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Genetic studies of seed longevity in hexaploid wheat using segregation and association mapping approaches

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Abstract Genebanks are entrusted with the storage, preservation and distribution of crop germplasm. Seed longevity is an important character in this context, but little is known regarding its genetic basis, largely because it is so strongly influenced by non-genetic factors. Here we present the outcome of a genetic dissection of seed longevity in bread wheat. We applied both a standard quantitative trait locus analysis based on segregation from a biparental cross, and an association analysis using a germplasm panel to detect marker trait associations. The latter revealed more loci than the former. Some of the genomic regions identified are known to contain genes determining spike architecture or aspects of biotic and abiotic stress responses. The results open perspectives for identification of favourable longevity alleles and the more accurate prediction of seed longevity in cereal germplasm collections.

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Introduction

Cropping lies at the heart of human innovation, and genetic diversity of crops developed by farmers over several millennia, aided over the last century by plant breeders and scientists is critical for its success. A sample of this diversity—in the major crop species at least—is maintained in collections curated in a variety of ex situ genebanks. Seeds (and in some cases other plant material) held in these collections provide the raw materials required for breeding crop varieties able to withstand environmental change and the ever-increasing demand for improved yield and quality (Anonymous 2001). Currently some 1,750 separate genebanks curate around 7.4 million accessions of crop species worldwide (FAO 2010).

Seed longevity is an important consideration in the context of ex situ conservation. Its non-genetic determinants are the ambient environment during seed development, the seed's moisture content and maturity of the seed at harvest, the presence of pathogenic microflora on the surface of, or within, the seed, the extent of mechanical damage to the seed and the post-harvest storage conditions (Copeland

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and McDonald 1995). Superimposed upon these variables is a genetic component of seed longevity; for example, seed of pea and alfalfa can remain viable for many decades, while those of other species (e.g., lettuce, onion, parsnip and rye) are short-lived (Walters et al. 2005; Nagel and Börner 2010). In addition there may be an effect of seed dormancy on longevity which is, so far, discussed controversially (Siddique et al. 1988; Miura et al. 2002).

Linkage analysis and, more recently, association mapping represent two very versatile methods for genetic dissection of quantitative traits (Zhu et al. 2008). The former relies on trait segregation in a population derived from a bi-parental cross, and has been used to identify QTL for seed longevity in *Arabidopsis thaliana* (Bentsink et al. 2000; Clerkx et al. 2004a, b), rice (Miura et al. 2002; Zeng et al. 2006; Xue et al. 2008), barley (Nagel et al. 2009) and oilseed rape (Nagel et al. 2011). The latter is a population-based method which relies on the detection of a linkage disequilibrium between a trait and a genetic marker (Jannink et al. 2001). As the test population consists of a set of unrelated accessions, no time needs to be expended for the construction of a mapping population. The approach has been applied with some success for the identification of marker trait associations (MTAs) in a range of plant species, including *A. thaliana* (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). However, seed longevity was not considered so far.

Here, we report the simultaneous analysis of a conventional (ITMI = International Triticeae Mapping Initiative) mapping population and an association mapping (AM) population, seeking to elucidate the genetic components of seed longevity in wheat. A dormancy test was performed in order to investigate the genetic relationship between longevity and dormancy.

Materials and methods

Plant materials

The ITMI mapping population used was derived from a cross between the variety 'Opata 85' and the synthetic hexaploid wheat 'W7984'. The parents of 'W7984' were *Aegilops tauschii* accession 'CIGM86.940' and durum (tetraploid) wheat variety 'Altar 84' (Börner et al. 2002). Two seed lots were available, one harvested in 2003 (consisting of 86 recombinant inbred lines lines (RILs)) and the other harvested in 2009 (99 RILs). Seventy-four of the lines were common to both years. After harvest the materials were stored in a cold chamber at 0°C. The AM population comprised a set of 96 winter wheat accessions assembled at the Institute of Field and Vegetable Crops, Novi Sad, Serbia; accessions were selected on the basis of their phenotypic diversity with respect to a group of key agronomic traits, and their provenance is spread over 21 countries. More detailed information of the AM population is provided elsewhere (Quarrie et al. 2003; Neumann et al. 2011). The material investigated here was regenerated in 2008/2009 in Novi Sad, Serbia.

Accelerated ageing (AA) and controlled deterioration (CD) tests

The purpose of AA tests was to mimic long term storage and hence allow for the evaluation of longevity. The tests subject the seeds to a combination of high temperature and high relative humidity, and this reflects longevity, as seeds with greater longevity deteriorate to a lesser extent than those with poor longevity (Hampton and TeKrony 1995). The AA test was applied to 200 seeds from each of the ITMI 2003 and 2009 RILs, and from each member of the AM population. The seeds were placed in a stainless metal cage sealed inside a glass jar containing 200 ml deionised water. The jars were held at 43 ± 0.5°C for 72 h, following International Seed Testing Association (ISTA) protocols (ISTA 2008). For the CD test, 200 seeds of each of the ITMI 2009 RILs and the AM population accessions were assessed for initial moisture content, following the ISTA protocols (ISTA 2008), and this was then adjusted to 18% by the addition of the appropriate volume of deionised water (Hampton and TeKrony 1995). After a 2 h equilibration at room temperature and a 22 h relaxation period at 7°C, the seeds were sealed in an aluminium foil bag and exposed to 43 ± 0.5°C for 72 h.

