Genetic architecture of seed longevity in bread wheat (*Triticum aestivum* L.)

MIAN ABDUR REHMAN ARIF[1,2], MANUELA NAGEL[1], ULRIKE LOHWASSER[1] and ANDREAS BÖRNER[1,*]

[1]*Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany*

[2]*Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan*

**Corresponding author (Email, boerner@ipk-gatersleben.de)*

The deterioration in the quality of *ex situ* conserved seed over time reflects a combination of both physical and chemical changes. Intraspecific variation for longevity is, at least in part, under genetic control. Here, the grain of 183 bread wheat accessions maintained under low-temperature storage at the IPK-Gatersleben genebank over some decades have been tested for their viability, along with that of fresh grain subjected to two standard artificial ageing procedures. A phenotype–genotype association analysis, conducted to reveal the genetic basis of the observed variation between accessions, implicated many regions of the genome, underling the genetic complexity of the trait. Some, but not all, of these regions were associated with variation for both natural and experimental ageing, implying some non-congruency obtains between these two forms of testing for longevity. The genes underlying longevity appear to be independent of known genes determining dormancy and pre-harvest sprouting.

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1. Introduction

Some 1,750 *ex situ* genebanks have been established over the last decades to combat the continuing erosion of genetic variation experienced by crop plants. They are estimated to currently curate >7.4 million accessions (FAO 2010), of which ~45% are cereal species (Börner *et al.* 2014). The loss in quality of stored seed can be slowed, but not stopped, by controlling the storage environment (temperature, relative humidity and gaseous composition). The deterioration in viability experienced during long-term low-temperature storage is due to damage to the membranes, to the DNA and to the action of a variety of enzymes and other proteins (Coolbear 1995; McDonald 1999). Species producing very long-lived seed have evolved a number of structural and chemical features to limit the rate of this decay (Bartosz 1981). Among the agents responsible for seed ageing identified to date, lipid peroxidation is the most well documented (Davies 2005), but oxidative damage to DNA and proteins has also been identified as being causal (Rao *et al.* 1987; Bailly *et al.* 2008).

Standard experimental ageing procedures have been elaborated by the International Seed Testing Association (ISTA) to speed the assessment of viability loss. Both the accelerated ageing (AA) and the controlled deterioration (CD) tests have been used to reveal the genetic basis of longevity in barley (Nagel *et al.* 2009, 2015), oilseed rape (Nagel *et al.* 2011) and wheat (Rehman Arif *et al.* 2012a). Although a proteomics-based analysis has shown that in aged *Arabidopsis thaliana* seed, the two tests highlight a number of common molecules (Rajjou *et al.* 2008), there is no consensus as yet as to whether the two protocols generate comparable outcomes (Walters 1998; McDonald 1999; Black *et al.* 2006).

The genetic basis of most of the important traits in both cereal and non-cereal crops is complex (Börner *et al.* 2002). Seed longevity belongs to this class of trait (Dickson 1980; Clerkx *et al.* 2004b); furthermore, it is

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strongly affected by the environment experienced by the mother plant during seed development and by the seed during both the immediate post-harvest period and the period of *ex situ* storage (Contreras *et al.* 2008, 2009). Two approaches are typically taken to determine the number and location of genes underlying variation in a quantitatively inherited trait: these are biparental linkage mapping and phenotype–genotype association analysis (Zhu *et al.* 2008). Both these approaches have been taken to address the inheritance of longevity in a number of crop species. Examples include *A. thaliana* (Bentsink *et al.* 2000; Clerkx *et al.* 2004a, b), rice (Miura *et al.* 2002; Zeng *et al.* 2006; Xue *et al.* 2008; Li *et al.* 2014), soybean (Singh *et al.* 2008), barley (Nagel *et al.* 2009, 2015), *Aegilops tauschii* (Landjeva *et al.* 2010), maize (Revilla *et al.* 2009), lettuce (Schwember and Bradford 2010), oilseed rape (Nagel *et al.* 2011), wheat (Rehman Arif *et al.* 2012a) and tobacco (Agacka *et al.* 2015). Dormancy – the inability of a mature cereal grain to germinate until it has aged sufficiently (Simpson 1990), and pre-harvest sprouting (PHS) – its tendency to germinate prematurely, have both been intensively studied at both the physiological and the genetic level (Kulwal *et al.* 2005; Lohwasser *et al.* 2005, 2013; Mares *et al.* 2009; Rehman Arif *et al.* 2012b). The relationship between seed longevity and either dormancy or PHS, however, has not been fully explored. In rice, Miura *et al.* (2002) concluded that dormancy and longevity are independent of each other, as was also the case for wheat (Rehman Arif *et al.* 2012a). Here, the objectives were to characterize variation for longevity in wheat grain samples stored *ex situ* over a long period, and to identify phenotype–genotype associations in grain which had been either stored long term or exposed to artificial ageing.

