Molecular Mapping of Loci Determining Seed Longevity in "*Brassica napus*"

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Mai Allam

Table of Contents

List of Figures:

(FA) and the related orthologous genes compeered with *Arabidopsis thaliana* (LOG).

List of Tables:

List of Supplementary Tables:

(odds ratio), Expected number of transcripts found associated with the GO term for enrichment (Exp-Count), Real number of transcripts found associated with the GO term (count), Population size of transcripts found associated with the GO term within the analysis (size) are listed for each candidate gene.

List of abbreviations

Abn	Abnormal Seedlings
AEM	Adjusted Entry Means
AFLP	Amplified Fragment Length Polymorphism
APX	Ascorbate Peroxidase
BC	Back crossed lines
CAT	Catalase
CD	Controlled Deterioration
CD	Controlled Deterioration test
CIM	Composite Interval Mapping model
CT	Control seeds
DArT	Diversity Array Technology
DH	Double Haploids
DNA	De-oxyribo Nucleic Acid
DSC	Differential Scanning Calorimetly
ExV	Express x V8
F2	Second filial generation
GLM	General Linear Model
GO	Gene Ontology
GR	Glutathione Reductase
GWAS	Genome Wide Association Study
h^2	Rrepeatabilities
HOLL	High Oleic And Low Linoleinic Acids
LD	Linkage Disequilibrium
LEAR	Low Erucic Acid Rapeseed-
LGs	Linkage Groups
LiCl	Lithium chloride
LOX	Lipoxygenase
LSD	Least Significant Difference
LSM	Least Squares Means
MC	Moisture Content
MLM	Mixed Linear Model
Mpa	Megabase
NIL	Near Isogenic Line
NS	Normal Seedlings
OSR	Oilseed Rape
PCA	Principle Component Analysis
PCoA	Principle Coordinate Analysis
PCR	Polymerase Chain Reaction
PGR	Plant Genetic Resources
PLDα1	Phospholipase D
PMA	Parallel Multiplex Assay
POD	Peroxidase
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RH	Relative Humidity
RIL	Recombinant Inbred Lines

RL	Root Length
ROS	Reactive Oxygen Species
RSH	Root/Shoot ratio
SD	Standard deviation
SGSV	Svalbard Global Seed Vault
SGT	Standard Germination Test
SHL	Shoot Length
SNPs	Single Nucleotide Polymorphism
SOD	Superoxide Dismutase
SPA	Sigle-Plex Assay
SRAP	Sequence-Related Amplified Polymorphism
SSR	Microsatellites or Simple Sequence Repeats
T10	Time required to reach 10% germination
T50	Time required to reach 50% germination
TAG	Triacyglycerol
TG	Total Germination
TSW	Thousand seed weight
USDA	United States Department of Agriculture

1. Introduction

Oilseed rape (*Brassica napus* L.) is the most widely cultivated crop species in crucifer family (*Brassicaceae*). The subspecies *B. napus* ssp. *napus* includes winter and spring oilseed, fodder and vegetable rape forms. Oilseed rape ranks the first oilseed crop in Europe. It ranks third after soybean and cotton seeds for meal (Snowdon et al. 2007). In the European Union the total area of winter and spring rapeseed production is 21.8 million ha in 2014-2015 (http://www.oilworld.biz) and with 27 million tons it ranks third in the global consumption of vegetable oils after oil palm and soybean oils (http://www.statista.com). Rapeseed oil is highly nutritional food oil and provides the raw materials for biodiesel productions. The oil content ranges from 40 to 45% and the residual meal is rich in protein and used mainly for animal feed and human food. Rapeseed is mostly produced for low erucic and low glucosinolate varieties or so-called canola (Canadian oil low acid) quality which contain the lowest level of saturated fatty acids recommended as healthy fatty acid profile. This composition maintains the balance of blood cholesterol and prevent heart disease risks (Snowdon et al. 2007). Due to the remarkable increase of oilseed rape production it becomes a focus for breeding and molecular genetics in recent years.

The modern plant breeding programs require easy accessibility for plant genetic recourses and the availability of variable and trustable seed resources. Conserving diversity of genotypes in *ex-situ* gene banks is considered as accessible gate for breeders to improve the desired traits in commercial crops. *Ex-situ* gene banks play a substantial role in preserving wild relatives of crop plants as well as local varieties in many diverse habitats across the world. Genetic resources provide the basic input to all plant breeding programs, whether traditionally or transgenically based (Börner 2006). Among worldwide plant genetic resources (PGR) collections 6% are oil crops, which is low comparing with cereals (45%) and legumes (15%) (FAO 2010). The oil crop collections in Federal *Ex-Situ* Genebank in Gatersleben include 6,615 accessions from different species such as flax, sunflower, soybean, oilseed rape and others (IPK 2014) which is 4.4 % of the total collection. Maintaining seeds in genebanks require several testing procedures to evaluate the seeds storability. Screening of 18 crop species stored under ambient conditions (20°C and 50 % relative humidity (RH)) in Gatersleben genebank revealed that germination rates for the different species after several periods of storage were species characteristic (Nagel and Börner 2010). They desplayed that oil seeds recourded less storability than cereals and legumes. The half viability period for Brassica ssp. seed was estimated to reach 7.3 years under ambient conditions (Nagel and

Börner 2010) and 23 years when stored under cold storage conditions (-18°C) (Walters et al. 2005c). Brassica seeds are orthodox seeds which can be dried without damage and extended by lowering seed moisture content (MC) and low temperature (Roberts 1973).

In general, the period seed can survive when stored under optimal conditions is termed seed longevity. It is a complex trait which is influenced by several factors as environment during seed development, seed moisture content and maturity at harvest, pathogenic infection, mechanical damage and the post harvest storage conditions (Rehman Arif et al. 2012). Seed longevity and the storage potential of seeds varies among or within species, even in the individuals belonged to same population (Copeland and McDonald 1995). These differences are in partly genetically determined (Nagel and Börner 2010; Walters et al. 2005c). Understanding factors which determines inter- and intra- specific variations would benefit for genebank management (Nagel and Börner 2010; Nagel et al. 2009).

Against the backdrop that canola types are the fourth dominant transgenic crops after soybean, Bt-corn and cotton grown on 3.6 million ha which equals 5% of the global transgenic area in 2003 *B. napus* seed longevity is particularly important as seed survival in the soil can be problematic (Snowdon et al. 2007). In addition, transgenic species and modern cultivars suffer a reduction in genetic diversity which increase the demand for conservation of plant genetic resources in genebanks (Börner 2006).

Variation of seed longevity within species offers the possibility to study its genetic bases. Thereby, mapping of quantitative trait loci (QTL) is based on the linkage between polymorphic markers and phenotypic values in a population of related individual lines and can be used to identify genomic regions responsible for trait variation. This method is commonly used to elucidate the genetic bases of complex traits with major constraint to crop breeding (Lukowitz et al. 2000). Following this seggregation mapping approach, the genetic bases of seed longevity in *B. napus* was firstly investigated in the YE2 population by applying experimental ageing (Nagel et al. 2011) which was previously used in other crops as Arabidopsis, rice, wheat, barley and lettuce (Bentsink et al. 2000; Clerkx et al. 2004; Miura et al. 2002; Nagel et al. 2009; Rehman Arif et al. 2012; Revilla et al. 2009; Schwember and Bradford 2010b; Singh et al. 2008a; Xue et al. 2008; Zeng et al. 2006). For comparison, the association mapping approach represents a more recent population based method of non-related individuals and relies on the linkage disequilibrium, between the simply inherited markers and genetic loci affecting the trait of interest (Jannink et al. 2001; Snowdon and Friedt 2004).

The present study attempts to quantify seed longevity in seed sets of two different genetic populations. For each seed set grown in different environments, control and experimental ageing treatments were performed in order to examine the seed longevity in a wide range of storage periods including ambient storage (natural ageing) and after experimental ageing treatments. Seed weight, viability, germination percentages, germination speed and shapes of abnormal plantlets were identified to characterise in detail seed deterioration. The results were conducted to QTL and association mapping analysis to identify genomic regions responsible for the different ageing mechanisms. The objectives of this study were listed as following:

1- Investigation of Express x V8 doubled haploid (ExV) population consisting of 122 lines harvested in three seasons 2005, 2009 and 2012 (ExV-5, ExV-9 and ExV-12) and stored under ambient conditions for six, two and one year(s), respectively. Standard germination tests were performed for each set in control conditions and after experimental ageing treatments which differed in ageing periods depending on the initial viability of each seed set. QTL mapping was performed using 475 simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers.

2- Investigation of the "ASSYST" population consisting of 215 cultivars which were genotyped with 4001 recently developed bi-allelic SNP (6K) markers. In addition to the mentioned traits plantlet performance was screened before and after experimental ageing by shoot and root length measurements. Identification of candidate genes associated with significant SNPs was performed depending on gene ontology analysis which screen the sequence of all significant associations and compare it with Arabidopsis thaliana or *B. napus* genome sequences.

This comprehensive study screens firstly all germination stages traits linked to seed longevity of *B. napus*. In order to underlie the genetic bases of such complex trait linkage and association mapping were performed under wide range of environments and ageing conditions

2. Review of literature

2.1. Cultivation and conservation of crop plants

2.1.1. Domestication and plant breeding

About 12,000 years ago first domestication events and begin of agriculture are known for the Fertile Crescent in the Near East Asia. In former eras small grains and grasses are considered as a main source for food (Damania 2008). The earlier farmers selected favourable plants with certain traits as a source of seeds to cultivate in the next season. This method enhanced the diversity of landraces especially when the natural gene pools of those landraces were surrounded (Damania 1994). At the time when these landraces were replaced with modern cultivars, the original gene pool of those landraces became lost and it became an urgent need to conserve the plant genetic resources (Lev-Yadun et al. 2000).

Modern plant breeding programs professionally started with the industrialisation in the 19th century, aiming to enhance the yield of crops and to produce desired commercial characteristics. Consequently, domestication and modern plant breeding narrowed down genetic variation and led to a decrease of landraces which were adapted to a wide range of biotic and abiotic stresses over centuries (Damania 2008; Gepts 2006; Tanksley and McCouch 1997). Furthermore, intensive agriculture resulted in a loss of native habitats by causing fundamental damage for the surrounding environmental components. Fertilization affects water quality and irrigation may enhance salinity of agricultural land (Gepts 2006). A short-term benefit of industrialized agriculture is the productivity increase in but on a long-term scale the loss of unique genotypes appeared in addition to ecosystem damages (Foley et al. 2005). Although the green revolution enhanced genetic erosion, the demographic transition in developmental countries and globalization of communications were other critical factors accelerating diversity loss (Sutherland 2003). Therefore, the conservation of PGR through seeds or plant tissues is required to maintaine the original genetic structure of desired plant species.

2.1.2. Conservation of plant genetic resources

The Russian botanist and ecologist Nicolay I. Vavilov was one of the first scientists who called for the conservation of PGR. He demonstrated that plants were not randomly domesticated in some regions (Fig.1). In Vavilov's theory, eight centres of origins exists which vary in the diversity of the major crops and its utilization of plant species belonging to the same family. These centres were divided into:

1. The tropical south Asiatic centre (coconuts, rice, sugarcane)
2. The east Asiatic centre (Chinese cabbage, soybean)
3. The south western Asiatic centre (cucumber, eggplant, pigeon pea)
4. Middle East centre (wheat, barley, oats)
5. The Mediterranean centre (almond, cabbage, olive)
6. The Abyssinian centre (coffee, sorghum)
7. The Central American centre and (maize, tomato)
8. The Andean (South American) centre (pepper, potato, rubber)

One or two centres of origin were defined for each crop depending on its first domestication and its dispersal to other areas in the world (Börner 2006; Dorofeyev 1992).

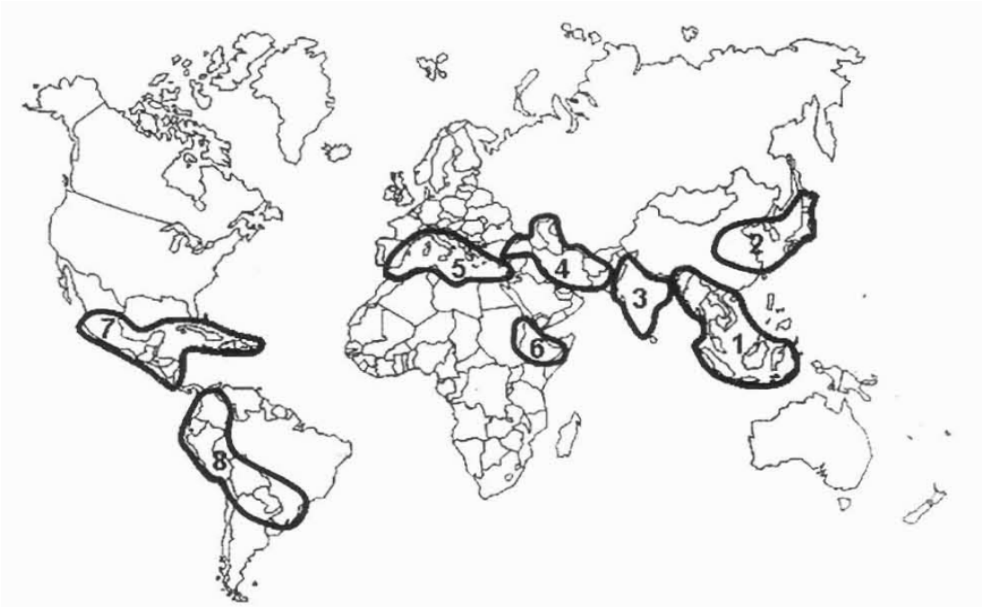


Fig. 1. The genetic centres of origin: According to Vavilov's theory the origins of major crops are explained by numbers from one to eight. Source: Börner 2006

Following the 'centers of origin' theory, several expeditions outside Soviet Union were started from 1916 to 1924 to collect plant genetic resources from Iran (1916), South America, United States(1921, 1930, 1932), Afghanistan (1924), Mediterranean, Lebanon, Syria, Ethiopia (1926 , 1927) and central Asia (1929) (Damania 2008). Additionally, in 1910 F. Cook has been sent by USDA to Syria and Palestine for collecting the wild type of durum wheat (*T. dicoccoides*) and later E. Schultes to Amazon River basin in South America for collecting rubber. The further benefits of PGR collections are the chances to find resistances of biotic and abiotic stresses and genotypes with higher yield potential. In this context, interesting exploration and utilization success story was mentioned by F. Knowles about his visit to Egypt in 1958. During his visit he collected lettuce germplasm which was known for

its high oil productivity and disease resistance and successfully cultivated in Upper Egypt, (Kena). The present *mo* allele then was transferred to four Californian lettuce cultivars and produced strong resistance to mosaic virus (Damania 2008).

The past collection missions encouraged recent scientists to search for optimum collecting procedures and conservation methods of unique stocks. In general, PGR can be conserved in two major ways *ex situ* and *in situ*. *Ex-situ* (off-site) conservation aims to conserve the genetic resources in gene banks using special facilities (Damania 2008; Gepts 2006). Gene banks are considered as a source of diversity for commercial crops which are aimed to support new breeding programs and research. Most cultivated plants are maintained as seeds which are considered as practical, cheap and accessible comparing with *in situ* conservation (Esquinas-Alcazar 2005). In addition, so-called seed banks, are considered as fundamental pillar for food security (Börner et al. 2014) . Till 1975 eight gene banks had been established worldwide (Damania 2008). Nowadays, about 1,750 gene banks house 7.4 million accessions (Fig. 2). The largest gene bank collections are hold in USA, China, India, Russia, Japan, Mexico, Germany, Syria and Philippines (FAO 2010). Most accessions are cereals which recorded 45% from total collections, followed by food legumes with 15%. Vegetables, fruits, forage crops, root crops, tuber crops and finally oil and fiber crops combine only 2 to 9%, respectively, from total collections until recently (Börner et al. 2014) (Fig. 3).

Fig. 2: Established crop gene banks around the world adjusted according to the accession numbers known by the FAO, 2010. The green column shows the Svalbard Global Seed Vault (SGSV).

The Svalbard Global Seed Vault (SGSV) represents a backup gene bank for all *ex situ* collections worldwide (Fig. 2). It was established at the Norwegian island of Spitsbergen in 2008 and was digged in the permafrost 130 meters deep in mountains. The security samples housed in SGSV reached 824,625 accessions of 4,740 species in 2014

(http://www.nordgen.org/sgsv/) . The majority of insured accessions were collected from Asia (307,501), North America (180,273) and Africa (125,320) (FAO 2010). Until recently, different methods were applied in *ex-situ* conservation such as dry ambient storage, cold storage, ultra-dry storage, vacuum storage, above saturated salt solutions or surrounds controlled gases e.g. nitrogen or CO2 (Copeland and McDonald 1995; FAO 1987).

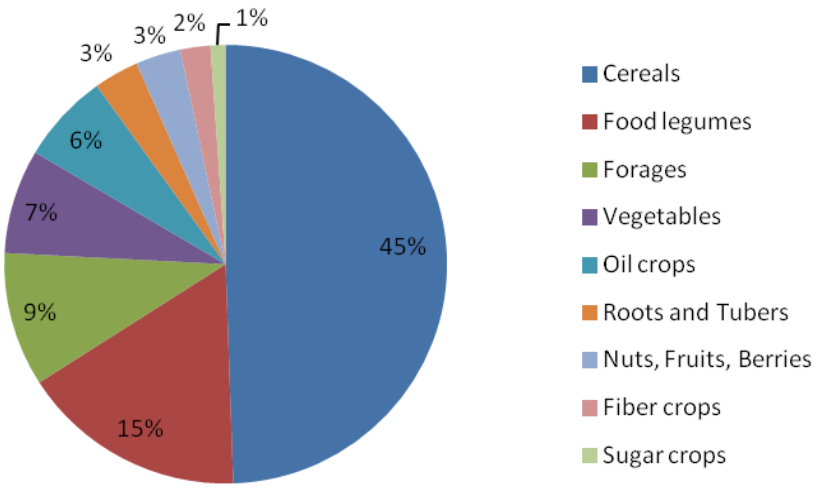


Fig. 3. Collections of major plant genetic resources maintained in gene banks worldwide (FAO 2010) .

The second conserving way of genetic resources is the *in-situ* (in-site) conservation which intended to maintain crop populations and its wild relatives in their natural ecosystem. Over centuries landrace populations were grown close to their wild relatives which allowed the exchange of genes and cause extreme wide diversity. That comprehensive diversity is difficult to be conserved in *ex-situ* collections which are only able to maintain 50 to 150 plant samples per site. However, ex situ collection, play central roles to conserve several varieties which have been disappeared from the field. (Damania 2008; Esquinas-Alcazar 2005).

In gene banks, seed materials are mostly conserved under cold storage conditions, because the majority of the materials are orthodox seeds that resist low temperatures (-20) and low relative humidities e.g. cereals, as wheat, barley, maize and rice, many legumes as bean, chickpea, lentil and soybean but also. onion and cabbages (3-7%) (McDonald 1999). In comparison, recalcitrant, non-desiccation-tolerant, seeds which include tropical and herbacouse plants from humid areas are conserved in field gene banks and/or *in-vitro* or by cryo-conservation (Börner et al. 2014). However, 90% of gene bank material is stored as

seeds. Therefore, seed longevity under different storage conditions is a matter of concern for scientists, nowadays. Seeds of different crops have different longevity and even seeds from the same species produced at the same conditions showed different germination behaviours after same storage period (Nagel and Börner, 2010; Walters et al. 2005c). These differences occurred according to different morphological, physiological, chemical, physical and genetical changes among the individual seed sets which will be discussed later.

2.2. Oilseed rape (*Brassica napus* L.)

2.2.1. Origin, characteristics and production

The *Brassicaceae* family comprises a wide genetic diversity which is varying but also resembles in their species characteristics. In total, it includes 3,709 species and 338 genera (Warwick et al. 2009). Among these species the genus *Brassica* occupies a highly important place as it comprises many economical crops such as oilseeds, condiments and vegetables. Domestication of *Brassica* crops widely took place in India (4000-1400 years BC), China (1,122 years BC) and in medieval Europe. Most common *Brassica* species include oilseed species such as oilseed rape (*Brassica napus*), turnip rape (*B. rapa*), Ethiopian mustard (*B. carinata*), Indian mustard, (*B. junica*) as well as *B. oleracea* which include cabbage, broccoli and cauliflower in addition to edible root species and condiment and spice plants such as *B. nigra* (King 2006; Snowdon et al. 2007).

The World production of oilseed rape reached 67.6 million tons in 2013-2014 which is second after soybeans with 218.6 million tons. The production is still increasing mostly in Canada and Europe. In Canada the optimum climatic conditions increased the production by 10% more than in 2011-2012. However, production increase was also reported in China and Australia (FAO 2013).

Advanced breeding programs aiming to produce edible rapeseed oil with low erucic and glucosinolate acids (Low Erucic Acid Rapeseed- LEAR) have greatly influenced the increase of oilseed rape production (Snowdon et al. 2007). More challenging was the breeding of high oleic and low linoleinic acids varieties (HOLL) which considered to be lower in saturated lipids favourable for food productions that needs high temperature requirements during cooking (Mailer 2009). As a result of *B. napus* breeding, Canola (Canadian oil) quality was originally produced which include all LEAR varieties that contain erucic amount lower than 2% (Kimber and McGregor 1995). Additionally to edible oils, oilseed rape has advanced

applications to produce biodiesel as a source of energy and rape meal for livestock feed having high protein amount (Snowdon et al. 2007).

2.2.2. Genomic and Genetic studies

The genetic variability within in oilseed rape is very narrow especially with the relatively limited geographical domain (Hasan et al. 2006). This dilemma increases gradually by increasing the attack of biotic and abiotic stresses as consequence of environmental and climatic changes which specifically intended *Brassica* crop. *Brassicaceae* family comprises diverse species which encourage studying genetic diversity by investigating the genetic relation between species to find new diversity sources (Gali and Sharpe 2012). Recent studies revealed wide diversity within *Brassica* genera and introduced new germplasm which could improve the important agronomic traits for oilseed rape breeding (Warwick et al. 2009). Comparison between relative species on the genetic level is now possible by the availability of high density markers which can be used for various advanced genetic mapping procedures. Additionally, the linkage between specific genetic markers and the desired breeding traits through marker-assisted selection technology allowed more efficient breeding programs. (Gali and Sharpe 2012). *B. napus* (AACC n=19) is an amphidiploid species derived from two original ancestors, the diploid Asian cabbage or turnip *B. rapa*, (AA n=10) and the Mediterranean cabbage *B. oleracea*, (CC n=9) which both has formed the *B. napus* genome of 1,130-1,240 Mbp (King 2006; Snowdon et al. 2007). Due to the high identity between genetic sequences of *B. napus*, their ancestors and Arabidopsis which occurred though duplications or chromosomal arrangements of a common ancestor (Parkin et al. 2005) comparative studies have been successfully applied. Additionally, linkage maps were extensively constructed to reveal the bases of evolutionary relationship between diploid and tetraploid species and revealed economical complex traits (Gali and Sharpe 2012). In 2014, *B. napus* genome was fully sequenced (Chalhoub et al. 2014) which allows the possibility to identify the favourable regions and genes controlling complex agronomic traits.

Genetic mapping

The phenotypic differences among species are regulated by complex genetic bases. These phenotypic variations are mainly influenced by functional groups of genes (genetic effect) and the environmental effects in addition to interaction between those genes and environment. Genetic mapping is a powerful tool to screen the genetic bases of complex traits and to determine the loci on a chromosome which controlling such traits. This tool is depending on

the link among population individuals in both genetic (segregation of polymorphic markers) and phenotypic (change in phenotypic behaviour) levels. The genetic map for a population is constructed based on calculations of frequency of loci recombination and the number of genetic markers along chromosomes (Collard et al. 2005).

The application of linkage mapping is depending on using genetic markers and suitable segregating mapping population. A cross between two individuals, from same or relative species, that differ in their appearance of certain traits, led to production of several mapping populations which produce progenies segregating differently according to the breeding method (Gali and Sharpe 2012). Most linkage studies are using recombinant inbred lines (RIL), double haploids (DH) or back crossing (BC) populations as immortal and homozygous genetic background. A single seed of F2 individual is used to produce RIL population with more than 99% homozygosity in the eighth or ninth generation in addition to high recombination level (Pink et al. 2008). DH is considered a fast alternative of RIL which produce highly homozygous individuals through *in vitro* propagation of anther or microspore of F1 plants which represents the desired phenotype for a trait of interest (Thiagarajah and Stringam 1993). By the repeated backcrossing and selection of best trait expression, the near isogenic population (NIL) is produced. According to the objective of mapping study the suitable population should be chosen depending on the advantages and disadvantages of each individual in regards of variability in meiotic events for each loci and percentage of chromosomes homozygosity. Disadvantages of RIL and DH are the difficulties to distinguish the distinct population lines for QTL studies due to the high homozygosity and inaccurate allele segregation records for certain loci. However, the advantage is that these populations are easily to produced and informative tools for QTL studies. On the other hand, NILs are difficult to produce but they are most appropriate for fine mapping and accurate to detect the minor QTLs which are disappeared using other mapping populations (Gali and Sharpe 2012).

The second step to arrange genetic linkage study is to construct a genetic map for the selected population using genetic markers for each individual in the tested population which cover part of their genome size with known distances between markers. The linkage between genetic and phenotypic behaviours of a certain individual may result in QTL that may affect the studied trait. There are different kinds of genetic markers which differ in their qualifications and upon them the resolution of the linkage map is ranged. Before common application of polymerase chain reaction technology (PCR) two genetic markers were used to construct genetic maps, Isozyme (protein-based marker) and restriction fragment length polymorphism

(RFLP), DNA-based markers (Botstein et al. 1980). Later the PCR-based markers were used depending on design of polymorphic DNA pieces which varied in size. The first group of marker types was random, untargeted, non-sequence based markers such as random amplified polymorphic DNA (RAPD) (Williams et al. 1990), amplified fragment length polymorphism (AFLP) (Vos et al. 1995) and simple sequence repeat (SSR) (Zietkiewicz et al. 1994). This group followed by more advanced markers, sequence-based and dominant such as diversity array technology (DArT) (Jaccoud et al. 2001) and sequence-related amplified polymorphism (SRAP) (Li and Quiros 2001). Recently, the sequence targeted markers is markedly used for producing high resolution linkage maps such as simple sequence repeat (SSR) and Single Nucleotide Polymorphism (SNP) markers. (Gali and Sharpe 2012).

