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ORIGINAL PAPER

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Development of candidate introgression lines using an exotic barley accession (*Hordeum vulgare* ssp. *spontaneum*) as donor

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Abstract In the present paper, we report on the selection of two sets of candidate introgression lines (pre-ILs) in spring barley. Two BC_2DH populations, S42 and T42, were generated by introgressing an accession of *Hordeum vulgare* ssp. *spontaneum* (ISR42-8, from Israel) into two different spring barley cultivars, Scarlett (S) and Thuringia (T). From these BC_2DH populations two sets with 49 (S42) and 43 (T42) pre-ILs were selected, and their genomic architecture as revealed by SSR marker analysis was characterised. The selected pre-ILs cover at least 98.1% (S42) and 93.0% (T42) of the exotic genome in overlapping introgressions and contain on average 2 (S42) and 1.5 (T42) additional non-target introgressions. In order to illustrate a potential application and validation of these pre-ILs, the phenotypic effect of the exotic introgression at the locus of the major photoperiod response gene *Ppd-H1* was analysed. Pre-ILs carrying the introgression at the *Ppd-H1* locus flowered significantly earlier than the elite parents, and the introgression maintained its effect across the two genetic backgrounds and across four tested environments. The selected pre-ILs represent a first promising step towards the assessment and utilization of genetic variation present in exotic barley. They may promote the breeding progress, serve for the verification of QTL effects and provide a valuable resource for the unravelling of gene function, e.g. by expression profiling or map-based cloning.

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Introduction

Most agronomic traits in crop plants are modulated by a large number of gene variants, which interact in complex and not easily predictable ways. Over the past decade, the application of molecular markers and genetic linkage maps has allowed the identification of specific regions of the genome controlling polygenic traits, called quantitative trait loci (QTLs), in all of the major crop species. Eshed and Zamir (1995) noted, however, that the considerable advances in the methodologies of mapping quantitative genes have not been matched by a parallel development of suitable population structures for precise QTL localisation, partly because the generation of genetic material requires a substantial effort. The key to the analysis of genes behind agronomically important traits is nevertheless plant genetic resources. In *Arabidopsis*, concerted efforts have led to the establishment of a number of valuable resources, i.e. mutant plant collections or panels of substitution lines. Given the limited synteny of *Arabidopsis* and crops, it is important to establish similar resources in crops in order to advance the understanding of the function of genes with agronomic relevance. It is important to, thus, identify the nature of functional variation in genes, which exist in the germplasm pools of crop species. Zamir (2001) proposed capitalizing on the genetic diversity in exotic germplasm and use it for breeding as well as gene discovery. To overcome problems associated with considerable phenotypic variation and linkage drag in interspecific crosses, Eshed and Zamir (1994) proposed modifying population structure. They established introgression lines (ILs) by systematic backcrossing and introgression of marker-defined exotic segments in the background of elite varieties. These ILs are a useful tool with which to address a number of questions. They enable the phenotypic analysis of specific QTL, offering a common genetic background in which direct comparison of two lines can be used to evaluate the phenotype conditioned by a single introgressed exotic segment (Tanksley et al.

1996). ILs facilitate fine mapping of QTLs, because the location of a QTL can be narrowed to a smaller genomic interval by evaluating a series of ILs that differ for overlapping regions of the genome (Paterson et al. 1990). The resolution and statistical power of QTL mapping is increased, because excluding extra genetic factors reduces phenotypic variation. Furthermore, ILs are useful for breeding purposes, as they contain a low percentage of exotic germplasm and favourable exotic alleles can thus be easily and rapidly isolated and transferred into the elite varieties. Finally, these ILs provide a valuable resource for the unravelling of gene function by expression profiling or map-based cloning.

Several sets of introgression lines have already been developed for various crops, like tomato (Young and Tanksley 1989; Chetelat and Meglic 2000; Eshed and Zamir 1995; Bernacchi et al. 1998; Monforte and Tanksley 2000), rice (Lin et al. 1998), lettuce (Jeuken and Lindhout 2004) and wheat (Pestova et al. 2001). Pillen et al. (2003, 2004) and Matus et al. (2003) analysed agronomic performance of exotic alleles in advanced backcross populations of barley. However, the systematic selection of ILs for barley providing a complete coverage of the exotic genome has not been described yet.

In the present paper, we report on the development of two sets of candidate lines for barley ILs (pre-ILs) by introgressing exotic segments from the exotic barley accession ISR42-8 (*Hordeum vulgare* ssp. *spontaneum*) into two spring barley genetic backgrounds (cultivars Scarlett and Thuringia). Our goal is to establish two sets of pre-ILs, which represent the entirety of the exotic genome with overlapping donor segments of different sizes and contain a low number of additional exotic segments. First, segregation pattern and recovery rate of the recurrent parent genome as revealed by molecular marker fingerprinting using SSRs are analysed for two generated BC_2DH populations. Subsequently, two selected sets of pre-ILs are presented and characterised. In order to illustrate a potential application and validation of these pre-Ils, they are used to evaluate the phenotypic effect of an exotic introgression at a specific genomic region known to carry a candidate gene for the trait days until heading (Hea). The selected chromosomal region includes the major photoperiod response gene, *Ppd-H1*, which was mapped on the short arm of chromosome 2H by Laurie et al. (1994). The mean phenotypic performance of the pre-ILs carrying an exotic introgression at the *Ppd-H1* locus was compared to that of the elite parents as control.

