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Seed longevity and dormancy in wheat (*Triticum aestivum* L.) – phenotypic variation and genetic mapping

Dissertation

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List of abbreviations

AA	Accelerated ageing
ABA	Abscisic acid
ABF3	Abscisic acid element binding protein
Abi3	*Abscisic acid insensitive3*
Aba1	*Abscisic acid deficient1*
Ale	*Aleurain*
AM	Association mapping
ANOVA	Analysis of variance
Awn	Awnedness
BIL	Backcross inbred line
bp	Base pair
CBP	Chitinase binding protein
CC-NBS-LRR	Coiled-coil nucleotide-binding site leucine-rich repeat
CD	Controlled deterioration
Chs	Chalcone synthase
CSSLs	Chromosome segment substitution lines
DArT	Diversity Arrays Technology
DHL	Double haploid line
DI	Dormancy index
DNA	Deoxyribonucleic acid
Dor	Dormancy
DR	Defense responsive
DREB	Dehydration responsive element binding protein
D10	Dormancy at 10°C
D20	Dormancy at 20°C
EMMA	Efficient mixed-model association
Enod	Early nodulin
FAO	Food and Agriculture Organization
Ft	Flowering time
F_1	First filial generation, produced by crossing two parental lines
F_2	Second filial generation, produced by selfing F_1 plant
$F_{2:3}$	Third filial generation, produced by selfing each single F_2 plant
GA	Gibberellic acid
GAA	Germination after AA
GCD	Germination after CD
GLM	General linear model
G1978	Germination in 1978
G1998	Germination in 1998
G2008	Germination in 2008
Hd	Heading time
HSP	Heat shock protein
Ht	Height
H_2O_2	Hydrogen peroxide
IAA	Indole acetic acid
IG	Initial germination
IPK	Leibniz Institute of Plant Genetics and Crop Plant Research
ISTA	International seed testing association

ITMI	International Triticeae Mapping Initiative
ITMI2003	International Triticeae Mapping Initiative population grown in 2003 season
ITMI2009	International Triticeae Mapping Initiative population grown in 2009 season
LD	Linkage disequilibrium
LOD	Logarithm of odds
LRR	Leucine rich repeats
MAP	Mitose-activated protein
MCMC	Markov Chain Monte Carlo
MLM	Mixed linear model
NADPH	Nicotinamid adenine dinucleotide phosphate
NBS	Nucleotide binding site
OS	Oligosaccharides
OWB	Oregon Wolfe Barley population
Per2	Peroxidase-2
PHS	Pre-harvest sprouting
PKS	Polyketide synthase
Pub	Pubescence
Q	Sub-populations
QTL	Quantitative trait loci
RGAA	Relative germination after AA
RGCD	Relative germination after CD
RH	Relative humidity
RFLP	Restriction Fragment Length Polymorphism
RIL	Recombinant inbred line
Rip	Ribosome inactivating protein
ROS	Reactive oxygen species
SIM	Simple interval mapping
SOD	Superoxide dismutase
Srg6	Stress responsive gene 6
SSR	Simple Sequence Repeat
S x M	Steptoe x Morex population
Tha1	Thaumatin 1
TKW	Thousand kernel weight
TLP	Thaumatin-like protein
UV	Ultra violet
WGA	Wheat germ agglutinin
Wip	Wound induced protein
%	Percentage

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1   Introduction

At the heart of agriculture is human innovation; and at the heart of this innovation is the vast diversity of crops that have been developed by farmers for millennia, and more recently by plant breeders and scientists as well. Today, much of this plant diversity is contained in plant collections - stored, nurtured and distributed by the world's crop genebanks. The seeds and other plant material kept in these genebanks provide the raw material for increased yield, improved quality, and greater diversity in the human diet. For example, genes from wild wheats have been incorporated in domesticated wheat to resist fungal diseases, drought, heat and cold (Anonymous 2001). Crop diversity is at the root of sustainable agriculture and provides environmental benefits through the use of crop varieties that are resistant to pests and diseases and do not require pesticides spray. Although, human beings cultivate 7,000 plants for food and other purposes (Padulosi 1999), yet just three crops - wheat, rice and maize - together provide more than half of humanity's global food supply. The world contains an estimated amount of 250,000 species of flowering plants, but one in 12 of them (8 %) now seem likely to disappear before 2025. The loss of crop wild relatives has profound implications for agriculture. Plant breeders and farmers depend on wild relatives of crops as essential source of genes (Anonymous 2001).

The realisation of the danger of extinction of plant genetic resources by Nicolai I. Vavilov (Maxted et al. 1997) led to the establishment of genebanks around the world (Linington and Pritchard 2001). Genebanks provide an excellent storage faciltiy for the long term *ex-situ* conservation of the plant germplasm (Probert et al. 2009). There are now more than 1,750 individual genebanks worldwide storing more than 7,000,000 accessions. Ten world-wide largest collections by crop are enlisted in Table 1 (FAO 2010).

Among these genebanks, the German national genebank (Federal *ex situ* collection) located at the Institute for Plant Genetics and Crop Plant Research (IPK) in Gatersleben houses 1,51002 accessions belonging to 3,212 species of 776 genera and provides a major contribution to the prevention of extinction (genetic erosion) of both cultivated plants and their related wild species. Over 40% of all the accessions are cereals. Wheat (*Triticum*) has the highest share among cereals with 28,111 accessions followed by barley (*Hordeum*) with 23,245 accessions in storage at the genebank in Gatersleben (Anonymous 2012).

Table 1: The ten largest world-wide germplasm collections by crop (FAO 2010)

Crop	Genus	Accessions
Wheat	*Triticum*	856,168
Rice	*Oryza*	773,948
Barley	*Hordeum*	466,531
Maize	*Zea*	327,932
Bean	*Phaseolus*	261,963
Sorghum	*Sorghum*	235,688
Soybean	*Glycine*	229,944
Oat	*Avena*	130,653
Groundnut	*Arachis*	128,465
Cotton	*Gossypium*	104,780

Seed longevity represents the period a seed remains viable (Barton 1961). The time for which stored seeds can remain viable is determined by the pre-storage and storage conditions, their genetic and physiological storage potential, and is affected by adverse events such as too high or too low temperatures or damage prior to or during storage (Roberts 1961). The long term storage of seeds, especially under unfavourable conditions, leads to loss of viability which is variable in its nature. Seed longevity has been related to various seed properties such as colour, weight and membrane composition. The correlation between seed longevity and these traits is often species or in some cases even variety specific (McDonald 1999). All parts of the seed including the seed coat and embryo are prone to physiological damage. The ability of seeds to withstand stresses that occur while stored is one aspect of seed longevity (Clerkx et al. 2004a).

Seed quality can be reduced on the parental plant due to adverse environmental conditions, premature germination (Coolbear 1995) and pathogens (McGee 2000). Damage to seed quality can be categorized broadly into two types. One is short term deterioration in the field which can be the deterioration of the seeds after they have attained the maximum dry weight such as deterioration on the mother plant. The second type is the long term deterioration which occurs in storage. This includes membrane and genetic damage, changes in respiratory activity and enzymes and protein damage (Coolbear 1995, McDonald 1999). All parts of seed deteriorate with time, the damage of which can be sustained by the chemical constituents of seeds and by the way these compounds interact to form biological structures. The integrity of DNA, proteins and membranes is especially important for maintaining seed viability (Clerkx et al. 2004b).

Seed deterioration in storage may involve many physical and chemical changes including the disrupted intracellular integrity, decreased activities of enzymes, lipid peroxidation and non-enzymatic reactions (Wettlaufer and Leopold 1991, Priestley 1986). Seed viability and vigour are dependent on the integrity of cellular macromolecules and the orderly compartmentalisation of the cell (Priestley 1986). Ageing involves an inexorable trend to disorder. Defense mechanisms innate to the seed - structural and chemical features that are characteristic of a particular species - may serve to limit the rate of this decay (Bartosz 1981).

Seed deterioration varies among seed populations. Certain varieties exhibit less deterioration than others. Even within a variety the storage potential of individual lots varies, and even within a seed lot, individual seeds have different storage potential (McDonald 1999). Broken, cracked, or even bruised seeds deteriorate more rapidly than undamaged ones (McDonald 1985, Priestley 1986). Environmental stresses such as deficiency of minerals (nitrogen, potassium and calcium) (Harrington 1960), water (Haferkamp et al. 1953) and temperature extremes (Justice and Bass 1978) during seed development and prior to physiological maturity can also reduce the longevity of seeds.

Immature and small seeds as well as large seeds within a seed lot do not store well (Wien and Kueneman 1981, Minor and Paschal 1982). Hard-seededness might play a role in extending seed longevity (Flood 1978, Patil and Andrews 1985). Since seed storage is often accompanied by progressive loss of vigour, storage conditions must be optimised for both the preservation of genetic resources and commercial applications. For orthodox seeds, low seed moisture content, low temperature, or cryopreservation seems to result an increase in storage life span (Abdalla and Roberts 1968, Walters 2004, Walters et al. 2004). Orthodox seed is a term introduced by Roberts (1973) for seeds that can be dried to moisture contents of 10% or less; in this condition they can be successfully stored at subfreezing temperatures. A thumb rule states that each 1% reduction in seed moisture content and each 5°C reduction in the temperature doubles the life of the seed (Harrington 1972). Similarly, based on the experiments initiated by Haberlandt regarding seed germination in storage in Vienna 139 years ago, Steiner and Ruckenbauer (1995) verified the feasibility of ultra-dry storage of seeds at ambient conditions.

A number of mechanisms of seed ageing have been identified (Kranner et al. 2010, Smith and Berjak 1995). Lipid peroxidation, resulting in membrane damage as well as the generation of toxic byproducts, is well documented in stored seeds (Davies 2005). Oxidative damage to DNA and protein can also be involved in seed ageing (Rao et al. 1987, Bailly et al. 2008). Formation of sugar–protein adducts (i.e. the Maillard reaction) or of isoaspartyl residues may be factors in loss of protein function during deterioration (Sun and Leopold 1995, Ogé et al. 2008, Rajjou et al. 2008). On the other hand, antioxidants, heat shock proteins (HSPs) and enzymes to repair protein damage may be involved in mitigating the effects of ageing on seed longevity (Kibinza et al. 2006, Prieto-Dapena et al. 2006, Ogé et al. 2008, Almoguera et al. 2009).

Seeds of some species are genetically and chemically equipped for longer storability than others under similar conditions e.g. seeds of *Canna* (Sivori et al. 1968) and *Lotus* (Shen-Miller et al. 1995) have been reported to be viable even after 1300 years. *Albizia*, *Cassia*, *Goodia* and *Trifolium* seeds are able to germinate after 100 years (Harrington 1972). Seeds of other species are characteristically short-lived such as lettuce (*Lactuca sativa*), onion (*Allium cepa*), and parsnip (*Pastinaca sativa*) and rye (*Secale cereale*) (Walters et al. 2005, Nagel and Börner 2010). Wheat exhibits intermediate storability compared to alsike clover which has excellent storability. Seed species that possess high oil content do not store well compared to those that contain less oil (Copeland and McDonald 1995). Nagel and Börner (2010) concluded that oil content seemed to influence longevity under open storage conditions and further analysis will be necessary to come to a solid conclusion. Seeds of different species may be chemically similar but have greatly different storability. For example, chewings fescue and annual ryegrass seeds are similar in appearance and chemical composition. However, ryegrass seeds store much better under comparable conditions (Copeland and McDonald 1995). Genetic differences in storage potential are not limited to seeds of different species but it can also occur among cultivars of the same species as the bean cultivar Black Valentine stores better than Brittle Wax (Toole and Toole 1953). Lindstrom (1942) reported 90% germination of some inbred lines of maize after 12 years stored at room temperature while others were completely dead after this time. Maternal effects also increased the seed storability in F_1 seeds of soybean, perhaps by influencing the

permeability of seed coat (Kueneman 1983). Seed longevity can vary between genotypes of a given species by as much as seven-fold (Roberts and Ellis 1977).

Studies of seed longevity under optimal storage conditions would take years to complete, so experimental ageing procedures as accelerated ageing (AA) or controlled deterioration (CD) conditions are utilised to speed the loss of viability. The CD test has been used to assess the vigour of seed lots and to predict their relative longevity by ageing seeds rapidly at elevated temperature and relative humidity (RH) (Delouche and Baskin 1973, Powell and Matthews 2005). Proteome analysis of *Arabidopsis thaliana* seeds revealed common features between the CD (85% RH at 40°C for up to 7 days) and conventionally (up to 11 years at 5°C) aged seeds (Rajjou et al. 2008).

Genetic studies of agronomic important traits in cereals have revealed that most of them are inherited quantitatively and difficult to detect within the genome (Börner et al. 2002). Linkage analysis and association mapping are the two most commonly used tools for dissecting complex traits (Zhu et al. 2008). A typical linkage mapping involves the development of a mapping population. Molecular markers are mapped on this population after which genetic mapping is performed. The most commonly used mapping populations in plants are Double Haploid Lines (DHLs) and Recombinant Inbred Lines (RILs) (Collard et al. 2005). There are various kinds of markers which have already been mapped in wheat on such populations as Restriction Fragment Length Polymorphism (RFLP) (Chao et al. 1989), Simple Sequence Repeat (SSR) (Röder et al. 1998) or Diversity Arrays Technology (DArT) (Akbari et al. 2006). Performing classical linkage mapping many Quantitative Trait Loci (QTL) have been identified over the last decade. Examples for wheat are given by Börner et al. (2002), Quarrie et al. (2005), Huang et al. (2006) and Kumar et al. (2007).

An alternative means of detecting QTL is based on correlating genotypes with phenotypes in natural populations or germplasm collections which is called association mapping. The underlying principle of this approach is that linkage disequilibrium (LD) tends to be maintained over many generations between loci which are genetically linked to one another. LD is defined as nonrandom association of alleles at different loci. That is, when a particular allele at one locus is found together with a specific allele at a second locus more often than expected if alleles at the loci were combining independently in a population, the loci are said

to be in LD (Oraguzie et al. 2007). High LD is expected to be observed between loci in tight linkage, as recombination events since the mutation should have eliminated LD between loci that are not in close distance (Breseghello and Sorrells 2006a). There are numbers of generic applications of association genetics. On the one hand, the higher resolution afforded by the use of unstructured populations allows the intriguing possibility of identifying the genes - or even the specific nucleotides - underpinning trait variation. On the other hand, the opportunity to use molecular markers to enhance rates of genetic gain, including the utilisation of specific genes from non-elite germplasm is more direct and efficient than was hitherto possible. Also, it provides the knowledge about the genetic architecture of extant variation in populations along with the opportunity to determine evolutionary phenomena that have led to existing population structures (Oraguzie and Wilcox 2007). Pioneering association mapping studies in plants began only a few years ago (Ivandic et al. 2002, Skøt et al. 2004, Aranzana et al. 2005). For some traits, the approach can be simplified by searching for LD between a number of candidate genes and the trait (the ''candidate gene approach''), which has been widely applied (e.g., Tommasini et al. 2007, Matthies et al. 2008). But where no such prior information is available, the whole genome needs to be scanned in a search for LD between a framework of mapped marker loci and the target trait (''genome-wide association analysis''). Genome-wide association mapping in cereals has not often been attempted to date (Breseghello and Sorrells 2006a, Kraakman et al. 2006, Roy et al. 2006, Crossa et al. 2007, Neumann et al. 2011), largely because of the impracticality of genotyping large numbers of entries at the required number of marker loci.

The development of high throughput systems such as Diversity Arrays Technology (DArT) (Jaccoud et al. 2001) has overcome this difficulty of high through-put genotyping, since it had provided a highly multiplexed platform, which allows rapid and cost-effective genome-wide analysis (Wenzl et al. 2004). DArT markers are bi-allelic dominant anonymous markers obtained by cloning random fragments of genomic representations. A first analysis of sequencing these markers showed that most of them are derived from the genespace (Wenzl et al. 2006).

Very little is known about the genetic basis of differences in seed quality because this trait is strongly affected by environmental factors during seed formation, harvest and storage, and is probably controlled by several genes and, therefore, is complex in genetic studies (Clerkx

et al. 2003). This can be illustrated by genetically identical seed lots in which individual seeds, even when grown under identical conditions or even when coming from the same plant, may lose their viability at different intervals after harvest. Seed vigour and longevity being quantitative traits (Dickson 1980, Clerkx et al. 2004b) are strongly affected by the environment during seed formation, harvest, and storage (Contreras et al. 2008, 2009) through variety of mechanisms whose understanding might enable us in future to greatly increase the seed longevity of agriculturally important species and varieties. This can enahnce our ability to preserve plant genetic resources for generations (Bewley and Black 1994). Using the linkage mapping approach, QTLs for seed longevity have been identified in *Arabidopsis* (Bentsink et al. 2000, Clerkx et al. 2004a, 2004b), rice (Miura et al. 2002, Zeng et al. 2006, Xue et al. 2008), soybean (Singh et al. 2008), barley (Nagel et al. 2009), *Aegilops* (Landjeva et al. 2010), maize (Revilla et al. 2009), lettuce (Schwember and Bradford 2010) and oilseed rape (Nagel et al. 2011).

Seed dormancy is defined as an inability of viable mature seeds to germinate under adequate conditions (Simpson 1990) whereas pre-harvest sprouting (PHS) is a phenomenon in which grain germination occurs in the spikes even before harvest. Germination of wheat kernels prior to harvest reduces the value of grain for both food and seed (Derera 1989). Many studies have been performed in wheat regarding dormancy and PHS (Flintham 2000, Flintham et al. 2002, Groos et al. 2002, Kato et al. 2001, Kulwal et al. 2005, Lohwasser et al. 2005, Mares et al. 2009, Mori et al. 2005, Osa et al. 2003). However, there has been no report so far addressing the relationship of seed longevity with dormancy and PHS in wheat, though, in rice one study had been reported that concluded that dormancy and longevity are governed by different alleles (Miura et al. 2002).

Linkage and association mapping have proven to be useful to map traits of economic as well as agronomic importance. Consequently, in the present study, both of these methods were used to conduct research on the genetics of seed longevity, dormancy and pre-harvest sprouting in wheat (*Triticum aestivum* L.)

Aim of the research

This study was conducted keeping in perspective the following objectives:

1- The identification of quantitative trait loci (QTLs) for seed longevity and dormancy analysing the bi-parental 'International Triticeae Mapping Initiative' (ITMI) mapping population applying 'accelerated ageing' (AA) and 'controlled deterioration' (CD) methods and dormancy test

2- The identification of marker trait associations (MTAs) for seed longevity after AA and CD as well as for dormancy and pre-harvest sprouting, respectively, in a collection of advanced germplasm

3- The screening of germplasm present in the Gatersleben genebank to assess the seed longevity behaviour after long term cold storage

4- The identification MTAs for seed longevity in genebank accessions after long term cold storage

5- The identification of MTAs for seed longevity after AA and CD as well as dormancy and pre-harvest sprouting, respectively, in the same but freshly regenerated genebank accessions

6- The comparison of the results obtained after long term cold storage with those obtained applying AA and CD

7- The analysis of the association of seed longevity with dormancy and pre-harvest sprouting

8- The analysis of the relationship between seed longevity and agronomic traits

The identification and characterisation of seed longevity, dormancy and pre-harvest sprouting loci/genes in wheat will not only help in broadening our knowledge about these traits but will also help to understand their interactions. In addition, this will improve the methods to conserve cereal seeds in genebanks.

2   Literature review

2.1   Brief history about the awareness of seed longevity

The history of awareness about seed ageing dates back quite long ago. Some of the oldest botanical writings are still extant - those of Theophrastus (372 BC-287 BC) - considering problems of seed deterioration to some degree. Theophrastus was well aware that some kinds of seed retained vitality longer than others: onions, for example rapidly lost viability, whereas millets had excellent powers of preservation. In general, seed was considered best for sowing when it was a year old and useless after four years. He also noted that seeds stored at high altitude in breezy locations could remain viable and free from pests for up to forty years. He explained the malady of seed ageing as 'decline in seed vigour was thought to be due to a prolonged elemental imbalance associated with loss of moist component'. Hard seeds, such as those of the date palm, retained this element more tenaciously and consequently served their viability longer (Priestley 1986).

Fan Sheng-zhi Shu, an agriculturistic book of China written in the 1st century B.C. (Shih 1959), clearly show that wheat and millet seeds best retain their viability if they are kept as dry as possible, cool and free from pests. It also stated that only large and solid ears of wheat should be chosen for sowing, and they should be dried as thoroughly as possible by the heat of the sun before they are placed in storage.

2.2   Classical genetic studies on seed longevity

The classical genetic studies for longevity have been best characterised in maize (*Zea mays*). It was pointed out by Weiss and Wentz (1937) that maize seeds which are homozygous for either the *luteus* 2 or *luteus* 4 genes were more susceptible to ageing and most died within two years under open storage. *Luteus* gene imparts yellow colour to maize seedling and seeds. Seed material homozygous for other *luteus* genes displayed unimpaired longevity. The physiological basis of this difference remains obscure.

Later, Lindstrom (1942, 1943) in two unrelated studies indicated that the long-lived character in maize appeared to be dominant, although a non-cytoplasmic maternal plant influence could also be identified. Rao and Fleming (1979) transferred the same nuclear genotype used by Lindstrom (1942) in genetically different cytoplasmic types. The seeds

were stored for 2 years under normal conditions of room temperature and humidity. Germination tests performed after 2 years showed a marked influence of cytoplasmic factors with respect to seed storability.

Haber (1950) also investigated long- and short-lived lines of maize. Evidence for the dominant character of the long-lived trait was obtained from an analysis of the storage characteristics of single cross hybrids.

Based on a survey that extends over 20 years of open storage of maize Yarchuk (1966) and Yarchuk and Leizerson (1972) reported that hard flint and dent varieties were found to remain viable longer than starchy and sweet form. Yarchuk (1974) also found that soft seeded maize varieties in open storage are probably more responsive to fluctuations in atmospheric humidity, and in consequence loss of viability is more rapid.

Moreno-Martínez et al. (1978) reported differences between maize lines stored at 17% moisture content and 25°C for period up to 120 days. However, enhanced longevity was clearly associable with resistance to invasion by pathogenic fungi of the genus *Aspergillus*.

The genetic basis of longevity in maize has also been addressed by Scott (1981), who selected seeds with pronounced resistance to ageing using experimental procedures (42°C and saturated humidity). After three selection cycles, sensitivity to accelerated ageing was significantly reduced which provides the evidence that in maize, genetic improvement for storability is feasible.

Van de Mey et al. (1982) also indicated that winter wheat stores better than spring wheat over periods of 15-20 years at 5°C. On the contrary Mackay and Tonkin (1967) reported no association between colour and longevity in wheat and Čuriová and Valasák (1984) have indicated that varieties of spring wheat are far more stable in accelerated ageing treatments (45°C, 95% RH) than winter wheat lines.

Hard seeds in legumes within a particular seed lot have been reported to retain viability longer in storage than their softer companions (Harrington 1916, Wahlen 1929, Flood 1978, Gō et al. 1979). According to Gvozdeva and Zhukova (1971), chickpeas with black seed coats store better than lighter coloured varieties and Van der Maesen (1984) also reported that pale-seeded "Kabuli" chickpeas are shorter lived than "Desi" types with thicker, harder

coats. In addition, Starzinger et al. (1982) found that black-seeded soybeans were more resistant to storage under conditions of high humidity than pale varieties, a trait that was associated with depression of fungal growth. Atabekova and Ermakova (1973) also claimed that alkaloid-containing strains of *Lupinus angutifolius* were harder-seeded and longer-lived than sweet forms.

In *Phaseolus vulgaris*, Nakayama and Saito (1980) used a diallele-cross-analysis of homozygous lines to identify parental genotypes capable of retaining high germinability over seven to eight years of storage. In three separate analyses, one varietal genotype clearly transmitted superior storability properties to its progeny, and the genes that conferred pronounced longevity were of dominant character.

In soybean, Kueneman (1983) was able to demonstrate by means of reciprocal crosses that the maternal plant exerted considerable influence on the longevity of F_1 seeds. The storability was assessed using an accelerated ageing regime (40°C, 75% RH) which minimises fungal effects. The identified maternal influence acts through characteristics of the seed coat being maternal in origin. Kueneman (1983) also distinguished a minor influence of the seed's own genotype on longevity.

Khoroshailov and Zhukova (1971) proposed that varieties of linseed (*Linum usitatissimum*) developed principally for oil (linseed) lost viability in storage faster than those cultivated primarily for fibre (flax).

In okra (*Abelmoschus* sp.), the heritability of seed longevity has been demonstrated by Kuwada (1980) at the species level when he studied and artificially created amphidiploid (2n=192) produced from *A. esculuntus* (2n=124) and *A. manihot* (2n=68). The new species displayed seed longevity characteristics similar to those of its *A. esculentus* parent and considerably superior to those of the other species involved.

Significant cultivar-by-ageing interactions have also been reported in cotton by Bourland and Ibrahim (1982) when they performed the germination tests of seeds that had previously been exposed to 45°C and saturated humidity for several days.

Contrasting reports exist as far as ploidy level is concerned in relation to longevity. Perdok (1970) came up with a conclusion that in beets (*Beta vulgaris*), polyploidy decreases seed

longevity. According to him, diploid seed is more resistant than triploid, triploid more than tetraploid. Murín (1972) claimed that increased ploidy in *Allium* was associated with lowered sensitivity to ageing.

The phenomenon of intraspecific variability in seed longevity has also been addressed by Nagel et al. (2009) when they studied 55 accessions of barley stored at the Gatersleben genebank since 1974. Germination tests after 35 years of storage indicated intraspecific variability of seed longevity within barley. The authors concluded that since these tested accessions were grown at the same time and handled the same way after harvest, therefore this difference must be due to genetic variation in seed longevity.

In a similar study in *Brassica napus* L., Nagel et al. (2011) tested 42 accessions for viability after 26 years of storage at ambient conditions. Despite having been grown simultaneously and subjected to the same post-harvest and storage conditions, the accessions nevertheless displayed variation with respect to seed viability which leads them to conclude that there is a genotypic component involved in the determination of seed viability. Interestingly, the same phenomenon of intra-specific variability towards longevity has been observed in *Sorghum bicolor* L., *Secale cereale* L. and *Linum usitatissimum* L. (Nagel et al. 2010).

2.3   Genetic mapping of seed longevity traits

Genetic mapping of seed longevity was initiated only recently when the arrival of molecular markers to study and improve important plant traits revolutionised the science of genetics and plant breeding. It started with studies on model plant *Arabidopsis* followed by rice, soybean, wheat (*Aegilops*), maize, barley, lettuce and oilseed rape. These are discussed in detail in the coming paragraphs.

2.3.1   *Arabidopsis*

Bentsink et al. (2000) analyzed the seed-soluble oligosaccharides (OS) (sucrose, raffinose, and stachyose) content of several *Arabidopsis* accessions and thus identified the genotype Cape Verde Islands having very low raffinose series OS content. By performing QTL mapping in an RIL population, they identified one major QTL responsible for the practically monogenic segregation of seed stachyose content. This locus also affected the content of the two other OSs, sucrose, and raffinose. Two candidate genes encoding respectively for galactinol synthase and raffinose synthase were located within the genomic region around this major

QTL. In addition, they identified three minor QTLs, each one specifically affecting the content of an individual OS. Seed storability was also studied in the same RIL population by measuring viability (germination) under two different seed aging assays: after natural ageing during 4 years of dry storage at room temperature without any control of humidity and after experimental aging induced by a controlled deterioration test. In total, four QTLs for storability were mapped. Comparison of the QTL genetic positions showed that the seed storability is not affected by the OS contents as the major QTLs for these two traits lie at different positions in the *Arabidopsis* genome.

A detailed investigation to study seed longevity in *Arabidopsis* was also reported by Clerkx et al. (2004a). 114 F_9 RILs derived from a cross of accessions 'Landsberg *erecta*' and 'Shakdara' were genotyped with 65 polymerase chain reaction-based markers and the phenotypic marker *erecta*. Dormancy, speed of germination, seed sugar content, seed germination after a controlled deterioration test, hydrogen peroxide (H_2O_2) treatment and abscisic acid treatment were investigated. For all traits, one or more QTLs were identified with some QTLs for different traits co-locating. For longevity, 3 QTLs on chromosomes 1, 3 and 4 were identified after controlled deterioration test.

2.3.2 Rice

QTLs for seed longevity in rice have been identified by Miura et al. (2002) using 98 backcross inbred lines (BILs) derived from a cross between an *Oryza sativa* ssp. *japonica* variety 'Nipponbare' and an *O. sativa* ssp. *indica* variety 'Kasalath'. Three putative QTLs for seed longevity on chromosomes 2 (*qLG-2*), 4 (*qLG-4*) and 9 (*qLG-9*) were identified. 'Kasalath' alleles increased the seed longevity. The authors also verified the effect of 'Kasalath' alleles for *qLG-9* using chromosome segment substitution lines.

Seed storability in rice has also been studied by Zeng et al. (2006). The authors used a set of 127 DHLs derived from anther culture of an *O. sativa* ssp. *indica* x *O. sativa* ssp. *japonica* hybrid ('ZYQ8'x'JX17'). Three QTLs for rice storability were detected on chromosomes 9 (*qLS-9*), 11 (*qLS-11*) and 12 (*qLS-12*). The authors also verified the effects of 'ZYQ8' alleles for *qLS-11* and *qLS-12* using chromosome segment substitution lines.

An attempt to study the role of hull, seed coat and embryo on the effect of rice storability QTL *qLG-9* along with *qLG-2* and *qLG-4* QTLs reported by Miura et al. (2002) was made by

Shigemune et al. (2008). The authors used chromosome segment substitution lines (CSSLs) derived from the cross between 'Koshihikari' and 'Kasalath'. In both hulled and unhulled seeds, *qLG-9* enhanced seed longevity whereas *qLG-2* and *qLG-4* had no effect. The germination test using unhulled seeds of the reciprocal crosses between 'Koshihikari' and 'SL226', the CSSL harbouring *qLG-9*, failed to reveal a maternal effect from the hull or seed coat on the longevity giving the indication that the increased longevity by *qLG-9* was associated primarily with embryonic and/or endospermic factors.

The identification of QTLs controlling seed storability in rice based on relative germination (%) using a saturated linkage map and RILs derived from a cross between *O. sativa* ssp. *japonica* cultivar 'Asominori' and *O. sativa* ssp. *indica* cultivar 'IR24' has been reported by Xue et al. (2008). Three QTLs (*qRGR-1*, *qRGR-3* and *qRGR-9*) were identified on chromosomes 1, 3 and 9, respectively. 'IR24' alleles enhanced seed storability in all the three QTLs. These QTLs were verified using 'IR24' CSSLs in the 'Asominori' genetic background. The authors also concluded that since *qRGR-9* was also detected in other studies of seed storability in rice, this region may become important for isolating the responsible gene.

2.3.3   Soybean

Association of SSR markers with seed longevity in soybean has been demonstrated by Singh et al. (2008a) in an $F_{2:3}$ population of a cross of 'Birsa soya-1' x 'JS 71-05'. 153 $F_{2:3}$ lines were subjected to accelerated ageing. In total, four (Satt538, Satt285, Satt600 and Satt434) independent SSR markers were declared to be significantly associated with seed longevity. These four markers were located on four different linkage groups namely A2 (158.63 cM), D1b (75.4 cM), H (105 cM) and J (25.51 cM), respectively as reported by Cregan et al. (1999). Singh et al. (2008b) reported that Satt538 and Satt434 were also associated with seed coat permeability in soybean.

2.3.4   Barley

Identification of seed longevity QTLs in barley and possible candidate genes responsible for increased seed life have been reported by Nagel et al. (2009). The authors used three DH populations: Steptoe x Morex ('S x M') population consisting of 150 lines of spring barley – 'OWB' mapping population consisting of 94 lines of spring barley and 100 lines of winter barley population 'W766'. For 'S x M', a major QTL for longevity on chromosome 5H was

identified. For 'OWB', 3 QTLs on chromosomes 2H, 5H and 7H were found, whereas 'W766' analysis yielded one QTL on chromosome 7H. *Nud* gene determining the hulled/naked caryopsis was identified as the candidate gene in 'W766' and 'OWB' populations segregating for that character. *Zeo1* gene responsible for very compact spikes with long awns and reduced fertility was identified as a candidate gene for longevity in OWB. In addition, *Ale* (*Aleurain*) was detected as a possible candidate gene to be involved to provide long life to seeds. The *aleurain* gene was syntesised from gibberellic acid-stimulated aleurone cell mRNA (Rogers et al. 1985) whose expression is regulated by the plant hormones gibberellic acid and abscisic acid which are known to take part in germination process.

2.3.5 Maize

Studies to identify genes related to germination in aged seeds in maize have been performed by Revilla et al. (2009). The authors used sweet corn inbred line 'P39' and the field corn inbred line 'EP44'. Bulks of living and dead seeds after 20 and 22 years of storage were compared by using SSRs and, when the bulks differed for a marker, the individual grains were genotyped. Differences between dead and living seeds could be explained by residual variability, spontaneous mutation, or ageing. Variability was larger for chromosome 7 than for other chromosomes, and for distal than for proximal markers, suggesting some relationships between positions in the genome and viability in aged seed. Polymorphic SSRs between living and dead seeds were found in six known genes including *pathogenesis-related protein 2*, *superoxide dismutase 4*, *catalase 3*, *opaque endosperm 2* and *metallothionein 1* that were related to germination, along with *golden plant 2*. In addition five novel candidate genes have been identified; three of them could be involved in resistance to diseases, 1 in detoxification of electrophillic compunds, and another in transcription regulation.

2.3.6 Wheat (*Aegilops*)

Currently no information is available about the nature of seed longevity in wheat which makes up the largest share of the seeds stored in the genebanks across the world. However, Landjeva et al. (2010) made one pilot investigation to study seed vigour, longevity and early seedling growth investigating a set of wheat/*Aegilops tauschii* introgression lines. The authors reported 5 QTLs for seed longevity on *Aegilops* chromosomes 1D and 5D giving the indication of existence for genetic variability in seed longevity in wheat.

2.3.7 Lettuce

In lettuce, Schwember and Bradford (2010) performed QTL mapping for seed longevity after conventional storage (30% RH and various temperatures for various durations) and controlled deterioration (CD) on RILs produced from a cross between *Lactuca satvia* cv. 'Salinas' x *Lactuca serriola* accession 'UC96US23'. They identified multiple QTLs under CD and conventional storage conditions. But they did not find a correlation between the results of CD and conventional storage which led them to conclude that CD conditions are not predictive for ageing in conventional storage conditions. Also, they did not find the same QTLs when seeds from different years of production were analysed giving the indication that seed longevity is strongly affected by the production environment.

2.3.8 Oilseed Rape

Recently, Nagel et al. (2011) examined a population of 153 doubled haploid lines of the winter oilseed rape 'YE2-DH' mapping population (Badani et al. 2006) to explore the genetic basis of seed longevity by applying three different artificial ageing treatments. They discovered 13 QTLs in total for longevity, most of which were treatment specific. In fact, only 1 QTL on chromosome N7 was common to all 3 AA treatments.

2.4 Seed longevity and dormancy

Relationship between seed longevity and dormancy is not clearly figured out yet. In *Arabidopsis*, Clerkx et al. (2004b) studied various mutants that had mutations in defined developmental or biochemical pathways. Reduced longevity was shown by mutants with *abscisic acid insensitive3* (*abi3*) and *abscisic acid deficient1* (*aba1*) mutations that may also be partially related to the seed dormancy phenotype for those mutants. Thus a positive association between longevity and dormancy can be hypothesised.

In contrast, in rice, Miura et al. (2002) performed QTL mapping for dormancy of the same population for which they have investigated seed longevity traits (see 2.3.2). Whereas the loci for seed longevity were located on chromosomes 2, 4 and 9, QTLs for dormancy were identified on chromosomes 1, 3, 5, 7 and 11 which led to the conclusion that these two traits seem to be controlled by different genetic factors.

2.5   Genetic mapping of dormancy and pre-harvest sprouting in wheat

Dormancy and pre-harvest sprouting are two extreme opposite phenomena. Dormancy is a seed characteristic, the degree of which defines what conditions should be met to make the seeds germinate (Vleeshouvwers et al. 1995). Dormancy is a situation when a seed fail to germinate under optimum conditions (Simpson 1990) and pre-harvest sprouting (PHS) is a situation when seeds start to germinate under conditions of high humidity and high temperature while still being on mother plant. These two phenomena are thought to be controlled by similar loci. Many studies have been performed in wheat to map loci for dormancy and pre-harvest sprouting. These included Anderson et al. (1993), Chen et al. (2008), Flintham et al. (2002), Groos et al. (2002), Kato et al. (2001), Kulwal et al. (2005), Lohwasser et al. (2005), Mares et al. (2005), Mori et al. (2005), Noda et al. (2002), Osa et al. (2003), Sorrells and Anderson (1996), Torada et al. (2005), Zanetti et al. (2000) for pre-harvest sprouting where 13 of 21 chromosomes of wheat were reported to harbour QTLs. These chromosomes were 1A, 2B, 3A, 3B, 3D, 4A, 5A, 5D, 6A, 6B, 7A and 7D. Similarly, PHS studies have been performed by Kato et al. (2001), Chen et al. (2008), Lohwasser et al. (2005), Mares et al. (2005), Noda et al. (2002) and Torada et al. (2005) where a major QTL on chromosome 4AL has been reported. On the other hand, Osa et al. (2003) and Mori et al. (2005) detected a major QTL on chromosome 3A.

3   Materials and methods

3.1   Plant materials

One bi-parental population and two association mapping panels were used in this study to investigate genetics of seed longevity and dormancy in hexaploid wheat. The bi-parental 'International Triticeae Mapping Initiative' (ITMI) mapping population was analysed to find 'Quantitative Trait Loci' (QTLs) whereas the two association mapping panels consisting of 96 advanced germplasm accessions and 183 genebank accessions, respectively, were used to find 'Marker Trait Associations' (MTAs).

3.1.1   ITMI mapping population

The ITMI mapping population was established in the early 1990s and used world-wide for joint mapping of RFLP or microsatellite markers. This mapping population is the most extensively used one for mapping molecular markers and to find genetic loci determining important agronomic traits in wheat. The first SSR-based genetic map of wheat was created using this population (Röder et al. 1998) and since then, many additional microsatellites have been mapped on this material.

The population was created by crossing the spring wheat variety 'Opata 85' with the synthetic hexaploid wheat 'W7984', generated via a cross of the *Triticum tauschii* accession 'CIGM86.940' (DD) with the tetraploid wheat 'Altar 84' (AABB). Dr. A. Mujeeb-Kazi made this interspecific cross at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico. Out of the 114 lines that originally were developed, a sub sample of seeds of 86 randomly selected lines regenerated in 2003 and stored at 0°C in glass jars (ITMI 2003) were used. The same population was regenerated in 2009 (ITMI 2009) in a glass house at IPK, Gatersleben and seeds from 99 randomly selected lines were available.
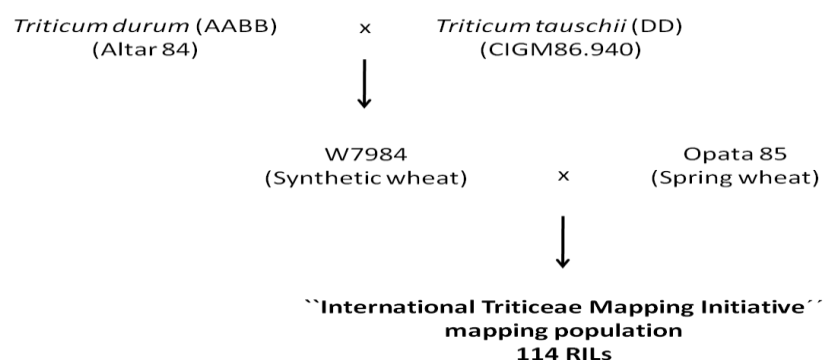
Figure 1: Pedigree of ITMI mapping population

3.1.2 Advanced germplasm collection

The advanced germplasm collection consisted of 96 winter wheats comprising entries from 21 countries assembled from a larger core collection created at the Institute of Field and Vegetable Crops (Novi Sad, Serbia) based on contrasting phenotypic expression for breeding relevant traits (Kobiljski et al. 2002, Quarrie et al. 2003) (Table 2). Population was regenerated in Serbia in 2009 in field plots and transferred to Germany in 2010 for longevity experiments. Whole collection was regenerated again in field plots in IPK-Gatersleben in 2010 and and fresh spikes and seeds were available for dormancy and PHS test in 2011.

Table 2: Advanced gemplasm collection along with country of origin

Name	Origin	Name	Origin	Name	Origin
Magnif 41	Argentina	UPI 301	India	Nov. Crvena	Serbia
Gala	Argentina	Acciaio	Italy	Nova Banatka	Serbia
Crook	Australia	Ai-bain	Japan	NS 33/90	Serbia
Kite	Australia	Norin 10	Japan	NS 46/90	Serbia
Min. Dwarf	Australia	Saitama 27	Japan	NS55-25	Serbia
Timson	Australia	*Triticum compactum*	Latvia	NS 74/95	Serbia
Triple Dirk B	Australia	Cajeme 71	Mexico	PKB Krupna	Serbia
Triple Dirk S	Australia	Inia 66	Mexico	Pobeda	Serbia
Triple Dirk B (bulk)	Australia	Mex. 120	Mexico	Sofija	Serbia
Rusalka	Bulgaria	Mex. 17 bb	Mexico	Tibet Dwarf	Tibet
Lambriego Inia	Chile	Mex. 3	Mexico	Tom Thumb	Tibet
Al-Kan-Tzao	China	S. Ceros	Mexico	Mironovska 808	Ukraine
Ching-Chang 6	China	Vireo "S"	Mexico	Centurk	USA
Peking 11	China	BCD 1302/83	Moldova	Lr 12	USA
Ana	Croatia	F 4 4687	Romania	Benni multifloret	USA
ZG 1011	Croatia	Donska polupat.	Russia	Florida	USA
ZG 987/3	Croatia	Bezostaja 1	Russia	Hays 2	USA
ZG K 3/82	Croatia	Ivanka	Serbia	Helios	USA
ZG K 238/82	Croatia	Mina	Serbia	Holly E	USA
ZG K T 159/82	Croatia	NS 22/92	Serbia	Hope	USA
Capelle Desprez	France	NS 559	Serbia	INTRO 615	USA
Durin	France	NS 602	Serbia	Lr 10	USA
Avalon	Great Britain	NS 63-24	Serbia	Norin10/Brev. 14	USA
Brigant	Great Britain	NS 66/92	Serbia	Phoenix	USA
Highbury	Great Britain	NS 79/90	Serbia	Purd./Loras	USA
TJB 990-15	Great Britain	Renesansa	Serbia	Purd.39120	USA
Bankut 1205	Hungary	Sava	Serbia	Purd.5392	USA
L-1	Hungary	Salvija	Serbia	Red Coat	USA
Szegedi 768	Hungary	*Tr. sphaerococcum*	Serbia	Semilia Eligulata	USA
Hira	India	L 1/91	Serbia	UC 65680	USA
Sonalika	India	L 1A/91	Serbia	VEL	USA
Suwon 92	India	Nizija	Serbia	WWMCB 2	USA

3.1.3 Genebank collection

The genebank collection consisted of 183 hexaploid wheat accessions including 129 spring and 54 winter wheats selected from the germplasm repository at the German Federal *ex situ* Genebank in Gatersleben, Germany. These accessions come from 32 countries across five continents of the world. Table 1 in Appendix I provides the list of the collection. Seeds harvested in 1974 and since then stored at 0°C in glass jars were tested for germination in 2008. Accessions were reproduced in 2010 in foil tunnel at IPK, Gatersleben (Figure 2). The material was hand threshed after manual harvest and seeds were available from 177 accessions for accelerated ageing (AA) and controlled deterioration (CD) tests to study longevity and other traits. Details about the population structure and sub-groups of this collection are presented in Appendix II.

Figure 2: Regeneration of the genebank collection in foil tunnel in 2010

3.2 Genotyping methods

3.2.1 ITMI mapping population

Data used for mapping of ITMI population consisted of 942 loci comprising mainly SSR and some RFPL markers. This data was kindly provided by Dr. Marion Röder, head of gene and genome mapping group, IPK-Gatersleben. The markers were uniformly distributed on the whole genome. Numbers of 290 markers were mapped to A, 354 to B and 298 to D genomes. In terms of homoeologous groups, 145 markers were mapped to group 1, 161 to

group 2, 126 to group 3, 106 to group 4, 144 to group 5, 106 to group 6 and 154 to group 7 chromosomes.

3.2.2 Advanced germplasm collection

Triticarte Pty. Ltd (Canberra, Australia; http://www.triticarte.com.au/) performed the diversity array technology (DArT) profiling assay. Each marker was designated by the prefix '*wPt'*. 525 markers were assigned to linkage groups based on Crossa et al. (2007). All chromosomes except 6D were covered. 24 markers were mapped to more than one position and considered as multilocus markers. In addition, there were 315 unmapped markers, of which chromosomal location of 177 markers was known. The whole map is available in Neumann et al. (2011).