SSR markers are co-dominant and locus-specific markers. They are produced based on PCR amplification of short repeats ranged from 2-6 nucleotides which differently characterized among related individuals or species (Schuelke 2000). SSRs are used for genetic mapping of *Brassica* to identify the sequence information of A, B and C genomes and their relations (Marquez-Lema et al. 2010). The increase of sequence information of *Brassicacea* provided wealthy information on more SSR markers which consequently helped finding orthologous loci in the same or related species (Cheng et al. 2009; Hasan et al. 2008; Iniguez-Luy et al. 2008; Ling et al. 2007). Mapping QTLs using SSRs alone or together with RFLP and AFLP markers have taken place widely for the last ten years. In *B. napus*, seed yield and their related traits have been investigated by (Quijada et al. 2006; Udall et al. 2006). DH population used by Cao et al. (2010) revealed new QTL representing erucic acid content on chromosome A8, in addition, seven seed oil content QTLs on the same chromosome were detected. Mineral accumulation in seeds has been investigated by screening ion uptake and transporter orthologs genes of *A. thaliana* and mapping them on *B. napus* chromosomes using *in silico* approach (Liu et al. 2009). This link was confirmed with association of 21 ion genes in *A. thaliana* with mineral accumulation QTLs in *B. napus* (Ding et al. 2010). Additionally, phosphorous stress genes in *A. thaliana* were detected in the interval of root morphology in *B. napus* (Yang et al. 2010) and 74 QTLs were detected under low and normal phosphorous conditions which controlled the seed yield and yield-related traits. 161 orthologous representing 45 QTL in *B. napus* were detected in *A. thaliana* controlling phosphorous amount and yield traits (Ding et al., 2012). Boron deficiency is one of the major reasons that affect yield limitation, depending on its important role to control root and shoot growth. Shi et al. (2012) screened the regulated QTLs of boron accumulation in shoot and root under normal

(27 QTL) and low boron (18 QTL) conditions. Nine QTLs represented the thousand seed weight (TSW) of *B. napus* in DH population which appeared on six linkage groups (LGs), while two of them were identified on LG A7 in both reproductive years of the population (Fan et al. 2010). In further study the silique traits were investigated by SSR and AFLP markers. In different environments, 26 QTLs were identified on 10 LGs from which the QTL *qSL.N8-1* was detected in four environments and other three and seven QTLs were overlapped in three and two environments respectively (Zhang et al. 2011). The same markers were used in recent study in combination to new added SSRs to colinear the homologous loci between the progenitors *B. rapa* and *B. oleracea* with *B. napus* in order to predict the candidate genes underlying seed size and weight through e-PCR and BLAST approaches using the available sequence data of both progenitors and *A. thaliana* (Cai et al. 2012). Yang et al. (2012) determined major QTL on LG A9 for silique length and seed weight of *B. napus*.

In contrast to SSR markers, SNPs are co-dominant markers which display the nucleotide variations within genome. Because of their abundance and genome covering they are widely applied. Utilization of SNPs allows the establishment of high resolution genetic linkage maps that are used for several applications such as fine mapping and map based-cloning (Hanemann et al. 2009).

Brassicaceae were the first group of plant species subjected to wide discovery with SNP markers specifically in *A. thaliana* using 37.000 SNPs to compare between accessions Colombia and Landsberg (Jander et al. 2002). The new sequencing technologies such as Illumina increased the chance to detect the genome-wide nucleotide differences by dense screening of transcriptome sequence. Trick et al. (2009) identified several thousands of SNPs in parental genotypes which showed 80% duplicated SNPs due to the duplicated nature of amphidiploid *B. napus* and suggested the need for more advanced analysis methods (Parkin et al. 2010).

Different genotyping methodologies are known for SNP markers. The single-plex assay (SPA) and the parallel multiplex assay (PMA) methodologies which can be used in wide range of applications. SPA for example is used in marker assisted selection between population individuals (Jena and Mackill 2008). However, PMA is used for generating high density maps and association mapping (Hyten et al. 2010). PMA SNPs are developed by GoldenGate and analysing high level of multiplexing such as PCR products specific to highly parallel alleles (www.illumina.com). The development of MassArray such as Illumina arrays

in Brassicaceae species allowed the accurate selection of nucleotide sequence variations in different individuals and species (Gali and Sharpe 2012).

Association mapping

Association mapping analysis is an approach established to link markers with the complex traits depending on linkage disequilibrium (LD) decay of natural diverse populations (Flient Garcia et al. 2003). The LD is the non-random association of alleles of two or more polymorphic loci. It is calculated using the genetic distance between loci and the number of generations in the population since it is originated (Mackay and Powell 2007). This methodology allows the application of highly accurate mapping by employing the ancient occurred recombination among population lines (Zhu et al. 2008). Association studies aim to analyse candidate genes in particular loci which are directly linked to the trait of interest and to conduct genome-wide association studies (GWAS) that screen the linked QTLs across the whole genome. The latter is mostly used for highly complex traits that are affected by different factors and governed by group of genes. The first association study in plants took place in maize in which the candidate genes linked to flowering time were investigated (Thornsberry et al. 2001).

In *Brassica spp.* first association mapping studies started in *B. rapa* (Zhao et al., 2007) followed by allelle-trait association studies using genome wide SSR markers in *B.napus* which identified the SSRs linked to glucosinolate content (Hassan et al. 2008). By the increase of *Brassica* genome sequence data and detection of SNPs across genomes, highly dense maps were constructed. In 20 genotypes of *A. thaliana* one million SNPs were detected by Clark et al 2007, while a GWAS study using 250,000 SNPs were used to screen inbred population (Atwell et al., 2010). This abundance and availability of array sequence and high throughput SNP genotyping shows the expected effect of screening the genetic bases of important agronomic traits. Till 2010 the application of GWAS was not used in *Brassica spp*.

2.3. Seed longevity

2.3.1. Factors affecting seed longevity

The seed deterioration process gradually starts after the complete maturation on the mother plant. The environmental conditions during growth and post-harvest influence stored seed compositions and the final vigour (Walters et al. 2005c). Unfavourable conditions during production led to the increase of seed damage subsequently, cause higher production of free

fatty acids that reduce the oil quality. As soon as seeds are in storage, the biological changes, so-called deterioration processes are initiated. These ageing mechanisms distinguish between genotypes of species even under the same storage conditions and emphasize the genetic reasons behind these results. Seed maturity is very critical factor for high seed quality. Mature dark oilseed rape seeds have higher germination than immature red/orange coloured seeds which conflict the importance of seed degree of maturity during harvest time on seed quality. (Stokes et al. 2000). Under storage condition the most important factor is the seed moisture content which is influenced by the hygroscopic properties especially the absorption and loss of water until the equilibration of the relative humidity. This induces in combination with the storage temperature enzymatic and biological activities (Robert 1972).

2.3.2. Biochemical and physiological changes

As previously mentioned, one of the most important factors influencing seed longevity is the content of water inside seeds. Seed hydration level can be categorized to five stages according to (Walters et al. 2005a). For orthodox seeds, in the first hydration level (water potential -200 Mpa) seeds are dried to lowest seed moisture contents, which enhance degradation reactions and decrease longevity. In the second hydration level (≥ -15) the seeds are still in the dry, glassy state of cytoplasm in which the molecular mobility is extremely decreased. This glass matrix is an amorphous, solid state which is formed depending on the special ability of carbohydrates to replace the hydrogen bonding of water and protect degradation of cell membranes. In the third hydration level with water potential from -15 to -5 Mpa the cells are not in the glassy state and loss their membrane integrity. Due to higher moisture content free radicals are produced from different sources. In the fourth hydration stage repair mechanism are initiated by synthesizing the antioxidants and heat shock proteins which prevent protein damage in addition to synthesis of DNA replication and translation enzymes. Fully hydrated seeds reached water potential of ≥ -1 which encourage the initiation of cell division and enhance the metabolic processes (Hydration level five). The glass transition temperature controls the transition from a glass to a liquid state and is influenced by the chemical composition and the seed moisture content. Strong glasses existent in hydration level one and two resist higher temperature before structural change in the matrix occur. At higher moisture contents seeds are affected by small changes in temperature and more oxidative reactions influence seed longevity.

Oil is the main seed storage compound in several plant species including sunflower and oilseed rape. The storage lipids are accumulated in oil bodies in the form of triacyglycerol (TAG) which are gathered during maturation and act afterwards as resource during germination and development (Graham 2008; Theodoulou and Eastmond 2012). Oilseed rape lipid consists of five acyle groups which varied in their composition between species and genotypes. These acyle groups are considered as the major components of the cell membrane and are named as following: palmitate (16:1), searate (18:0), oleate (18:1), linoleate (18:2) and α-linolenate (Starner et al. 1999). The type and composition of fatty acids in oil seeds influence the occurred chemical reactions and their extent during storage (Morelló et al. 2004). Oilseed rape has lowest amount of saturated fatty acids and moderate content of poly-unsaturated fatty acid. Free, unsaturated fatty acids encourage chemical reactions occurring at the double bonds and determine oil susceptibility to oxidation process and consequently peroxidative degradation (Ghasemnezhad and Honermeier 2007).

Lipid peroxidation is mostly the reason behind seed viability loss and cause the reduction of lipid quality during storage. The increase of lipid peroxidation damages cell membrane and results to accumulation of toxic sub-products which speed the seed deterioration (Schwember and Bradford 2010b). The initiation of lipid peroxidation is caused by free radicals which contain unpaired electrons and appeared as active form of oxygen named as reactive oxygen species (ROS). Those can work in forms of superoxide radical (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH°) (del Rio et al. 2002; Tabak et al. 1999). ROS can be enhanced by the increase of biotic and abiotic stresses and interact with the poly unsaturated fatty acids which led to extreme oxidative damage of cellular membrane and nucleic acids (McDonald 1999; Smith and Berjak 1995).

The degradation of fatty acids was firstly observed in seed stored at high temperatures and relative humidities (RH) e.g. ambient storage conditions (Mladen et al. 2012). Fresh maize, sunflower and soybean seeds stored for four years at moderate and high temperature in combination with high relative humidity (75% RH - 25^{o}C & 60%RH - 12^{o}C) dramatically decreased the seed oil content. A significant decline in oil content was remarked among other species and the genotypes within one species (Šimic et al. 2007). de Souza Abreu et al. (2013) displayed that the accelerated deterioration of oil seeds depends mainly on the storage environment and the chemical composition of each species. Cold storage at low seed moisture contents was the most effective environment for sunflower seeds. In this context, Walters et al. (2005b) demonstrate that lipid composition, lipid content, proportion of saturated and

unsaturated fatty acids and level of free fatty acids were significantly changed in accelerated aged sunflower seeds for 5 days at 45°C and 100% RH comparing with untreated seeds, while a decrease in poly unsaturated fatty acids and increase in free fatty acids was observed in double aged seeds under same conditions, where no germinated seeds were resulted. Therefore, the TAG-peroxidation considered as indirect consequence of seed deterioration and loss of viability, because the increase of poly unsaturated fatty acids are usually detected in highly deteriorated or died seeds (Walters 1998).

Seed deterioration process concur a gradual decrease in saturated fatty acid content and inversely high amount of malondialdehyde. The low expression of membrane-lipid hydrolysing phospholipase D (PLDα1) in Arabidopsis improved the oil stability and seed longevity (Devaiah et al. 2007). In addition, the suppression of PLDα in transgenic soybean seeds enhanced the germinability from 30 to 50 % comparing with the wild type seeds (Lee et al. 2012).

2.3.3. Protein changes

The damaging of membrane proteins is caused by lipid peroxidation, and lead to the reduction of enzyme activity by oxidation of protein thiol group. Additional, a non-enzymatic chemical reaction which decrease seed viability and proceed under very low moisture content is the Maillard reaction. This reaction is induced by glycosylation and led to reduced sugar and protein compounds which change the available forms to amines groups and glycated protein, respectively (McDonald 1999). Loss of seed viability and vigour by increase of accumulated Maillard products was observed in long-term storage (Sun and Leopold 1995) and after high temperature and relative humidity storage (Murthy et al. 2002).

2.3.4. Antioxidants changes

Antioxidants play an important role to keep the high seed vigour and viability. They prevent lipid peroxidation by enhanced ROS scavenging and prevent their reaction (Bailly et al. 2002). The reactions speed is widely varied according to species properties which are affected by surrounded temperature and seed moisture content (Walters et al. 2010). The protection mechanisms involving peroxide-scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) which react with ROS and control their effects on cellular biological processes (Foyer et al. 1991). Superoxide dismutase is the major enzyme that is responsible for regulation of radical cellular

concentration to avoid lipid peroxidation damage (Bowler et al. 1992). Peroxidase (POD), CAT and APX play an important role to reduce hydrogen peroxide and prevent oxidation stress (Weckx and Clijsters 1996). The efficiency of radical scavenging is considered as important factor for high seed vigour. A correlation between lack of germination and seed degradation during accelerated ageing and decrease of SOD, APX and LOX in peanuts and CAT and GR in sunflower was remarked by (Bailly et al. 1998; Sung and Jeng 1994).

Non-enzymatic antioxidants such as tocopherol are lipid-soluble compounds which produced just in photosynthetic organisms. Tocopherols are activated to protect the poly unsaturated fatty acids from oxidation damage by inhibiting the peroxide formation. Oilseed rape contain four different forms of tocopherols which divided to major types α-tocopherol and γ-tocopherol (64% and 34% respectively) which contributed with 800 mg/Kg oil, while β-tocopherol and δ-tocopherol contribute with very minor amount (about 1%) (Olejnik et al. 1997; Ratnayake and Daun 2004). The amount of α-tocopherol and γ-/α-tocopherol ratio was decreased in stored seeds (Goffman and Mollers 2000). The loss is higher when seed moisture content increases to 15% and temperature to 30°C while α-tocopherol decreases to a higher rate than γ-tocopherol. In addition 24% loss of plastochromanol-8 amount (PC-8), which is combined with tocopherols facilitate the loss of lipid stability (Gawrysiak-Witulska et al. 2011). Storage of crushed canola and flax seeds for 60 days in ambient and cold conditions had decreased the tocopherol to 50%.

2.3.5. Biophysical changes

The production of vigorous seeds is a combined process which is initiated with seed development. Orthodox seeds develop in three stages named embryogenesis, reserves accumulation and desiccation of mature seeds (Kermode 1995). Each of these stages is organized and regulated by different cellular signals and events which led ultimately to formation of high quality, vigorous and viable seeds under wide range of environments. The drying phase of mature seeds is believed to have a critical role for seed vigour (Ellis 1996), where metabolic processes are reduced, carbohydrates accumulated and several unique cellular events take place as a result of decrease in seed moisture content (smc) to reach 30 to 35 % (Vertucci and Farrant 1995).

TAGs which are considered the main composition of lipids in oil seeds (90 to 95%) are stored as oil bodies in the cotyledons (Fig. 4-A). During cooling at 10°C min^{-1}, TAGs crystallized to three forms α, β′ and β which differ in their stability depending on their melting points and

backing density (Lehner et al. 2006). The biochemical and molecular lipid changes occurred in the dry, glass state is accompanied by biophysical changes which affect the size and organization of oil bodies. These changes could be screened in the three developmental stages of immature, filled and mature sunflower seeds using the differential scanning calormetry (DSC) and subjecting seeds from each stage to cooling and thawing cycles. Oil bodies of immature seeds varied dramatically in size and sub-cellular organelles had high metabolic activity which helps the filling of oil bodies in the following stages (Fig. 4-B). Enlargement of oil and protein bodies increased rapidly till the end of seed filling stage, while amount of visible cytoplasm was still available and disappeared in the complete mature seeds by occupation of oil bodies of the complete cell space (Lehner et al. 2006).

Re-warming of cotyledon cells to study TAGs thermal behaviour revealed both endo- and exothermic changes in filled and mature seeds which was not the case for immature seeds. The formation of α crystals which is highly stable occur just in mature seed which melt at about -75°C, while filled and immature witnessed formation of β′ crystals which is less stable and less melting point (-40°C to 0°C). The temperature at which transition from glass to rubber or liquid state (Tg) is decreased is dependent on the increase of smc. It ranges from 80°C to 30°C in highly moistened seeds to extrem dry sunflower seeds, respectively. The recorded transistion temperature is 10°C higher in mature seeds than those recorded in immature and filled seeds, which confirmed more physical stability in dry seeds (Lehner et al. 2006).

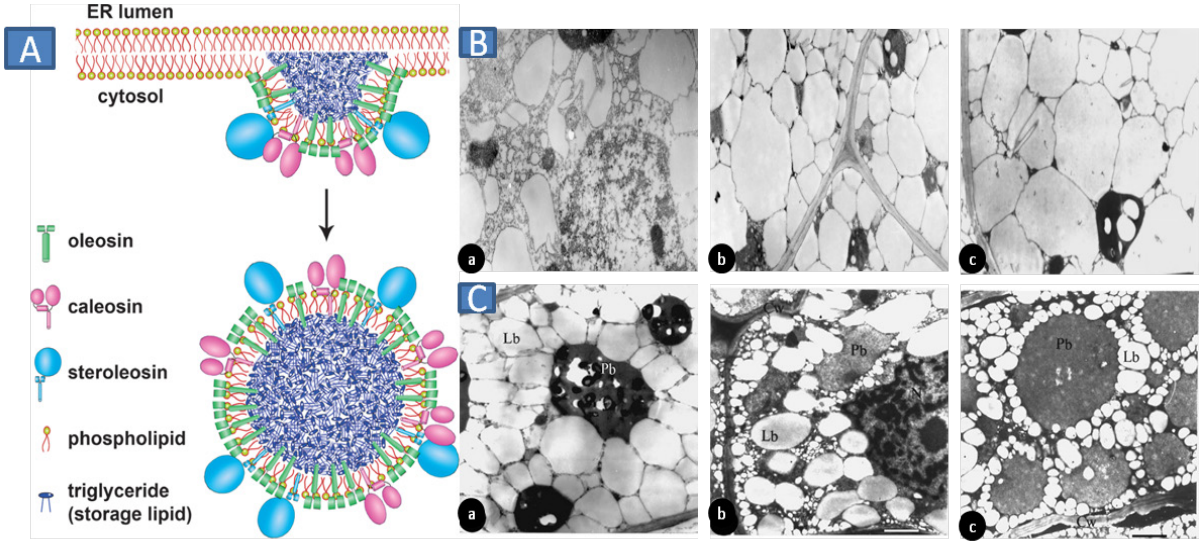


Fig. 4. A: the structural formation of oil bodies in seeds ((Shimada and Hara-Nishimura 2010). B: Electron micrograph of cotyledon cells of sunflower seeds during developmental stages before maturation and after maturation. a- b & c respectively after 29, 42 and 58 days after anthesis (Lehner et al. 2006). C: Electron micrograph of cotyledon cells in sunflower dry seeds of a- control, b- primed and c- aged seeds (Walters et al. 2005b).

A similar study took place to compare the physical properties of fresh dry seeds and aged hydrated seeds. In hydration level 4 which describe the imbibed seeds of water potential between -2MPa to -5 MPa, seeds could behave both ways of faster germination (priming) or faster deterioration depending on the surrounding temperature (Walters et al. 2005b). The priming take place at low temperature (about 15°C) and higher water potential which enhance the metabolic processes (-5 Mpa), while seed deterioration or accelerated ageing occur at lower water potential (-2 Mpa) which is low enough to prevent starting of germination. Remarkable TAGs are considered as major storage reserves within the seeds which are highly affected by seed deterioration processes especially ROS attack (Smith and Berjak 1995).

The results also revealed visual demonstration of changes occurred in lipids in both priming and ageing. In untreated seeds, majority of cotyledon cells was occupied by protein and lipid bodies where two sizes dispersed in the cytoplasm of primed seed cotyledons (Fig. 4-C). Additionally, the oil and protein bodies in aged cotyledons compared with primed cotyledons contained less dry matter reserves and led to conclusion of less mobilization of dry matters during seed ageing (Walters et al. 2005b). Different forms of crystalline were obtained according to temperature of transition and tendency of crystallization as following, α crystals which melting between -110°C and -80°C, glass-like which turns to baseline shift at -65°C and β crystals which melting between -35°C and 5°C. 90% of the lipids crystallized as β crystals and α and glass-like structures are not stable under annealing stage at -60°C.

The thermal behaviour of lipids during ageing could be detected by the increase in melting temperature of β crystals (-33°C to -28°C) in which the crystallinity percentage increases when the crystallization rate decreases. In addition, the increase of non-lipid components into TAGs induces the ageing procedure by increasing the β crystals melting temperature and affects the kinetics of crystal formation (Walters et al. 2005b). To conclude, a case study in sunflower seed displayed that success of germination is mainly dependent on the availability of oil bodies which control the supplementary of carbohydrates necessary for seedling development. Changing architecture of these oil bodies during ageing is preventing the availability of lipids and cause lack of mobilization which cause germination delay.

2.3.6. Genetics

Genetic studies of important complex traits were started by the availability of diverse molecular markers which allow the screening of variable alleles for advanced breeding programs. The genetic bases of seed longevity were examined firstly in Arabidopsis where RIL population was screened for seed-soluble oligosaccharides and seed viability after controlled deterioration (CD) experiments (Bentsink et al. 2000). Major QTLs were identified for both traits and were detected in different chromosomal positions which indicated that seed longevity is not depending on oligosaccharides content. Additionally, another RIL population was investigated for several traits such as seed germination, germination speed, dormancy and sugar content after CD experiment (Clerkx et al. 2004). Three QTLs were detected on chromosomes one, three and four related to seed longevity after CD.

Additional oil crops were investigated such as soybean, lettuce and oilseed rape. In soybean, SSR markers were used to screen the F2:3 populations after accelerated ageing test (AA). Four QTL on different chromosomes were significantly detected (Singh et al. 2008a), whereby two of them Satt538 and Satt434 found to be associated with seed coat permeability (Singh et al. 2008b). QTL mapping was performed for lettuce RIL population after storing seed under several temperatures and constant RH of 30% and they compared the seed behaviour with other CD experiment (Schwember and Bradford 2010a). No overlap was found between QTLs in both experiments which suggested that CD could be governed by different gene groups than conventional storage. Seed longevity was identified using YE2-DH population of winter oilseed rape (Nagel et al. 2011). Three different experimental ageing methods were performed in 153 lines which resulted in 13 QTLs related to seed longevity. Although majority of QTL were treatment specific and the comparison with Arabidopsis showed a co-localized QTL-8 on loci which regulated genes for germination process and stresses tolerance. This result draws an attention for the link between seed longevity and their ability to avoid surrounding stresses. In rice, QTLs for seed longevity were identified using backcross inbred lines (BILs). The investigations revealed three QTLs linked with seed longevity on chromosome two (Miura et al. 2002), while another three QTLs were detected on chromosome nine (Zeng et al. 2006). The QTL *qRGR-9* on chromosome nine was confirmed in another study (Xue et al. 2008) which underlie this QTL as common QTL for seed longevity in rice. Several studies also took place in cereals such as barley, maize and wheat which underly seed longevity QTL specific for these crops (Landjeva et al. 2010; Nagel et al. 2009; Rehman Arif et al. 2012; Revilla et al. 2009)

3. Materials and methods:

3.1. Genetic bases of seed longevity in oilseed rape "*Brassica napus* L."- Linkage QTL mapping on DH-ExV population

3.1.1. Plant material

The mapping population was comprised of 122 DH lines from the cross of the inbred lines "Express 617" (derived from the German cultivar Express) and the high erucic acid line "V8" (Basunanda et al. 2007). Seed lots harvested in 2005 (ExV-5) and 2009 (ExV-9) were maintained under ambient conditions (20°C and 50% relative humidity at Giessen University until early 2011 and kept under same conditions in the Federal *Ex-situ* Genebank in Gatersleben, Germany, from 2011 until 2013 for further seed tests. In 2012, a third set of material (ExV-12) was multiplied and stored under ambient conditions for one year until tested in 2013.

3.1.2. Experimental design and phenotyping

The standard germination test (SGT) was applied to control seeds (CT) and after experimental ageing treatments following the International Seed Testing Association (ISTA, 2011) protocol. Three replicates of 50 seeds per seed lot were germinated on moistened filter paper and held for 16 h photoperiod at 23°C (light) and 25°C (dark). SGT was applied to CT seeds of ExV-5 in 2011 and 2013, representing six (ExV-5_CT1) and eight years (ExV-5_CT2) of ambient storage and to ExV-9 and ExV-12 (ExV-9_CT and ExV-12_CT) representing two and one years of ambient storage, respectively. Seeds of ExV-5, ExV-9 and ExV-12 were exposed to experimentally ageing treatments by applying controlled deterioration test (CD) as described by Hay et al. (2008). Here, seeds were equilibrated above 8.7 molar non-saturated lithium chloride (LiCl) solution at 20°C to reach 47% relative humidity (RH). After 14 days seeds were placed above 7.1 molar LiCl solution at 45°C to increase the RH to 60% (Fig. 5). Physiological germination is initiated with seed imbibition and terminates with radical emergence; while afterwards the seedling is established and considered as post-germination stage. For wide covering of traits related to seed longevity several parameters were measured before germination (seed weight), during germination (total germination of both normal and abnormal plantlets (TG) and germination speed) and after physiological germination (post-germination) concentrating on discrimination of normal and abnormal seedlings (Table 1).

The experimental ageing period is depended on the initial germination (CT) (Ellis and Roberts 1980), and was calculated according to following formula: $v = Ki - \frac{p}{10^{K_E - C_W \log m - C_H t - C_Q t^2}}$,

where *v* represents the viability (in probit) after *p* days of treatment, *m* represents the seeds % moisture content, *t* represents the temperature in °C, *Ki* represents the viability (in probit) at *p* = 0 days, *CH* and *CQ* are species-specific temperature constants, and *KE* and *CW* are the species-specific moisture content constants. The period used for ExV-5_CD was seven, for ExV-9_CD1 and ExV-12_CD1 forty and that for ExV-9_CD2 and ExV-12_CD2 fifty days (Table 1).

Table 1. Treatments and traits overview of ExV mapping population: In total nine traits were investigated: TSW before germination, TG, AUC, T_{50}, T_{10} during germination process and NS, Abn, R and RS during seedling development. The standard germination test was applied to control seeds (CT) before and after ageing by controlled deterioration test (CD).

