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Discovery of loci determining pre-harvest sprouting and dormancy in wheat and barley applying segregation and association mapping

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Abstract

Three wheat and two barley populations were studied in order to find loci responsible for dormancy and pre-harvest sprouting. A classical quantitative trait loci analysis was combined with an association mapping approach. Many quantitative trait loci and marker trait associations could be detected on all seven chromosome groups of wheat and on the chromosomes 2H, 3H, 5H, 6H, and 7H of barley. Especially, the known regions on chromosomes 3A and 4A for wheat and 5H for barley were confirmed. Putative functions could be found *via* a candidate homologues search and *via* expressed sequence tag annotation. On chromosome 3A, the *viviparous1* gene is located which is associated to pre-harvest sprouting and dormancy. On chromosome 4A, a protein is detected which belongs to the aquaporin family. In barley, an association with the *aleurain* gene on chromosome 5H was found. The expression of *aleurain* is regulated by abscisic acid and gibberelic acid. An influence of both hormones on dormancy and pre-harvest sprouting is known. It can be concluded that dormancy and pre-harvest sprouting are very complex traits regulated by multigenes and/or quantitative trait loci.

Additional key words: abscisic acid, gene function, gibberellic acid, *Hordeum vulgare*, quantitative trait loci, *Triticum aestivum.*

Introduction

Pre-harvest sprouting (PHS) is a phenomenon of cereal crops when germination of grains occurs in the spikes before harvest and therefore it is a big problem in wheat and barley production. It reduces crop yield to the point of a total damage of the harvest. Seed dormancy can prevent sprouting but can also delay the malting process in cultivated barley (Sato *et al*. 2009). Many factors and mechanisms affect PHS and dormancy which have been described and reviewed in several articles (Gerjets *et al.* 2010, Kulwal *et al.* 2010). On the molecular background, much information based on a lot of mapping populations is available for wheat (Chang *et al*. 2011, Kulwal *et al.* 2012) and barley (Ullrich *et al*. 2009). Wheat loci for dormancy and PHS are known from nearly all chromosomes, mainly from 2A, 2B, 2D, 3A, 3B, 3D, 4A, 4B, 6B, 6D, and 7D. Especially, on homoeologous chromosome groups 3 and 4, there are important regions often described in the literature (Flintham 2000, Kato *et al*. 2001, Flintham *et al*. 2002, Groos *et al*. 2002, Osa *et al*. 2003, Kulwal *et al*. 2005, 2010, Lohwasser *et al*. 2005, Mori *et al*. 2005, Mares *et al*. 2009). New studies present the influence of the *viviparous-1* gene on pre-harvest sprouting tolerance. It is important for seed maturation processes and it plays an important role also in other species like maize, rice, and oat (Xia *et al*. 2008, Chang *et al*. 2011). Furthermore, the importance of red-kernel colour genes as marker for resistance to PHS is widely accepted (Foley and Fennimore 1998, Flintham *et al*. 2002). But also PHS resistance in white wheat could be mapped (Liu *et al*. 2011, Kulwal *et al.* 2012). Another essential role plays the aleurone layer which is regulated by abscisic acid (ABA) and gibberellins (GAs). The enzymes of the aleurone layer have been identified as a contributor to damage that occurs during PHS in all

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Abbreviations: ABA - abscisic acid; AM - association mapping; CS - Chinese Spring; D10 - dormancy at 10 °C; D20 - dormancy at 20 °C; DArT - diversity array technology; DI - dormancy index; EST - expressed sequence tag; GA - gibberellins; GLM - general linear model; IPK - Leibniz Institute of Plant Genetics and Crop Plant Research; ITMI - International Triticeae Mapping Initiative; LOD - logarithm of odds; MLM - mixed linear model; SOD - superoxide dismutase; MTA - marker trait association; NCBI - National Centre for Biotechnology Information; OWB - Oregon Wolfe barley; PHS - pre-harvest sprouting; QTL - quantitative trait loci.

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cereals (Bethke *et al*. 2002). Also, sensitivity of the embryo to ABA has been reported to be important for seed dormancy (Noda *et al*. 2002).

In barley, detection of QTL with significant dormancy effects is known from chromosome 5H and minor effects from chromosomes 4H and 7H (Foley and Fennimore 1998). Further studies show a major QTL controlling both PHS and dormancy on the long arm of 5H (Han *et al*. 1999, Gao *et al*. 2003, Li *et al*. 2004, Prada *et al*. 2004). For comparison, Ullrich *et al*. (2009) found dormancy QTL located on six barley chromosomes and PHS QTL on five chromosomes. Similarly to wheat, ABA plays an important role for dormancy of barley (Benech-Arnold *et al*. 1999). Most of the studies are based on biparental populations with standard genetic mapping. Now, new methods like transcript maps with expressed sequence tag (EST)-based markers and association mapping (AM) approaches are available. EST markers allow a search for putative biological functions and the identification of candidate genes at agronomically important loci (Stein *et al*. 2007). AM is a population based survey with a much larger and more representative gene pool used to identify trait-marker relationships. Recently, association mapping approaches have been successfully applied in the identification of various marker trait associations in *Arabidopsis* (Aranzana *et al*. 2005), rice (Agrama *et al*. 2007), barley (Kraakman *et al*. 2004, 2006), and wheat (Breseghello and Sorrells 2006, Crossa *et al*. 2007, Neumann *et al*. 2011, Kulwal *et al.* 2012).