Germination and dormancy tests

Four replicates of 50 seeds each for control, AA and CD were subjected to a standard ISTA germination test, in which the seeds were placed between two layers of wet filter paper, formed into rolls and stood on a Jacobsen apparatus at 25 ± 1°C during the day and 23 ± 1°C during the night. The germination percentages were recorded on the eighth day (ISTA 2008). Apart from the absolute germination (in both non-treated and treated seed lots), a relative germination was determined by dividing the rates obtained after treatment by that of the non-treated control. A dormancy test was conducted in which a sample of 60 freshly harvested seeds per ITMI 2009 RIL was germinated at either 20°C for 7 days or 10°C for 14 days, both under a regime of 12 h light/12 h darkness and the proportion of non-germinated seeds at both temperatures were recorded. A dormancy index (DI), given by (2 × % dormant seed at 10°C + % dormant seed at 20°C)/3 was calculated following Strand (1965).

Statistical analyses

For the analysis of the ITMI population, genotypic data previously gathered for 942 loci consisting of mainly SSR and some RFLP loci were used. The data were kindly provided by Dr. M. Röder, IPK Gatersleben, and discussed in detail by Ganal and Röder (2007). QTL for both longevity (based on relative germination) and dormancy were detected using QGENE software (Nelson 1997), applying single marker analysis. The QTL obtained were classified into major (LOD > 3.0) and minor (LOD 1.5–3.0). The existence of a QTL was declared when a significant ($P < 0.01$) marker-trait association was recorded in at least two of the four replicates and the mean.

A DArT (**D**iversity **Ar**ray **T**echnology)-based linkage map was available for the AM population. DArT genotype profiling was done by Triticarte Pty. Ltd (Canberra, Australia; http://www.triticarte.com.au). The map consists of 525 markers with known chromosome and map positions, and 315 of unknown location (of which, however, 177 had been assigned a chromosomal location). Details of the AM population (e.g., population structure, linkage disequilibrium) are described by Neumann et al. (2011). Marker trait associations (MTAs) for relative germination for each replicate separately and for overall line means were performed within TASSEL v2.1 software (Yu et al. 2006). Both the general linear (GLM) and mixed linear models (MLM) were applied. An MTA was declared significant when a P value of less than 0.05 was obtained in at least 2 of the 4 replicates and the mean or highly significant when a P value of less than 0.01 was obtained in at-least 2 of the 4 replicates and the mean.

Results

ITMI mapping population

Germination tests showed a clear effect of the harvest year (Table 1). Seeds from the 2009 harvest germinated less readily, and showed a greater degree of between RIL variation (75.96 ± 15.89%) than did the 2003 set (83.85 ± 7.51%). The AA treatment reduced the germinations to 32.87 ± 22.78% and 62.63 ± 13.20%, respectively. The germination of seeds from 2009 subjected to CD reached 25.37 ± 24.89. The relative germination of 2009 seeds after AA treatment was 0.41 ± 0.25 and after the CD treatment was 0.31 ± 0.28. The AA treatment of the 2003 seeds revealed a relative value of 0.75 ± 0.10. The analysis of variance indicated that AA and CD significantly reduced the germinations compared to the control. It also indicated that there were significant differences among the RILs.

The predicted QTL are presented in Table 2 and Fig. 1. Following the AA test on the 2003 seeds, one major QTL (*QLng.ipk-2A*) was identified in the centromeric region of chromosome 2AS, linked to *Xcdo1281*. This QTL (LOD score 3.22) explained 20% of the phenotypic variance, with the favourable allele present in 'W7984'. Two minor QTL were detected, *QLng.ipk-2D* mapping to chromosome arm 2DL and *QLng.ipk-1D.1* to 1DL. The LODs for these two QTL were, respectively, 1.91 and 1.57, and the relevant linked markers and proportions of phenotypic variance explained were, respectively, *Xgdm6* (16.4%) and *Xcmwg695* (8.1%). The favourable allele at the chromosome 2D locus was present in 'W7984', and at the 1D locus in 'Opata'.