Materials and methods

Plant materials

The development of two BC_2DH populations was conducted according to the advanced backcross strategy of Tanksley and Nelson (1996). An exotic accession of *H. vulgare* ssp. *spontaneum* from Israel (ISR42-8) was crossed with two spring barley cultivars (*H. vulgare* ssp. *vulgare*). The German spring barley cultivars Scarlett and Thuringia were selected as high-performing malting varieties and obtained from the breeders Saatzucht Josef Breun GdbR and Saaten-Zentrum Schöndorf. The wild barley accession, ISR42-8, from Eastern Lower Galilee, Israel, was provided by Prof. G. Fischbeck, Weihenstephan. The recurrent parents, Thuringia and Scarlett, were used as the female and the donor as the male parent to generate the F_1 generation. A single F_1 plant (maternal) was backcrossed to the respective cultivars (paternal). From each initial cross 12 BC_1F_1 plants were backcrossed a second time with the two cultivars. Two BC_2DH populations for convenience designated as S42 (derived from the cross Scarlett × ISR42-8) and T42 (derived from the cross Thuringia × ISR42-8) were developed by anther culture (in the lab of the Saaten-Union Resistenzlabor, Leopoldshöhe, Germany). The two BC_2DH populations counted 301 and 84 BC_2DH lines derived from 76 (S42) and 43 (T42) BC_2F_1 plants, respectively.

Molecular characterisation

The DNA of the BC_2DH lines was extracted from foliage of 10- to 14-day-old seedlings grown in a greenhouse. DNA from parental and BC_2DH lines was isolated according to the protocol described by Saghai Maroof et al. (1984).

A total of 220 SSR markers were screened for polymorphism between the parent pairs. The primer sequence information was primarily taken from the following published sources: Becker and Heun (1995), Liu et al. (1996), Russell et al. (1997), Struss and Plieske (1998), Ramsay et al. (2000), Pillen et al. (2000) and Thiel et al. (2003). One hundred polymorphic SSR markers were selected for genotyping, which consistently yielded PCR products, produced minimal stutter bands and were fairly evenly distributed over the chromosomes.

The PCR amplification was performed in 20-µl final volume reactions containing 5 µl template DNA (ca. 50 ng), 0.1 µl *Taq* polymerase (5 U/µl, Promega, Mannheim, Germany), 1.5 µl 10× PCR-buffer [500 mM KCl, 100 mM Tris-HCl (pH 9.0), 1% Triton X-100], 1.5 µl 25 mM $MgCl_2$, 0.75 µl dNTP (2 mM) and 0.075 µl of the forward and reverse oligonucleotide primers (10 µM). Each forward primer was tailed by adding the M13 universal forward primer sequence at the 5′ end and 0.5 µl (1 µM) of the M13 universal forward primer was added to the reaction. The M13 primer was labelled with either IRD700 or IRD800 at the 5′ end for visualization, and a LI-COR DNA Sequencer 4200 (LI-COR, Bad Homburg) was used as an automated DNA detection device. The amplification profile started with ten cycles of denaturing at 94°C for 1 min, annealing at 64–55°C (touch-down PCR) for 1 min and extension for 1 min at 72°C, followed by 30 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C, with a final extension step at 72°C for 5 min.

Linkage map construction

Eleven new SSR markers (MGB) derived from EST sequences were assigned to chromosomes on the basis of results from barley wheat addition assays (Islam et al. 1981). Linkage between the MGB markers was estimated in an F_2 population derived from the cross Thuringia × ISR42-8 or in the Oregon Wolfe Barleys population [(OWB) Costa et al. 2001], using the MAPMAKER software (Lander et al. 1987). The MGB-primer sequences are available upon request and will be published soon (M. von Korff et al., submitted).

Simple sequence repeat markers from different mapping studies were integrated, and their relative order was inferred with the program MapManager QTX (Manly et al. 2001), which allows for linkage mapping in advanced backcross populations. Linkage was calculated on the basis of: (1) the genotype data of the two BC_2DH populations, (2) on a priori chromosome assignments derived from barley wheat addition line assays or from published sources and (3) on fixed marker order taken from Ramsay et al. (2000). The linear order was established with the command *make linkage groups* and verified with the commands *ripple* and *distribute*. The Kosambi mapping function was applied for map distance calculation. The sequence order of markers, whose relative position could not be resolved with the BC_2DH populations due to a lack of recombination, was estimated after linkage analysis in the OWB population (Costa et al. 2001).

By using the data of the generated linkage map, graphical genotypes were obtained from each BC_2DH plant, using the software program Graphical GenoTypes [(GGT) Van Berloo 1999]. Based on the genotype representations with GGT, the size and the number of introgressions as well as the percentage of donor genome were determined. For the calculation of segment length and genome ratios, the half-intervals flanking a marker locus were considered to be of the same genotype as implemented by the GGT software. For missing marker data, plants were assumed to have the genotype of the two flanking markers if these had identical genotypes for a line. However, if the two flanking markers showed contrasting genotypes, then the data were recorded as missing.