Searching for dormancy and sprouting genes can help to prevent these effects. As they are complex traits with high genetic variation, it can be assumed that both traits are controlled by multigenes or quantitative trait loci (QTL). Simultaneously, environmental effects especially during the ripening stage and storage conditions influence both traits making it difficult to evaluate under field conditions (Torada and Amano 2002). Nevertheless, it is important to identify functional genes for cereal breeding.

In the present study, five cereal populations, two biparental and one multiparental wheat populations and two biparental barley populations with EST markers, were investigated in order to detect QTL and marker trait associations for PHS and dormancy and search for putative functions.

Materials and methods

Plants: Three wheat (*Triticum aestivum* L.) and two barley (*Hordeum vulgare* L.) populations were used to study pre-harvest sprouting and dormancy. All material was cultivated in Gatersleben, Germany, on experimental fields, in greenhouses, and/or in a foil tunnel in different years.

For wheat, two biparental populations and one multiparental population were investigated. One of them, the ITMI (International Triticeae Mapping Initiative) mapping population was derived from a cross between the cv. Opata 85 and the synthetic hexaploid wheat W7984. The parents of W7984 are *Aegilops tauschii* accession CIGM86.940 and durum wheat cv. Altar 84 (Börner *et al*. 2002). Here, 114 recombinant inbred lines and a map with 942 markers (Ganal and Röder 2007) are available. Second wheat population developed in IPK (Pestsova *et al*. 2001, 2006) contains 85 D genome introgression lines and a set of 80 markers. The parents of this population are cv. Chinese Spring and a genotype Synthetic 6x which was created by McFadden and Sears (1947) from a cross between a tetraploid wild emmer and *Aegilops tauschii*. The genome of *Ae. tauschii* is fully represented in these lines. The third population is based on 183 genebank accessions of *Triticum aestivum* primarily selected for seed longevity. Four hundred and twenty nine mapped DArT (Diversity Array Technology) markers and 1 837 unmapped DArT markers are available for an association mapping approach. Further detailed information about this population and subgroup structure is provided by Rehman Arif (2012).

For barley, two biparental populations were studied, the Oregon Wolfe barley (OWB) with the parents OWB_{DOM} (two-row barley) and OWB_{REC} (six-row barley) (Costa *et al*. 2001), and Steptoe × Morex, a cross between a feeding barley and a malting and brewing barley (Kleinhofs *et al*. 1993). Each of the populations contains 94 doubled haploid lines and a marker set of 737 and 442 EST markers, respectively (Stein *et al*. 2007).

Pre-harvest sprouting test: Five ears per line directly harvested at maturity were placed in a plate full of wet sand for 14 d. For the interpretation of the data, a rating of seven score points was used (Fig. 1). A replication followed after 14 d with ripened ears. A mean value of both scoring results was calculated.

Dormancy test: Sixty seeds per line directly harvested at maturity were tested under a 12-h photoperiod (irradiance of 200 μmol m^{-2} s^{-1}), relative humidity of 90 %, and two different temperatures: 20 °C for 7 d and 10 °C for 14 d. The evaluation of the seed dormancies was conducted according to the germination testing rules of the International Seed Testing Association (ISTA 2010). The percentages of dormant seeds at 10 °C and at 20 °C were computed. In addition, the dormancy index (DI) was calculated: DI = [2 × (% dormant seed at 10 °C) + % dormant seed at 20 °C] / 3 (Strand 1965).

Statistical analysis: QTL analysis of the biparental populations was conducted with the programme *QGene* (Nelson 1997) using single marker analysis to identify marker loci linked with the phenotypic variation and interval analysis to identify genomic regions with significant impacts on each trait. Only QTL with a LOD

threshold > 3.0 ($P < 0.001$) were determined as significant. The putative functions of QTL and marker trait associations located in key genomic regions in wheat were established *via* candidate homologue searches using the deletion maps of wheat cv. Chinese Spring (CS) with candidate gene identities assigned to the corresponding chromosome bins based on *NCBI* (National Centre for Biotechnology Information) Genbank non-redundant database matches to nucleic acid and peptide sequences (http://wheat.pw.usda.gov/pubs/ 2004/Genetics/Bioinfo/).

For the two barley populations, EST based markers are available. Only markers in a ± 10 cM interval detected by single marker analysis and having LOD values > 3.0 were considered. The sequence information of the barley ESTs are stored in the *CR-EST* database (*IPK Crop EST* database, *v. 1.5*) (http://pgrc.ipk-gatersleben.de/cr-est). Annotation of the ESTs was performed by *BLASTX* (Basic Local Alignment Tool) similarity search against the public non-redundant protein database from *NCBI*.

For phenotype-genotype association analysis, the software programme *TASSEL 2.1* was used to calculate associations between the markers and each trait. Two model approaches, the general linear model (GLM) and the mixed linear model (MLM), were employed in order to determine marker trait associations (MTAs). A MTA was considered only if it occurred in both models at $P < 0.01$.

Results

The performance of the parents and the lines of the three wheat and two barley populations is summarized in Table 1. For all populations, a good diversity and segregation within the traits PHS and dormancy at 20 °C (D20) and at 10 °C (D10), as well as for the dormancy index (DI) could be observed.

The outcomes of the QTL analysis for wheat and barley are presented in Tables 2 and 3 showing the LOD value as well as the explained variation and the donor of the favourable allele. In Table 4, the marker trait associations together with the putative functions are accessible.