Table 1 Germinations before and after AA and CD treatments and relative germination in the ITMI RIL population and AM panel (means ± standard deviation)

	Initial germination (%)			Germination after AA (%)			Mean relative germination after AA (%)	Germination after CD (%)			Mean relative germination after CD (%)
	Max	Min	Mean	Max	Min	Mean		Max	Min	Mean	
ITMI 2003	97.0	59.0	83.85 ± 7.51	90.5	27.0	62.63 ± 13.20	0.75 ± 0.10	–	–	–	–
ITMI 2009	98.0	24.5	75.96 ± 15.89	92.0	0.5	32.87 ± 22.78	0.41 ± 0.25	90.0	0.5	25.37 ± 24.89	0.31 ± 0.28
AM	98.5	68.0	93.72 ± 5.05	60.5	0	10.97 ± 12.80	0.12 ± 0.13	95.0	5.5	61.05 ± 25.33	0.64 ± 0.26

Table 2 Single marker QTL analysis of the ITMI RIL population from grain harvested in 2003 and 2009

Year	Treatment	QTL designation	Marker	Chromosome	LOD score	Source	Phenotypic variation explained (%)
2003	AA	*QLng.ipk-2A*	*Xcdo1281*	2AS	3.22	W7984	20.1
		QLng.ipk-2D	*Xgdm6*	2DL	1.91	W7984	16.4
		QLng.ipk-1D.1	*Xcmwg695*	1DL	1.57	Opata	8.1
2009	AA	*QLng.ipk-3B*	*Xgwm376*	3BS	2.33	Opata	16.1
		QLng.ipk-7A	*Xbcd1066*	7AS	2.21	Opata	10.8
		QLng.ipk1D.2	*Xbcd1930a*	1DL	1.84	Opata	9.0
2009	CD	*QLng.ipk-1A*	*Xgwm99*	1AL	2.89	Opata	12.6
		QLng.ipk-3D	*Xbcd515*	3DL	2.45	Opata	11.6
		QLng.ipk-1D.3	*Xgwm1291*	1DS	2.11	W7984	13.5
		QLng.ipk-6B	*Xgwm935a*	6BS	2.06	W7984	14.0
2009	Dormancy	*QDor.ipk-4A*	*Xksug12b*	4AL	4.40	W7984	17.2

The AA and CD tests yielded contradictory results for the 2009 harvested RILs (Table 2). The former identified three minor longevity QTL, marked by *Xgwm376* on chromosome 3BS (explaining 16.1% of the phenotypic variance, LOD score 2.33), *Xbcd1066* in the centromeric region of chromosome 7AS (10.8% of the phenotypic variance, LOD score 2.21) and *Xbcd1930a* on chromosome 1DL (explaining 9.0% of the phenotypic variance, LOD score 1.84). The favourable alleles were all present in 'Opata'. Meanwhile, the CD test revealed four minor QTL, marked by *Xgwm99* (1AL; 12.6% of the phenotypic variance, LOD score 2.89), *Xbcd515* (3DL; 11.6% of the phenotypic variance, LOD score 2.45), *Xgwm1291* (1DS; 13.5% of the phenotypic variance, LOD score 2.11) and *Xgwm935a* (6BS; 14% of the phenotypic variance, LOD score 2.06). The favourable alleles at the chromosomes 1A and 3D loci were present in 'Opata', and at the other two QTL in 'W7984'. When the same set of material was tested for dormancy, a major QTL was detected on chromosome arm 4AL. This locus was marked by *Xksug12b* (LOD score 4.40) and explained 17.2% of the phenotypic variance for the trait. The favourable allele (high dormancy) was present in 'W7984'.

AM population

The mean germination of the entire set was 93.72 ± 5.05% (Table 1), but this fell to 10.97 ± 12.80% after AA treatment, and to 61.05 ± 25.33% after the CD treatment. The respective relative germinations were 0.12 ± 0.13 and 0.64 ± 0.26. The reductions in germination after AA and CD were significant as were differences among accessions of the AM population.

Marker coverage of the D genome was relatively poor, and chromosome 6D was not marked at all