2. Materials and methods

2.1 *Plant material*

A set of 183 hexaploid wheat (129 spring type, 54 winter type) accessions (supplementary table 1) was selected from the collection maintained at the IPK genebank and last multiplied in 1974. These were the oldest seed lots available in the storage. The grain has been stored at 0 ±1°C and the grains' moisture content (GMC) maintained at 8±2%. Historical germination data are available from 1978 and 1998, and a new set of data was generated in 2008. The majority of the collection (177 accessions) was grown out to produce fresh material in 2010. Each accession was represented by 30 plants arranged as five plants per pot; the plants were manually harvested and hand-threshed.

2.2 *Standard germination test*

The conventional germination test procedure followed the standard protocol (ISTA 2008), in which the grains are laid between two layers of moist filter paper, which is then formed into a roll and held in a Jacobsen apparatus. The temperature of the water bath of the equipment was 25 ±1°C during the day and 23±1°C during the night. Germination success was recorded after eight days. The test was applied to a sample of 100 grains from the 1974 harvest, and to three replicates of 100 grains per treatment from the materials regenerated in 2010.

2.3 *AA and CD tests*

For the AA test, three replicates of 100 grains per accession were laid on a rack within a sealed glass jar, which contained 200 mL deionized water to ensure the maintenance of near 100% relative humidity. The jars were exposed to 43±0.5°C for 72 h, after which the grains' ability to germinate was tested by an standard germination test (SGT) (ISTA 2008). For the CD test, initial GMC was first determined using an ISTA approved protocol, then increased to 18% by the addition of an amount of deionized water based on the expression, $mH_2O=[100–GMC_I\ (\%)/100-GMC_T\ (\%)] \times W_I$, where mH_2O is the quantity of water added; GMC_I is the initial GMC (%); GMC_T is the target GMC (18%) and W_I the initial weight of the 100 grain sample (g). The grain was left to equilibrate for 2 h at room temperature after which they were chilled to 7°C for 22 h. Three single replicates of 100 grains per accession were then sealed in an aluminium foil pouch and held at 43±0.5°C for 72 h. Finally, the grains' ability to germinate was tested by an SGT. Longevity was expressed by dividing the rate recorded following the treatment (AA or CD) by that of the non-treated control to give a relative AA (RAA) and a relative CD (RCD).

2.4 *Dormancy and PHS tests*

Spikes were harvested at Zadoks stage 92 over a period of 10 days. To assay for dormancy, a sample of 60 fresh grains per accession was laid on moist filter paper in a plastic box held under a 12 h photoperiod for either 7 days at 20°C (D20) or 14 days at 10°C (D10). A dormancy index (DI) (Strand 1965) was derived from the expression (2×D10+D20)/3, where D10 and D20 represented the proportion of successfully germinated grains at each temperature. For the PHS test, five freshly harvested spikes per accession were laid over wet sand and held for 14 days in two replicates which followed each other. For the interpretation of the data, a rating of seven score points was

used where one score meant no sprouting and seven score meant complete sprouting. Each accession's PHS score was derived from the mean of these two replicates.