In the wheat ITMI population, four QTL could be identified related to dormancy and two related to PHS. The loci for dormancy and PHS were distributed on chromosomes 1D (1 QTL for D20), 3A (1 for D20), 4A (1 for D20, 1 for DI), and 1A (1 QTL for PHS) and 4A (1 for PHS), respectively. With respect to dormancy and

Table 1. Phenotypic values of parents and the lines of the populations for the dormancy at 10 °C (D10), dormancy at 20 °C (D20), dormancy index (DI), and pre-harvest sprouting (PHS) calculated according to Fig. 1.

ITMI	Opata 85	W 7984	RILs mean	SD	min	max
D10 [%]	1.67	1.67	1.29	3.39	0.00	30.00
D20 [%]	26.67	81.67	64.02	33.42	0.00	100.00
DI	10.00	28.33	22.20	11.85	0.00	53.33
PHS	6.45	2.05	4.00	1.72	1.00	7.00
Introgression Lines	Chinese Spring	Synthetic 6x	lines			
D10 [%]	33.33	25.00	23.83	16.81	0.00	73.33
D20 [%]	61.67	90.00	77.57	18.19	21.67	100.00
DI	42.78	46.67	41.74	14.37	10.56	80.56
PHS	1.60	4.00	3.87	1.09	1.50	7.00
Ass. mapping population			lines			
D10 [%]			11.97	17.12	0.00	95.00
D20 [%]			75.67	29.07	0.00	100.00
DI			33.21	17.31	0.00	95.56
PHS			4.01	1.49	1.20	6.90
OWB	DOM	REC	DH lines			
D10 [%]	11.67	30.00	20.04	23.15	0.00	95.00
D20 [%]	91.67	83.33	65.70	32.21	1.67	100.00
DI	38.33	47.78	35.26	22.53	1.11	96.67
PHS	3.00	3.80	4.79	1.76	1.00	7.00
S × M	Steptoe	Morex	DH lines			
D10 [%]	96.67	0.00	23.08	26.59	0.00	95.00
D20 [%]	100.00	6.67	69.95	35.60	0.00	100.00
DI	97.78	2.22	38.71	26.20	0.00	96.67
PHS	2.10	6.70	4.69	1.31	1.70	7.00

Table 2. QTL for the dormancy at 20 °C (D20), dormancy at 10 °C (D10), dormancy index (DI), and pre-harvest sprouting (PHS) traits in wheat (ITMI and introgression lines, IGL)

	Trait	QTL symbol	Chromosome	Nearest markers	LOD	Explained variation [%]	Donor of favourable allele
ITMI	D20	*QD20.ipk-1D*	1D	GWM458	3.01	53.7	synthetic
		QD20.ipk-3A	3A	ATPased	3.52	22.1	Opata
		QD20.ipk-4A	4A	WG622	5.79	22.8	synthetic
	D10			KSUG12b	5.43	20.0	synthetic
				GWM894	4.69	26.6	synthetic
				GWM937	4.24	24.6	synthetic
	DI	*QDI.ipk-4A*	4A	WG622	5.78	22.8	synthetic
				KSUG12b	5.36	19.8	synthetic
				GWM894	4.43	25.3	synthetic
				GWM937	4.00	23.4	synthetic
	PHS	*QPHS.ipk-1A*	1A	GWM135	3.22	20.1	Opata
		QPHS.ipk-4A	4A	KSUG12b	11.41	38.3	Opata
				GWM894	9.37	46.0	Opata
	D10			WG622	8.90	33.6	Opata
				GWM937	7.42	39.0	Opata
				GWM397	5.41	31.4	Opata
				BCD402b	4.07	18.3	Opata
IGL	D20	*QD20.ipk-6D*	6D	GDM98	3.80	18.8	synthetic
	DI	*QDI.ipk-6D*	6D	GWM1401	3.02	15.3	synthetic

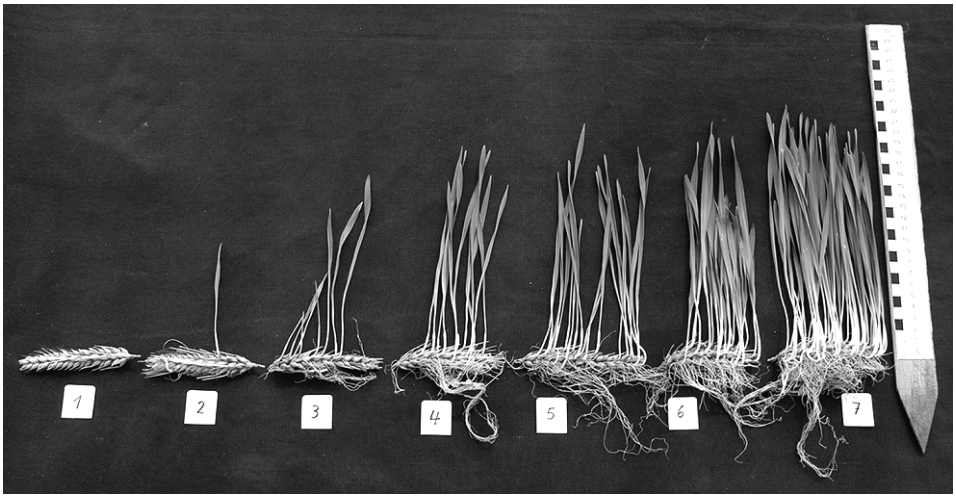

Fig. 1. Scoring the PHS test (1 = no coleoptile, 7 = all seeds germinated).

sprouting, a major region on chromosome 4A could be localised. Interval mapping shows a region on chromosome 4A between the markers *Xgwm731* and *Xmwg549b* and a second region on the long arm of chromosome 3A between the markers *Xgwm1159* and *Xgwm32* only for dormancy (Fig. 2). The location on chromosome 4A fell into bin C-4AL12-0.43 which contains the γ-type tonoplast intrinsic protein of wheat belonging to the aquaporin family. The region on 3A coincided with bin 3AL5-0.78-1.00 which includes the *viviparous1* gene.