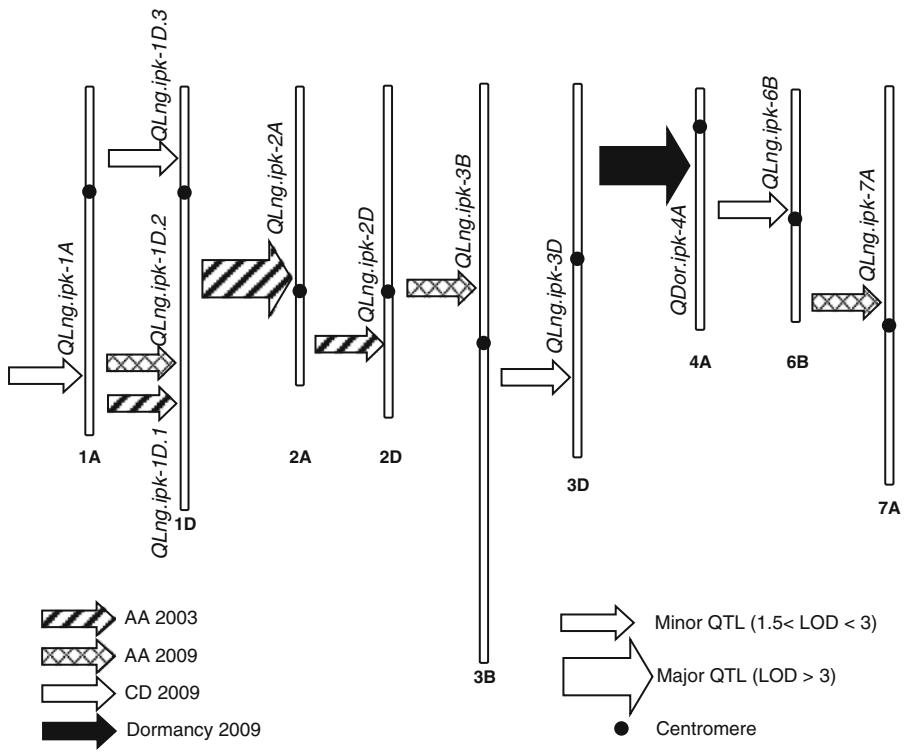

Fig. 1 QTL for seed longevity and dormancy

(Neumann et al. 2011). Association mapping revealed markers associated with longevity on 14 chromosomes (Fig. 2). Significant MTAs were associated with 38 mapped markers, 20 markers of known chromosomal location and 15 unmapped markers (Fig. 2; Table 3). Of the 38 MTAs with mapped markers, 18 and 20 were revealed after AA and CD, respectively. One MTA was revealed with both treatments. Among the unmapped markers with known location, the AA treatment gave 14 and CD treatment six significant associations. In cases of markers without known chromosomal locations, eight MTAs were detected after AA and seven after CD treatment. One marker gave a significant MTA with both the AA and CD treatments.

The highest number of markers associated with longevity was detected on homoeologous group 4 chromosomes where 13 markers gave significant associations. Map positions of two markers remained unknown. This was followed by homoeologous group 7 chromosomes with 12 significant MTAs, including 7 with mapped loci and 5 with unmapped loci. Eight markers were in significant associations on chromosomes of each of homoeologous groups 1 and 2, but only ten of these 16 MTAs had been assigned map locations. Six loci were associated with significant MTA on chromosomes of each of homoeologous groups 3 and 6, but the chromosomal locations of only nine of these 12 loci were known (Fig. 2; Table 3). Finally, the least number of MTAs were detected on homoeologous group 5 chromosomes where five markers had significant associations of which only one was associated with a mapped locus. A comparison among A, B and D genomes was not considered due to the unequal marker distribution per genome (Neumann et al. 2011).

Discussion

Seed longevity is an important trait in the context of ex situ conservation (Xue et al. 2008), and it is clear that the management of seed banks would benefit from a clearer understanding of the factors which determine both inter- and intraspecific variation in this trait (Nagel et al. 2009, 2010; Nagel and Börner