The selection of pre-ILs was based on the following criteria: (1) the selected lines should be characterised by a low percentage of donor genome and (2) contain a low number of additional donor segments and, (3) the donor segments in the selected lines should be overlapping and covering the entire exotic genome.

Evaluation of days to Hea

Phenotypic evaluation of the BC_2DH plants was carried out under field conditions at four different locations in the season 2003. The test locations were the experimental station of the University of Bonn (West Germany), and the breeders' experimental stations in Gudow (Nordsaat Saatzucht, northern Germany), Morgenrot (Saatzucht Josef Breun, eastern Germany) and Irlbach (Dr. J. Ackermann, southern Germany). The field experiment was designed in randomised plots without replications. As a control, the recurrent parents were tested with 20 (Scarlett) and ten (Thuringia) replications per block. Plot size (6–10 m^2), seedling rate (300–330 kernels/m^2) and field management were in accordance with the local practice. The trait days to Hea was recorded as number of days from sowing until emergence of 50% of spikes on main tillers.

Data analysis

Fragment sizes of the SSR markers were visualised by the LI-COR e-seq software, and genotype scoring was carried out manually. Fragment sizes were translated into homozygous elite or homozygous exotic genotypes according to the two possible fragment sizes, revealed by the elite and exotic barley parents, respectively. The proportion of exotic alleles (P [exotic]) was calculated as the percentage of exotic alleles present in a single BC_2DH line according to the formula:

$$P[\text{exotic}] = \frac{[aa]}{[AA] + [aa]}.$$

In the above formula $[AA]$ and $[aa]$ correspond to the number of the homozygous elite and the homozygous exotic genotypes, respectively. All marker loci were subjected to a chi-square goodness-of-fit test for segregation analysis.

Effect of different introgressions at 2HS on the trait days until Hea

In order to examine whether the introgression on chromosome 2HS had an effect on the trait days to Hea, S42 and T42 pre-ILs were selected. These carried different introgressions on the chromosome arm 2HS, where the major photoperiod response gene *Ppd-H1* is located. The phenotypic variance of the trait days until Hea was partitioned with the GLM procedure (SAS Institute 1999). The GLM model included the introgression genotype at 2HS (selected pre-ILs with introgressions at 2HS) and the genetic background (Scarlett or Thuringia) as fixed effects, and the environment and the respective interactions with the environment as random effects (mixed model). Significance was established on the basis of a 0.01 probability threshold.

Comparison of pre-ILs carrying different introgressions on chromosome 2HS

The least square means [(LSMEANS) SAS Institute 1999] across environments were calculated for each selected pre-IL carrying an introgression on chromosome

2HS. A Dunnett multiple comparison of LSMEANS differences of the selected pre-ILs with Scarlett and Thuringia as respective controls was implemented (Dunnett 1955).

Results and discussion

Generation of the BC2DH populations

Two advanced backcross populations with 301 (S42) and 84 (T42) BC_2DH lines were generated from 12 BC_1F_1 plants each. The final number of BC_2DH lines in each population was mainly determined by different survival rates during tissue culture and by the number of successfully produced fertile DH plants. From 196 (S42) and 207 (T42) BC_2F_1 plants, 645 and 158 DH plants were produced, of which 301 (S42) and 84 (T42) were fertile. The number of fertile DH plants was three times higher in the Scarlett-derived population than in the Thuringia-derived population. Of the vital DH plants, 47% (S42) and 53% (T42) were fertile. No further selection was carried out during the generation and propagation of the BC_2DH lines.

Linkage map construction

In order to infer the sequential order of SSR markers genotyped in this study, they were integrated using the program MapManager QTX (Table 1). Calculations were based on a priori chromosome assignments, and the SSR markers mapped by Ramsay et al. (2000) were used as anchors. Linkage groups verified with the command *distribute* of the MapManager QTX program corresponded well with the published linkage groups with the exception of EBmac684 (5H, Ramsay et al. 2000) and BMS02 (1H, Russell et al. 1997), which were grouped with SSR markers of chromosome 2H and 5H, respectively. The order of the markers returned by the program MapManager QTX was in agreement with that in the map by Ramsay et al. (2000). However, there were the exceptions of Bmac32, Bmag105 and Bmag381, which were located further downstream on the respective chromosomes than in the published map, and of the markers HVM60 and GMS116 whose order was inversed. Map positions of markers were estimated by translating the distances calculated by MapManager QTX into new distances relative to the map positions by Ramsay et al. (2000). The sequential order of the newly mapped markers (MGB) and of those deviating from the map by Ramsay et al. (2000) was verified by mapping data from the OWB population or the F_2 population Thuringia × ISR42-8.

The inferred marker linkage map covered 1,089 cM (S42) and 990 cM (T42), respectively, with an average marker density of 11.2 cM and 14.7 cM (Table 1). When the genotyped markers were assigned to bins according to information by Kleinhofs and Graner (2001) and by the OWB population (http://barleyworld.org), 82% of the bins contained at least one genotyped SSR marker.

Genetic constitution of the BC2DH populations

The BC_2DH populations were successfully genotyped with 97 (S42) and 65 (T42) SSR markers. Among the BC_2DH lines, the percentage of exotic germplasm ranged from 0% to 36.9% (S42) and from 0% to 30.5% (T42). The average percentage of exotic germplasm was 13.9% for population S42, which indicated a higher-than-expected recovery of the donor-parent genome. The average fraction of exotic germplasm in T42 was 10.8%, thus below the expected 12.5% of exotic germplasm. The BC_2DH lines carried 0–11 (S42) and 0–7 (T42) introgressed segments with an average of four and three introgressions in S42 and T42, respectively.