Multiplication year		**2005 (ExV-5)**			**2009 (ExV-9)**			**2012 (ExV-12)**		
Treatment		**CT**	**CD**	**CT**	**CT**	**CD1**	**CD2**	**CT**	**CD1**	**CD2**
Storage/ Ageing period		**6y**	**7d**	**8y**	**2y**	**40d**	**50d**	**1y**	**40d**	**50d**
Abbreviation		**ExV-5_CT1**	**ExV-5_CD**	**ExV-5_CT2**	**ExV-9_CT**	**ExV-9_CD1**	**ExV-9_CD2**	**ExV-12_CT**	**ExV-12_CD1**	**ExV-12_CD2**
Thousand seed weight	TSW	x	-	-	x	-	-	x	-	-
Total germination	TG	x	x	x	x	x	x	x	x	x
Normal seedlings	NS	x	x	x	x	x	x	x	x	x
Abnormal seedlings	Abn	x	x	x	x	x	x	x	x	x
Root abnormality/ TG	R	x	x	x	x	x	x	x	x	x
Root - shoot abnormality/ TG	RS	x	x	x	x	x	x	x	x	x
Area under the curve (150 h)	AUC	x	x	x	x	x	x	x	x	x
Time to 50 % germination	T_{50}	x	x	x	x	x	x	x	x	x
Time to 10 % germination	T_{10}	x	x	x	x	x	x	x	x	x

The thousand seed weight (TSW) was measured prior germination. The follow-up SGT for each seed batch recorded the proportion of normal seedlings (NS), abnormal seedlings (Abn), and the proportion of TG. Germination had been recorded daily for 14 days, and data were used to analyse germination speed i.e. the time required to reach 50% germination (T50), the time required to reach 10% germination (T10) and the area under the germination time curve after 150 hours (AUC).

It was important to screen three speed parameters to investigate the high and low aged lines in each population, where T_{10} consider highly aged seeds, T_{50} considers the vigorous seeds and AUC after 150 hours which is the maximum period of germination test according to ISTA and

the curve area display the relation between the increase in germination and the increase in time.

Root abnormalities were classified into defective primary and secondary roots and the total disappearance of root system. Root and shoot (hypocotyls) abnormalities included seedlings having deficiencies in root system in addition to shoot (hypocotyls) defects such as dwarfed, ,infected, weak, broke or total disappearance of shoots (Fig. 6). The proportion of seedlings showing root abnormalities and root-shoot (hypocotyls) abnormalities were divided by the total number of germinated seed for calculating the traits R and RS respectively.

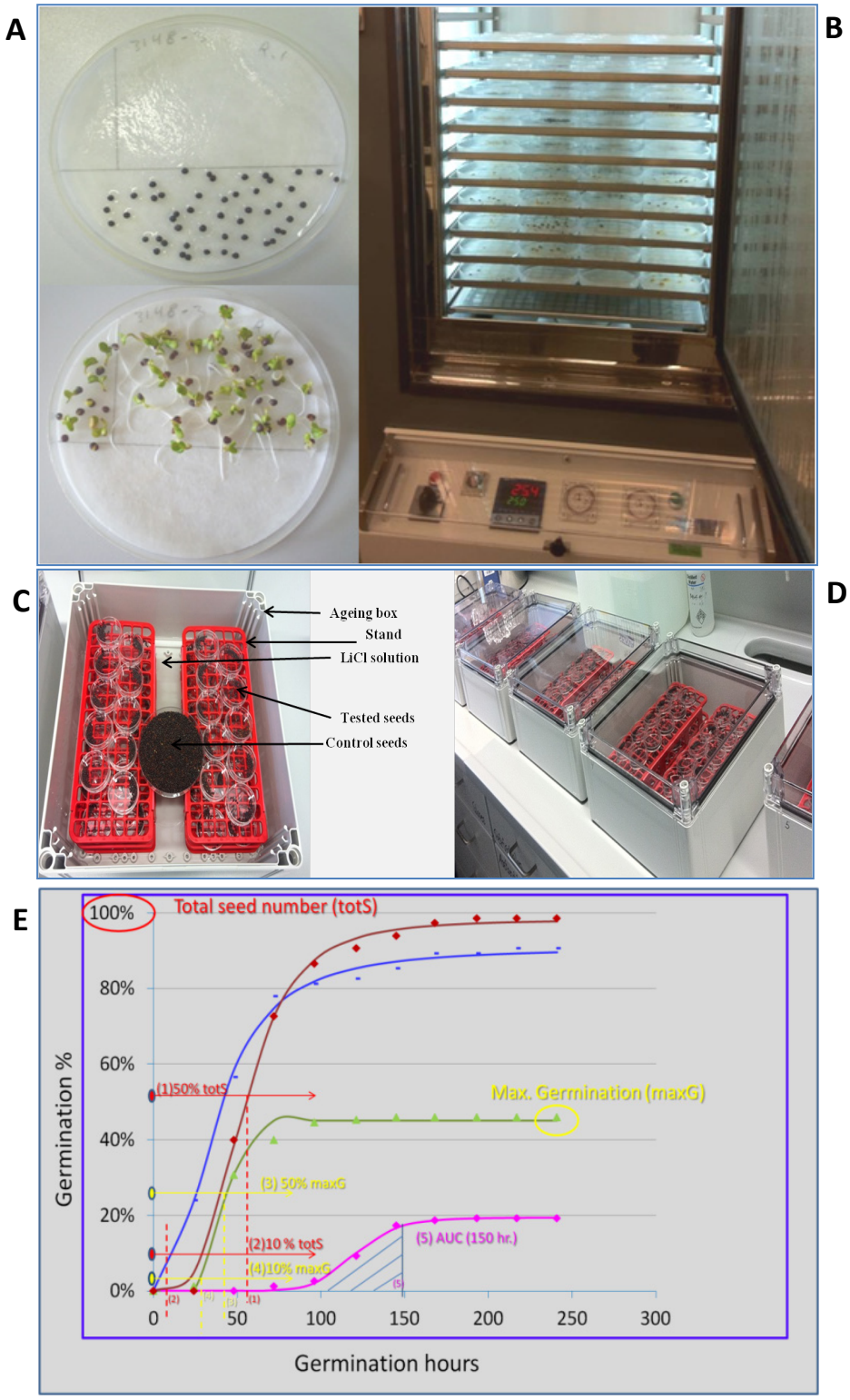


Fig. 5. The controlled seed deterioration procedure: A. germination test showing seeds after one day (upper Petri dish) and germinated seeds transferred to the empty half till complete germination (lower dish). B. The germination chamber using 20°C. C. Air-tight boxes filled with non-saturated LiCl solution. D. Seeds placed above LiCl. E. The germination curve for some ExV lines from GERMINATOR software shows 5 speed parameters: Two red arrows showing the time to reach 10% (T_{10}, lower) and 50% (T_{50}, upper) germination, the yellow arrows showing the time that genotype takes to reach 10% (lower) and 50% (upper) of their max germination and finally the area under the curve (AUC) after 150 hours of germination. In recent study only T50, T10 and AUC were considered.

3.1.3. Statistical analysis

GERMINATOR software (Joosen et al. 2010) was used to calculate the germination speed parameters AUC, T50 and T10. Data from 122 lines ExV-DH population were tested for significance by *t-test* and analysis of variance (ANOVA) followed by Holem-Sidak post hoc analysis or, if normality test failed, Wilcoxon signed-rank test and Kruskal-Wallis (ANOVA) on ranks followed by Dunn's analysis, as appropriate. The Standard deviation (SD) and Fisher's least significant difference (LSD) were determined to test the significance of means at level of 0.05. Further, normality of data was checked graphically by histogram of the observed frequency distribution. Correlation of coefficient was calculated to investigate relationships between the quantitative traits observed in each environment for the mean of all experiments. Calculation of traits repeatabilities (h^2) within each treatment replicates was calculated from the estimates of genetic variances using PlabStat software (Utz 2000). The phenotypic data were prepared before QTL analysis in two analysis steps: The first step was the calculation of adjusted entry means (AEM) for each of control and treatments separately considered as nine different environments (env). The second step was the calculation of combined AEM (CAM) for the genotypes across all treatments based on PlabStat standard procedure (Utz 2000).

Additional confirmation for QTL detection across all treatments (second step), least squares means (LSM) were calculated for the mean values of the three replicates in each experiment using the PROC MIXED procedure (SAS 2008), considering the fixed effects for genotype and deterioration regime plus their interaction as well as the random effects for storage duration, the year of harvest and the interaction of genotype with storage duration and year of harvest, respectively.

3.1.4. QTL analysis

The genotypic data used to construct the ExV linkage map involved 310 AFLP (Amplified fragment length polymorphism) and 165 SSR markers (Basunanda et al. 2010; Basunanda et al. 2007; Mladen Radoev 2008), spread over the 19 chromosomes. The linkage groups referred to N1-N19 correspond to A1-A10 (A genome) and C1-C9 (C genome) according to the new standardized nomenclature (http://www.brassica.info). The composite interval mapping model (CIM) was conducted for env, CAM and LSM means using QTL cartographer v2.5 software (Zeng 1994). Permutation tests using 1000 iterations were used to confirm the significance level at a LOD score threshold of three (Doerge and Churchill 1996). The estimated additive effect (Add) and the percentage of explained phenotypic variations (R^2)

were shown for each QTL. The obtained QTL for each treatment, year (env-QTL) and for adjusted means across treatments (CAM-QTL) and (LSM-QTL) were compared with published comparative maps constructed for agronomic traits of oilseed rape.

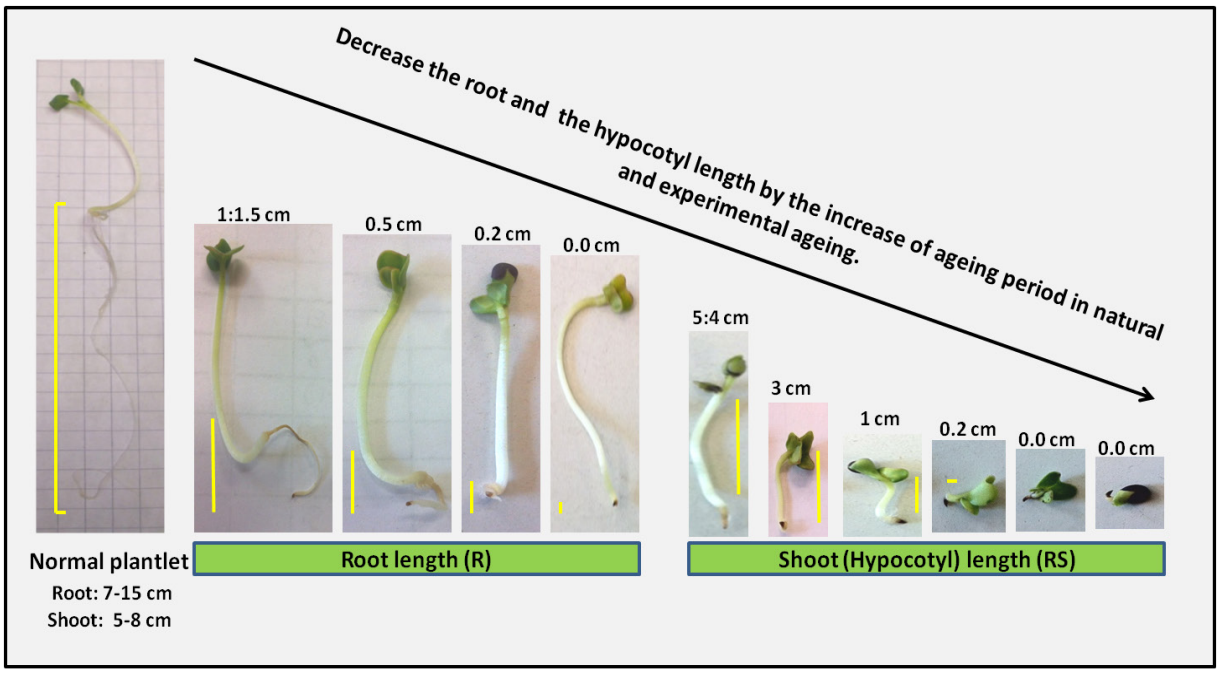


Fig. 6. Root and shoot abnormalities in ExV population. First abnormalities appear in primary and secondary roots followed by short roots. Increasing seed age or ageing period results in gradual dwarfing of hypocotyls and root loss. Extreme deteriorated seeds show neither root nor shoot only leaf extension.

3.2. Genome wide association study (GWAS) for seed longevity of oilseed rape *Brassica napus* L. - Screening of ERANET-ASSYST population

3.2.1. Seed materials and molecular markers

A wide diversity set of *B. napus* consisting of 509 inbred lines (ERANET-ASSYST) were previously investigated for genetic diversity and decay of linkage disequilibrium (LD) using set of SSR markers (Bus et al. 2011). In our recent study we used a subset of 215 inbred lines, regenerated at Giessen University in 2012, combined 173 winter oilseed rape (OSR) lines, 20 winter fodder lines, 2 spring OSR lines, 9 vegetable lines and 11 unspecified inbred lines (Table S5). Further, 136 winter OSR genotypes were categorized according to levels of erucic acid and GSL named as ++ (17, high level of erucic acid and seed glucosinolate), 0+/+0 (11, low level of erucic acid and high level of seed glucosinolate/high level of erucic acid and low level of seed glucosinolate) and 00 (108, low level of erucic acid and seed glucosinolate). Additionally, 90 winter OSR were classified into four groups of release dates: 1954-1979 (11), 1980-1989 (15), 1990-1999 (25) and 2000-2007 (39) (Table S5). After harvest seeds were stored at ambient storage for one year until experiments were conducted.

3.2.2. Experimental design and phenotyping

Germination tests and experimental ageing were conducted at same conditions (60% RH, 45°C) applied to ExV population. Due to the high initial viability of ASSYST seeds CD period was extended to 70 days. The traits TG, NS, Abn, R, RS, T10, T50 and AUC were recorded after control germination test (CT) and the controlled deterioration test (CD). Additionally, the shoot length (SHL), the root length (RL) of the normal plantlets and the root/shoot ratio (RSH) were recorded.

3.2.3. Genotyping

Plant Genomic DNA for SNP genotyping was extracted from fresh young leafs (Bus et al. 2011) and the genotyping of the SNPs for available diversity panel (*n= 215*) was performed at Agriculture and Agri-Food Canada using custom Illumina Infinium 6K array (http://aafc-aac.ca/ASSYST/). 5,506 SNP loci were assayed from which 2,854 SNP loci have been physically positioned on *B. oleracea* genome sequence (Parkin et al. 2014) and 2,652 SNP loci have been positioned on *B. rapa* genome sequence (Wang et al. 2011b). In present study, 4,001 SNPs of 215 individuals having a minor allele frequency of > 10% were used for association study.

3.2.4. Statistical analysis

GERMINATOR software was used to calculate germination speed parameters (Joosen et al. 2010). SD, LSD, ANOVA, AEM and correlation of coefficient between control and treatment repeatabilities and heritabilities were calculated for 215 ASSYST winter oilseed rape diversity (Table S5) using standard procedure of PLABSTAT software (Utz 2000) as explained earlier in ExV. Associations among tested traits were visualized using the principle component analysis (PCA) calculated by software PAST (Hammer et al. 2001) depending on the correlation between traits.

3.2.5. Association analysis

The software program TASSEL 2.1 (Bradbury et al. 2007) was used for association mapping analysis. The general linear model (GLM) and the mixed linear model (MLM) methods (Yu et al., 2006) were performed for twelve traits. Kinship was calculated using principle coordinate analysis (PCoA) described by (Price et al., 2006). The final associations was considered as significant when the $-\log_{10}(p)$ is ≥ 3 ($P < 0.001$) in both models (GLM and MLM).

3.2.6. Population structure

The population structure of the ASSYST subset of 215 individuals was studied using STRUCTURE 2.3.4 software (Pitchard et al., 2000) and 4,001 SNP markers. The admixture model was applied using 5000 iterations and 5000 MCMC (Markov Chain Monte Carlo approach) and the duration for testing the Q values ranged from 1 to 10 using 200 replicates each.

3.2.7. Gene ontology and annotation analysis

For physical localization of SNP markers (*This part of analysis have been done by the help of Dr. Birgit Samans, Max Plank Institute*), flanking sequences were blasted onto *B. napus* Darmor-bzh reference genome sequence assembly version 4.1 (Chalhoub et al. 2014). For each SNP, the BLAST hit with highest score was selected. Predicted gene sequences were annotated using Blast2GO approach http://www.blast2go.com/ (Conesa et al. 2005) with screening the significant SNPs regions ($> 3 -\log_{10}$) and their surrounding areas of plus and minus 500,000 bp. The second methodology for screening the selected sequences is gene ontology (GO) enrichment analysis (hypergeometric test) which was performed using bioconductor package GOstat approach (Beissbarth and Speed 2004). The putative candidate genes for our tested traits were detected and compared with their homologues' in *Arabidopsis thaliana*.

4. Results

4.1. QTL mapping of Express x V8 population

4.1.1. Phenotypic variation

The distributions of phenotypic values for each trait among the 122 lines and nine treatments are shown in Figure 6. All investigated traits were normally distributed and showed transgressive variation in ExV population at both CT and CD conditions All traits were highly variable among the three seed sets of ExV, whether the seed were ambient stored or experimentally aged. TG in ExV-5_CT2 and _CD showed a normal distribution with a range from 0 to 100% and the majority of tested genotypes had TG between 60 to 80 %. This was not the case in ExV-5_CT1 which showed TG of 100% for the majority of tested genotypes. Similarly, most of genotypes showed 100% TG in all treatments of ExV-9_CT and ExV-12_CT. In ExV-5_CT1, NS ranged between 20 to 40%, whereas in ExV-5_CT2 and _CD more than 90% of genotypes had 0% NS. Traits Abn, R, RS, T50 and T10 clearly showed a gradually increase from CT to CD1 and CD2 in all three years. Oppositely, the TG, NS and AUC were decreased gradually after CD1 and CD2. Generally, the range of trait values became more extended as the seeds deteriorated (Fig. 7, Table 2).

The ExV-9 lines showed a higher mean TG and NS than those of ExV-5 or ExV-12. Both traits decreased gradually as the seed aged, either after ambient storage or experimentally (Table 2). The comparison between ExV-5_CT1 and _CT2 showed the large effect of two years of ambient storage: TG decreased from 80.5% to 56.5%, NS from 29.2% to 3.4%, and AUC from 63.9 to 25.3 (Table 2). In ExV-5, R was lower in the CT2 and CD than in CT1, respectively (34.0%, 47.2% and 51.4%). In the opposite direction, RS was increasing as the seeds aged in CT2 and CD compared to CT1 (respectively: 57.7%, 46.4% and 12.6%). Similarly, T50 and T10 increased in the aged seeds however AUC dropped down (Table 2). The lowest R and RS were experienced in ExV-9_CT whereas the highest was recorded in ExV-5_CT1 and _CT2, respectively. The highest and lowest germination speeds were observed in ExV-9_CT and ExV-5_CT2, respectively. In ExV-9_CT, seed lots which had been aged in ambient storage for two years, had higher TG, NS and produced fewer Abn, R and RS than did the one year ambient aged seeds from ExV-12_CT. However, the average of NS was higher and T10 was less rapid in ExV-9 than in ExV-12 for the seed exposed to CD1 or CD2. Significant differences between genotypes in each treatment was noted for each trait. A moderate to high level of repeatability was recorded among the nine seed lots for all the traits.

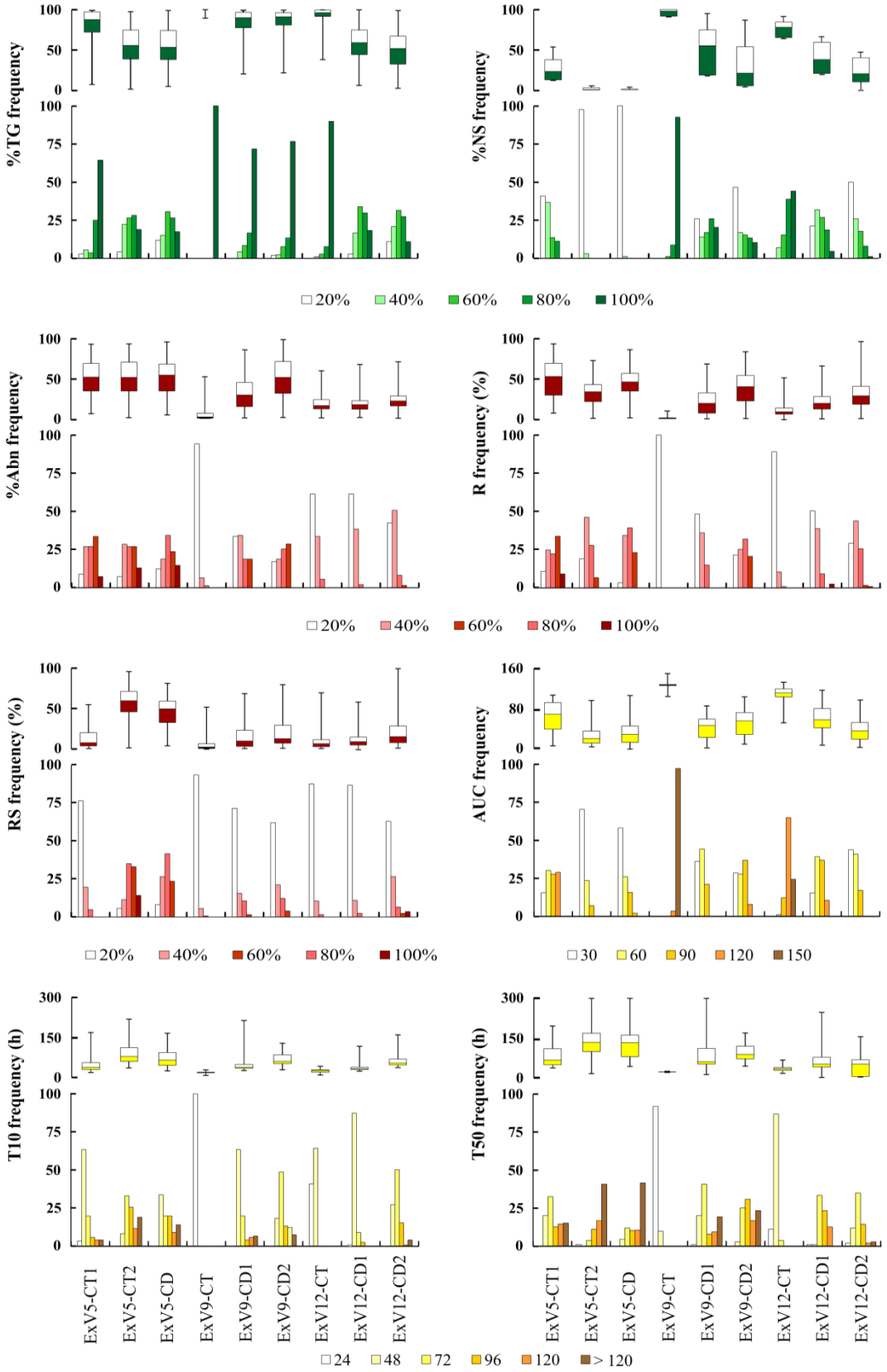


Fig. 7. Distribution of eight germination traits indicating seed longevity in nine environments. The range of seed performance is shown by histogram and box plots: control germination (CT) and controlled deterioration (CD) experiments are shown in three seed sets from three seasons ExV-5, ExV-9 and ExV-12. Normal seedlings (NS), total germination (TG), abnormal shoots (Abn), abnormal roots (R), ratio of root to shoot abnormalities (RS), area under the curve (AUC), time to 50% germination (T50), time to 10% germination (T10) and thousand seed weight (TSW).

TSW varied from 2.1 to 7.4 g (Fig. 8, Table S1). In ExV-5_CT1, TSW was positively correlated with TG (r = 0.523**). Additionally, TG was positively correlated with AUC (r = 0.815**) and negatively with T10 and T50 (r = -0.738 and -0.747 respectively). This high correlation with AUC, T10 and T50 was repeated with TG in all other treatments in the same directions (Table S1). R was displayed a negative correlation with all of RS, T10 and T50 in ExV-5-CT2 & -CD (-0.672**, -0.371** and -0.425**) and (-0.885**, -0.420**, -0.491**) respectively and positive correlation with AUC (0.475** and 0.556**) respectively. On the other hand RS showed a positive correlation with T10 and T50 and negative with AUC in ExV-9 and ExV-12 after CD.

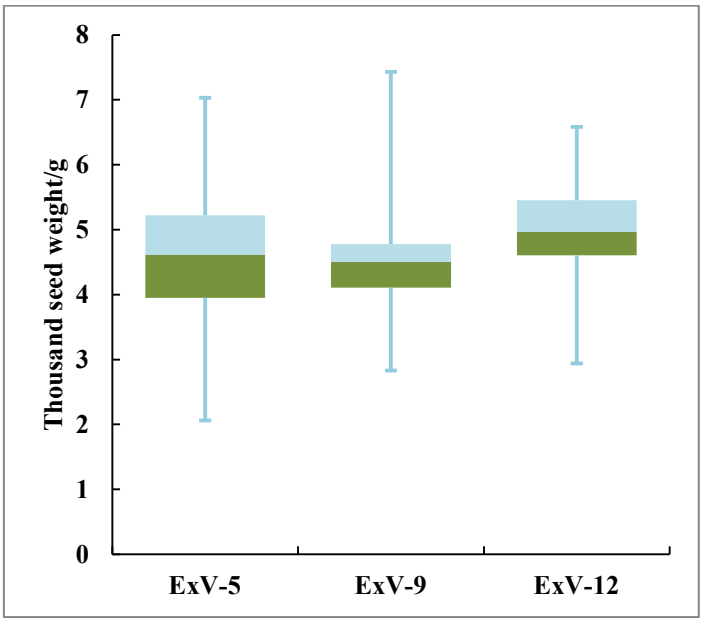


Fig. 8. Variation of thousand seed weight in different harvest years of Express x V8 population (ExV): Box plots represent medians, lower and upper quartiles and whiskers show the 98th percentile of 122 double haploid lines produced in 2005, 2009, 2012.

Table 2. Phenotypic distribution of germination traits in the ExV mapping population. ExV-5, ExV-9 and ExV-12 were tested for control (CT) or after controlled deterioration (CD) for percentage of normal seedlings (NS), of total germination (TG), of abnormal shoots (Abn), of abnormal roots (R), of the ratio of root to shoot abnormalities (RS), for the area under the curve (AUC), time to 50% germination (T50), time to 10% germination (T10) and for thousand seed weight (TSW). The LSD5%, least significant difference at *P* <5 % Repeatability was calculated for each trait in 9 treatments.