The population structure analysis revealed two sub-groups within the whole collection. So, each accession was assigned to one of the two sub-groups. There was one large sub-group (Q1) composed of 76 accessions and one small sub-group (Q2) consisting of the rest of 20 accessions. Majority of the non-Serbian accessions lied in the first sub-group (Neumann et al. 2011).

The average r^2 value for all pairs of markers was 0.069. The critical value for r^2 for the whole collection was 0.263, whereas the r^2 values for Q1 and Q2 were 0.279 and 0.535, respectively. Beyond this critical value of r^2, LD is likely to be caused by genetic linkage. Further details for this collection can be found in Neumann et al. (2011).

3.2.3 Genebank collection

For genebank accessions, genotyping for 183 accessions (Table 1, Appendix 1) was also performed by Triticarte Pty. Ltd (Canberra, Australia; http://www.triticarte.com.au/). For that purpose, DNA from seeds produced in 1974 and since then stored in the genebank storage facility at 0°C in glass jars was used. In total, 2,266 DArT markers were genotyped. Crossa et al. (2007) was used to assign 429 DArT markers to their respective linkage groups. 1,837 markers remained unmapped, of which the chromosomal locations of 928 markers were provided by Triticarte Pty. Ltd, whereas the rest remained unknown. The whole genetic map of genebank accessions is provided in Appendix III.

3.3 Phenotyping methods

To study seed longevity experimental or artificial ageing tests by Hampton and TeKrony (1995) were used along with standard germination test devised by International Seed Testing Association (ISTA 2008). In addition, dormancy was investigated for the ITMI population regenerated in 2009 and dormancy and pre-harvest sprouting were investigated in the association mapping panels. In the genebank collection, further traits as heading time, flowering time, plant height, 1000 grain weight and the presence of awns and pubescence were recorded at the time of regeneration.

The number of seeds and replicates used in seed tests are provided in Table 3. Germination after AA (GAA) and CD (GCD) was calculated as healthy and normal seedlings from the total seeds (ISTA 2008). Relative germination (%) after AA or CD (RGAA or RGCD) was calculated and used for mapping seed longevity loci in all the three populations except for long term cold stored seeds of genebank accessions where absolute germination of 2008 was used. This material was aged 'naturally'.

Relative germination was determined in the following way:

$$RGAA \ or \ RGCD \ (\%) = \frac{GAA \ or \ GCD \ (\%)}{Germination \ before \ AA \ or \ CD \ (\%)} \times 100$$

Table 3: Number of seeds and replicates used in seed ageing, dormancy and pre-harvest sprouting tests

Population	Long term cold storage ageing	Standard germination test	Accelerated ageing (AA) test	Controlled deterioration (CD) test	Dormancy test	Pre-harvest sprouting test
ITMI2003	-	4 replicates of 50 seeds each	4 replicates of 50 seeds each	-	-	-
ITMI2009	-	4 replicates of 50 seeds each	4 replicates of 50 seeds each	4 replicates of 50 seeds each	60 seeds at 10°C and 60 seeds at 20°C	-
Advanced gemplasm collection	-	4 replicates of 50 seeds each	4 replicates of 50 seeds each	4 replicates of 50 seeds each	60 seeds at 10°C and 60 seeds at 20°C	5 spikes per plant at 2 weeks interval
Genebank collection	1 replicate of 100 seeds	3 replicates of 100 seeds each	3 replicates of 100 seeds each	3 replicates of 100 seeds each	60 seeds at 10°C and 60 seeds at 20°C	5 spikes per plant at 2 weeks interval

3.3.1 Standard germination test

In the standard germination test, 50 seeds were germinated between two layers of moistened filter papers. These were formed to rolls standing on a Jacobsen apparatus at 25±1°C during day and 23±1°C during the night (14 hours of day and 10 hours of night). The number of normal, healthy seedlings (Figure 3) from the proportion of germinated seedlings was counted according to ISTA (2008) on the eighth day. Figure 4 shows germinated seedlings but only healthy and green seedlings with proper root-shoot ratio (normal germination) were taken into account for genetic mapping whereas the rest which were germinated were recorded as abnormal seedlings. These were not included in mapping for loci related to longevity.
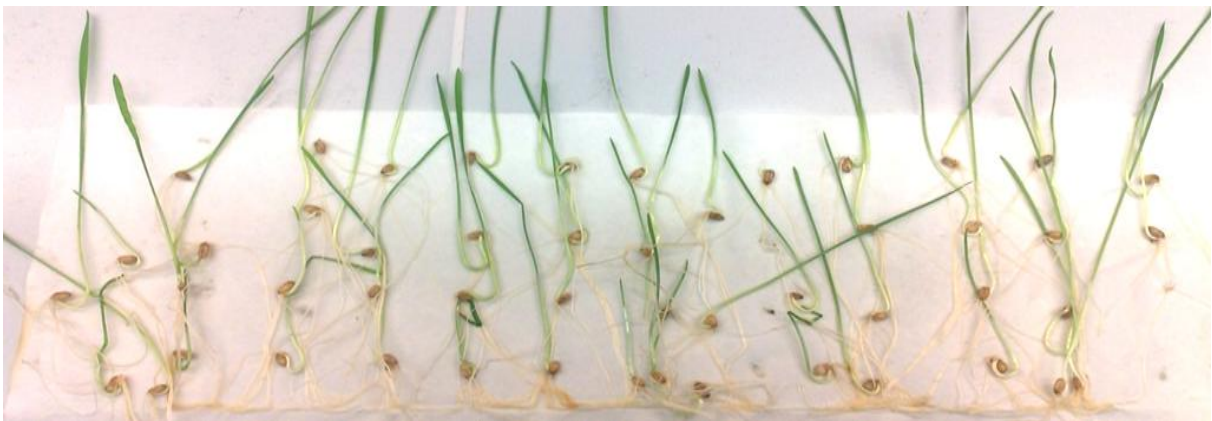
Figure 3: Wheat seedlings on moistened filter paper during standard germination test (ISTA 2008) on eighth day
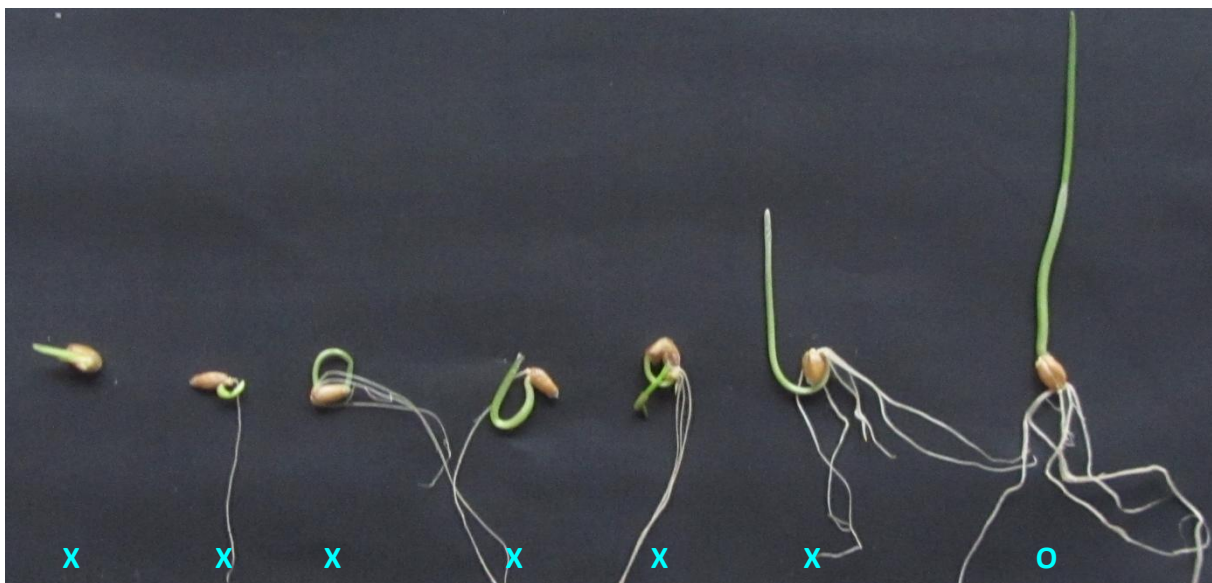
Figure 4: Wheat seedlings performance after standard germination test (ISTA 2008). X = abnormal seedlings; O = normal germination

3.3.2 Seed ageing

Seed ageing was either achieved naturally through long term cold storage as in case of the genebank collection association mapping panel or through different laboratory protocols such as experimental/artificial ageing.

Experimental ageing tests were performed keeping in view the principle that high temperature and high relative humidity deteriorate seeds rapidly. In these tests, seeds are subjected to these two environmental variables, which lead to changes in the seeds resulting in loss of viability in terms of reduced germination or abnormal seedlings. More vigorous seeds deteriorate to a lesser extent and at a slower rate than the seeds which are unequipped with any defense mechanism against these variables which deteriorate at a much faster rate and to a greater extent (Hampton and TeKrony 1995). Two different kinds of experimental/artificial ageing tests were used in this study: accelerated ageing (AA) test and controlled deterioration (CD) test.

3.3.2.1 Long term cold storage ageing

Long term cold storage ageing was achieved by placing the seeds of 1974 reproduction in glass jars sealed and kept at 0°C for 35 years. This long term cold storage is 'natural' ageing under practical genebank conditions.

3.3.2.2 Accelerated ageing (AA) test

The steps to perform accelerated ageing tests are given below:

1. 50 seeds each in case of ITMI 2003, ITMI2009 and advanced germplasm collection and 100 seeds (free from any impurity and soil debris) in case of the genebank collection were placed in sterilised cages rested on a steel stand above 200 ml of distilled water in glass chambers.

2. The glass chambers were sealed air tight (Figure 5a) and placed in a climatic chamber for 72 hours at 43±0.5°C (Figure 5b).

3. Standard germination tests were performed after 72 hours following the ISTA (2008) protocols.

| (a) | (b) |

Figure 5: (a) Steel cages containing seeds placed on steel stands above 200 ml distilled water and sealed in glass jars and **(b)** Glass jars are placed in a climatic chamber at 43°C for 72 hours

3.3.2.3 Controlled deterioration (CD) test

Following steps were performed:

1. Initial moisture content (ISTA 2008) of the seeds was determined as follows:

    a. Two replicates each of approximately 15 g seeds were ground to powder using seed grinder (Figure 6a) and then placed in oven for 2 hours at 130±5°C.

    b. Ground seeds were weighted again after two hours to find the moisture contents of the seeds using the formula

$$smc_1 = \left(1 - \frac{X_1}{X_2}\right) \times 100$$

Where smc_1 = initial seed moisture content

X_1 = weight of seeds before drying and

X_2 = weight of seeds after drying.

The moisture content of the seeds to be subjected to CD test was raised to 18% as recommended by Hampton and TeKrony (1995) by the addition of distilled water. The amount of water was calculated using the formula:

$$m_{H2O} = (100 - smc_1/100 - smc_T) \times m_1$$

Where m_{H2O} = added water (g),

smc_1 = initial seed moisture content (%),

smc_T = target seed moisture content (%) and

m_1 = initial seed weight (g).

2. Seeds were shaken for two hours after addition of water to ensure uniform distribution and thereafter sealed in aluminum bags (Figure 6b) to prevent any moisture loss and kept at 7°C for additional 22 hours. After 22 hours, seeds were placed in climatic chamber for 72 hours at 43±0.5°C.

3. Standard germination tests were performed after 72 hours following the ISTA protocols (ISTA 2008).

(a)   (b)

Figure 6: (a) Seed grinder to grind seeds to determine moisture % of seeds and **(b)** Wheat seeds sealed in a sealer to keep constant moisture contents

3.3.3 Dormancy and pre-harvest sprouting test

To measure dormancy, 60 seeds per line were harvested and germinated immediately after maturity using two different temperatures: 20°C for 7 days and 10°C for 14 days on moist filter papers placed in plastic boxes under a light regime of 12 h light/12 h dark. The evaluation of the seeds was conducted according to ISTA (2008). The percentage of dormant seeds at 20°C and at 10°C (Figure 7) was calculated. The dormancy parameter which is also

called as the dormancy index (DI) (Strand, 1965) was calculated and used for mapping in the following way:

$$DI = [2(\% \ dormant \ seeds \ at \ 10°C) + \% \ dormant \ seeds \ at \ 20°C]/3$$

Figure 7: Seed germination at 10°C after 14 days during dormancy test

To test pre-harvest sprouting, five ears per line were harvested at maturity from intact plants. These were placed immediately in plates full of wet sand for 14 days. Plates were placed in glass houses under a temperature regime of 20-22°C. Plates were kept moistened by regular addition of tap water. After 2 weeks, data was recorded based on a scale of 1-7 where 1 stands for no sprouting and 7 stands for sprouting of all the spikelets of an individual spike (Figure 8 a and b). The same procedure was repeated after 2 weeks. A mean of these two scores was used for further analysis.

(a)   **(b)**
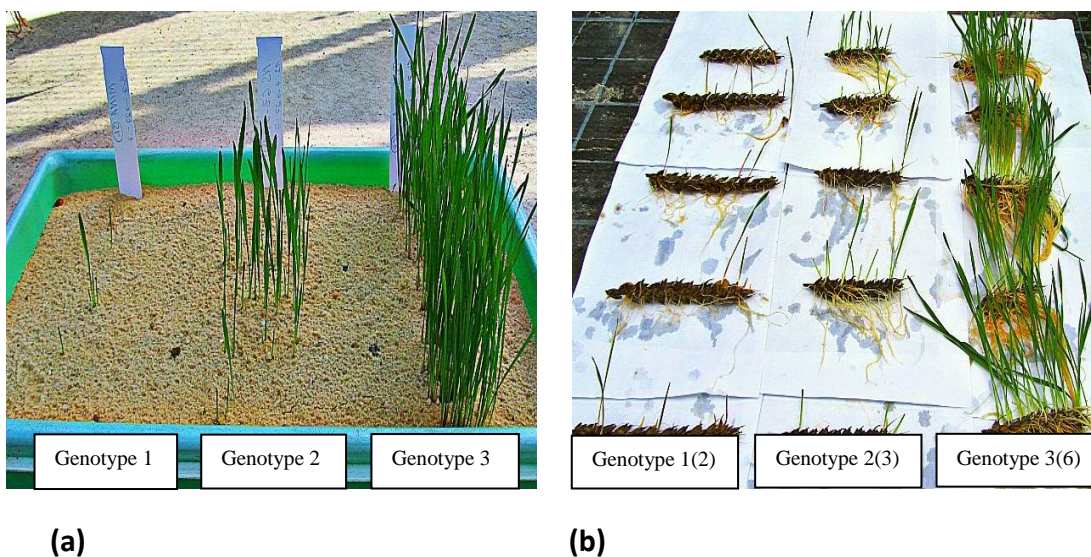
Figure 8: (a) Spikes germination during pre-harvest sprouting test after 14 days and **(b)** Spikes scoring from 1-7 (scores in brackets) days during pre-harvest sprouting test after 14 days

3.3.4 Agronomic traits characterization

Heading time was recorded as the number of days from date of sowing to the appearance of complete spikes having left the flag leaf of 50% of plants per genotype.

Days to flowering were determined as the number of days from date of sowing to the first anthers exertion of 50% of the ears per genotype of all plants.

Heights of all the plants were measured from the soil surface to the tip of the main ear at maturity per genotype (excluding awns).

Thousand grain weight was measured using standard balance of 1000 seeds.

Awns were scored either as present (1) or absent (0) before harvest.

Flag leaf pubescence was also scored either as presence (1) or absence (0) of tiny hair at the base of the flag leaf.

3.4 Statistical analysis and tools

Since one bi-parental and two association mapping populations were used to map genetic loci responsible for seed longevity, different statistical tools were applied in this study.

3.4.1 SPSS 10.0

Software SPSS version 10.0 (SPSS Inc. 1999) was used to perform descriptive statistics, correlations and t-tests. The same software was used for construction of box-plots of various traits.

3.4.2 QGENE

Relative germination after AA and CD was used for QTL identification in ITMI population. QTL analysis was performed using QGENE software (Nelson 1997) by applying the single marker regressions analysis and simple interval mapping (SIM) approach. Single marker analysis indicates the chromosome location of QTL (R^2) and source of allele. SIM makes use of linkage maps and analyses intervals between adjacent pairs of markers along the chromosomes simultaneously. The LOD (logarithmic of odds) score is used to identify the most likely position for a QTL where the highest LOD value is obtained. The QTL with a LOD value 3.0 and above was regarded as major QTL whereas the QTL with an LOD value of 1.5 < 3.0 was determined as minor QTL.

3.4.3 STRUCTURE and PAUP

The software STRUCTURE (Pritchard et al. 2000) was employed to determine the population structure of genebank accessions, prior to analyses for marker trait associations (MTAs) to avoid false positives for marker trait associations. A subset of unrelated mapped markers was used to assign accessions to different sub-groups. Options of admixture model, a burn-in of 10,000 iterations and 10,000 MCMC (Markov Chain Monte Carlo) durations were used to test for a K value in the range of 1-16.

The software PAUP (Swofford 2002) was used to construct the dendrogram for the genebank collection and to verify the results of STRUCTURE.

3.4.4 TASSEL 2.01

The software TASSEL 2.01 (Bradbury et al. 2007) was used to calculate associations between the markers and each trait in turn, employing the general linear model (GLM) based on the chosen Q-matrix derived from STRUCTURE. Mixed Linear Model (MLM) (Yu et al. 2006) was used using Q-matrix and the Kinship-matrix, which was also, calculated using the version 2.01 in TASSEL considering all markers. The Kinship-matrix is generated by TASSEL through converting the distance matrix calculated from TASSEL's Cladogram function to similarity matrix. The EMMA method (efficient mixed-model association) (Kang et al. 2008) was applied and the MLM parameters were left at the default settings from TASSEL. Markers that gave significant ($p \leq 0.05$) or highly significant associations ($p \leq 0.01$) in 2 out of 4 replicates in advanced germplasm collection and 2 out of 3 replicates in genebank accessions along with mean with always both general linear model (GLM) and mixed linear model (MLM) were declared as significant or highly significant associations, respectively.

4   Results

4.1   ITMI mapping population

4.1.1   Phenotyping

Figure 9 gives the dispersion of ITMI2003 and 2009 germination before and after AA and CD and of DI of 2009 seeds.

4.1.1.1 ITMI 2003

The seeds of year 2003 showed a germination % before ageing (IG2003) with mean germination of 83.85±7.5% (Appendix 1, Table 2). The highest germination was 97% whereas the lowest germination was 59%. Upon accelerated ageing, the germination percentages of the lines dropped variably and independently from initial germination. The mean germination after artificial ageing (GAA2003) was dropped to 62.63±13.2% with the highest and the lowest germination being 90.5 % and 27%, respectively. The mean relative germination after AA (RGAA2003) ranged from 42.58% to 97.53% with the mean value of 74.52±13.5%.

4.1.1.2 ITMI 2009

The initial germination for the seeds produced in 2009 (IG2009) ranged from 24.5% to 98% with the mean of 75.96±15.9%.

The germination percentage after AA (GAA2009) ranged from 0.5 to 92% with the mean value of 32.87±24.0%. The relative germination rate after AA (RGAA2009) ranged from 0.86 to 97.5% with the mean of 40.69±24.9%.

The highest value of germination after CD (GCD2009) was 90% and the lowest was 0.5%. The mean germination after CD was less as compared to mean germination after AA with 25.37±24.4%. The mean relative germination rate after CD (RGCD2009) was 31.57±27.6% and ranged from 0.68 to 91.8%.

The percentage of dormant seeds after 20°C (D20) treatment was 75.63±26.8% with 27.0 and 90.5% being the lowest and highest values whereas the percentage of dormant seeds after 10°C (D10) was 9.63±11.3% with 0 and 58.0% lowest and highest dormancy %, respectively. DI (dormancy index) value ranged from 1.11 to 72.22 with the mean of 31.63±13.6 (Appendix 1, Table 3).
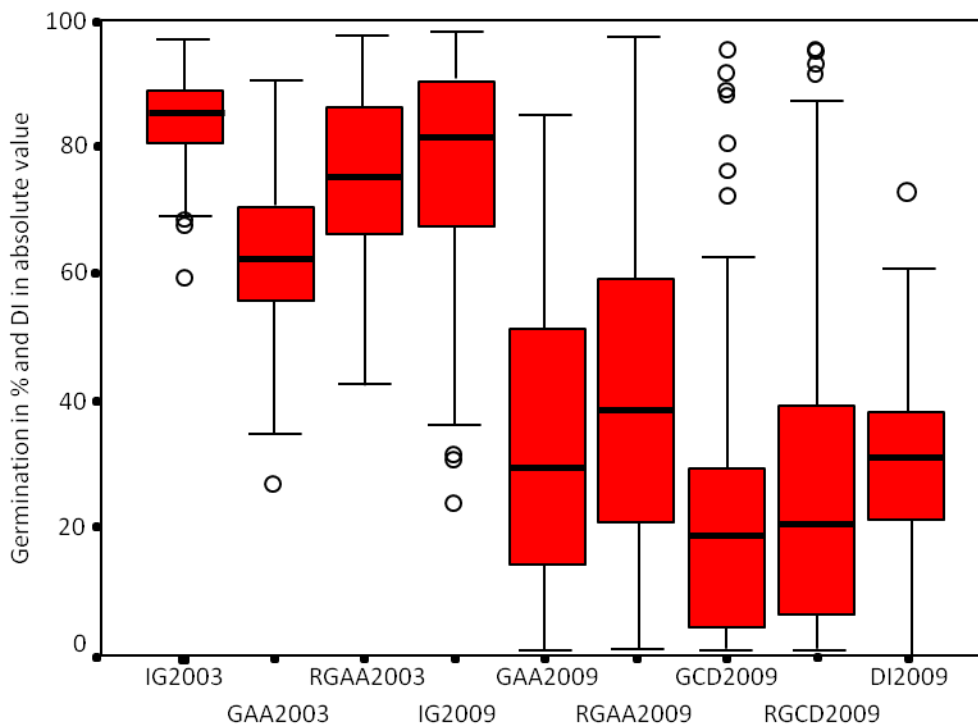
Figure 9: Boxplots of IG2003, GAA2003, RGAA2003, IG2009, GAA2009, GCD2009, RGAA2009, RGCD2009 and DI2009 of ITMI. IG, GAA, RGAA, GCD, RGCD and DI stand for initial germination, germination after AA, relative germination after AA, germination after CD, relative germination after CD, and dormancy index, respectively. 2003 and 2009 indicate regeneration years

4.1.2 Correlations

There was a correlation of 0.54** between the initial germination and germination after AA for the seeds produced in 2003. The correlations between initial germination and germinations after AA and CD for 2009 seeds were 0.63** and 0.46**, repectively. RGAA2009 and RGCD2009 showed a low correlation of 0.30** between each other. But there was no correlation observed between RGAA2003 and RGAA2009 or RGCD2009. Similarly no correlation of dormancy was observed with either initial seed germination or germination after AA and CD. Table 4 shows the correlations between the traits measured/calculated.

Table 4: Correlations among longevities and dormancy of ITMI

	IG2003	GAA2003	RGAA2003	IG2009	GAA2009	GCD2009	RGAA2009	RGCD2009
GAA 2003	0.54**							
RGAA 2003	0.13	0.90**						
IG 2009	0.15	0.01	-0.06					
GAA 2009	0.00	0.05	0.07	0.63**				
GCD 2009	0.1	0.02	-0.04	0.46**	0.43**			
RGAA 2009	-0.07	0.03	0.09	0.51**	0.97**	0.36**		
RGCD 2009	0.1	0.02	-0.03	0.32**	0.33**	0.97**	0.30**	
DI 2009	-0.19	-0.29*	-0.24*	0.13	0.17	0.09	0.16	0.09

IG2003 = initial germination of 2003 seeds, GAA2003 = germination after AA of 2003 seeds, RGAA2003 = relative germination after AA of 2003 seeds, IG2009 = initial germination of 2009 seeds, GAA2009 = germination after AA of 2009 seeds, GCD2009 = germination after CD of 2009 seeds, RGAA2009 = relaltive germination after AA for 2009 seeds, RGCD2009 = relative germination after CD for 2009 seeds, DI2009 = dormancy index of 2009 seeds, * = significant correlation at $p < 0.05$ (2-tailed)and ** = significant correlation at $p < 0.01$ (2-tailed)

4.1.3 QTL mapping

The QTLs detected for seed longevity using relative germinations after AA and CD and dormancy using DI in ITMI population are summarized in Table 5 and Figure 10.

4.1.3.1 ITMI2003

One major QTL (LOD ≥ 3.0) for seed longevity associated with the marker *Xcdo1281* on the short arm of chromosome 2A near to the centromeric region was found in ITMI2003 analysis. This QTL was highly significant with the *p*-value of 0.0002 and LOD score of 3.22 explaining 20.1% of phenotypic variation for longevity after AA. There were two additional minor QTLs (1.5 ≤ LOD < 3.0) observed on chromosomes 2DL and 1DL. The markers associated with these QTLs were *Xgdm6* and *Xcmwg695* with LOD values of 1.91 and 1.57 explaining 16.4 and 8.1% phenotypic variation, respectively. The positive effect on longevity for chromosomes 2AS and 2DL were provided by 'W7984' whereas 'Opata' contributed for the positive effect for chromosome 1DL.

4.1.3.2 ITMI2009

Quantitative analysis performed to find the QTLs for seed longevity in the ITMI2009 revealed three minor QTLs after AA on chromosomes 3BS, 7AS and 1DL. The LOD values for these QTLs ranged from 2.3 (chromosome 3BS) to 1.8 (chromosome 1DL). The closest markers associated with these QTLs were *Xgwm376* (3BS) responsible for 16.1% variation, *Xbcd1066*

(7AS) responsible 10.8% variation and *Xbcd1930a* (1DL) responsible for 9.0% variation. The positive alleles in all cases were provided by 'Opata'.

Seed treatment with CD yielded four minor QTLs for chromosomes 1AL, 3DL, 1DS and 6BS. The highest LOD among those QTLs was 2.89 on chromosome 1AL, followed by 2.45 on 3DL and 2.11 on 1DS and the lowest LOD value was 2.06 observed for 6BS. The variations explained by these QTL were variable. The variation associated with longevity was 12.6% explained by marker *Xgwm99* on chromosome 1AL. There was 11.6% variation associated with marker *Xbcd515* on chromosome 3DL and 13.5% variation for marker *Xgwm1291* on chromosome 1DS whereas 14% of variation was associated with marker *Xgwm935a* on chromosome 6BS. 'Opata' contributed favorable effects on seed longevity for chromosomes 1AL and 3DL whereas 'W7984' contributed to seed longevity on chromosomes 1DS and 6BS.

DI calculated from 2009 seeds and subjected to QTL analysis revealed one major QTL for dormancy on chromosome 4AL. The LOD value for this QTL was 4.40 and the marker closely associated with this QTL was *Xksug12b* which explained a phenotypic variation of 17.2%. The source for the positive effects on dormancy was 'W7984'.

Table 5: Results of single marker analyses of germination and dormancy treatments for ITMI2003 and ITMI2009 are shown including QTL (quantitative trait loci) designation, marker, chromosome, LOD (logarithm of odds), source and phenotypic variation explained.

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.3	*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.1	*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 %

LOD = logarithm of odds, AA = accelerated ageing, CD = controlled deterioration, AS = A genome short arm, AL = A genome long arm, BS = B genome short arm, DS = D genome short arm and DL = D genome long arm
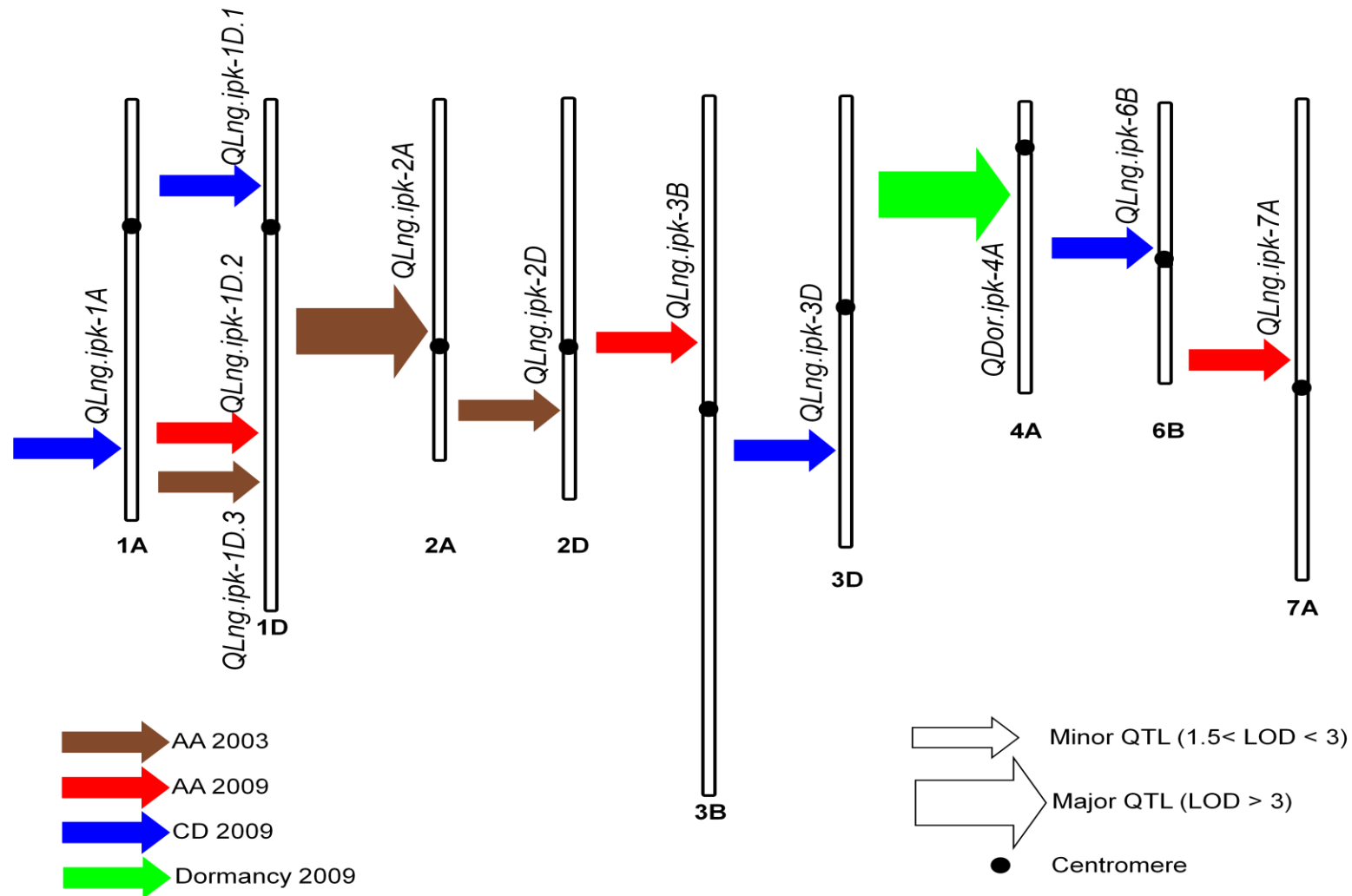
Figure 10: Positions of various QTLs for longevity and dormancy for ITMI2003 and ITMI2009. Color code is given in figure. AA 2003 = accelerated ageing for 2003 seeds, AA 2009 = accelerated ageing for 2009 seed lot, CD 2009 = controlled deterioration for 2009 seed lot and Dormancy 2009 = dormancy of 2009 seed lot

4.2 Advanced germplasm collection

4.2.1 Phenotyping

4.2.1.1 Standard germination and seed longevity after experimental seed ageing

The distribution of IG, GAA, RGAA, GCD and RGCD of this collection is shown in Figure 11. Table 4 (Appendix I) displays the minimum, maximum and mean values for the above mentioned traits along with standard deviations for the whole collection, Q groups as well as geographical groups.

The population consisted of 2 groups, Q1 and Q2 based on STRUCTURE analysis. However, this collection could also be divided into four sub-groups based on geographical distribution. These groups included (1) 24 European accessions excluding Serbian material, (2) 22 Serbian accessions, (3) American accessions consisting of 21 US accessions, 6 Mexican accessions, 2 accessions from Argentina and 1 accession from Chile; thus in total 30 accessions and (4) Asian (12 accessions) and Australian (8) accessions with 20 accessions in total.

In general, the whole collection of advanced germplasm showed a high percentage of initial germination (IG) with the mean of 93.70±5.1%. The initial germinations of the 2 Q groups as well as all the 4 geographical groups were very similar and, therefore, not considered further. AA had a dramatic effect on the performance of this collection and reduced the mean germination (GAA) to 10.97±12.1%. The minimum value of germination was 0% and maximum was 60.5%. The mean relative germination after AA (RGAA) was 11.55±12.8% that ranged from 0 to 67.98%

CD treatment also had profound effect on the germination of seeds (GCD) as evident from the mean germination after CD which was reduced to 61.05±25.3% with the minimum of 5.5% and maximum of 95%. The mean relative germination after CD (RGCD) was 64.57±25.8% with minimum and maximum values of 6.47% and 99.47%, respectively.

4.2.1.2 Dormancy and pre-harvest sprouting

Dormancy at 10°C of seeds regenerated in 2011 ranged from 0 to 41 with the mean dormancy of 3.57±6.32. Dormancy at 20°C was higher with mean of 24.7±16.53 and minimum and maximum of 0 and 59, respectively. DI ranged from 0 to 73.33 with the mean value of 17.69±13.08. High sprouting was observed in this collection which was in the range

of 2.1 to 7 with the mean of 4.5±1.3. Table 5 (Appendix I) shows the percentage of dormancy at 10°C and 20°C, dormancy index (DI) and pre-harvest sprouting (PHS) values for Q groups, geographical groups as well as whole collection whereas boxplots for D10, D20, DI and PHS are provided in Figure 11.
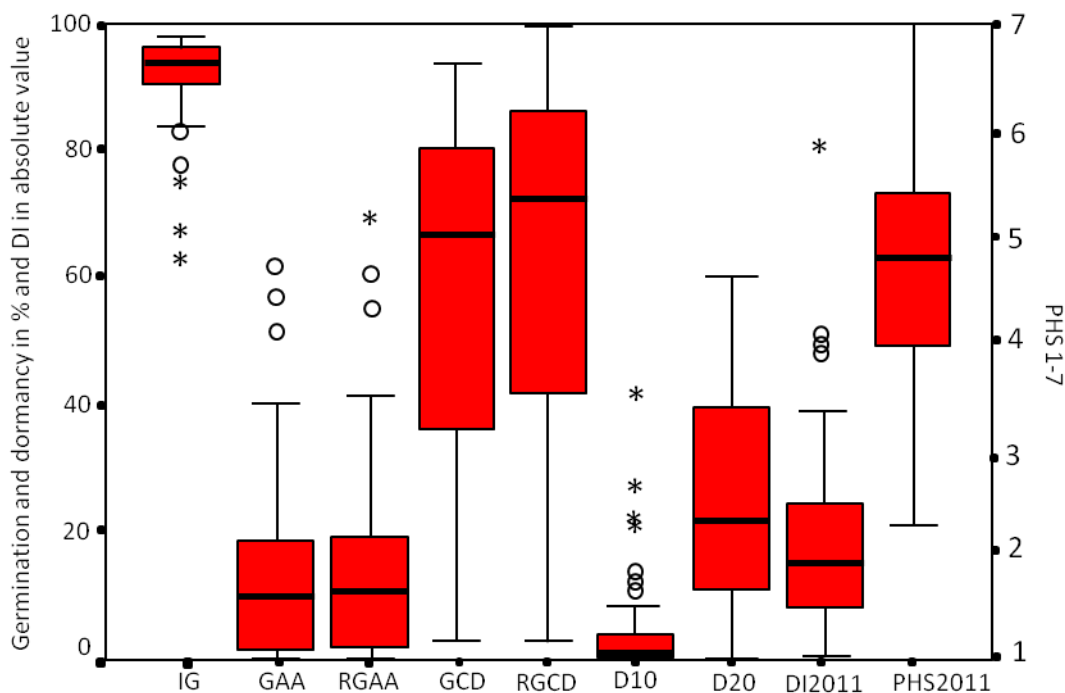
Figure 11: Boxplots of IG (initial germination), GAA (germination after AA), RGAA (relative germination after AA), GCD (germination after CD), RGCD (relative germination after CD), D10 (% dormant seeds at 10°C), D20 (% dormant seeds at 20°C), DI (dormancy index) and PHS (pre-harvest sprouting) of advanced germplasm collection

4.2.2 Correlations

Table 6 shows the correlation between the germination after AA and CD. IG gave a correlation of 0.25* with GAA and 0.23* with RGAA. The correlation of IG with GCD and RGCD was 0.48** and 0.41**, respectively. GAA and GCD showed correlation of 0.38** with each other and the correlation of RGAA with RGCD was 0.37**. DI and PHS showed no correlation with longevity but showed r of -0.50** with each other.

Table 6: Correlations among longevity, dormancy and PHS of advanced germplasm collection

	IG	GAA	RGAA	GCD	RGCD	DI
GAA	0.25*					
RGAA	0.23*	0.99**				
GCD	0.48**	0.38**	0.37**			
RGCD	0.41**	0.37**	0.37**	0.99**		
DI	0.02	0.18	0.19	0.08	0.08	
PHS	0.00	-0.11	-0.11	-0.03	-0.036	-0.50**

IG = initial germination, GAA = germination after AA, RGAA = relative germination after AA, GCD = germination after CD, RGCD = relative germination after CD, DI = dormancy index and PHS = pre-harvest sprouting, * = significant correlation at $p < 0.05$ (2-tailed) and ** = significant correlation at $p < 0.01$ (2-tailed)

4.2.3 Marker Trait Associations (MTAs)

The MTAs for seed longevity after AA and CD with mapped and unmapped markers are presented in Figure 12 and Table 7, respectively.

4.2.3.1 MTAs for seed longevity after AA

Considering GLM and MLM simultaneously, 16 mapped markers gave significant ($p \leq 0.05$) and 2 markers gave highly significant ($p \leq 0.01$) associations with seed longevity. There were 16 and 6 unmapped markers which were in significant and highly significant association with seed longevity, respectively. Chromosomes of 8 significant and 6 highly significant associated unmapped markers were known whereas 8 of them remained unknown.

4.2.3.2 MTAs for seed longevity after CD

After CD, 15 significant and 5 highly significant MTAs for seed longevity were observed with mapped markers. In addition, 10 significant and 3 highly significant MTAs for seed longevity with unmapped markers were observed. Chromosomes of 4 significant and 2 highly significant MTAs were known whereas others were not assigned to any chromosome.

4.2.3.3 Chromosome-wise comparison of MTAs for seed longevity

In total, 73 MTAs (40 MTAs after AA and 33 MTAs after CD including 2 MTAs detected for both AA and CD) were detected for seed longevity in this collection. The mapping positions of the significantly associated markers were known for 37 markers (Figure 15). Among the unmapped markers, chromosomal location of 20 markers was available.

In general, all the homoeologous groups gave associations for the traits of interest. Among groups, group 4 chromosomes showed the highest number of associations with 13 MTAs including two MTAs with unmapped markers. It was followed by group 7 chromosomes with 12 MTAs (seven MTAs with mapped markers and five MTAs with unmapped markers). Group 1 and group 2 chromosomes gave eight MTAs each. One MTA among group 1 and five MTAs of group 2 chromosomes were detected with unmapped markers. One marker of group 1 MTAs was significant with both AA and CD. Each of group 3 and 6 chromosomes gave six MTAs but one marker in group 3 and two markers in group 6 remained unmapped. Finally, group 5 chromosomes gave the least number of five MTAs for seed longevity including one with mapped and four with unmapped markers. The genome-wise comparison was not considered because of the unequal distribution of the markers which was highest for B genome, followed by A and D genomes. The latter was poorly covered.

The highest number of MTAs on one single chromosome was observed for 4A where 9 markers were in significant association with longevity, 8 of which were mapped and 1 unmapped. It was followed by chromosome 2A where 7 markers were in association with longevity. These included 2 mapped and 5 unmapped markers. Six markers (5 mapped and 1 unmapped) were in association with longevity on chromosome 7B. 4 MTAs each were associated with longevity on five chromosomes (1A, 4B, 5B, 6B and 7D). These MTAs were observed with 4, 3, 1 and 1 mapped marker of chromosomes 1A, 4B, 5B and 6B and 1, 3, 3 and 4 unmapped markers of chromosomes 4B, 5B, 6B and 7D. There were 3 MTAs observed on each of chromosomes 1B and 3B. Only 1 marker in case of chromosome 1B was unmapped. Chromosomes 3A, 6A and 7A showed 2 MTAs each. All MTAs were mapped except one MTA on chromosome 6A which was with an unmapped marker. Finally, each of chromosomes 1D, 2D, 3D, 5A and 7D showed 1 MTA. The MTAs of 1D, 2D and 7D were with mapped markers and the MTAs of 3D and 5A were with unmapped marker. Chromosomes 2B, 5D and 6D did not give any MTA.

4.2.3.4 MTAs for dormancy and pre-harvest sprouting

Dormancy revealed 70 MTAs out of which 45 were mapped and 25 unmapped but chromosome numbers of 14 MTAs of unmapped markers were known. Eleven MTAs with mapped markers and five MTAs with unmapped markers were highly significant.

There were less MTAs observed for pre-harvest sprouting than for dormancy. In total, 34 MTAs were revealed with 21 mapped and 13 unmapped markers. In case of unmapped markers, chromosome numbers of seven MTAs were available. Four MTAs with mapped markers and three with unmapped markers were highly significant.

4.2.3.5 Chromosome-wise comparison of MTAs for dormancy and pre-harvest sprouting

For dormancy, homoeologous group 1 showed maximum number of MTAs where 21 markers were in significant associations. Eight MTAs were observed for group 2 chromosomes whereas seven MTAs were observed for group 7 chromosomes. Finally, each of group 3, 4, 5 and 6 chromosomes exhibited six MTAs.

In terms of single chromosomes 1B gave the highest number of 17 MTAs for dormancy, followed by chromosome 6B with six MTAs. Chromosome 5B showed five MTAs. Each of chromosomes 2A and 7A showed four MTAs. There were four chromosomes (1A, 3A, 4A, and 4B) that gave three MTAs each. Two MTAs were shown individually by chromosomes 2B, 2D, 3B and 7B. Finally, 3D, 5D and 7D showed only one MTA for dormancy.

With respect to pre-harvest sprouting, homoeologous group 2 showed eight and homoeologous group 7 showed seven MTAs each which were the highest followed by group 5 and group 1 chromosomes with six and four MTAs, respectively. Two MTAs were present on group 6 and one MTA was shown by group 4 chromosomes. No MTA was observed on homoeologous group 3.

