. spontaneum* 41-1, 3 = *H. spontaneum* 41-1/Tadmor (1), 4 = Hml (Union/C103576/Coho), 5 = WI2291, 6 = SLB05-96, 7 = Zanbaka (Pure line from Arabi Aswad), 8 = SLB45-40/*H. spontaneum* 41-5, 9 = *H. spontaneum* 41-5, 10 = SLB39-39, 11 = Arta, 12 = Arabi Abiad/Arar, 13 = Sara (Arar/Arabi Aswad, 14 = *H. spontaneum* 41-1/Arta, 15 = SLB39-01, 16 = Moroc9-75, 17 = Moroc9-75//*H. spontaneum* 41-1/Tadmor, 18 = Moroc9-75/ Arabi Aswad, 19 = Clipper, 20 = JLB37-74, 21 = Harmal-2, 22 = Sel160, 23 = Rihane-2, standard ladder (50 bp). Varieties 19 and 23 were used as a susceptible control

only around 30 genes, seems feasible. Karakous *et al.* (2003) emphasized that it is important to find more than one marker linked to the locus for the efficient monitoring of the trait. Three highly informative SSR markers per trait locus would be good for MAS.

In this study, we confirmed that marker Bmag0006 is located on the long arm of the chromosome 3H close to the centromere at a distance of 0.9 cM from the *Rrs1* gene. This is contrary to the findings of Hofmann *et al.* (2013) who reported

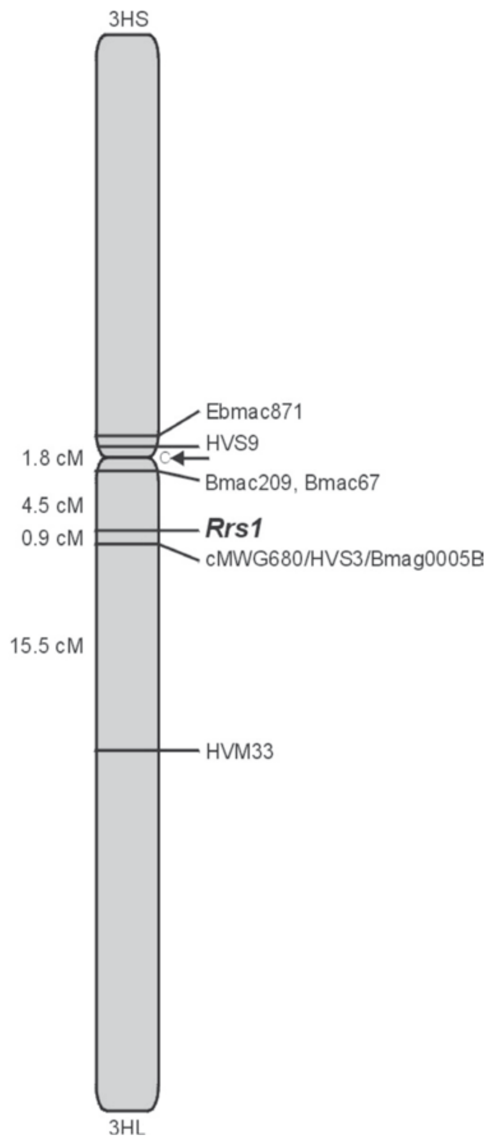

Fig. 4. Partial linkage map of barley chromosome 3H according to Genger *et al.* (2003), with some modification of adding two markers (Ebmac871 and Bmag0005) showing the location of the five markers (Ebmac871, Bmag0006 = Bmag0005B, Bmac209, Bmac67 and HVS3). The SSR markers Bmac209 and HVM33 (The James Hutton Institute, Scotland), the STS marker cMWG680 (Graner and Tekauz 1996), the SCAR markers HVS3 and HVS9 (Genger *et al*. 2003). The approximate position of the centromere is indicated by an arrow

marker Bmag0006-SSR on the short arm of chromosome 3H at about 5 cM from the *Rrs1* gene. Therefore, marker Bmac0006 matched perfectly with the *Rrs1* gene or its most dominant allele in our study and can be used for MAS.

Hofmann *et al.* (2013) suggested that five markers which developed (11_1476, 11_0010, 11_0823, 11_0205 and 11_0315) were converted into cleaved amplified polymorphic sequences (CAPS) markers and were mapped on two populations and that four of them mapped into the gap between *Rrs1* and the closest SSR Bmag0006 or STS (Falcon and STSagtc17) markers, whereas SNP11_1476 mapped together with the closed proximal SSR marker GBM1242. The genetic distance in the consensus map of the interval comprising the resistance locus was 2.1 cM. Four markers closely linked to single nucleotide polymorphism (SNP) markers were converted to easily accessible CAPS markers. Therefore, they focused on identifying polymorphic markers in the small interval between markers 11_1476 and 11_0205 (which flank the QTL with great certainty in their populations) and used a bulked segregant transcriptome analysis (BSTA) approach and production of marker-selected recombinant $F_{2:3}$ lines. The analyses of map-based candidate genes is in progress. In this study, we were able to confirm these perfect markers which match the *Rrs1* gene. A test panel of 23 barley varieties and Syrian landraces was used as a model for assessing the usefulness of the DNA markers (SSR and SCAR) linked to resistance gene for MAS. The analysis indicated that the success of monitoring a donor trait with a DNA marker (Ebmac871 as an example) in a variety of crosses is also possible (Fig. 5).