	ExV-5				ExV-9				ExV-12			
Traits	Average	LSD	Range	Repeat%	Average	LSD	Range	Repeat%	Average	LSD	Range	Repeat %
	CT1 (6y)				**CT (2y)**				**CT (1y)**			
TSW	4,5	0,4	2,06 - 7,03	95,6	4,5	0,2	28 - 7,43	97,8	5,0	0,2	2,9 - 6,6	97,0
TG	80,5	8,0	7,2 - 100	94,9	99,6	1,1	88,7 - 100	82,2	92,5	5,3	38,5- 100	91,3
NS	29,2	18,8	0 - 82,7	73,4	94,0	3,7	47,3- 100	94,0	73,3	18,7	22,2- 98,3	67,6
Abn	51,1	19,2	6,7 - 94	73,4	5,5	3,2	0 - 52,7	95,0	18,9	16,4	1,67 -60	44,3
R	51,4	22,5	6,8- 94,4	69,8	0,8	2,2	0 - 11	53,8	11,3	11,3	0 - 51,9	47,0
RS	12,6	12,4	0 - 54,7	70,8	5,1	5,4	0 - 51,3	84,7	8,9	7,6	0 - 69,1	81,4
T_{50}	79,6	11,8	36,6-191,6	96,4	20,6	2,2	0 - 27,1	94,5	32,7	9,8	15,6 - 66,4	57,0
T_{10}	47,4	9,5	19,4 - 163,9	95,3	18,4	2,4	0 - 24,3	91,2	25,6	12,1	11,3- 41,9	22,0
AUC	63,9	8,7	2,2 - 107,2	96,9	128,1	2,6	104 - 150	93,6	108,3	11,6	50,9 - 133	82,2
	CT2 (8y)				**CD1 (40d)**				**CD1 (40d)**			
TG	56,5	16,5	1,7 -97,5	82,1	83,9	10,7	20,4 - 100	86,6	59,1	12,7	6,7 - 99,2	87,2
NS	3,4	4,4	0 - 31,9	76,3	50,4	13,4	0 - 96,7	92,8	40,9	13,4	2,5 - 92,5	87,3
Abn	52,5	16,0	1,7 - 93,3	81,4	33,5	13,1	1,7 - 85,8	86,8	19,1	11,4	2,2 - 67,8	60,3
R	34,0	22,2	0 - 73,7	50,9	22,4	11,4	0 - 69,1	84,2	23,0	17,4	0 - 66,9	56,6
RS	57,7	23,9	1,3 - 95,6	60,1	16,0	11,5	0 - 68,4	84,9	11,4	10,9	0 - 57,6	67,7
T_{50}	134,9	34,6	15,2- 3,2,3	82,4	82,5	22,2	11,5- 324,6	93,0	61,7	14,0	0 - 247,5	93,7
T_{10}	87,5	24,0	31,5 - 215,2	86,0	56,7	25,1	24,8 - 210,9	81,1	36,4	9,2	23,9 - 112	79,8
AUC	25,3	10,8	0 - 95,9	89,8	40,4	16,0	0 - 85,1	83,4	59,5	13,1	5,8 - 116,8	90,1
	CD (7d)				**CD2 (50 d)**				**CD2 (50d)**			
TG	54,8	13,6	5,6 - 99,2	88,6	84,1	9,2	6,9 - 100	91,1	50,6	15,5	2,5- 98,3	84,2
NS	2,5	3,3	0 - 33,5	80,8	33,3	11,6	0 - 96,7	94,5	26,2	12,7	0 - 95	86,3
Abn	52,0	13,2	4,96 - 95,8	88,3	50,3	15,1	2,5 - 98,4	87,4	23,7	13,9	0 - 71,1	58,0
R	47,2	27,5	0 - 87,2	39,1	40,7	18,3	1,7 - 84,1	76,7	30,3	18,9	0 - 97,5	59,7
RS	46,4	28,2	3,4 - 80,99	41,4	19,9	14,0	0 - 79,6	81,3	20,3	14,9	0 -100	80,4
T_{50}	124,4	19,2	40,6 - 347,9	95,9	95,0	14,1	41,8 - 168,1	92,9	99,8	42,3	0 - 218,3	74,9
T_{10}	71,3	15,8	9,5 - 164,1	92,8	68,7	12,2	26,8 - 126,9	92,0	61,7	20,8	36,6 - 157,9	71,3
AUC	32,3	11,0	0 - 106,14	93,0	51,8	9,6	8,2 - 103,3	95,1	36,6	12,4	0 - 97,03	89,1

4.1.2. Seed longevity QTL

QTL analysis of the ExV-5, ExV-9 and ExV-12 seed lots revealed 107, 127 and 60 QTL, respectively, associated with seed longevity. QTLs of ExV-5 were distributed across 14 linkage groups (LGs), while those derived from ExV-9 and ExV-12 were located on eight and nine LGs, respectively. The CAM-QTL and the LSM-QTL confirmed 71 and 49 of the loci on eight LGs, respectively (Table 3). Most QTL were concentrated on LGs A8, A9, A10, C3 and C6 (Fig. 9 & 10).

ExV-5

A total of 44 QTL emerged from CT1: these formed one single QTL and ten clusters (Table 3), involving seven LGs. The percentage of R^2 of these QTL ranged from 8 to 19.4, while the LOD scores varied from 3.1 to 6.0. QTL for five traits were detected in CT1, there were four loci affecting NS and two and three QTL affecting each of Abn and TG, respectively. The marker interval 25.1-28.6 on LG A7 harboured loci affecting both Abn and TG, while the interval 34 - 51.5 on LG A5 contained a NS QTL associated with a highest LOD score of 6.0. Following the CAM-QTL analysis, seven loci were validated, mapping to LGs A5, A7, C3 and C6. The marker interval 34 - 51.5 on LG A5 harboured QTL underlying R and Abn which significantly were confirmed by LSM-QTL and CAM-QTL, while, in env-QTL some markers in the same location were detected for Abn and R. The latter interval was the largest one which covers 12 markers, the same region was detected in ExV-9_CT (AUC: 43.7 cM) and in ExV-9_CD2 (Abn: 34 cM). In ExV-5, the analysis of the CD and CT2 data revealed additional QTL on LGs A1, A8, C1, C2 and C7 which were not detected in CT1. The largest QTL interval of CT2 with a R^2 of 14.6 and a LOD score of 5.4 was associated with R and mapped to the marker interval 30.4 – 53.4 on LG A9. This interval was detected in a part with RS (3.04 – 37). Two QTL were identical for ExV-5 (CT and CD) on A7 and A9 which is shown in table 3 by the positive confirmation signs (+++). Five QTL were repeated in ExV-9 and four in ExV-12 in addition to 13 loci confirmed by CAM-QTL.

ExV-9

QTL for all tested traits were detected in ExV-9. Twenty seven of in total 32 QTL intervals were confirmed by the CAM-QTL and 14 QTL were also found in ExV-5 and ExV-12 (Table 4). For TSW one interval was detected on LG A10 (LOD = 7.21; R^2 = 18.57%). Some markers of this interval were found in _CD1 (AUC, NS and R) and in _CD2 (Abn, AUC and NS) and confirmed by CAM-QTL for the traits R and NS. The highest LOD score (6.9) across all three experiments was detected for R in CD2 on LG A5 (60.6-71.5). The QTL in

A10 (48 - 68.3cM) and in C6 (64.5 – 88.2 cM) were single loci for CD1 and CD2. The second interval was detected mainly for germination speed parameters AUC, T10 and T50.

ExV-12

Sixty QTL were detected for ExV-12. Two, three and one QTL on A8, C2 and C4, respectively were individual to _CT. In addition, four QTL on A9 were specific to CD1 and five others were found on A9, C7 and C9 in _CD2. The LG A10 interval 0.0-19.9, harboured three QTL for AUC, NS and TG in _CT (LOD = 3.3 to 3.9), two QTL for AUC and TG in CD1 (LOD = 3.8 and 6.1) and one QTL for AUC in CD2 (LOD = 4.1). This interval was identical between traits of CT and CD treatments. In total eight QTL were confirmed by CAM-QTL, five QTL appeared in ExV-5 and three in ExV-9 (Table 5). The interval 87.8 – 104.2 was found in five treatments across ExV-5, ExV-9 and ExV-12. The marker BRAS039b (89.1 cM) was common in those repeated intervals. This locus was validated by CAM-QTL for both traits AUC and T10.

Table 3. Genomic regions harbouring seed longevity QTL derived from the ExV-5_CT and _CD. LOD scores (> 3.0) and explained phenotypic variances R² are shown. (a) QTL names comprise the trait abbreviation followed by an experiment or treatment identifier. (b) The number of markers mapped within each interval (c) LOD threshold calculated by 1000 permutation times at the standard CIM. (d) The additive effects contributed by the parent Express 617 (positive) by the parent V8 (negative). (e) QTL identified in multiple experiments: 3 signs of minus (---) or plus (+++) explaining the absence or presence of the same QTL respectively, in the three experiments of ExV-5 (CT1, CD & CT2), ExV-9 (CT, CD1 & CD2) and ExV-12 (CT, CD1 & CD2), the LSM-QTL and the CAM-QTL.

QTL[a]	LG	Interval marker	Position	Peak	#M[b]	Td	LOD	R^2 %	Add[d]	Confirmed QTL[e]				
										ExV-5	ExV-9	ExV-12	LSM-QTL	CAM-QTL
TG_CT1_5	A3	Na12H06 -E44M50_362	0 - 13,6	0	5	3,2	4,9	13,5	-8,3	+--	---	---		
Abn_CT1_5	A5	CB10574 - Na12E01b	42,8 - 51,5	49,9	6	3,3	4,0	10,8	-6,9	+--	+--	---	+	R
NS_CT1_5	A5	Na10E02 - Na12E01b	34 - 51,5	42,8	12	3,0	6,0	14,8	8,1	+--	--+	---	+	Abn-R
R_CT1_5	A5	MD21 - Na12E01b	41,6 - 51,5	41,6	4	3,4	3,9	10,5	-7,6	+-+	--+	---		Abn - R
Abn_CT1_5	A7	Ra2G08 - Na12B02	25,1 - 28,6	25,1	2	3,3	3,5	10,0	7,0	+++	---	---		Abn
TG_CT1_5	A7	Ra2G08 - Na12B02	25,1 - 28,6	28,6	2	3,2	5,0	12,8	8,2	+++	---	---		Abn
NS_CT1_5	A9	E35M60_540 - Na10B11	17,9 - 22,8	22,8	2	3,0	4,2	10,0	-6,9	+++	---	---		
T10_CT1_5	C3	Na10C01a - BRAS039b	87,8 - 89,1	89,1	2	3,0	3,8	19,4	29,1	+--	+++	--+		AUC-T10
Abn_CT1_5	C6	E45M48_404	107,7	107,7	1	3,3	3,1	8,1	-6,1	+--	---	---	+	
NS_CT1_5	C6	E43M62_168 - E32M59_285	102,5 - 116,1	107,7	5	3,0	4,3	10,6	6,9	+--	---	-++	+	TG-NS
NS_CT1_5	C9	E45M51_80 - CB10266	0 - 14,8	14,8	2	3,0	3,4	8,0	5,9	+--	---	---		
T50_CT2_5	A1	E46M62_52	39,6	39,6	1	3,2	3,4	9,6	25,2	++-	-+-	+--		T50
T50_CT2_5	A1	E42M51_661 - E33M54_65	68,6 - 81,9	77,9	5	3,2	4,3	12,6	29,3	-+-	---	---		T50
Abn_CT2_5	A7	E39M49_307 - Ra2G08	0 - 25,1	0	4	3,3	4,9	15,3	8,9	+++	---	---		Abn
TG_CT2_5	A7	E39M49_307 - Ra2G08	0 - 25,1	0	3	3,1	4,8	14,4	9,2	+++	---	---		Abn
AUC_CT2_5	A8	E44M48_414 - E39M47_181	34,5 - 52,1	42,1	7	3,1	5,3	13,6	-7,6	-+-	---	---	+	T10
AUC_CT2_5	A9	E35M60_540 - Na10B11	17,9 - 22,8	22,8	2	3,1	5,5	13,5	-8,1	+++	---	---		
R_CT2_5	A9	GMR013a - Na14C12	30,4 - 53,4	46	9	3,2	5,4	14,6	-6,4	-++	---	-+-		
RS_CT2_5	A9	GMR013a - E46M59_87	30,4 - 37	37	2	3,2	3,3	9,5	6,6	-++	---	-+-		
T50_CT2_5	C1	E33M50_414 - E31M55_102	22,5 - 35,6	22,5	3	3,2	4,0	19,4	34,7	-+-	---	---		
AUC_CT2_5	C2	E34M60_94	58,8	58,8	1	3,1	3,1	7,4	7,3	-+-	---	---		
R_CT2_5	C7	E35M62_246	36,5	36,5	1	3,2	3,3	8,7	-5,1	--+	---	---		
RS_CT2_5	C7	E31M62_355 - E43M51_58	85,3 - 90	90	2	3,2	3,7	11,3	-8,3	--+	---	---		
Abn_CD_5	A7	E39M49_307	0	0	1	3,2	3,2	10,7	7,8	+++	---	---		
T50_CD_5	A7	Ra2G08 - Na12B02	25,1 -28,6	28,6	2	3,1	4,2	12,7	28,7	+++	---	---		Abn
T10_CD_5	A8	E31M53_515 - HMR582	24 -30,8	24	2	3,2	3,2	10,4	12,1	--+	---	---	+	
Abn_CD_5	A9	E36M51_53	56,7	56,7	1	3,2	3,7	10,4	10,5	--+	---	---		
AUC_CD_5	A9	E35M60_540 - Na10B11	17,9 - 22,8	22,8	2	3,2	3,6	9,9	-8,5	+++	---	---		
TG_CD_5	A9	Na12E06B - E36M51_53	49,5 - 56,7	56,7	3	3,3	4,4	11,3	-8,4	--+	---	---		
R_CD_5	C2	HMR364	67,9	67,9		3,2	3,4	9,5	5,2	--+	---	---		
NS_CD_5	C7	E35M62_246 - E43M51_58	36,5 - 90	38,6	7	2,4	5,6	16,0	-2,1	--+	---	---		

Table 4. Genomic regions harbouring seed longevity QTL derived from the ExV-9_CT and _CD. LOD scores (> 3.0) and explained phenotypic variances R² are shown. (a) QTL names comprise the trait abbreviation followed by an experiment or treatment identifier. (b) The number of markers mapped within each interval. (c) LOD threshold calculated by 1000 permutation times at the standard CIM. (d) The additive effect contributed by the parent Express 617 (positive) and by the parent V8 (negative). (e) QTL identified in multiple experiments: 3 signs of minus (---) or plus (+++) explaining the absence or presence of the same QTL respectively, in the three experiments of ExV-5 (CT1, CD & CT2), ExV-9 (CT, CD1 & CD2) and ExV-12 (CT, CD1 & CD2), the LSM-QTL and the CAM-QTL

QTL[a]	LG	Interval marker	Position	Peak	#M[b]	Td[c]	LOD	R^2 %	Add[d]	Confirmed QTL[e]				
										ExV-5	ExV-9	ExV-12	LSM-QTL	CAM-QTL
AUC_CT_9	A5	E43M52_320	43,7	43,7	1	2,7	3,08	8,48	1,88	+--	+--	---	+	R
Abn_CT_9	A8	E33M47_216 - E31M53_515	0 - 24	18,7	11	2,8	5,54	15,49	-3,37	--+	+--	+--		
NS_CT_9	A8	E33M47_216 - E31M53_515	0 - 24	16,9	11	2,9	5,42	14,39	3,59	--+	+--	+--		
R_CT_9	A8	E33M47_216 - HMR353a	0-20,8	9,5	6,0	2,8	5,12	12,72	-2,91	---	+--	+--		
TSW_9	A10	E46M59_252 - Na12H04	50,4 - 72,2	59,9	6	3,1	7,21	18,57	-0,31	---	+++	---	+	NS-RS
T10_CT_9	C3	E46M62_122 - E44M47_239	59,9 - 86,4	77,8	11	2,7	5,95	17,62	2,48	+--	+++	--+		AUC-T10
TG_CT_9	C5	CB10374 - Na12C01	3,5 - 20,9	20,9	2	2,0	5,62	17,83	1,02	---	+-+	---		NS
NS_CD1_9	A1	E46M51_99	36,8	36,8	1	3,4	3,01	7,28	-8,19	++-	-+-	+--		T50
Abn_CD1_9	A10	E43M51_349 - Ol10B11	48 - 68,3	50,4	5	3,2	6,62	18,45	-9,40	---	-++	---		NS-RS
AUC_CD1_9	A10	E46M59_252 - E44M48_137	50,4 - 62,3	59,9	3	3,3	3,79	8,73	7,02	---	+++	---	+	NS-RS
NS_CD1_9	A10	E46M59_252 - Na12E09	50,4 - 59,9	59,9	2	3,4	3,30	8,03	8,83	---	+++	---	+	NS-RS
R_CD1_9	A10	E46M59_252 - Na12E09	50,4 - 59,9	59,9	2,0	3,2	3,29	9,63	-5,34	---	+++	---	+	NS-RS
TG_CD1_9	C3	Na10C01a - BRAS039b	87,8 - 89,1	89,1	2	3,1	3,80	9,54	-5,45	+--	+++	--+		AUC-T10
T10_CD1_9	C3	Na10C01a - BRAS039b	87,8 - 89,1	89,1	2	2,9	3,95	11,44	27,36	+--	+++	--+		AUC-T10
NS_CD1_9	C3	MR86b - Ol10E05	0 - 2,3	0	2	3,4	3,41	8,43	-8,91	---	-++	---	+	NS - R
AUC_CD1_9	C6	E34M54_45 - E31M49_112	64,5 - 88,2	79,8	5	3,3	4,33	11,14	7,91	+--	-++	---	+	T10-AUC-TG-NS-R
TG_CD1_9	C6	E32M51_225 - E32M51_350	75,9 - 97,8	88,2	5	3,1	6,17	16,58	7,21	+--	-++	---	+	T10-AUC-TG-NS-R
T10_CD1_9	C6	E34M54_45 - E45M48_151	64,5 - 79,8	75,9	3	2,9	4,06	12,06	11,81	---	-++	---	+	T10-AUC-R
RS_CD1_9	C6	E34M54_45 - E45M48_151	64,5 - 79,8	75,9	3	3,3	6,56	17,21	-7,34	---	-++	---	+	T10 - AUC
Abn_CD2_9	A5	Na10E02	34	34	1	3,3	5,15	13,56	-9,59	+--	--+	---	+	Abn-R
R_CD2_9	A5	E32M59_162 - E42M55_166	60,6 - 71,5	60,6	2	3,2	7,35	24,59	-11,01	--+	---	---		
T50_CD2_9	A8	Na12B05 - Ra1F06	59,4 - 72,5	72,5	4	3,2	6,01	15,18	14,32	---	-++	---		
Abn_CD2_9	A10	E46M59_252 - E44M48_137	50,4 - 62,3	59,9	3	3,3	4,58	11,90	-8,86	---	+++	---	+	NS-RS
AUC_CD2_9	A10	E43M51_349 - Ol10B11	48 - 68,3	59,9	5	3,3	5,48	12,33	9,72	---	-++	---	+	NS-RS
NS_CD2_9	A10	E46M59_252 - Ol10B11	50,4 - 68,3	59,9	4	3,3	6,46	15,47	12,16	---	+++	---	+	NS-RS
RS_CD2_9	A10	Ra1F06 - E44M48_137	72,5 - 62,3	62,3	3	3,2	4,11	11,01	-6,83	---	+++	---	+	NS-RS
NS_CD2_9	C3	MR86b - E33M62_153	0 - 4,3	0	3	3,3	4,54	10,63	-10,12	---	-++	---	+	NS -R
T10_CD2_9	C3	Na10C01a	87,8	87,8	1	3,1	3,22	7,89	7,58	+--	+++	--+		AUC-T10
AUC_CD2_9	C6	E34M54_45 - E45M48_151	64,5 - 79,8	75,9	3	3,3	4,67	11,16	9,22	---	-++	---	+	T10-AUC-R
TG_CD2_9	C6	E32M51_225 - E31M49_112	75,9 - 88,2	88,2	4	3,0	3,33	9,46	5,85	+--	-++	---	+	T10-AUC-TG-NS-R
T50_CD2_9	C6	E31M61_195 - E32M51_225	74,6 - 75,9	64,5	3	3,2	3,28	9,36	-11,07	---	-++	---	+	T10-AUC-R
T10_CD2_9	C6	E34M54_45 - E45M48_151	64,5 - 79,8	75,9	3	3,1	3,67	9,88	-8,52	---	-++	---	+	T10-AUC-R

Table 5. Genomic regions harbouring seed longevity QTL derived from the ExV-12_CT and _CD. LOD score (> 3.0) and explained phenotypic variances R² are shown. (a) QTL name comprise the trait abbreviation followed by an experiment or treatment identifier. (b) The number of markers mapped within each interval. (c) LOD threshold calculated by 1000 permutation times at the standard CIM. (c) The additive effects contributed by the parent Express 617 (positive) and by the parent V8 (negative). (e) QTL identified in multiple experiments: 3 signs of minus (---) or plus (+++) explaining the absence or presence of the same QTL respectively, in the three experiments of ExV-5 (CT1, CD & CT2), ExV-9 (CT, CD1 & CD2) and ExV-12 (CT, CD1 & CD2), the LSM-QTL and the CAM-QTL

QTL[a]	LG	Interval marker	Position	Peak	#M[b]	Td[c]	LOD	R^2 %	Add[d]	Confirmed QTL[e]				
										ExV-5	ExV-9	ExV-12	LSM-QTL	CAM-QTL
Abn_CT_12	A8	E33M47_216 - HMR353a	0 - 20,8	20,8	3	3,1	3,14	8,22	-3,1	---	+--	+--		
NS_CT_12	A8	E46M59_160	9,50	9,5	1	3,1	3,02	7,64	5,1	---	+--	+--		
AUC_CT_12	A10	E34M62_109 - E39M59_142	9,3 - 19,9	17,6	3	3,2	3,85	10,34	5,6	---	---	+++	+	
NS_CT_12	A10	E46M59_241	17,6	17,6	1	3,1	3,27	8,29	5,7	---	---	+++	+	
TG_CT_12	A10	E34M62_109 - E39M59_142	9,3 - 19,9	17,6	3	2,9	3,54	9,17	3,5	---	---	+++	+	
NS_CT_12	C2	E33M50_93	43,40	43,4	1	3,1	3,26	8,37	-5,4	---	---	+--		
TG_CT_12	C2	E44M62_128	19,10	46,9	1	2,9	5,20	13,79	-5,1	---	---	+--		
TG_CT_12	C2	E34M59_71 - E32M48_462	33,6 - 51	46,9	8	2,9	5,20	13,79	-5,1	---	---	+--		
TSW_12	C4	Na10C01c - E32M59_142	37,4 - 48,6	48,6	4	3,2	4,27	12,15	-0,3	---	---	+--		
Abn_CD1_12	A9	E39M59_186 - E44M51_350	0 - 13,2	3,7	3	3,0	4,95	16,14	-5,3	---	---	-+-	+	TSW-T10
Abn_CD1_12	A9	GMR013a	30,40	3,7	1	3,0	3,27	9,12	4,5	-+-	---	-+-		
Abn_CD1_12	A9	HMR602	69,10	3,7	1	3,0	4,47	12,15	-3,9	---	---	-+-		
T50_CD1_12	A9	E32M48_148 - E44M51_350	3,7 - 13,2	13,2	2	3,0	3,30	9,36	12,4	---	---	-+-		TSW-T10
AUC_CD1_12	A10	E44M60_114 - E39M59_142	0 - 19,9	17,6	4	3,3	6,13	17,25	11,1	---	---	+++	+	
TG_CD1_12	A10	E34M62_109 - E46M59_241	9,3 - 17,6	9,3	2	3,3	3,82	11,84	7,8	---	---	+++	+	
NS_CD1_12	C4	E39M61_383 - HMR542	0 - 7,5	7,5	2	3,2	3,24	8,68	7,1	---	---	++-		
NS_CD1_12	C6	E32M59_285	116,10	116,1	1	3,2	3,72	9,86	7,1	+--	---	-++	+	NS
R_CD1_12	C6	E32M59_285	116,1	116,1	1	3	3,11	8,91	-3,2	---	---	-++	+	NS
R_CD2_12	A9	E34M51_122 - CB10373	94,6 - 97,6	97,6	2	3,1	3,38	9,88	-5,1	---	---	--+	+	R
T50_CD2_12	A9	E38M49_139 - HMR360	41,9 - 44,6	44,6	2	3,2	3,96	11,53	-23,7	-++	---	--+		
AUC_CD2_12	A10	E34M62_109 - E46M59_241	9,3 - 17,6	17,6	2	3,4	4,12	11,77	8,2	---	---	+++		
Abn_CD2_12	C3	Na12B07b	111,10	111,1	1	3,0	3,04	9,73	-3,6	---	---	--+		
AUC_CD2_12	C3	BRAS039b - E43M62_147	89,1 - 104,2	96,5	3	3,4	3,64	10,59	-7,4	+--	+++	--+		AUC-T10
NS_CD2_12	C6	E32M59_285	116,10	116,1	1	3,2	3,58	9,99	6,5	+--	---	-++		NS
TG_CD2_12	C6	E32M59_285	116,10	116,1	1	3,2	3,41	9,19	7,0	+--	---	-++		NS
RS_CD2_12	C7	E38M49_507 - Na12H07a	60,8 - 70,1	70,1	2	3	3,23	9,64	-6,5	---	---	--+		
T50_CD2_12	C7	E44M49_242	103	103	1	3,2	3,23	9,51	19,6	---	---	--+		
T50_CD2_12	C9	E32M49_386	99,5	99,5	1	3,2	3,35	10,64	-20,3	---	---	--+		

Fig. 9. The genetic map of A genome of Express x V8 DH population indicating QTLs related to seed longevity: Trait abbreviations are explained in table 2. Blue traits represent QTLs for ExV-5, the red traits QTLs for ExV-9 and green QTLs for ExV-12. QTLs confirmed across all tested environments by LSM-QTL and CAM-QTL are shown in dark green. Underlined marker represent SSR marker constructed from known genes from *Arabidopsis thaliana* and their functions are listed in table S4.