Chromosome 5B showed highest number of five MTAs for pre-harvest sprouting on one single chromosome. There were four MTAs observed individually on chromosomes 2D and 7A. Chromosome 1B and 2B gave three MTAs each and chromosome 7B gave two MTAs. One MTA was detected on seven chromosomes (1A, 2A, 4A, 5D, 6A, 6B and 7D). No MTA was found on chromosomes 1D, 3A, 3B, 3D, 4B, 4D, 5A and 6D.
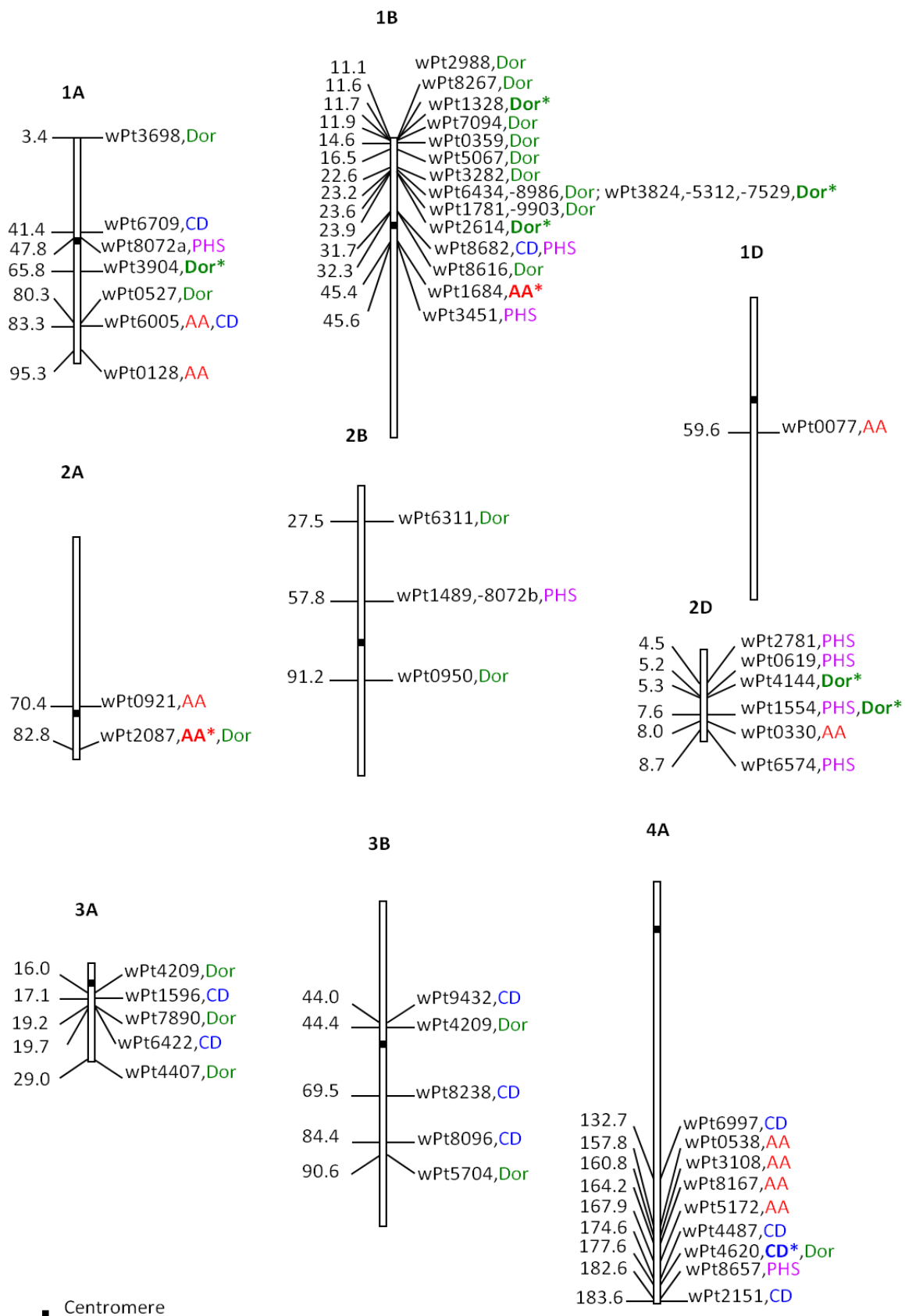
Figure 12: A genetic map showing MTAs significant at p ≤ 0.05 or p ≤ 0.01 (traits in bold with asterisk) for seed longevity after AA (accelerated ageing; red) and CD (controlled deterioration; blue) and for dormancy (Dor; green) and pre-harvest sprouting (PHS; pink) detected in the advanced germplasm collection
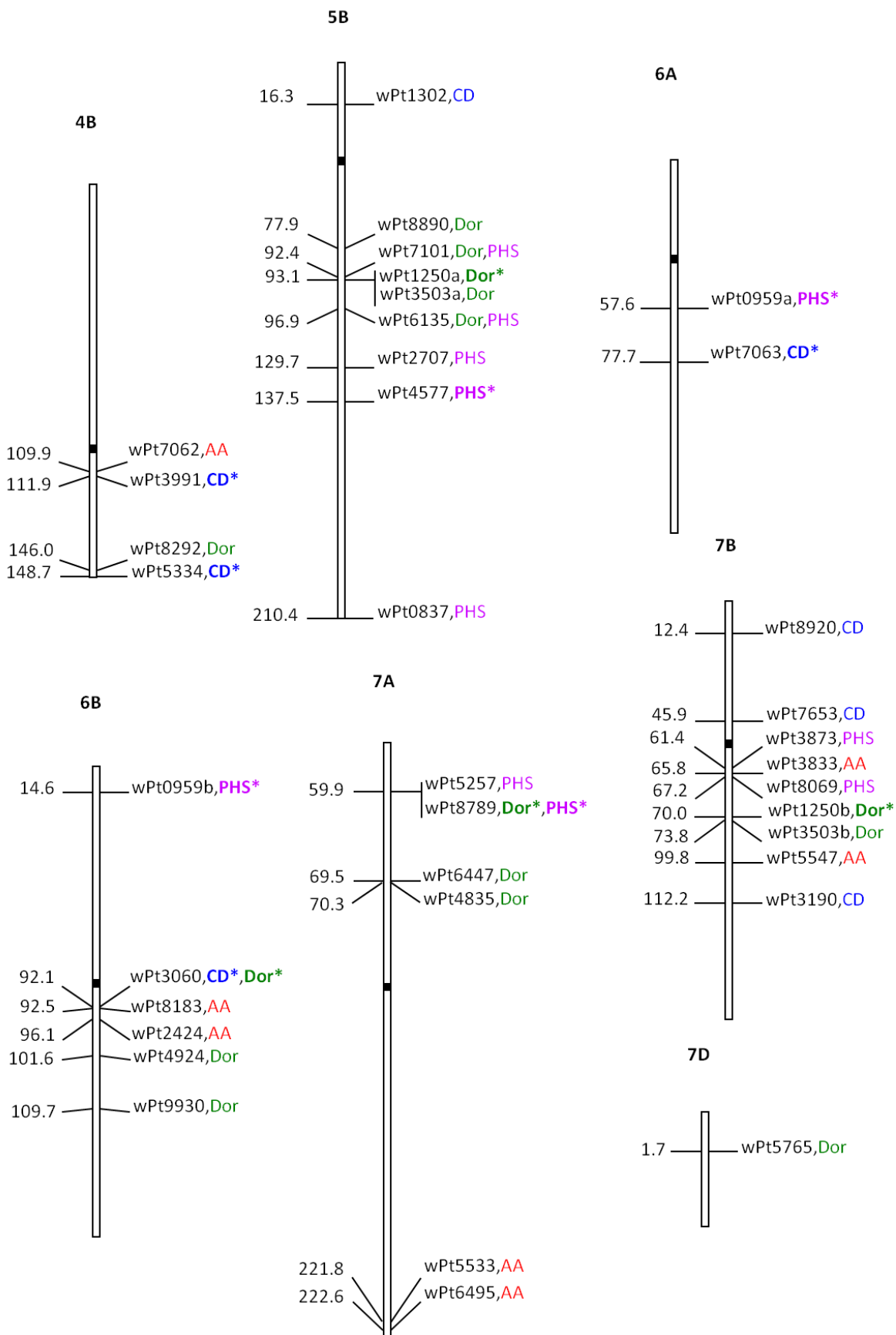
Figure 12: *Continued*

Table 7: MTAs significant at p ≤ 0.05 or p ≤ 0.01 (traits in bold with asterisk) for seed longevity after AA (accelerated ageing; red) and CD (controlled deterioration; blue) and for dormancy (Dor; green) and pre-harvest sprouting (PHS; pink) with unmapped markers detected in the advanced germplasm collection. –, unknown

Locus	Chromosome	Trait	Locus	Chromosome	Trait
wPt6012	1B	**CD***,PHS	wPt0170	–	**Dor***
wPt7708	1B	**Dor***	wPt0276	–	**Dor***,PHS
wPt1368	2A	**AA***,Dor	wPt0745	–	CD
wPt2185	2A	**AA***	wPt0773	–	Dor
wPt3976	2A	**AA***	wPt1022	–	Dor
wPt5865	2A	Dor, PHS	wPt1176	–	Dor
wPt6207	2A	**AA***, Dor	wPt2448	–	AA ,Dor
wPt7187	2A	**AA***	wPt2464	–	AA ,CD
wPt0079	2B	PHS	wPt2883	–	Dor
wPt4569	3D	AA	wPt4164	–	AA
wPt5506	3D	Dor	wPt4515	–	CD
wPt2777	4A	Dor	wPt4648	–	CD
wPt9901	4A	**Dor***	wPt5776	–	Dor
wPt9183	4A	AA	wPt5803	–	PHS
wPt5265	4B	Dor	wPt6160	–	**CD***,**PHS***
wPt8756	4B	**AA***,Dor	wPt6200	–	CD
wPt7769	5A	**CD***	wPt6457	–	**Dor***
wPt2373	5B	AA	wPt6878	–	AA
wPt4551	5B	AA	wPt7595	–	Dor
wPt6880	5B	AA	wPt7636	–	AA
wPt5870	5D	Dor,**PHS***	wPt7739	–	**PHS***
wPt3581	6B	Dor	wPt8275	–	AA
wPt4662	6B	CD	wPt8539	–	PHS
wPt4930	6B	Dor	wPt8456	–	AA
wPt9881	6B	Dor	wPt8713	–	Dor,PHS
wPt1695	6D	AA	wPt8938	–	AA
wPt5153	7A	Dor	wPt9986	–	CD
wPt9552	7A	PHS			
wPt9651	7A	PHS			
wPt6936	7B	CD			
wPt3923	7D	CD			
wPt5674	7D	CD			
wPt3923	7D	PHS			
wPt7842	7D	AA			
wPt8343	7D	AA			

4.3 Genebank collection

4.3.1 Phenotyping

4.3.1.1 Germination after long term seed storage

The mean germination of all genebank accessions of 1974 harvest in 2008 was 56.45±23.3% with 0 and 94 being the lowest and the highest values. Additionally, historical data from the germination tests performed on these accessions in 1978 and 1998 were available. Figure 13 shows the dispersion of germination in 1978 (G1978), 1998 (G1998) and 2008 (G2008) and Table 6 in Appendix I shows descriptive statistics of germination of this collection. The mean germination in 1978 for this collection was 87.13±9.1% with 57% minimum and 99% maximum germinations. Germination data from the tests in 1998 of these lines indicated a slight increase in the performance with the mean of 91.12±12.4% and minimum and maximum values of 22 and 100%, respectively.
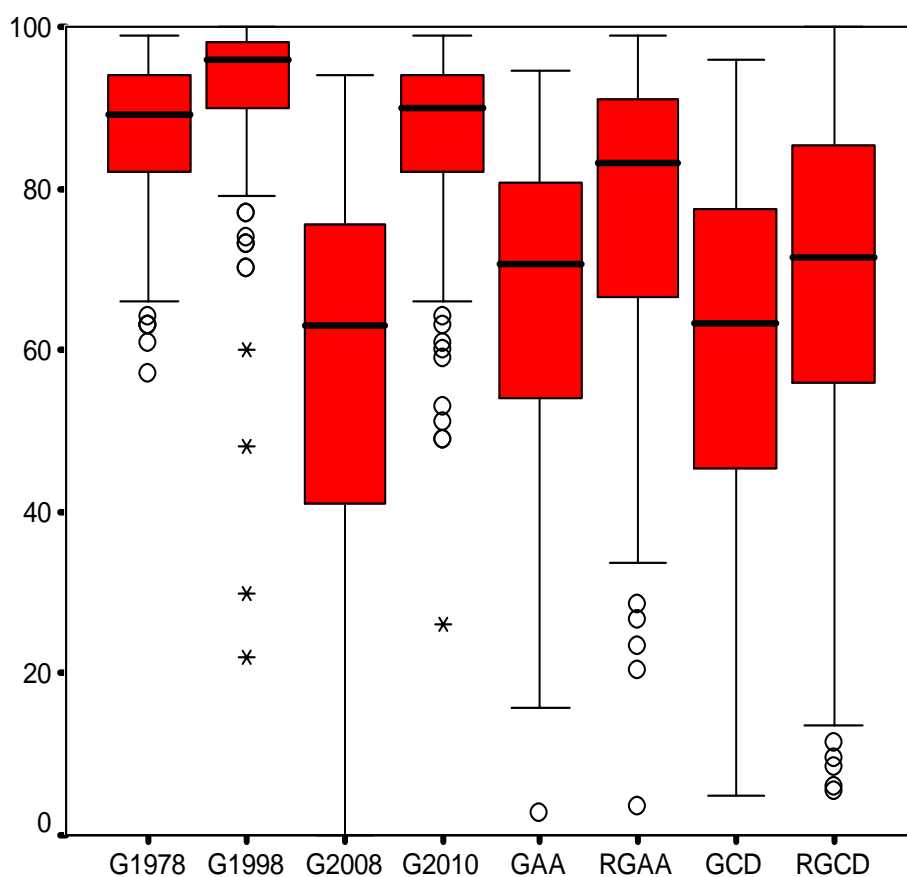
Figure 13: Boxplots of germination in 1978 (G1978), 1998 (G1998), 2008 (G2008), 2010 (G2010) and GAA (germination after AA), RGAA (relative germination afer AA), GCD (germination after CD) and RGCD (relative germination after CD) of 2010 seed lot of genebank collection

The genebank collection was composed of spring and winter wheat that originated from diverse locations belonging to different varieties. Therefore, these accessions could be divided into (a) according to their origin to Asia, South European, remaining European and South and North American accessions, (b) according to varieties of these accessions to *aestivum*, *lutescens*, *ferrugineum* and 'others' varieties and (c) according to their growth habit to spring and winter type. The graphical representation of germination during 1978, 1998 and 2008 of all the accessions as well as individual groups are presented in Figure 14 (a to k).

Mean germination of this collection (G2008) was dropped to 56.45±23.31 after 34 years of storage with the minimum and maximum values being 0 and 94%, respectively. The G2008 was significantly different from G1978 and G1998 germination at α = 0.01 (Appendix I, Tables 7 and 8). But there was no difference in G2008 of Asian, South European, rest of the European and North and South American accessions (Appendix I, Table 9) as well as in variety groups (*aestivum*, *lutescens*, *ferrugineum* and 'others') at α = 0.01 (Appendix I, Table 10). However, a significant difference was observed for G2008 between spring and winter wheat (Appendix I, Table 11).

Based on above mentioned observations, it was concluded that seed longevity in wheat is independent of species type and geographical origin but might depend on growth habit. Even within spring and winter type wheat, there was huge variability in terms of survival over longer periods of time. Since, these accessions were treated exactly the same way after growing at the same time, and stored at identical conditions i.e., 0±1°C and 8±2% moisture contents; it was assumed that there are genetic factors responsible for contrasting behaviours in longevity of these accessions.
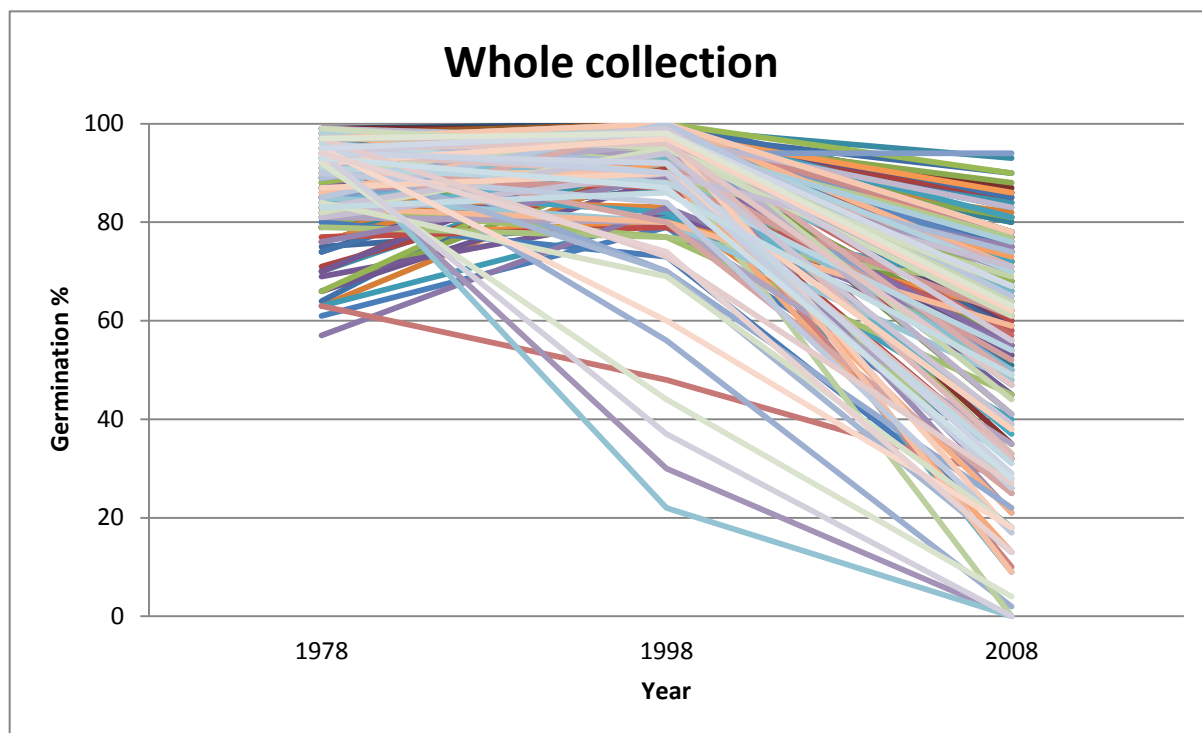
(a)
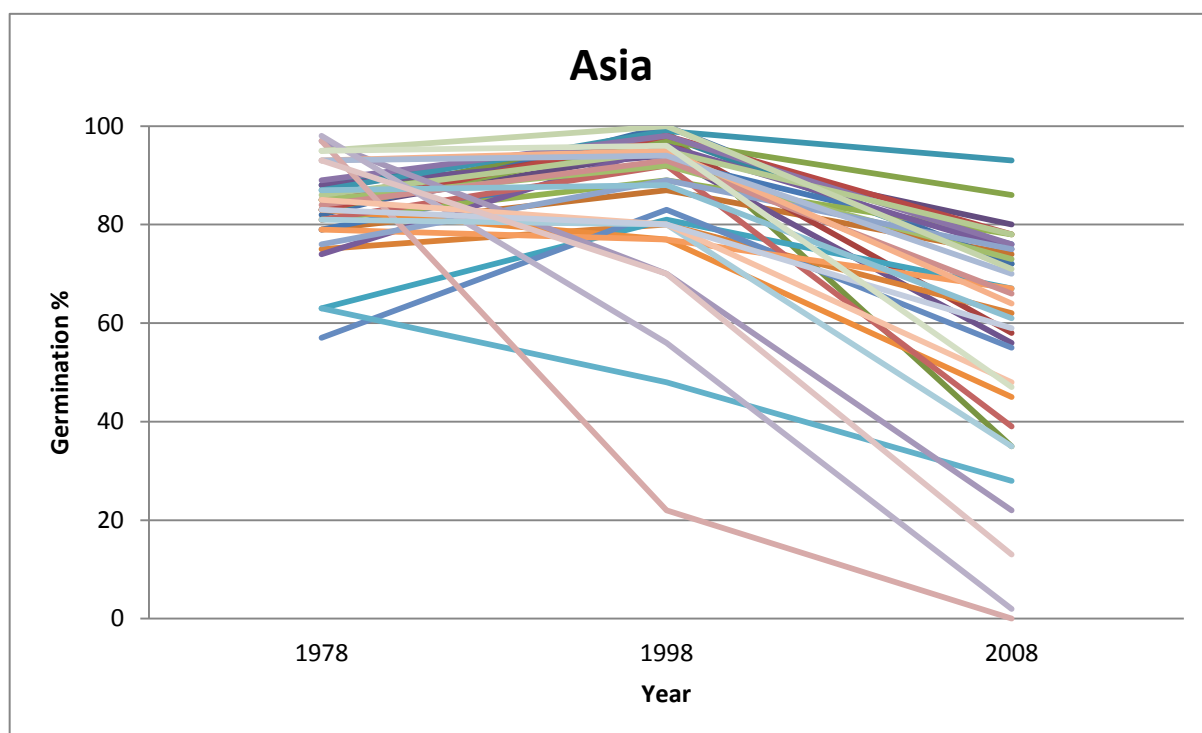
(b)

Figure 14: Graphical representation of germinations from 1978, 1998 and 2008 years of **(a)** whole collection, **(b)** Asian accessions, **(c)** South Europe accessions, **(d)** remaining of European accessions, **(e)** North and South American accessions, **(f)** var. *aestivum,* **(g)** var. *lutescens,* **(h)** var. *ferrugineum* **(i)** var. 'others', **(j)** spring wheat and **(k)** winter wheat

(c)

(d)

Figure 14: *Continued*

North and South America

(e)

Triticum aestivum* var. *aestivum

(f)

Figure: 14 *Continued*

Triticum aestivum var. *lutescens*

(g)

Triticum aestivum var. *ferrugineum*

(h)

Figure 14: *Continued*

Triticum aestivum (var. 'others')

(i)

Spring wheat
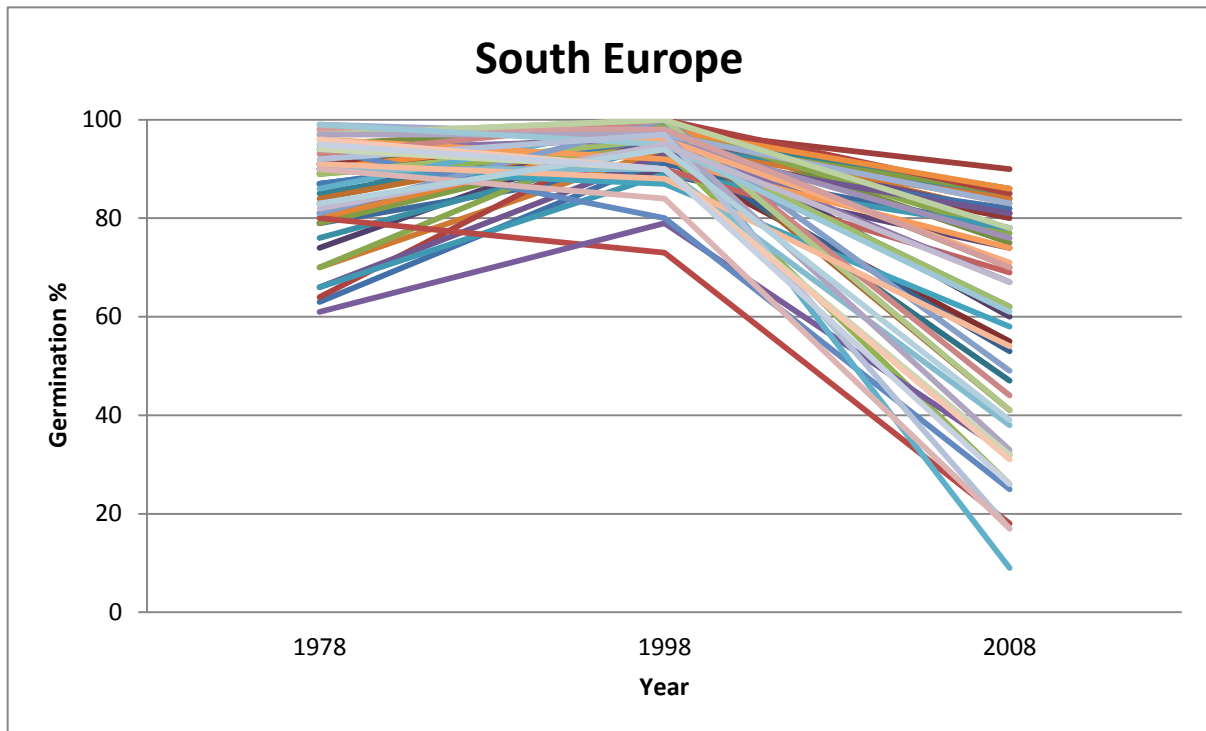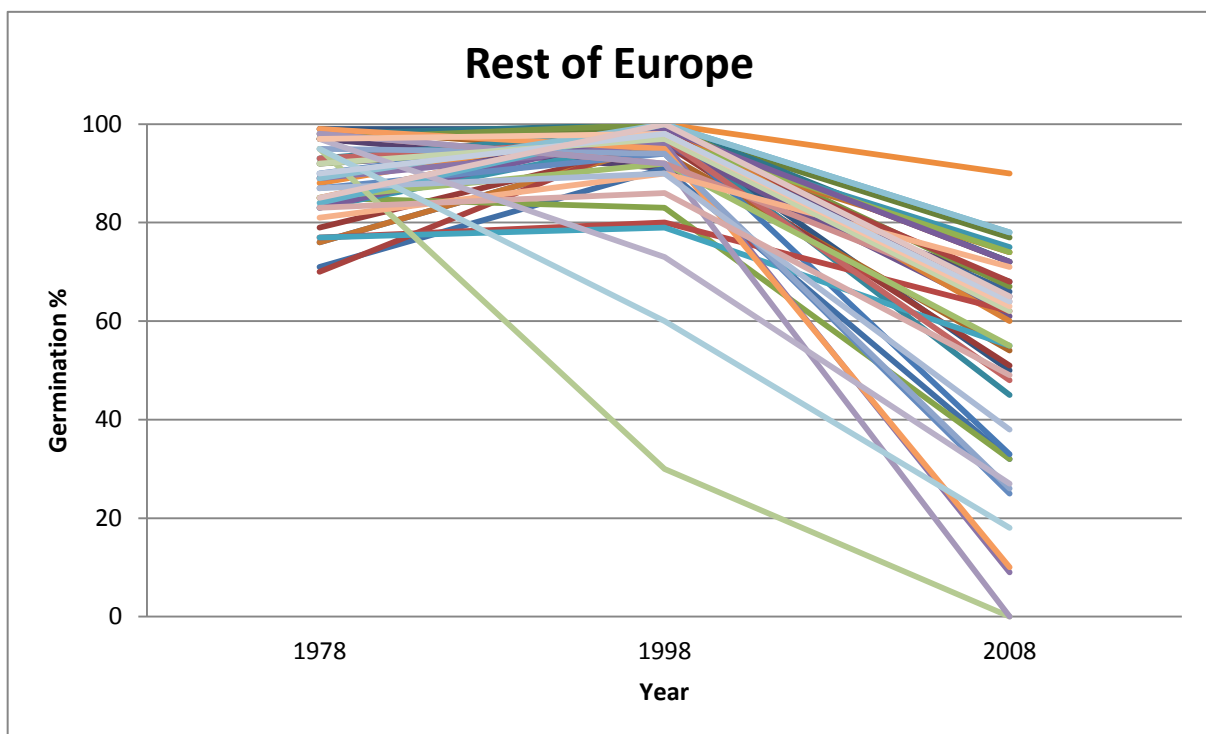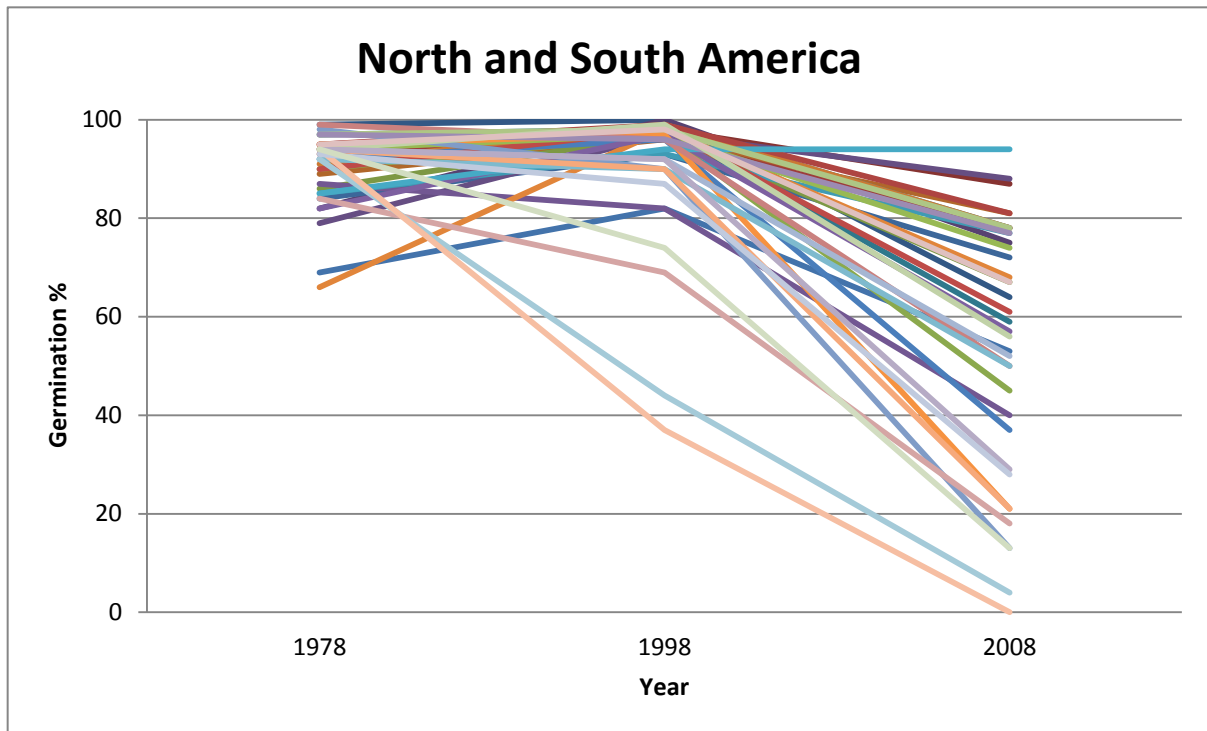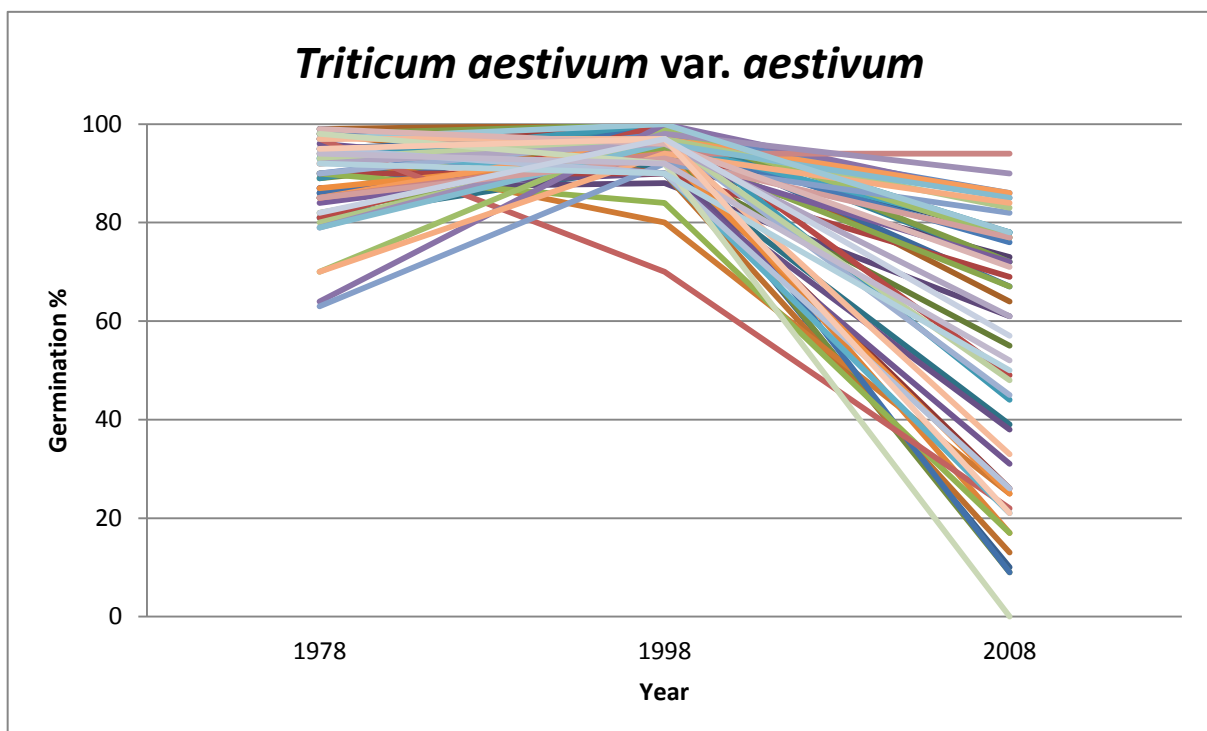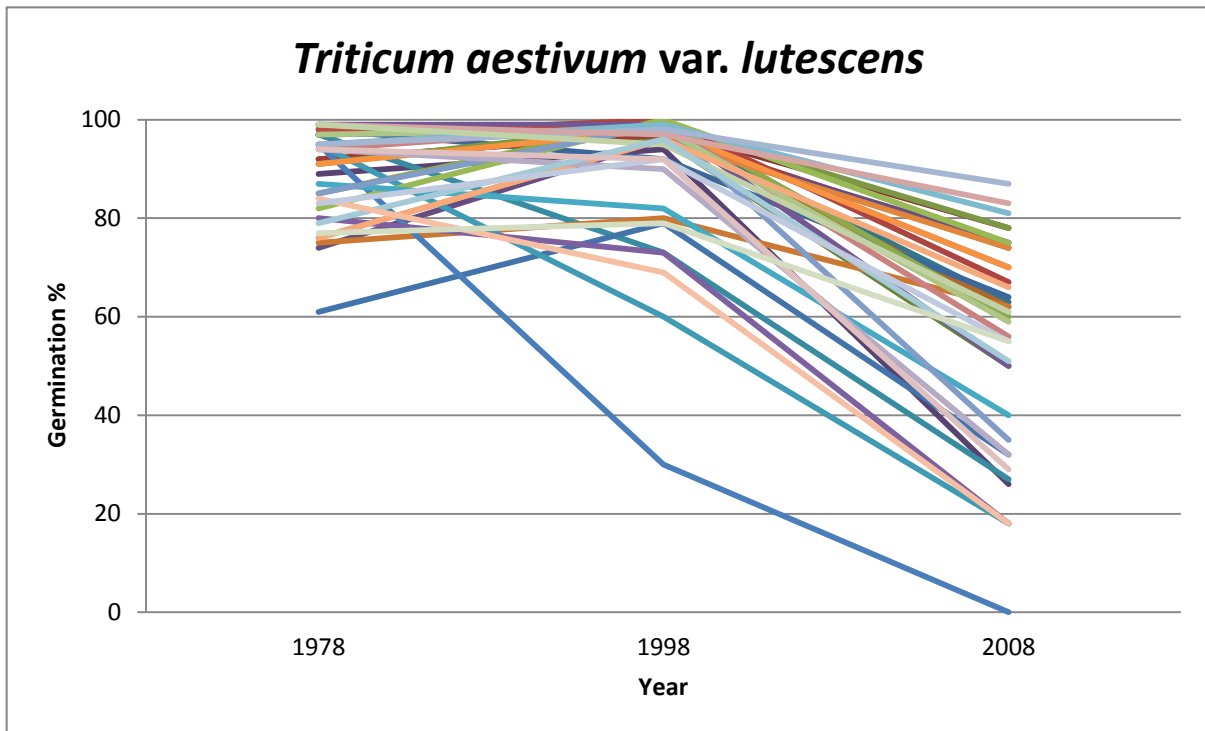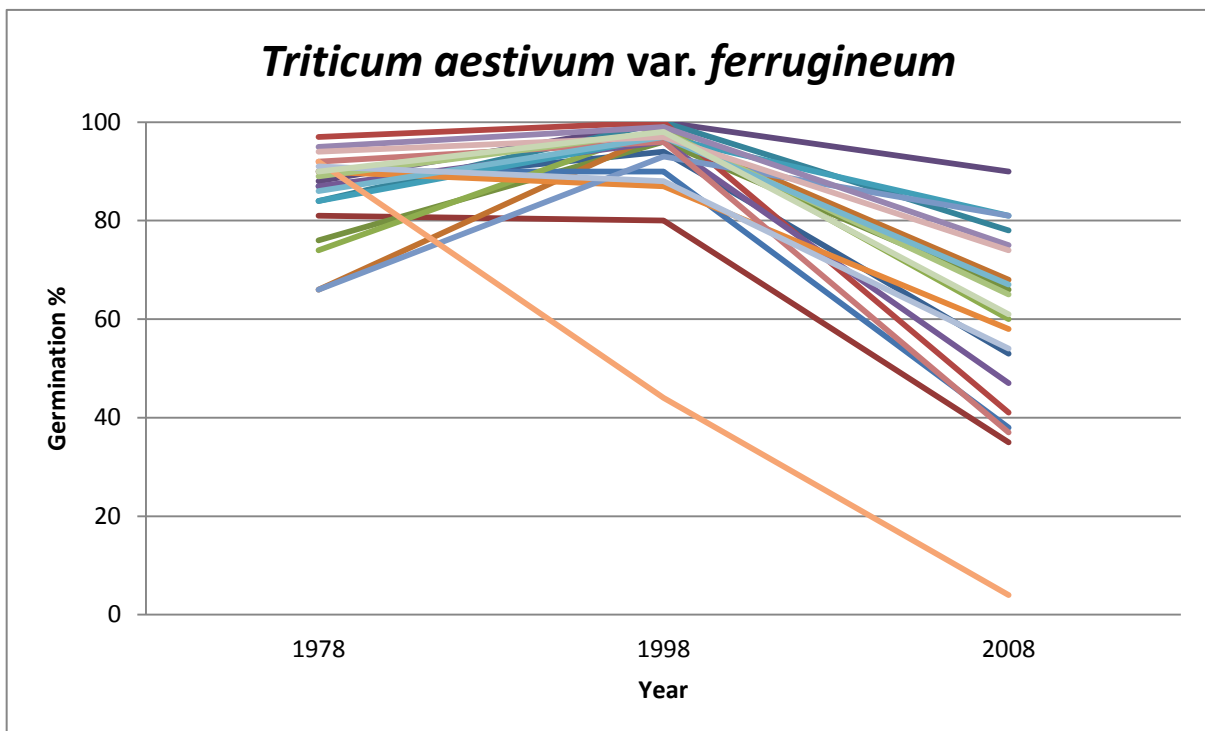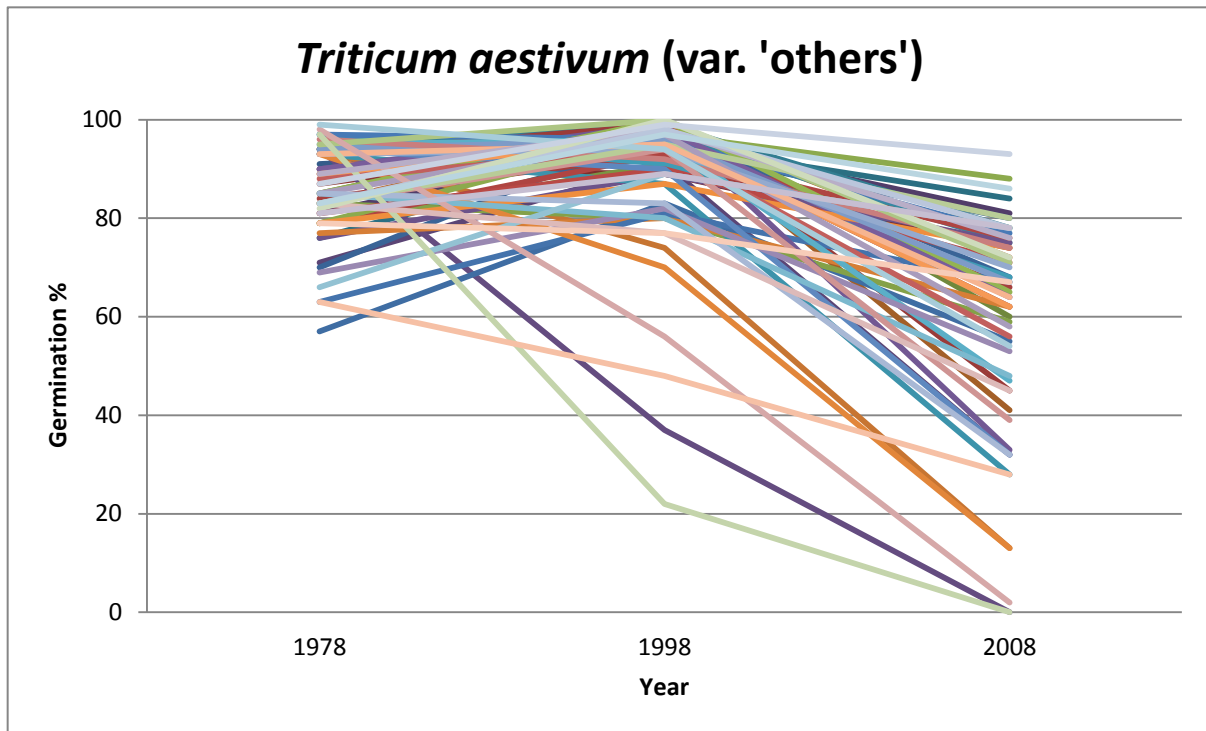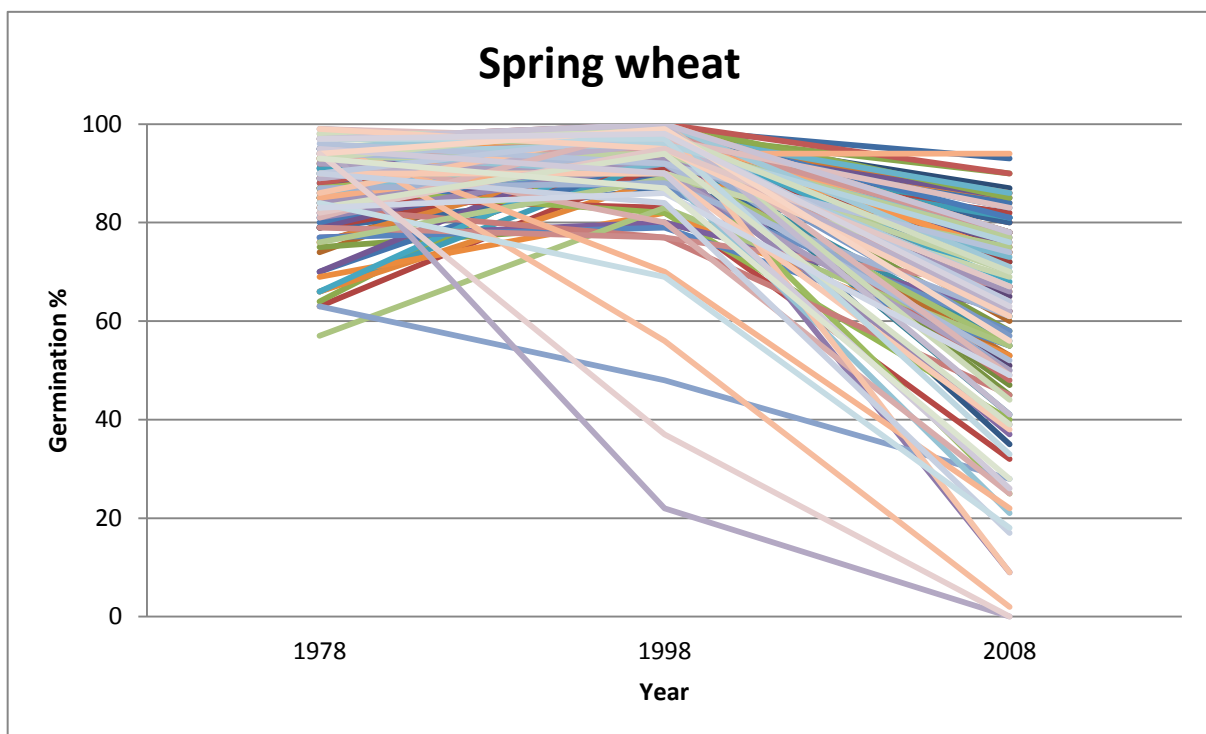
(j)

Figure 14: *Continued*
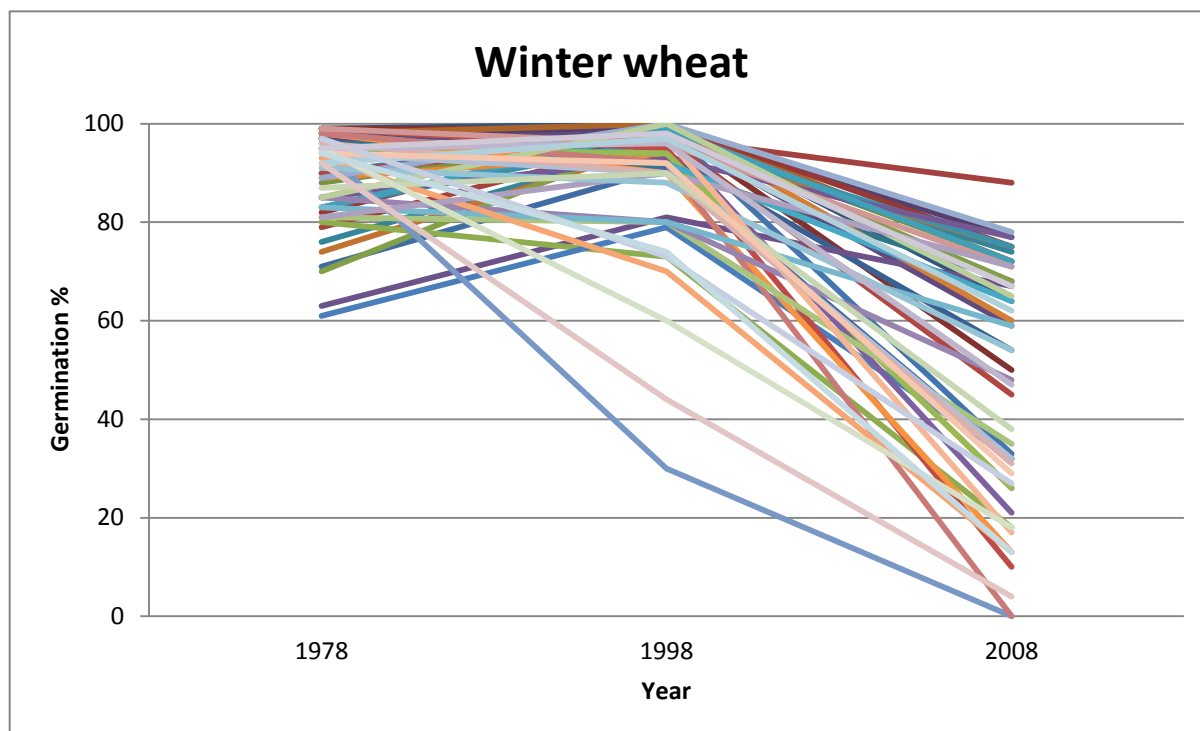
(k)

Figure 14: *Continued*

4.3.1.2 Investigations on fresh seeds after regeneration

4.3.1.2.1 Standard germination and seed longevity after accelerated seed ageing

The mean germination of genebank accessions regenerated in 2010 (G2010) was 85.75±11.8% and ranged from 26% to 99%. There was only little difference in the initial germination of various sub groups within this collection. The only notable difference was shown by Q6 among Q groups (sub-groups within genebank collection as identified by STRUCTURE). Figure 13 shows the boxplots of 2010 germination and relative germination percentage after AA and CD and Table 12 (Appendix I) shows the descriptive statistics of various Q groups, geographical groups, variety groups, growth habit groups and total accessions of the genebank collection.