The second biparental wheat population (D genome introgression lines) shows only one region on chromosome 6D with only one QTL for D20 and DI each. Looking for a putative function, an association with bin 6DL6-0.29-0.47 could be found in which the betaine-aldehyde dehydrogenase involved in abiotic stress tolerance is located.

In addition, a multiparental population was studied for an association mapping approach. Only the mapped markers were included in the calculation. In total, 36 MTAs could be detected, 12 for PHS and 24 for dormancy which can be separated in seven for D20, six for D10, and 11 for DI. Five loci show joined MTAs for PHS and dormancy. Comparing the MTAs with known loci from the literature (Francki *et al*. 2009), an assignment to chromosomal bins is also possible (Table 4). As important putative functions, again the *viviparous1* gene on chromosome 3AL could be detected but also proteins involved in biotic and abiotic stresses.

Regarding barley, the OWB population and crosses

Steptoe × Morex (S×M) were investigated. In the OWB population, QTL could be found on the chromosomes 2H (1 QTL for D20, 1 for PHS), 3H (1 for D10, 1 for DI) 5H (1 for D10, 1 for DI, 1 for PHS), 6H (1 for D20, 1 for DI), and 7H (1 for D20, 1 for DI, 1 for PHS) whereas in S×M, loci with a high LOD score occurred, 1 QTL for D10, 1 for D20, 1 for DI, and 1 for PHS only on the chromosome 5H (Table 3). Interval analysis shows a very large region on chromosome 5H in both populations (Fig. 3, shown for S×M). The annotation of the ESTs of both populations is given in a supplementary Table S1 (*see on-line*) but not from all markers ESTs are known. As biological functions especially in barley, the proteins iron/ascorbate-dependent oxidoreductase and β-hordothionin which both are involved in stress response could be detected. Another interesting region is on chromosome 7H, the *nud* gene which is responsible for naked and hulled grains. All QTL and MTAs for wheat and barley are combined in Fig. 4 and supplementary Fig. S1 (*see on-line*). Only on two chromosomes (1DL; 3AL), QTL and MTAs were in the same region. This demonstrates the complexity of the PHS and dormancy traits.