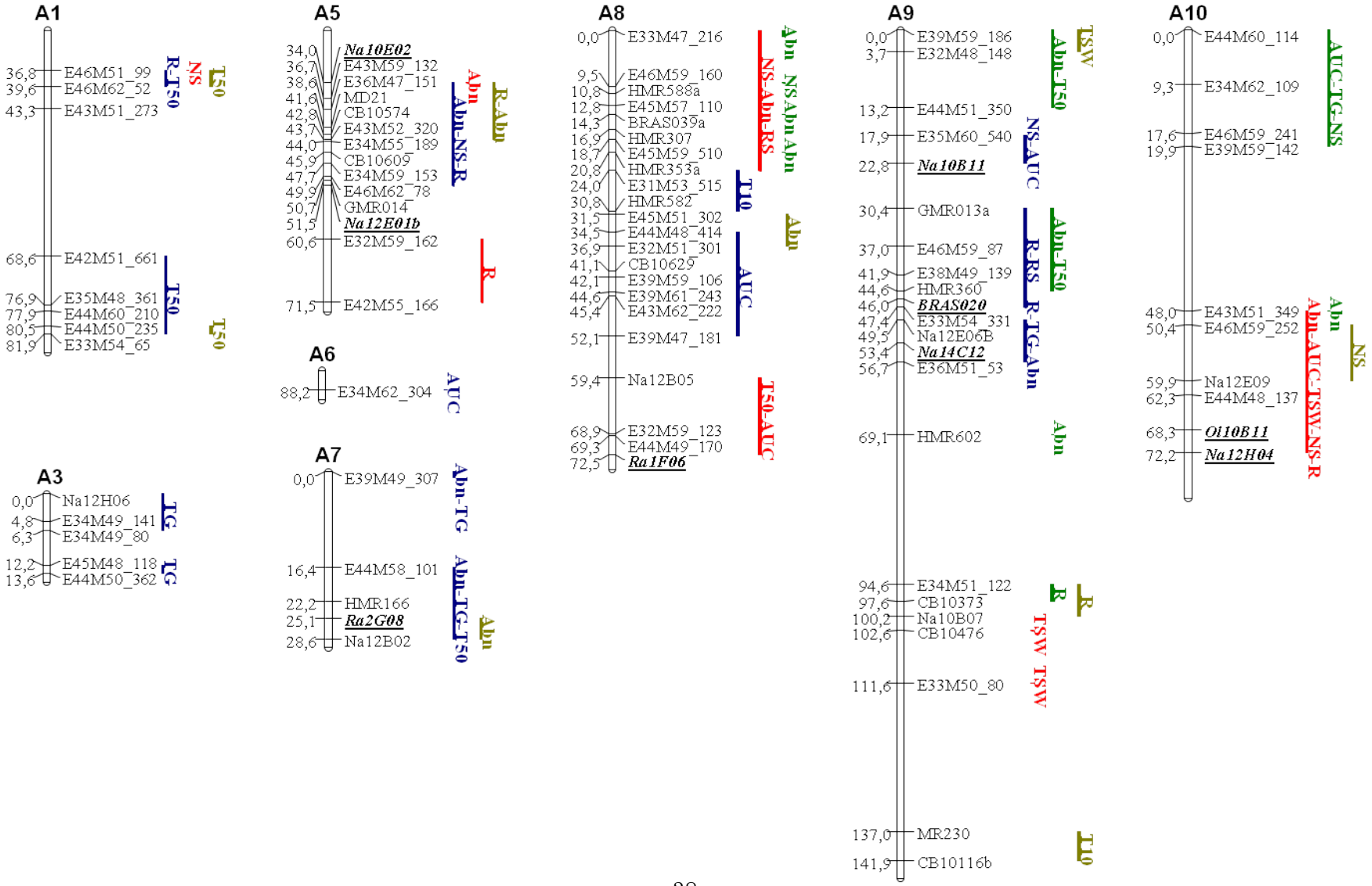
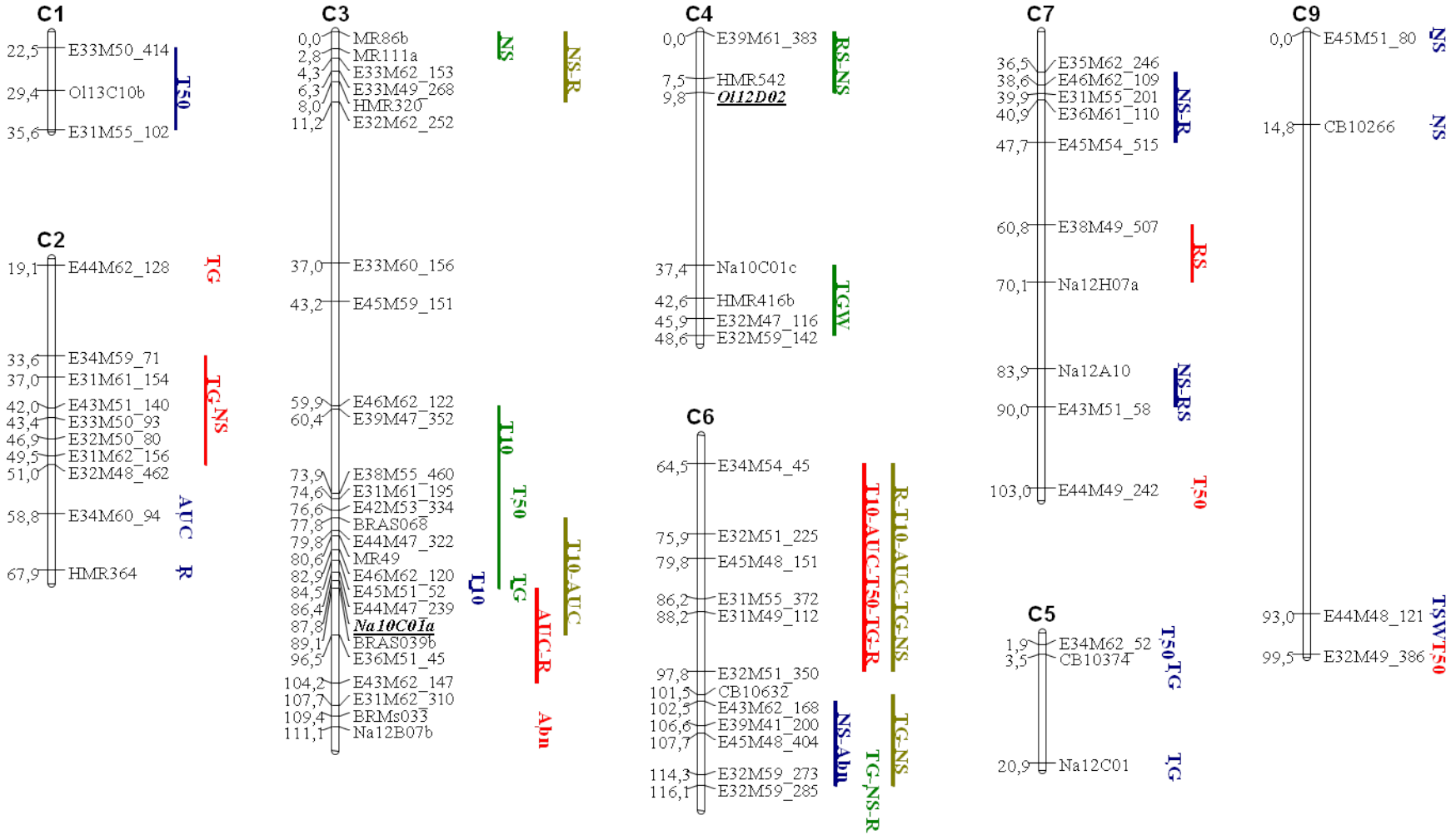


Fig. 10. The genetic map of C-genome of Express x V8 DH population indicating QTLs related to seed longevity: Trait abbreviations are explained in table 2. Blue traits represent QTLs for ExV-5, the red traits QTLs for ExV-9 and green QTLs for ExV-12. QTLs confirmed across all tested environments by LSM-QTL and CAM-QTL are shown in dark green. Underlined marker represent SSR marker constructed from known genes from *Arabidopsis thaliana* and their functions are listed in table S4.



4.2. Association mapping

4.2.1. Phenotypic distribution of the traits

Seed longevity was investigated by examining twelve traits in the set of 215 ASSYST inbred lines. Distributions were much varied among traits and treatments (Fig. 11). Majority of genotypes showed TG and NS between 81 and 100% in CT. After CD most genotypes had high TG while NS dropped to a range between 41 and 60%. TSW ranged between 4.1 and 6 g for 74% of genotypes whereas the highest and lowest weights were 7.8 g and and 1.4 g, respectively (Fig. 11-A). Abn, R and RS distributed between 0 and 20% in CT (Table 6). After CD these traits were distributed between three groups 20, 40 and 60%, where the majority of Abn distributed between 0-20% and the majority of R and RS were distributed between 20 to 40% (Fig. 11-B). The AUC clearly increased after the fifth day of germination (97-120 h), while more than 90% of genotypes reached T10 and T50 within 24 and 48 h respectively. T10 was highly distributed from 48 to 120 h after CD, in the meanwhile low AUC (24 h) and high T50 (>120 h) were observed (Fig. 11-C).

The normal distribution shape clearly appeared in RL after CD (Fig. 11-D), while the majority of genotypes for SHL, RL and RSH were centred between 6.1 and 8.0 cm. In CT, SHL was shorter than in CD while most genotypes showed between 2.1 and 3.0 cm in CT and 4.1 to 5.0 cm in CD, which reveal shoot elongation under stress conditions of experimental ageing (Fig. 11-D). The opposite was the case in RL, which ranged in CT between 8.1 and 10 cm and dropped after CD to 4.1 to 8.0 cm (Fig. 11-D). The gradual elongation of SHL and the shortness in RL affect negatively the final RSH and result to unbalanced ratio of plantlets root and shoot systems as a consequence of seed ageing. High repeatability was recorded in most traits in CT and CD experiments (> 70%), while TG recorded 98.9 and 92.7 in CT and CD respectively (Table 6). Similarly, RS, AUC and T50 in CT and NS, Abn, R, RS, AUC and T50 in CD recorded repeatability higher than 70%. However, moderate repeatability (30 – 60%) was mainly observed in CT with R, Abn, NS, T10, SHL and RL. While low repeatability (< 30%) was observed in RSH in both CT and CD. Very wide range were found among population individuals. This range is clearly increased after CD especially in germination speed parameters (Table 6).

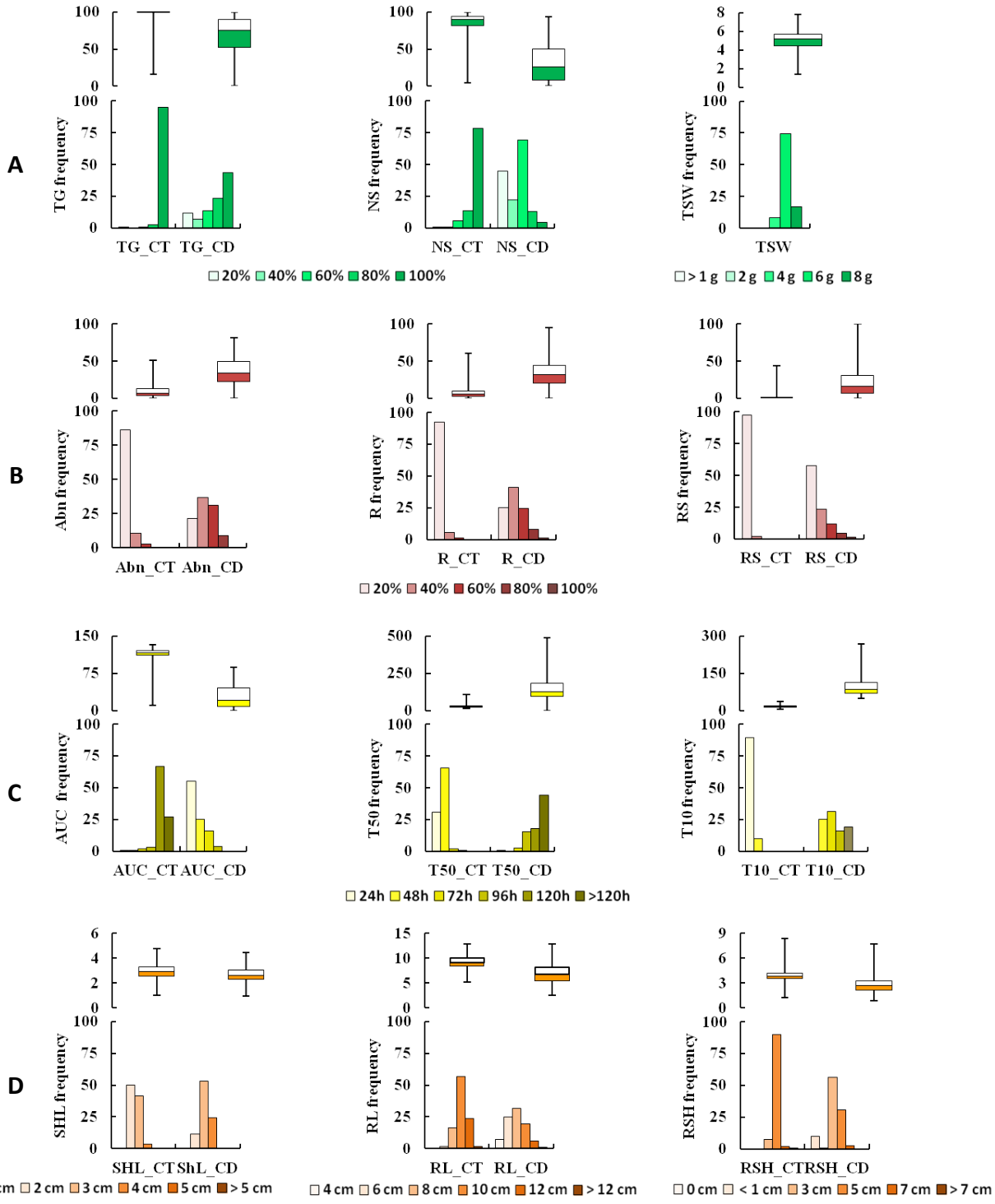


Fig. 11. Trait distribution (lower chart) of twelve traits recorded in ASSYST population Box Plot (higher chart) shows max, 25% quartile, median, 75% quartile and min. A: shows the distribution of total germination (TG) and normal seedlings (NS) and thousand seed weight (TSW). B: shows the total abnormality (Abn), root abnormality (R) and root-hypocotyle abnormality (RS). C: shows the speed traits, Area under the curve of 150 h (AUC), time to 50% germination and time to 10%germination. D: shows shoot length (SHL), root length (RL) and root-shoot ratio.

Table 6. Germination traits representing seed longevity. The average of three replicates and the range of recorded max and min values are shown. The LSD 5% and the repeatability 1% for control and experimental ageing (CD) are shown.

Traits	Unit	Control germination (CT)				Controlled deterioration (CD)			
		Average	LSD	Range	Repeatability%	Average	LSD	Range	Repeatability%
TSW	g	5.10	0.00	1.40 - 7.80	-	5.10	0.00	1.40 - 7.80	-
TG	%	96.70	1.70	12.5 - 100.0	98.90	66.40	12.80	0.0 -100.0	92.70
NS	%	84.90	17.20	0.0 - 100	60.50	31.00	14.90	0.0 - 95.7	88.20
Abn	%	10.40	11.30	0.0 - 57.5	65.20	35.50	16.40	0.0 - 87.5	74.80
R	%	8.00	11.70	0.0 -60.0	49.70	33.00	18.70	0.0 - 100	71.40
RS	%	2.70	1.60	0.0 - 45.2	97.50	22.10	15.90	0.0 - 100	78.90
AUC	h	114.20	9.20	0.0 - 135.9	86.80	25.90	10.40	0.0 - 91.5	92.00
T50	h	28.50	4.60	12.6 - 107.8	89.70	107.60	0.00	0.0 - 495.7	84.70
T10	h	17.00	5.80	4.0 - 41.1	68.30	100.00	27.10	41.2 - 260.4	-
SHL	cm	2.90	0.70	0.7 - 5.3	60.40	2.60	1.20	1.1 - 6.1	22.80
RL	cm	9.20	2.30	4.4 - 15.7	34.60	6.80	2.60	1.0 - 15.3	54.50
RSH	-	3.80	1.40	1.2 - 14.7	22.00	2.80	2.00	0.7 - 9.99	26.50

In CT, correlation between traits in different treatments displayed positive significances between NS, TG and AUC (respectively 0.707** and 0.889**), Abn and R (0.737**), Abn and RS (0.570**) and T50 and T10 (0.630**). Additionally negative correlations were detected among NS and Abn, R and RS (respectively, 0.657**, 0.616**, 0.707**) and between SHL and RSH (0.680**). In CD, a highly positive correlation was displayed between AUC and both of TG, NS (respectively, 0.714** and 0.822**) and between RSH and RL (0.700**), while negative correlation was found among T10, T50 and AUC (respectively, -0.767** and -0.629**). Additionally, RL and RSH correlated positively with NS and negatively with R. While T10 correlated negatively with all of AUC, TG and NS (Table 7).

Table 7. Coefficient of correlation for twelve traits in control experiment (CT) after controlled deterioration (CD) representing seed longevity investigations: The red cells highlighted the highly significant positive and negative correlations.

1.0 / 0.9 / 0.8 / 0.7 / 0.6 / 0.5 / 0.4 / 0.3 / 0.2 / 0.1 / 0.0	CT	TSW	TG	NS	Abn	R	RS	AUC	T50	T10	SHL	RL	
	RSH	0.046	0.427**	0.419**	-0.156*	-0.182**	-0.317**	0.378**	-0.229**	-0.09	-0.680**	0.310**	
	RL	0.319**	0.131	0.105	0.021	-0.05	-0.09	0.177**	-0.228**	-0.157	0.156*		
	SHL	0.139*	-0.365**	-0.413**	0.224**	0.214**	0.263**	-0.332**	0.174*	0.11		0.042	TG
	T10	-0.016	-0.287**	-0.210**	0.021	-0.03	0.166*	-0.512**	0.630**		0.757**	-0.126	NS
	T50	-0.178**	-0.732**	-0.496**	0.1	0.288**	0.214**	-0.890**		-0.197**	0.469	0.240**	Abn
	AUC	0.127	0.889**	0.707**	-0.159*	-0.222**	-0.500**		0.625**	-0.383**	0.069	0.217**	R
	RS	0.171*	-0.490**	-0.707**	0.570**	0.228**		0.064	0.153*	-0.555**	-0.395**	0.253	RS
	R	0.037	-0.276**	-0.616**	0.737**		-0.488**	-0.215**	-0.009	0.822**	0.714**	-0.025	AUC
	Abn	0.219**	-0.145*	-0.657**		-0.629**	0.361**	0.251**	0.348**	-0.438**	-0.274**	0.005	T50
	NS	-0.009	0.762**		0.485**	-0.767**	0.527**	0.337**	-0.065	-0.623	-0.696**	0.075	T10
	TG	0.081		-0.276**	-0.039	0.162*	-0.297**	0.049	0.126	0.149*	0.244**	0.165*	SHL
			0.356**	-0.456**	-0.310**	0.532**	-0.418**	-0.593**	-0.402**	0.663**	0.430**	0.009	RL
		0.700**	-0.,322**	-0.280**	-0.285**	0.430**	-0.256**	-0.551**	-0.426**	0.563**	0.319**	-0.132	RSH
		RL	SHL	T10	T50	AUC	RS	R	Abn	NS	TG	TSW	CD

4.2.2. Population structure and genetic clusters

Population structure analysis of 215 ASSYST individuals revealed 197 genotypes in three major groups (65 in Q1, 99 in Q2 and 33 in Q3) clustered mainly according to the oil quality level of seeds while 18 genotypes were not grouped (Fig. 12). Twelve individuals from ++ oil quality were grouped in Q1, while two individuals were grouped in each Q2 and Q3 from the same oil quality level (Table 8). 76 from total 95 individuals from 00 oil quality, while 18 were listed in Q1 and just one individual belonged to Q3. Oil quality individuals group of +0/0+ were divided between Q1 (5 individuals) and Q2 (4 individuals) while the genotyped with unspecified quality (USQ) were divided in two groups Q1 and Q3 (Table 8).The population structure did not show clear relation with the origin of genotypes, since most of genotypes were winter OSR originated from Europe. However, majority of genotypes originated in Germany, France and UK were clustered in Q2 and majority of Swedish genotypes were found in Q1. Mix of various origins was clustered in Q3 (Table S.5). Most of vegetable genotypes and winter fodder were clustered in Q3 while majority of winter OSR were clustered in Q2 followed by Q1 (Table 8).

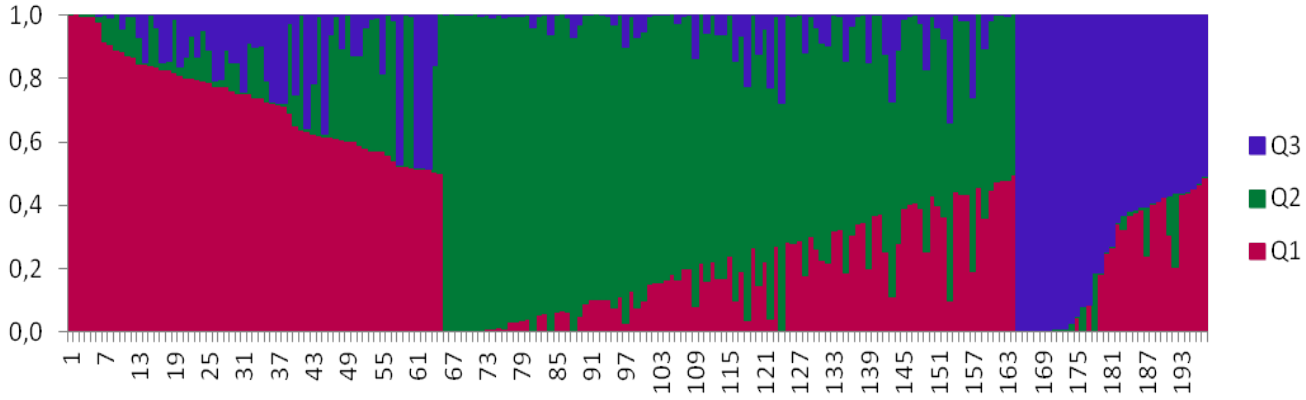


Fig. 12. The population structure of 215 genotypes (ASSYST population) of oilseed rape originated mainly from Europe. The population structure to three groups depending mainly on oil quality of seeds. The supplementary table S1 gives details about genotypes in each group.

Table 8. Types of 197 genotypes of ASSYST population clustered in three structured groups. The low and moderate oil quality seeds were grouped in Q1 and the high oil quality seeds (00) were grouped in Q2 and the rest were clustered in the third group.

Subgroups	Origin	Seed quality of winter OSR	Unspecified quality (USQ)	Spring OSR	Unspecified	Vegetable	Winter fodder	Winter OSR
Q1 (65)	16 countries	35 HQ : 18 (**00**), 12(++), 5 (+**0**/**0**+)	30 **USQ**	1	0	1	5	55
Q2 (99)	9 countries	82 HQ : 76(**00**),2(++) & 4(+**0**/**0**+)	17 **USQ**	0	1	1	3	93
Q3 (33)	18 countries	3 HQ : 1(**00**) & 2(++)	30 **USQ**	1	4	7	11	9

Additional clusters of genetic relatedness calculated using the principal coordinate analysis (PCoA) applied to 215 genotypes with minor allele frequency more than 10%, in which the first PCo represented 39% of genetic variations and the second 24%. The proportions of PCo1 and PCo2 are shown according to the three clusters identified in population structure analysis (Fig. 12). PCo1 and 2 displayed close relation of most of genotypes where all genotypes were gathered in the positive quarter of PCo1 and PCo2, however, definite groups were clustered (Fig. 13). Q1 and Q2 displayed in two different dimensions in positive quarter PCo1 and positive quarter PCo2, respectively. Q3 was distributed widely comparing with the Q1 and Q2 which is in a part close related to Q1 genotypes. Q2 considered as the largest group consisted of 99 genotypes showing narrow distribution comparing with Q1 and Q3. The 18 ungrouped genotypes are distributed in the middle between the three clusters but they showed close cluster to Q2 (Fig. 13)

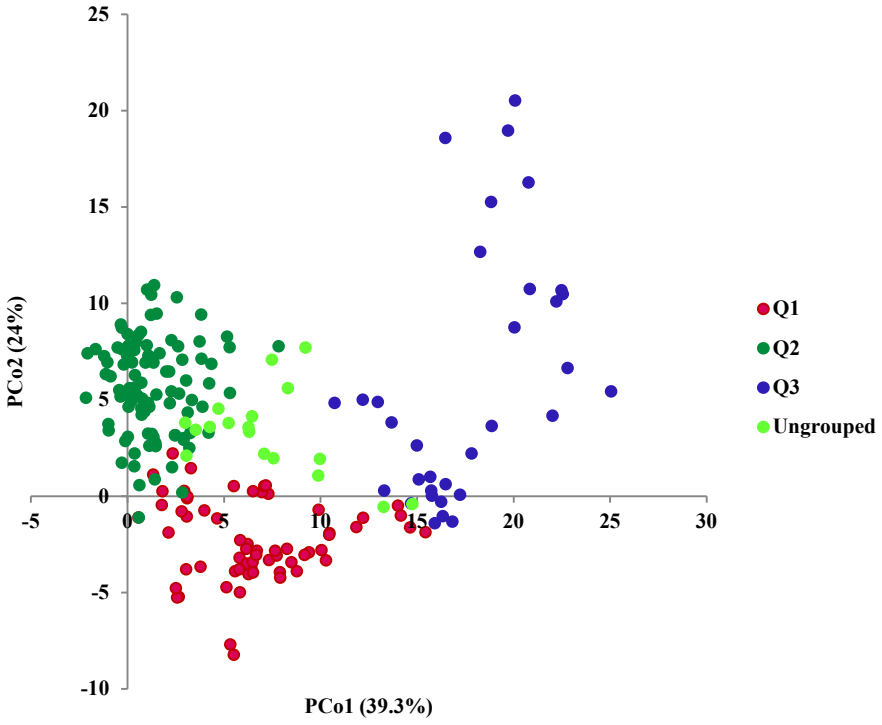


Fig. 13. The principal coordinate analysis showed the genotypic variations based on the first PCo's among 215 genotypes of ASSYST population regarding the first two coordinates. Different clusters were represented with different colours. The red, green and blue points are representing the genotypes in the three structured groups Q1, Q2 and Q3, respectively.

4.2.3. Genetic contribution of tested traits

Genome-wide association study (GWAS) revealed 172 Marker-trait associations (MTAs) across traits and treatments. 132 MTAs were detected on A-linkage groups (A1-A10) and C-linkage groups (C1-C9). The rest of positions were detected on the random positions of genome A (Ann) and genome C (Cnn) in addition to some sequence sets with unknown positions (UKN) (Fig 14, 15, 16, and 17). MTAs were detected on all linkage groups (LGs) except on C2. The highest numbers of MTAs presented on chromosomes A5 and A9 (12 MTA) followed by A3 and C4 (11 MTAs), C2 and C7 (9 MTAs) and A4, A8 and C1 (8 MTAs) for all tested traits. Additionally, 35 MTAs detected from UKN, six from Ann and only one from Cnn. Fourteen loci were identical to one position for two or more traits which differ in significance level and R^2 (Table 9).