Selection and characterisation of pre-ILs

A selection of pre-ILs with the least number of introgressions was made with the intention to accelerate the generation of pure isogenic lines. A minimal number of BC_2DH lines were selected to represent the entire exotic genome in overlapping segments of about 20 cM. In order to investigate the effect of the same donor introgression in two different elite backgrounds, two sets of pre-ILs with 49 lines for S42 (Fig. 1) and 43 lines for T42 were selected. Altogether 45 (S42) and 41 (T42) different pre-ILs were selected of which four (S42) and two (T42) were chosen twice, because they contain two target introgressions each. The pre-ILs contained, on average, 3.0 (S42) and 2.5 (T42) introgressed segments and 10.8% (118 cM) and 9.8% (97 cM) of exotic germplasm, respectively (Table 2). The percentage of introgressed segments ranged from 3.1% (34 cM) to 28.1% (306 cM) in S42 and from 0.7% (7 cM) to 20.5% (203 cM) in T42. Three and 12 pre-ILs contained a single introgression in S42, and T42, respectively. Based on the level of genome coverage provided by the investigated SSR markers, these can be considered as isogenic lines of Scarlett and Thuringia. In barley, Pillen et al. (2003, 2004) and Matus et al. (2003) generated backcross populations using an exotic accession. However, they worked with large sets of above 130 unselected BC_2-derived lines. The selection of a minimal number of pre-ILs sufficient to represent the entire exotic genome, as demonstrated in this study, has several advantages compared to working with the complete population. First, selecting pre-ILs with a low number of additional introgressions can accelerate elimination of non-target introgressions, and further cycles of backcrossing may be accomplished with a considerably reduced set of lines, thereby saving resources and efforts. Second, costly analyses of quality parameters or of proteome and expression profiles may be carried out with a reduced set of lines, while the sampling of the complete exotic genome is still guaran-

Table 1 Map positions of SSR loci assayed in the S42 and T42 populations and in the candidate introgression lines (pre-ILs)

No.[a]	Chr.	Pos.[b]	Pop.[c]	SD[d]
1H				
1	MGB402	0	B	V_S
2	GMS21	14	B	V_S
3	S53707	20	B	V_S
4	GBM1007	28	S	V_S
5	GBM1042	39	S	
6	MGB325	52	S	
7	HVALAAT	63	B	
8	HVM20	65	B	
9	Bmag211	68	B	
10	Bmag149	70	B	
11	Bmag105	75	S	
12	HVGLUEND	85	B	
13	Bmac32	105	S	S_S
14	GBM1061	115	S	S_S
15	HVABAIP	130	S	S_S
2H				
16	HVM36	17	B	
17	GBM1035	27	B	
18	GBM1052	42	B	
19	MGB391	67	B	
20	EBmac684	80	B	S_S
21	GMS3	86	B	S_S
22	HVTUB	92	S	S_S
23	Bmag381	107	S	S_S
24	Bmag125	122	B	S_S
25	GBM1016	139	S	V_S
26	HVM54	143	B	V_S
27	EBmac415	146	B	V_S
28	MGB334	159	B	V_S
3H				
29	HVLTPPB	25	B	V_S
30	EBmac705	30	B	V_S
31	HVITR1	49	S	V_S
32	MGB410	65	B	V_S
33	Bmag603	70	S	
34	HVM33	94	S	
35	GMS116	100	S	
36	HVM60	110	B	S_S V_T
37	GBM1043	130	S	S_S
38	HV13GEIII	155	B	S_S V_T
39	HVM62	165	B	V_T
40	MGB358	175	B	
41	Bmac29	190	B	
4H				
42	HVM40	14	B	S_S
43	HVOLE	21	S	S_S
44	HVB23D	25	B	S_S
45	HVKNOX3	31	B	S_S
46	HVPAZXG	44	S	S_S
47	HVM13	55	B	S_S
48	GMS89	57	B	S_S
49	EBmac775	80	B	S_S
50	MGB396	95	B	S_S
51	TACMD	125	S	S_S
52	EBmac701	130	B	S_S
53	EBmac635	131	B	S_S
54	EBmac679	132	B	S_S
55	EBmac788	150	B	S_S
56	GBM1015	170	S	S_S
57	HVJASIP	180	B	S_S
58	HVM67	180	B	S_S
59	HDAMYB	190	B	S_S
5H				
60	MGB384	0	B	
61	BMS02	12	S	
62	Bmac0163	24	B	
63	Bmag337	43	B	
64	Bmag357	48	S	
65	Bmag223	69	S	
66	MGB338	85	B	
67	GMS061	126	B	V_S
68	AF04394A	137	B	V_S
69	MGB318	150	T	
70	Bmag0222	162	B	V_S
71	MGB357	165	S	V_S
6H				
72	Bmac316	6	B	S_T
73	GBM1049	40	S	
74	GMS06	96	B	S_S
75	HVM74	103	B	S_S
76	EBmac624	107	B	S_S
77	Bmag613	112	B	S_{ST}
78	GBM1008	135	B	S_S
79	GBM1022	145	S	S_S
80	Bmac40	155	S	S_S
7H				
81	Bmag206	19	S	
82	Bmag7	27	S	V_S
83	EBmac603	50	B	V_S
84	AF22725A	59	T	
85	HVSS1	62	B	V_S
86	HVA22S	75	B	V_S
87	Bmag11	93	S	
88	HVACL3	94	S	
89	Bmag321	100	S	
90	GMS46	120	B	
91	GMS56	133	B	
92	BMS64	146	B	
93	Bmag120	152	S	
94	MGB317	155	B	
95	EBmac755	166	B	
96	HVM49	178	B	S_T
97	Bmag135	180	S	
98	HVCHI26A	181	B	S_T