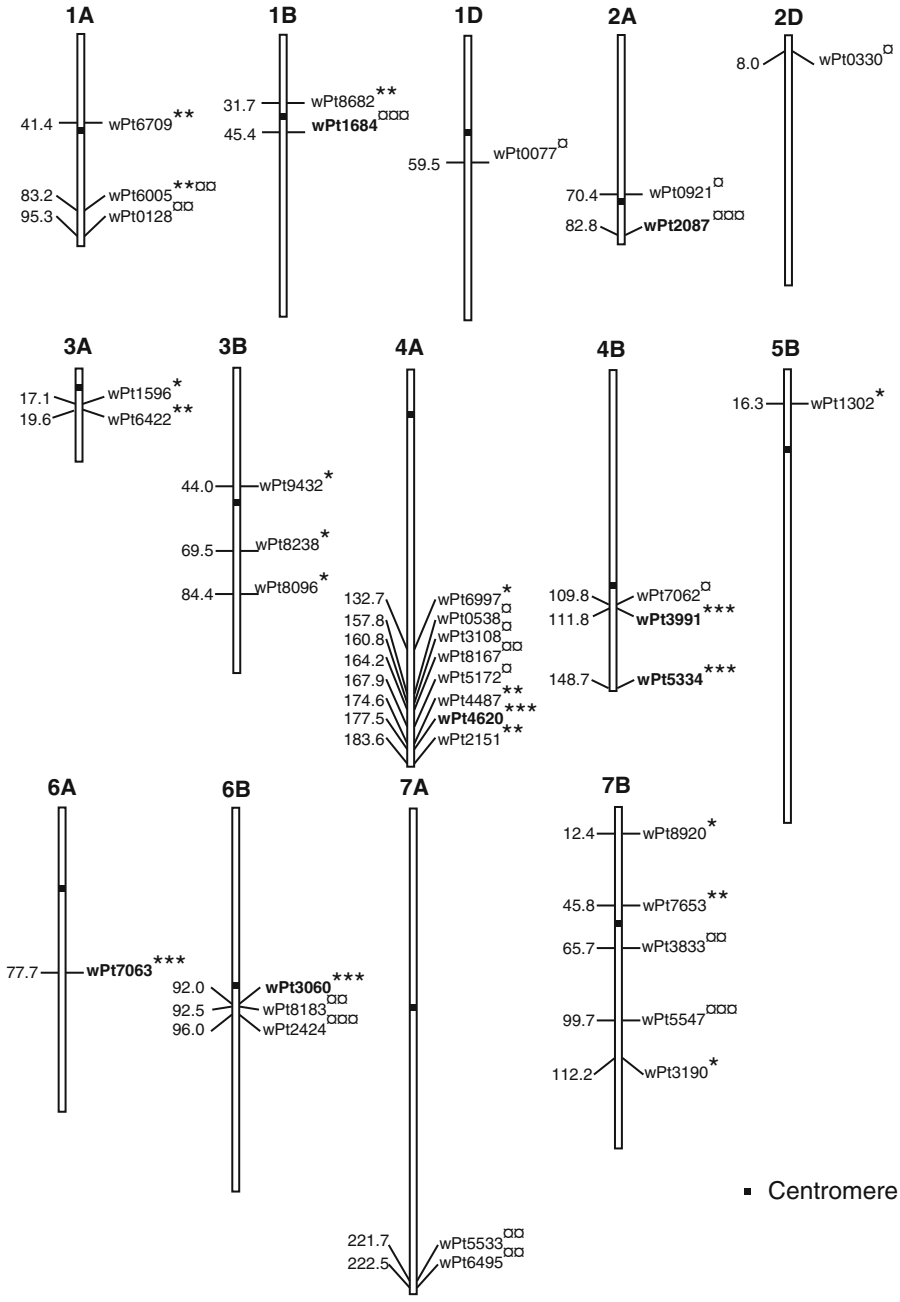

Fig. 2 A genetic map showing only markers involved with a seed longevity MTA. ¤ and *: MTA significant at $P = 0.05$ in two replicates and the mean after AA and CD, respectively; ¤¤ and **: MTA significant at $P = 0.05$ in at least three replicates and the mean after AA and CD, respectively; ¤¤¤ and ***: MTA significant at $P = 0.01$ in three or more replicates and the mean after AA and CD, respectively (markers in *bold*)

2010). Here, we attempted to define the genetic basis for variation in wheat by a joint analysis of a segregating population and a representative core collection.

ITMI mapping population

The bi-parental ITMI mapping population has been used extensively for both the construction of marker-based maps (Röder et al. 1998; Pestsova et al. 2000; Gupta et al. 2002; Somers et al. 2004; Song et al. 2005; Ganal and Röder 2007) and detection of loci important for the determination of key agronomic traits, including seed characters such as weight, size or dormancy (Börner et al. 2002; Lohwasser et al. 2005; Landjeva et al. 2008). The genetic basis of seed longevity has not as yet been investigated in bread wheat, although some relevant data have been

Table 3 Significant MTAs for seed longevity involving unmapped markers

Markers	Chromosome	Association
wPt6012	1B	***
wPt1368	2A	¤¤¤
wPt2185	2A	¤¤¤
wPt3976	2A	¤¤¤
wPt6207	2A	¤¤¤
wPt7187	2A	¤¤¤
wPt4569	3D	¤¤¤
wPt9183	4A	¤¤
wPt8756	4B	¤¤¤
wPt7769	5A	***
wPt2373	5B	¤¤¤
wPt4551	5B	¤¤¤
wPt6880	5B	¤¤
wPt4662	6B	**
wPt1695	6D	¤¤
wPt6936	7B	***
wPt3923	7D	*
wPt5674	7D	*
wPt7842	7D	¤¤
wPt8343	7D	¤¤
wPt0745	X	***
wPt2448	X	¤¤
wPt2464	X	** ¤¤
wPt4164	X	¤
wPt4515	X	***
wPt4648	X	***
wPt6160	X	***
wPt6200	X	*
wPt6878	X	¤¤¤
wPt7636	X	¤
wPt8275	X	¤¤
wPt8456	X	¤
wPt8938	X	**
wPt9986	X	¤¤

¤ and *: MTA significant at $P = 0.05$ in two replicates and the mean after AA and CD, respectively; ¤¤ and **: MTA significant at $P = 0.05$ in at least three replicates and the mean after AA and CD, respectively; ¤¤¤ and ***: MTA significant at $P = 0.01$ in three or more replicates and the mean after AA and CD, respectively (markers in bold). Chromosomal location of the markers is given, where known

gathered from an analysis of a set of bread wheat/*Aegilops tauschii* introgression lines (Landjeva et al. 2010) where segments of chromosomes 1D and 5D were shown to be important sites for genes affecting seed longevity related traits.