2.5 *Genotyping*

DNA was extracted from three grains per accession of the 1974 material. The grains were crushed and the DNA extracted using a commercial kit (QIAGEN, Germany). The DNA was transferred to Triticarte Pty. Ltd. (now Diversity Arrays Technology Pty Ltd.) Canberra, Australia. for DArT (Diversity Array Technology) genotyping. All information about sequences and chromosomal location of the markers are given by *http://www.diversityarrays.com*.

2.6 *Population structure and phenotype/genotype association analysis*

A sub-set of 161 DArT markers, chosen to define a set of 5 cM intervals (*http://www.diversityarrays.com*) was used to characterize the population structure of the germplasm panel, based on STRUCTURE software (Pritchard *et al.* 2000). The admixture model with a burn-in period of 10,000 iterations and 10,000 MCMC (Markov Chain Monte Carlo) approach duration to test for K over the range of 1–15 was used. PAUP software (Swofford 2002) was used to portray genetic relationships between accessions. Associations between individual markers and each trait were calculated using TASSEL v2.01 software (Bradbury *et al.* 2007) assuming either the general linear model (GLM) or the mixed linear model (MLM) (Yu *et al.* 2006). The EMMA method (efficient mixed-model association) (Kang *et al.* 2008) was applied and default settings were chosen for the MLM parameters. Markers assigned a significant ($p \leq 0.05$) or highly significant ($p \leq 0.01$) association with both GLM and MLM models were designated as credible for all traits except for RAA and RCD where an association was declared as significant or highly significant when it appeared in 2 out of 3 replicates and the mean.

2.7 *Statistical analysis*

All statistical analyses of the data were performed using SPSS v17.0 software (SPSS Inc. 1999). Candidate genes mapping within a genomic region associated with a trait were identified from the deletion bin maps prepared in wheat cv. Chinese Spring *(http://wheat.pw.usda.gov/pubs/2004/Genetics/Bioinfo/)*.

3. Results

3.1 *Genomic distribution of DArT markers*

The 2,134 polymorphic DArT markers used for genotyping cover a genetic distance of 2,875 cM (supplementary figure 1); they were non-uniformly distributed between the wheat sub-genomes: 931 were B genome loci, 824 A genome loci and 379 D genome loci. A total of 166 markers defined more than one locus (156 defined two loci, seven three loci and three four loci). The most well represented homoeologous group was group 6 (382 markers) and the least well represented was group 4 (150 loci).

3.2 *Population structure*

The STRUCTURE analysis recognized six groups (Q1–Q6) (figure 1). Q1 clustered 30 accessions of mainly Asian descent (25 spring and five winter types), and were clustered in the PAUP (Phylogenetic Analysis using Parsimony)-based phylogenetic tree (supplementary figure 2). Q2 harboured 48 accessions (14 spring, 34 winter) of diverse provenance outside of Asia. The PAUP tree assigned most of these to the 'winter wheat worldwide' section, although eight belonged to the 'winter wheat temperate region' section and four to the 'spring wheat temperate region' section. The provenance of most of the 19 accessions in Q3 (15 spring, four winter) was from the Eastern Mediterranean; in the PAUP tree, these accessions clustered within the 'spring wheat South Europe' section. The accessions within Q4 (36 spring, one winter) were bred in Italy and other temperate countries; most clustered within the 'spring wheat South Europe' section, but seven fell into the 'winter wheat worldwide' and two into the 'winter wheat temperate region' sections. Accessions in Q5 (18 spring, one winter) had diverse provenance; the PAUP analysis placed 11 in the 'spring wheat temperate region' section, six in the 'spring wheat Middle/East Asia' section and one each in the 'spring wheat temperate region' and 'winter wheat temperate region' sections. Finally, the small Q6 (seven spring, one winter) harboured exclusively material bred in Greece, all of which were included in 'spring wheat South Europe' section. The remaining 23 accessions (17 spring, six winter) formed a 'mixed group' and were scattered throughout the PAUP tree.