[a] 1H–7H are chromosomal locations of the target introgression. SSR markers are numbered consecutively according to their relative order starting with chromosome arm 1HS and ending with 7HL

[b] CentiMorgan positions based on calculations with MapManager QTX (Manly et al. 2001) and on Ramsay et al. (2000)

[c] SSR markers are genotyped: *B* in both population, *S* in S42 only, *T* in T42 only

[d] Segregation distortion ($P < 0.01$): V_S in favour of the elite allele in S42; V_T in favour of the elite allele in T42; S_S, S_T and S_{ST} in favour of the exotic allele in S42, T42 and in both, S42 and T42, respectively

teed. Other studies, which demonstrated the introgression of a complete exotic genome into a cultivar, have also worked with a reduced set of lines. Eshed et al. (1992) and Eshed and Zamir (1994) established 21 ILs and 50 ILs, respectively, in tomato; Jeuken and Lindhout (2004) selected 28 introgression lines in lettuce. These sets of introgression lines can be considered as permanent resources from which certain lines may be selected for further analysis according to the focus of the study or according to the genome segment of interest.

The average length of the target introgressions was 46.0 cM in S42 and 48.1 cM in T42. The average sizes of all introgressions in the pre-ILs were 35 cM in S42 and

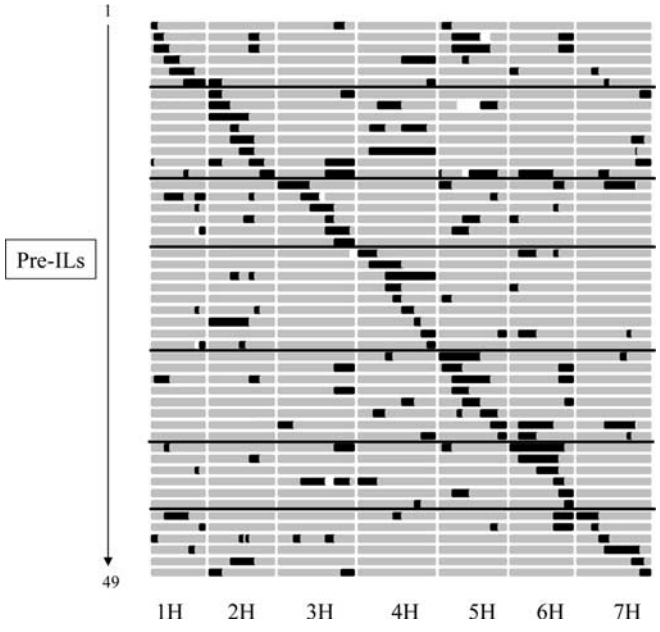

Fig. 1 Graphical genotypes for 49 candidate introgression lines (*pre-ILs*) selected from 45 BC_2DH lines in S42. *Four lines* are represented twice as they carry two different target introgressions. The *vertical bars* represent the seven chromosomes of barley and the pre-ILs are depicted *horizontally*. The *black areas* indicate the *Hordeum vulgare*. ssp. *spontaneum* introgressions, *grey* the elite genotype and *white* missing data

35.4 cM in T42. This corresponds to the predictions by Fulton et al. (1997), who calculated from simulations mean segment lengths of 50 cM in a BC_1 and 34 cM in a BC_2 for an unselected population. Matus et al. (2003) found similar introgression sizes of 38.6 cM on average in a BC_2F_6 population of an elite × exotic barley cross.

From the S42 population, pre-ILs with overlapping donor segments could be selected for all but one genomic region. For the marker interval between EBmac603 and HVSS1 on the short arm of chromosome 7H, no line spanning the entire interval was present in the population. For two marker intervals in T42, no introgressions could be detected. These were on chromosome 2HS (GBM1052-MGB391) and 3HS (EBmac705-MGB410). The lack of lines with introgressions at these particular genomic regions might be due to the strong segregation distortion in favour of the elite genotype at these loci (data unpublished). Except for the aforementioned intervals, donor segments for all genomic regions were represented by at least one pre-IL, indicating that at least 98.1% and 93.0% of the exotic genome were selected in S42 and T42, respectively.