AA reduced the mean germination of the seeds (GAA) to 66.32±19.6% with the minimum and maximum germinations being 2.67% and 94.67%, respectively. Significant differences were observed among Q and variety groups for GAA (Appendix I, Tables 13 and 14). Relative germination after AA (RGAA) ranged from 3.56% to 99.58% with the mean value of 76.87±19.5%. Again significant differences were observed among Q groups and geographical groups for RGAA (Appendix I, Tables 15 and 16).

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The mean germination after CD (GCD) was 58.68±22.7% which was lower than the mean germination after AA with 5% and 96% minimum and maximum values, respectively. Significant differences were observed among Q and geographical groups for GCD (Appendix I, Tables 17 and 18). Relative germination after CD (RGCD) of whole collection was 67.60±22.6% which ranged from 5.49% to 100%. RGCD was significantly different only among geographical groups (Appendix I, Table 19).

4.3.1.2.2 Dormancy and pre-harvest sprouting

There were less seeds dormant at 10°C compared to 20°C. The minimum percentage of dormant seeds at both 10°C and 20°C was 0 and the maximum was 95 at 10°C and 100 at 20°C, respectively. Mean percentage of dormant seeds at 10°C and 20°C was 11.97±17.1% and 75.67±29.1%, respectively. DI ranged from 0 to 95.55 with the mean value of 33.11±17.3. Pre-harvest sprouting showed the opposite trend in relation to dormancy. The mean score for PHS was 4.0±1.5 with the minimum of 1.2 and the maximum of 6.9 score. Box plots and descriptive statistics of dormancy at 10°C (D10) and 20°C (D20), dormancy index (DI) and pre-harvest sprouting (PHS) are provided in Figure 15 and in Appendix I, Table 20.

4.3.1.2.3 Agronomic traits

Figure 16 shows the box plots of heading time (Hd), flowering time (Ft), plant height (Ht), and thousand kernel weight (TKW) and Table 21 in Appendix I shows the descriptive statistics for these traits.

Minimum and maximum values for Hd were 109 and 150 and for Ft 119 and 155 days, respectively. The means were 130.80±6.2 days and 138.10±5.9 days for Hd and Ft, respectively. There was a great variation among these accessions in Ht which ranged from 51.5 to 179.5 cm with the mean of 131.91±21.9 cm. Mean value for TKW was 45.14±6.2g with 29.19g and 61.09g being the minimum and maximum values, respectively.
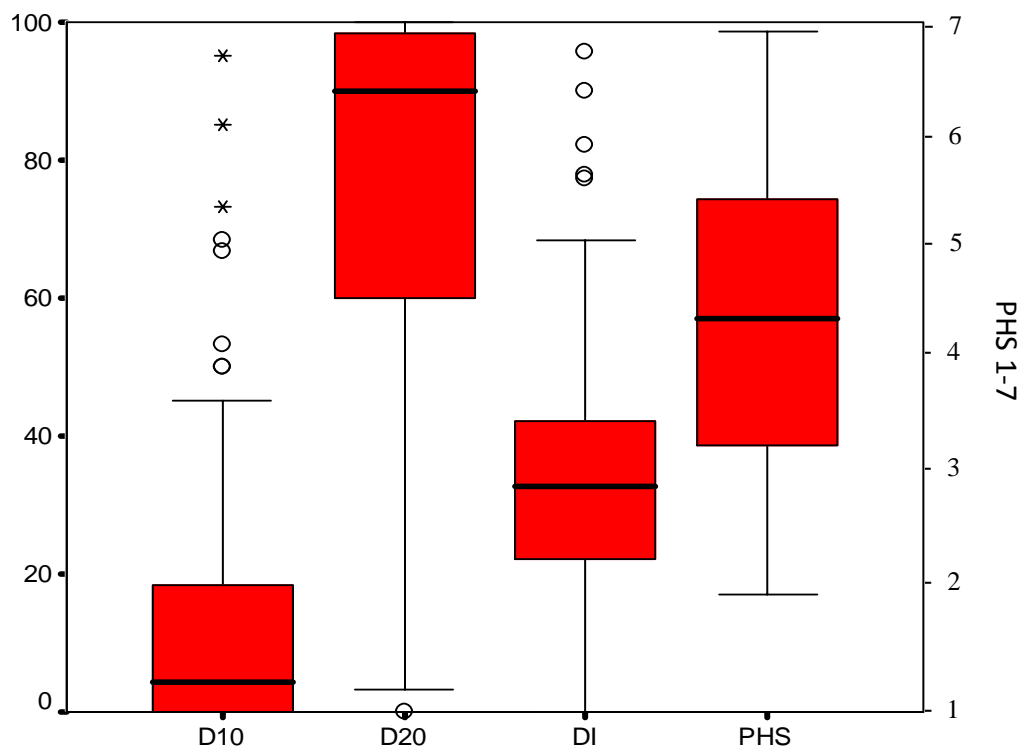
Figure 15: Boxplots of mean dormant seeds at 10°C (D10), mean dormant seeds at 20°C (D20), dormancy index (DI) and pre-harvest sprouting (PHS) of genebank collection
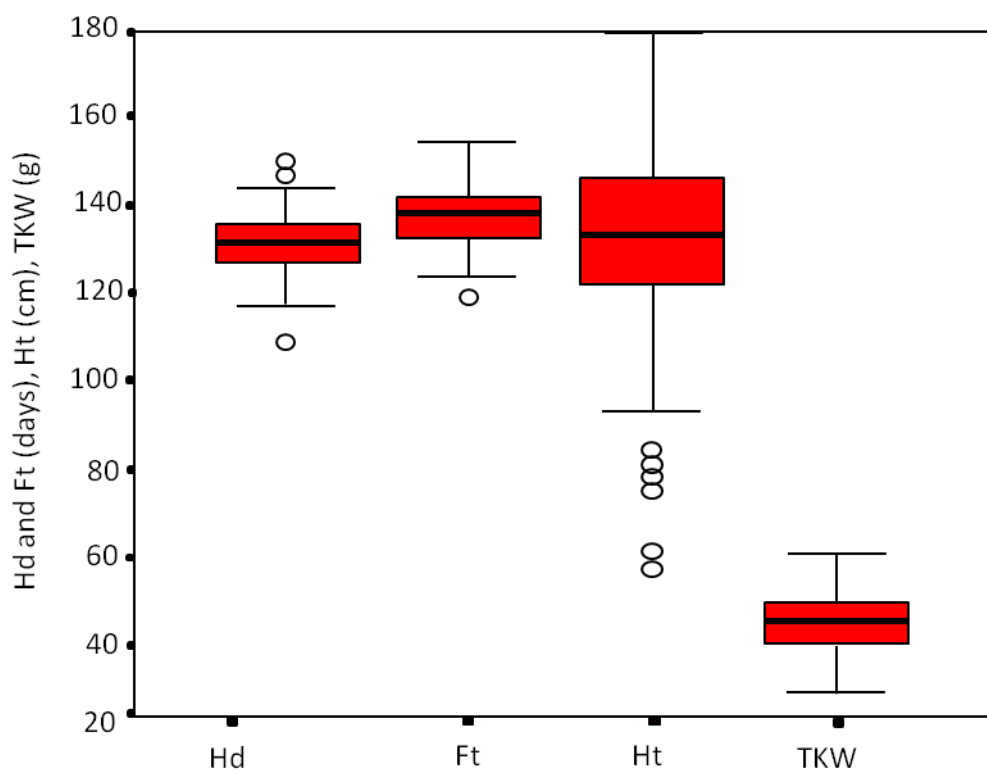
Figure 16: Boxplots of heading date (Hd), flowering time (Ft), height (Ht) and thousand kernel weight (TKW) of genebank collection

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4.3.2 Correlations

4.3.2.1 Correlation between germination after long term seed storage and artificial ageing

Table 8 shows the correlations among the germinations of 1978, 2008, 2010, GAA2010, RGAA2010, GCD2010 and RGCD2010. There was a slight significant negative correlation of -0.22** between G1978 and G2008 germination. But there was no correlation between G1978 and G2010. There was also no correlation between G2008 and GAA2010 or RGAA2010. Similarly, there was no correlation observed G2008 and GCD2010 or RGCD2010. But there was a moderate correlation of 0.35** between the relative germination after AA and CD.

Table 8: Correlations among longevities of genebank collection

	G1978	G2008	G2010	GAA2010	GCD2010	RGAA2010
G2008	-0.22**					
G2010	-0.07	-0.01				
GAA2010	0.01	0.10	0.54**			
GCD2010	0.05	0.14	0.54**	0.55**		
RGAA2010	-0.01	0.12	0.10	0.89**	0.36**	
RGCD2010	0.01	0.16*	0.14	0.43**	0.95**	0.35**

G1978 = germination in 1978, G2008 = germination in 2008, G2010 = germination of 2010 seeds in 2010, GAA2010 = germination after AA of 2010 seeds, GCD2010 = germination after CD of 2010 seeds, RGAA2010 = relative germination after AA of 2010 seeds, RGCD2010 = relative germination after CD of 2010 seeds, * = correlation significant at $p < 0.05$ level (2-tailed); ** = significant correlation at $p < 0.01$ level (2-tailed)

4.3.2.2 Correlations between seed longevity and other traits

Table 9 shows the correlations of longevity of seeds harvested in 2010 with the agronomic traits of the mother plants during regeneration. GAA showed a positive correlation with height among agronomic traits. RGAA did not show any correlation with any of the agronomic traits. GCD showed significant but low correlation with plant height, heading time and flowering time. RGCD showed positive correlation with plant height only. Among other traits, dormancy and PHS exhibited a strong negative correlation between each other. But there was no correlation of dormancy and PHS with GAA, RGAA, GCD and RGCD. Thousand kernel weight (TKW) also gave no correlation with seed longevity.

Table 9: Correlations of seed longevity with agronomical traits of genebank collection in 2010

	IG	GAA	RGAA	GCD	RGCD	DI	PHS	Ht	Hd	Ft
GAA	0.54**									
RGAA	0.1	0.89**								
GCD	0.54**	0.55**	0.36**							
RGCD	0.27**	0.43**	0.35**	0.95**						
DI	-0.51**	-0.18	0.05	-0.19*	0.04					
PHS	0.35**	-0.01	-0.19	0.01	-0.11	-0.68**				
Ht	0.41**	0.25**	0.09	0.40**	0.29**	-0.39**	0.32**			
Hd	0.51**	0.11	-0.13	0.28**	0.13	-0.49**	0.38**	-0.39**		
Ft	0.56**	0.14	-0.11	0.26**	0.08	-0.50**	0.41**	0.45**	0.87**	
TKW	-0.01	0.00	0.02	-0.10	-0.12	0.00	0.09	-0.13	-0.09	0.15

IG = initial germination, GAA = germination after AA, RGAA = relative germination after AA, GCD = germination after CD, RGCD = relative germination after CD, DI = dormancy index, PHS = pre-harvest sprouting, Ht = height, Hd = heading time, Ft = flowering time, TKW = thousand kernel weight, * = correlation significant at $p < 0.05$ level (2-tailed) ** = significant correlation at $p < 0.01$ level (2-tailed)

4.3.3 Marker trait associations (MTAs)

Figure 17 and Appendix I, Table 22 show the MTAs detected in the genebank collection for germination after long term storage, AA, CD, dormancy, PHS and other agronomic traits considering mapped and unmapped markers.

4.3.3.1 MTAs for seed longevity after long term cold storage

There were 134 MTAs in total observed for germination after long term cold storage (G2008). Fourteen of them were recorded with mapped markers that also included 3 highly significant MTAs. These MTAs were located on chromosomes 1DC (one MTA), 2AS (2 MTAs), 2BL (two MTAs), 3AL (one MTA), 4AL (three MTAs), 5BL (three MTAs), 6BS (one MTA) and 7D (one MTA). The remaining 120 MTAs were related with unmapped markers including 38 highly significant MTAs. Chromosomal positions for 64 unmapped markers were known.

4.3.3.2 MTAs for seed longevity after AA

Seventy eight MTAs were observed altogether for seed longevity after AA. Fourteen of them were observed with mapped markers, three of which were highly significant. The MTAs with mapped markers were located on chromosomes 1AS (one MTA), 1BL (two MTAs), 2BS (one MTA), 4BS (one MTA), 5BS (one MTA), 5BL (one MTA), 6AC (one MTA), 6BS (two MTAs), 7AS (two MTAs), 7BS (one MTA) and 7D (one MTA). In case of unmapped markers that were in association with longevity after AA, chromosomes of 31 markers were known. Sixteen MTAs with unmapped markers were also highly significant.

4.3.3.3 MTAs for seed longevity after CD

The numbers of MTAs observed for seed longevity after CD were more than for AA. In total, 128 MTAs were observed. Eighteen of them were observed with mapped markers of which six were highly significant. The MTAs with mapped markers were located on 2AL (one MTA), 2BS (one MTA), 3BS (three MTAs), 4AL (four MTAs), 4B (one MTA), 5B (three MTAs), 6BL (two MTAs) and 7BL (three MTAs). Chromsomes of 63 MTAs observed with unmapped markers were known. Numbers of 43 MTAs with unmapped markers were highly significant.

4.3.3.4 Chromosome-wise comparison of MTAs for longevity

Numerous multilocus markers were involved in MTAs for longevity. Unmapped multilocus markers on the same homoeologous group were considered only in comparison among different homoeologous groups but not considered in comparison among individual

chromosomes whereas when they were located on different groups, they were not at all considered.

When homoeologous groups were considered, it was group 3 that gave the highest number of 51 MTAs for seed longevity, four of which were common to both G2008 and AA and another four MTAs were common to AA and CD. It was followed by group 2 chromosomes with 39 MTAs. In this group, six MTAs were common to all ageing treatments i.e, G2008, AA and CD, four were common to G2008 and AA and two were common to AA and CD. There were 28 MTAs observed for group 7 chromosomes with two MTAs common to G2008 and CD followed by group 1 with 26 MTAs including two MTAs common to AA and CD. Group 6 exhibited 22 MTAs with two common MTAs. The common MTAs were associated with G2008 and CD. Finally, groups 5 and 4 showed 18 and 14 MTAs, respectively. Two MTAs in case of 5B were common to G2008 and AA whereas two MTAs in case of group 4 were common to G2008 and CD.

From an individual chromosome perspective, it was chromosome 3B that gave the highest number of 39 MTAs for seed longevity followed by chromosome 2B with 20 MTAs. Each of Chromosome 2A and 5B exhibited 15 MTAs. There were 14 MTAs observed on chromosome 7B. Chromosomes 6B and 1B showed 13 and twelve MTAs, respectively. Ten MTAs were shown by chromosome 4A. Chromosomes 3A and 6A gave eight MTAs each. Chromosomes 7A and 7D gave seven MTAs each whereas chromosome 1A exhibitied six MTAs. Each of chromosomes 1D and 4B showed four and chromosomes 3D and 5A showed two MTAs. Finally, there was one MTA observed on each of chromosomes 2D, 5D and 6D whereas no MTA was observed on chromosome 4D.
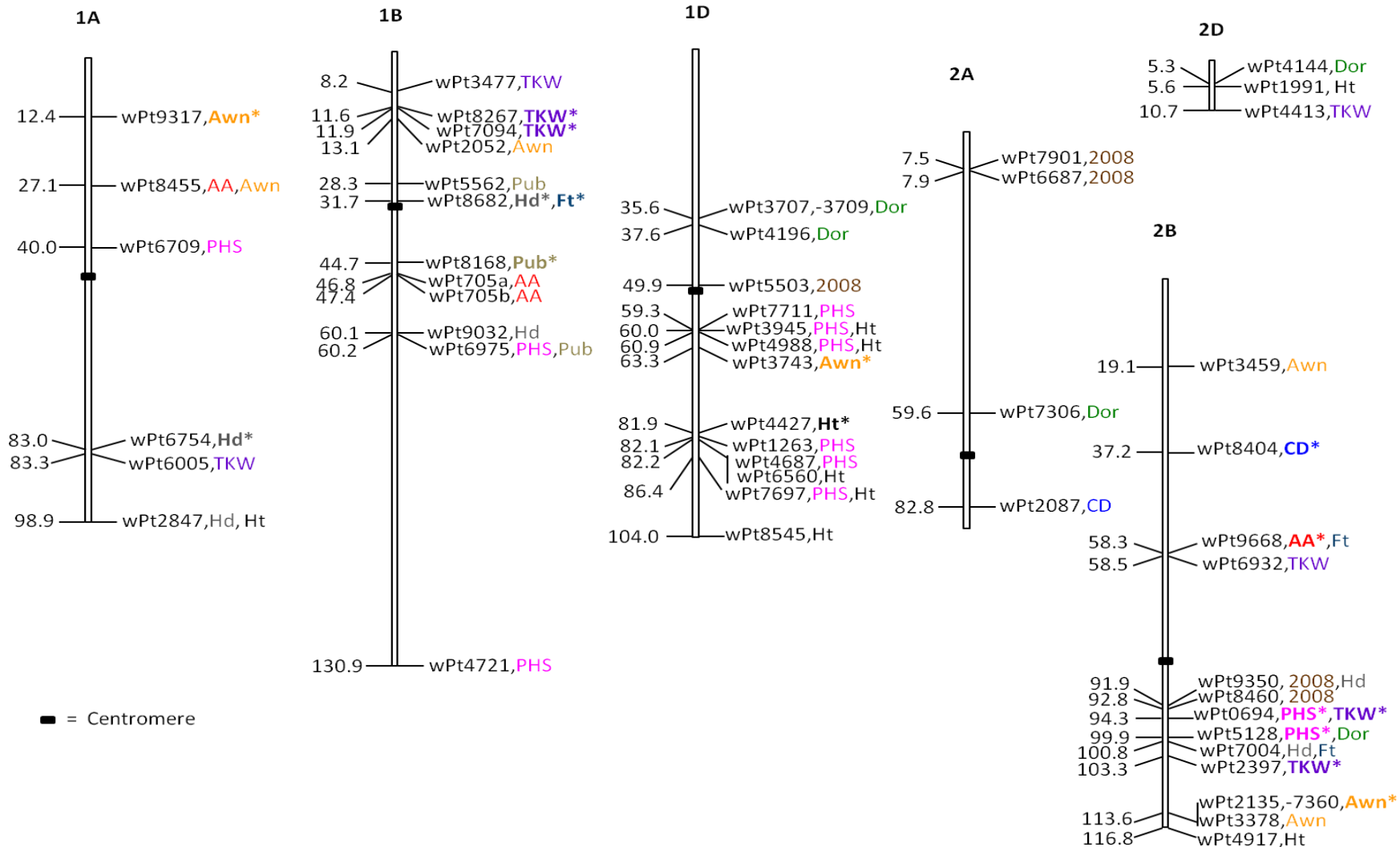
Figure 17: A Genetic map showing MTAs significant at p ≤ 0.05 or p ≤ 0.01 (traits in bold and with asterisk) for seed longevity after long term seed storage in 2008 (brown), AA (red) and CD (blue) and for PHS (pink), Dor (green), Hd (grey), Ft (navy blue), Ht (black), TKW (purple), Pub (light brown) and Awn (orange) detected in the genebank collection

3A

19.3 — wPt1562,**Dor***,**Ht***

44.7 — wPt3816,**Dor***,**Ht***
49.3 — wPt9422,**2008***,PHS,Dor

3B

17.1 — wPt8855,Ft
23.2 — wPt7212,CD

wPt9432,CD
wPt0995,**Pub***
wPt2748,Ft
wPt7015,**PHS***,Hd*,**Ft***
wPt7266,Pub
wPt8983,**Pub***
44.0 — wPt8352,**Pub***
44.4 — wPt9510,CD
46.2 — wPt5390,**PHS***
49.4 — wPt9170,**PHS***,Dor,Hd*,**Ft***
52.7 — wPt6047,Awn
53.0 — wPt5105,**PHS***,Dor,Hd*,**Ft***
53.9 — wPt0438,**Pub***

54.2 — wPt6973,**PHS***,Hd*,**Ft***

63.3 — wPt0280,Pub

74.9 — wPt3921,Awn

3D

6.0 — wPt2757,**Ft***

4A

52.8 — wPt3638,Ft,Ht,**TKW***

132.7 — wPt6997,Ft

143.7 — wPt5825,**Pub***

161.1 — wPt2084,Ft
161.4 — wPt3795,Ft

161.7 — wPt7807,**Ft***
162.8 — wPt5003,Ht
wPt6440,Hd,Ft,**Ht***
163.3 — wPt1155,CD,Dor,Awn
164.3 — wPt8167,Hd,Ft,**Ht***
165.5 — wPt8271,Hd,Ft,**Ht***
167.9 — wPt5172,TKW
174.1 — wPt3491,PHS
174.6 — wPt4487,Ht,Pub
175.4 — wPt4680,2008
176.6 — wPt0610,2008,**PHS***,**Dor***,Hd*
177.0 — wPt4424,CD,Dor,Awn
177.6 — wPt4620,**2008***,CD,TKW
178.6 — wPt2533,Pub
180.1 — wPt8091,Pub
181.4 — wPt0150,Hd,Pub
182.4 — wPt9418,**CD***,Dor,Awn
182.6 — wPt8657,Ft,Ht

4B

6.0 — wPt0246,CD,TKW
6.3 — wPt9118,**AA***,Pub

9.7 — wPt1046,-5559,Pub
11.9 — wPt1272,Pub

111.9 — wPt3991,**Dor***,TKW

144.6 — wPt5564,Pub

Figure 17: *Continued*

Figure 17: *Continued*

7A

60.3 — wPt7151,TKW
69.6 — wPt6447,TKW,**Awn***
70.3 — wPt4835,TKW,Awn
73.8 — wPt0008,AA,**TKW***
74.3 — wPt7034,AA

7B

12.4 — wPt8920,**Awn***

45.9 — wPt7653,AA

56.0 — wPt4863,PHS,Ht
61.5 — wPt3873,Ht
64.7 — wPt1149,**TKW***
65.8 — wPt3833,Awn
66.2 — wPt6372,PHS,Ht

89.2 — wPt9925,**Hd***,Ft

101.5 — wPt0194,Dor,Ft,Ht
106.6 — wPt2356,Pub
108.0 — wPt6156,CD,**Ht***
112.9 — wPt3093,Hd
114.3 — wPt0600,Hd

120.9 — wPt5138,CD

128.3 — wPt2677,Hd,**Ft***,Ht
129.3 — wPt6484,**CD***,TKW
136.4 — wPt4875,PHS
138.0 — wPt5816,Hd
       wPt9813,**TKW***
144.0 — wPt6869,Ft

7D

1.3 — wPt2565,AA

97.0 — wPt4555,2008,TKW

Figure 17: *Continued*

4.3.3.5 MTAs for dormancy and pre-harvest sprouting

One hundred and eighteen MTAs were observed for dormancy in total. These were observed with 23 mapped markers and 95 unmapped markers. Chromosomes for 43 of these unmapped markers were known. The MTAs with mapped markers were found on 1DS (three MTAs), 2AS (one MTA), 2BL (one MTA), 2D (one MTA), 3AL (three MTAs), 3BC (one MTA), 3BL (one MTA), 4AL (four MTAs), 4BL (one MTA), 5AS (one MTA), 5BS (one MTA), 5BL (two MTAs), 6BL (two MTAs) and 7BL (one MTA). Five MTAs with mapped markers and 18 with unmapped markers were highly significant.

For pre-harvest sprouting, 193 markers gave significant associations. These included 30 MTAs with mapped markers and 163 MTAs with unmapped markers. The mapped MTAs were located on 1AS (one MTA), 1BL (two MTAs), 1DL (six MTAs), 2BL (two MTAs), 3AL (one MTA), 3BS (two MTAs), 3BC (one MTA), 3BL (two MTAs), 4AL (two MTAs), 5AS (one MTA), 5BL (one MTA), 6AS (three MTAs), 6BS (two MTAs), 6BL (one MTA), 7BC (one MTA) and 7BL (two MTAs). Eight MTAs with mapped markers and 64 MTAs with unmapped markers were highly significant.

4.3.3.6 Chromosome-wise comparison of MTAs for dormancy and pre-harvest sprouting

Both dormancy and pre-harvest sprouting (PHS) MTAs were considered simultaneously in comparison and the same criteria that were used in comparing seed longevity MTAs were kept in view.

The highest number of MTAs for dormancy and PHS were 32 (16 for dormancy and 16 for PHS) recorded on homoeologous group 3 chromosomes followed by 28 MTAs recorded on homoeologous group 1 and 6 (eight for dormancy and 20 for PHS on both 1 and 6) chromosomes each. There were 26 MTAs observed on group 7 (four for dormancy and 22 for PHS). Both homoeologous group 2 and 4 chromosomes showed 20 MTAs each with nine and eleven MTAs for dormancy and PHS, respectively, in case of group two and ten MTAs each for dormancy and PHS in case of group 4 chromosomes. The least number of MTAs for dormancy and PHS were observed on homoeologous group 5 chromosomes where 18 MTAs (ten for dormancy and eight for PHS) were observed.

When single chromosomes were considered, highest number of MTAs for dormancy and PHS were detected on chromosome 3B where 24 MTAs (eleven for dormancy and 13 for PHS) were observed. Chromosomes 4A and 6B, both of which revealed 17 MTAs each followed. Nine and eight MTAs on 4A and six and eleven MTAs on 6B were detected for dormancy and PHS, respectively. A number of 14 MTAs (four for dormancy and ten for PHS) were observed on chromosome 1D and 13 MTAs (two for dormancy and eleven for PHS) were found on chromosome 7B. Two chromosomes i.e., 2A and 6A, gave eleven MTAs each. Five MTAs of 2A and two MTAs of 6A were detected for dormancy whereas the remaining six MTAs of 2A and 9 MTAs of 6A were revealed for PHS. There were nine MTAs (five for dormancy and four for PHS) observed on chromosome 5B. Eight MTAs were shown by five different chromosomes (1B, 2B, 3A, 5A and 7A), individually. These included two, three, five, four and one MTA for dormancy and six, five, three, four and seven MTAs for PHS detected on chromosomes 1B, 2B, 3A, 5A and 7A, respectively. Six MTAs (two for dormancy and one for PHS) were revealed on 1A and four MTAs (one for dormancy and three for PHS) were detected on 7D. Finally, chromosome 4B showed one and two MTAs for dormancy and PHS, respectively whereas chromosomes 2D and 5D gave one MTA (both for dormancy) each. No MTA was observed on chromosomes 3D, 4D and 6D.

4.3.3.7 MTAs for agronomic traits

For heading time (Hd), 149 MTAs were detected. These MTAs were recorded with 24 mapped and 125 unmapped markers but chromosomal locations of 58 unmapped markers were available. The MTAs with mapped markers were found on 1AL (two MTAs), 1BS (one MTA), 1BL (one MTA), 2BL (two MTAs), 3BS (one MTA), 3BC (one MTA), 3BL (two MTAs), 4AL (fove MTAs), 5BL (one MTA), 6BL (three MTAs) and 7BL (five MTAs). Seven MTAs with mapped markers and 25 MTAs with unmapped markers were highly significant.

Flowering time (Ft) gave 111 MTAs including 24 MTAs with mapped and 87 MTAs with unmapped markers. Among those unmapped markers, chromosome number was known for 43 markers. The MTAs with mapped markers were observed on 1BS (one MTA), 2BS (one MTA), 2BL (one MTA), 3BS (three MTAs), 3BC (one MTA) 3BL (two MTAs), 3D (one MTA), 4AS (one MTA), 4AL (eight MTAs), 6BL (one MTA) and 7BL (four MTAs). Nine MTAs with mapped markers and 30 MTAs with unmapped markers were highly significant.

The highest number of MTAs among agronomic traits was detected for plant height (Ht) where 188 MTAs were observed. These MTAs were revealed by 29 mapped and 159 unmapped markers including 80 markers with known chromosome. The MTAs observed with mapped markers were located on different chromosomes. These included 1AL (one MTA), 1DL (six MTAs), 2BL (one MTA), 2D (one MTA), 3AL (two MTAs), 4AS (one MTA), 4AL (six MTAs), 5AL (one MTA), 5BL (one MTA), 6BL (three MTAs), 7BC (two MTAs) and 7BL (four MTAs). Nine MTAs with mapped markers and 48 MTAs with unmapped markers were highly significant.

When TKW was considered, 125 MTAs were detected. These MTAs were observed with 26 mapped markers and 101 unmapped makrers. MTAs with mapped markers were located on chromosomes 1AL (one MTA), 1BS (three MTAs), 2BS (one MTA), 2BL (two MTAs), 2D (one MTA), 4AS (one MTA), 4AL (two MTAs), 4BS (one MTA), 4BL (one MTA), 6AS (four MTAs), 6AL (one MTA), 7AL (four MTAs), 7BL (three MTAs) and 7D (one MTA). However, chromosome numbers of 69 markers of the unmapped markers were known. Eight MTAs with mapped markers and 29 MTAs with unmapped markers were in highly significant associations.

For flag leaf pubescence (Pub), 25 MTAs were revealed with mapped markers and 106 MTAs with unmapped markers but chromosomes of 52 unmapped markers were known. The MTAs with mapped markers were located on chromosomes 1BS (one MTA), 1BL (two MTAs), 3BS (four MTAs), 3BL (two MTAs), 4AL (five MTAs), 4BS (four MTAs), 4BL (one MTA), 5AS (one MTA), 5BL (one MTA), 6AC (one MTA), 6BS (one MTA), 6BL (one MTA) and 7BL (one MTA). Out of these total 139 MTAs, 27 were highly significant. These highly significant MTAs were recorded with 6 mapped and 21 unmapped markers.

Considering the presence/absence of awns (Awn), 164 MTAs were observed in total. These were with 21 mapped markers and 143 unmapped markers. In case of unmapped markers, chromosomes of 73 markers were available. The mapped MTAs were found on 1AS (two MTAs), 1BS (one MTA), 1DL (one MTA), 2BS (one MTA), 2BL (three MTAs), 3BL (two MTAs), 4AL (three MTAs), 5AS (one MTA), 6AC (one MTA), 6BL (two MTAs), 7AS (two MTAs), 7BS (one MTA) and 7BL (one MTA). Seven MTAs with mapped and 50 MTAs with unmapped markers were also highly significant.

4.3.3.8 Chromosome-wise comparison of MTAs for agronomic traits

The highest number of MTAs on homoeologous groups for agronomic traits other than dormancy and PHS were 86 observed on each of groups 1 and 7 chromosomes. They were followed by groups 2 and 3 chromosomes with 85 MTAs each. There were 71 MTAs observed on homoeologous group 4 chromosomes whereas 64 MTAs were shown by chromosomes of homoeologous group 6. Finally, the least number of MTAs were 29 exhibited by group 5 chromosomes.

In terms of individual chromosomes, 2B and 3B gave highest numbers of 59 MTAs for agronomic traits followed by chromosome 4A, which gave 58 MTAs. Chromosome 7B gave 43 and chromosome 6B gave 40 MTAs. Chromosomes 1A and 1B gave 36 and and 35 MTAs, respectively. Chromosome 7A showed 31 MTAs. Each of chromosomes 2A and 3A exhibited 19 MTAs. Eighteen MTAs were shown by chromosome 5B whereas chromosome 6A showed 17 MTAs. Chromosomes 4B and 5A carried 13 and six MTAs, respectively. Only 42 MTAs were shown by D genome which were distributed on chromosomes 1D (twelve MTAs), 2D (six MTAs), 3D (three MTAs), 5D (four MTAs), 6D (seven MTAs) and 7D (ten MTAs). No MTAs were observed on chromosome 4D.

5   Discussion

Wheat is an allohexaploid (2n=6x=42) with the three genomes A, B and D and has an extremely large genome size of 16 x 10^9 bp/1C (Bennet and Smith 1976). Wheat has very limited level of polymorphism in its DNA (Cadalen et al. 1997), that has led to the use of populations derived from wide crosses for gene and genome mapping (Röder et al. 1998). The ITMI population was the first and since then has been used extensively for 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 for the detection of loci important for the determination of key agronomic traits including seed characters as weight, size or dormancy (Börner et al. 2002, Lohwasser et al. 2005, Landjeva et al. 2008). With the arrival of association mapping in plants (Breseghello and Sorrells 2006b, Neumann et al. 2011), search for loci affecting complex traits in unrelated germplasm has opened new horizons in quantitative genetics. However, genetic basis of seed longevity has not as yet been investigated in bread wheat, although some relevant data have been 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.

Genetic markers are very useful to identify regions and genes involved in seed longevity. However, so far, there are no such markers to determine seed longevity, though some candidate genes have been identified to influence the trait (Debeaujon et al. 2000, Clerkx et al. 2004b, Sattler et al. 2004, Xu et al. 2004, Bentsink et al. 2006, Prieto-Dapena et al. 2006, Devaiah et al. 2007, Ogé et al. 2008, Rajjou et al. 2008, Almoguera et al. 2009). Experimental ageing tests are frequently utilised to check for potential long life of seeds. The purpose of experimental ageing tests is to mimic long term storage and hence allow for the evaluation of seed longevity. To reveal the genetic nature of seed longevity, behavior of seeds was studied after two different experimental ageing protocols during this investigation. These expose the seeds to high temperature and high humidity; the two most important factors that determine seed vigour (Hampton and TeKrony 1995). These approaches were adopted and used because a study by Rajjou et al. (2008) revealed that similar molecular events accompany artificial and natural seed ageing and Long et al. (2008) has also demonstrated that seed persistence of many weeds in the field may be predicted by laboratory-controlled

ageing. Consequently, similar genes/genetic areas should be involved in both natural and artificial ageing. Whether the same holds true for plant species other than *Arabidopsis* or weeds, especially in crop plants is yet to be proven. Three mapping populations of hexaploid wheat were used to study seed longevity in wheat. In addition dormancy, pre-harvest sprouting and agronomic traits and their relationship to seed longevity were investigated.

5.1 Seed longevity

5.1.1 Genetic mapping

5.1.1.1 Segregation mapping approach

Recombinant inbred lines of bi-parental ITMI mapping population reproduced in two different years were used. The contradictory QTL outcomes from the 2003 and 2009 RILs showed that the 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 predicted different QTL, indicating that seed moisture content during the treatment process clearly has a major effect on the trait. A similar phenomenon with respect to slight differences in temperature during ageing was described for *Brassica napus* L. (Nagel et al. 2011). The authors exposed the seeds to three artificial ageing conditions and they found 13 significant QTLs related to longevity. However, there was only one QTL that was common to two different ageing treatments.

The many genomic regions contributing to the genetic determination of seed longevity underline the polygenic nature of the inheritance of this trait. The major longevity QTL *QLng.ipk-2A* mapped to a region of chromosome 2AS which 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 may play a role in longevity of seeds can only be speculated. The minor QTL *QLng.ipk-3B* maps to a region of chromosome 3B and is implicated in the control of 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 be able to withstand an unfavourable storage environment.

Of the four QTLs revealed by the CD test, three mapped to regions already associated with the determination of various grain traits. *QLng.ipk-1A* mapped close to a QTL for spike

compactness (Sourdille et al. 2000) and its associated marker has also been linked to QTL for the number of grains 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).Compact spikes, however, are more prone to ear diseases. No explanation for this opposite behaviour can be given at this stage. The second QTL of interest was *QLng.ipk-1D.1* which mapped within a 5cM distance to 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 a grain weight QTL (Börner et al. 2002, Quarrie et al. 2005). Therefore, longevity might have been associated with a superior seed development and performance.

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 (Hrstková et al. 2006, Aparicio et al. 2002). Similarly, in a recent study of seed longevity in lettuce, Schwember and Bradford (2010) concluded that a strong genotype x environment interaction existed, as it was not possible to recover the same QTL from seed lots produced in different years. It is concluded that genotype by environment interaction played a significant role in our study as it was also performed on the same population but seeds were regenerated in two different years.

5.1.1.2 Association mapping approach

Genome-wide association mapping approach has emerged as a promising methodology in mapping genes and genomic regions that underlie complex traits in cereals, and particularly in wheat and has only been possible recently after the development of high-throughput genotyping systems such as DArTs (Jaccoud et al. 2001). DArT markers are bi-allelic dominant markers which are obtained by cloning random fragments of genetic representation (Akbari et al. 2006). The two collections used in this investigation (advanced germplasm and genebank collection) to study longevity through association mapping were genotyped with DArT markers. They are discussed simultaneously. The combined map of these two collections showing the MTAs of seed longevity but also dormancy and pre-harvest sprouting (see 5.2) along with the possible positions of seed longevity and dormancy QTLs of bi-parental ITMI population is shown in Figure 18.
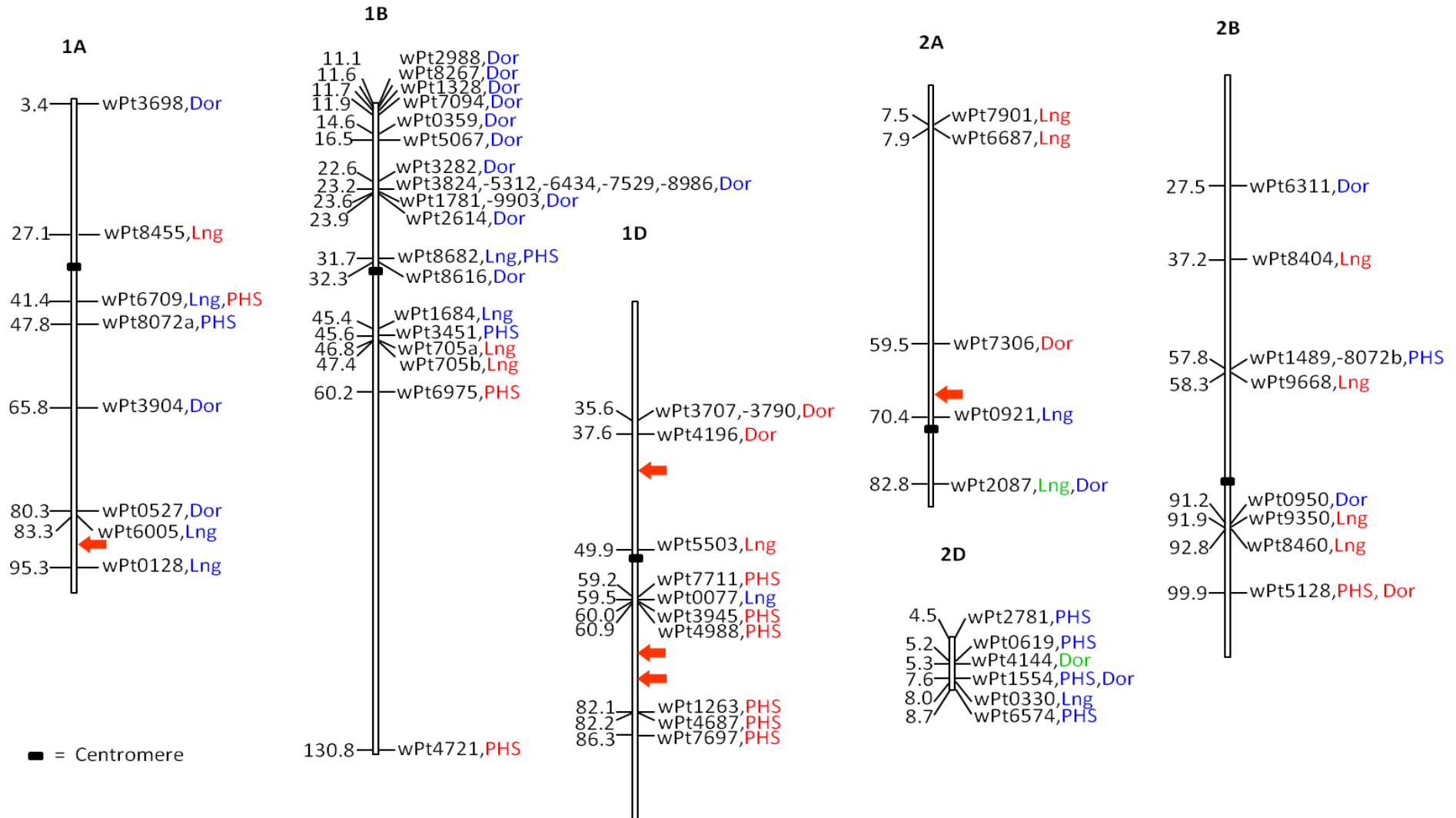
Figure 18: A consensus genetic map showing the MTAs for longevity (Lng), dormancy (Dor) and pre-harvest sprouting (PHS) detected in either advanced germplasm collection (blue) or genebank collection (red) or both (green). Longevity (red arrows) and dormancy (green arrow) QTLs of ITMI population are also shown
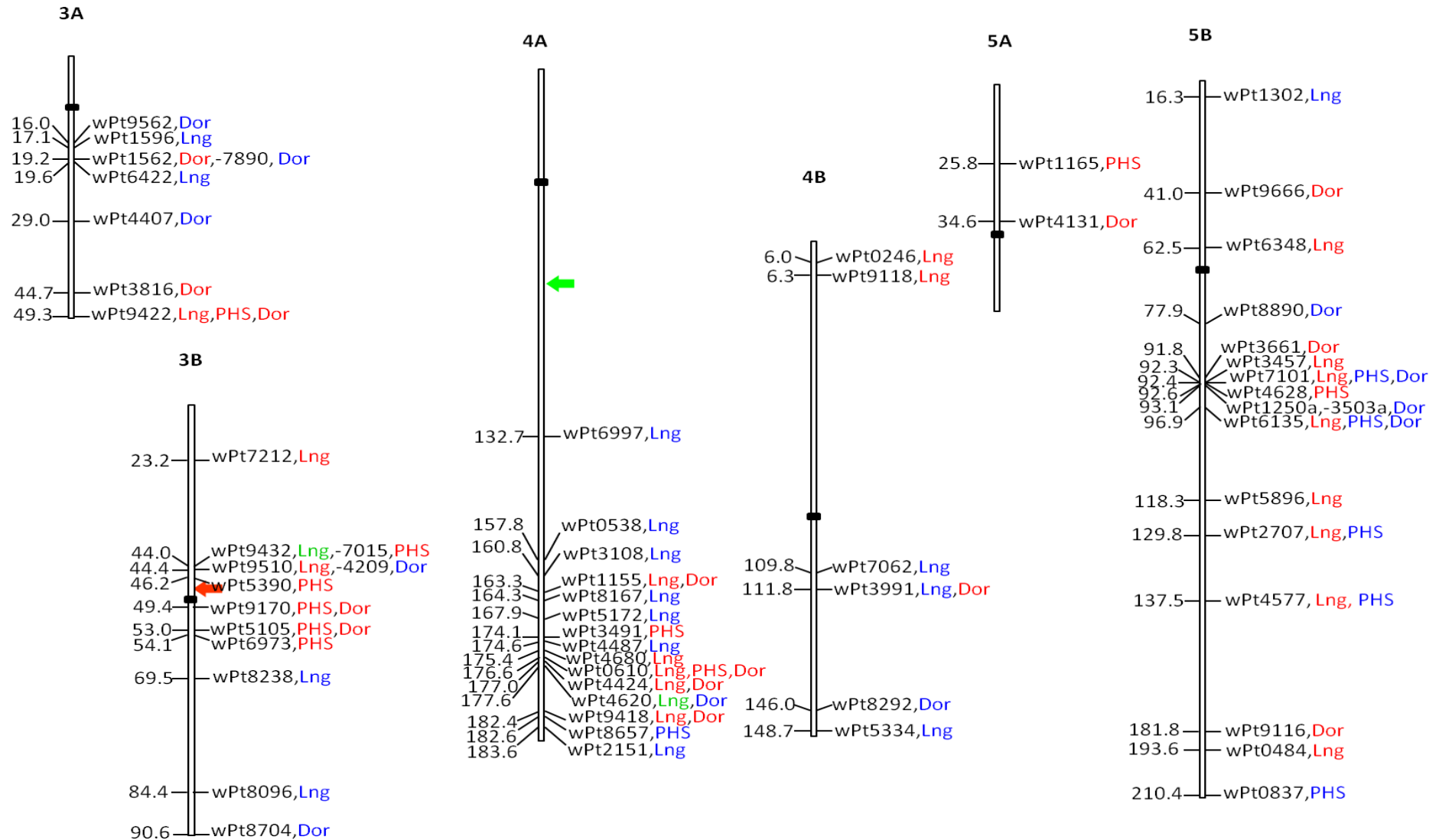
Figure 18: *Continued*
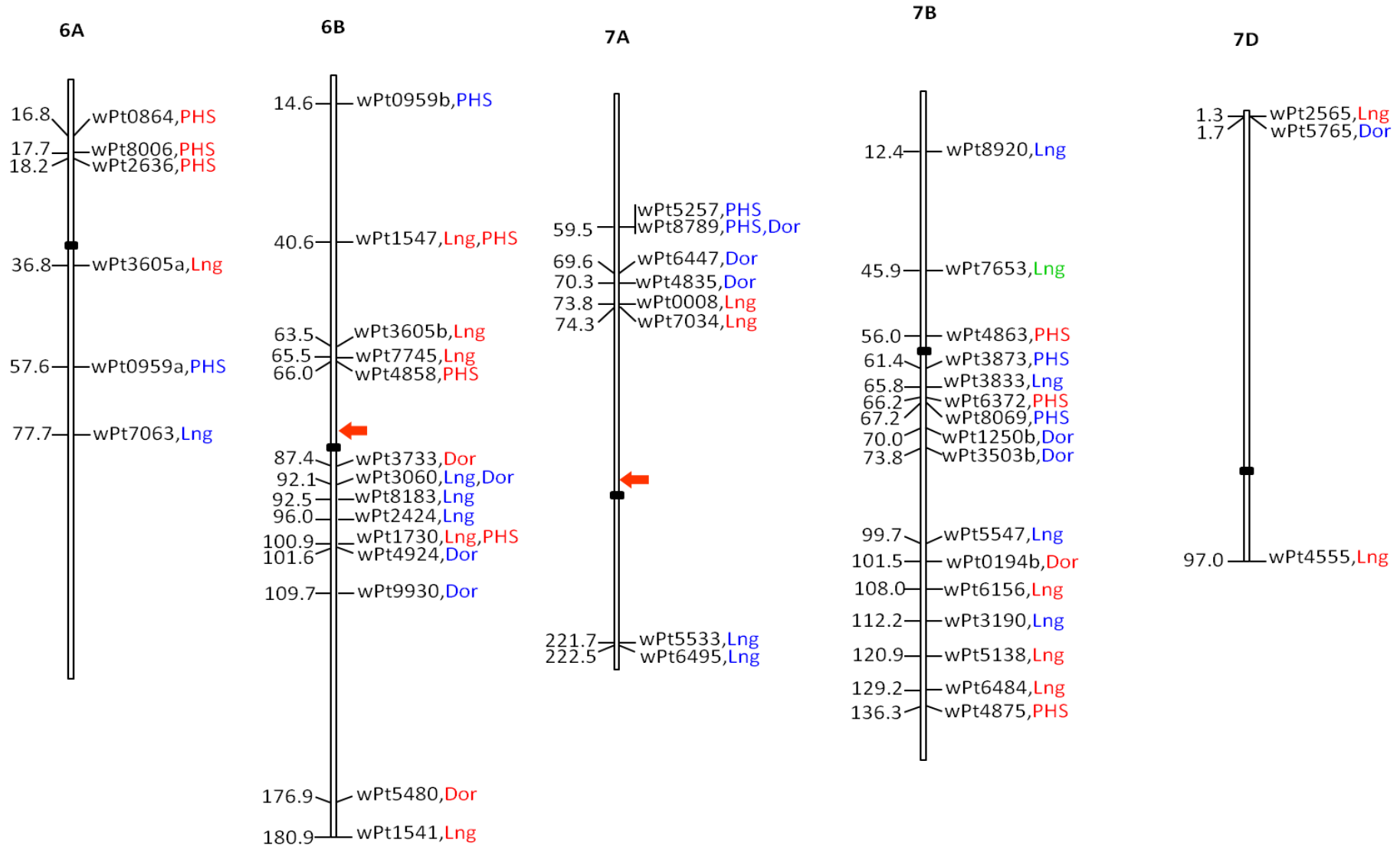
Figure 18: *Continued*

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 2006a), disease resistance and yield (Crossa et al. 2007) and for a number of key agronomic traits (Neumann et al. 2010). Here, for the first time this approach is used to study the trait seed longevity in wheat exploiting DArT markers. A direct comparison to other surveys is not possible at this stage. However, a comparison between various genetic and cytogenetic wheat maps constructed from either DArT- or SSR-based genotypic data has 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 (Mantovani et al. 2008, Peleg et al. 2008) wheats. Distribution of DArT markers in DH lines of wheat has also been reported (Semagn et al. 2006). Akbari et al. (2006) has reported the first combined map of DArT with other marker systems of hexaploid wheat. All these resources were used to place the longevity MTAs in the deletion bins described by Sourdille et al. (2004).