3.3 *Variation for the ability to germinate*

The mean germination rate of all accessions as tested by the SGT carried out in 2008 was 56.4±1.72% (range: 0–94%). A comparison with the outcomes of the 1978 and 1998 SGTs is shown in figure 2 and supplementary table 2. In 1978, the germination rate was substantially higher (87.1±0.67, range:

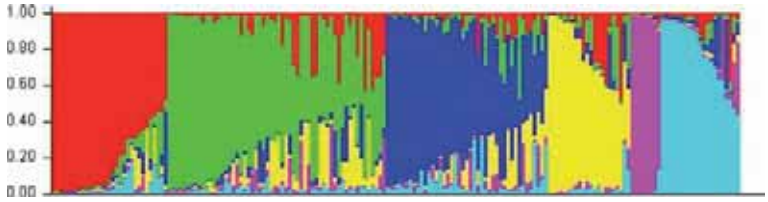

Figure 1. STRUCTURE-based analysis of the germplasm set.

57–99%), and the rate remained high in 1998 (91.1±0.91%, range: 22–100%). Variation for germination capacity as seen from the perspective of the accessions' provenance (Asia, South Europe, Rest of Europe, and Americas), botanical variety (*aestivum*, *lutescens*, *ferrugineum* and 'others'), and vernalization requirement (spring/winter) is illustrated in supplementary figure 3. While there was no influence of either of the former two categorizations on performance, it appeared that the accession's vernalization requirement does exert an influence. For grain harvested in 2010, the overall mean germination rate was 85.7±0.88% (range: 26–99%) (supplementary table 3).

The AA treatment reduced the global mean germination rate to 66.3±1.47 (range: 3–95%), and the Q group means also differed significantly from one another. The mean RAA was 76.8±1.46 (range: 4–100%), with significant differences between both Q group and geographical provenance group means. Similarly, the CD treatment reduced the global germination to 58.6±1.70 (range: 5–96%), once again with significant differences between both Q group and geographical provenance group means. The mean RCD was 67.6 ±1.69 (range: 5–100%) but here there was a significant difference between geographical provenance group means, but not between Q group means.

As summarized in supplementary table 4 and figure 3, the mean percentage dormancy rates (D10 and D20) were, respectively, 11.9±1.28 (range: 0–95%) and 75.6±2.18 (range: 0–100%). The DI ranged from 0 to 96 with mean of 33.1 ±1.30. For PHS, the mean score for was 4.0±0.11 (range: 1.2–6.9).

3.4 *Association analysis*

Altogether, the TASSEL analysis identified 101 marker-trait associations (MTAs) in the STG test carried out in 1978 (supplementary figure 4), of which 25 were highly significant ($p<0.01$). The homoeologous group 2 chromosomes harboured the highest number of MTAs (29), while the group 4 chromosomes only harbored four MTAs. Analysis of the 2008 STG data revealed 103 MTAs (29 highly significant), distributed throughout the genome. In this case the homoeologous group harbouring the highest number of MTAs was group 6 (24), and the

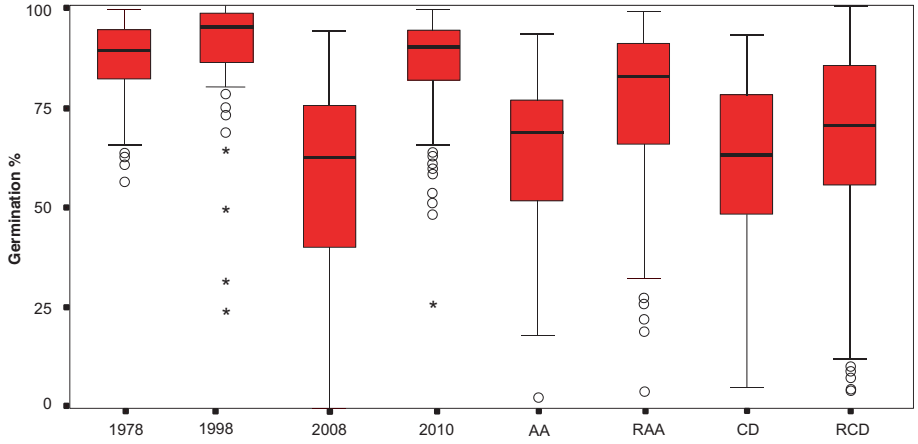

Figure 2. Box plots illustrating the variation recorded for germination % in SGTs conducted in 1978, 1998 and 2008 of materials harvested in 1974 and of fresh grains (2010), along with the germination performance following after artificial ageing.