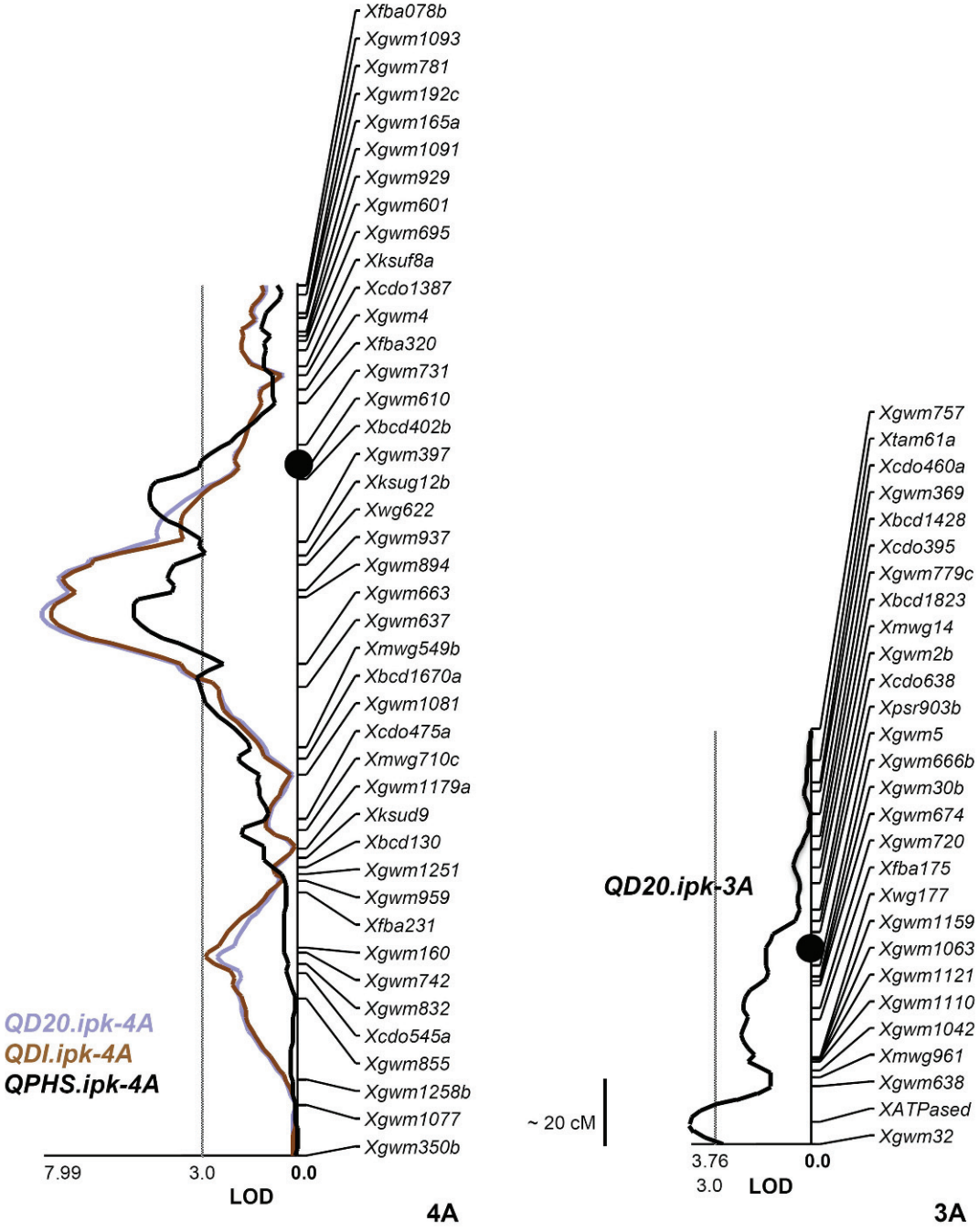

Fig. 2. Interval mapping of ITMI population.

Table 3. QTLs for the dormancy at 20 °C (D20), dormancy at 10 °C (D10), dormancy index (DI), and pre-harvest sprouting (PHS) traits in barley. Only markers with putative functions are shown.

	Trait	QTL symbol	Chromosome	Nearest markers	LOD	Explained variation [%]	Donor of favourable allele
OWB	D10	*QD10.ipk-3H*	3H	GBR1792	3.96	18.3	DOM
			3H	GBR1138	3.68	17.5	DOM
		QD10.ipk-5H	5H	GBS0318	4.21	19.2	REC
			5H	GBS0042	4.08	19.2	REC
			5H	GBR188	3.68	18.1	REC
	D20	*QD20.ipk-2H*	2H	GBR1682	4.42	19.8	REC
			2H	GBS0003	4.24	19.1	REC
			2H	GBS0864	4.14	18.5	REC
			2H	GBS0236	3.83	17.3	REC
		QD20.ipk-6H	6H	GBR504	4.80	22.5	DOM
			6H	GBM1027	4.47	19.8	DOM
			6H	GBR458	4.13	19.0	DOM
			6H	GBR1597	3.68	17.2	DOM
		QD20.ipk-7H	7H	GBR1478	4.21	20.2	DOM
			7H	GBR283	4.15	19.9	DOM
			7H	Nud	3.94	18.1	DOM
			7H	GBM1359	3.59	17.5	DOM
			7H	GBS0040	3.52	17.8	DOM
			7H	GBR159b	3.45	16.9	DOM
	DI	*QDI.ipk-3H*	3H	GBR1792	3.65	17.0	DOM
			3H	GBR1138	3.43	16.4	DOM
		QDI.ipk-5H	5H	GBS0318	4.67	21.1	REC
			5H	GBR188	4.29	20.7	REC
			5H	GBS0042	4.13	19.4	REC
			5H	GBR1057	3.93	19.2	REC
		QDI.ipk-6H	6H	GBR504	3.59	17.3	DOM
			6H	GBS0617	3.24	14.8	DOM
		QDI.ipk-7H	7H	GBR159b	3.53	17.2	DOM
			7H	GBR1478	3.07	15.2	DOM
	PHS	*QPHS.ipk-2H*	2H	GBM1462	3.15	15.1	DOM
			2H	GBS0105	3.11	14.6	DOM
		QPHS.ipk-5H	5H	GBR188	6.55	29.9	DOM
			5H	GBS0242	5.63	24.3	DOM
			5H	GBS0330	5.54	24.5	DOM
			5H	GBR1349	5.45	24.4	DOM
			5H	GBR0986	5.28	24.4	DOM
			5H	GBS0318	4.97	22.2	DOM
			5H	GBR1564	4.93	23.7	DOM
			5H	GBS0286	4.81	21.2	DOM
			5H	GBS0019	4.49	19.9	DOM
			5H	GBS0042	4.01	18.9	DOM
			5H	GBR518	3.71	18.8	DOM
			5H	GBS0283	3.66	16.6	DOM
			5H	GBR1082	3.57	17.0	DOM
			5H	GBM1173	3.05	14.2	DOM
		QPHS.ipk-7H	7H	GBR159b	3.71	18.0	REC
			7H	Nud	3.32	15.5	REC
			7H	GBR1478	3.01	14.9	REC
S × M	D10	*QD10.ipk-5H*	5H	GBS0892	8.97	37.5	Steptoe
			5H	GBS0613	7.36	34.6	Steptoe
			5H	GBR1543	6.02	29.0	Steptoe
			5H	Ale	5.59	27.8	Steptoe
			5H	GBR1041	4.99	23.0	Steptoe
			5H	GBR0482	3.56	18.1	Steptoe
			5H	GBS0561	3.42	16.4	Steptoe
			5H	GBS0462	3.24	15.6	Steptoe
	D20	*QD20.ipk-5H*	5H	GBS0892	17.55	60.1	Steptoe
			5H	GBS0613	13.76	54.7	Steptoe
			5H	Ale	8.92	40.5	Steptoe

cont.