We explored seeds of randomly selected RILs of the ITMI mapping population reproduced in two different years. The contradictory QTL outcomes from the 2003 and 2009 RILs confirmed that growing and/or storage conditions exert a large influence on longevity. Furthermore, the two seed treatments (AA and CD) applied to an identical seed lot identified different QTL, indicating that seed moisture content during the treatment process had a major effect on the trait. A similar phenomenon with respect to slight differences in temperature during aeging was described for *Brasicca napus* L. (Nagel et al. 2011).

The many genomic regions contributing to the genetic determination of longevity underline the polygenic nature of this trait. The major longevity QTL *QLng.ipk-2A* mapped to a region of chromosome 2AS that also carries several pathogen defence response genes; in particular, the QTL is flanked by *Per2* (peroxidase) and *Wip* (wound-induced protein) (Li et al. 1999). Whether these stress response genes play a role in longevity of seeds can only be speculated. The minor QTL *QLng.ipk-3B* mapped to a region of chromosome 3B also implicated in the yield components grains per ear and thousand grain weight (Quarrie et al. 2005). Although other QTL for various yield components map elsewhere in the genome, it is reasonable to speculate that relatively large grains are more likely to withstand an unfavourable storage environment.

Of the four QTL revealed by the CD test, three mapped to regions already associated with the determination of grain traits. *QLng.ipk-1A* mapped close to a QTL for spike compactness (Sourdille et al. 2000) and its associated marker was also linked to a QTL for grain number per ear (Quarrie et al. 2005). The compact spike may protect the developing grain from environmental extremes, which can result in a lowering of seedling vigour (McDonald 1999). The second QTL of interest was *QLng.ipk-1D.1* which mapped within 5 cM of the SSR locus *Xgwm337*, which is also associated with grain weight (Groos et al. 2003). Finally, *QLng.ipk-6B* mapped to a region which also mirrored the location of a grain weight QTL (Börner et al. 2002; Quarrie et al. 2005). Therefore, longevity might be associated with superior seed development and performance. Consequently, it is necessary to use good quality initial seed to study the trait.

A likely explanation as to why different QTL emerged from the two different harvests lies in the condition of the seeds prior to the AA or CD treatment, since both vigour and longevity are known to be affected by the conditions under which seeds were produced and stored, as well as the size of the seed itself (Aparicio et al. 2002; Hrstková et al. 2006). Similarly, in a recent study of seed longevity in lettuce, Schwember and Bradford (2010) concluded that a strong genotype × environment interaction existed, as it was not possible to recover the same QTL from seed lots produced in different years. We also believe that genotype by environment interaction played a significant role in our study as it was performed on the same population but seeds were regenerated in two different years.

Apart from the previously mentioned analysis of wheat/*Ae. tauschii* introgression lines (Landjeva et al. 2010), no other studies of seed longevity in wheat have been reported. A single common marker (*Xgwm1291*), mapping to chromosome arm 1DS, was linked to the trait in both the current study and that of Landjeva et al. (2010). Analogous analyses have, however, been performed in both rice (Miura et al. 2002; Zeng et al. 2006; Xue et al. 2008) and barley (Nagel et al. 2009). In rice, seed longevity QTL were identified on seven of the 12 chromosomes, although only one of these—on chromosome 9—appeared repeatedly. With respect to homoeology, chromosome 1 of rice is comparable with wheat group 3, 2 with group 6, 3 with group 1, 4 with group 2 and 9, 11 and 12 with group 5 chromosomes of wheat (Kurata et al. 1994; Salse et al. 2008). Hence some similarity exists between comparable genomic regions in both cereals in regard to control of seed longevity. However, further detailed comparable mapping studies are necessary to confirm postulated homoeologous relationships for the target trait. In barley, regions of chromosomes 2H, 5H and 7H were identified as sites of genes responsible for seed longevity. QTL *QLng.ipk-7H* was mapped to the centromeric region of 7HS, although the confidence interval of this location spanned both sides of the centromere (Nagel et al. 2009). Thus, there is a chance that it is an orthologue of the minor QTL present in the centromeric region of chromosome arm 7AS detected from seeds subjected to the AA test in the present study.

The existence of a relationship between longevity and dormancy remains controversial. In rice it was assumed that the two traits are related (Siddique et al. 1988). On the other hand Miura et al. (2002) showed that longevity and dormancy QTL mapped to different loci, with the former mapping to chromosomes 2, 4 and 9, and the latter to chromosomes 1, 3, 5, 7 and 11. In Arabidopsis seeds of mutants affecting testa pigmentation and/or structure exhibited both reduced dormancy and faster deterioration (Debeaujon et al. 2000). Here also, QTL for the two traits were not coincident. Whereas a major dormancy QTL mapped to chromosome 4AL, no longevity QTL appeared on that chromosome arm in either the 2009 or 2003 seed lots. This suggests strongly that seed dormancy and seed longevity are independently controlled. When the 2003 seed lot was also used to analyse variation in pre-harvest sprouting (Lohwasser et al. 2005), a QTL was identified in the same region as *QDor.ipk-4A*. The coincidence of these two loci not only indicates the accuracy of the QTL mapping approaches, but also a higher heritability of seed dormancy than seed longevity.