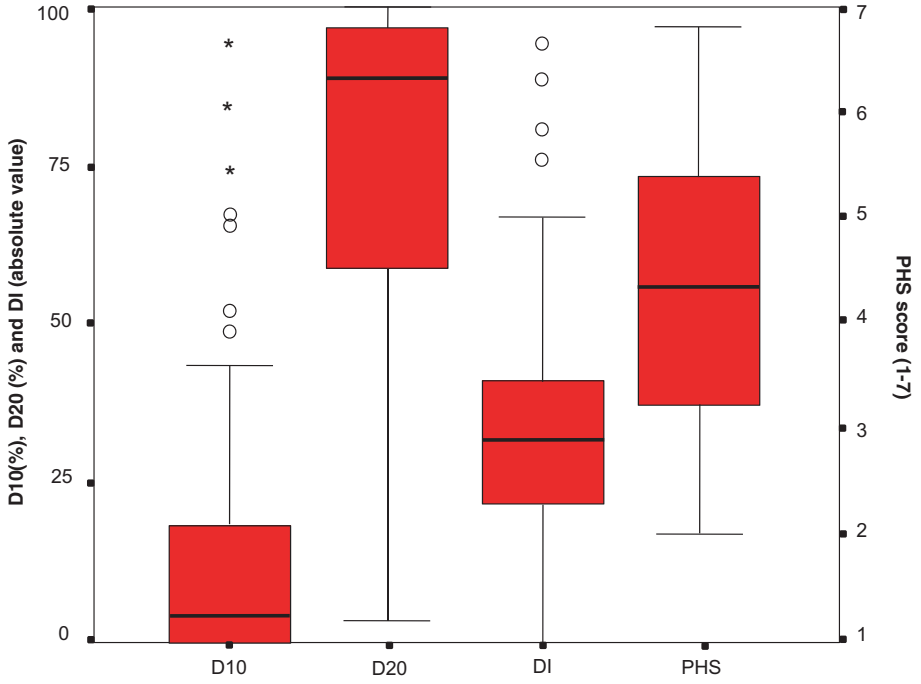

Figure 3. Box plots illustrating the variation recorded for dormancy (D10, D20, DI) and PHS.

least well populated one was again group 4 (three). The same analysis applied to the STG carried out with grain harvested in 2010 revealed 95 significant MTAs (11 highly significant); here, the most well populated homoeologous group was group 6 (26) and the least well populated was group 5 (one).

Since there was very high correlation between AA and RAA as well as CD and RCD (data not shown), only