		5H	GBS0561	6.83	30.1	Steptoe
		5H	GBS0462	6.29	28.1	Steptoe
		5H	GBR0482	5.75	27.6	Steptoe
		5H	GBR1543	5.62	27.4	Steptoe
		5H	GBR1041	5.37	24.5	Steptoe
		5H	Ltp1	5.23	24.2	Steptoe
DI	*QDI.ipk-5H*	5H	GBS0892	15.86	56.4	Steptoe
		5H	GBS0613	12.57	51.5	Steptoe
		5H	Ale	8.71	39.8	Steptoe
		5H	GBR1543	7.60	35.1	Steptoe
		5H	GBR1041	6.57	29.1	Steptoe
		5H	GBS0561	5.82	26.2	Steptoe
		5H	GBM1041	5.59	27.0	Steptoe
		5H	GBR0482	5.48	26.5	Steptoe
		5H	GBS0462	5.43	24.7	Steptoe
		5H	Ltp1	4.35	20.6	Steptoe
PHS	*QPHS.ipk-5H*	5H	GBS0892	9.20	38.2	Morex
		5H	GBS0613	6.35	30.6	Morex
		5H	Ale	4.66	23.8	Morex
		5H	GBR1543	4.42	22.2	Morex
		5H	GBR1041	3.90	18.4	Morex
		5H	GBR0097	3.31	18.2	Morex
		5H	GBS0561	3.16	15.2	Morex
		5H	GBR0287	3.06	14.8	Morex

Table 4. MTAs and putative functions of wheat association mapping population (markers with *an asterisk* are considered as one marker trait association)

Trait	MTA symbol	Chromosome	Marker	Bin	Function (*Triticum*)
PHS	*MPHS.ipk-1A*	1A	wpt6709	1AS3-0.86-1.00	γ-gliadin Ω-gliadin storage protein gene
D20; DI; PHS	*MD20.ipk-1B; MDI.ipk-1B; MPHS.ipk-1B*	1B	wpt6975	-	
D10; DI	*MD10.ipk-1D; MDI.ipk-1D*	1D	wpt3707	-	
PHS	*MPHS.ipk-1D.1*	1D	wpt3945*	1DL4-0.18-0.41	type V thionin precursor
PHS	*MPHS.ipk-1D.1*	1D	wpt4988*	-	
PHS	*MPHS.ipk-1D.2*	1D	wpt7697	-	
DI; PHS	*MPHS.ipk-2B; MDI.ipk-2B*	2B	wpt0694*	-	
DI; PHS	*MPHS.ipk-2B; MDI.ipk-2B*	2B	wpt5128*	2BL6-0.89-1.00	MnSOD blue copper-binding protein homolog cystatin
DI	*MDI.ipk-2B*	2B	wpt3378*	-	
DI	*MDI.ipk-2B*	2B	wpt2135*	-	
DI	*MDI.ipk-2B*	2B	wpt7360*	-	
D10; D20; DI	*MD10.ipk-3A.1; MD20.ipk-3A.1; MDI.ipk-3.1*	3A	wpt1562	3AL5-0.78-1.00	viviparous1 repressor protein (Dr1) (1-3)-β-glucanase peroxidase 1
D10; D20; DI	*MD10.ipk-3A.2; MD20.ipk-3A.2; MDI.ipk-3A.2*	3A	wpt3816*	C-3AL3-0.42	-
DI	*MDI.ipk-3A.2*	3A	wpt9422*	-	
PHS; DI	*MDI.ipk-3B.1; MPHS.ipk-3B.1*	3B	wpt7015*	3BS9-0.57-0.78	oxalate oxidase 1-*cis*-peroxiredoxin
PHS; DI	*MDI.ipk-3B.1; MPHS.ipk-3B.1*	3B	wpt5390*	-	

cont.

PHS; DI	*MDI.ipk-3B.1; MPHS.ipk-3B.1*	3B	wpt9170*	3BS9-0.57-0.78	oxalate oxidase
					1-*cis*-peroxiredoxin
PHS; DI	*MDI.ipk-3B.1; MPHS.ipk-3B.1*	3B	wpt5105*	3BS9-0.57-0.78	oxalate oxidase
					1-*cis*-peroxiredoxin
PHS; DI	*MDI.ipk-3B.2; MPHS.ipk-3B.2*	3B	wpt6973	C-3BL2-0.22	-
D10; DI; PHS	*MD10.ipk-4A; MDI.ipk-4A; MPHS.ipk-4A*	4A	wpt0610	-	
D10; DI	*MD10.ipk-4B; MDI.ipk-4B*	4B	wpt3991	-	
D20	*MD20.ipk-4D*	4D	wpt4572	-	
PHS	*MPHS.ipk-5A*	5A	wpt1165	-	
D20	*MD20.ipk-5B*	5B	wpt3661	-	
PHS	*MPHS.ipk-6A*	6A	wpt0864*	-	
PHS	*MPHS.ipk-6A*	6A	wpt8006*	6AS1-0.35-0.65	thiosulfate transferase
					α-gliadin storage protein
D10; DI	*MD10.ipk-6B; MDI.ipk-6B*	6B	wpt3733	-	
D20	*MD20.ipk-6B*	6B	wpt1730	-	
D20	*MD20.ipk-7A*	7A	wpt7763	7AS8-0.45-0.59	p34cdc2
PHS	*MPHS.ipk-7B.1*	7B	wpt4863	-	
PHS	*MPHS.ipk-7B.2*	7B	wpt4875	-	

Discussion

On all seven chromosome groups of wheat and on the chromosomes 2H, 3H, 5H, 6H, and 7H of barley, QTL and/or MTAs for PHS and dormancy were detected. Putative functions were described and if possible, interactions with dormancy and PHS explained.