Table 23 in Appendix 1 shows the sources used to assign bins to markers in association with longevity in the two populations under investigation. This bin information has allowed determining 22 markers associated with longevity in advanced germplasm collection. They include seven mapped DArT markers (*wPt2087, wPt2151, wPt4487, wPt5334, wPt5547, wPt6709* and *wPt7063*) and one having only a known chromosomal location (*wPt3976*) through Francki et al. (2009). Five further mapped markers (*wPt3991, wPt4620, wPt6422, wPt8682* and *wPt9432*) and one unmapped marker (*wPt4569*) were assigned based on Mantovani et al. (2008). Four markers (*wPt2424, wPt3060, wPt6005* and *wPt8183*) were placed based on Hai-Chun et al. (2009). Two more markers (*wPt7062, wPt8167*) were given bins through Semagn et al. (2006). Finally Peleg et al. (2008) helped in placing two mapped markers (*wPt0538* and *wPt8920*) in their respective bins. All these markers were placed in 14 bins located on 1AS, 1AL, 1BS, 2AS, 2AL, 3AL, 3BS, 3DS, 4AL (2 bins), 4BL, 6AL, 6BL and 7BL.

In case of genebank collection, 39 markers associated with longevity are placed in bins. Five mapped (*wPt1541, wPt2087, wPt5138 and wPt5896* and *wPt8460*) and 9 unmapped markers (*wPt1859, wPt2933, wPt3268, wPt3879, wPt3976, wPt4476, wPt7096, wPt7340* and *wPt8446*) were assigned through direct comparison with Francki et al. (2009). Semagn et al. (2006) was used in placing 12 mapped markers (*wPt0008, wPt0610, wPt1155, wPt1547,*

wPt3605b, wPt5503, wPt6135, wPt7034, wPt7101 wPt9418, wPt9422, and *wPt9510*) in their bins. Mantovani et al. (2008) provided the information to place 4 mapped (*wPt4424, wPt4620, wPt9432* and *wPt9350*) and 3 unmapped (*wPt0398, wPt2883* and *wPt8721*) markers. Comparison with Hai-Chun et al. (2009) helped in placing 3 unmapped markers (*wPt1480, wPt6711* and *wPt8826*). One mapped marker (*wPt8404*) and one unmapped marker (*wPt2416*) were also assigned to bins through Peleg et al. (2008). In the end Akbari et al. (2006) was used to place one mapped marker (*wPt1730*) in its bin. Twenty two bins were identified in total and eight of them were common to the bins of advanced germplasm collection. These bins were located on chromosomes 1DS, 2AS (2 bins), 2AL (2 bins), 2BS, 2BL, 3AL (2 bins), 3BS (2 bins), 3DS, 4AL, 5BL (2 bins), 6BS, 6BL, 6DS, 7AL, 7BS, 7BL and 7DL (2 bins). The gene contents of these bins are provided in Table 24 in Appendix 1.

Bins 1AS-0.86-1.00, 1AL3-0.61-1.00, C-1BS10-0.50 and C-1DS3-0.48 that carried four mapped associated DArT markers of homoeologous group 1 chromosomes contain the genes *Glu-A3* and its orthologues, *Histone H4* and encoders of a seed maturation protein, disease resistance proteins, plasma membrane-associated proteins and a number of enzymes (hexokinase, 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 can be chalcone synthase, lipoxygenase and cellulose synthase. Chalcone synthase (*Chs*) along with another defense responsive gene, flavanol 7-*O*-methyl transferase, has been mapped to the short arms of chromosomes 1B and 1D by Li et al. (1999). It is difficult to say that MTAs for longevity on 1B and 1D in this study correspond to these genes. Nevertheless, they seem to be in comparable locations in comparison to Li et al. (1999). Chalcone synthase is an ubiquitous plant enzyme that belongs to the super family of type III polyketide synthases (PKSs). It catalyses the first committed step in flavanoid biosynthesis by performing the sequential decarboxylative addition of three acetate units from malonyl-CoA to *p*-coumaroyl-CoA, this is then followed by intramolecular cyclisation and aromatisation events leading to the formation of chalcones (Wu et al. 2008). Chalcones are chemoprotectants that may be a consequence of their antioxidant properties, mediated via inhibition or induction of metabolic enzymes, by an anti-invasive effect or a reduction in nitric oxide production (Go et al. 2005).

Four mapped and three unmapped markers belonging to homoeologous group 2 chromosomes were assigned to 2AS5-0.78-1.00, 2AL1-0.85-1.00, C-2AL1-0.85, 2BS3-0.84-1.00 and C-2BL2-0.36 which carry *elicitor-responsive gene, Vrga1, Enod* (early nodulation gene) related protein, ABA responsive protein, DNA repair protein, pathogenesis related proteins, stem rust resistance protein Rpg1, heat shock protein HSP26, cold shock proteins along with 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 defense responsive 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) also found QTLs for longevity on chromosome 2H of barley where they reported a dehydration responsive element binding protein (DREB) to be candidate gene for longevity. One particularly important gene in relation to longevity of the above mentioned genes is *Sod*. SODs are in the first line of defence against reactive oxygen species (ROS) which are produced in both stressed and unstressed cells (Alscher et al. 2002) at any location where an electron transport chain is present (Elstner 1991). This include mitochondria, chloroplasts, micorsomes, glyoxysomes, peroxisomes, apoplasts and the cytosol. ROS can also form as a result of drought, injury, pesticides, ozone, plant metabolic activity, nutrient deficiencies, photoinhibition, temperature extremes, toxic metals, and UV or gamma rays (Nicholas 1993, Raychaudhuri and Deng 2000). Generation of ROS leads to the peroxidation of membrane lipids (Mead 1976), breakage of DNA strands (Brawn and Fridovich 1981) and inactivation of enzymes (Fucci et al. 1983). SODs are produced by *Sod* genes present in all these subcellular locations or produced in response to the above mentioned stresses. They can be distinguished into three types: iron SOD which is present in chloroplasts, manganese SOD which is present in mitochondria and peroxisomes, and copper-zinc SOD which is present throught out the plant cell (Alscher et al. 2002). These metalloenzymes protect cells from superoxide radicals by catalyzing the dismutation of superoxide radical to molecular O_2 and H_2O_2 (Wu et al. 1999).

Nine homoeologous group 3 associated DArT markers that included 4 mapped and 5 unmapped markers were assigned to bins C-3AL3-0.42, 3AL5-0.78-1.00, 3BS8-0.78-1.00, 3BS1-0.33-0.57 and 3DS6-0.55-1.00, which contain genes like *gliadin*, and others encoding a chloroplast ribosomal protein, a biostress resistance related protein, a putative plasma membrane protein, a lipid transfer protein, a putative late embryogenesis protein, a hypersensitivity related protein, stress enhanced protein, along with others responsible for the production of enzymes involved in amino acid synthesis. ITMI mapping population analysis also showed longevity QTLs on chromosomes 3BC and 3DL. As per defense responsive genes are concerned, Li et al. (1999) mapped chitin binding protein (*Cbp2*), chitinase 1a (*Cht1a*), oxalate oxidase 2 (*Oxo2*) and many others to homoeologous group 3. Lohwasser et al. (2005) also found a dormancy QTL on 3AL which is carrying *Vp1* gene. This gene plays a role in control of seed maturation, dormancy and germination (Hoecker et al. 1995). Seed maturation is associated with the activation of a variety of genes encoding storage proteins and various hydrophilic late embryogenesis proteins that probably function as desiccation protectants (Dure et al. 1989, Skriver and Mundy 1990). Thus, seed longevity and seed maturation can be related to each other because optimally mature seeds tend to last long and can sustain stresses longer than immature seeds of the same plant.

The associated group 4 deletion bins include C-4AL12-0.23, 4AL4-0.80-1.00 and C-4BL1-0.71 that carried seven mapped and two unmapped longevity associated DArT markers. They contain genes encoding heat and cold shock proteins, disease resistance proteins, kelch-containing protein, seed maturation protein, abscisic acid responsive proteins, a thaumatin like protein, an oxalate oxidase precursor, a ubiquitin activating enzyme and many other related enzymes. Group 4 chromsomes are of particular importance in relation to longevity because 4A carried MTAs common to both experimental ageings and long term cold storage. Some of these MTAs were also in association with dormancy, PHS and other agronomic traits giving the implication of a gene rich region on chromosome 4A. Bi-parental ITMI population analysis also showed the QTL for dormancy centromere region of chromosome 4AL. Li et al. (1999) reported thaumatin (*Tha2*) and chitinase 2 (*Cht21*) genes to be closely linked on 4AL, and 14-3-3 protein *(1433a)* and oxalate oxidase (*Oxo2*) genes to be tightly linked near the centromere of 4A whereas *Oxo2*, *1433a* and *Cht21* were all clustered in the distal region of 4BS. Thus, MTAs for seed longevity on 4AL might correspond to the former group and the

two MTAs on the distal end of 4BS might correspond to latter group of genes. Important genes from longevity perspective include oxalate oxidase and thaumatin like protein. Oxalate oxidase is an enzyme that degrades oxalate to hydrogen peroxide and carbon dioxide. It was first descrbided by Zaleski and Reinhard (1912) in a study of wheat flour and has since been shown to be widespread in microbes and plants (Dunwell 1998). Oxalate oxidase is a germin like protein that is under the conrol of a germin gene (wheat *gf*-2.8) (Berna and Bernier 1999). It has also been demonstrated that a wide range of external stimuli contribute to the expression of this gene in addition to the endogenous developmental signal (Berna and Bernier 1999). Thaumatin-like protein is (TLP) a PR-5 gene that possesses antifungal activity in response to pathogen attack or biotic stress (Datta et al. 1999). TLP also possesses some sequence homologies with the *osmotin* gene that accumulates in plant cells during adaptation of cells to high osmotic stress including salt or polyethylene glycol (Singh et al. 1987). Nagel et al. (2009) also identified a TLP as a candidate gene for longevity in barley caryopsis. Osmotic adjustment is a major component of abiotic stress tolerance in plants and contributes to pressure potential maintenance (Cherian et al. 2006). It will be interesting to fine map the 4AL chromosome of wheat for more precise location of the loci for seed longevity, dormancy, PHS and traits located here.

Three mapped markers of genebank accessions on chromosome 5B were assigned to bins 5BL1-0.5-0.75 and 5BL9-076-079. Cell division control protein, heat shock protein, NBS-LRR type resistance protein, viviparous-14 protein and IAA1 protein are encoded by genes present in these bins. Enzymes encoded by genes constituting these bins are lipoxygenase, protein kinase and glucose and succinate dehydrogenase. Li et al. (1999) mapped 2 defense genes on each of homoeologous group 5 chromosomes. One MTA on distal end of 5BS in this investigation might correspond to the *Tha3* gene whereas one of the MTAs on 5BL might correspond to *Lpx*. Lipoxygenase is also identified when the gene contents of bins carrying MTAs of 5B and group 1 chromosomes were searched. The wheat lipoxygenase gene is rapidly and transiently induced by touch and wind wounding (Mauch et al. 1997). Lipoxygenases are the family of enzymes that catalyse the introduction of molecular oxygen into the cis, cis-1,4-pentadiene structure of polyunsaturated fatty acids (Vick and Zimmermann 1987, Siedow 1991). Lipoxgenases have been implicated in plant growth and development, mobilisation of lipid reserves, senescence, ABA biosynthesis, wound responses

and resistance against pathogens and pests (Ocampo et al. 1986, Vick and Zimmerman 1987, Creelman et al. 1992, Croft et al. 1993). The primary products of lipoxgenase catalyzed reactions, fatty acid hydroperoxides, are often metabolized into molecules with known or suspected regulatory activity such as jasmonic acid and traumatic acid (Vick and Zimmermann 1987, Vick 1991, Hamberg 1993). Jasmonic acid and its methyl ester have been shown to affect many physiological processes (Koda 1992, Staswick 1992), including the induction of a large number of genes (Sembdner and Parthier 1993). In particular, jasmonates have been implicated as signal transduction molecules in the response of plants to stress in the form of wounding and pathogen attack (Creelman et al. 1992, Farmer et al. 1992).

Ten mapped markers of homoeologous group 6 chromosomes associated with longevity were placed in C-6AL4-0.55, 6BS-Sat, C-6BL3-0.36 and 6DS-0.99-1.00 bins. The main constituents of these bins include CC-NBS-LRR resistance protein MLA13, *Enod* related protein, chloroplast translational elongation factor, and ABA induced protein ABA7, prohibitin, pescadillo like protein, nuclear cap binding protein and many enzymes. One minor QTL was also observed on chromosome 6BC in ITMI population. The distal end of 6BL carried two defense responsive genes i.e., *Tha1* and chitin binding protein (*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 (Li et al. 1999). *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 because of dearth of information. The role of *Tha1* has been described above. Chitinase binding proteins (CBP) denote the family of proteins containing one or more chitin-binding domains. The best characterised CBP is wheat germ agglutinin (WGA) (Raikhel and Lee 1993). It has been shown that WGA synthesis is under the control of ABA and that ABA accelerates synthesis of WGA in both developing embryos (Triplett and Quatrano 1982, Raikhel and Quatrano 1986,) and wheat root caps (Raikhel et al. 1986, Cammue et al. 1988). This study indicated that CBP also play role in enhancing seed life by the regulation of ABA or some other action. Future studies might help to unleash the role of CBPs in seed longevity.

Finally, eight DArT markers of group 7 were placed in C-7AL1-0.39, 7BS1-27-1.00, 7BL10-0.78-1.00, 7DS4-0.61-1.00 and 7DL2-0.61-0.82 bins. These contain *Enod*, tetra-ubiquitin,

lipoprotein, heat shock protein 80, *Srg6* (stress responsive gene 6) protein, DNA and RNA binding proteins, membrane proteins, along with catalase isozymes, oxidases and alcohol dehydrogenase. A cluster of defense responsive (DR) genes have been reported to occur on the long arm of group 7 chromosomes, especially *Tha2*, *Cht1b*, *Tha1* 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) lie in comparable locations. Similarly two MTAs on 7AL might correspond to *Tha2* gene (Li et al. 1999). *CAT* (catalases) play a crucial role in elimination of H_2O_2 (Scandalios et al. 1997) which accumulates in wheat leaves in response to drought (Luna et al. 2005) and other stimuli. Plant catalases are encoded by a small gene family usually composed of three isozyme genes which exhibit fairly complex spatial and temporal patterns of expression throughout the plant life cycle (Scandalios et al. 1997). It is concluded that catalases play an important role in seed longevity by the removal of H_2O_2 which is being produced constantly in seeds in response to environmental stimuli.

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 small clusters of two or more MTAs such as on 2AS, 2BL, 4AL, 4BS and 7AS whereas the others were distributed throughout the entire chromosomes. There are also other 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) which along with others function 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. It is also affected by the treatments that is given to seeds prior to their storage as evident from the different results of long term cold storage and experimental ageing as well as by the enzymatic systems/genes that constitute the genetic system of the seeds.

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. Furthermore, the effects of maternal environment on seed longevity as well as the genetic background seem to be important contributors towards seed life. Therefore, seed longevity analysis using different populations will prove useful in validating current findings. The goal is to develop methods which are more predictive for the longevity of seeds so that germplasm management can be carried out more effectively. The identification of favorable alleles offers perspectives for the prediction of longevity in germplasm collections.

5.1.2 Seed longevity after long term cold storage versus experimental ageing

The low correlations between germination after long term cold storage and artificial ageing treatments were quite unexpected, since 100% RH (AA) or 18% moisture contents (CD) and 43±1°C is a common treatment to check the vigour of wheat seeds recommended by ISTA (2008). In the present case, these tests did not predict the deterioration that occurs under long term storage conditions. There can be many reasons attributed to this unexpected result. First, there was no correlation between the initial germination of seeds produced during two different growing seasons of 1974 and 2010 (Table 8). Second, different deterioration mechanisms may be involved in artificial ageing and conventional storage (Walters 1998, McDonald 1999). Studies performed in soybeans indicated a slight increase in free radical levels of conventionally aged seeds but a doubling of free radicals in accelerated aged seeds (Priestley and Leopold 1983, Priestley et al. 1985). Powell and Harman (1985) questioned whether the physiological processes occurring under accelerated ageing conditions mimic those found during conventional storage of pea (*Pisum sativum*) seeds. In contrast, Likhatchev et al. (1984) made a conclusion that physiological changes that occur in seeds when they are exposed to accelerated ageing conditions were the same as those under conventional storage conditions, with the exception of the rate at which they occur. Longevities of *Arabidopsis* seeds were also well correlated when seeds from various ecotypes, mutants and RILs were aged at temperatures ranging between ambient conditions up to 85% RH at 50°C (Clerkx et al. 2004a, b). Furthermore, when proteome analysis of *Arabidopsis* was performed by Rajjou et al. (2008), it was found that the extent of protein carbonylation was strongly increased by both storage conditions but the protein targets of carbonylation were nearly the same. Contrary, Schwember and Bradford (2010) did not find any overlapping QTLs for naturally aged and artificially aged seeds of lettuce which led them to conclude that species-specific processes that are involved in the loss of viability may be

independent of (i.e. *Arabidopsis*) or dependent on (i.e. lettuce, soybean) the environment in which the seeds are stored. The authors also concluded that while the CD tests of lettuce seeds may be useful for comparisons among seed lots, an alternative ageing method is needed for studying the mechanisms responsible for seed deterioration under conditions more closely resembling to conventional storage conditions.

In this study, there were 25 MTAs common between long term storage and experimental ageing in genebank collection. These MTAs were distributed on chromosomes 1B, 2A, 2B, 3B, 4A, 5B, 6B and 7B. Fourteen common MTAs remained unmapped. Chromosomes 4AL and 5BL also carried long term cold storage and AA/CD MTAs in similar regions. There were 6 deletion bins that carry the natural as well as artificial ageing MTAs (either in advanced germplasm collection or in genebank collection). These bins were identified on chromosomes 3AL, 3DS, 4AL, 5BL, 6BS, 7BS and 7BL. Thus, it can be concluded that in general, long term cold storage might not be similar to artificial ageing, yet there are some genomic areas in particular which are of equal importance in both phenomena. Therefore, the common MTAs are in accordance with Rajjou et al. (2008) for *Arabidopsis* but contrary to Bradford and Schwember (2010) for lettuce. It should be kept in mind that an AM population that encompassed huge genetic variation in wheat compared to the bi-parental mapping population of lettuce was used in this study that unraveled numerous loci including those common to both natural and artificial ageing. It will be interesting to compare proteomic profiles of naturally as well as artificially aged seeds and same results as that of Rajjou et al. (2008) may be expected in wheat. A physiological study pertaining to enzymes actions and interactions might shed more light on the mechanics of longevity during long term storage and experimental ageing.

5.1.3 Synteny of longevity in wheat, barley and rice

Analogous analyses on seed longevity have been performed in both rice (Miura et al. 2002, Sasaki et al. 2005, Zeng et al. 2006, Xue et al. 2008) and barley (Nagel et al. 2009) as well as in wheat/*Aegilops tauschii* introgression lines (Landjeva et al. 2010). Chromosomes 1DS (a cluster of QTLs), 2DS, 4DC, 5DL, 7DS and 7DL showed longevity QTLs in the investigation carried out by Landjeva et al. (2010). In this study, longevity QTLs/MTAs were observed on 1DS, 2DS and 7DS and 7DL. QTL on the proximal end of 1DS in this study can be compared

with that observed by Landjeva et al. (2010) (Fig 19) which supports the results of this investigation.

Chromosomes 2H, 5H and 7H of barley are known to carry QTLs for seed longevity (Nagel et al. 2009). MTAs for longevity of genebank collection on chromosome 5B are distributed all along the long arm of that chromosome and can be compared with the positions of the longevity QTLs on 5HL in barley (Figure 20a). Heat shock, thaumatin like and APETALA2 like proteins along with different enzymes were reported to be candidate genes on chromosome 5H of barley. Similarly, the minor QTL of ITMI and MTA of advanced germplasm collection in the centromeric regions of 7A and 7B, respectively, can be compared with the longevity QTL on 7H of barley, although the confidence interval of this location in barley spanned both sides of the centromere (Figure 20b). *Nud* gene and ethylene-responsive element binding protein and its transcription factor were discovered to reside on that area of the chromosome. Thus, there is a chance that related genes might be present on the wheat genome where longevity QTLs and MTAs identified in this study lie on the orthologous regions.

In rice, QTLs for seed longevity have been identified on 7 of the 12 chromosomes. These included chromosomes 1,3, 2, 4, 7, 9, 11 and 12 (Miura et al. 2002, Sasaki et al. 2005, Zeng et al. 2006, Xue et al. 2008). 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 barley and wheat (Kurata et al. 1994, Salse et al. 2008). Figure 20a shows the homoeologous relation between chromosome 5B and rice chromosomes 3, 9 and 12 with respect to loci of longevity. It can be stated that some similarity does exist for comparable genomic regions between both the cereals that control seed longevity.

1D of wheat   1D of *Aegilops*
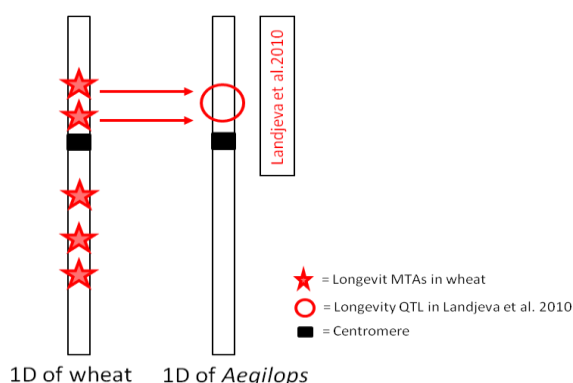
Figure 19: Comparison of seed longevity in wheat with *Aegilops*
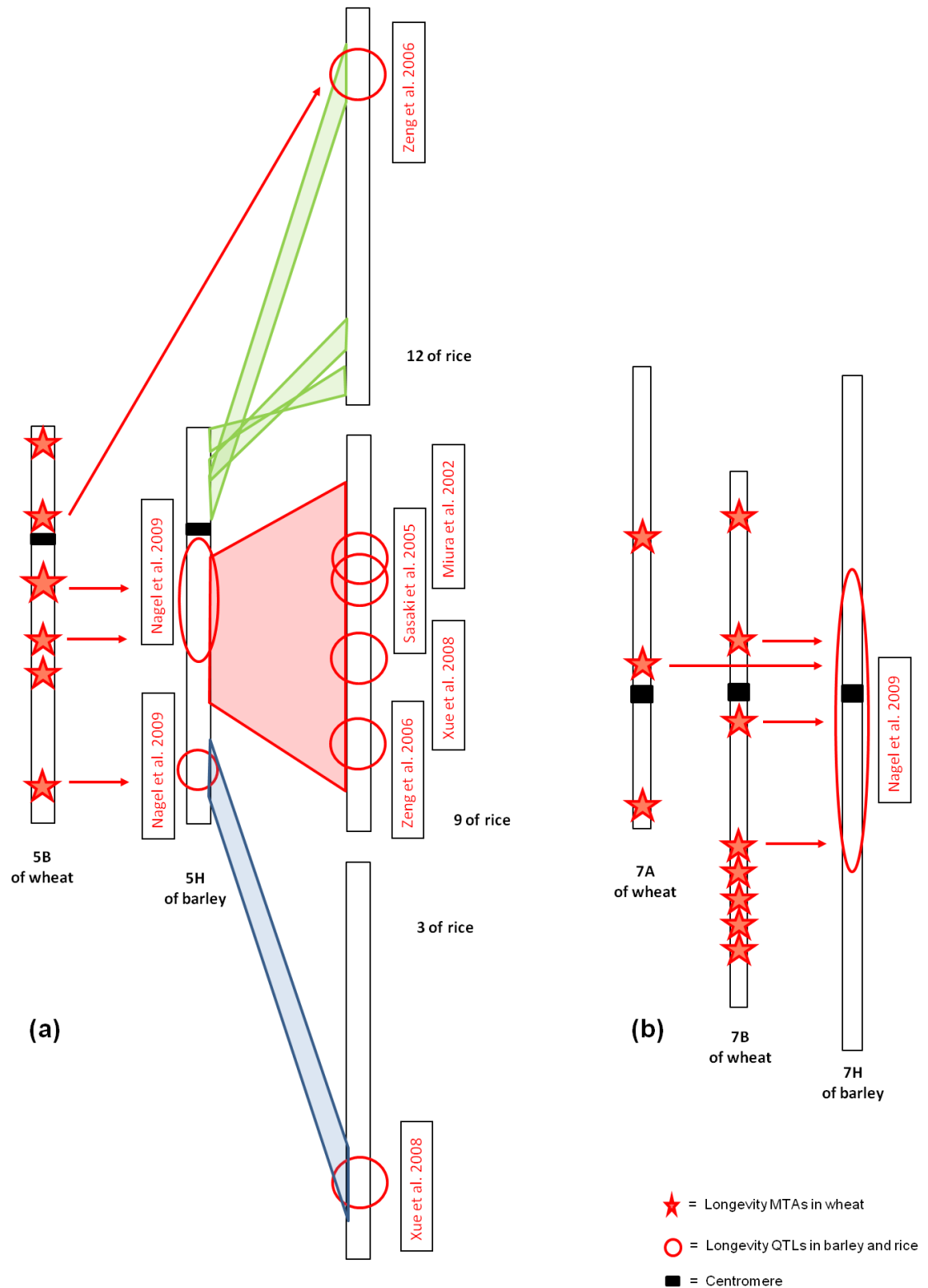
Figure 20: Comparison of longevity in wheat with barley and rice. **(a)** Longevity MTAs of wheat chromosome 5B compared to barley longevity QTLs of 5H and rice longevity QTLs of chromosomes 3, 9 and 12 and **(b)** longevity MTAs and QTLs of group 7 chromosomes of wheat compared with barley longevity QTLs of chromosome 7H. Stein et al. (2007) is used to compare rice with barley

5.2 Dormancy and pre-harvest sprouting

5.2.1 Genetic mapping

The major QTLs for dormancy have been mapped to chromosomes 4A (Kato et al. 2001, Noda et al. 2002, Mares et al. 2005, Torada et al. 2005, Chen et al. 2008) and 3A (Osa et al. 2003, Lohwasser et al. 2005, Mori et al. 2005). In the current study, a major QTL for dormancy on chromosome 4AL of wheat is identified in ITMI mapping population. Investigation of the association mapping panels led to the identification of numerous MTAs for dormancy on all the chromosomes except 4D and 6D. Thus, the results for dormancy match with the previous studies. In addition, several new loci were detected for dormancy on homoeologous group 1 and group 2 chromosomes. Other regions that may be of interest in future investigations for dormancy studies are group 5 and 6 chromosomes. It is also interesting to note that although homoeologous group 5 chromosomes were poorly covered, yet they revealed MTAs for dormancy on all 3 homoeologous chromosomes.

Many studies have been performed to identify the genetic regions/QTLs for seed dormancy and PHS in wheat. QTLs for PHS have been mapped to chromosomes 2BS, (http://maswheat.ucdavis.edu/), 3A (Kulwal et al. 2005), 4AL (Anderson et al. 1993, Sorrells and Anderson 1996, Flintham et al. 2002), 3B, 5A, 6A and 7B (Zanetti et al. 2000), 1AS, 3BL, 4AL, 5DL and 6BL (Anderson et al. 1993, Sorrells and Anderson 1996), 6B and 7D (Roy et al. 1999), 5A and all group 3 chromosomes (Groos et al. 2002). In this investigation, the PHS MTAs were also detected on all chromosomes of wheat except chromosomes 3D, 4D and 6D. These findings are in accordance with the previous studies.

Like seed longevity, dormancy/PHS mapping has not been conducted using association genetics and DArT markers so far. Therefore, in order to look deeper into the genomic areas associated with the two traits, 38 associated markers linked to dormancy and 29 markers linked to PHS were assigned to 33 possible deletion bins using various available resources (Appendix I, Table 23). The gene contents of these bins are provided in Appendix I, Table 24.

Using Francki et al. (2009), seven dormancy associated mapped DArT markers (*wPt1328, wPt2087, wPt3282, wPt3824, wPt5067, wPt6311* and *wPt6434*) and one unmapped DArT marker (*wPt4930*) in the advanced germplasm collection and five mapped DArT markers (*wPt1562*, *wPt4131*, *wPt5105*, *wPt9170* and *wPt9666*) and one unmapped DArT marker

(*wPt3976*) in the case of genebank collection were assigned to their respective deletion bins. The same resource was used in assigning three PHS associated mapped DArT markers i.e., *wPt0837*, *wPt1489* and *wPt5765* of advanced germplasm collection as well as seven PHS associated mapped DArT markers i.e., *wPt2636, wPt3945, wPt5105, wPt5128, wPt6709, wPt7015, wPt9170* and four unmapped markers i.e., *wPt0934, wPt3976, wPt5049, wPt9207* of the genebank collection to their respective bins.

Twelve dormancy associated mapped DArT markers of advanced germplasm collection which include *wPt0950, wPt1250, wPt3904, wPt4144, wPt4407, wPt4835, wPt4924, wPt5704, wPt6135, wPt6447, wPt7101* and *wPt8292* and six mapped DArT markers of genebank collection which included *wPt0610, wPt1155, wPt4144, wPt7306, wPt9418* and *wPt9422* were assigned to their bins using Semagn et al. (2006). Three PHS associated DArT markers of advanced germplasm collection including *wPt6135*, *wPt7101* and *wPt8072* and six PHS associated mapped markers of genebank collection including *wPt0610*, *wPt1547*, *wPt4721, wPt4863, wPt6975* and *wPt9422* were also placed in their bins using the same resource.

Mantovani et al. (2008) was utilised in placing one dormancy associated marker *wPt4620* and 1 PHS associated marker *wPt8682* of advanced germplasm collection in their bins. Four more dormancy associated markers *wPt0194*, *wPt3816*, *wPt3991* and *wPt4424* and three PHS associated markers *wPt0864*, *wPt5390* and *wPt8006* of genebank collection were given the bins using Mantovani et al. (2008). All of the assigned markers in those cases were mapped.

Marker *wPt3060* associated with dormancy in advanced germplasm collection was assigned to a bin based on Hai-Chun et al. (2009). Furthermore, *wPt3661* which was in association with dormancy in genebank collection and *wPt8657* which was in association with PHS in advanced germplasm collection were placed using Peleg et al. (2008). Finally, *wPt1730* which was in association with PHS in genebank collection was placed with the help of Akbari et al. (2006).

In the following discussion bins already discussed in 5.1.1.2 related to seed longevity are not considered again. Group 1 dormancy and PHS mapped MTAs were distributed in seven different bins with three, three and one bins on A, B and D genomes, respectively. Two of

them were common to bins of longevity MTAs and five of them were unique. The bins unique to dormancy and PHS were 1AS-0.47-0.86, C-1AL-0.17, 1BL2-0.69-0.85, 1BL3-0.85-1.00 and 1DL4-0.18-0.4. These carried genes encoding for triticin, type V thionin and mitochondrial chaperonin CPN60-2 precursors, various enzymes like CTP synthase, cellulase synthase-1, glutathione-S-transferase 3, glutamate synthetase 1 and cyanate hydratase. Others included transcription factors like MADs box transcription factor and complex proteins or their homologues such as NBS-LRR disease resistance protein homologoue, Ras related protein Rab11D, porin VDAC2, transmembrane protein, MAP-kinase like protein, and eukaryotic translation initiation factor 4E. One particular gene of interest among the above mentioned ones is thionin precursor. 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). Thionins are widely distributed cysteine-rich plant polypeptides that are toxic to plant microbial pathogens and might be involved in redox regultions (García-Olmedo et al. 1992). Seed specific thionins of cereals, in addition to their possible role as storage protein, are assumed to perform general-purpose defense toxins and might protect the starchy endosperm against invasive bacteria and fungi (Fernandez de Caleya et al. 1972).

There were eight deletion bins identified for group 2 dormancy and PHS DArT markers in either advanced germplasm collection or genebank collection. Four of them including 2AS5-0.78, C-2BS1-0.53, 2BL6-0.89-1.00 and 2DS5-0.47-1.00 were unique to dormancy and PHS only whereas other four also contained seed longevity MTAs. These unique bins carried genes for 60S ribosomal protein L27a, transcription factor APF1, Na+/H+ antiporter, NADH dehydrogenase, ferrodoxin NADPH oxidoreductase, blue-copper binding homologue, cystatin and manganese superoxide dismutase (MnSOD). MnSOD is an essential antioxidant enzyme and plays a role for environmental stresses such as chilling, freezing, oxidative stress, aluminium toxicity, etc. (Baek et al. 2006). The blue copper binding protein is involved in defence against oxidative stress possibly by preventing synthesis of hydroxyl radicals through binding of copper ions, thereby reducing deleterious reactive oxygen species (Jansen et al. 2005). Cystatin is an important determinant of flour quality which is reduced by PHS (Kuroda et al. 2001).

Group 3 dormancy and PHS loci were distributed in three bins but one of them was unique and carried genes that encoded for various dehydrogenases such as malate, cinnamyl

alcohol and allyl alcohol dehydrogenases. Others included laccase, histidine kinase, chitinase 1, metallothioneine and *gliadin*. These enzymes play roles in stress responses.

MTAs of dormancy and PHS belonging to group 4 chromosomes lie in the region where QTLs for dormancy and PHS have already been discovered (Li et al. 2004, Lohwasser et al. 2005). These MTAs were assigned to three bins, two on chromosome 4AL and one on chromosome 4BL, but none of them were unique to dormancy or PHS only. Analysis of ITMI population also revealed a major QTL for dormancy on 4AL signifying the importance of this chromosome with respect to dormancy and PHS.

Although group 5 chromosomes were least covered by genetic markers, yet the MTAs for this group were distributed on five bins with three only carrying dormancy and PHS MTAs whereas others carrying MTAs for seed longevity also. The unique bins were 5AS3-0.75-0.98, 5BS6-0.81-1.00 and 5BL16-0.79-1.00. Genes constituting these bins are P-type ATPase, lipoxygenase, and oxalate oxidase, RNA binding proteins, grain softness protein, methionine synthase protein, small GTP binding proteins, chlorophyll a/b-binding protein WCAB precursor, APETALA-2 like protein and drug resistance-associated proteins. *Aleurain* gene is also located in the centromere region of 5H of barley whose regulation is controlled by gibberlic acid and abscisic acid. Both of these hormones influence PHS and dormancy (Noda et al. 2002). It can be speculated that orthologues of these genes are located in wheat which influence PHS and dormancy the same way as they do in barley (Lohwasser et al. unpublished).

MTAs of PHS and dormancy belonging to group 6 chromosomes were distributed on three bins but one of them was unique named 6AS5-0.65-1.00 that carried cationic peroxidase, α gliadin, senescence associated proteins, BAX inhibitor 1 and ABA7 protein. This ABA7 protein is influenced by abscisic acid levels which influence PHS and dormancy (Noda et al. 2002). BAX inhibitor 1 has been identified as an evolutionary conserved protein that act as a key regulator for a common cell death pathway in animals and plants (Watanabe and Lam 2004).

In case of group 7, five bins were identified carrying PHS and dormancy MTAs. Only bin 7AS1-0.89-1.00 was unique carrying a PHS MTA. The gene contents of this bin included lipoxygenase, 60S ribosomal protein L9, glutathione transferase and putative disease resistance protein. No obvious relationship of these genes can be associated with PHS yet.

5.2.2 Synteny of dormancy and pre-harvest sprouting in wheat, barley and rice

QTLs for dormancy in barley have been identified on chromosomes 1HL, 2HS, 2HL, 3HS, 5HC, 6HS, 5HL and 7HC (Takeda 1996, Ullrich et al. 2002, Li et al. 2003, Hori et al. 2007, Bonnardeaux et al. 2008, Ullrich et al. 2009, Lohwasser et al. unpublished). Similarly, QTLs for dormancy in rice have been reported in chromosomes 1, 2, 3, 5, 6, 7 and 11 (Miura et al. 2002, Guo et al. 2004, Wan et al. 2005, Wan et al. 2006, Xue et al. 2008).

Synteny is observed between the dormancy/PHS QTLs/MTAs of wheat in this study with barley or rice (Figure 21). For dormancy MTAs on 1DS identified in this study can be compared with dormancy QTL on chromosome 5 shown by Miura et al. 2002 (Figure 21a). Dormancy QTL identified by Lohwasser et al. (unpublished) in barley on 2H is at a similar position as MTAs for dormancy on 2B found in this study (Figure 21b). MTAs for dormancy on 4A of wheat can be compared with dormancy QTLs on chromosome 1 of rice as reported by Miura et al. (2002) and Wan et al. (2005, 2006) (Fig 21c). In barley, Lohwasser et al. (unpublished) reported one QTL for dormancy on chromosome 5H and one on 6H which are in comparable locations to the dormancy MTAs on 5BL and 6BL, respectively identified in this study (Figure 21d and 21e). Centromere region of chromosome 5H of barley has also shown consistent QTLs in eight different populations in a study performed by Hori et al. (2007). The MTA on chromosome 7B of wheat is comparable to that on 7H of barley (Lohwasser et al. 2011) as shown in Figure 21f.

For PHS, the MTAs near to centromere of 1D in this investigation can be compared with PHS QTL in rice on chromosome 5 by Gao et al. (2008). Other MTAs on chromosome 1DL are related to dormancy in rice (Guo et al. 2004, Wan et al. 2006)(Figure 21a). One MTA for PHS on 2B of wheat can be compared to PHS QTL of 2H of barley (Lohwasser et al. 2011) (Fig 21b). One more MTA on 7A in wheat near to centromere can be considered to be similar to 7HS QTL of PHS in barley (Lohwasser et al. 2011). The centromere region of 7A of wheat is orthologous to rice chromosome 8 where Gao et al. (2008) found a QTL for PHS as well (Fig 21f).

The previous studies to dissect dormancy and PHS were conducted using the bi-parental mapping populations and resulted in the detection of fewer loci. In comparison, the current study was conduted using a collection of germplasm preserved in the genebank alongside a

collection of advanced germplasm. This indicates that the current association mapping panels are useful to dissect complex traits of economic as well as physiological importance. The comparable locations of dormancy and PHS QTLs/MTAs in wheat, barley and rice indicate that the three cereals share similarities among each other to some extent. It can be speculated that similar genes might be present at those locations governing these complex traits.