The availability of highly polymorphic markers for the discrimination of breeding material would be extremely useful for barley breeders to select for the trait at the DNA level rather than relying on phenotypic expression and infection reaction. The development of a set of polymorphic markers will be useful in pedigree analysis, population genetics and other basic genetic analysis and it will provide a good step towards the identification of candidate genes.

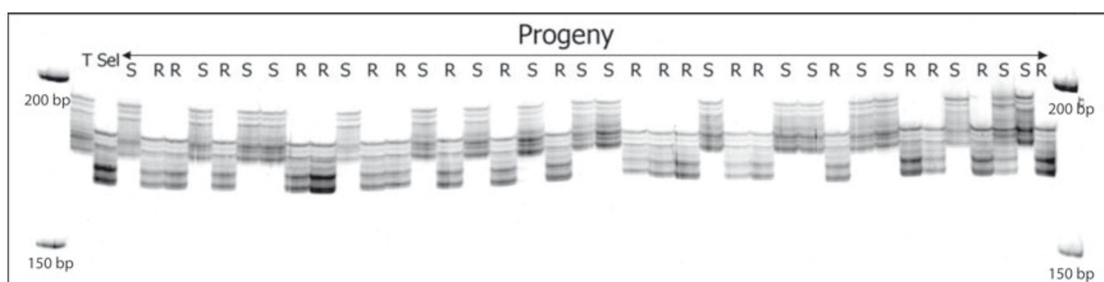

Fig. 5. Screening of Tadmor/Sel160 population for *Rrs1* gene on chromosome 3H, with the microsatellite marker Ebmac871. The resistant allele 182 bp and the susceptible allele 199 bp

Table 4. The pedigree of the 23 barley varieties that have been used in the breeding program at ICARDA. Scald screening for *Rrs1*/3HL gene with the five markers (HVS3, Ebmac871, Bmag209, Bmag0006, and Bmac67). (R) resistant line, (S) susceptible line

No.	Parents	Pedigree	*Rrs1*/3HL
1	Tadmor	(Pureline from A.Aswad)	S
2	*H. spontaneum* 41-1	Pureline from *H. spontaneum*	R
3	*H. spontaneum* 41-1/Tadmor (1)	ICB87-1833-165AP-0BO	S
4	Hml (Union/C103576//Coho)	SEL-09L-12AP-0AP	R
5	WI2291	Australian cultivar	R
6	SLB05-96	(Pureline from A.Aswad)	S
7	Zanbaka (Pureline from A.Aswad)	SLB62-64	R
8	SLB45-40/H. spont41-5	ICB87-1003-109AP-0BO	R
9	*H. spontaneum* 41-5	Pureline from *H. spontaneum*	R
10	SLB 39-39	(Pureline from A.Aswad)	S
11	Arta	(Pureline from A.Aswad) SLB39-58	S
12	ArabiAbiad/Arar	ICB84-1741-0AP-8AP-1APH-0AP	S
13	Sara (Arar/ArabiaAswad)	ICB84-1730-4Ap-0AP-4APH-0AP	R
14	*H. spontancum*41-1/Arta	ICB88-1290-1518AP-F6	S
15	SLB39-01	(Pureline from A.Aswad)	S
16	Moroc9-75	Moroccan cultivar	R
17	Moroc9-75//*H. spont*.41-1/Tadmor	ICB91-0389-5AP-1AP-0AP	S
18	Moroc9-75/ArabiAswad	ICB90-0391-3BO-1AP-0AP	R
19	Clipper	Australian cultivar	S
20	JLB37-74	cultivar	S
21	Harmal-2	cultivar	R
22	Sel160	selected line from (Tadmor/W12291) cross	R
23	Rihane-2	cultivar	S

Table 5. Three populations of barley (Arta/*H. spontaneum*, Tadmor/WI2291 and Tadmor/Sel160) revealing scald resistance and susceptible for the *Rrs1* gene on chromosome 3HL, using Bmag0006 marker as marker assisted selection for breeding

Barley populations	Populations size	Resistant (R) Allele A	Susceptible (S) Allele B	F_R	F_S	χ^2
Arta/H. spontaneum	184	86	98	0.5	0.5	0.7*
Tadmor/Sel160	242	111	131	0.5	0.5	1.5*
Tadmor/WI2291	71	31	40	0.4	0.6	0.9*

F_R – allele frequency from resistant parents (*H. spontaneum*, WI2291 and Sel160); F_S – allele frequency from susceptible parents (Arta and Tadmor); χ^2 – Chi-square values; *significantly segregated at 0.05 ($\chi^2_{(t)} = 3.84$)

We found that the result of scald inoculation coincided with the result of marker genotyping, while in other studies in Australia they found one cultivar Hindmarsh which carries the gene *Rrs*1 but was otherwise susceptible to scald (Wallwork *et al*. 2014). This suggests that the gene *Rrs1* is dominantly responsible for scald resistance in semi-dry and dry areas in the West Asia and North Africa region (WANA) but not so in other environments.

In this study, we added new SSR markers for *Rrs1* scald resistance tagging. The scald resistance gene *Rrs1* described here is of value in the WANA environment (semi and dry areas) and linked markers will facilitate pyramiding and marker-assisted selection in breeding programs.

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