For wheat, a homoeologous region for PHS and dormancy on the long arm of chromosomes 1A, 1B, and 1D near to the centromere were detected. For the region of chromosome 1B, a thionin precursor is known which is involved in plant reactions against microbial infections and microbial pathogens toxic to plants. Thionins located in the endosperm of wheat are encoded by genes on all three group 1 chromosomes (Sanchez-Monge *et al*. 1979, Romero *et al.* 1997). The region on chromosome 1D is also known from another association panel responsible for PHS (Rehman Arif *et al.* 2012). One additional MTA on 1AS is associated with a gliadin protein which is one of the major storage proteins of wheat. For the other MTAs on the short and long arms of 1D, no putative function could be found.

As concerns chromosome group 2, only on the long arm of 2B, MTAs for dormancy and PHS are located. For this region, the MnSOD, blue copper-binding protein homologue, and cystatin are mentioned. MnSOD is the primary protective enzyme for oxidative stress in mitochondria and plays a possible role in tolerance to environmental stresses, such as chilling, freezing, oxidative stress, aluminum toxicity, *etc.* (Baek *et al*. 2006). The blue copper-binding protein is involved in defence against oxidative stress. One possible role could be prevention of the synthesis of hydroxyradicals by binding copper ions, thereby reducing deleterious reactive oxygen species (Jansen *et al*. 2005). The third protein, cystatin, is an important determinant of the flour quality which is reduced by PHS (Kuroda *et al*. 2001). A major QTL for PHS resistance which was found in white wheat on 2B could not be confirmed (Munkvold *et al*. 2009).

In barley, on chromosome 2H, two QTL were located, one for dormancy on the short arm and one for PHS on the long arm. The dormancy QTL shows an association to some proteins detected in rice and wheat, and for the sprouting QTL, no result was found based on EST annotation. Describing the proteins, only a peroxidase is of special interest for our studies which is also involved in oxidative stress response (Higa *et al*. 2001).

On chromosome 3AL of wheat two major regions for dormancy could be ascertained. On 3A, the *viviparous-1* gene which is also involved in oxidative stress response (Higa *et al*. 2001) is located (Chang *et al*. 2011, Xia *et al*. 2008). For the second region, no functions are available but it is possible that this is the same region as described by Kulwal *et al*. (2005) and Rehman Arif *et al.* (2012). On chromosome 3B, two areas with MTAs having the same putative functions were detected. The proteins oxalate oxidase and 1-Cys peroxiredoxin were assigned. Oxalate oxidase has been found to be concentrated in the surface tissues of wheat embryos and grains might have an influence on plant defence (Lane 2000). 1-Cys peroxiredoxin accumulates in the nucleus of seed cells suffering with oxidative stress but its primary function may be to avoid damage to DNA and nuclear structures (Pulido *et al*. 2009). For barley, two dormancy QTL on 3HS were detected but the EST based annotations show no clear connection with PHS and dormancy.

Important loci could be detected on chromosome 4 of wheat. The location of chromosome 4AL near to the

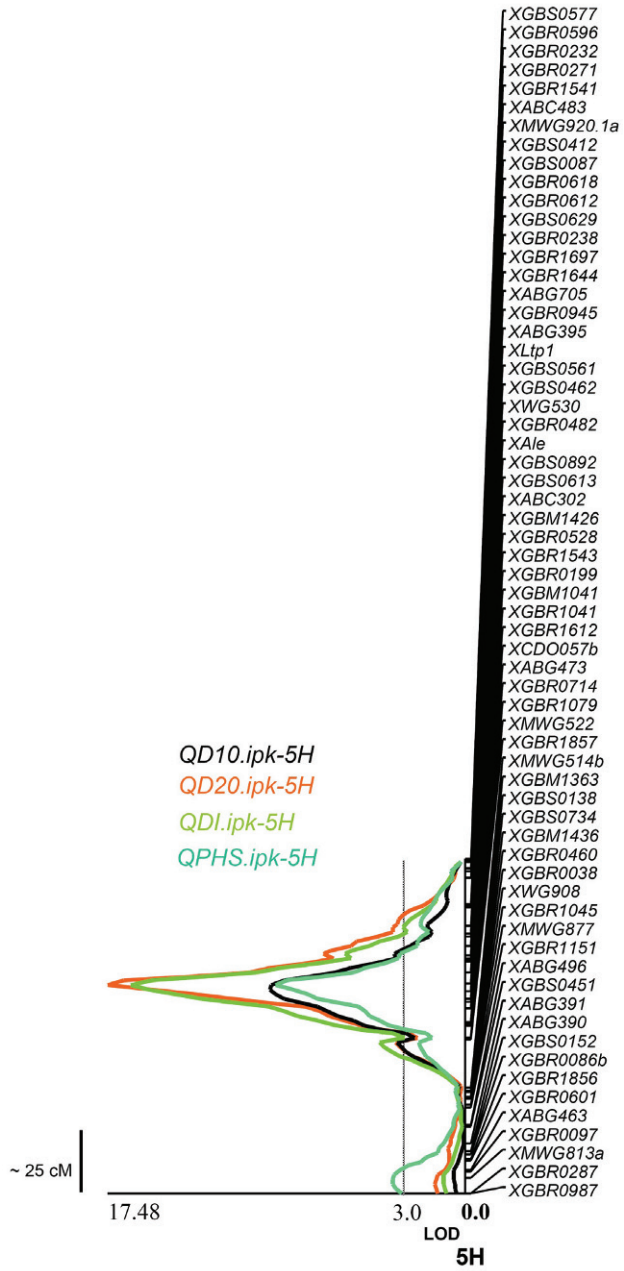

Fig. 3. QTLs from interval mapping for S × M on chromosome 5H.