It is evident that although dormancy and PHS are quite complex and governed by several genes the effect of which can be variable depending on the nature of the populations, and the environment where they are produced, yet the majority of the QTLs in wheat, barley and rice are assumed to be conserved. Although there are complex rearrangements in the wheat genome in comparison to rice, yet some areas are of particular interest to dissect dormancy and PHS further in future. Chromosome 4AL of wheat has shown consistent QTLs in lots of independent studies (Li et al. 2004, Lohwasser et al. 2005, Chen et al. 2008). Chromosome 5B has also shown MTAs for dormancy and PHS in both association mapping panels highly comparable to loci on the orthologous barley chromosome 5H. Both these chromosomes may provide a solid basis to clone these QTLs. In addition, clusters of DArT markers in association with dormancy are observed on short arms of chromosomes 1B and 2D. Similarly, scattered clusters of PHS associated markers are observed on long arms of chromosomes 1D and 6A. These areas have not been considered for dormancy and PHS studies so far. It is proposed that these areas should be investigated using different germplasm to confirm these findings. Nevertheless, this study establishes a strong relationship among the QTLs and genes influencing dormancy and PHS in wheat, barley and rice.
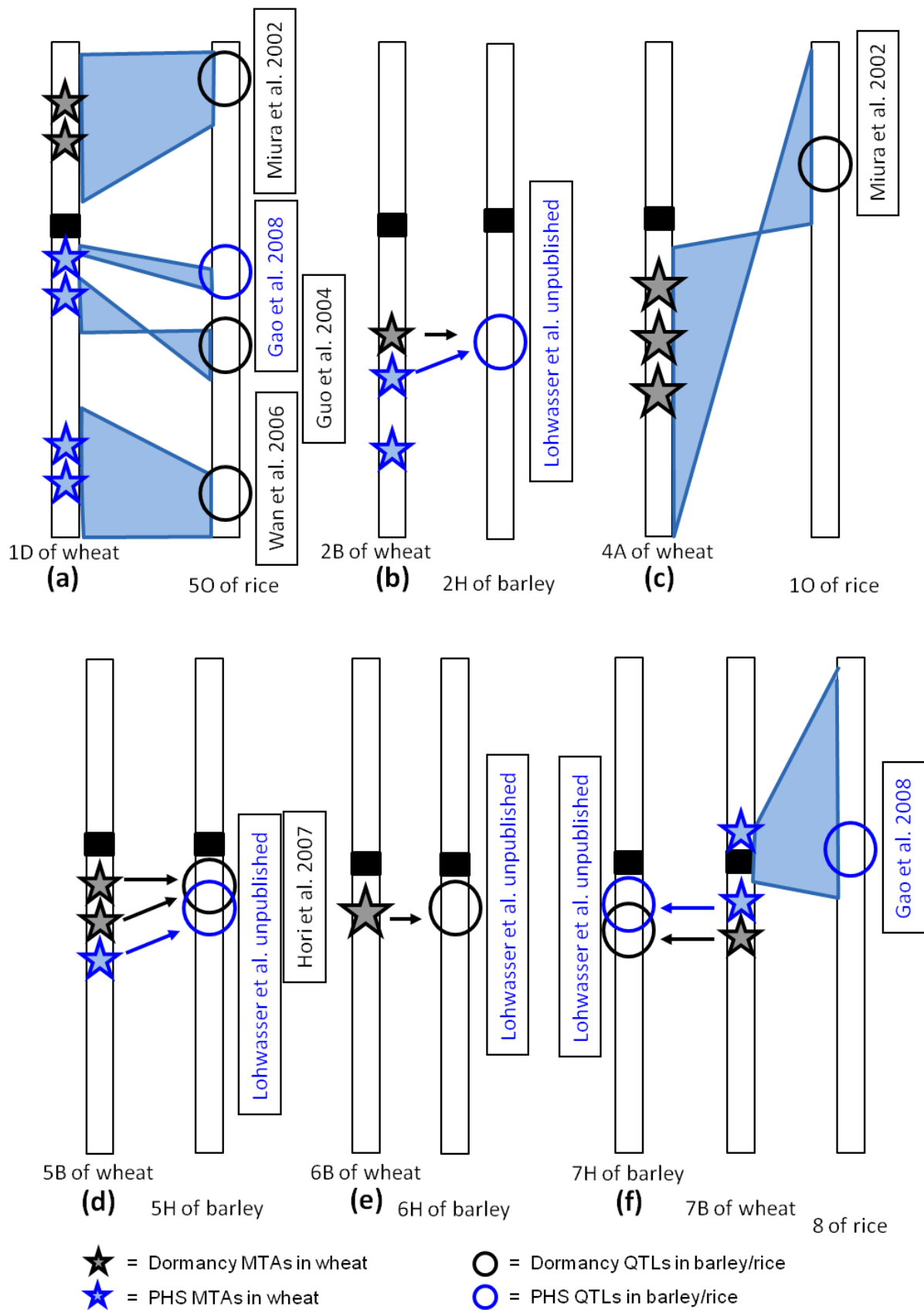
Figure 21: Comparison of dormancy and PHS MTAs/QTLs among wheat, barley and rice. **(a)** Chromosome 1D of wheat and 5 of rice, **(b)** 2B of wheat and 2H of barley **(c)** 4A of wheat and 1 of rice, **(d)** 5B of wheat and 5H of barley **(e)** 6B of wheat and 6H of barley and **(f)** 7B of wheat, 7H of barley and 8 of rice. Stein et al. (2007) is used to compare rice with barley

5.3 Seed longevity in relation to dormancy and pre-harvest sprouting

The existence of a relationship between longevity and dormancy/PHS remains controversial. In rice it was assumed that the two traits are connected to each other (Siddique et al. 1988). On the other hand Miura et al. (2002) showed that longevity and dormancy QTL mapped to distinct loci, with the former mapping to chromosomes 2O, 4O and 9O and the latter to chromosomes 1O, 3O, 5O, 7O and 11O. In *Arabidopsis* seeds of mutants affecting testa pigmentation and/or structure exhibited both reduced dormancy and faster deterioration (Debeaujon et al. 2000). In our study the QTL for the two traits were not coincident in bi-parental ITMI population. Whereas a major dormancy QTL mapped to chromosome 4AL, no longevity QTL appeared on that chromosome arm in either the 2009 or the 2003 seed lots. This suggests strongly that seed dormancy and seed longevity are independently controlled. Note that when the 2003 seed lot was also used to genetically analyse variation in pre-harvest sprouting (Lohwasser et al. 2005), a QTL was identified in the same segment into which *QDor.ipk-4A* mapped. The coincidence of these two loci indicates the accurateness of the QTL mapping approaches but also a higher heritability of the trait seed dormancy in comparison to seed longevity.

When longevity MTAs of both association mapping panels were compared to those for dormancy/PHS, a different picture emerged. In total, 51 MTAs were common to longevity and dormancy/PHS in both the advanced germplasm and genebank collection. Seventeen of them were with mapped and 34 with unmapped markers. In terms of deletion bins, there were 18 bins common to both longevity and dormancy/PHS. These bins were located on chromosomes 1AS, 1BS, 2AS, 2AL, 2BS, 2BL, 3AC, 3BS, 4AL, 4BC, 5BL (2 bins), 6BS, 6BC, 7AC, 7BS, 7BL and 7DS. There are numerous genes described in these bins and the functional mechanisms by which these genes interact to influence longevity and dormancy are not known at this stage. However, there are some genetic areas orthologous to barley for which possible longevity and dormancy genes have been identified. These included 5BL of wheat which is comparable to 5H of barley and 7AL and centromere region of 7B of wheat comparable to 7H of barley. QTLs of longevity on both of these barley chromosomes have been identified (Nagel et al. 2009).

Genes identified for 5H included hydrolase, epoxide hydrolase, thaumatin-like protein TLP5, barperm1, heat shock protein, APETALA-2 like protein, asparate aminotransferase and for

7H included ethylene responsive element binding protein, ethylene-responsive transcription factor and putative enoyl-ACP reductase. Deletion bins of 5BL identified in this study carrying longevity and dormancy/PHS MTAs also contained heat shock 70 KD protein and APETALA-2 like protein giving the indication that both of them affect seed dormancy and longevity in their own distinct ways.

Deletion bin of 7AC in this study carried EREBP transcription factor known to affect longevity in barley. Thus, in wheat EREBP might also be responsible for longevity and dormancy/PHS. In fact, Lohwasser et al. (unpublished) have recently identified QTLs for dormancy and PHS on 5H and 7H of barley at the locations where Nagel et al. (2009) have already found longevity QTLs.

Thus, this study alongside Lohwasser et al. (unpublished) might confirm that loci controlling longevity and dormancy/PHS in wheat seem to be identical on certain locations such as 5BL and 7BL. These loci might influence these two phenomena either independently or in a collective manner. Common loci also indicate that both longevity and dormancy might be controlled by similar genes in the upstream such as ABA-7 and viviparous-1. This study further establishes that dormancy and PHS along with longevity are not oligogenic but polygenic traits. To look further in to these traits physiological and biochemical approaches should be combined in parallel to the genetic analysis to counteract the limitations in genetical approaches and to reach a solid conclusion. This will not only pave the way towards further identification of loci controlling dormancy, PHS and longevity but also might lead towards the cloning of respective genes.

5.4 Agronomic traits, genetic mapping and relation to longevity

Most agronomic traits are quantitative in nature and controlled by many genes. Therefore, it becomes necessary to have multi-year and multi-location trials to compare the results of agronomic traits like height, heading time, flowering time and grain weight.

Not all of the loci mapped for heading time, flowering time, plant height and TKW identified in this study match with previous studies performed in wheat for these traits (Börner et al. 2002, Huang et al. 2006, Kumar et al. 2007, Neumann et al. 2011). One reason can be that this study was conducted a a foil tunnel experiment to prevent the potential loss of seedlings or adult plants grown from seeds after 34 years of storage. So, the results might not be

comparable with the results of field trials. The other reason can be the application of an association mapping strategy using a germplasm collection of the genebank of IPK, Gatersleben. Association mapping studies in wheat are rare. Neumann et al. (2011) conducted such a study using 96 diverse cultivars for mapping agronomic traits with DArT markers whereas this study was performed by using 183 accessions. The third reason can be the map saturation. In this study, many markers remained unmapped due to the lack of a consensus map of DArT markers in wheat. Nevertheless some of the loci detected in this study for these traits can be compared with previous investigations.

QTLs for heading time have been found on chromosomes 1A, 1B, 2A, 2B, 2D, 3B, 4A, 5A, 5B, 5D, 6B, 6D and 7D (Araki et al. 1999, Kato et al. 1999a, Börner et al. 2002, Gervais et al. 2003, Marza et al. 2006, Zhang et al. 2009). In this study MTAs for heading time were found on all the above mentioned chromosomes except 5A and 5D, the reason for which can be the poor coverage of group 5 chromosomes.

Flowering time has been reported to be controlled by genes located on homoeologous group 3, 5 and 6 chromosomes (Börner et al. 2002, Nemoto et al. 2003, Pánková et al. 2008). Chromosomes of group 3 and 5 are known to carry *Eps* and *Vrn* genes, respectively. Numerous MTAs identified in this study on 3B might correspond to the flowering time gene identified by Pánková et al. (2008).

Classical genetic studies indicated the genetic control of height in bread wheat is complex and most chromosomes harbour factors that can affect it (Law et al. 1973). Almost all of the 21 chromosomes were found to contribute to genetic variation for Ht in the case of substitution lines Cappelle-Desprez into Chinese Spring (Snape et al. 1977). This finding has been confirmed by various other reports of QTL detection for plant height in wheat (Kato et al. 1999b, Keller et al. 1999, Börner et al. 2002, Huang et al. 2003, 2004, Liu et al. 2005, McCartney et al. 2005, Huang et al. 2006, Marza et al. 2006, Klahr et al. 2007, Sourdille et al. 2007, Zhang et al. 2008, Chu et al. 2008, Wang et al. 2009, Wang et al. 2010, Mao et al. 2010, Wu et al. 2010). Therefore, it was not surprising that, during the course of this investigation, many MTAs were detected on almost all of the 21 chromosomes.

TKW is a complex trait like other yield traits and QTLs for TKW have been reported on almost all 21 wheat chromosomes. The MTA for TKW on 1A can be compared to the yield QTL

reported by Cuthbert et al. (2008). Another MTA on 2D can be compared with thousand kernel weight QTL reported by Huang et al. (2006). One MTA on 4B with *wPt3991* was also associated with TKW in Neumann et al. (2011) as well. MTAs on 6A for TKW can be compared with the QTLs for grain weight observed by Sun et al. (2009) in three different environments. It cannot be said at this moment that the other MTAs are novel or not.

Inheritance of 'awnedness' in wheat, *Triticum aestivum*, has been well studied since the beginning of twentieth century. The genetic control of awns was generally found to be simple, and only a few genes were involved in the differences between awned and awnless varieties (Sourdille et al. 2002). Three dominant inhibitors are described (McIntosh et al. 1998): *Hd* (Hooded), *B1* and *B2* (tipped 1 and 2). *Hd* is located on chromosome 4AS, *B1* on 5AL and *B2* on 6BL. In this study, no MTA has been found associated with awns on 4AS and 5AL except one MTA on 5A with an unmapped marker. The reason for this can be that only presence or absence of awns was taken into account in this study whereas Sourdille et al. (2002) used three criteria (awn length at the base, the middle and the top of the ear) to map genes for awnedness.

Leaf pubescence in common wheat has received much attention because of its possible agronomic merits, such as insect resistance (Ringlund & Everson 1968) and drought tolerance (Erdei et al. 1990). MTAs common to flag leaf pubescence and longevity were observed on chromosomes 1A (2 MTAs), 3A, 3B, 4BS, 6AL, 6BS and 6BL. All of them were unique to both traits except the one on 6BL clustering to other traits as well.

Among the agronomic traits, only height has been found to be in a positive correlation with longevity. No reason can be given for such behaviour at this stage. Börner et al. (2002) and Neumann et al. (2011) argued that peduncle length can be of some importance in the context of ear diseases. It can be speculated here that height played its role in longevity by providing protection to the spikes against various ear diseases such as *Fusarium* head blight. Some MTAs of TKW on chromosomes 2BS, 4AL, 4BS, 7AS, 7BL and 7D were common or very near to longevity MTAs, giving the indication of the superiority of relatively heavier grains towards the stresses that affect long lives of seeds in wheat. Two MTAs on 6BL associated with presence of awns were found to be associated with longevity which leads to the speculation that seeds coming from awned varieties might be useful for good quality grains

in wheat which contribute towards the longevity of wheat grains. But leaf pubescence might be independent of longevity because of unique MTAs.

A comparison was made between the DArT markers associated with longevity with those highlighted in the association studies made by Crossa et al. (2007) and Neumann et al. (2011). Nine and eleven longevity associated DArT markers in case of advanced germplasm collection and genebank collection, respectively, were associated with grain yield or fungal resistance by Crossa et al. (2007) (Table 10). Similarly, four and six markers in advanced germplasm and genebank collection, respectively, were associated with yield traits and disease resistance in Neumann et al. (2011) study. This leads to the conclusion that superior agronomic performance may influence seed longevity.

Table 10: Markers associated with seed longevity and also associated with grain yield (GY) and leaf rust (LR), stripe rust (SR), yellow rust (YR) and powdery mildew (PM) resistances in advanced germplasm (blue) and genebank collection (red) or both (green)

Locus	Chrom	Traits					Cited by
		GY	LR	SR	YR	PM	
wPt0128	1A	X	X	X		X	Crossa et al. 2007
wPt6709	1A	X					Crossa et al. 2007
wPt8455	1A		X				Neumann et al. 2011
wPt5503	1D	X					Neumann et al. 2011
wPt2087	2A	X			X		Crossa et al. 2007
wPt7901	2A	X			X		Crossa et al. 2007
wPt9350	2B				X		Crossa et al. 2007
wPt8096	3B	X	X				Crossa et al. 2007
wPt9510	3B				X		Crossa et al. 2007
wPt0610	4A	X				X	Neumann et al. 2011
wPt1155	4A	X					Neumann et al. 2011
wPt2151	4A	X					Neumann et al. 2011
wPt4487	4A			X		X	Crossa et al. 2007
wPt4620	4A		X				Crossa et al. 2007
wPt5172	4A	X	X			X	Crossa et al. 2007
wPt9418	4A	X					Neumann et al. 2011
wPt5896	5B	X	X				Crossa et al. 2007
wPt7101	5B	X					Crossa et al. 2007
wPt7063	6A	X		X			Crossa et al. 2007
wPt1541	6B	X		X	X		Crossa et al. 2007
wPt1547	6B	X					Crossa et al. 2007
wPt2424	6B		X				Neumann et al. 2011
wPt6495	7A			X			Crossa et al. 2007
wPt6156	7B	X					Crossa et al. 2007
wPt2565	7D			X			Crossa et al. 2007
wPt4555	7D				X		Neumann et al. 2011
wPt5249	-	X					Neumann et al. 2011
wPt9986	-	X					Neumann et al. 2011

5.5 Segregation mapping approach versus association mapping approach

Three mapping populations were used in the present study. For seed longevity the bi-parental ITMI mapping population revealed the least number of 10 loci (1 major and 9 minor QTLs) on chromosomes 1A, 1D, 2A, 2D, 3B, 3D, 6B and 7A. The other two populations were employed to map loci for longevity using association mapping approach. Advanced germplasm collection revealed 73 MTAs on all the chromosomes except 2B, 3D, 4D, 5A, 5D and 7D (6D was not covered). In case of the genebank collection, 340 MTAs were identified in association with seed longevity after either long term cold storage or experimental ageing

on all the chromosomes of wheat except 4D. Thus, the number of loci identified for seed longevity increased from a bi-parental mapping population to an association mapping population. It also increased in the association mapping approach when the number of accessions was doubled as the genebank accessions were almost twice as much as in the advanced germplasm collection. The number of accessions used by Maccaferri et al. (2011) in a durum wheat association mapping study was twice as much as large as those surveyed by Chao et al. (2007) and Somers et al. (2007) where the authors detected a higher number of loci. Similar results are obtained in this study when genebank collection (183 accessions) was investigated alongside advanced germplasm collection (96 accessions) to investigate seed longevity.

There are many regions where the longevity loci of at least 2 of the 3 populations lie at comparable position (Figure 18). These include distal ends of 1AL, 4AL and 7BL and proximal ends of 2AS, 3BS and 6BL. In fact, the QTLs of the ITMI population and MTAs of either advanced germplasm or genebank collection in case of 2AS, 3BS and 6B are concentrated in the centromeric regions giving the impression that these loci are conserved. Longevity markers at the distal ends of chromosomes 3AL, 3BL, 4AL, 5BL, 6BL and 7AL seem to be in agreement with the theory that associates telomere stability and longevity (Monaghan and Haussmann 2006, Revilla et al. 2009).

Dormancy and PHS results indicate similar observations. On the one hand ITMI population was able to demonstrate the presence of only one major QTL for dormancy on chromosome 4A, advanced germplasm collection anaylsis comprising 96 lines identified much more loci for dormancy as well as PHS. In the case of the genebank collection, many other loci for dormancy and PHS were detected. Some of them were novel and some were identical to the loci identified in advanced germplasm collection. The regions where both association mapping populations shared dormancy and/or PHS MTAs included chromosome 2D and proximal ends of 3AL, 4AL, 5BL, 6BL.

5.6 Outlook

D-genome map in both association mapping panels was poorly covered. There were also a large number of markers that remained unmapped. Map positions of these markers will be available soon which will make it easy to compare this study with those already performed.

In future, following work should be continued:

1. Fine mapping of chromosome 4AL to reveal interesting information about longevity, dormancy and PHS

2. Validation of MTAs/QTLs found on chromosomes 2AL, 3BC, 4AL and 7B in order to strengthen the hypothesis about the genes located here

3. Further longevity studies of already available wheat mapping populations

4. Development of new mapping populations by crossing long lived and short lived genebank accession

5. Confirmation of syntenic relationships regarding longevity, dormancy and PHS among cereals (wheat, barley and rice) with common markers

6. Further physiological studies e.g., ABA sensitivity for better understanding of longevity, dormancy/PHS mechanisms

7. Usage of new ageing methods to mimic long term storage

6   Summary

This study was undertaken to map quantitative trait loci (QTLs) and marker trait associations (MTAs) controlling seed longevity in wheat by applying experimental ageing methods using one bi-parental and two association mapping populations. Other aims of this study were to compare seed longevities of long term cold stored and experimentally aged seeds and to investigate the relationships between seed longevity and dormancy/pre-harvest sprouting as well as agronomic traits.

QTL analysis of bi-parental 'International Triticeae Mapping Initiative' population reproduced in two different seasons resulted in the identification of one major and nine minor QTLs for seed longevity. The major QTL located on chromosome 2A was closely linked to *Per2* (peroxidase-2) and *Wip* (wound induced protein) genes giving the indication that peroxidases might play a role in seed longevity. One minor QTL on chromosome 1AL mapped close to spike compactness and grain number QTLs detected in previous studies. One minor QTL on 3BS coincided with yield component loci whereas other minor QTLs on 1DL and 6BS match with grain weight QTLs. There was one major QTL for dormancy observed on chromosome 4AL. The location of this QTL does not match with any of the longevity QTLs. Thus longevity and dormancy are controlled by different loci in this population.

A set of 96 winter wheat accessions (advanced gemplasm collection) and another set of 183 accessions consisting of a mixture of spring and winter wheat (genebank collection) revealed 73 and 340 MTAs for seed longevity, respectively. Of the 340 MTAs, 134 MTAs were observed after long term cold storage but the others after experimental ageing. The associations were distributed over all the wheat chromosomes except 4D which was not covered with markers.

Results obtained after long term cold storage and experimental ageing did match only to some extent in this study which could be due to different quality of seeds produced during different seasons. However, it also can be proposed that different mechanisms are involved during deterioration in cold storage over long periods and decaying during artificial ageing treatments.

When compared with barley and rice, it was found that longevity QTLs in barley on chromosomes 5H and 7H and rice on chromosomes 3, 9 and 12 coincide with the MTAs observed on chromosome 5B and homoeologous group 7 chromosomes of wheat. Thus, it can be assumed that wheat, barley and rice share commonalities towards genes governing seed longevity.

For dormancy, there were 68 and 118 MTAs identified in the advanced germplasm collection and genebank collection, respectively, in addition to one major QTL on chromosome 4A in the ITMI population. Similarly, pre-harvest sprouting revealed 32 and 193 MTAs in case of advanced germplasm collection and genebank accessions, respectively.

Co-linearity was found among wheat, rice and barley for loci influencing dormancy and PHS. For example, MTAs of chromosome 1D revealed in this investigation match with the QTLs for dormancy in rice on chromosome 5, chromosome 4AL MTAs in wheat match with the QTLs on rice chromosome 1 whereas 7B MTAs can be compared with the PHS MTA observed on chromosome 8 of rice. Similar associations were observed for barley where MTAs of chromosomes 2B, 5B, 6B and 7B of wheat are on comparable locations in the barley genome. This again gives us the idea about the conservation of genes affecting dormancy and PHS across the three cereals.

A relationship was observed between loci that govern seed longevity and PHS/dormancy in the two association mapping populations examined in this study. Fifty-one MTAs were common to both phenomena. MTAs on chromosomes 5B and 7B in particular were in regions where longevity and dormancy/PHS QTLs have been identified. This proves that genes located here influence both longevity and dormancy/PHS either through modifying morphological traits or enzymes that govern these two independent phenomena.

Finally, number of detected loci increased dramatically from a shift from segregation mapping towards association mapping. This is in principle because association mapping utilises the past recombination events and does not require a defined population which is the case in segregation analysis having only a few recombinations. However, for the validation of detected loci, bi-parental segregation mapping remains necessary.

7   Zusammenfassung

Ziel der vorliegenden Arbeit war es, quantitative vererbte Genorte (Quantitative Trait Loci; QTLs) und Marker-Merkmal-Assoziationen (Marker Trait Associations; MTAs), welche das Merkmal Langlebigkeit von Samen im Weizen kontrollieren, unter Verwendung von experimentellen Alterungsmethoden zu identifizieren. Ein Schwerpunkt und Alleinstellungsmerkmal war hierbei der Vergleich der Langlebigkeit von Samen nach experimenteller Alterung mit langzeit-kühlgelagertem Saatgut. Des Weiteren wurde die Beziehung zwischen Saatgut-Langlebigkeit und den Merkmalen Dormanz bzw. Auswuchs sowie anderen agronomischen Eigenschaften untersucht.

Die Analyse der bi-parentalen ITMI (International Triticeae Mapping Initiative) Population, reproduziert in zwei verschiedenen Vegetationsperioden, führte zur Identifizierung von einem Major- und neun Minor-QTLs für Langlebigkeit von Samen. Der Major-QTL befand sich auf dem Chromosom 2A, eng gekoppelt zu den Genen *Per2* (*Peroxidase-2*) und *Wip* (*Wound induced protein*), was vermuten lässt, dass Peroxidasen einen Einfluss auf die Langlebigkeit von Samen haben könnten. Ein Minor-QTL auf Chromosom 1AL kartierte in einer Region in der in früheren Studien Loci für die Merkmale Ährendichte und Kornzahl pro Ähre gefunden wurden. Weitere Minor-QTLs auf den Chromosomen 3BS, 1DL und 6BS befanden sich in Regionen, in denen QTLs für Ertragskomponenten wie Korngewicht bekannt waren. Für das Merkmal Dormanz wurde ein QTL detektiert. Die Lokalisierung dieses Major-QTLs auf Chromosom 4AL stimmte mit keinem der QTLs für Langlebigkeit überein. Das führte zu dem Schluss, dass in der untersuchten Population die Merkmale Langlebigkeit von Samen und Dormanz von verschiedenen Genen kontrolliert werden.

Eine Serie von 96 Winterweizen-Akzessionen (Advanced Germplasm Collection) und ein weiterer Satz von 183 Akzessionen bestehend aus Sommer- und Winterweizen (Genebank Collection) führten zum Auffinden von 73 beziehungsweise 340 MTAs für Langlebigkeit von Samen. Von den 340 MTAs wurden 134 nach Langzeit-Kühllagerung, alle übrigen nach experimenteller Alterung detektiert. Die Marker-Merkmal-Assoziationen waren mit Ausnahme von Chromosom 4D, welches nicht kartiert war, auf allen Chromosomen verteilt.

Die Ergebnisse, erzielt mit langzeit-kühlgelagertem Saatgut, stimmten nur zum Teil mit denen nach experimenteller Alterung überein. Ursächlich könnte eine unterschiedliche

Qualität der Samen sein, die während verschiedener Vegetationsperioden produziert wurden. Zudem liegen der Alterung unter kühlen Bedingungen über einen langen Zeitraum und der experimentellen Alterung möglicherweise verschiedene Mechanismen zu Grunde.

Vergleicht man die vorliegenden Ergebnisse mit Studien in Gerste und Reis, so sind dort detektierte QTLs für Langlebigkeit auf den Chromosomen 5H und 7H (Gerste) sowie 3, 9 und 12 (Reis) vergleichbar mit den hier aufgefundenen MTAs auf Chromosom 5B und den Chromosomen der homöologen Gruppe 7. Folglich teilen Weizen, Gerste und Reis mögliche gemeinsame genetische Mechanismen für die Langlebigkeit von Samen.

Für das Merkmal Dormanz wurden 68 (Advanced Germplasm Collection) und 118 (Genebank Collection) MTAs identifiziert. Hinzu kommt ein Major-QTL detektiert in der ITMI Population. Vergleichbar wurden für das Merkmal Auswuchs 32 (Advanced Germplasm Collection) und 193 (Genebank Accessions) MTAs gefunden.

Auch für Dormanz und Auswuchs konnte Ko-Linearität zwischen Weizen, Gerste und Reis gefunden werden. So sind MTAs, lokalisiert auf dem Weizenchromosom 1A in dieser Studie, vergleichbar mit QTLs auf dem Reischromosom 5, MTAs von 4AL mit QTLs auf dem Reischromosom 1 oder MTAs auf Chromosom 7B mit MTAs auf dem Reischromosom 8. Ähnliche Beziehungen existieren zur Gerste. MTAs auf den Weizenchromosomen 2B, 5B, 6B und 7B haben vergleichbare Loci im Gerstengenom. Wiederum kann geschlussfolgert werden, dass die genannten Getreide gemeinsame genetische Mechanismen für Dormanz und Auswuchs teilen.

In der vorliegenden Arbeit konnte gezeigt werden, dass Genloci für Langlebigkeit und Dormanz/Auswuchs zueinander in Beziehung stehen. Insgesamt ergaben sich 51 gemeinsame MTAs hauptsächlich auf den Chromosomen 5B und 7B. Die verantwortlichen Gene beeinflussen die Merkmale Langlebigkeit und Dormanz/Auswuchs gemeinsam möglicherweise auf Grund morphologischer Merkmale oder durch Enzyme, welche beide Phänomene steuern.

Abschließend sei noch erwähnt, dass sich die Anzahl aufgefundener Loci in der assoziationsgenetischen Studie im Vergleich zur bi-parentale Analyse deutlich erhöht hat. Die Assoziationskartierung nutzt hierbei eine Vielzahl von Rekombinationen der

Vergangenheit wohingegen die bi-parentalen Populationen nur wenige Rekombinationen aufweisen können. Für die Validierung aufgefundener MTAs wird eine nachfolgende bi-parentale Kartierung dennoch weiterhin notwendig bleiben.

8 References

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Appendix I

Table 1: Genebank collection with identification number, botanical name, origin, annuity and Q group as identified by STRUCTURE (Pritchard et al. 2000) analysis. TRI followed by a number is the genebank identification number of wheat accessions, var. stands for variety, USA stands for United States of America and NZL stands for New Zealand

Genebank ID	Botanical name	Origin	Annuity	Q-group
TRI 27	*Triticum aestivum* L. var. *aestivum*	USA	Winter	Q2
TRI 144	*T. aestivum* L. var. *lutescens*	Russia	Winter	Q2
TRI 147	*T. aestivum* L. var. *aestivum*	Russia	Winter	Q2
TRI 181	*T. aestivum* L. var. *aestivum*	Hungary	Winter	Q2
TRI 207	*T. aestivum* L. var. *villosum*	France	Winter	Q2
TRI 287	*T. aestivum* L. var. *aestivum*	Germany	Winter	Q2
TRI 390	*T. aestivum* L. var. *lutescenscompactoides*	Europe	Winter	Q2
TRI 403	*T. aestivum* L. var. *lutescens*	USA	Spring	Q5
TRI 2513	*T. aestivum* L. var. *lutescens*	China	Spring	Q5
TRI 2607	*T. aestivum* L. var. *suberythrospermum*	Nepal	Spring	Q1
TRI 2619	*T. aestivum* L. var. *suberythrospermum*	Nepal	Spring	Q1
TRI 2656	*T. aestivum* L. var. *milturum*	India	Spring	Q5
TRI 2679	*T. aestivum* L. var. *aestivum*	India	Spring	Q1
TRI 2835	*T. aestivum* L. var. *meridionale*	Afghanistan	Spring	Q1
TRI 2839	*T. aestivum* L. var. *pyrothrix*	India	Spring	Mix
TRI 2889	*T. aestivum* L. var. *lutescens*	Nepal	Spring	Q1
TRI 3126	*T. aestivum* L. var. *milturum*	Portugal	Spring	Mix
TRI 3242	*T. aestivum* L. var. *lutescens*	USA	Spring	Mix
TRI 3438	*T. aestivum* L. var. *ferrugineum*	Germany	Spring	Q2
TRI 3477	*T. aestivum* L. var. *lutescens*	NZL	Spring	Q4
TRI 3492	*T. aestivum* L.	Nepal	Spring	Q1
TRI 3511	*T. aestivum* L. var. *lutescens*	Portugal	Spring	Mix
TRI 3513	*T. aestivum* L. var. *lutescens*	Britain	Spring	Mix
TRI 3526	*T. aestivum* L. var. *lutescens*	Portugal	Spring	Q4
TRI 3564	*T. aestivum* L. var. *ferrugineum*	Portugal	Spring	Q4
TRI 3569	*T. aestivum* L. var. *lutescens*	Uruguay	Spring	Q4
TRI 3570	*T. aestivum* L. var. *aestivum*	India	Spring	Q1
TRI 3631	*T. aestivum* L. var. *lutescens*	Canada	Spring	Q5
TRI 3633	*T. aestivum* L. var. *lutescens*	Canada	Spring	Q5
TRI 3664	*T. aestivum* L. var. *lutescens*	Germany	Spring	Q2
TRI 3831	*T. aestivum* L. var. *ferrugineum*	Portugal	Spring	Q4
TRI 3839	*T. aestivum* L. var. *ferrugineum*	France	Spring	Q4
TRI 3842	*T. aestivum* L. var. *milturum*	Portugal	Spring	Q4
TRI 3850	*T. aestivum* L. var. *ferrugineum*	Portugal	Spring	Q1
TRI 3874	*T. aestivum* L. var. *milturum*	Italy	Spring	Q4
TRI 3881	*T. aestivum* L. var. *ferrugineum*	Italy	Spring	Q4
TRI 3895	*T. aestivum* L. var. *aureum*	France	Spring	Q4
TRI 3904	*T. aestivum* L. var. *milturum*	Portugal	Spring	Mix
TRI 3925	*T. aestivum* L. var. *aestivum*	Uruguay	Spring	Q4
TRI 3926	*T. aestivum* L. var. *aestivum*	Uruguay	Spring	Q4

TRI 3929	*T. aestivum* L. var. *pyrothrix*	China	Spring	Q2
TRI 3931	*T. aestivum* L. var. *subhostianum*	China	Spring	Q1
TRI 3955	*T. aestivum* L.	Nepal	Spring	Q1
TRI 3964	*T. aestivum* L. var. *aestivum*	Nepal	Spring	Q1
TRI 3987	*T. aestivum* L. var. *pyrothrix*	India	Spring	Q1
TRI 4013	*T. aestivum* L. var. *aestivum*	Italy	Spring	Q4
TRI 4020	*T. aestivum* L. var. *pseudokazvinicum*	Nepal	Spring	Q1
TRI 4041	*T. aestivum* L. var. *ferrugineum*	Canada	Spring	Q5
TRI 4042	*T. aestivum* L. var. *lutescens*	China	Spring	Q2
TRI 4056	*T. aestivum* L. var. *villosum*	India	Spring	Q5
TRI 4081	*T. aestivum* L. var. *ferrugineum*	Portugal	Spring	Q4
TRI 4112	*T. aestivum* L. var. *fulvocinereumcompactoides*	Afghanistan	Spring	Q1
TRI 4113	*T. aestivum* L. var. *ferrugineum*	Afghanistan	Spring	Q1
TRI 4116	*T. aestivum* L. var. *aureum*	Italy	Spring	Q4
TRI 4117	*T. aestivum* L. var. *aestivum*	Italy	Spring	Q4
TRI 4118	*T. aestivum* L. var. *aestivum*	Italy	Spring	Q4
TRI 4126	*T. aestivum* L. var. *milturum*	Italy	Spring	Q4
TRI 4130	*T. aestivum* L. var. *milturum*	Italy	Spring	Q4
TRI 4141	*T. aestivum* L. var. *aestivum*	Italy	Spring	Q4
TRI 4144	*T. aestivum* L. var. *aestivum*	Italy	Spring	Q4
TRI 4145	*T. aestivum* L. var. *aestivum*	Italy	Spring	Q4
TRI 4148	*T. aestivum* L. var. *aestivum*	Italy	Spring	Q4
TRI 4149	*T. aestivum* L. var. *aestivum*	Italy	Spring	Q4
TRI 4152	*T. aestivum* L. var. *alborubrum*	Italy	Spring	Mix
TRI 4171	*T. aestivum* L. var. *aestivum*	Italy	Spring	Q4
TRI 4177	*T. aestivum* L. var. *lutescens*	Germany	Winter	Q2
TRI 4179	*T. aestivum* L. var. *lutescens*	Nepal	Winter	Q2
TRI 4186	*T. aestivum* L. var. *milturum*	Germany	Winter	Q2
TRI 4233	*T. aestivum* L. var. *villosum*	USA	Winter	Q2
TRI 4249	*T. aestivum* L. var. *milturum*	Germany	Winter	Q2
TRI 4253	*T. aestivum* L. var. *villosum*	China	Winter	Mix
TRI 4540	*T. aestivum* L. var. *melanopogon*	Russia	Spring	Q5
TRI 4545	*T. aestivum* L. var. *aestivum*	Austria	Spring	Q5
TRI 4547	*T. aestivum* L. var. *milturum*	Chile	Spring	Q2
TRI 4549	*T. aestivum* L. var. *lutescens*	Chile	Spring	Q2
TRI 4551	*T. aestivum* L. var. *leucospermum*	Chile	Spring	Mix
TRI 4554	*T. aestivum* L. var. *lutescens*	Chile	Spring	Q5
TRI 4563	*T. aestivum* L. var. *milturum*	Italy	Spring	Q2
TRI 4599	*T. aestivum* L. var. *aureum*	Albania	Winter	Q2
TRI 4723	*T. aestivum* L. var. *milturum*	France	Winter	Q2
TRI 4748	*T. aestivum* L. var. *villosum*	Germany	Winter	Q2
TRI 4839	*T. aestivum* L. var. *aestivum*	Argenitna	Winter	Q5
TRI 4896	*T. aestivum* L. var. *lutescens*	Chile	Spring	Q4
TRI 4919	*T. aestivum* L. var. *graecum*	Austria	Spring	Q5
TRI 4930	*T. aestivum* L. var. *lutescens*	Italy	Spring	Q4
TRI 4940	*T. aestivum* L. var. *aestivum*	USA	Spring	Q5
TRI 4942	*T. aestivum* L. var. *ferrugineum*	USA	Spring	Q2

TRI 4943	*T. aestivum* L. var. *lutescens*	Sweden	Spring	Q2
TRI 5069	*T. aestivum* L. var. *milturum*	Germany	Winter	Q2
TRI 5231	*T. aestivum* L. var. *lutescens*	Italy	Winter	Q2
TRI 5241	*T. aestivum* L. var. *lutescens*	Portugal	Winter	Q2
TRI 5262	*T. aestivum* L. var. *lutescens*	Estonia	Winter	Mix
TRI 5304	*T. aestivum* L. var. *ferrugineum*	Sweden	Spring	Q2
TRI 5310	*T. aestivum* L. var. *aestivum*	France	Spring	Q4
TRI 5315	*T. aestivum* L. var. *ferrugineum*	Argenitna	Spring	Q4
TRI 5325	*T. aestivum* L. var. *ferrugineum*	Argenitna	Spring	Mix
TRI 5332	*T. aestivum* L. var. *ferrugineum*	USA	Spring	Mix
TRI 5333	*T. aestivum* L. var. *ferrugineum*	Spain	Spring	Q4
TRI 5342	*T. aestivum* L. var. *aestivum*	Germany	Spring	Q5
TRI 5357	*T. aestivum* L. var. *aestivum*	Britain	Spring	Q1
TRI 5386	*T. aestivum* L. var. *aestivum*	Bulgaria	Spring	Q2
TRI 5425	*T. aestivum* L. var. *aestivum*	Spain	Spring	Q4
TRI 5426	*T. aestivum* L. var. *ferrugineum*	Sweden	Spring	Q2
TRI 5438	*T. aestivum* L. var. *aestivum*	USA	Spring	Mix
TRI 5603	*T. aestivum* L. var. *submeridionale*	Iran	Spring	Q1
TRI 5645	*T. aestivum* L. var. *aureum*	Iran	Spring	Q1
TRI 5653	*T. aestivum* L. var. *aestivum*	Iran	Spring	Q1
TRI 5692	*T. aestivum* L. var. *aestivum*	Iran	Spring	Q1
TRI 5984	*T. aestivum* L. var. *delfii*	Iran	Spring	Q3
TRI 6052	*T. aestivum* L. var. *suberythrospermum*	Iran	Spring	Q1
TRI 6094	*T. aestivum* L. var. *subgraecum*	Iran	Spring	Q1
TRI 6108	*T. aestivum* L. var. *graecum*	Iran	Spring	Q1
TRI 6129	*T. aestivum* L. var. *graecum*	Iran	Spring	Q1
TRI 6148	*T. aestivum* L. var. *aestivum*	Iran	Spring	Q1
TRI 10032	*T. aestivum* L. var. *aestivum*	Mexico	Spring	Q4
TRI 10134	*T. aestivum* L. var. *aestivum*	Romania	Winter	Q2
TRI 10135	*T. aestivum* L. var. *aestivum*	Romania	Winter	Q2
TRI 10165	*T. aestivum* L. var. *aestivum*	USA	Winter	Mix
TRI 10173	*T. aestivum* L. var. *lutescens*	Belgium	Winter	Q2
TRI 10211	*T. aestivum* L. var. *aestivum*	USA	Winter	Q2
TRI 10256	*T. aestivum* L. var. *lutescens*	USA	Winter	Q2
TRI 10259	*T. aestivum* L. var. *lutescens*	USA	Winter	Q2
TRI 10261	*T.* species (Population)	USA	Winter	Q2
TRI 10290	*T. aestivum* L. var. *lutescens*	Britain	Spring	Q2
TRI 10296	*T. aestivum* L. var. *lutescens*	Mexico	Spring	Mix
TRI 10297	*T. aestivum* L. var. *aestivum*	Brazil	Spring	Q4
TRI 10310	*T. aestivum* L. var. *aestivum*	India	Spring	Q4
TRI 10311	*T. aestivum* L. var. *aestivum*	Japan	Spring	Q2
TRI 10336	*T. aestivum* L. var. *variabile*	China	Spring	Q5
TRI 10338	*T. aestivum* L. var. *hostianum*	China	Spring	Q5
TRI 10339	*T. aestivum* L. var. *magnificum*	China	Spring	Q5
TRI 10340	*T. aestivum* L. var. *leucospermum*	China	Spring	Mix
TRI 10341	*T. aestivum* L. var. *turanicum*	China	Spring	Q5
TRI 10349	*T. aestivum* L. var. *aestivum*	Peru	Spring	Mix
TRI 10420	*T. aestivum* L. var. *aestivum*	Romania	Winter	Mix

TRI 10478	*T. aestivum* L. var. *ferrugineum*	Afghanistan	Winter	Q1
TRI 10485	*T. aestivum* L. var. *meridionale*	Afghanistan	Winter	Q1
TRI 10486	*T. aestivum* L. var. *graecum*	Afghanistan	Winter	Q1
TRI 10504	*T.* species (Population)	Afghanistan	Winter	Q1
TRI 10556	*T. aestivum* L. var. *aestivum*	Cyprus	Spring	Q3
TRI 10591	*T. aestivum* L. var. *aestivum*	Cyprus	Spring	Q3
TRI 10593	*T. aestivum* L. var. *murinumcompactoides*	Cyprus	Spring	Q3
TRI 10620	*T. aestivum* L. var. *murinumcompactoides*	Cyprus	Spring	Q3
TRI 10625	*T. aestivum* L. var. *aestivum*	Cyprus	Spring	Q3
TRI 10654	*T. aestivum* L. var. *murinumcompactoides*	Cyprus	Spring	Q3
TRI 10679	*T. aestivum* L. var. *aestivum*	Greece	Spring	Q3
TRI 10688	*T. aestivum* L. var. *aestivum*	Greece	Spring	Q3
TRI 10692	*T. aestivum* L. var. *ferrugineum*	Greece	Spring	Q3
TRI 10693	*T. aestivum* L. var. *aestivum*	Greece	Spring	Q3
TRI 10697	*T. aestivum* L. var. *ferrugineum*	Greece	Spring	Q3
TRI 10699	*T. aestivum* L. var. *aestivum*	Unknown	Winter	Q3
TRI 10700	*T. aestivum* L. var. *aestivum*	Greece	Winter	Mix
TRI 10702	*T.* species (Population)	Unknown	Winter	Q6
TRI 10703	*T. aestivum* L. var. *lutescens*	Greece	Spring	Q6
TRI 10704	*T. aestivum* L. var. *lutescens*	Greece	Spring	Q4
TRI 10705	*T. aestivum* L. var. *aestivum*	Greece	Spring	Q6
TRI 10707	*T.* species (Population)	Unknown	Spring	Q6
TRI 10709	*T. aestivum* L. var. *aestivum*	Greece	Spring	Q6
TRI 10710	*T. aestivum* L. var. *lutescens*	Greece	Spring	Q6
TRI 10712	*T.* species (Population)	Unknown	Spring	Q4
TRI 10714	*T. aestivum* L. var. *ferrugineum*	Greece	Winter	Mix
TRI 10770	*T. aestivum* L. var. *aestivum*	Greece	Winter	Q3
TRI 10780	*T. aestivum* L. var. *aestivum*	Greece	Spring	Q3
TRI 10781	*T. aestivum* L. var. *lutescens*	Greece	Spring	Q6
TRI 10785	*T. aestivum* L. var. *aestivum*	Greece	Spring	Q3
TRI 10786	*T. aestivum* L. var. *hostianum*	Greece	Spring	Q3
TRI 10807	*T. aestivum* L. var. *aestivum*	Greece	Winter	Q3
TRI 10811	*T. aestivum* L. var. *aestivum*	Unknown	Winter	Q3
TRI 10813	*T. aestivum* L. var. *aestivum*	Greece	Winter	Q6
TRI 10853	*T. aestivum* L. var. *suberythrospermum*	Nepal	Winter	Q1
TRI 11003	*T. aestivum* L. var. *lutescens*	Mexico	Spring	Mix
TRI 11020	*T. aestivum* L. var. *lutescens*	USA	Spring	Q5
TRI 11068	*T. aestivum* L. var. *pyrothrix*	Russia	Winter	Q2
TRI 11070	*T. aestivum* L. var. *lutescens*	France	Winter	Q2
TRI 11073	*T. aestivum* L. var. *lutescens*	USA	Winter	Mix
TRI 11075	*T. aestivum* L. var. *lutescens*	Belgium	Winter	Q2
TRI 11076	*T. aestivum* L. var. *ferrugineum*	USA	Winter	Mix
TRI 11077	*T. aestivum* L. var. *aureum*	Canada	Winter	Q2
TRI 11078	*T. aestivum* L. var. *graecum*	USA	Winter	Q2
TRI 11079	*T. aestivum* L. var. *lutescens*	France	Winter	Q2
TRI 11080	*T. aestivum* L. var. *aestivum*	USA	Winter	Q2
TRI 11081	*T. aestivum* L. var. *aureum*	USA	Winter	Q2
TRI 11082	*T. aestivum* L. var. *lutescens*	Germany	Winter	Q2

Table 2: IG, GAA and RGAA, GCD and RGCD for ITMI with standard deviations

	Initial germination (%) (IG)			Germination after AA (%) (GAA)			Mean relative germination after AA (%) (RGAA)	Germination after CD (%) (GCD)			Mean relative germination after CD (%) (RGCD)
	Min	Max	Mean	Min	Max	Mean		Min	Max	Mean	
ITMI 2003	59.0	97.0	83.85± 7.5	27.0	90.5	62.63± 13.2	74.53±13.5	-	-	-	-
ITMI 2009	24.5	98.0	75.96± 15.9	0.5	92.0	32.87± 24.0	40.69±24.9	0.5	90.0	25.37± 24.4	31.57±27.6