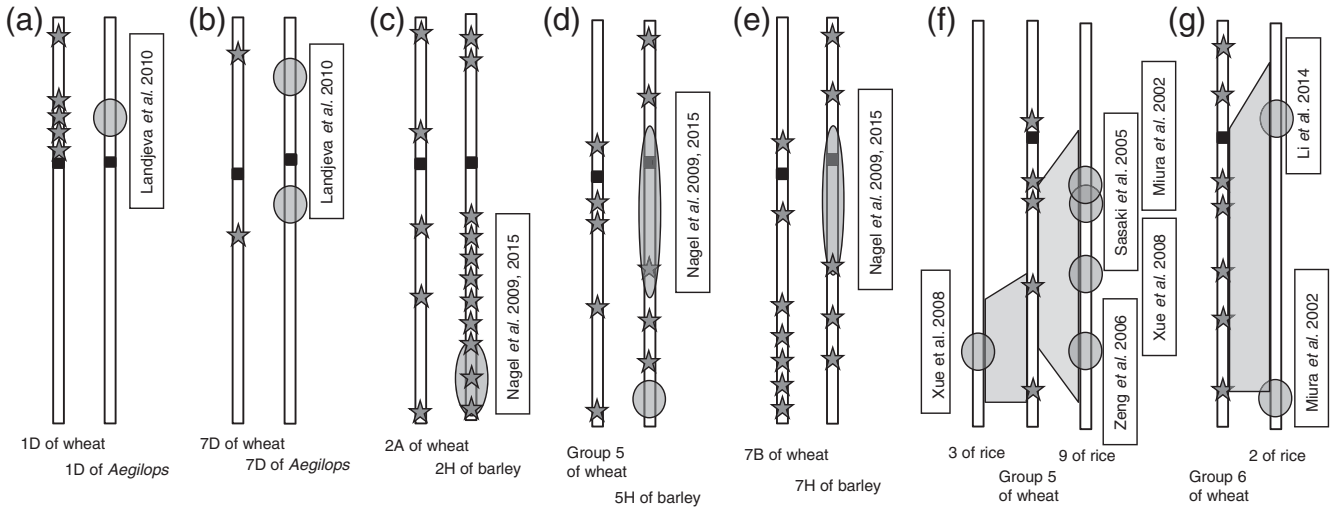

Figure 4. Synteny of longevity loci between (**a**) chromosomes 1D of wheat and 1D of *Aegilops*, (**b**) 7D of wheat and 7D of *Aegilops*, (**c**) chromosomes 2A of wheat and 2H of barley, (**d**) homoeologous group 5 of wheat and chromosome 5H of barley, (**e**) chromosomes 7B of wheat and 7H of barley, (**f**) homoeologous group 5 of wheat and chromosomes 3 and 9 of rice and (**g**) homoeologous group 6 of wheat and chromosome 2 of rice. Small squares indicate centromeres on wheat and barley. Stars on wheat chromosomes represent areas in association with longevity detected in this study and on barley detected in Nagel *et al.* (2015). Shaded circles indicate seed longevity QTLs identified in previous studies either in barley or rice. Stein *et al.* (2007) is used to compare wheat with rice.

associations with RAA and RCD are presented. Analysis of the RAA germination data identified 74 significant MTAs (ten highly significant), with the most well represented homoeologous group being group 3 (23) and the least well represented one group 5 (four). For RCD, there were 97 significant MTAs (11 highly significant), with 38 mapping to the group 3 chromosomes and just two to the group 4 chromosomes. With respect to DI, 78 significant MTAs (19 highly significant) were uncovered, of which 20 mapped to the group 6 chromosomes and only four to each to groups 1 and 4 chromosomes. Finally, for PHS, 110 significant MTAs (30 highly significant) were identified, of which 36 mapped to the group 2 chromosomes and only seven to the group 7 chromosomes.

4. Discussion

A core responsibility of genebanks is to conserve crop germplasm, which is increasingly threatened by genetic erosion. This implies a regular assessment of germination capacity (Nagel *et al.* 2009). The rate of deterioration of a given seed lot's quality depends on many factors, both environmental and genetic. An example of how variable this can be can be seen in the behaviour of the materials multiplied in 1974 and tested 34 years later: some accessions failed to germinate, while others were almost 100% viable (supplementary table 2). Since the materials had all been handled identically, it is assumed that most of this variation reflects genetic variation for seed longevity. The association analysis revealed that many regions of the genome harbour genes influencing longevity, whether this was assayed by either natural or experimental ageing.

The combination of genetic and physical mapping in wheat has generated a series of deletion bins, each associated with a set of DArT markers (Francki *et al.* 2009; Semagn *et al.* 2006; Mantovani *et al.* 2008; Hai-Chun *et al.* 2009). Altogether, 15 bins were identified as likely containing genes influencing longevity (supplementary table 5). Bins 1AS-0.86-1.00 and C-1DS3-0.48 that carried two associated DArT markers of homoeologous group 1 chromosomes contain the genes *Glu-A3* and its orthologues and a number of enzymes (various dehydrogenases, chalcone synthase, lipoxygenase, cellulose synthase, protein kinases, NADH-oxidoreductase, reverse transcriptase and some ethylene forming enzymes). Of particular interest with respect to longevity could be chalcone synthase, lipoxygenase and cellulose synthase. Chalcone synthase (*Chs*) along with another defense responsive (DR) gene, flavanol 7-*O*-methyl transferase, has been mapped to the short arms of chromosomes 1B and 1D by Li *et al.* (1999).