AM population

Association mapping is proving to be an effective means of unravelling the genetic basis of complex traits (Flint-Garcia et al. 2003). In wheat, it has been used to identify MTAs for grain size and milling quality (Breseghello and Sorrells 2006), disease resistance and yield (Crossa et al. 2007), and a number of key agronomic traits (Neumann et al. 2011). Here, for the first time we used the approach to study the trait seed longevity in wheat exploiting DArT markers. A direct comparison to other surveys is not possible at present. However, a comparison between various genetic and cytogenetic wheat maps constructed from either DArT- or SSR-based genotypic data permitted DArT markers to be assigned to specific deletion bins (Francki et al. 2009). Similarly, DArT-SSR joint maps have been generated for both diploid (Hai-Chun et al. 2009) and tetraploid (Montovani et al. 2008) wheats. This bin information has allowed 15 of the 73 DArT markers associated with longevity to be genetically characterized (Table 4), including five mapped DArT markers (*wPt6709, wPt4487, wPt5547*, *wPt7063, wPt5334*) and two having only a chromosomal location (*wPt3976, wPt4569*). Four further markers (*wPt8682, wPt6422, wPt9432, wPt8167*) were assigned based on

Table 4 Gene content of deletion bins containing MTA

Marker	Chromosome	Chromosomal bin	BLASTX candidate genes
wPt6709	1A	1AS-0.86-1.00	Glutenin A3 gene, seed maturation protein, disease resistance proteins, plasma membrane associated proteins, replication protein homolog, receptor kinase, gamma gliadin, mitochondrial aldehyde dehydrogenase, lipoxygenase, chalcone synthase
wPt6005	1A	1AL3-0.61-1.00	Histone H4 gene, disease resistance protein, MAP kinase like protein, protein translation factor, DNA binding protein homolog, ATP synthase gamma sub unit, CTP synthase, threonine synthase, malate dehydrogenase, cytochrome reductase, aldehyde dehydrogenase, ethylene forming enzyme, ferrodoxin precursor
wPt8682	1B	C-1BS10-0.50	Protein kinase, NADH oxidoreductase, cellulose synthase, phosphoglycerate dehydrogenase
wPt3976	2A	2AS5-0.78-1.00	Ribosomal protein, abscisic acid element binding protein (ABF3), putative resistance complex protein, putative disease resistance protein, cold shock protein, barley stem rust resistance protein, seven transmembrane protein, cytochrome P450, glutamate dehydrogenase, pyruvate decarboxylase, peroxidase and chaperonin
wPt6422	3A	C-3AL3-0.42	Chloroplast ribosomal protein, malate dehydrogenase, beta glucosidase, lipase like protein, allinase, threonine synthase, NADP specific isocitrate dehydrogenase
wPt9432	3B	3BS1-0.33-0.57	Biostress resistance related protein, putative plasma membrane protein, lipid transfer protein, putative late embryogenesis protein, peroxysomal multifunctional protein, histidine-containing phosphotransfer protein, putative tetra-functional protein of glyoxysomal fatty acid beta oxidation
wPt4569	3D	3DS6-0.55-1.00	Gigantea like protein, stem rust resistance protein, disease resistance protein, peroxysomal multifunctional protein, lipid transfer protein precursor, cytochrome P450, asparagine synthetase 2, phospholipase D alpha 1, triose phosphate isomerase, nodulin
wPt8167, -4487	4A	C-4AL12-0.43	Putative abscisic acid responsive protein, stem rust resistance protein, elongin like protein, ribosomal proteins, thumatin like protein, DNA binding protein, oxalate oxidase precursors, oxaloglutarate/malate translocator, allene oxide synthase, ubiquitin activating enzyme
wPt5334	4B	C-4BL1-0.71	Heat shock protein, low temperature induced protein, resistance protein, seed maturation protein, nucleic acid binding protein, kinases, glutamate decarboxylase isozyme, protein disulfide isomerase, cytochrome c oxidase, peroxidases, lipases, hydrolases
wPt7063	6A	C-6AL4-0.55	Chloroplast translational elongation factor, protein kinase, senescence related proteins, fatty acid elongase, catalase, oxidase, alcohol dehydrogenase
wPt3060, -8183, -2424	6B	C-6BL3-0.36	Prohibitin, pescadillo like protein, protein kinase, nuclear cap binding protein, cytochrome
wPt5547	7B	7BL10-0.78-1.00	Resistance protein, barley stem rust resistance protein, membrane protein, catalase isozyme, ammonium transporter, cytochrome, putative dehydrogenases, chloroplast nucleotide DNA binding protein, lectin precursor, chalcone synthase

Montovani et al. (2008) and four (*wPt6005, wPt3060, wPt8183, wPt2424*) on Hai-Chun et al. (2009). BLASTX searches of the gene content within these bins identified sets of candidate genes (Table 4).