Several studies have already demonstrated the generation of isogenic lines employing different strategies. The majority of them used marker-assisted selection (Chetelat and Meglic 2000; Eshed and Zamir 1994; Monforte et al. 2001; Fulton et al. 1997; Bernacchi et al. 1998). Others employed random backcrossing followed by genetic characterisation and selection of isogenic lines (Jeuken and Lindhout 2004; Lin et al. 1998; Matus et al. 2003). The number of backcrosses and selfings varied between the different studies. For example, introgression lines have been developed from BC_3S_1 (Bernacchi et al. 1998), BC_2S_3 (Monforte and Tanksley 2000), BC_1F_5 (Lin et al. 1998), or a number of different generations, $BC_{(1-2)}F_{(2-6)}$ (Chetelat and Meglic 2000) and $BC_{(1-5)}S_1$ (Jeuken and Lindhout 2004). Jeuken and Lindhout (2004) argued that using more cycles of backcrossing and less selfing generations was more efficient to establish introgression lines than using fewer backcross and more selfing generations.

In this study, random backcrossing was used to establish two BC_2DH populations. These populations with 301 and 84 BC_2DH plants were constructed from 76 (S42) and 43 (T42) BC_2F_1 lines. Despite the considerable difference in sample size, in both populations overlapping segments for almost the entire exotic genome could be detected. However, each population is derived from 12 BC_1 plants. Cox (1984) has already demonstrated that the additive genetic variance depends on the number of F_1 plants backcrossed in each previous generation rather than on the size of the derived population analysed. He also showed that increasing the number of BC_1F_1 beyond 12 individuals produced little change of additive genetic variance in BC_1F_1-derived lines.

Introgression lines generated without marker-assisted selection are generally based on a large number of BC_1F_1 plants. Lin et al. (1998) constructed a population of backcross-inbred lines in rice from 98 BC_1F_1. Matus et al. (2003) used 143 BC_1F_1 plants to establish recombinant chromosome substitution lines in barley and employed single-seed descent only from the BC_2F_3 onwards. However, some forms of *H. vulgare* ssp. *spontaneum* may have rather open flowers and larger anthers and are thus adapted to a higher rate of outbreeding (Brown et al. 1978). It is thus very likely to sample more than one exotic barley allele per locus if backcross populations are established from more than one F_1 plant. In the polymorphism survey conducted for this study, more than 30% of all markers amplified two or three exotic alleles for the same locus. We thus preferred to use a single F_1 plant for the first backcross and then to employ the maximal number of BC_1F_1 plants obtainable from one F_1 plant.

Phenotypic comparison among selected pre-ILs

Effect of the introgression at 2HS on the trait days until Hea

In order to test for association between a particular exotic segment and a phenotype, S42 and T42 pre-ILs were selected. These carried an introgression on the short arm of chromosome 2H where the major photoperiod response gene *Ppd-H1* is located (Fig. 2). The exotic accession ISR42-8 was introgressed into two different elite varieties and evaluated in field trials at four different locations in Germany. We could thus perform a three-factorial ANOVA with the introgression genotype at 2HS (selected pre-ILs, Fig. 2), the genetic background

Table 2 Selection of two sets of 49 and 43 pre-ILs from S42 and T42, respectively