Two associated markers belonging to homoeologous group 2 chromosomes were assigned to 2AS5-0.78-1.00 and 2AL1-0.85-1.00 that carried *elicitor-responsive gene, Vrga1, Enod* (early nodulation gene) and genes influencing the production and level of enzymes like NADH dehydrogenase, glutamate dehydrogenase, pyruvate decarboxylase, peroxidases, superoxide dismutase (SOD) and chaperonins. Li *et al.* (1999) reported *per2* (peroxide 2), *sod* (superoxide dismutase), *wip* (wound induced protein) and other DR genes to be located on all the three homoeologous group 2 chromosomes. In fact, genes *cbp1* and *cbp2* (chitinase binding proteins) were mapped to the long arms of 2A and 2B that might correspond to the MTAs on long arms of 2A and 2B in this investigation. Nagel *et al.* (2009 and 2015) also found QTLs and associations for longevity on chromosome 2H of barley where they reported a dehydration responsive element binding (*DREB*) protein probably to be a candidate gene for longevity.

Nine associated markers of group 3 chromosomes were assigned to bins 3AS2-0.23, 3AL5-0.78-1.00, 3BS1-0.33-0.57, 3BL7-0.63-1.00 and 3DS6-0.55-1.00, which contain genes such as a biostress resistance related protein, a putative plasma membrane protein and others responsible for the production of enzymes involved in amino acid synthesis. Biparental mapping has also identified loci affecting longevity on chromosome 3B near centromere and 3DL (Rehman Arif *et al.* 2012a). The chromosome 4B bin 4BS4-0.37 harbours genes encoding a protein kinase like protein, a dehydrin/LEA group 2 like protein and putative a phosphoesterase. While no bin identification was possible for the group 5 chromosomes, defence genes have been mapped on each of the homoeologous group 5 chromosomes (Li *et al.* 1999). The MTA on 5BS in this investigation might correspond to the *Tha3* gene whereas one of the MTAs on 5BL might correspond to *Lpx*. Two markers of homoeologous group 6 chromosomes associated with longevity were placed in 6BS-Sat and C-6BL3-0.36. The distal end of 6BL carries two DR genes (*Tha1* and *Cbp1*) (Li *et al.* 1999). Polyphenol oxidase (*Ppo*) was mapped to both 6A and 6D chromosomes and hydroxyl-proline rich protein (*Hrp*) and ribosome inactivating protein (*Rip*) were mapped to chromosomes 6A and 6D, respectively. *Tha1* and *Cbp1* seem to be in comparable location to the MTA on the distal end of 6B but it could not be assigned to any deletion bin.

Finally, 5 DArT markers of group 7 were placed in 7AS1-0.89.1.00, 7BS1-27-1.00, 7BL10-0.78-1.00 and 7DS4-0.61-1.0 bins. A cluster of DR genes have been reported to occur on the long arm of group 7 chromosomes, especially *Tha1, Tha2*, *Cht1b*, and *Cat* reside within a relatively small segment on the distal end of 7BL (Li *et al.* 1999). Faris *et al.*

(1999) found a strong QTL for tan spot infection on 7BL corresponding to the same cluster of DR genes. Longevity MTAs on 7BL and genes reported by Li *et al.* (1999) are in comparable locations. Similarly, two MTAs on 7AL might correspond to *Tha2* gene (Li *et al.* 1999).