TSW presented only two MTAs which were not close to any of other associations of the studied traits. Twenty MTAs associated with TG (-CT and -CD) located on nine LGs, where four, three two and two MTAs were presented on A4, A9, A7 and A10, respectively. The range of $-\log_{10}$ (*p-value*) in TG-MTAs was 3.00 – 3.94 and R^2 was 0.05 - 0.07 (Table, S6) .UKN marker Bn-p16321 (Site 711) was associated with TG-CT and TG-CD and recorded $-\log_{10}$ (*p-value*) range of 3.6 - 4.0. Additionally, two subsequent associations

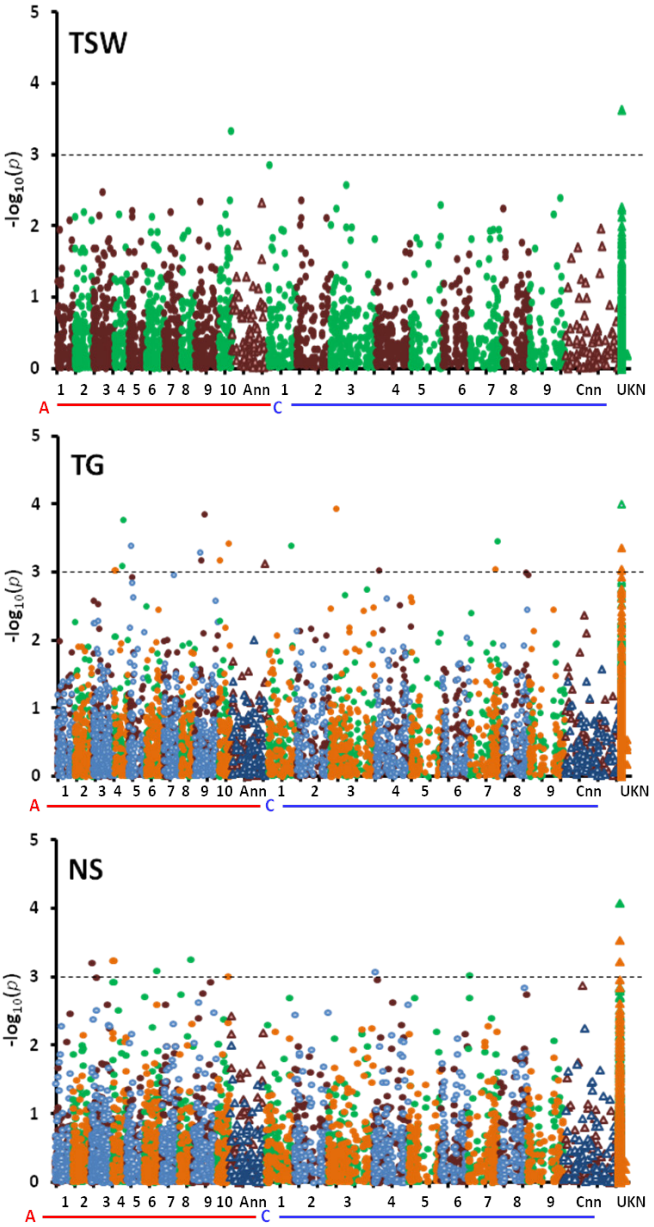


Fig. 14. Manhattan plots showing associations for thousand seed weight (TSW), total germination (TG) and normal seedlings (NS). The significant threshold -log $_{10}$ ($P < 0.001$) = 3. 0. Different in colours explain different linkage groups of A and C genomes of *B. napus*. Dots in brown and green show associations from control germination (CT) and dots in blue and orange show associations from the controlled deterioration (CD). Triangles explain random genomic regions which belong to A-genome (Ann), C-genome (Cnn) or to unspecified regions (UNK).

linked with TG-CT were presented on A4 on positions 10299790- 12207782 (Sites 54 and 56). The highest R^2 in TG-CT was detected on C3 (7.05) with the marker Bn-p5282 and physical position 7069134 and the highest in TG-CD (6.47) was detected on marker Bn-p209445 and position 13822514 (Table, S6).

NS presented 12 associations on seven chromosomes and UKN which were different in each of CT and CD treatments, however, UKN marker Bn-p16321 (site 711) was detected in both TG-CD and NS-CT (Fig. 14 & Table 9).

Abn showed 12 significant associations for both CT and CD (Fig. 15). On A8 two subsequent markers Bn-p1249 (site 14) and Bn-p2547584 (site 15) were linked with Abn-CT and Abn-CD respectively. While in C5, C7 and C8 MTAs were detected for both of CT and CD but in different positions. R and RS displayed 21 and 11 loci in CT and CD, respectively, where R-MTAs were widely located on 13 LGs and three different UKN markers. On C1 a significant SNP was found associated with both RS-CT and RS-CD detected by the marker Bn-p3110 (Site 119) and the subsequent marker Bn-p3167 (Site 118) was detected with RS-CD (Table 9).

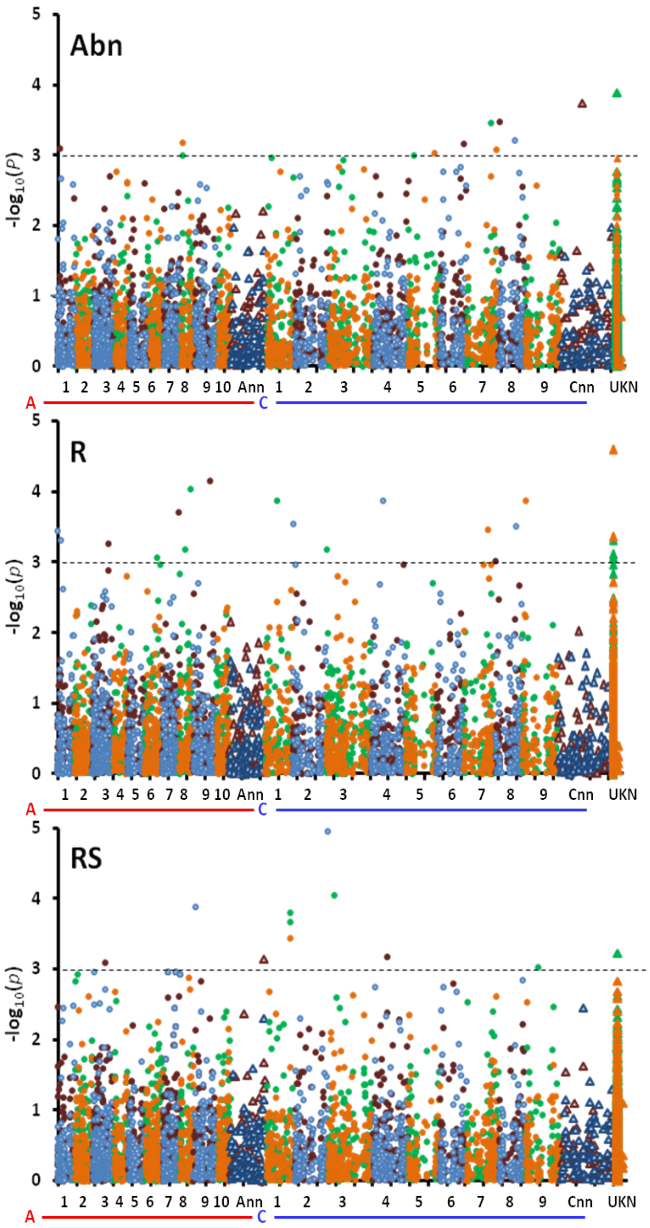


Fig. 15. Manhattan plots showing associations for total abnormal plantlets (Abn), root abnormalities (R) and root and shoot abnormalities (RS). The significant threshold -log $_{10}$ ($P < 0.001$) = 3. 0. Different colours explain different linkage groups of A and C genomes of *B. napus*. Dots in brown and green show associations from control germination (CT) and dots in blue and orange show associations from the controlled deterioration (CD). Triangles explain random genomic regions which belong to A-genome (Ann), C-genome (Cnn) or to unspecified regions (UNK).

T10, T50 and AUC detected 11, 21 and 20 positions in CT and CD, respectively, (Fig. 16). T50-CT recorded the highest significant level associated with marker Bn-p8356 (Site 63) equalled to –log10 (P-value) = 7.98 and positioned on LG C4. T50-CT was also detected with two lower significant markers on the same chromosome, Bn-p2198 and Bn-p12663 (sites 50 and 30), which confirm the strong association of this trait on this position of chromosome C4. Generally, eight highly significant SNPs were detected in T50 (CT and CD) which recorded –log10 (p-value) more than four.

SHL and RSH revealed four and fourteen significant associations. While RL detected the highest numbers of significant SNPs (24 MTAs) with $-\log_{10}$ (*p-value*) ranged from 3.0 to 4.92 and R^2 ranged from 0.04 to 0.07 (Fig. 17, Table S6).

Unique positions were revealed for the exact traits in 14 positions across the whole genome. These positions were localized mostly on A-genome (Table, 9).

A couple of traits were closely linked and repeated together in same positions such as T50 and AUC which repeated on A5, A9 and C4.

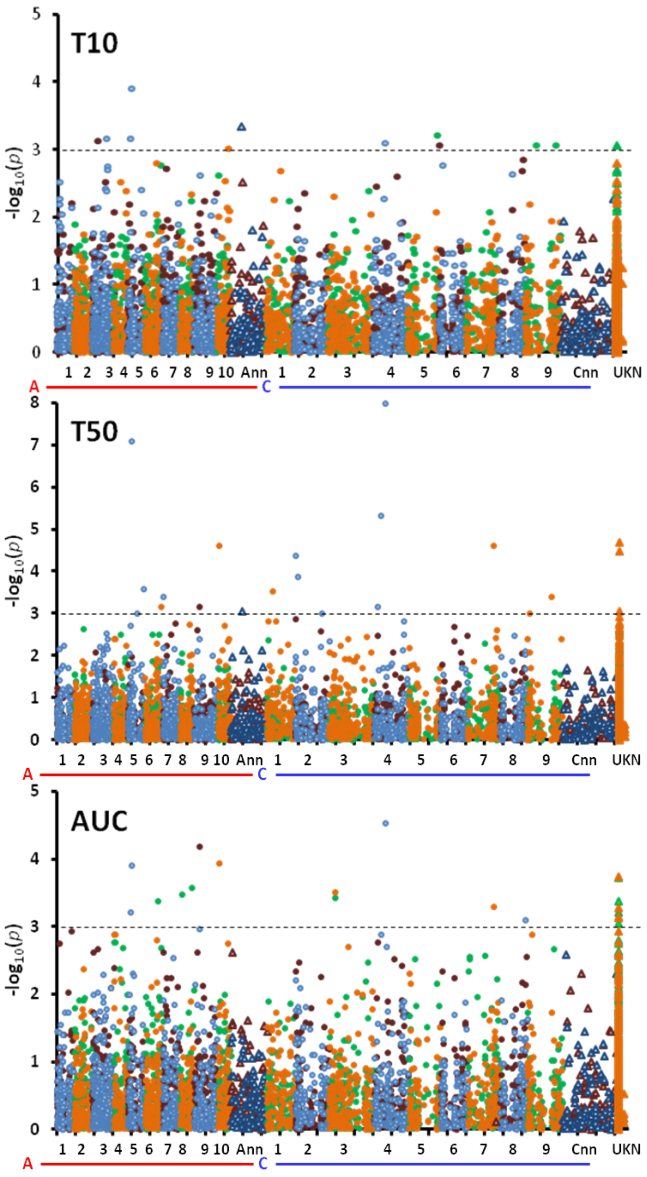


Fig. 16. Manhattan plots showing associations for time to reach 10% and 50% of germination (T10 and T50) and area under the curve after 150 hours of germination (AUC). The significant threshold -log $_{10}$ ($P < 0.001$) = 3. 0. Different in colours explain different linkage groups of A and C genomes of *B. napus*. Dots in brown and green show associations from control germination (CT) and dots in blue and orange show associations from the controlled deterioration (CD). Triangles explain random genomic regions which belong to A-genome (Ann), C-genome (Cnn) or to unspecified regions (UNK).

Additionally, T50 and TG were localized in same position with T10 on A5. AUC, T50 and TG were remarked together on extra two positions on A10 and C7 sites 16 and 92 respectively. The speed parameters T10, T50 and AUC were found in site 51 of LG A5.

Furthermore, TG and NS were found together in site 1 and 17 of LG A4. Meanwhile, on A6 and A8 the traits AUC, NS and R of CD were detected with sites 106 and 86, respectively. RS was remarked on both CT and CD on same position on C1 in addition to TG-CD which found on same position. However, T10 were co-localized twice in combined positions with NS in case of CD and with T50 and AUC in case of CT. Additionally RL and RSH were detected in site 58 on A3. The highest R^2 of 13.79% was recorded with T50 which on LG C4 with $-\log_{10}(P)$ of 7.98 (Table, 9). The complete set of R^2 which revealed in all traits and treatments are listed in table S6.

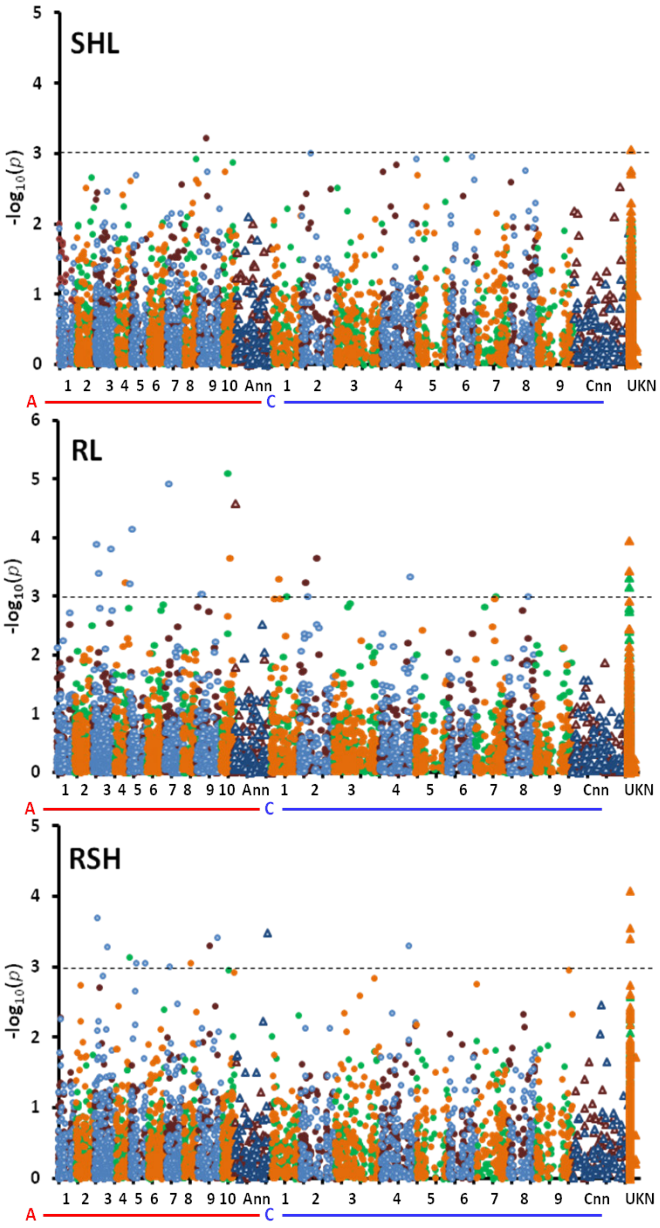


Fig. 17. Manhattan plots Showing associations for plantlet shoot length (SHL), plantlets root length (RL) and plantlets root-shoot ration (RSH). The significant threshold -log $_{10}$ ($P < 0.001$) = 3. 0. Different in colours explain different linkage groups of A and C genomes of *B. napus*. Dots in brown and green show associations from control germination (CT) and dots in blue and orange show associations from the controlled deterioration (CD). Triangles explain random genomic regions which belong to A-genome (Ann), C-genome (Cnn) or to unspecified regions (UNK).

Table 9. Overlapping positions in two or more traits which suggest a link between these traits: R^2 represents the explained phenotypic variation and $-\log_{10}$ represents the significance level of association. Ten positions were detected on seven linkage groups (LGs) from A-genome and four positions were detected on three LGs from C-genome

Trait	R^2	$-\log_{10}$	Locus	LG	Site	Position
RL_CT	6,06	3,88	Bn-ctg7180014774289-p2882	A03	58	3483561
RSH_CT	5,61	3,70				
NS_CD	4,62	3,00	Bn-Scaffold000039-p674225	A03	91	6775781
T10_CD	5,61	3,12				
NS_CT	5,53	3,24	Bn-Scaffold000016-p141266	A04	1	16958
TG_CT	5,28	3,03				
NS_CT	5,53	3,24	Bn-Scaffold000016-p1309884	A04	17	1265282
TG_CT	5,28	3,03				
AUC_CT	5,4	3,21	Bn-Scaffold000004-p2925914	A05	39	2982867
TG_CT	6,16	3,39				
AUC_CT	6,68	3,91	Bn-Scaffold000004-p5075668	A05	51	4896407
T10_CT	6,27	3,91				
T50_CT	12,48	7,09				
AUC_CD	5,46	3,37	Bn-Scaffold000044-p810737	A06	106	16749821
NS_CD	4,8	3,09				
R_CD	4,83	3,05				
AUC_CD	5,73	3,57	Bn-Scaffold000020-p568288	A08	86	15644722
NS_CD	5,07	3,26				
R_CD	6,42	4,02				
AUC_CD	7,06	4,19	Bn-Scaffold000058-p205581	A09	45	8308215
T50_CD	6,67	3,15				
AUC_CT	6,6	3,93	Bn-Scaffold000002-p4278788	A10	16	1798853
T50_CT	7,86	4,62				
TG_CT	5,57	3,17				
RS_CD	6,13	3,79	Bn-ctg7180014743660-p3110	C01	119	31396196
RS_CT	5,83	3,45				
TG_CD	5,06	3,38				
T50_CT	5,16	3,15	Bn-ctg7180014744894-p12663	C04	30	5163438
TG_CD	4,51	3,03				
AUC_CT	7,64	4,53	Bn-ctg7180014733601-p8356	C04	63	15071482
T50_CT	13,79	7,98				
AUC_CT	5,39	3,29	Bn-ctg7180014746444-p5025	C07	92	35327200
T50_CT	7,81	4,61				
TG_CT	5,28	3,04				

4.2.4. The annotation of significant SNPs in the association study:

Annotation of traits associated SNPs revealed 19,794 genes within a distance of 500,000 bp (Table 10) up and downstream depending on Blast2GO procedure. The detected genes for twelve traits per treatment were counted in table 10. The matching of those genes with their orthologue genes in *Arabidopsis thaliana* has been screened using recently published *B. napus* genome data (Chalhoub et al. 2014). Detected genes were divided depending on their functions to three groups: 1) gene functions in biological processes (BP), 2) genes acting in molecular functions (MF) and 3) genes playing a role on cellular components (CC). In addition some genes detected with unknown functions (UKN). Genes could be either found in BP or serve in several functions in MF and CC. Numbers of genes functions were displayed for each trait (CT and CD) in Fig.18. Generally, the BP recorded the highest number in all tested traits comparing with MF and CC although the three functions are varied for each trait in CT and CD. The BP functions were higher in TG-CT than TG-CD while the opposite was observed in NS, Abn, R, RS. This observation suggested more biological functions take place in case of seed vigour (NS) which is closely linked with abnormality traits. In R-CT, very few genes and functions took place while in R-CD more than 4000 BP and 2000 CC were detected, in meanwhile RL-CD and RSH-CD precede low BP, MF and CC comparing with CT. Similarly, T50-CD detected very few genes and genetic functions which could relate to less of functional processes during the extreme ageing and cell death especially that several individuals after CD did not reach T_{50} and produced very weak roots comparing with CT. The hypergeometric test (GOstat), a second annotation procedure, revealed BP as a major genes group followed by MF and finally CC (Fig. 19). Genes were filtered by *p-value* of 0.05 and listed by their major function which belonged to one of these three groups (BP, MF or CC). While the BP group recorded the highest gene number, the highly significant genes (*p-value* < 0.01) were selected and utilized to explain our tested traits (Table S7).

Table 10. Number of detected genes out from annotation analysis using Blast2GO procedure by analyzing positions of trait associated SNPs plus and minus 500,000 bp.

	CT	CD
TSW	240	
TG	1240	1154
NS	582	948
Abn	583	1053
R	870	1345
RS	440	694
T10	813	752
T50	2272	144
AUC	1178	832
SHL	258	120
RL	2057	611
RSH	1313	295

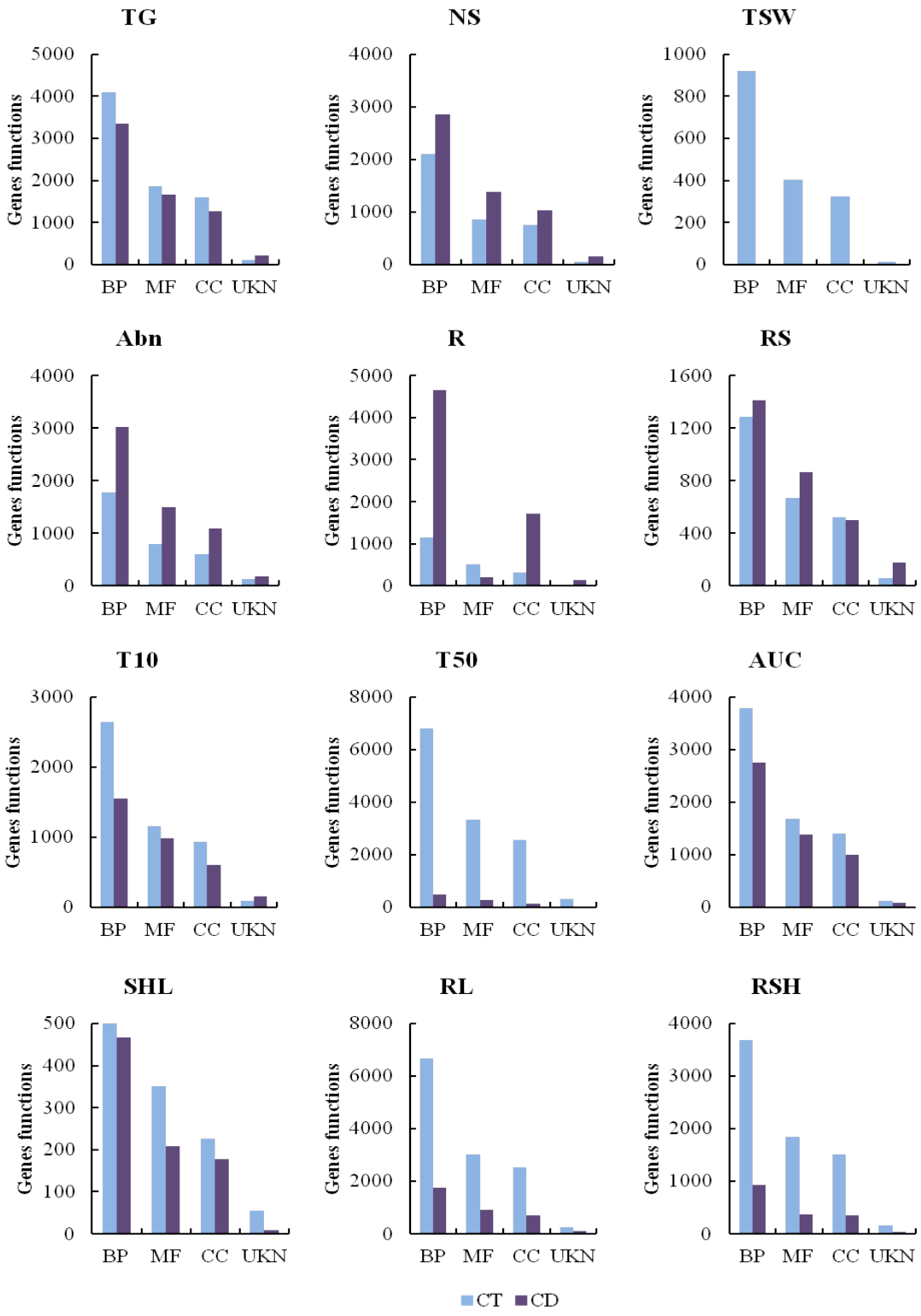


Fig. 18. Annotation results of trait-associated SNPs: Blast2GO analysis revealed 19,794 genes, each of these genes has one or several function in direction of biological processes (BP), molecular function (MF) or cellular components (CC). Some detected genes with unknown functions (UKN). Traits were previously explained in Fig. 10. Genes were detected for each trait after control germination (CT) and controlled deterioration (CD).

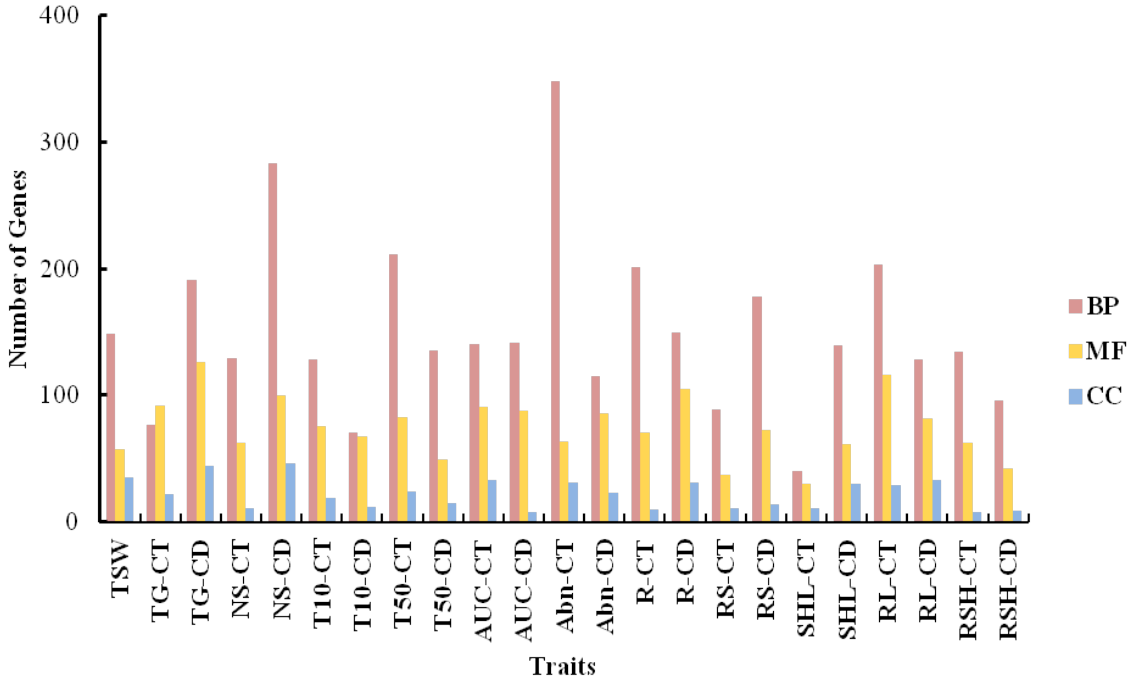


Fig. 19. The gene annotated of the significant SNPs using GOstat procedure: TSW plus 11 seed longevity traits examined by control germination (CT) and controlled deterioration (CD). Majority of annotated genes function in the biological processes (BP) followed by molecular functions (MF) and cellular components (CC).