Name	Chr.[a]	Interval[b]	No.[c]	Size[d]	Percentage [e]
S42 Pre-ILs					
Sca040-1	1H	1–2	3	17.0	6.6
Sca300-1		2–4	4	19.5	14.6
Sca299-2		2–5	4	38.5	18.0
Sca332-1		5–10	3	39.0	12.6
Sca050-2		6–13	3	64.5	9.1
Sca118-2		12–15	4	50.0	9.0
Sca196-1	2H	16–17	3	17.5	6.2
Sca062-2		16–18	3	37.5	13.0
Sca244-1		16–22	1	82.5	7.8
Sca212-1		19	3	19.0	11.8
Sca169-1		19–23	2	60.0	8.5
Sca204-2		20–23	3	41.0	18.7
Sca175-1		23–25	5	41.5	15.2
Sca285-2		25–28	7	28.5	28.1
Sca074-1	3H	29–33	4	57.0	18.5
Sca236-2		32–35	5	48.0	13.3
Sca088-1		34–37	3	60.5	8.0
Sca227-1		37	4	22.5	10.2
Sca030-1		37–40	3	62.5	10.4
Sca035-2		38–41	1	47.5	4.4
Sca059-1	4H	42–46	2	35.5	8.7
Sca214-1		45–50	1	82.0	7.5
Sca213-1		49–59	3	121.5	14.4
Sca220-1		49–50	2	41.5	5.4
Sca217-1		50	2	22.5	4.6
Sca235-1		51–54	3	31.0	5.5
Sca221-2		55	2	19.0	9.6
Sca161-2		56–59	3	30.0	9.5
Sca101-1		57–59	2	15.0	3.7
Sca277-1	5H	60–66	3	105.5	12.9
Sca034-2		61–64	3	52.5	12.1
Sca299-2		63–67	4	98.0	18.0
Sca042-1		63–65	2	43.5	8.4
Sca229-1		65–66	3	47.0	8.5
Sca291-1		67–68	3	44.0	8.1
Sca073-1		68–71	4	33.5	19.7
Sca161-2		70–71	3	9.0	9.5
Sca017-1	6H	72–78	4	134.0	20.3
Sca312-2		73–77	2	83.5	11.8
Sca051-2		74–77	2	55.5	6.2
Sca002-1		77–78	3	30.5	15.5
Sca036-1		78–80	2	31.5	6.9
Sca199-1		79–80	2	5.0	3.1
Sca245-1	7H	81–83	4	37.0	15.3
Sca246-1		83	4	17.5	8.1
Sca297-2		85–86	5	28.0	9.6
Sca186-1		86–94	2	92.0	9.8
Sca169-1		92–95	2	32.5	8.5
Sca196-1		95–98	3	20.5	6.2
Average S42			3.0	46.0	10.8
T42 Pre-ILs					
Thu012-1	1H	1–3	4	41.5	16.6
Thu011-1		1–12	3	85.0	20.5
Thu001-1		2–12	3	78.0	18.6
Thu035-2		3	4	24.5	10.8
Thu016-2		7–12	1	43.5	4.4
Thu044-1	2H	16–18	3	37.5	14.7
Thu077-1		19–22	1	45.5	5.6
Thu078-1		20–21	1	30.5	3.0
Thu036-2		20–24	1	59.0	5.4
Thu046-1		24–28	3	55.0	8.3
Thu052-1		26–28	1	26.5	2.7
Thu093-1		28	1	6.5	0.7
Thu084-1	3H	29–30	3	22.5	6.6
Thu070-1		32–39	4	122.5	19.7
Thu086-1		38	4	20.0	17.7
Thu010-1		39–41	6	30.0	14.6
Thu064-1		40–41	3	20.0	3.9
Thu090-1	4H	42–44	1	14.0	1.4
Thu069-1		42–45	2	23.5	3.7
Thu059-2		44–49	1	68.0	6.9
Thu058-1		45–48	2	40.5	8.6
Thu056-1		45–50	5	84.5	18.0
Thu038-1		50	3	25.0	5.3
Thu039-1		50–59	1	102.5	10.4
Thu034-2		59	4	5.0	10.8
Thu073-1	5H	60–62	4	33.5	11.1
Thu043-2		60–63	3	64.0	13.8
Thu088-1		62–68	4	125.5	18.2
Thu085-1		66–68	1	79.5	8.0
Thu045-1		66–70	2	99.5	10.7
Thu048-1		69–70	3	18.5	7.6
Thu092-1	6H	72	2	45.0	6.6
Thu014-1		72–77	3	117.5	16.6
Thu044-1		74–76	3	58.5	14.7
Thu009-1		77–78	1	25.5	2.6
Thu015-1		78	2	2.5	9.2
Thu065-1	7H	83	2	4.5	4.6
Thu034-2		83–86	4	47.5	10.8
Thu022-1		85–98	3	120.5	19.3
Thu020-1		85	1	9.0	0.8
Thu026-R		90–98	2	83.5	12.8
Thu004-1		95–98	2	20.5	6.6
Thu005-1		96–98	2	9.0	6.5
Average T42			2.5	48.3	9.8

[a]Chromosomal location of the target introgression
[b]SSR marker interval (see Table 1 for numeric code of SSR markers)
[c]Total number of introgressions in each pre-IL
[d]Size of the target introgression in centiMorgans
[e]Percentage of exotic germplasm per pre-IL

(Scarlett or Thuringia), the environment and the respective interactions as factors. The effect of the introgression genotype at 2HS and the effect of the environment were significant ($P < 0.01$). The effects of the genetic background and of all possible interactions were not significant. The introgression on chromosome 2HS maintained its effect across the two genetic backgrounds and across the tested environments. Interactions of introgressions and genetic background can be a critical issue when QTL alleles discovered in one genetic background are to be transferred into another genetic background (Melchinger et al. 1998). Low interactions of introgression and genetic background are advantageous, because these introgressions may then be transferred into a broad range of cultivars and still exhibit the desired effect.

Comparison of pre-ILs carrying an introgression on chromosome 2HS

The mean values for the trait days to Hea of the selected pre-ILs were compared with those of the corresponding control genotypes, Scarlett and Thuringia, using a