Bins 1AS-0.86-1.00, 1AL3-0.61-1.00 and C-1BS10-0.50 contain the genes *Glu-A3* and its orthologues, and *Histone H4*, and encode a seed maturation protein, disease resistance proteins, plasma membrane-associated proteins and a number of enzymes (hexokinase, various dehydrogenases, lipoxygenase, cellulose synthase and some ethylene forming enzymes). An unmapped marker was assigned to the 2AS5-0.78-1.00 bin, which carries genes encoding ribosomal proteins, an abscisic acid element binding protein (ABF3), putative disease resistance proteins, cold shock proteins and barley stem rust resistance, along with genes influencing the production and level of glutamate dehydrogenase and pyruvate decarboxylase.

Table 5 Markers associated with seed longevity, and also significantly associated with grain yield (GY) and leaf rust (LR), stripe rust (SR) and powdery mildew (PM) resistances

Locus	Chromosome	Traits				Cited by
		GY	LR	SR	PM	
wPt6709	1A		X			Crossa et al. (2007)
wPt0128	1A	X	X	X	X	Crossa et al. (2007)
wPt8096	3B	X	X			Crossa et al. (2007)
wPt5172	4A				X	Crossa et al. (2007)
wPt4487	4A			X	X	Crossa et al. (2007)
wPt4620	4A		X			Crossa et al. (2007)
wPt7063	6A	X		X		Crossa et al. (2007)
wPt2424	6B		X			Neumann et al. (2011)
wPt6495	7A			X		Crossa et al. (2007)
wPt5249	–	X				Neumann et al. (2011)
wPt9986	–	X				Neumann et al. (2011)

X indicates an association

Homoeologous group 3-associated DArT markers were assigned to bins C-3AL3-0.42, 3BS1-0.33-0.57 and 3DS6-0.55-1.00, which contain genes encoding a chloroplast ribosomal protein, a biostress resistance related protein, a putative plasma membrane protein, a lipid transfer protein, and a putative late embryogenesis protein, along with others responsible for the production of enzymes involved in amino acid synthesis. The associated group 4 deletion bins contain genes encoding heat and cold shock proteins, disease resistance proteins, abscisic acid responsive proteins, a thaumatin like protein, an oxalate oxidase precursor and a ubiquitin activating enzyme. Finally, the group 6 and 7 DArT markers lie in bins containing genes encoding senescence related proteins and membrane proteins, along with catalase isozymes, oxidases and alcohol dehydrogenase (Table 4).

Peroxidases (Gulen and Eris 2004), glutamate dehydrogenase (Skopelitis et al. 2006), alcohol dehydrogenase (Kato-Noguchi 2001) and aldehyde dehydrogenase (Sunkar et al. 2003) along with other proteins function to protect plants against various kinds of stress (Houde et al. 2006). Consequently seeds expressing these enzymes more effectively may be better equipped to live longer and maintain their genetic and membrane integrity.

Using the association mapping approach more loci were detected than in the biparental ITMI cross. This is partly due to the higher number of genotypes (96) involved in association mapping. The major QTL for seed longevity on chromosome 2A as well as the minor QTL on chromosome 3B may be comparable in both studies; however, common markers are necessary to confirm this.

Another comparison was made between the DArT markers identified here with those highlighted in the association studies made by Crossa et al. (2007) and Neumann et al. (2011). Eight markers were associated with grain yield or fungal resistance in the study by Crossa et al. (2007), whereas three were associated with either grain yield or disease resistance in the Neumann et al. (2011) study (Table 5). This leads to the conclusion that superior agronomic performance may influence seed longevity.

Some of the MTAs identified here correspond to genomic regions in barley and rice where longevity QTL have been located. The wheat group 4 chromosomes are related to rice chromosome 3 (Kurata et al. 1994; Salse et al. 2008), in which Xue et al. (2008) mapped a storability QTL. Similarly, the segment on chromosome 7B associated with longevity matches the location of the longevity QTL mapped to barley chromosome 7H (Nagel et al. 2009). This gives some indication that wheat may share some loci for seed longevity with its relatives barley and rice.

The genetics of seed longevity, particularly in wheat, is still at an early stage. An improved understanding will require the analysis of variation for the trait across a more diverse range of germplasm. The goal is to develop methods which are

more predictive of the longevity of seeds so that germplasm management can be carried out more effectively. The identification of favourable alleles offers perspectives for the prediction of seed longevity in germplasm collections.

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