5. Discussion

5.1. Genotype variation in seed longevity

Long-term seed storage behaviour varies among species, genotypes and even within seeds of an individual seed lot (Kochanek et al. 2009; Nagel and Börner 2010; Revilla et al. 2009; Revilla et al. 2006; Walters et al. 2005c). The seed storage behaviour can be categorized into short, medium and long life spans. *Brassica* spp. seeds were considered to have a medium life span which lose about 50% of their viability (P50) after 7.3 years of ambient storage at 20°C and 50% RH (Nagel and Börner 2010) and after 23 years of cold storage (-18°C) (Walters et al. 2005c). Ultra-dry storage, storage below 3% seed moisture content, of oilseed rape showed a high viability with more than 90% seeds after 38 years of storage (Perez-Garcia et al. 2008). In general, the period oil seeds reach 50% of their viability ranged for linseed, sunflower and cabbage (*B. oleracea*) from 10.4 years in linseed to 4.5 years in sunflower (Fig. 20). A lower P50 was recorded for chive (1.9 years) whereas higher P50s were recorded for non-oilseed crops as cucumber, pea and maize (14.9, 13.9 and 12.3 years, respectively) (Nagel and Börner 2010) . Differences between genotypes of a species could be shown by historic seed viability data from the Federal *Ex-situ* Genebank in Gatersleben. After five years ambient storage and a comparable high initial viability, most *B. oleracea* accessions kept their high germination percentage while others dropped to less than 25% (Fig. 21). However, seed longevity became trait of interest for seed science researchers since it has been an urgent need for future food security. Understanding mechanisms and genetics of seed deterioration could improve long-term conservation of our plant genetic resources. Due to the complexity of the trait seed longevity and germination which is linked to several agronomic traits and influenced by several genes, linkage mapping is a promising technique to discover the genetic basis.

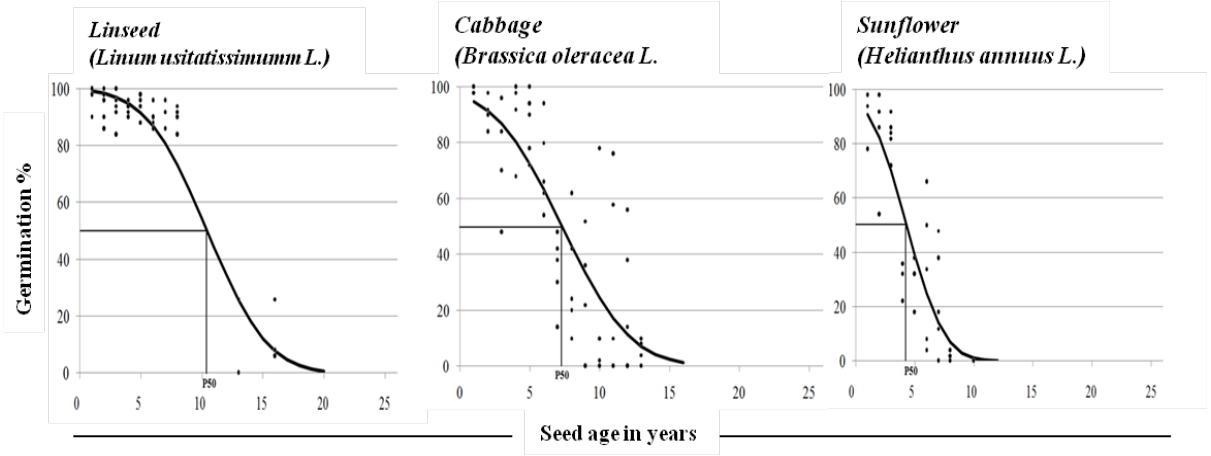


Fig. 20. Determination of the half-viability period for some oil crops stored under ambient conditions (20C, 50% RH). The results revealed medium longevity of oil crops which ranged between 4.5 to 10.4 years in sunflower and linseeds while *Brassica* spp. recorded 7.3 years. Modified after Nagel and Börner 2010.

In the recent study, control seed sets of 122 ExV-DH lines, three harvested seasons, were screened by SGT (CT) and (CD). Seeds harvested in 2005, were ambient stored for eight and six years, seeds harvested in 2009 were stored for two years and those harvested in 2012 for one year until CD was applied. For each seed set TG and NS decreased gradually after CD, however some lines kept their high values > 80% and others dropped down to zero (Fig. 21).

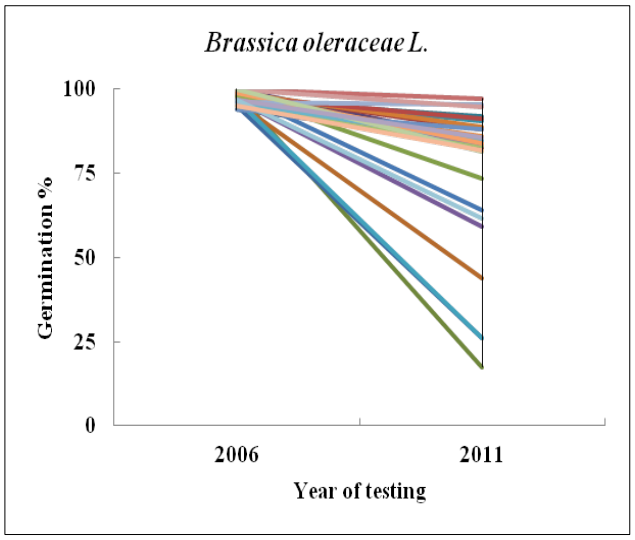


Fig. 21. Seed longevity variation within thirty *B. oleracea* accessions stored in Federal *Ex-Situ* Genebank in Gatersleben: The variance in decreasing the germination level of seed lots from same species and stored under same conditions for five years tend to genetic difference among seed lots.

Seed stored over a prolonged time are well known to suffer a gradual loss in their viability due to the physiological storage potential and deterioration events which determine the seed life-span (Bewley 1997; Rajjou and Debeaujon 2008). In the present research this gradual viability loss appeared in the degradation after six and eight years of ambient storage in ExV-5 seed lots. Further, deterioration processes are obvious when these extreme aged seeds of ExV-5_CT were compared with fresher seeds of ExV-9_CT (stored for two years under ambient conditions).

Wide variation in the initial seed viability, represented by TG, was observed among three harvests of ExV, where TG recorded 80.5, 99.6 and 92.5 in ExV-5, ExV-9 and ExV-12, respectively. It is clearly known that less stored seeds will have better initial viability. However, unexpectedly TG was lower in ExV-12_CT than in ExV-9_CT (92.2% and 99.6%, respectively) and similarly NS was recorded 73.3% and 94%, respectively. Nevertheless, comparing with the initial viability for each reproductive year, ExV-12 recorded the highest TG, NS and showed faster germination speed after CD when compared with ExV-9 and ExV-5. (Fig. 7 & Table 2). The unfavourable environmental conditions during seed development and/or harvest could be the reason behind the loss of ExV-12 viability which may cause internal damage in seed compositions and led to less TG and NS. However, the proportion of germinated seeds were very powerful, since they show faster germination after CD. This remark led us to conclude that initial viability is not an accurate parameter to judge the seed

longevity, while germination speed parameters (T50 & T10) are directly linked with seed vigour and real germination capacity.

In addition, the experimental ageing is a highly appreciated test in such evaluation. Where the highly vigour seeds are able to avoid the stress conditions and appeared after germination as normal seedlings. For more classification for vigorous level, the proportions of R and RS abnormality are indicators. From these discussed points we can conclude that only a combination of normal seedling proportion, germination speed and screening of radical and coleoptiles abnormalities may indicate seed longevity behaviour. In this context Mendes et al. (2009); Mladen et al. (2012) concluded that seed vigour tests are necessary to predict seed storage potential and provide more information than standard germination test alone by displaying details of deterioration processes.

5.2. Effect of environment on seed longevity

Seed longevity was recently studied in combination with secondary dormancy (Schatzki et al. 2013). In this experiment, 28 black-seeded winter oilseed rape cultivars produced at six different German locations chosen on basis of large differences in seed oil content were invisigated.

TG was calculated after accelerated ageing (AA: 42°C, 100% RH for 3 days) to estimate seed longevity. Large significant effects of the locations were observed for seed longevity, where TG after AA ranged from 35.2% to 74.4%. Thus, genotype x location interactions effects was consequently large for seed longevity and indicates that environmental factors as nutrient supply and growth conditions of the mother plant affect longevity of the harvested seeds (Kochanek et al. 2011). Additionally, harvest in different maturation stages was also discussed as a reason affecting seed longevity and dormancy in these seed sets (Schatzki et al. 2013) (Fig. 22). Variance components for the genotypes were comparatively large for secondary seed dormancy and thousand-kernel weight, whereas large effects of the locations were observed for seed longevity and oil and protein content. Genotype x location

Fig. 22. Six different locations of winter oilseed rape experimental materials were used for experimental ageing to test seed longevity: high effect of different locations was observed.

interaction effects was large for germination rate and seed longevity. Heritability was high for all traits investigated, except for germination rate and seed longevity (Table 11).

Table 11. Variance components and heritabilities for secondary seed dormancy (SD, %), germination rate (GR, %), seed longevity (SL, %), thousand kernel weight (TKW, g), for oil and protein content of the seed (in % seed dry matter) of 28 current winter oilseed rape cultivars tested in field experiments at 6 locations

Source of variance	SD	GR	SL	TKW	oil	protein
location (L)	49.2**	0.43+	192.5**	0.05**	6.9**	5.22**
genotype (G)	125.5**	4.22**	46.5**	0.10**	0.9**	0.36**
G x L	25.6	10.83	113.1	0.04	0.6	0.33
heritability	0.97	0.70	0.71	0.94	0.90	0.87

**, + Significant at P = 1%, 10% (F-test, ANOVA)

5.3. Seed composition in relation to seed longevity

Seed longevity is influenced by various factors. Next to environmental factors during seed development, also seed composition, seed size and structure affect final storability. Spearman's rank correlation coefficients between traits revealed significant positive correlations between seed longevity and thousand seed weight and protein content, while, oil were significantly negative correlated. (Schatzki et al. 2013) This observed weak negative relationship between oil content and seed longevity is supported by previous findings of Walters et al. (2005c) and of Nagel and Börner (2010).

A weak correlation was also observed among the examined traits in ExV-9 and the protein content, oil content, and oil composition (C18:1, C18:3 and C22:1) of the same seed sets (Data provided from Prof. Rod Snowdon Giessen University) (Table S3). We suggest that the time of evaluation for those traits influence the final correlation. Whereas oil and protein contents were recorded directly after harvest the ageing experiments started two years afterwards which exposure the seeds to gradual loss in seed moisture content. In addition, the oxidation reactions under ambient storage conditions could influence the oil compositions during these two years which require completely new estimations. Similarly, no correlation was found between the examined traits in ASSYST population (association panel tested for control and CD after reproduction in 2012) between longevity and oil (DW) and protein (DW) contents, lignin content, cellulose and hemicelluloses, fibre and the start and end of flowering were (Data provided by Prof. Rod Snowdon and published by (Körber et al. 2012).

5.4. QTL for seed longevity in different ExV harvests and ASSYST lines

The three harvests of ExV were studied for several traits related to seed longevity and generated a large number of QTLs. Many were specific to one harvest and/or to only one seed treatment of a harvest. The highest numbers of QTL were found on C3, A8 and A9 with 26, 22 and 22 loci, respectively. On those chromosomes traits across all three ExV reproductive years were displayed (Tables 3, 4 & 5). In ExV-5 (Table 3) unique QTL for CT1 of ExV-5 was mapped on LGs A3, A5, C3, C6 and C9, whereas for CT2 on A1, A8, C1 and C2 and for CD on A3, A5, C3, C6 and C9. However, identical QTL for all treatments could be detected and were located on A7 (0.0 -28.6 cM) and A9 (17.9 -22.8 cM). On A7 the SSR marker Ra2G08 (25.1 cM) detected with Abn and TG in _CT1 and CT2 and with T50 in _CD, was linked in previous studies with seed weight (Basunanda et al. 2010). On chromosome A9 one locus (30.4 -53.4 cM) was mapped for CT2 and CD in ExV-5 representing R and T10. The same locus was detected in ExV-12_CD1 which suggests similar physiological stage and deterioration processes could take place across different environments. The major QTL for ExV-5, namely T10_CT1_5, T50_CT2_5 and NS-CD_5 accounted for 19.4%, 19.4% and 16.0% of total phenotypic variation were unique QTLs for each treatment and indicate specific characteristics for each ageing stage. In this context, QTL unique for a harvest or a treatment may reflect biological differences between seed lots due to environmental differences during growing seasons and/or are caused by different mechanisms occurring during seed deterioration processes.

ExV-9 showed highest germination speed and initial viability comparing with other seasons in addition to similar seed sizes and weights among all individual lines. Identical loci for each treatment were on A5 and A8 for CT, on A1 for CD1 and on A5 and A8 for CD2, while, common loci for all treatments were represented on A10 and C3 and for CD on C6. Twenty seven loci from total 32 were confirmed by CAM-QTL and LSM-QTL which suggests stability of genetic effects across different environments. ExV-5 and ExV-12 had less confirmed QTL after QTL-CAM which suggests the high environmental influence on seed deterioration processes in these two years.

Further, a comparable loci were found in ExV-9_CD1 and _CD2 on LG C3 (87.8 – 89.1 cM) and shared the same markers with ExV-12_CD2 (89.1 – 104.2 cM) and with ExV-5_CT1 (87.8 – 89.1cM). Appearance of these loci in several treatments specifically may suggest that these seed lots are in the same deterioration stage and similar mechanisms were active. QTLs

for strong positive or negative correlated traits overlapped in each treatment. For example, Abn and TG are positively correlated and were mapped on similar loci on A7 and A9 of all ExV-5 treatments. In addition, germination and abnormality traits (Abn, NS, R) and germination speed traits (AUC, TG) did overlap at several loci. However, most overlaps were produced on A10 and C6 of ExV-9 where max four traits were found. TSW, mapped on two loci in the three harvests, share similar loci and AFLP marker E46M59_252 (50.4 cM) with AUC, NS and R in ExV-9. The positive correlation and co-localization of QTLs propose same regulation processes of germination for such traits.

5.4.1. Mother plant nutrition and seed longevity

Production side and environmental effects have strong effects on plant growth and development. These conditions are optimal if the mother plant is able to accumulate required amount of nutrition, energy and water necessary for vigorous, high-yielding seed production. Nutritional elements (inorganic ions or ionomes) act as regulators, cofactors or structural components which are essential to plant cell (Baxter 2009). Accumulation of minerals in seeds is a complex phenomenon, which is controlled by number of genes. The movements of elements from soil to seeds involve their mobilization from soils, uptake by roots, translocation to shoot, redistribution within the plant and deposition in seeds (White and Broadley 2009). Phosphorus deficiency is one of the major limitations for crop production, since it is essential macronutrient for plants and its high adsorption capacity in soils (e.g. adsorbed by Al-, Fe-oxides, Ca- and P complexes) (Ding et al. 2010). Mineral elements such as phosphorus (P), calcium (Ca), iron (Fe), magnesium (Mg) manganese (Mn) and zinc (Zn) are very essential for plant growth and development in addition to their act in various functions in plant cells (White and Broadley 2009). A strong relationship exist between P uptake and accumulation of P and other elements in seeds (Ding et al. 2010). P deficiency is considered as major limitation for crop production worldwide. In addition, Boron (B) is an essential micronutrient for plant growth and development (roots and shoots formation). The main function of B is in formation and structural integrity of plant cell wall (Zhang et al. 2014). In tropics and subtropics B deficiency is major factor limiting oilseed rape yield. However, most plant species cannot retranslocate B efficiently, and thus require a continuous supply of B through their life. Deficiency of B causes oxidative damage which is a major cause of cell death (Shi et al. 2012). Boron deficiency is major constraint to crop production worldwide (Shorrocks 1997). Stopping of root elongation, defect in active absorption area in root system, inhibition of young leaves growth and reduce fertility are effects of B deficiency

which decline the crop yield and quality (Shi et al. 2012). Oilseed rape has high requirement for B and P for its optimum seed quality and is sensitive to their deficiency.

Several genetic studies were performed to characterize genetic loci controlling the accumulation of mineral elements in seeds under different concentrations of P in soil (Ding et al. 2012; Ding et al. 2010; Shi et al. 2013; Yang et al. 2010) and the effect of B accumulation in mother plant on shoot and root formation of developed plantlets (Shi et al. 2012; Zhang et al. 2014). Our recent study on seed longevity revealed linkage among nutrition accumulation traits and TG, NS, R, Abn T50 and AUC (Fig. 23).

QTL detected under low boron (LB) conditions for root and shoot development of oilseed rape seedlings shared same markers with our tested traits in A1, A5 and C3. Shoot boron accumulation (SBA) was detected at the same location in A1 (70-80 cM) with trait T_{50} (Shi et al. 2012) Under low phosphorous supply (LP) QTLs for Mg and P elements were also detected in the comparable loci (Table S2) (Ding et al. 2010). These findings show that similar genetic mechanisms are activated for the availability of boron in the surrounding area and phosphorous accumulation in seeds as a critical demand for uniform germination and seedling development. Additionally, two putative candidate genes govern boron efficiency in Arabidopsis were localized at the same locus (A1) using *in silico* mapping (Shi L., 2012). The first homologe coding for HCO3 protein, a member of transporter family function in anion exchanger activity (AT5G25430.1) and the second is mutant displays sucrose-dependent seedling development and reduce the lateral root production (AT5G25760.2). On A5, increment of primary root length (IPRL) under LB was linked with NS, Abn and R under normal phosphorous supply (NP) for P, Ca, Mg elements (Ding et al. 2010; Shi et al. 2012)These finding suggest that formation of primary root or shoot in seedling development is linked with the lake in B and P availability which could be directly linked with producing abnormal seedlings and root defects during germination. Some Abn QTLs detected in ExV-5 and ExV-12 were at comparable locus with Fe QTLs under LP supply on A9. *LPR1* candidate gene responsible for controlling low Pi-triggered root growth inhibitor was detected on same locus by (Shi et al. 2013). QTLs for several seedling development traits under LP and LB were linked with TG, NS and R in C2 and C3. Additionally, Mn, Zn and Cu accumulation in seeds co-localized QTL under LP which were in same location with TG, NS and AUC on A10. Inheritance of B efficiency is a quantitative trait and it is highly linked by plant growth traits (IPRL, SDW and RDW) and B uptake traits (SBA and RBA). The QTLs that overlapped among these traits and the co-localization of seed longevity tested traits on the

same loci suggests that these physiological processes are regulated at the similar molecular level.

It is noteworthy that bases of plant ionome were genetically screened in ASSYST population for more understanding to the control of nutrient transport and accumulation in oilseed rape (Bus et al. 2014). Comparing this study with our results we found very close SNPs associated with R_CT (P5331) which was also detected for Zn concentration (P5336) on C2. Additionally, two other SNPs for Na concentration were found in comparable distance on C9 and A9 associated with T10_CD and RSH_CT. In this context, Bus et al. (2014) suggested that mineral concentration may be a predictor for seedling development more than for seed quality. However, we suggest close link between the ionome data from field trials and nutrition accumulation in mother plant could predict agronomic traits and seed quality data including seed longevity.

5.4.2. Seed weight and longevity

Oilseed rape yield is directly influenced by silique length (SL), seed per silique (SS) and seed weight (SW) because the silique is the main organ for photosynthesis and yield production (Zhang et al. 2011). The comparison of different QTL mapping for silique traits revealed intraspecific variation for the three traits, however, majority of their detected QTL in several genetic backgrounds were linked (Zhang et al. 2011). When comparing our recent study with literature the major QTLs for seed weight were selected according to their high LOD value or their stability across different environments and populations and were compared with seed longevity traits (Fig. 24, Table S2). Seed weight common QTLs were identified on A5 (52.9 – 60.8 cM) and A7 (0.0 – 21.2 cM) by a number of previous studies (Cai et al. 2014; Cai et al. 2012; Fan et al. 2010; Quijada et al. 2006; Radoev et al. 2008; Shi et al. 2009; Yang et al. 2012; Zhang et al. 2011). On A5, the interval 45.9 – 47.7 detected by NS and Abn in ExV-5_CT1 was linked with TSW and candidate gene was detected in same interval by (Cai et al. 2012) which involved in regulating amino acid accumulation and the synthesis of myo-inositol and raffinose during plant adaptation to long days. Additionally at A5 four QTL were detected in ExV-5_CT1 representing R and Abn and in ExV-9 representing R in the interval (51.5 - 71.5 cM). Two candidate genes were detected in this region encoding Acyl-coA protein and phosphatase protein 2A (PP2A). At A7 five QTL were detected in ExV-5_CT2 and _CD in two positions 0.0 and 16.4 that represented the traits TG and Abn mainly in CT and CD of ExV-5. Seed weight QTLs were co-localized on A1 which were linked with T50 in

highly natural aged seeds of ExV-5. Candidate gene was detected on 70.2 cM position which involved in the formation of nematode-induced syncytia in roots of *Arabidopsis thaliana*. In A8, NS, Abn and RS loci were detected across 0.0 – 10.8 cM which were linked with SL and SS. Unique QTLs for seed weight were confirmed on A7, A8 and C3 (Zhang et al. 2011) which were directly linked with our tested traits. The SSR marker Ra2-G08 was reported for TSW by (Basunanda et al. 2010; Fan et al. 2010) which was detected on A7 linked with TG, Abn and T50. On same locus a consensus QTL qSW.A7-2 was reported by Shi et al. (2009) which confirm the stability of TSW QTL expressed in different backgrounds and environments.

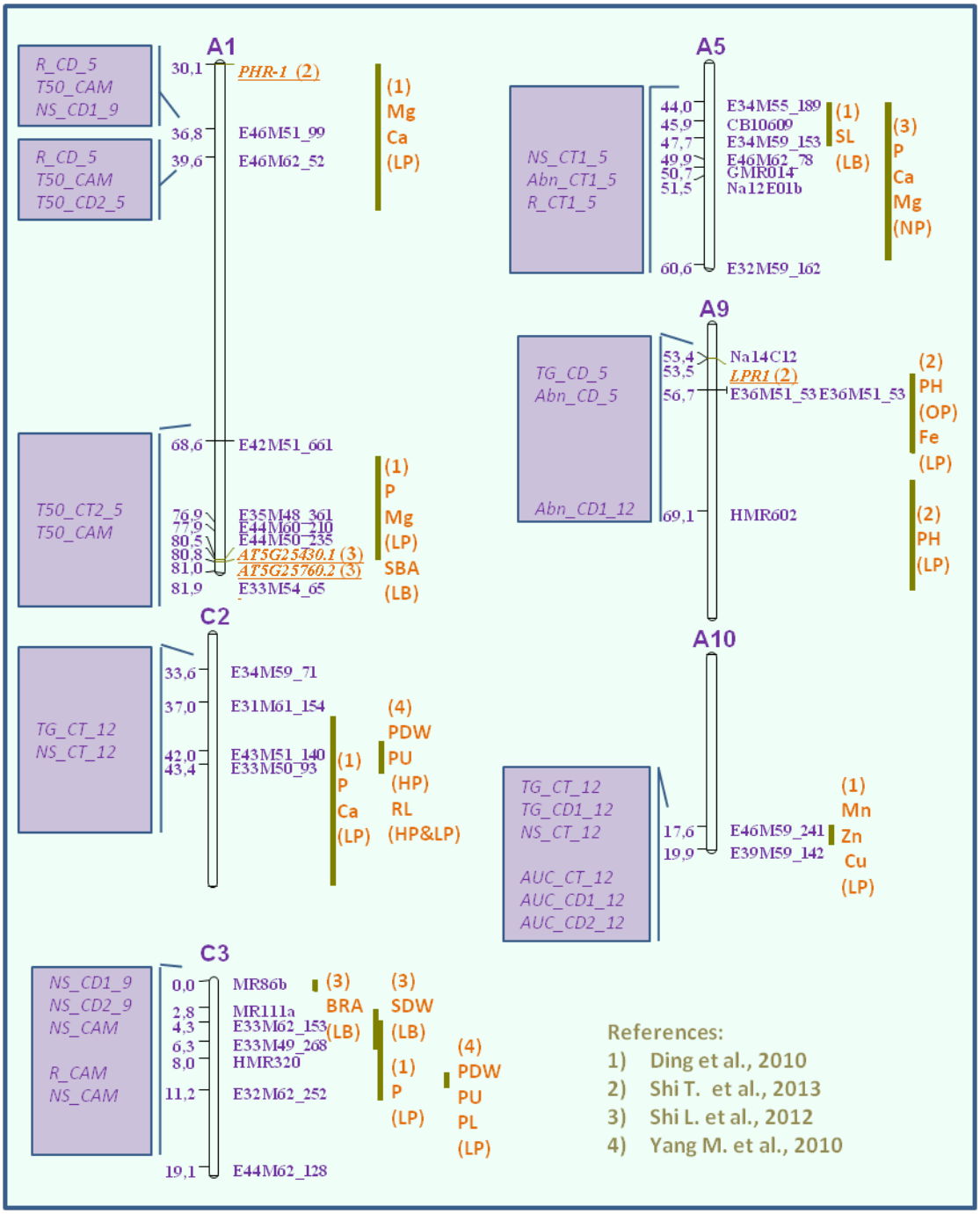


Fig. 23. QTL comparison among seed longevity QTLs produced by ExV population and the published linkage maps which screens the accumulation of plant nutrition in seeds and mother plant under LP: low phosphorus, HP: high phosphorus fertilization, OP: optimal phosphorus and LB: low boron fertilization and for following tested traits: SL: primary shoot length, SBA: shoot boron accumulation, SDW: shoot dry weight, PU: phosphorus uptake, PH: plant height , RL: root length and PDW: total plant dry weight.