Li *et al.* (1999) found that most of the DR genes in the genome are present in clusters along the distal regions of the chromosomes. The same is observed in this study i.e., some seed longevity MTAs are present in clusters of two or more MTAs such as on 1AS, 2AL, 2BS, 2DL, 3AL, 3D, 4BS, 5AL, 6AL, 6D, 7AS, 7BL and 7DS whereas the others were distributed throughout the entire chromosomes (supplementary figure 4). Rehman Arif *et al.* (2012a) also assume enzymes like peroxidases (Gulen and Eris 2004), glutamate dehydrogenase (Skopelitis *et al.* 2006), alcohol dehydrogenase (Kato-Noguchi 2001) and aldehyde dehydrogenase (Sunkar *et al.* 2003) to be responsible for seed longevity which along with other functions protecting 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. It can be concluded that seed longevity is in part under the control of the morphological features of the seeds such as cell wall and cell membrane.

The associated markers with the germination rate following long term low temperature storage and the artificial ageing treatments were not similar. The implication is that different deterioration mechanisms are involved in these processes (Walters 1998; McDonald 1999). *A. thaliana* seed responds quite predictably to a range of ageing regimes (Clerkx *et al.* 2004a, 2004b), while proteomic comparisons have revealed that the extent of protein carbonylation is strongly increased whatever the storage conditions are, and the carbonylation targets mostly overlap (Rajjou *et al.* 2008). In contrast, linkage mapping in lettuce has failed to show any overlap between the loci responsible for deterioration resulting from natural and artificial ageing (Schwember and Bradford 2010). Here, ten MTAs were shared between long term storage and experimental ageing (six with AA and four with CD) (supplementary figure 4). The conclusion is that in wheat, deterioration during long term low temperature storage is dissimilar from that induced by artificial ageing, although a few genes are likely important for both. In all, 23 genomic locations were identified where the 2008 SGT- and the AA/CD- MTAs lay within 5 cM of one another.

The genetics of cereal grain longevity has actively been researched in rice (Miura *et al.* 2002; Sasaki *et al.* 2005, 2015; Zeng *et al.* 2006; Xue *et al.* 2008), barley (Nagel *et al.* 2009, 2015) and in a set of wheat/*Aegilops tauschii* introgression lines (Landjeva *et al.* 2010). The latter study identified loci which match in location to the MTAs identified here on the proximal ends of the 1DS (figure 4a) and 7DL (figure 4b) chromosome arms. The barley data include similar locations to the MTAs identified on chromosomes 2A (figure 4c), 5A and 5B (figure 4d) and 7B (figure 4e). In rice, longevity loci have been identified on eight of its 12 chromosomes (Miura *et al.* 2002; Sasaki *et al.* 2005; Zeng *et al.* 2006; Xue *et al.* 2008), while Li *et al.* (2014) have identified MTAs with respect to both conventional and high temperature ageing. The syntenic relationship between wheat chromosome 5A and 5B and rice chromosomes 3 and 9, and between chromosome 6B and rice chromosome 2 (figures 4f and g) highlights some related genomic regions which harbour genes underlying longevity.

The nexus between longevity and dormancy/PHS remains controversial. In rice, Siddique *et al.* (1988) argued that the connection is real, while Miura *et al.* (2002) were able to show by linkage mapping that the relevant genes are non-identical. In *A. thaliana* mutants affecting testa pigmentation and/or structure show both a reduced level of dormancy and a more rapid rate of deterioration (Debeaujon *et al.* 2000). Here, only 11 of the dormancy and 19 of the PHS MTAs overlapped those for longevity, indicating only a limited degree of similarity with respect to the genetic determination of these traits.

Genome-wide association mapping can provide a powerful means of revealing the genetics of complex, quantitatively inherited traits. The present investigation of the genetic basis for the longevity of the wheat grain has shown that the deterioration in viability does have a genetic component. The recognition that distinct sets of genomic regions harbouring the genes active during the process of natural and induced ageing indicates that the mechanisms involved in the artificial ageing protocols do not fully mirror those operating during long term low temperature storage, although the sharing of some genomic regions does imply that experimental ageing can to, an extent, be predictive. In wheat, there appears to be little evidence for either dormancy or PHS to be associated with longevity.

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