Table 3: D10, D20 and DI of ITMI2009 with standard deviations

	D10			D20			DI		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
ITMI 2009	0	58	9.63±11.3	27.0	90.5	75.63±26.8	1.11	72.22	31.63±13.6

D10 = percentage dormant seeds at 10°C, D20 = percentage dormant seeds at 20°C, DI = dormancy index

Table 4: IG, GAA, RGAA, GCD and RGCD of Q-groups, geographical groups and all accessions of advanced germplasm collection with standard deviations

	No. of acc.	Initial Germination (%) (IG)			Germination after AA (%) (GAA)			Relative germination after AA (%) (RGAA)			Germination after CD (%) (GCD)			Relative germination after CD (%) (RGCD)		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Q1	76	68	98.5	93.32±5.4	0	60.5	11.51±12.9	0	67.98	12.14±13.7	5.5	95	61.11±26.2	6.47	99.47	64.84 ± 26.6
Q2	20	85	99	95.18±3.2	0.5	33.5	8.95 ± 8.1	0.5	34.18	9.33±8.3	6	91.5	60.83±22.5	7.06	95.81	63.57 ± 23.2
European acc.	24	86	98.5	94.90±2.8	0	29	9.5±9.7	0	29.74	9.9±10.1	22	93.5	70.54±20.7	22.80	99.47	74.13 ± 21.1
Serbian acc.	22	75.5	99	93.82±5.8	0	33.5	8.61±8.1	0	34.18	8.94±8.3	6	93.5	51.59±25.2	7.06	95.41	54.37 ± 25.3
American acc.	30	88.5	98.5	94.52±2.6	0.5	60.5	12.82±14.2	0.5	67.98	13.64±15.4	9.5	95	66.83±24.5	10.16	97.94	70.68 ± 25.7
Asian & Aust. acc.	20	68	97.5	90.93±7.7	0	57	12.58±14.7	0	58.46	13.28±15.3	5.5	89.5	51.40±26.6	6.47	92.75	55.17 ± 26.3
Total	96	68	99	93.70±5.1	0	60.5	10.97±12.1	0	67.98	11.55±12.8	5.5	95	61.05±25.3	6.47	99.47	64.57 ± 25.8

Table 5: D10, D20, DI and PHS of Q-groups, geographical groups all accessions of advanced germplasm collection with standard deviations

	Dormant seeds % at 10°C (D10)			Dormant seeds % at 20°C (D20)			Dormancy Index (DI)			Pre-harvest sprouting (PHS)		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Q1	0	41	3.95±6.94	0	59	25.84±16.74	0	73.33	18.75±13.81	2.1	7	4.6±1.4
Q2	0	8	2.15±2.71	2	53	20.42±15.37	1.11	33.33	13.74±9.18	2.2	6.2	4.2±1.1
Asia	0	8	1.38±2.22	0	58	22.44±17.63	0	35.56	14.01±11.21	1.4	7	4.5±2.0
Serbia	0	22	4.18±5.49	4	57	28.77±15.68	3.33	46.11	20.63±12.14	1.4	6.8	3.7±1.3
Europe	0	27	3.81±6.15	0	59	23.45±19.26	0	47.22	17.27±13.60	2.3	7	4.2±1.2
North and South America	0	41	4.28±8.47	2	52	23.03±14.02	1.11	73.33	17.55±14.52	2.1	7	4.6±1.3
Total	0	41	3.57±6.32	0	59	24.7±16.53	0	73.33	17.69±13.08	2.1	7	4.5±1.3

Table 6: G1978, G1998 and G2008 in geographical groups, varietal groups, growth habit groups and total accessions of the genebank collection 1974 harvest with standard deviations. N: number of accessions tested

		G1978				G1998			G2008		
		N	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Geographical location	Asia	45	57	98	84.15±9.2	22	100	85.89±16.2	0	93	56.66±22.7
	South Europe	58	61	99	86.33±10.0	73	100	94.11±5.5	9	90	59.89±22.7
	Rest of Europe	41	70	99	88.65±8.2	30	100	91.90±12.9	0	90	52.24±23.4
	North and South America	39	63	99	90.15±7.4	37	100	90.92±13.9	0	94	55.05±25.5
Species	*T. aestivum* L. var. *aestivum*	60	63	99	88.85±8.6	70	100	94.33±5.4	0	94	55.08±26.9
	T. aestivum L. var. *lutescens*	40	61	99	89.20±9.0	30	100	90.11±14.3	0	87	55.43±20.9
	T. aestivum L. var. *ferrugineum*	24	66	97	85.66±8.7	44	100	92.71±12.3	4	92	57.6±19.91
	'Others'	67	57	99	84.86±9.4	22	100	88.46±15.0	0	93	57.59±22.5
Growth Habit	Spring Wheat	129	57	99	85.96±8.7	22	100	91.845±12.1	0	94	60.26±21.8
	Winter Wheat	54	61	99	89.90±9.5	30	100	89.55±13.1	0	88	47.01±24.1
	Total	183	57	99	87.13±9.1	22	100	91.12±12.4	0	94	56.45±23.31

G1978 = germination in 1978, G1998 = germination in 1998 and G2008 = germination in 2008

Table 7: ANOVA between G1978 and G2008 germination of genebank collection at α=0.01

ANOVA   (α = 0.01)

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	85897.4	1	85897.4	274.19	0.000	6.70
Within Groups	114030.8	364	313.27			
Total	199928.2	365				

Table 8: ANOVA between G1998 and G2008 germination of genebank collection at α=0.01

ANOVA   (α = 0.01)

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	106738.5	1	106738.5	301.24	0.000	6.70
Within Groups	125432.7	423	354.33			
Total	232171.20	355				

Table 9: ANOVA among G2008 of geographical groups (Asia, South Europe, rest of Europe and North and South American accessions) of genebank collection at α = 0.01

ANOVA   (α = 0.01)

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1491.07	3	497.02	0.918	0.43	3.89
Within Groups	96844.84	179	541.03			
Total	98335.91	182				

Table 10: ANOVA among G2008 of varietal groups (*aestivum*, *lutescens*, *ferrugineum* and 'others') of genebank collection at α = 0.01

ANOVA   (α = 0.01)

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	258.84	3	86.28	0.15	0.92	3.89
Within Groups	98077.07	179	547.91			
Total	98335.91	182				

Table 11: ANOVA for G2008 between spring and winter wheat of genebank collection at α = 0.01

ANOVA   (α = 0.01)

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	6677.89	1	6677.89	13.18	0.001	6.77
Within Groups	91658.02	181	506.39			
Total	98335.91	182				

Table 12: IG, GAA, RGAA, GCD and RGCD of Q-groups, geographical groups, varietal groups, growth habit groups and all accessions of genebank collection 2010 harvest with standard deviations

		N	Initial Germination (%) (IG)			Germination after AA (%) (GAA)			Relative germination after AA (%) (RGAA)			Germination after CD (%) (GCD)			Relative germination after CD (%) (GCD)		
			Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
STRUCTURE	Q1	30	53	99	83.96±10.9	15.67	92	58.26±20.0	20.35	99.58	69.51±22.0	5	78.33	45.84±25.5	5.49	89.47	55.04±29.7
	Q2	46	69	98	89.02±7.10	14.33	90.67	64.90±18.2	15.75	96.67	72.88±19.5	6.67	96	62.73±22.5	8.33	100	69.74±22.9
	Q3	19	66	97	90.83±7.7	54	94.33	79.01±11.8	62.79	98.26	86.89±10.5	23.33	91	68.32±21.0	27.13	94.79	74.89±20.9
	Q4	36	45	96	85.22±12.6	19.67	91.67	71.87±16.7	38.56	97.52	82.29±14.4	22.67	87.33	62.34±18.0	29.96	94.22	72.34±15.6
	Q5	17	64	96	83.23±9.7	37.67	92	68.58±15.0	54.29	98.92	81.91±13.6	29.33	96	58.53±18.4	37.13	98.47	69.96±19.0
	Q6	8	26	96	64.87±22.7	24.33	85.67	53.70±23.2	44.97	95.24	82.67±15.9	7.37	84	41.75±24.9	28.36	87.5	59.05±20.1
	Mixed	21	60	99	87.49±10.5	2.67	94.67	63.22±26.4	3.55	98.61	71.69±27.5	22.67	89.33	60.06±21.6	23.86	97.10	67.58±20.4
Gerographical origin	Asia	43	53	98	84.86±9.2	15.67	92	62.17±18.6	20.35	99.58	73.23±20.4	5	89.33	48.16±25.2	5.49	98.47	56.97±28.9
	South Europe	55	26	97	86.37±12.9	19.66	94.33	72.71±17.0	38.56	98.26	83.58±13.7	7.37	91	65.11±19.8	15.29	94.92	74.23±17.7
	Rest of Europe	42	49	99	86.57±12.4	14.33	92	66.90±18.8	15.75	96.45	77.19±17.7	18.33	96	63.29±20.4	20.37	100	72.15±18.1
	North and South America	37	45	99	84.97±12.3	2.67	94.67	60.87±23.1	3.55	98.61	70.48±24.7	6.66	87.92	56.13±22.0	8.33	94.07	64.91±21.0
Varieties	Var. *aestivum*	54	45	99	87.90±10.1	27.66	92.66	72.36±14.5	28.52	97.54	81.68±15.0	6.66	91	61.78±20.1	8.33	94.79	69.68±20.1
	Var. *lutescens*	38	26	98	80.78±14.8	2.66	90.66	57.21±21.4	3.55	96.66	70.35±21.7	7.37	88.33	50.54±22.1	14.11	92.01	61.42±21.5
	var. *ferrugineum*	20	77	99	90.95±5.7	15.67	94.66	76.56±18.4	20.35	98.61	83.57±18.5	7.33	96	69.78±19.6	9.52	100	76.08±20.3
	Var. 'Others'	65	49	98	85.29±11.5	14.33	93	63.58±20.0	15.75	99.58	74.69±20.5	5	89.67	57.36±24.3	5.49	98.47	66.80±25.2
Growth habit	Spring	128	26	99	84.68±12.6	19.66	94.66	66.70±18.9	23.44	99.58	78.27±18.1	5	96	57.61±21.8	5.49	100	67.27±21.7
	Winter	49	53	98	88.57±8.7	2.66	92.66	65.36±21.6	3.55	97.54	73.24±22.6	6.66	91	61.52±24.8	8.33	97.10	68.47±25.1
	Total	177	26	99	85.75±11.8	2.67	94.67	66.32±19.6	3.56	99.58	76.87±19.5	5	96	58.68±22.7	5.49	100	67.60±22.6

Table 13: ANOVA for GAA among Q groups of genebank collection at α = 0.01

ANOVA α = 0.01

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	7742.8	6	1290.47	3.64	0.001	2.91
Within Groups	59766.4	169	353.64			
Total	67509.2	175				

Table 14: ANOVA for GAA among variety groups of genebank collection at α = 0.01

ANOVA α = 0.01

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	7624.39	3	2558.13	7.33	0.000	2.65
Within Groups	59834.82	172	347.87			
Total	67509.21	175				

Table 15: ANOVA for RGAA among Q groups of genebank collection at α = 0.01

ANOVA α = 0.01

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	6555.28	6	1092.55	3.08	0.006	2.91
Within Groups	59802.75	169	353.86			
Total	66358.03	175				

Table 16: ANOVA for RGAA among geographical groups of genebank collection at α = 0.01

ANOVA α = 0.01

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	4503.37	3	1501.12	4.17	0.006	3.89
Within Groups	61854.66	172	359.62			
Total	66358.03	175				

Table 17: ANOVA for GCD among Q groups of genebank collection at α = 0.01

ANOVA α = 0.01

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	10260.44	6	1710.74	3.62	0.002	2.9
Within Groups	78673.31	167	471.09			
Total	88933.75	173				

Table 18: ANOVA for GCD among various geographical groups of genebank collection at α = 0.01

ANOVA α = 0.01

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	8123.5	3	2707.83	5.69	0.000	3.90
Within Groups	80810.3	170	475.35			
Total	88933.8	173				

Table 19: ANOVA for RGCD among geographical groups of genebank collection at α = 0.01

ANOVA $\qquad$ α = 0.01

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	8379.25	3	2793.08	5.92	0.000	3.89
Within Groups	80205.75	170	471.80			
Total	88585	173				

Table 20: D10, D20, DI and PHS of Q-groups, geographical groups, varietal groups, growth habit groups and all accessions of genebank collection 2010 harvest with standard deviations

		Dormant seeds % at 10°C (D10)			Dormant seeds % at 20°C (D20)			Dormancy Index (DI)			Pre-harvest sprouting (PHS)		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
STRUCTURE	Q1	0	50	9.41±13.2	0	100	57.55±31.2	0	61.66	24.96±16.0	2.1	6.9	5.0±1.4
	Q2	0	95	10.25±18.0	3.33	100	66.64±33.2	1.11	95.55	29.04±19.0	1.2	6.7	4.5±1.5
	Q3	0	36.66	5±10.1	5	100	78.60±25.8	1.66	54.54	29.53±12.1	2.2	5.4	3.7±1.0
	Q4	0	85	12.95±18.1	31.66	100	90.65±13.5	12.77	90	38.85±13.5	1.4	6.4	3.3±1.3
	Q5	0	45	17.73±13.5	10	100	89.08±23.7	3.33	62.77	41.51±13.6	1.8	6.0	3.3±1.0
	Q6	0	73.33	31.14±29.0	81.67	100	92.60±7.0	28.33	82.22	51.63±20.5	1.7	4.8	3.1±1.3
	Mixed	0	53.33	12.04±15.9	18.33	100	75.69±28.3	7.22	68.33	33.26±17.6	1.2	6.6	3.9±1.6
Geographical origin	Asia	0	50	10.57±12.4	0	100	62.09±29.7	0	61.66	27.39±15.4	1.6	6.9	4.5±1.5
	South Europe	0	95	14.06±21.3	5	100	87.87±18.6	1.66	95.55	38.67±16.8	1.2	6.1	3.6±1.2
	Rest of Europe	0	68.33	10.48±15.6	3.33	100	69.80±34.6	1.11	77.77	30.25±18.46	1.5	6.7	4.2±1.5
	North and South America	0	85	12.17±16.9	13.33	100	80.07±26.7	6.66	90	34.80±16.8	1.2	6.6	3.9±1.5
Varieties	var. *aestivum*	0	85	8.39±14.3	10	100	78.93±23.4	3.33	90	31.90±13.7	1.5	6.6	4.0±1.3
	var. *lutescens*	0	73.33	17.09±17.9	13.63	100	87.40±22.9	4.54	82.22	40.53±16.2	1.4	6	3.5±1.3
	var. *ferrugineum*	0	66.66	8.82±17.3	0	100	64.47±39.6	0	77.22	27.37±20.0	1.4	6.9	4.3±1.6
	var. 'Others'	0	95	12.85±18.2	3.33	100	69.60±30.5	1.11	95.55	31.54±18.9	1.2	6.9	4.3±1.6
Growth habit	Spring	0	95	13.99±18.1	5	100	78.50±26.9	1.66	95.55	35.49±17.1	1.2	6.9	3.9±1.4
	Winter	0	50	6.73±13.1	0	100	68.33±33.1	0	66.66	26.95±16.6	1.2	6.9	4.3±1.5
	Total	0	95	11.97±17.1	0	100	75.67±29.1	0	95.55	33.11±17.3	1.2	6.9	4.0±1.5

Table 21: Hd (heading time), Ft (flowering time), Ht (height) and TKW (thousand grain weight) of Q-groups, geographical groups, varietal groups, growth habit groups and all accessions of genebank collection 2010 harvest with standard deviations

		Heading time (Hd)			Flowering time (Ft)			Height (Ht) in cm			Thousand grain weight (TKW) in grams		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
STRUCTURE	Q1	117	141	128.96±6.2	127	146	136.63±5.8	108.25	167.75	137.94±16.2	29.19	61.09	47.21±7.6
	Q2	123	150	136.76±5.8	124	155	142.60±6.1	78.50	179.50	132.15±25.4	34.01	51.50	42.60±4.4
	Q3	124	139	130.05±4.5	131	146	138.79±4.3	100.50	172.50	137.71±17.7	40.22	55.61	49.08±4.6
	Q4	109	136	128.30±4.2	119	142	135.83±4.5	57.25	156.50	129.72±22.7	34.75	57.12	45.37±6.1
	Q5	124	133	129.53±2.2	129	141	137.41±3.5	75.50	153.75	130.39±16.9	33.52	53.44	42.62±5.7
	Q6	121	128	124.25±2.6	129	138	131.75±3.7	115.25	127.50	121.12±4.7	45.20	53.17	48.33±2.7
	'Mixed'	117	138	128.85±5.8	126	144	136.57±5.9	51.50	158.50	126.55±28.3	33.47	55.01	44.42±7.1
Gerographical origin	Asia	117	141	129.46±5.3	127	147	137.44±5.2	57.25	167.75	133.33±21.0	29.19	61.09	46.37±7.1
	South Europe	121	139	128.69±4.1	126	146	136.30±4.6	81.50	172.50	131.58±16.3	34.75	57.12	47.78±5.5
	Rest of Europe	121	150	135.40±6.7	124	155	141.70±6.7	84.25	179.50	135.63±24.0	33.84	52.61	43.55±4.5
	North and South America	109	141	130.27±6.8	119	147	137.43±6.1	51.50	159.25	126.48±27.0	33.47	51.72	41.37±5.3
Varieties	var. *aestivum*	109	141	129.68±6.2	119	147	137.35±5.7	51.5	172.50	128.48±27.2	33.84	55.61	45.69±5.6
	var. *lutescens*	117	144	131.36±6.0	127	150	138.23±5.8	75.50	174.00	124.01±22.5	33.47	52.61	43.02±5.9
	var. *ferrugineum*	123	141	130.45±4.2	131	146	138.00±4.2	118.5	164.00	144.29±11.6	35.14	54.98	44.48±6.4
	var. 'Others'	121	150	131.50±6.8	124	155	138.67±6.7	93.50	179.50	135.54±16.4	29.19	61.09	46.09±6.5
Growth habit	Spring	109	140	128.84±5.1	119	148	136.54±5.2	51.50	167.75	131.00±21.1	33.47	61.09	45.44±6.2
	Winter	124	150	135.91±6.2	124	155	142.16±5.9	84.25	179.50	134.29±23.9	29.19	55.61	44.35±6.0
	Total	109	150	130.80±6.2	119	155	138.10±5.9	51.50	179.50	131.90±21.9	29.19	61.09	45.14±6.2

Table 22: MTAs significant at p < 0.05 (X) or p < 0.01 (**X***) for seed longevity after long term seed storage i.e., 2008 (brown), AA (red) and CD (blue) and for PHS (pink), Dor (green), Hd (grey), Ft (navy blue), Ht (black), TKW (purple), Pub (light brown) and Awn (orange) detected in the genebank collection

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt0497	1A							X				
wPt1011	1A									**X***		
wPt1426	1A						X					
wPt1709	1A					**X***						
wPt1786	1A|1B	**X***									X	
wPt1906	1A			**X***		**X***						
wPt1924	1A		X									
wPt3198	1A					X						
wPt3347	1A				**X***							
wPt3560	1A										X	
wPt3836	1A|7A			X						X		
wPt4177	1A											X
wPt4408	1A						X		X			
wPt4676	1A											**X***
wPt5077	1A										X	
wPt5367	1A								X			
wPt5411	1A	**X***							X			X
wPt5660	1A						X		X			
wPt6280	1A								X			
wPt6654	1A									X	X	
wPt6853	1A									**X***		

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt7147	1A									X		
wPt7215	1A|1D	X*										
wPt7339	1A			X*			X	X				
wPt7951	1A						X*	X*				
wPt8172	1A										X	
wPt8347	1A								X			X
wPt9429	1A											X
wPt9938	1A						X		X			
wPt9985	1A										X	
tPt5413	1A		X		X							
tPt6091	1A|1B	X*									X*	
wPt0308	1B					X*						
wPt0441	1B							X				
wPt1139	1B											X
wPt1248	1B										X	
wPt1573	1B										X	
wPt1726	1B							X				
wPt1782	1B				X	X	X*	X				
wPt2019	1B										X	
wPt2744	1B										X	
wPt2861	1B		X*			X						X
wPt3103	1B										X	
wPt4325	1B										X	
wPt4726	1B				X				X	X*		

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt5006	1B					X						
wPt5313	1B|3D											X
wPt5385	1B										X	
wPt5485	1B										X	
wPt6012	1B|3A						X				X	
wPt6370	1B									X		X*
wPt7066	1B						X					
wPt7242	1B										X	
wPt8280	1B									X		
wPt8320	1B	X										
wPt9028	1B		X	X								
wPt9490	1B									X		X
wPt9524	1B	X										
wPt9605	1B	X		X								
rPt4471	1B|1D										X	
rPt7906	1B											X
tPt0283	1B	X										
tPt5249	1B			X								
tPt7980	1B		X*									
tPt8929	1B										X	
wPt1685	1D								X			
wPt2206	1D											X
wPt2839	1D								X*			
wPt3855	1D											X

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt4647	1D		X									
wPt4711	1D					X						
wPt4942	1D				X							
wPt4971	1D		X									
wPt5320	1D	X										
wPt6503	1D					X						
wPt7057	1D					**X***			**X***			
wPt7437	1D|6B	X										
wPt7491	1D					X						
wPt0115	2A			**X***	**X***							
wPt0553	2A			**X***		X						X
wPt0568	2A			X					X			
wPt1480	2A			X								
wPt1722	2A						X					
wPt2435	2A				X				X			
wPt3244	2A											X
wPt3976	2A			X	X	X	**X***	**X***				
wPt4450	2A			X		X						
wPt5029	2A					X						X
wPt5251	2A									**X***		X
wPt5498	2A											X
wPt6207	2A				**X***							
wPt6711	2A			X					X			
wPt7187	2A					X						

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt8068	2A			X					X			
wPt8490	2A								X		X	
wPt8596	2A											X
wPt8826	2A			X				X				
wPt9320	2A	X	X									X*
tPt9405	2A								X			
tPt5584	2A			X		X						
wPt1499	2A|2D						X					
wPt6064	2A|2D	X*										
wPt0471	2B		X									
wPt0697	2B						X		X*			
wPt0906	2B			X								
wPt0948	2B									X*		
wPt1919	2B											X
wPt1024	2B								X			
wPt1140	2B								X*			
wPt2274	2B											X*
wPt2430	2B			X								X
wPt2327	2B									X*		
wPt3272	2B								X			
wPt3592	2B											X
wPt3807	2B											X
wPt3949	2B						X*	X*				
wPt4166	2B	X										

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Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt4199	2B						X					
wPt4350	2B											X
wPt4368	2B					X			X			
wPt4664	2B									X		
wPt4889	2B									X		
wPt5250	2B		X*	X*						X*		
wPt5878	2B						X					
wPt5934	2B											X
wPt5960	2B	X										
wPt5736	2B								X	X		
wPt5989	2B									X		
wPt6174	2B	X	X*	X*					X			
wPt6120	2B											X
wPt6242	2B				X							
wPt6271	2B					X						
wPt6471	2B	X*	X*									
wPt6627	2B											X
wPt6575	2B											X
wPt6970	2B											X
wPt7312	2B											X
wPt7320	2B					X						
wPt7506	2B									X*		
wPt7672	2B									X		X
wPt8263	2B											X

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Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt8349	2B									X		
wPt8424	2B											X
wPt8521	2B											X
wPt8548	2B	X	X	X*					X			
wPt8629	2B				X							
wPt8693	2B	X*										
wPt8737	2B											X
wPt9230	2B											X
wPt9257	2B						X		X			
rPt6122	2B								X	X	X	X
tPt6504	2B			X								
tPt7321	2B	X										
wPt0079	2B|4A				X*	X*	X		X			
wPt1964	2B|4D			X*								
tPt4125	2B|4B									X*		
tPt4627	2B|4D			X*								
wPt3995	2B|5B										X	
wPt0153	2D									X*		
wPt3757	2D						X					
wPt6780	2D									X		
wPt6850	2D	X										
wPt7466	2D											X
wPt0286	3A				X*				X*			
wPt0398	3A			X*								

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt1694	3A	X				X			X			
wPt1923	3A		X								X	X
wPt2144	3A											X
wPt2478	3A								X			
wPt2942	3A											X
wPt3278	3A	X*					X	X				
wPt4128	3A											X
wPt4398	3A	X							X			
wPt5133	3A				X		X	X				
wPt5173	3A		X									
wPt7340	3A|3B		X				X	X*				
wPt8146	3A											X*
wPt8876	3A					X*						
wPt9160	3A			X*								
tPt7492	3A						X	X				
tPt9901	3A											X
wPt0013	3B			X						X		
wPt0264	3B	X										
wPt0327	3B						X					
wPt0644	3B				X							
wPt0895	3B			X*						X		
wPt0912	3B					X						
wPt1158	3B						X*	X*				
wPt1171	3B		X									

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt1306	3B	X										
wPt2091	3B			X*	X*		X*	X*	X*			
wPt2193	3B			X*						X		
wPt2202	3B			X*		X*						
wPt2298	3B			X*						X		
wPt2416	3B		X				X					
wPt2557	3B							X				
wPt2766	3B			X*						X		
wPt3260	3B	X									X	
wPt3609	3B											X
wPt4048	3B		X	X*	X							
wPt4608	3B			X	X*		X*	X*	X*			
wPt4842	3B											X
wPt4974	3B							X				
wPt5522	3B	X										
wPt5786	3B			X*								
wPt5836	3B			X*		X*	X	X		X*		
wPt5939	3B			X								
wPt6020	3B		X	X*	X							
wPt6132	3B	X										
wPt6961	3B	X	X*									
wPt7152	3B			X*								
wPt7486	3B		X									
wPt7688	3B	X							X			

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt8056	3B	X			X	X*						
wPt8141	3B										X*	
wPt8356	3B|3D			X								
wPt8446	3B	X										
wPt8915	3B						X					
wPt9368	3B					X						
wPt10005	3B								X			
wPt10176	3B						X	X*	X*			
wPt10291	3B|3D						X				X	
wPt10323	3B				X	X	X	X				
wPt10462	3B			X					X			
wPt10945	3B			X*				X	X*			
wPt10948	3B									X		
wPt10991	3B	X										
wPt11295	3B				X	X*						
rPt5396	3B						X					
tPt1366	3B				X	X*	X*	X*				
tPt4541	3B	X	X*									
tPt5281	3B			X						X		
tPt6487	3B								X			
tPt9267	3B	X*										
wPt2367	3D											X*
wPt3134	3D											X*
wPt4292	3D	X										

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt4476	3D	X										
wPt0023	4A				X			X				
wPt0162	4A					X						
wPt0447	4A				X*			X*				
wPt0763	4A					X			X			
wPt0764	4A							X	X			
wPt1007	4A										X*	
wPt1161	4A										X	
wPt1743	4A								X			
wPt2903	4A					X	X*	X*	X*			
wPt2985	4A						X	X	X*			
wPt3515	4A					X	X*	X	X*			
wPt4828	4A|7B	X*								X		
wPt5428	4A	X										
wPt5578	4A										X*	
wPt5857	4A				X							
wPt6502	4A								X*			
wPt6757	4A	X*										
wPt6867	4A				X	X		X*				
wPt7096	4A|7A		X									
wPt7327	4A		X									
wPt7612	4A					X*			X			
wPt7876	4A								X*			
wPt8144	4A								X			

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt9445	4A				X					X		
wPt9641	4A|7A					X						
wPt9738	4A									X		
rPt0238	4A									X		
rPt7987	4A|7A								X		X	
wPt1101	4B					X						
wPt3608	4B											X
wPt3917	4B										X	
wPt4280	4B										X	
wPt4607	4B			**X***								
wPt4962	4B							X				
wPt6016	4B					X						
wPt8796	4B											**X***
wPt8892	4B|6B						X	X		X	X	
tPt4214	4B	X										X
wPt3563	5A								X			
wPt5096	5A	X										
wPt5421	5A										X	
wPt7185	5A					X						
wPt7769	5A				**X***							
wPt9094	5A	X										
tPt2429	5A											**X***
tPt4184	5A				X	X						
tPt6495	5A				X	X						

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt0312	5B|7B					X						
wPt0927	5B|5D								X			
wPt1304	5B									**X***		
wPt1409	5B											X
wPt2607	5B	X							X			
wPt2992	5B|7A									X		
wPt3085	5B										X	
wPt4418	5B			X								
wPt4551	5B								X	**X***		
wPt4736	5B	**X***	X				X					
wPt4900	5B|6B									**X***		
wPt5118	5B								**X***			
wPt5514	5B					**X***						
wPt5792	5B				X							
wPt6191	5B			**X***								
wPt6465	5B	X										
wPt7006	5B									X		
wPt7237	5B					X						
wPt7238	5B					X						X
wPt7400	5B								X	**X***	X	
wPt8449	5B									X		
wPt9724	5B				X							
tPt3144	5B										X	
tPt8942	5B	**X***										

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt0596	5D				X			X*			X*	
wPt2256	5D								X*			
wPt5870	5D										X	
wPt7323	5D	X										
wPt0139	6A											X
wPt1981	6A					X					X	
wPt2632	6A						X					
wPt2822	6A					X						X
wPt3308	6A					X						
wPt5696	6A					X				X		
wPt5964	6A		X									
wPt7548	6A			X*								
wPt7572	6A				X							
wPt8266	6A				X*	X				X		
wPt9113	6A	X						X				
wPt9132	6A											X
wPt9584	6A					X						X
rPt6189	6A			X*								
rPt9324	6A			X								
tPt3786	6A			X								
tPt8557	6A			X						X		
wPt1307	6B					X*						
wPt1756	6B										X	
wPt1761	6B									X	X*	

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Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt2000	6B											X*
wPt2537	6B		X						X			
wPt2564	6B								X			
wPt3168	6B							X	X	X*		
wPt3203	6B					X	X*	X	X			
wPt3207	6B	X*										
wPt3284	6B					X*	X	X	X*			
wPt4119	6B			X	X*	X						
wPt4218	6B										X	
wPt4648	6B								X			
wPt4742	6B									X		
wPt6039	6B				X				X			
wPt6585	6B				X							
wPt6674	6B								X			
wPt7489	6B						X					X
wPt7540	6B									X		
wPt7576	6B					X						
wPt7642	6B|7D			X*						X		
wPt7935	6B									X		
wPt8560	6B											X*
wPt8721	6B		X									
wPt8894	6B	X				X						X
wPt9195	6B	X		X								
wPt9952	6B	X										

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
rPt1040	6B									X		
tPt8161	6B				X				X		X	
tPt4887	6B					X						
tPt3506	6B					X						
wPt2864	6D						X		X*		X	
wPt3879	6D			X			X*	X	X*			
wPt5114	6D											X
wPt0275	7A									X*		
wPt0303	7A|7D					X			X			
wPt0514	7A	X								X		
wPt0961	7A		X									X*
wPt1706	7A											X*
wPt3425	7A										X*	X
wPt3782	7A						X					
wPt4172	7A									X		
wPt4515	7A					X					X*	
wPt4553	7A		X									X*
wPt4877	7A					X						
wPt4960	7A					X				X*		
wPt5524	7A											X
wPt5558	7A						X					
wPt6013	7A						X					
wPt6273	7A									X		
wPt6417	7A											X

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt6768	7A				X		X		X			
wPt6959	7A	X*										
wPt6967	7A					X				X		
wPt7113	7A|7B									X*		
wPt7122	7A						X	X				
wPt8043	7A					X				X		
wPt8149	7A					X				X		
wPt9207	7A					X*			X*	X		
tPt6221	7A			X								
wPt1196	7B								X*			
wPt2407	7B									X		
wPt2572	7B					X						
wPt2883	7B	X		X								X
wPt2933	7B	X										
wPt3004	7B			X*								
wPt3447	7B	X				X						
wPt3676	7B					X*						
wPt3785	7B									X		
wPt4025	7B							X	X	X		
wPt4045	7B									X		
wPt4120	7B					X						
wPt4309	7B				X	X*	X		X*		X	
wPt5283	7B	X										
wPt5462	7B							X				

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt6936	7B					X	X		X		X	
wPt7080	7B					X						
wPt7925	7B			X*		X*					X	
wPt8211	7B										X*	
wPt8246	7B							X				
wPt8803	7B	X										
wPt8938	7B			X					X			
wPt9515	7B						X					
wPt9877	7B						X					
tPt9518	7B			X								
tPt8504	7B						X					
wPt0231	7D	X										
wPt0366	7D										X	
wPt0789	7D				X		X*	X*	X*			
wPt0934	7D					X			X			
wPt1859	7D	X										
wPt2258	7D			X*						X		
wPt3268	7D	X					X		X			
wPt3359	7D					X						
wPt4315	7D											X*
wPt5049	7D					X*						
wPt7508	7D	X										
wPt1557	NA		X	X								
wPt4394	NA		X*									

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt6054	NA				X	X	X	X				
wPt7812	NA											
wPt663751	NA							X				
wPt663792	NA										X	
wPt663840	NA							X				
wPt663849	NA					X*	X					
wPt663989	NA					X*						
wPt663992	NA					X*	X					
wPt664020	NA					X*						
wPt664047	NA					X*						
wPt664193	NA				X			X				
wPt664250	NA		X*									
wPt664251	NA											X*
wPt664276	NA											X
wPt664286	NA					X*	X					
wPt664290	NA			X*						X		
wPt664309	NA					X*						
wPt664317	NA					X*						
wPt664393	NA				X	X*	X*	X*				
wPt664400	NA	X										
wPt664412	NA					X*				X		
wPt664438	NA			X			X		X*			
wPt664488	NA									X		
wPt664517	NA	X		X								X

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt664589	NA	X										
wPt664593	NA	X*			X						X	
wPt664666	NA										X*	
wPt664703	NA	X									X*	
wPt664733	NA											X*
wPt664735	NA			X								
wPt664745	NA				X							
wPt664746	NA										X	
wPt664778	NA											X*
wPt664788	NA											X
wPt664888	NA					X						X*
wPt664937	NA						X*					
wPt664968	NA										X*	
wPt664971	NA					X*						
wPt664972	NA					X*						
wPt665027	NA						X*	X*				
wPt665112	NA	X		X								X
wPt665260	NA		X			X*						
wPt665293	NA						X					
wPt665304	NA											X
wPt665342	NA		X									
wPt665360	NA								X			
wPt665398	NA				X*						X	
wPt665545	NA											X*

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt665550	NA									X		
wPt665613	NA			X*		X*						
wPt665687	NA	X										
wPt665724	NA								X			
wPt665749	NA								X			
wPt665757	NA									X		
wPt665784	NA			X*		X*						
wPt665868	NA					X						
wPt665927	NA					X		X		X		
wPt665936	NA	X										
wPt666095	NA	X										
wPt666104	NA					X*						
wPt666142	NA	X		X								
wPt666215	NA								X	X	X	
wPt666424	NA				X*					X		
wPt666436	NA											X
wPt666456	NA										X	
wPt666464	NA	X										
wPt666518	NA									X		
wPt666564	NA					X						
wPt666616	NA				X							
wPt666676	NA											X*
wPt666724	NA									X		
wPt666728	NA								X			

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt666738	NA						X*				X*	
wPt666776	NA											X*
wPt666850	NA		X									
wPt666931	NA						X	X*				
wPt666963	NA								X			
wPt666987	NA				X							
wPt666988	NA											X
wPt667005	NA						X	X	X*		X*	
wPt667054	NA											X*
wPt667058	NA				X			X				
wPt667083	NA	X										
wPt667155	NA											X*
wPt667153	NA	X*										X
wPt667162	NA	X										
wPt667170	NA											X*
wPt667172	NA						X	X				X
wPt667180	NA											X
wPt667266	NA								X			
wPt667294	NA				X							
wPt667312	NA				X							
wPt667324	NA		X									
wPt667328	NA					X					X	
wPt667413	NA						X*	X*				
wPt667430	NA											X*

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt667472	NA											X
wPt667485	NA	X*					X		X			
wPt667562	NA								X			
wPt667593	NA			X								
wPt667662	NA						X					
wPt667740	NA					X						
wPt667765	NA									X*		
wPt667771	NA	X										
wPt667780	NA											X
wPt667817	NA					X				X		
wPt667978	NA					X			X			
wPt668079	NA								X			
wPt668205	NA										X	
wPt668214	NA	X*		X			X	X*	X*			
wPt668236	NA						X	X				X
wPt668307	NA								X		X	
wPt669103	NA								X			
wPt669154	NA					X*						
wPt669199	NA						X		X*			
wPt669203	NA		X									
wPt669294	NA							X				
wPt669315	NA				X							
wPt669328	NA						X					
wPt669385	NA										X	

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt669484	NA		X									X
wPt669517	NA							X	X			
wPt669526	NA										X*	
wPt669693	NA						X		X			
wPt669755	NA									X		
wPt671558	NA			X		X*	X		X	X		
wPt671561	NA	X										
wPt671568	NA							X				
wPt671650	NA								X			
wPt671698	NA									X		
wPt671701	NA											X*
wPt671707	NA					X*						
wPt671708	NA				X	X*						
wPt671711	NA				X							
wPt671740	NA	X										
wPt671742	NA						X		X*			
wPt671790	NA											X*
wPt671798	NA						X	X				
wPt671801	NA	X		X	X				X			X
wPt671808	NA					X					X	
wPt671823	NA						X					
wPt671947	NA								X			
wPt671956	NA	X		X	X				X			
wPt671987	NA	X					X					

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt672030	NA			X*								
wPt672034	NA			X*							X	
wPt729839	NA			X*								
wPt729904	NA								X			
wPt729920	NA	X										
wPt729972	NA				X*					X		
wPt730136	NA				X*					X		
wPt730148	NA	X*					X	X*	X*			
wPt730208	NA				X		X	X				
wPt730303	NA		X	X*						X	X	
wPt730338	NA									X		
wPt730356	NA			X				X	X			
wPt730368	NA					X						
wPt730427	NA			X								
wPt730475	NA					X						
wPt730591	NA					X						
wPt730605	NA					X						
wPt730613	NA								X*			
wPt730651	NA											X*
wPt730718	NA					X*			X	X		
wPt730729	NA					X						X
wPt730794	NA											X*
wPt730879	NA					X		X				
wPt730885	NA						X		X			

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt730913	NA					X*			X*			
wPt731002	NA								X			
wPt731010	NA											X
wPt731020	NA					X			X*			
wPt731130	NA	X		X					X			
wPt731250	NA											X*
wPt731282	NA										X*	
wPt731336	NA	X*										
wPt731374	NA								X		X*	
wPt731409	NA				X							
wPt731413	NA					X						
wPt731445	NA									X*	X	X
wPt731512	NA		X									
wPt731617	NA			X*	X	X*						
wPt731699	NA					X	X					
wPt731806	NA	X*		X			X	X*	X*			
wPt731807	NA	X*					X	X*	X*			
wPt731869	NA		X	X								
wPt731910	NA								X			
wPt731941	NA				X		X	X	X*			
wPt732040	NA		X*						X			
wPt732044	NA				X	X*	X*	X*				
wPt732061	NA	X										
wPt732260	NA								X			

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt732263	NA									X		
wPt732270	NA				X				X			
wPt732330	NA			X						X*		
wPt732377	NA										X	
wPt732386	NA									X*		
wPt732448	NA										X*	
wPt732504	NA											X
wPt732520	NA					X						
wPt732546	NA	X*		X			X	X*	X*			
wPt732575	NA					X			X*			
wPt732602	NA		X									
wPt732603	NA				X		X	X	X*			
wPt732760	NA											X
wPt732881	NA										X	
wPt732923	NA	X*					X					
wPt732942	NA									X*		
wPt732946	NA			X*		X*						
wPt733007	NA	X*					X	X*	X*			
wPt733038	NA											X
wPt733087	NA				X	X*	X*	X*	X*			
wPt733104	NA		X									
wPt733151	NA				X	X						
wPt733267	NA											X*
wPt733322	NA								X		X	

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt733357	NA					X						
wPt733363	NA										X*	
wPt733374	NA											X
wPt733461	NA					X						
wPt733464	NA	X*		X			X	X*	X*			
wPt733477	NA					X						
wPt733544	NA			X*						X		
wPt733567	NA				X							
wPt733572	NA	X*		X*			X		X			
wPt733649	NA										X	
wPt733725	NA	X*		X					X			
wPt733764	NA											X*
wPt733779	NA	X*					X*	X*	X*			
wPt733811	NA						X		X			
wPt733858	NA							X	X			
wPt733882	NA				X							X
wPt733904	NA						X		X			
wPt734004	NA	X										
wPt734027	NA											X
wPt734054	NA								X			
wPt734074	NA									X*		
wPt734078	NA			X								
wPt734081	NA											X
wPt734107	NA											X

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt734132	NA					X						
wPt734140	NA										X	
wPt734141	NA		X*									
wPt734145	NA									X*		
wPt734161	NA							X				
wPt734169	NA	X				X						
wPt734202	NA				X							
wPt734216	NA	X*		X			X	X*	X*			
wPt734288	NA										X	
wPt734301	NA			X*		X*						
wPt734310	NA										X*	
wPt734314	NA	X										X
wPt734315	NA		X									
wPt740544	NA				X							
wPt740564	NA	X										
wPt740584	NA	X*										
wPt740658	NA								X			
wPt740739	NA										X	
wPt740822	NA		X									
wPt740854	NA		X									
wPt740860	NA	X			X	X				X*		
wPt740897	NA							X				
wPt740957	NA											X*
wPt741026	NA								X			

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt741128	NA	X										
wPt741164	NA					X						
wPt741170	NA	X										
wPt741172	NA		X									
wPt741189	NA											X*
wPt741192	NA		X*									
wPt741202	NA											X*
wPt741208	NA	X*										
wPt741216	NA											X
wPt741230	NA											X*
wPt741302	NA					X			X			
wPt741331	NA											X*
wPt741357	NA											X*
wPt741440	NA											X*
wPt741495	NA											X
wPt741529	NA											X*
wPt741547	NA			X								
wPt741598	NA											X*
wPt741630	NA								X			
wPt741683	NA											X*
wPt741685	NA											X*
wPt741686	NA										X*	
wPt741733	NA											X
wPt741750	NA		X									

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt741812	NA		X*									
wPt741816	NA				X							
wPt741848	NA				X							
wPt741921	NA					X						
wPt741943	NA											X*
wPt741949	NA											X*
wPt741953	NA										X	
wPt741976	NA				X							
wPt741986	NA				X							
wPt742025	NA					X					X	
wPt742039	NA				X							
wPt742051	NA						X		X			
wPt742052	NA					X						
wPt742118	NA				X							
wPt742141	NA					X*						
wPt742171	NA											X
wPt742206	NA		X									
wPt742220	NA											X
wPt742222	NA						X*					
wPt742244	NA					X*	X		X*			
wPt742266	NA											X*
wPt742396	NA										X	
wPt742405	NA											X*
wPt742431	NA	X										

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt742448	NA											X*
wPt742486	NA				X							
wPt742488	NA	X										
wPt742491	NA	X										
wPt742576	NA	X*										
wPt742587	NA					X					X	
wPt742569	NA											X*
wPt742665	NA				X							
wPt742676	NA											X
wPt742689	NA	X										
wPt744786	NA						X					
wPt742499	NA						X	X*	X*			
wPt742641	NA		X*									
wPt742705	NA		X*									
wPt742732	NA		X									
wPt742840	NA										X	
wPt742855	NA		X									
wPt742858	NA										X	
wPt742911	NA										X	
wPt742914	NA			X			X		X			
wPt742925	NA		X		X*							
wPt742982	NA						X					
wPt743061	NA			X								
wPt743096	NA		X			X*						

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt743099	NA				X							
wPt743140	NA										X	
wPt743380	NA					X*						
wPt743384	NA										X	
wPt743269	NA					X*	X					
wPt743306	NA										X	
wPt743331	NA					X						
wPt744366	NA			X			X		X			
wPt743412	NA						X*	X				
wPt743435	NA			X								
wPt743549	NA					X						
wPt743551	NA					X						
wPt743589	NA					X						
wPt743651	NA					X*						
wPt743661	NA					X	X					
wPt743666	NA										X	
wPt743671	NA										X	
wPt743749	NA		X									
wPt743777	NA								X			
wPt743790	NA					X*						
wPt743858	NA				X							
wPt743860	NA					X*						
wPt743889	NA								X			
wPt743909	NA				X							

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt743999	NA		X									
wPt744088	NA			X			X		X			
wPt744124	NA										X	
wPt744129	NA			X			X		X*			
wPt744175	NA				X			X				
wPt744217	NA				X			X				
wPt744251	NA	X										
wPt744256	NA						X					
wPt744265	NA					X*						
wPt744271	NA					X*						
wPt744290	NA	X*										
wPt744388	NA					X*						
wPt744434	NA				X*							
wPt744533	NA						X*	X				
wPt744612	NA			X			X		X*			
wPt744613	NA									X*		
wPt744595	NA										X	
wPt744632	NA									X		
wPt744643	NA		X*	X						X*		
wPt744675	NA					X*						
wPt744512	NA					X*						
wPt744577	NA								X			
wPt744595	NA										X	
wPt744596	NA								X			