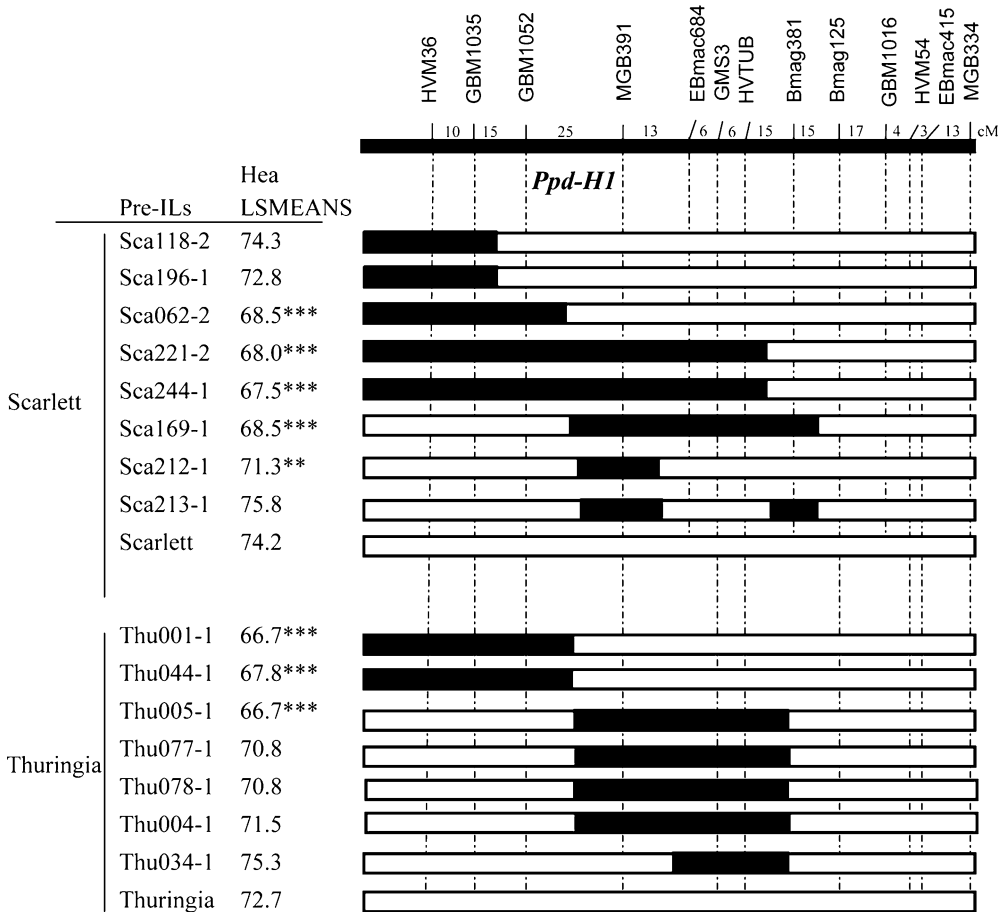

Fig. 2 Graphical genotypes for pre-ILs (S42, T42) carrying an exotic introgression on the chromosome arm 2HS. The chromosome 2H linkage map is depicted at the top and marker positions are indicated by *vertical dotted lines*. The assumed location of the *Ppd-H1* gene is indicated *below* the chromosome. CentiMorgan distances between adjacent markers are given. Three SSR markers were only used in the S42 population, and *dotted lines* for these markers do not extend beyond the pre-ILs derived from S42. A *black solid bar* indicates the exotic introgressions. The effect of introgressions on the chromosome arm 2HS on the trait days to heading is given by least square means values next to the pre-IL's code names and significant trait deviations from the respective control, cultivars Scarlett or Thuringia, are indicated with *asterisks* (**$P < 0.01$, ***$P < 0.001$)

Dunnett test (Fig. 2). Pre-ILs with an introgression at the marker locus GBM1052, Sca062-2, Sca221-1, Sca244-1, Thu001-1 and Thu044-1, flowered significantly earlier, on average 6 days (S42, T42), than the respective recurrent parents (Fig. 2). However, some of the pre-ILs carrying an introgression only at the adjacent marker MGB391, Sca169-1, Sca212-1 and Thu005-1, also flowered significantly earlier. This allows the assumption that the putative exotic locus, which promotes early flowering, is located within the marker interval GBM1052 and MGB391 on 2H. Since the exact recombination sites within this marker interval cannot be detected, we assume that recombination sites differ between the tested lines. Pre-ILs, which show earlier flowering, carry the introgression at the *Ppd-H1* locus in contrast to the pre-ILs, Sca213-1, Thu077-1, Thu078-1 and Thu004-1, which do not show early flowering. In these pre-ILs, the introgression distal to MGB391 might be smaller and therefore not extend to the locus promoting early flowering. In order to determine the position of the putative flowering gene in this interval, additional markers should be mapped in this region. Thiel et al. (2003) have collocated the SSR marker GBM1052 with the RFLP marker MWG858, which falls into the bin 4 on chromosome 2H, according to Kleinhofs and Graner (2001). Our findings correspond to results by Hayes et al. (1993), Pan et al. (1994) and Kjaer et al. (1995), who mapped a QTL for heading time at ABG318 in bin 4.

Conclusion

In this study, the generation and constitution of two sets of pre-ILs introgressed with the same exotic donor are described. We have demonstrated that pre-ILs can already serve for the verification of QTLs, i.e. through the analysis of segments carrying candidate genes. The effect of the introgression at a locus known to carry the photoperiod response gene *Ppd-H1* was verified in two genetic backgrounds and in four different environments. Pure introgression lines are currently generated by

marker-assisted backcrossing from the pre-ILs presented in this study. An additional set of pre-ILs is currently established using a winter barley cultivar as the recipient and a different exotic accession as the donor. These sets of introgression lines will facilitate the assessment and utilization of exotic barley with the purpose to promote the breeding progress and to enable studies of gene function. The two barley cultivars used here as recipients are leading malting varieties, and derived lines may be the basis for breeding a new cultivar. Additional perspectives for ILs are to use them for locating new DNA markers on the genome, and for fine mapping of genes. Near isogenic lines have been successfully used to perform fine mapping in maize and teosinte (Dorweiler et al. 1993), in tomato (Alpert and Tanksley 1996; Yates et al. 2004) and in rice (Yamanoto et al. 1998; Takeuchi et al. 2003). ILs are a first step towards the map-based cloning of QTLs (Frary et al. 2000, Yano et al. 2000). ILs may also be applied for genetic analyses of transcriptome and proteome variations. Studying differential expression in introgression lines allows the analysis of specific genome segments, which have previously been associated with a particular phenotype. Differences in transcript or proteome profiling of these ILs are directly attributable to the specific introgressed segments.

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