centromere falls into bin C-4AL12-0.43 which contains the γ-type tonoplast intrinsic protein of wheat belonging to the aquaporin family. Aquaporins are responsible for flow of water through the cell membrane and play a vital role in all aspects of plant-water relations and osmotic stress response (Forrest and Bhave 2010). The other regions on the long arm of 4A (three MTAs for dormancy and sprouting) and on the short arm of 4D (one MTA for dormancy) are not connected to bins but possibly also associated with aquaporins which are known on both chromosome loci (Forrest and Bhave 2010). The QTL on 4A and the MTAs on 4B are in similar regions and may be homologous. The locus on 4AL is also described by Kato *et al*. (2001) as important for controlling seed dormancy and by Lohwasser *et al*. (2005) for PHS. Especially, the SSR marker *GWM 937*, which could be detected in the ITMI population, is linked to a major QTL for PHS resistance as well as strongly associated with seed dormancy (Ogbonnaya *et al.* 2008).

Concerning chromosome group 5, only two MTAs, one on 5AS and one on 5BL, could be detected in wheat. No link to a bin could be found. Especially for 5A, the map is highly unsaturated; only four mapped markers are available. For barley, two important regions can be localised, one in the centromere region and one on 5HL. In the centromere region the *aleurain* gene is located. The expression of *aleurain* is regulated by the plant hormones gibberelic acid and abscisic acid. Both hormones have also an influence on PHS and dormancy (Noda *et al*. 2002). An effect on seed longevity in barley (Nagel *et al*. 2009) which could be also influenced by sprouting and dormancy is known. Other annotations found connections to the iron/ascorbate-dependent oxido-reductase which is known as stress response gene, and β-hordothionin which belongs to the group of thionins which are involved in plant reactions against microbial infections and pathogens (see also chromosome group 1; Sanchez-Monge *et al*. 1979, Romero *et al* 1997). The region on 5HL is linked with an ankyrin kinase. This large protein family is involved in plant growth, development, and signal transduction (Huang *et al*. 2009). Both regions are also described by Ullrich *et al*. (2009) as important for dormancy and PHS in barley.

On chromosome 6AS, one MTA for PHS could be localised. This locus is associated with the gliadin storage protein which is also found on 1AS. Its second function is a thiosulfate transferase, an enzyme catalyzing sulfide oxidation to thiosulfate (Hildebrandt and Grieshaber 2008). Here, no clear connection to PHS and/or dormancy is visible, nevertheless, it could also play a role in stress response. Looking on chromosome 6B, two MTA regions are located, one in the centromere section and one on 6BL. No associations with bins could be detected but in both regions, aquaporins are positioned (Forrest and Bhave 2010). On 6DL, two QTL for dormancy are detected but no function could be designated to them. For barley, two QTL, especially for dormancy, could be placed on 6HS. An association with phenylalanine ammonia lyase was found out. Phenylalanine ammonia lyase catalyses the conversion of phenylalanine to trans-cinnamic acid which is the first step in the biosynthesis of phenylpropanoids leading to a diverse group of plant secondary metabolites including lignins, phytoalexins, and flavonoids. Increased lignification has been suggested to be an important component of induced defence responses in cereals (Kervinen *et al*. 1998). Possibly, the regions on 6B and 6H are homoeologous.

For group 7, we found one MTA on 7AL, two MTAs on 7BS and 7BL, and three QTL on 7HL near the centromere region. The MTAs on 7AL and 7BL could be

in a homoeologous region. For the MTA on 7AL, an association with a bin shows a connection to p34cdc2 which is cell cycle control protein in wheat leaves. For both MTAs on 7B no association could be found but on 7BS aquaporin is located in a similar region (Forrest and Bhave 2010). For barley, the QTL found only in the OWB population is connected to the *nud* gene, determining the hulled or naked character of the caryopsis in barley which is an important trait for edibility (Kikuchi *et al*. 2003). Another annotation leads to the putative transcription factor EREBP1 which plays a role in pollen development known from *Arabidopsis thaliana*.

Many putative functions have been determined during this study which gives an idea of the complexity of PHS and dormancy. One general problem is the number of big gaps in the map of the association mapping population. Only 429 markers are mapped which leads to no result in potential regions that are known from other studies. For the next investigation, a wheat consensus map which will be better saturated is necessary. Nevertheless, association mapping is important for identifying QTL. Furthermore, other traits should be included in the research to look for correlations with other morphological and agronomical characters. In conclusion, dormancy and pre-harvest sprouting are very complex traits. They are regulated by multigenes and/or many QTL. Further studies are necessary to identify responsible genes and markers for a marker-assisted selection.

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