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt744656	NA					X*						
wPt744736	NA										X	
wPt744769	NA			X								
wPt744782	NA			X								
wPt744796	NA						X		X*			
wPt744846	NA						X		X			
wPt744851	NA										X	
wPt744866	NA										X	
wPt744900	NA				X*		X					
wPt744914	NA	X*		X								
wPt744960	NA							X				
wPt744976	NA					X*						
wPt744987	NA			X								
wPt745008	NA					X*						
wPt745062	NA					X*						
wPt745068	NA										X	
wPt745070	NA										X	
wPt745110	NA						X					
wPt745121	NA										X	
wPt798213	NA						X*	X	X			
wPt798333	NA				X							X*
wPt798604	NA								X			
wPt798970	NA				X		X					
wPt800131	NA								X			

Marker	Chrom	2008	AA	CD	Dor	PHS	Hd	Ft	Ht	TKW	Pub	Awn
wPt800213	NA			X								X
wPt800425	NA	X*										
wPt800634	NA							X				
wPt800707	NA	X*										

Table 23: Sources of deletion bin assignment to markers in significant association with longevity, doramcny and PHS in advanced germplasm (blue) and genebank collection (red) or both (green)

Mapped			Unmapped			Source
Longevity	Dormancy	PHS	Longevity	Dormancy	PHS	
wPt1541, -2087, -2151, -4487, -5138, -5334, -5547, -5896, -6709, -7063, -8460	wPt1328, -1562, -2087, -3282, -3824, -4131, -5067, -5105, -6311, -6434, -9170, -9666	wPt0837, -1489, -2636, -3945, -5105, -5128, -5765, -6709, -7015, -9170	wPt1859, -2933, -3268, -3879, -3976, -4476, -7096, -7340, -8446	wPt4930, -3976	wPt0934, -3976, -5049, -9207	Francki et al. (2009)
wPt0008, -0610, -1155, -1547, -3605b, -5503, -6135, -7034, -7062, -7101, -8167, -9418, -9422, -9510	wPt0610, -0950, -1155, -1250, -3904, -4144, -4407, -4835, -4924, -5704, -6135, -6447, -7101, -7306, -8292, -9418, -9422	wPt0610, -1547, -4721, -4863, -6135, -6975, -7101, -8072, -9422				Semagn et al. (2005)
wPt3991, -4424, -4620, -6422, -8682, -9432, -9350	wPt0194, -3816, -3991, -4424, -4620	wPt0864, -5390, -8006, -8682	wPt0398, -2883, -4569, -8721			Mantovani et al. (2008)
wPt3060, -6005, -8183, -2424	wPt3060		wPt1480, -6711, -8826			Hai-Chun et al. (2009)
wPt0538, -8404, -8920	wPt3661	wPt8657	wPt2416			Peleg et al. (2008)
wPt1730		wPt1730				Akbari et al. (2006)

Table 24: Gene content of the deletion bins associated with an MTA for longevity, dormancy and PHS in diverse germplasm (blue) and genebank collection (red) or both (green)

Chr.	Longevity	Dormancy	PHS	Chromosomal bin	BlastX candidate genes
1A			*wPt8072*	1AS1-0.47-0.86	Triticin precursor, cytoplasmic ribosomal protein L18, 40S ribosomal protein S4, probable cytochorme-C oxidase, serine proteinase, cellulose synthase-1, serine/threonine protein phosphatase, glutathione-S-transferase 2, casein-kinase like protein, carboxypeptidase D, NBS-LRR disease resistance protein homologue, MADS box transcription factor
1A	*wPt6709*		*wPt6709*	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
1A		*wPt3904*		C-1AL1-0.17	Thiolase, Ras related protein Rab11D, Chaperonin CPN60-2, mitochondrial precursor, glutamine synthetase 1, cyanate hydratase
1A	*wPt6005*			1AL3-0.61-1.00	*Histone H4* gene, disease resistance protein, MAP kinase like protein, protein translation factor, DNA binding protein homolog, CTP synthase, threonine synthase, malate dehydrogenase, cytochrome reductase, aldehyde dehydrogenase, ethylene forming enzyme, ferrodoxin precursor
1B	*wPt8682*	*wPt1328, -3282, -3824, -5067, -6434*	*wPt8682*	C-1BS10-0.50	Protein kinase, NADH oxidoreductase, cellulose synthase, phosphoglycerate dehydrogenase
1B			*wPt6975*	1BL2-0.69-0.85	Alpha-tubulin, thymidine kinase, cyclophilin, porin VDAC2, hexokinase 1, calcium sensor homologe, hypothetical transemembrane protein
1B			*wPt4721*	1BL3-0.85-1.00	CTP synthase, glucose 1 phosphate adenylyltransferase large subunit 1, chloroplast precursor, late embryogenesis abundant protein, threonine synthase, MAP-kinase like protein
1D	*wPt5503*			C-1DS3-0.48	Probable tocopherol cyclase, putative NADH oxidoreductase and lipoamide dehydrogenase, transcription factor similar to bHLH (Basic-

					helix-loop-helix), reverse transcriptase, MCB2 protein, cellulose synthase-1, asparaginyl-tRNA synthetase, protein kinase, chloroplast precursor (sucrose export defective 1), dihydrolipoamide dehydrogenase precursor, disulfide-isomerase precursor
1D			*wPt3945*	1DL4-0.18-0.41	Proline rich protein, type V thionin precursor, glutamine synthetase 1, cyanate hydratase, glutathione S-transferase, eukaryotic translation initiation factor 4E
2A	*wPt3976*, *6711*	-	*wPt3976*	*wPt3976*	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
2A			*wPt7306*		C-2AS5-0.78	60S ribosomal protein L27a, transcription factor APF1, Na+/H+ antiporter, zinc-finger helicase, ribulose biphosphate carboxylase small chain precursor, alanine transaminase
2A	*wPt1480*				2AL1-0.85-1.00	Early nodulin gene (*Enod*) related protein, cold shock protein¸ barley stem rust resistance protein, pyruvate kinase, dihydrofolate reductase, thymidylate synthase, apoplastic invertase 2, putative aldolase, protein phosphatase 2C, superoxide dismutase, peroxidase 2, cystatin, lipoxygenase, succinate dehydrogenase subunit 3, Mn precursor, auxin response factor 8
2A	*wPt2087*, *8826*	-	*wPt2087*		C-2AL1-0.85	*Elicitor-responsive gene 3*, ABA-responsive protein, pathogen-related protein, RAD50 DNA repair protein, poly(A)-binding protein, membrane protein, nucleic acid binding protein (alfin-1), reverse transcriptase, L-ascorbate peroxidase, glycolate oxidase, probable oxoglutarate dehydrogenase, epoxide hydrolase, alternative oxidase, prohibitin, translation initiation factor, P-type ATPase, aquaporin, thioredoxin H, putative acyl CoA thioesterase, protein kinase, casein kinase II alpha, wall-associated kinase 2, sphingosine kinase, potasium transporter, monosaccharide transporter 3, peptide chain release factor subunit, cis-

					zeatin O-glucosyltransferase 2, phenylalanine ammonia-lyase, chloroplast precursor, chitinase IV precursor, acidic pathogenesis-related protein 1a precursor
2B	*wPt8404*	*wPt6311*		2BS3-0.84-1.00	*Vrga1*, early nodulin gene (*Enod*) related protein, stem rust resistance protein Rpg1, abscisic acid-induced protein ABA7, Mad-related protein, dihydrofolate reductase, glucosyl transferase, triosephosphat-isomerase, chitinase 2, arginase, thymidylate synthase, cinnamoyl CoA reductase, allyl alcohol dehydrogenase and pyruvate dehydrogenase E1 alpha subunit
2B			*wPt1489*	C-2BS1-0.53	Cytochrome P450, acyl desaturase, NADH dehydrogenase, flavanone 3-hydoxylase, calcium-dependent protein kinase 2, ferrodoxin NADP(H) oxidoreductase, thioredoxin H, sucrose synthase type 1, heat shock protein HSP26, putative elongation factor 1 beta, ASAPETALA 1, LRR1,
2B	*wPt8460, -9350*	*wPt0950*		C-2BL2-0.36	phosphoinositide-specific phospholipase C, putative protein kinase, casein kinase II alpha, translation initiation factor
2B			*wPt5128*	2BL6-0.89-1.00	GTP-binding protein, ferrichrome-iron receptor, manganese superoxide dismutase (SOD) precursor, dehydration-responsive AP2 domain transcriptional activator, blue copper-binding protein homologue, beta-glucosidase, cystatin
2D		*wPt4144*		2DS5-0.47-1.00	Apyrase, transcription factor APF1, glutamate dehydrogenase, DNA polymerase A-family protein, elongation factor 1-beta, benzothiodiazole-induced protein, RuBisCO subunit binding-protein alpha subunit, ferrodoxin NADP(H) oxidoreductase, ribulose-biphosphate carboxylase, pyruvate decarboxylase, chaperonin 10
3A	*wPt6422, wPt9422*	*wPt1562, -3816, -4407, -9422*	*wPt9422*	C-3AL3-0.42	Chloroplast ribosomal protein, lipase like protein, malate dehydrogenase, beta glucosidase, threonine synthase, NADP specific isocitrate dehydrogenase, putative H+-transporting ATPase, mandelonitrile lyase, casein kinase I, allinase IAA-Ala hydrolase, carbamoyl phosphate synthetase and RNA helicase
3A	*wPt0398, -*			3AL5-0.78-1.00	*Gliadin*, GTP-binding protein, histone deacetylase HD2-like protein,

	7340				putative RING-H2 finger protein, viviparous 1 protein, ubiqutin-like protein SMT3, leaf senescence related protein, Ras-related GTP-binding protein, stress enhanced protein, barley stem rust resistance protein, repressor protein, phosphatidylserine decarboxylase, sterol delta-7 reductase, phosphoethanolamine methyltransferase, proteinase inhibitor, histidine kinase, auxin response factor 2, cinnamyl-alcohol dehydrogenase, allyl alcohol dehydrogenase, phosphoglycerate dehydrogenase, phosphogluconate dehydrogenase (decarboxylating), ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit, transaldolase, cytochrome c1 precursor, (1-3)-beta-glucanase, putative beta-fructofuranosidase glyoxalase II, dehydration-responsive protein RD22 precursor, H+-transporting two-sector ATPase beta chain, esterase D, dehydrogenase, DNA polymerase I, cytochrome B5, sucrose-phosphate synthase, peroxidase 1
3B	*wPt8446*			3BS8-0.78-1.00	Stem rust resistance protein Rpg1, hsr201 (hypersensitivity-related protein), disease resistance gene and protein, leaf senescence related protein, putative ethylene-responsive RNA helicase, serine/threonine kinase, acetoacyl-CoA-thiolase, 1-deoxy-D-xylulose 5-phosphate reductoisomerase, hydrolase, lactate dehydrogenase, putative phytochelatin synthetase, lipid transfer protein and its precursor, putative nodulin, fatty acyl CoA reductase, cyclin-dependent kinase inhibitor
3B	*wPt9432*, - *9510*	*wPt5105*, - *9170*	*wPt5105 , -5390, -7015, - 9170*	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
3B	*wPt2416*	*wPt5704*		3BL7-0.63-1.00	Malate dehydrogenase, laccase, G protein alpha subunit, histidine kinase, auxin repressor factor 2, cinnamyl alcohol dehydrogenase, ally alcohol dehydrogenase, cytosolic starch phosphorylase, (1-3)-beta-glucanase, dehydrogenase, chitinase 1, triacyl glyceroal lipase, alpha

					glucan phosphorylase, transposase, metallothioneine 2, *gliadin*
3D	*wPt4476, -4569*			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*
4A	*wPt8167,-4487*			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
4A	*wPt0538, -0610, -1155, -2151, -4424, -4620, -7096, -9418*	*wPt0610, -1155, -4424, -4620, -9418*	*wPt0610, -8657*	4AL4-0.80-1.00	Kelch-containing protein, cycloartenol synthase, disease resistance protein, sucrose synthase 2, glutathione transferase, ubiquitin-conjugating enzyme, NBS-LRR disease resistance protein homologue
4B	*wPt3991, -5334, -7062*	*wPt3991, -8292*		C-4BL1-0.71	Heat shock protein, low temperature induced protein, seed maturation protein, nucleic acid binding protein, kinases, glutamate decarboxylase isozyme, protein disulfide isomerase, cytochrome c oxidase, peroxidases, lipases, hydrolases
5A		*wPt4131*		5AS3-0.75-0.98	Thiosulfate sulfurtransferase, P-type ATPase, lipoxygenase, oxalate oxidase, ethylene-responsive elongation EF-Ts precursor, RNA binding protein, glutathione transferase F4, grain softness protein, sucrose-6F-phosphate phosphohydrolase SPP1, methionine synthase protein
5B		*wPt9666*		5BS6-0.81-1.00	Lipoxygenase, glutathione transferase F4, hydrolase, sucrose-6F-phosphate phosphohydrolase SPP1, small GTP-binding protein,
5B	*wPt7101*	*wPt1250, -3661, -7101*	*wPt7101*	5BL1-0.55-0.75	Heat shock 70 KD protien, membrane protein, NBS-LRR type resistance protein, allyl alcohol dehydrogenase, glyoxalase II, putative cinnamoyl-CoA reductase, cyl-thioesterase, beta-glucosidase isozyme 2 precursor, ADH-ubiquinone oxidoreductase subunit 1 and vacuolar sorting receptor
5B	*wPt6135, -5896*	*wPt6135*	*wPt6135*	5BL9-0.76-0.79	Cell division control protein, RNA-binding protein, viviparous-14 protein, IAA1 protein, gibberellin-stimulated transcript 1 like protein,

					lipoxygenase, IAA amidohydrolase, sucrose transporter 2, UDP-glucose dehydrogenase, succinate dehydrogenase, flavoprotein alpha subunit, ferredoxin--NADP reductase, root isozyme, chloroplast precursor, Type V thionin precursor, globulin-2 precursor, beta-D-glucan exohydrolase, protein kinase, glucosyltransferase
5B			*wPt0837*	5BL16-0.79-1.00	Glutamate decarboxylase isozyme 1, putative reductase, chlorophyll a/b-binding protein WCAB precursor, ECA1 protein, growth inhibitory protein, APETALA2-like protein, putative fructokinase II, serpin homologue WZS3, multi drug resistance-associated protein, barley stem rust resistance protein
6A			*wPt0864, -2636, -8006*	6AS5-0.65-1.00	Calcium-binding protein kinase, cationic peroxidase, alpha gliadin, histone H2A.2, chlorophyll a/b-binding protein WCAB precursor, BAX inhibitor 1, RACB protein, ABA7 protein, acid invertase, fructan 1-exohydrolase, senescence association protein
6A	*wPt7063*			C-6AL4-0.55	Chloroplast translational elongation factor, protein kinase, senescence related proteins, fatty acid elongase, catalase, oxidase, alcohol dehydrogenase
6B	*wPt1547, -3605b*		*wPt1547*	6BS-Sat	CC-NBS-LRR resistance protein MLA13, *Enod* related protein, acetohydroxyacid synthase, alpha-gliadin, histone H2A.2, BAX inhibitor 1, cellulose synthase-1, plastidic ATP/ADP-transporter, fructan 1-exohydrolase, ferredoxin-NADP(H) oxidoreductase, group 5 allergen precursor, NADPH-dependent mannose 6-phosphate reductase
6B	*wPt1541,-1730, -2424, -3060, -8183, -8721*	*wPt3060, -4924, -4930*	*wPt1730*	C-6BL3-0.36	Prohibitin, pescadillo like protein, protein kinase, nuclear cap binding protein, cytochrome
6D	*wPt3879*			6DS6-0.99-1.00	Alpha-amylase inhibitor Ima1 precursor, abscisic acid-induced protein ABA7, NADH-dependent hydroxypyruvate reductase¸ F-box protein family
7A			*wPt9207*	7AS1-0.89.1.00	Glycolate oxidase, lipoxygenase, 60S ribosomal protein L9, glutathione

					transferase, transketolase-like protein, putative disease resistance protein RPR1, peroxidase 1
7A	*wPt0008*, - *7034*	*wPt4835*, - *6447*		C-7AL1-0.39	Stress responsive gene 6 (*Srg6*) protein, UDP-galactose 4-epimerase-like protein, RNA-binding protein, AP2/EREBP transcription factor BABY BOOM2¸ DNA topoisomerase I, copper homeostasis factor, superoxide dismutase, peptide synthetase NRPS0
7B	*wPt2883*, - *8920*		*wPt4863*	7BS1-0.27-1.00	*Enod*, COP1(constitutive photomorphogenic 1)-interacting protein 7 (CIP7), heat shock protein 80, low temperature and salt responsive protein, proline-rich extensin-like protein, zinc-finger protein R2931, protein serine/threonine kinase, putative DNA-binding protein phosphatase 2C, beta-expansin, Tam3-transposase, protein kinase homolog, gamma-lyase, glycine dehydrogenase and sucrose synthase
7B	*wPt2933*, - *5138*, *-5547*	*wPt0194*		7BL10-0.78-1.00	Barley stem rust resistance protein, membrane protein, catalase isozyme, ammonium transporter, cytochrome, putative dehydrogenases, chloroplast nucleotide DNA binding protein, lectin precursor, chalcone synthase
7D	*wPt3268*		*wPt0934 , -5049, - 5765*	7DS4-0.61-1.00	*Enod*, biogenesis protein PEX1, ribosomal protein L35A, NBS-LRR type resistance protein, auxin-regulated GH3 protein, chloroplast Cpn21 protein, peroxisome, peroxidase, lipoxygenase, reverse transcriptase, diphosphonucleotide phosphatase 1, cytosolic chaperonin, DNA cytosine methyltransferase Zmet3, aldehyde dehydrogenase ALDH2, 6-phosphogluconate dehydrogenase isoenzyme B, plasma membrane H+-ATPase, UDP-glucosyltransferase BX9, blue copper-binding protein homolog and ubiquitin-specific protease
7D	*wPt1859*			7DL2-0.61-0.82	RNA helicase, tetra-ubiquitin, lipoprotein, peroxidase, MADS box-like protein, beta-glucosidase, cysteine protease, ATP citrate lyase, galactokinase, eukaryotic initiation factor 4A, ribosomal RNA apurinic site specific lyase, cinnamoyl-CoA reductase and auxin response factor 10

Appendix II

Population Structure

To determine population structure of genebank collection, a set of 161 markers which were at-least 5 cM apart were used to determine the population structure of this population. K was set from 1 to 15. The bar plot derived from the STRUCTURE 2.1 is shown in Figure 1. The average number of K subgroups (L (K)) along with the standard deviation is shown in Figure 2.

From the bar plot, it is clear that the population is composed of 6 sub-groups along with some lines that were not classified into any of these groups. This is mostly consistent when the data about the geographical origin and growth habit of various wheat accessions in this population was considered. The same was difficult to decide by looking at Figure 2 which gives the highest peak at 14. To verify the results, neighbour-joining tree with Paup* 4.0b10 (Swofford 2002) after Nei and Li (1979) distance coefficient was generated (Figure 3).

The PAUP tree (Figure 3) showed 6 groups which were consistent with the data available for these lines. The discussion in the following paragraph is about the groups obtained from STRUCTURE with reference to the PAUP tree.
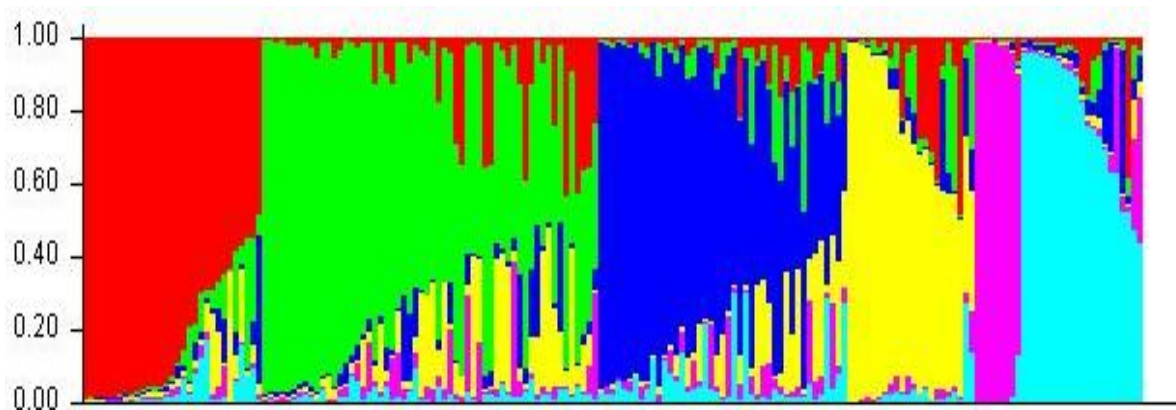
Figure 1: Bar plot derived from STRUCTURE 2.1 showing the distribution of the population into 6 groups.
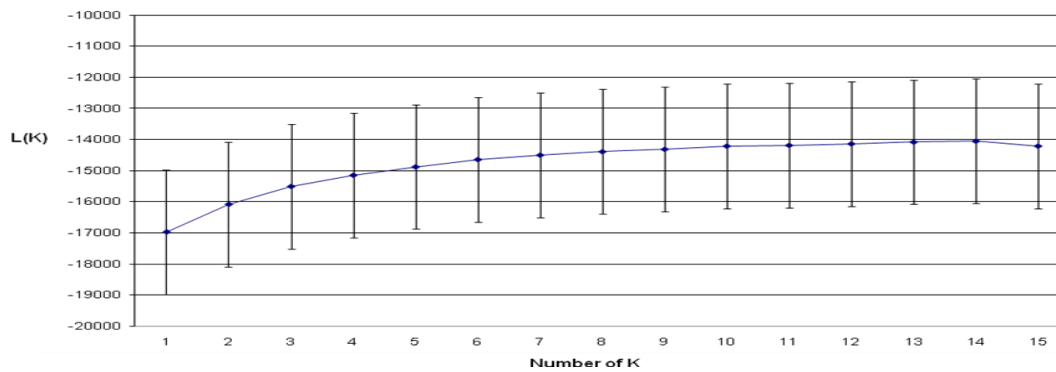
Figure 2: Log probabilities of data, L (K), averaged over the replicates along with the standard deviation

The population could be divided into 6 distinctive groups designated as Q1, Q2, Q3, Q4, Q5 and Q6 based upon STRUCTURE analysis. Q1 was composed of 30 accessions mainly from Asian descent including 25 spring and five winter wheat lines. All of them were present in 'spring wheat Middle/East Asia' in PAUP tree. Q2 was composed of the highest number of accessions with 48 accessions including 34 winter and 14 spring accessions coming from around the world excluding Asia. Majority of these came from the group designated as 'winter wheat worldwide' in the PAUP tree. But eight of these accessions were from the group 'winter wheat temperate region' and four were from 'spring wheat temperate region'. Q3 carried 15 spring and four winter wheat lines (total 19 lines) coming mainly from Cyprus and Greece. In the PAUP tree, these accessions are included in the 'spring wheat South Europe' group. Q4 constituted the second largest group comprising 36 spring and 1 winter wheat accession coming from Italy and other temperate countries. Majority of these are coming from 'spring wheat South Europe' but seven of them were from 'winter wheat worldwide' and two from winter wheat temperate region' in PAUP tree. Q5 was composed of 18 spring and one winter accession from diverse locations. Eleven of them are from 'spring wheat temperate region', six from spring wheat Middle/East Asia' and the last two accessions were included in 'spring wheat temperate region' and 'winter wheat temperate region'. Group 6 comprised of seven spring and one winter accession coming from Greece, all of which are included in 'spring wheat South Europe'. There were 23 accessions which were not classified into any of these groups and were regarded as 'Mixed group'. These accessions were originating from 13 countries. Seventeen of them were spring wheat and six were winter wheat. In the PAUP tree they were distributed in all the six groups. Thus, the results of STRUCTURE analysis were in agreement with the geographical origin and the type of these accessions.
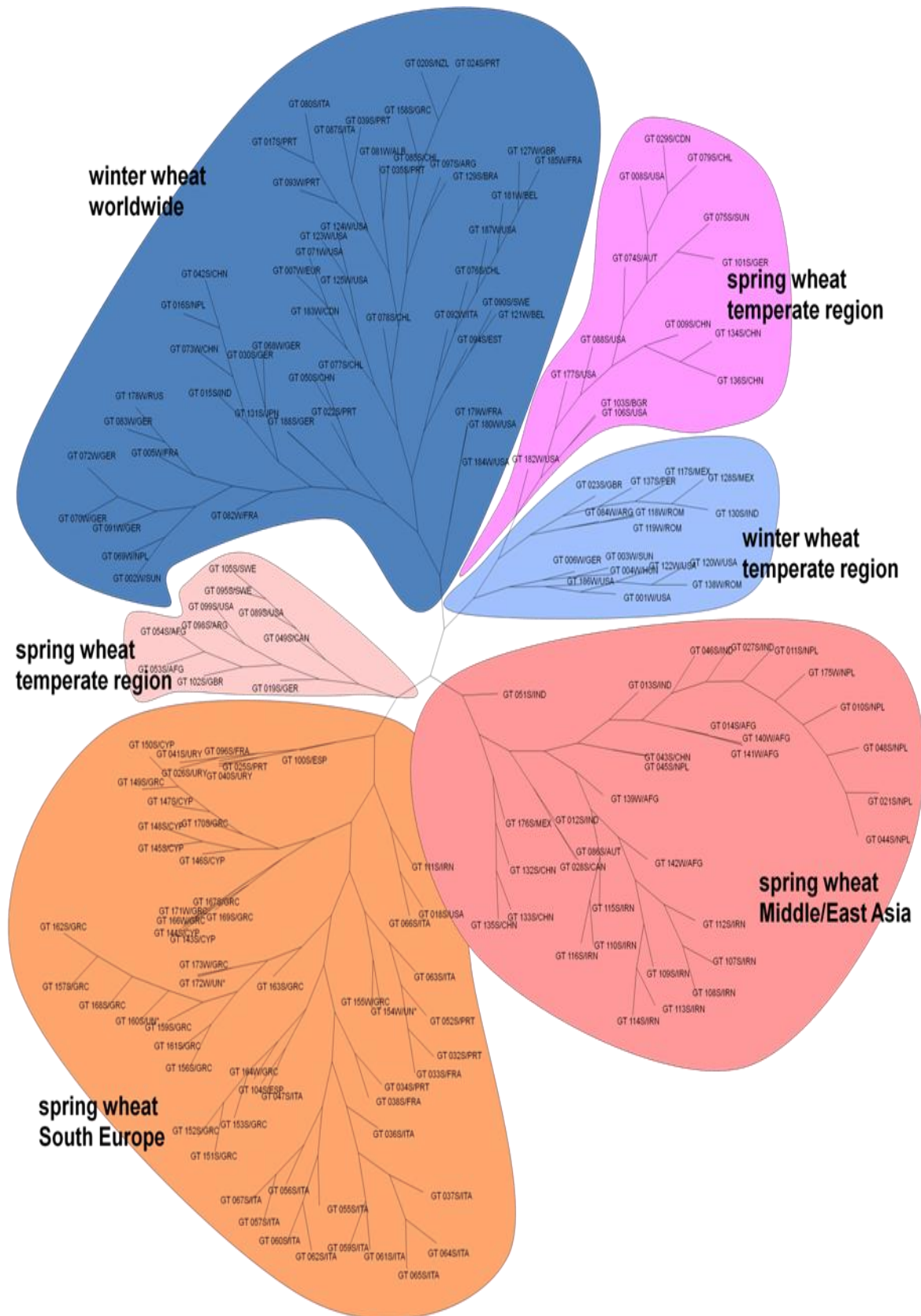
Figure 3: PAUP generated dendrogram of 183 accessions of genebank collection. The six groups are differentiated according to the growth habit and origin

Appendix III
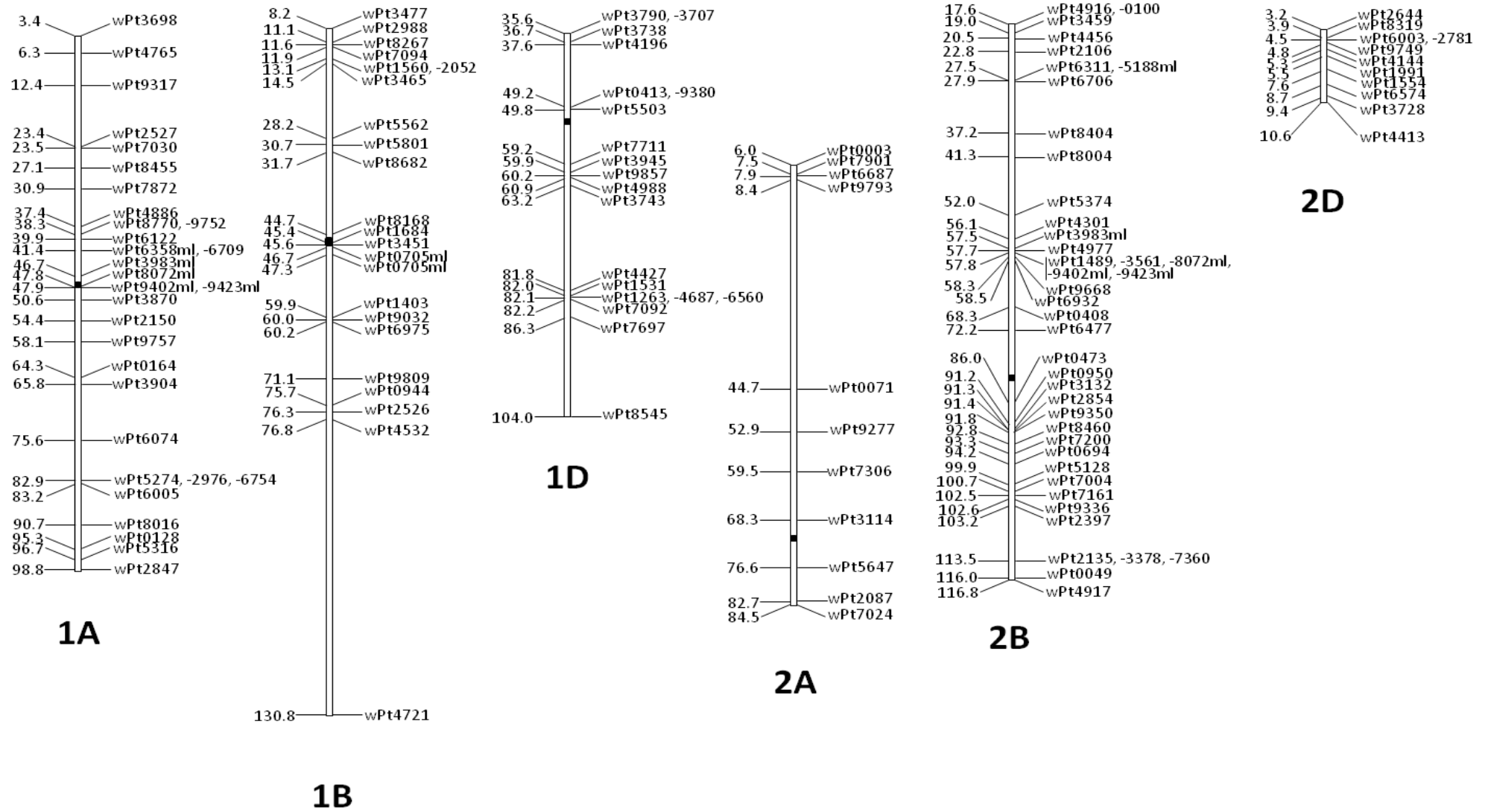
Figure 1: A genetic map of 183 wheat accessions of genebank collection
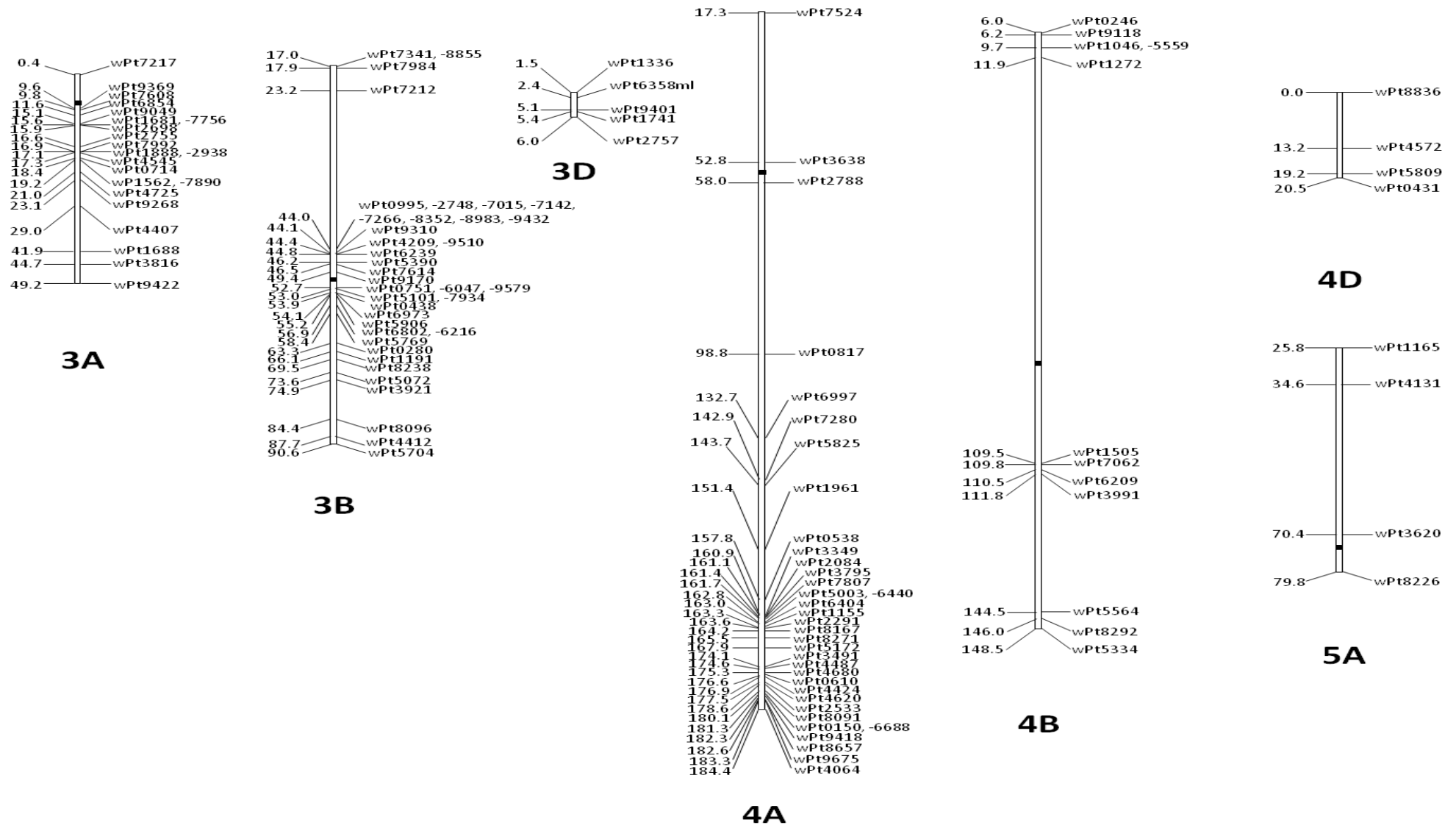
Figure 1: *Continued*
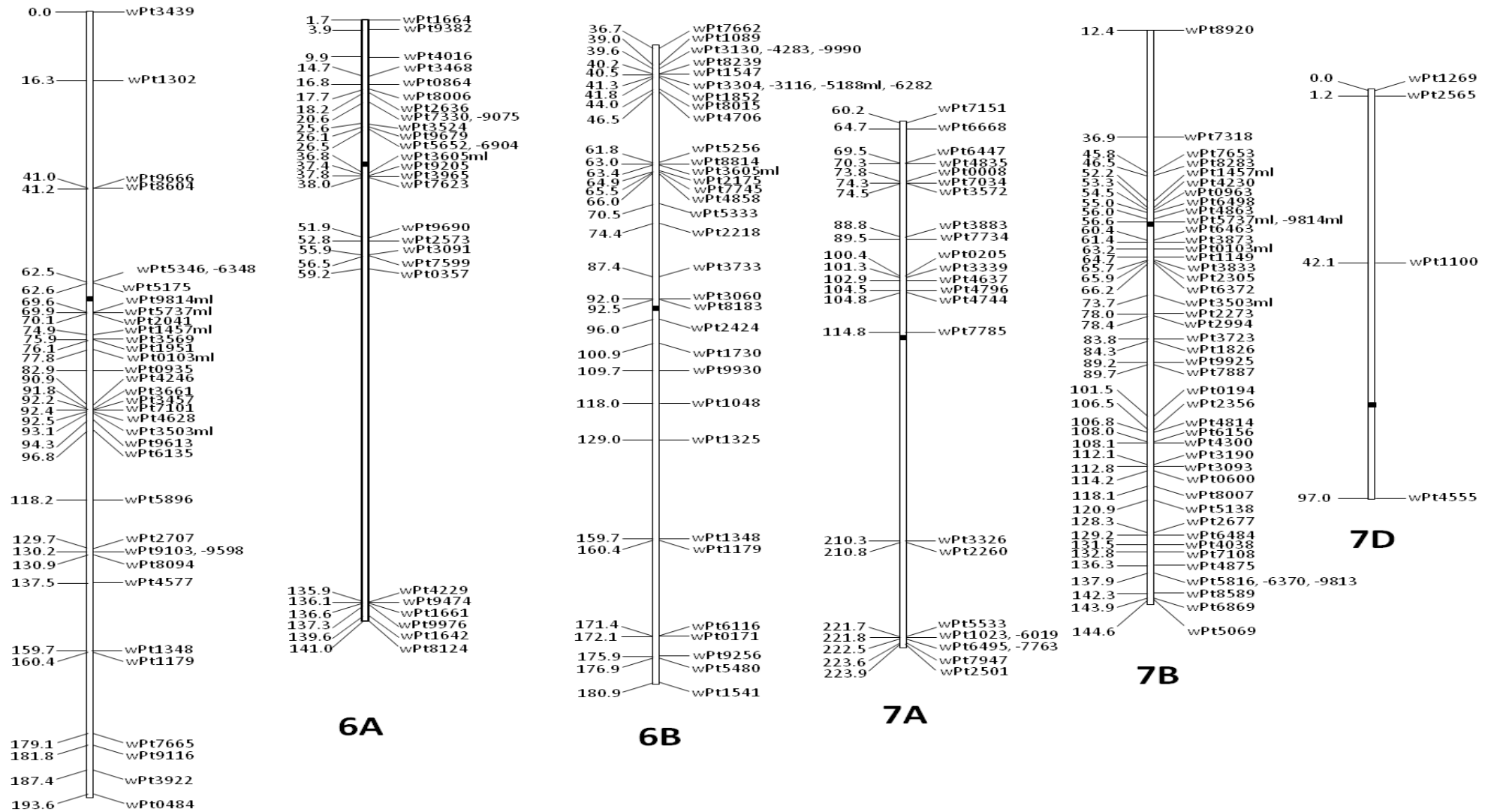
Figure 1: *Continued*

Acknowledgements

Curriculum Vitae

Personal data

Name	Mian Abdur Rehman Arif
Date of birth	22-11-1984
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1999	Secondary School Certificate (SSC), Sadeq Public School, F-Block, Rawalpindi, Pakistan
2001	Intermediate Certificate, Government Gordon College, Rawalpindi, Pakistan
2001-2005	B.Sc (hons) Agriculture with major in Plant Pathology, University of Arid Agriculture, Rawalpindi, Pakistan
2005-2007	M.Sc (hons) Agriculture in Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan
2008 till present	Ph. D student, Resources Genetics and Reproduction (RGR), Department of Genebank, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

Employment Experience

2007-2008	Agriculture Officer (Extension), Government of Punjab, Pakistan

Publications

Peer reviewed:

1- **Rehman Arif MA**, Neumann K, Nagel M, Lohwasswer U, Börner A (2012) An association mapping study of dormancy and pre-harvest sprouting in wheat. (accepted)

2- **Rehman Arif MA**, Nagel M, Neumann K, Kobiljski B, Lohwasser U, Börner A (2011) Genetic studies of seed longevity in hexaploid wheat exploiting segregation and association mapping approaches. Published online in Euphytica on 18-06-2011. DOI: 1010.1007/s1068101104715

3- Lohwasser U, **Rehman Arif MA**, Börner A (2011) Discovery of loci determining pre-harvest sprouting and dormancy in wheat and barley using QTL and association mapping (submitted to Euphytica)

4- **Rehman Arif MA**, Nagel M, Lohwasser U, Börner A (2011) Long-term seed storability in genebank collections – genetic studies in wheat. In: Veisz O. (ed.): Climate change: Challenges and opportunities in agriculture. Agricultural Research Institute of the Hungarian Academy of Sciences: 102-105

5- Börner A, Khlestkina EK, Chebotar S, Nagel M, **Rehman Arif MA**, Neumann K, Kobiljski B, Lohwasser U, Röder MS (2011) Maintenance and exploitation of genetic resources for future plant breeding. In: Veisz O. (ed.): Climate change: Challenges and opportunities in agriculture. Agricultural Research Institute of the Hungarian Academy of Sciences: 26-31

6- **Rehman Arif MA**, Nagel M, Lohwasser U, Börner A (2010) Seed longevity in wheat (*Triticum aestivum* L.) - genetics and beyond. Berichte der Gesellschaft für Pflanzenbauwissenschaften, Bd. 5, 11-15

7- Nagel M, **Rehman Arif MA**, Rosenhauer M, Börner (2010) Physiologische und genetische Aspekte zur Qualität von langzeitgelagertem Weizensaatgut, 45, DGQ, Vortragstagung, Berlin-Dahlem, 53-54

8- Nagel M, **Rehman Arif MA**, Rosenhauer M, Börner A (2009) Longevity of seeds and intraspecific differences in the Gatersleben genebank collections. In: Proceedings of the 60th conference of plant breeders and seed traders association in Austria, Raumberg-Gumpenstein, 179-181

Other abstracts:

1. Börner A, Antonova EV, Haile JK, Khlestkina EK, Kobiljski B, Kollers S, Lohwasser U, **Rehman-Arif MA**, Röder MS, Tikhenko N, Zaynali Nezhad K (2011) Items from Germany. Annual Wheat Newsletter (Accepted)

2. Börner A, Joshi AK, Khlestkina EK, Kobiljski B, Kranner I, Kumar U, Landjeva S, Leonova IN, Lohwasser U, Nagel M, Navakode S, Neumann K, Paliwal R, **Rehman-Arif MA**,

Röder MS, Tikhenko N, Weidner A, Zaynali Nezhad K (2010) Items from Germany. Annual Wheat Newsletter 56:47-52

3. Börner A, Khlestkina EK, Kobiljski B, Kumar U, Landjeva S, Lohwasser U, Nagel M, Navakode S, **Rehman Arif MA**, Röder MS, Weidner A, Xia LQ, Zaynali Nezhad K (2009) Items from Germany. Annual Wheat Newsletter 55:53-58

Conference presentations

1. 7th Plant Science Student Conference held at Institute of Plant Biochemistry, Halle (14-06-2011 to 17-06-2011), Abstract number: **T06**, Seed Conservation in *Ex situ* Genebanks-Genetic Studies on Longevity in Wheat

2. Agirsafe Final Conference held at Budapest , Hungary (21-3-2011 to 23-3-2011).

3. 6th Plant Science Student Conference held at Leibniz institute of Plant Genetics and Crop Plant Research, Gatersleben (15-6-2010 to 18-6-2010), Abstract number **T11**, Seed Longevity in Wheat (*Triticum aestivum* L.): genetics and beyond

4. BotanikerTagung held at Leipzig University (6-09-2009 to 10-09-2009), Abstract number **S12-5**, Genetics of Seed Longevity in Wheat

Poster presentations:

1. **Rehman-Arif MA**, Nagel M, Neumann K, Kobiljski B, Lohwasser U, Börner A (2011) Genome-wide association mapping of seed longevity, dormancy and pre-harvest sprouting in bread wheat (*Triticum aestivum* L.) in EWAC-EUCARPIA Cereals Section Conference held at Novi Sad, Serbia from 7-11 November, 2011

Erklärung

Hermit erkläre ich, dass mit dieser wissenschatlichen Arbeit noch keine vergeblichen Promotionsversuche unternommen wurden.

Dir eingereichte Dissertation mit dem Thema: "Genetics of Seed Longevity and Dormancy in Wheat" habe ich selbständig und nur unter Verwendung der angegeben Literatur und Hilfsmittel angefertigt.

Des weiteren erkläre ich, dass keine Strafverfahren gegen mich anhängig sind.

Halle/Saale, den 10-1-2012

Mian Abdur Rehman Arif