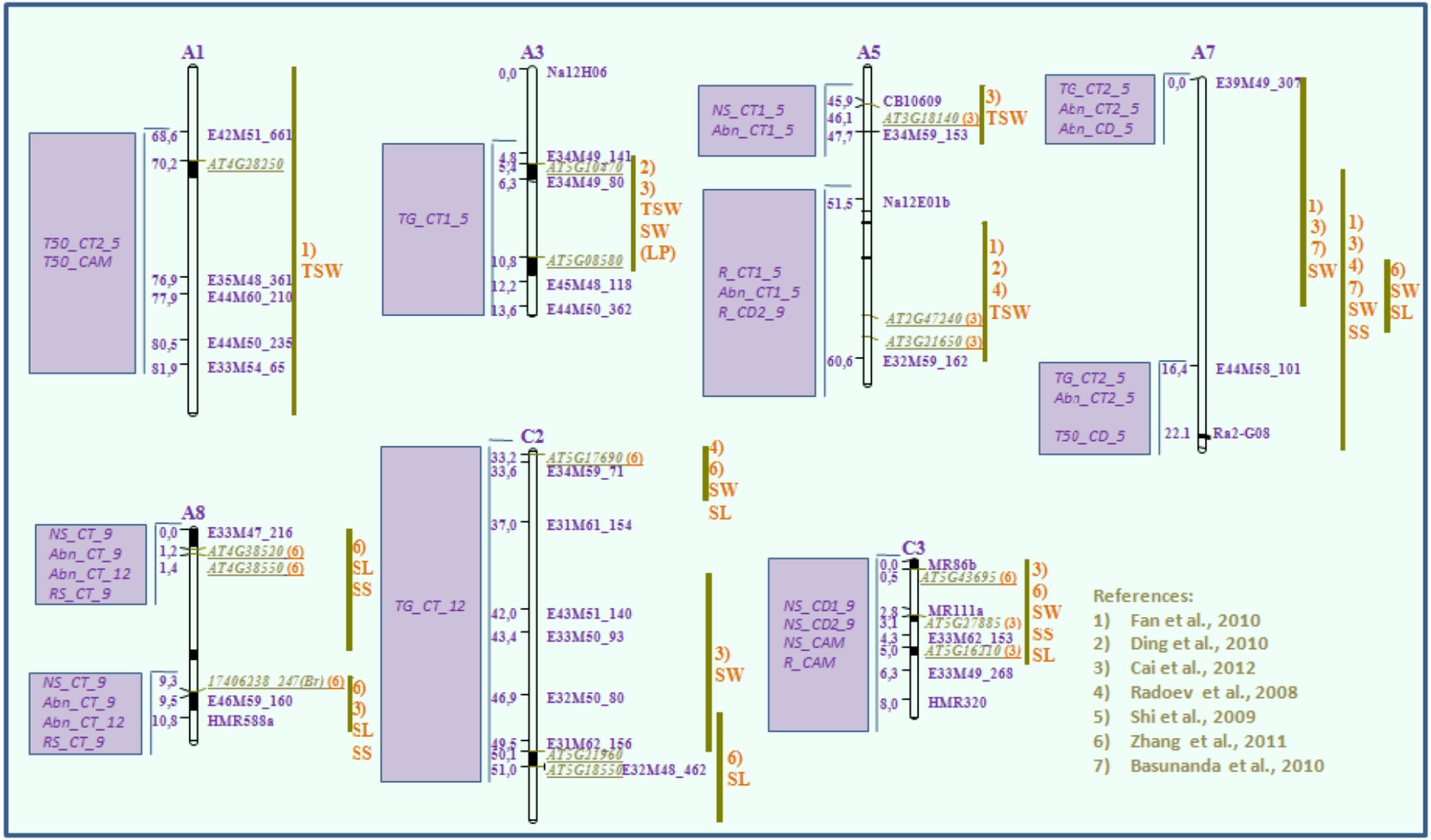


Fig. 24. QTL comparison among seed longevity QTLs produced by ExV population and the published linkage maps which screens seed weight related traits: thousand seed weight (TSW), silique length (SL) and seed per silique (SS).

Although comparing QTLs of ExV with literature displayed evidence that TSW and longevity traits are linked (Fig. 24), the GWAS study of ASSYST population did not confirm these findings, where two MTAs were detected for TSW which were unlinked with any of our tested traits. Confirming these results (Hatzig et al. 2015) found no correlation between TSW and germination performance, germination rate and radical growth. Furthermore, no attribution of TSW was found on total germination and time to absolute germination in *B. oleraceae* (Bettey et al. 2000).

5.4.3. Oil content and longevity

Lipid peroxidation is the most frequent cause for seed deterioration with oxidation of lipids and polyunsaturated fatty acids (Schwember and Bradford 2010b). Recently, several studies displayed the genetic bases of oil content (OC), fatty acid compositions (FA) (Delourme et al. 2006; Qiu et al. 2006; Wang et al. 2013; Zhao et al. 2008; Zou et al. 2010)and lipid orthologous genes (LOG) in *Arabidopsis thaliana* and oilseed rape (Zhao et al. 2012).

Oil content QTLs in published literature were distributed in all seed longevity loci in various tested traits detected with ExV. (Fig. 25 & Table S2). However, most QTL for OC and FA in oilseed rape were mainly found on A3, A8 and A9. Plantlets abnormality traits (Abn, R, RS) were detected in the upper loci of A8, A7 and lower loci on A9, where TG was concentrated on A3 which were linked to two strong loci representing OC and FA (Fig 24). The interval (41.1 - 45.4 cM) represented FA and OC and linked with AUC_CT2_5 on A8 while AUC_CT1_5 was detected on A6 in close interval for OC associated loci. AUC_CT2_5 and AUC_CD_5 were also linked on A9 with FA and OC. From this comparison we speculate about a genetic relation between abnormality, germination speed and OC and FA. However, no correlation was found for oil content and tested traits in ExV and ASSYST populations.

Some candidate genes were detected according to Zhao et al. (2012). On A1- AT3G13062 a lipid transport protein, on A7- AT2G19690 lipid signalling , A8- AT1G30120 lipid synthesis in plastids, A9- AT5G47670 protein coding gene EC1 (Leafy Cotyledon1) which functions as a regulator of embryo development.

Oil content influence seed longevity under open storage conditions, while at -20°C a rapid ageing was confirmed when the surrounding relative humidity is not constant (H.W. and J.B. 2004). Same findings were confirmed by (Nagel and Börner 2010; Walters et al. 2005c). Changes in lipid peroxidation and membrane integrity was recently studied in *Brassicaceae*

wild species (Mira et al. 2014) during ageing at 45°C and 90% RH. The results showed that the malondialdehyde accumulation, as a product for lipid peroxidation, was not associated with loss of seed viability under ageing conditions. The seed materials in mentioned experiment were stored previously under 5°C in silica gel which could prevent oxidation reactions and keep the high viability of seeds.

Our tested materials (ExV and ASSYST populations) were stored under ambient conditions which allow more reactions in seeds and deteriorate it faster than in cold storage. The comparison in Fig. 25 show that the linked QTL with OC or FA mostly associated with deteriorated seeds, where eleven loci were determined from ExV-5 which is six to eight years of storage in control and treatment (CT1, CT2 and CD) in addition to two QTL were determined in ExV-12_CD1. It is also clear that most detected traits are Abn, R and RS which is representing the seedlings abnormality. These observations indicates a relation between deteriorated seeds and OC or FA in the genetic level, which could led finally to abnormal performance of germinated seedlings.

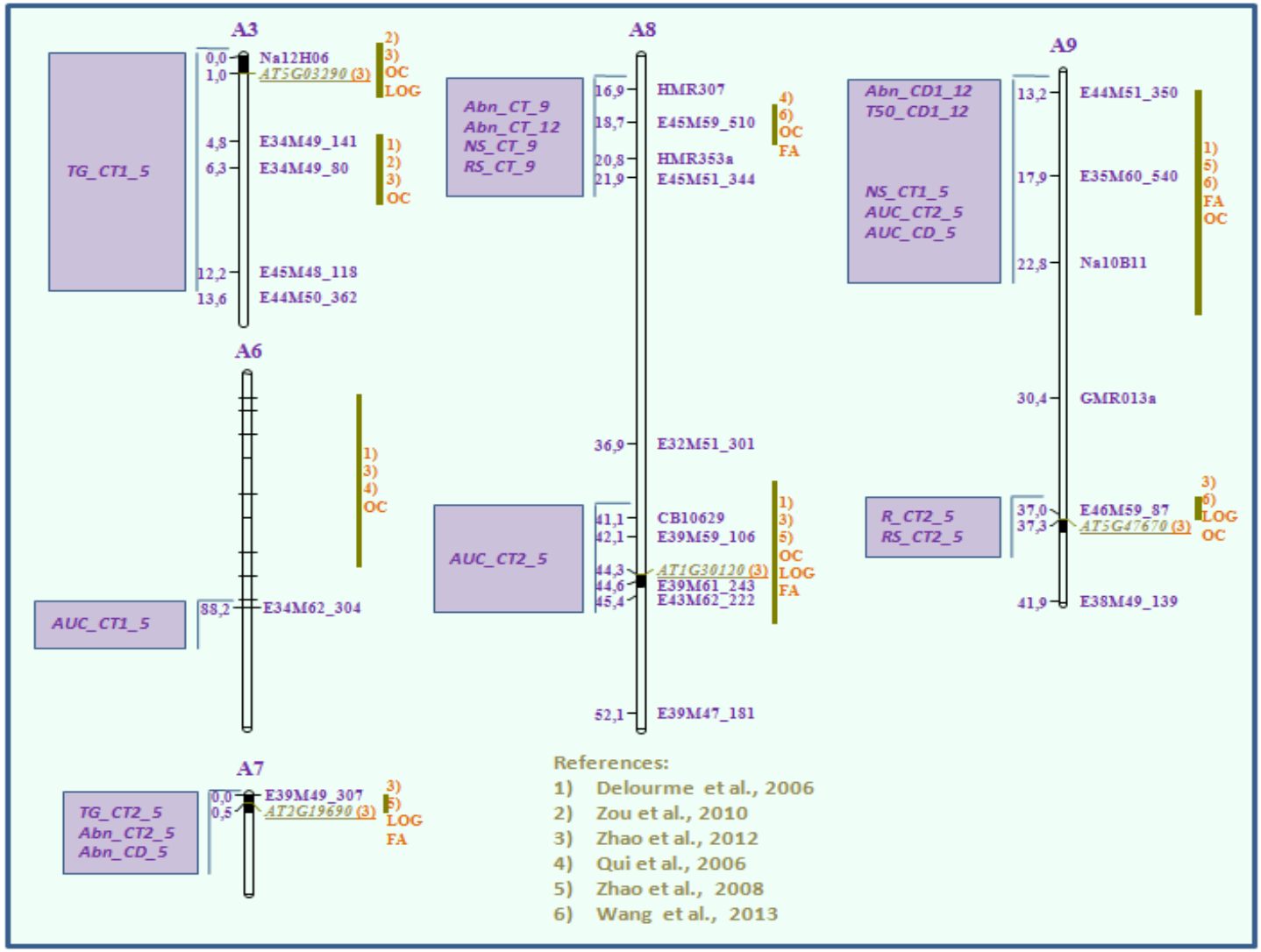


Fig. 25. QTL comparison among seed longevity QTLs produced by ExV population and the published linkage maps which screens oil content (OC), fatty acid compositions (FA) and the related orthologous genes compeered with *Arabidopsis thaliana* (LOG).

5.5. Candidate genes of seed longevity

Putative SSR candidate genes in ExV

Some SSR markers used for ExV mapping were designed from known gene hits of *Arabidobsis thaliana*. Comparing with available marker data in (at http://www.brassica.info) and with studies of Delourme et al. (2006); Lowe et al. (2004); Piquemal et al. (2005); Qiu et al. (2006); Suwabe et al. (2008); Wang et al. (2011a) we found eleven SSR markers linked to known genes which assumed to be linked with seed longevity. These SSR markers were detected on chromosomes A5, A7, A8, A9, A10, C3 and C4 of ExV-5, ExV-9 and ExV-12 (Table S4).

Variances of R and RS between different seed lots were specific for each environment and storage treatment. Stages of low, mediate and high germination are displayed by the gradual decrease in root length, extreme shortening of the root length till complete root loss and led to an increase of root/shoot ratio. More than 80% of abnormalities were obtained in the root system. QTL for R and RS in ExV-5, ExV-9 and ExV-12 were linked to the three homologues, AT1G12220.1, AT1G15120.1 and AT3G62980.1. These genes function as: resistance gene against bacterial pathogen, electron transporter and Auxin regulation. These three functions could give us a reason behind the root abnormality in the plantlets of the highly aged seeds which could be caused due to the bacterial infection in seeds or the lack of electron transportation during transcription and translation of certain genes. The Auxin is known as rooting hormone which is highly regulated under stress conditions (Overvoorde et al. 2010).

The homolog gene *PP2*-A10 (AT1G10155.1) was detected close to NS and confirmed by Abn on LG A5. This gene is responsible for long distance transport and binding function of RNA (Dinant et al. 2003; Vicente and Gustavo 2013). Another transporter family member was detected on A7, MtN21 (Gamas et al. 1996), which is linked to the marker Ra2G08 and associated with TG, Abn and T50 in ExV-5_CT1, _CT2 and _CD.

The LG A10 marker Na12H04 and LG C4 marker Na10C01a were associated with TSW in ExV9 and ExV12, respectively. These two markers are associated with genes promoting flowering and transporter element belonging to copia-like transporter family, which play critical role in RNA intermediate transcription and with membrane functional proteins (White et al. 1994).

Four candidate genes were detected that are related to avoide stresses. As mentioned before the homologe AT1G122201.1 describing a resistant gene against pathogens. Additionally, AGB31 gene encoding a plasma membrane arabinogalactan in *Arabidopsis* (Hijazi et al 2012) which have a role in vascular tissue function during both the defence response and development (Liu and Mehdy 2007). AGB31 was detected on A9 closely related to AUC. The third gene the heat shock factor related protein (AT4G19633.1) was linked to NS and Abn in ExV-5_CT1 on LG A5. While, the cyclase family protein involved in response to salt stress was linked to TG on ExV-5_CD on LG A9. Therefore, we can conclude that resistance to stresses is related to seed longevity as has been postulated by Clerkx et al. (2004); (Nagel et al. 2011).

Gene ontology analysis depending on GWAS

Annotation of MTAs of ASSYST population produced several highly significant genes directly linked with associated SNPs. We selected the highly significant SNPs –log10 (P) ≥4 to screen the most important genes related to seed longevity. T50-CT recorded the highest significance level in addition to six associated SNPs with –log10 (P) > 4. The annotation analysis using GOstat revealed some genes underlying the tested traits (Table S7). For T50-CT some genes were responsible for developmental processes, for example: GO:0010154, GO:0048316, GO:0052386, GO:0003006 were responsible, respectively, for fruit development, seed development, cell wall thickening and developmental process involved in reproduction. Additionally some genes were responsible for transportation of elements especially for the auxin, for example: GO:0010541 (acropetal auxin transport), GO:0060918 (auxin transport), GO:0009926 (auxin polar transport), GO:0010315 (auxin efflux), GO:0009914 (hormone transport), GO:0051180 (vitamin transport), GO:0015884 (folic acid transport).

RL-CD recorded highly significant SNP on A10 (-log10 (P) = 5.3), while RL-CT recorded two SNPs on A7 and A5 (-log (P) = 4.9 and 4.1 respectively). Forty four GO genes were listed for RL-CD (P-value < 0.01). For this trait, some genes were detected responsible for developmental processes like, while system RL-CD recorded highly significant SNP on A10 (-log10 (P) = 5.3), while RL-CT recorded two SNPs on A7 and A5 (-log (P) = 4.9 and 4.1 respectively). Forty four genes were listed for RL-CD (P-*value* < 0.01). For this trait, some genes were detected responsible for developmental processes like embryonic seed morphogenesis, regulation of seed germination or shoot system development. Some other

genes function for the negative regulation of seed germination or multicellular organismal processes in addition some genes detected are responsible for defense response and avoiding stresses such as osmotic stress, microbial phytotoxin. However, in RL-CT 16 genes were detected responsible for positive regulations for RNA-metabolic, DNA-templated and transcription, gene expression, ATPase activity and metabolic processes, cellular biosynthesis and response to stimulus. This group of genes display active phase of normal root development process.

RS-CT associated with highly significant SNP on C2 –log (p) = 4.95 as well as RS-CD which associated with significant SNP equal to –log (P) = 4 detected on C3. Most interesting genes represented in RS-CT were regulation genes for cell division and cell shape in addition to response to hypotonic salinity and UV and bacterium detection genes. RS-CD revealed number of positive regulation genes especially in response to stresses such as oxidative stress, fungus, salinity and leaf senescence process. Additionally, three genes were function in nitrogen biosynthesis (GO:0051173, GO:0080028 and GO:0050898). R-CD associated with highly significant SNPs on A9 (–log (p) = 4.15) and on A8 (–log (p) = 4). Most of presented genes for R-CD are responsible for metabolic processes, biosynthesis, photosynthesis and transport processes of root nutrition such as iron, manganese in addition to sugar phosphotransferase, pigment, glycerolipid. In addition five genes are function with RNA, mRNA stabilization and modification. A kind of recovery processes took place with help of these functional genes; such conditions suggest the ongoing machinery for root repair under extreme stresses.

In present study after the wide screening of seed longevity traits we can conclude that seed longevity is a very complex trait which is governed by different genes for each seed ageing stage. The effect of environmental conditions including growth atmosphere and mother plant nutrition is highly linked with seed quality which in turn affects seed vigour under long- term storage. Annotation of trait-associated sequences is a very powerful tool which brought closer some expectations about candidate genes linked to our traits of interests and put an imagination of which functions took place under certain physiological conditions. For more confirmation of the direct related candidate genes further analysis are required depending on LD blocks surrounding the significant SNP. Additionally, coding and non-coding regions of selected candidate genes and pathway relationship between those genes could be detected.

6. Summary

Gene banks are considered as a source of diversity for commercial crops which are aimed to support new breeding programs and research. Most cultivated plants are maintained as seeds which are considered as practical, cheap and accessible comparing with *in situ* conservation. Therefore, the seed survival period, also termed seed longevity, under different storage conditions is a matter of concern for scientists. Understanding factors which determine inter- and intraspecific variations of seed longevity would be beneficial for gene bank management but also seed industry.

Mapping of quantitative trait loci (QTL) is based on the linkage between polymorphic markers and phenotypic values in a population of related individual lines and can be used to identify genomic regions responsible for trait variation. In comparison the association mapping approach represents a more recent population based method of non-related individuals and relies on the linkage disequilibrium between a trait and a genetic marker. The present study attempts to quantify seed longevity in two different mapping panels. The Express x V8 doubled haploid (ExV) population consists of 122 lines harvested in the three seasons 2005, 2009 and 2012 and was analysed using 475 SSR and AFLP markers. The second "ASSYST" population is an association mapping and consists of 215 cultivars which were genotyped with 4001 bi-allelic SNP markers. Both populations were treated by controlled deterioration (CD) at 60% RH, 45°C and tested on traits elucidating seed longevity e.g. normal seedling appearance and germination speed. For each seed set grown in different environments, control (CT) and experimental ageing treatments were performed in order to examine the seed longevity in a wide range of conditions including ambient storage and different CD intervals.

In ExV, many QTLs were specific to one harvest and/or to only one seed treatment of a harvest. Identical QTL in ExV harvested in 2005 for CT and CD were detected on A7 (0.0 - 28.6 cM) and A9 (17.9 -22.8 cM) and in ExV harvested in2009 on A10 and C3. Common loci were found for CD treatments of ExV of 2009 and 2012 and CT of 2005 on chromosome C3 (89.1 – 104.2 cM). The appearance of these loci in several treatments indicates that these seed lots were in the same deterioration stage and similar mechanisms were active. In the combined QTL anaylsis across all treatments twenty seven loci from a total of 32 were confirmed, and suggest a stability of genetic effects across different environments. Comparison with literature revealed a close link between seed longevity QTLs and the major

QTLs detected for accumulation of plant nutrition, seed composition, seed weight and oil content.

Genome-wide association study (GWAS) revealed 172 marker-trait associations (MTAs) across traits and treatments. 132 MTAs were detected on both linkage groups (A1-A10 and C1-C9). Common loci were revealed for 14 MTAs across the whole genome. Germination speed parameter T50 (time to 50% germination), AUC (area under germination curve) and root length (RL) produced highly significant MTAs. Thereby, MTA associated with root abnormality on chromosome C2 was associated with Zn concentration. Further annotation of 12 traits associated SNPs using Blast2GO procedure revealed 19,794 genes. Majority of genes have functions in biological processes. Further, highly significant genes associated with T50 were involved in seed development and cell wall thickness, wheras RL was linked to genes playing roles in embryonic seed morphogenesis and regulation of seed germination.

The comprehensive genetic study on seed longevity revealed clear variances among genotypes cultivated in different seasons and stored at different conditions. This results emphasized high effects of the environment. However, comparable QTLs among seasons and storage treatments were detected. Germination speed parameters are considired as very helpful to evaluate seed longevity in addition to the abnormality levels of seedlings.

7. Zusammenfassung

Genbanken besitzen entscheidende Bedeutung bei der Erhaltung und Zurverfügungstellung der Diversität von Kulturpflanzen für Züchtungsprogramme und Forschung. Aufgrund hoher Praktikabilität, geringer Kosten und einfachem Zugang im Vergleich zur *in situ* Erhaltung werden die meisten Kulturpflanzen in Form von Saatgut konserviert. Dabei ist der Samenüberlebenszeitraum, auch als Samenlanglebigkeit bezeichnet, in Abhängigkeit von den Lagerungsbedingungen im besonderen Fokus der Wissenschaft; und Kenntnisse von Faktoren, die inter- und intraspezifischen Veränderungen der Langlebigkeit beeinflussen, vorteilhaft für ein effizientes Genbankmanagement, und die saatgutindustrie.

Die Kartierung von quantitativen Merkmalsloci (QTL) basierend auf dem Zusammenhang zwischen polymorphen Markern und phänotypischen Merkmalen in einer Population verwandter Individuen bietet die Möglichkeit genomische Regionen zu identifizieren, die für die Ausprägung des Merkmals verantwortlich sind. Dieses Ziel wird auch mit dem moderneren assoziationsgenetischen Ansatz verfolgt, der im Vergleich auf das Kopplungsungleichgewicht (Linkage Disequilibrium) genetischer Marker basiert und nicht-verwandte Individuen nutzt.

Die aktuelle Studie widmete sich in zwei Kartierungspanels der Identifizierung von Loci, die Merkmale der Samenlanglebigkeit von Rapssamen kontrollieren. Hierbei wird Saatgut der Doppelhaploidenpopulation Express x V8 (ExV), bestehend 122 Linien und genotypisiert mit 475 SSR und AFLP Markern, untersucht, welches in den drei Jahren 2005, 2009 und 2012 geerntet wurde. Die zweite “ASSYST” Population ist ein assoziationsgenetisches Panel bestehend aus 215 Rapssorten, welches mit 4001 biallelischen SNP Markern genotypisiert wurde. Beide Populationen wurden der kontrollierten Alterung (CD) bei 60% relativer Luftfeuchte und 45°C zugeführt und auf Merkmale geprüft, die mit der Samenlanglebigkeit in Verbindung stehen wie z.B. Prozent normal gekeimter Samen und Keimgeschwindigkeit. Für jedes Saatgutset aus verschiedenen Erntejahren erfolgte die Prüfung der Merkmale nach Kontrolle und experimenteller Alterung, die in Folge Rückschlüsse über die Samenlanglebigkeit über ein weites Spektrum an Umwelt- und Lagerungsbedingungen zulassen.

Hierbei ergibt die Kartierung der Ergebnisse der ExV population eine Reihe an QTL, die entweder spezifisch für ein Erntejahr oder eine Erntejahr-Merkmals-Kombination sind. Spezifische QTL für das Erntejahr 2005 wurden nach Kontrolle und CD auf Chromosom A7 (0,0 -28,6 cM) and A9 (17,9 -22,8 cM) und für das Erntejahr 2009 auf A10 und C3 ermittelt. Des Weiteren wurden gemeinsame Loci der CD Behandlungen in den Erntejahren 2009 und

2012 und der Kontrollbehandlung in dem Erntejahr 2005 auf Chromosom C3 im Bereich von 89,1 – 104,2 cM detektiert. Das Auftreten dieser genomischen Regionen in verschiedenen Behandlungen deutet auf eine Ähnlichkeit der Alterungsstadien und –mechanismen der verschiedenen Saatgutsets hin. Die Kombination der Resultate aus allen Entwicklungsumwelten bestätigte 27 der bisher 32 ermittelten QTL und lässt eine Beständigkeit des genetischen Effektes über verschiedene Umwelten vermuten. Weiterführend deuten die Samenlanglebigkeit QTL auf einen engen Zusammenhang zwischen Samenalterung und der Akkumulation von Pflanzennährstoffen, inhaltsstoffliche Samenzusammensetzung, Tausendsamengewicht und Öl Gehalt hin.

Die genomweite Assoziationsstudie (GWAS) ergab über alle Merkmale und Behandlungen insgesamt 172 Marker-Merkmals-Assoziationen (MTA). 132 MTAs wurden auf den beiden Kopplungsgruppen (A1-A10 und C1-C9) detektiert, wobei 14 MTAs Loci darstellten, die für mehrere Merkmale gleichzeitig kartiert wurden. Insbesondere ergaben die Keimgeschwindigkeitsparameter T50 (Zeit bis 50% Keimung) und AUC (Fläche unter der Keimungskurve) sowie die Wurzellänge (RL) hochsignifikante MTAs. Die MTA für Wurzelanomalität auf Chromosom C2 weist des Weiteren auf einen Zusammenhang mit der Zn Konzentration im Samen hin. Insgesamt ergab die Annotation von 12 Merkmalen in Verbindung mit den SNPs 19.794 Gene bei der Blast2GO Analyse, deren Mehrheit in biologische Prozesse eingebunden sind. Hoch signifikant annotierte Gene assoziert mit T50 sind in die Samenentwicklung und Zellwandverdickung involviert, wobei Gene verbunden mit RL besondere Bedeutung für die embryonische Samenmorphogenese und für der Regulation von Keimungsprozessen besitzen.

Die umfassende genetische Studie zur Untersuchung der Samenlanglebigkeit ergab deutliche Unterschiede zwischen Genotypen in Abhängigkeit von Erntejahren und Lagerungsbedingungen. Nichtsdestotrotz wurden vergleichbare QTLs über alle Wachstums- und Lagerungsumwelten hinweg detektiert. Dabei waren besonders Keimgeschwindigkeitsparameter in Kombination mit der Identifikation von abnormalem Keimlingswachstum hilfreich um die Samenlanglebigkeit von Rapssamen zu evaluieren.

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9. Appendix

Table S1: Coefficient of correlation of ExV population in nine environments representing germination test for control (CT) and after controlled deterioration (CD). Experimental design and abbreviations are represented in table 1. A, B and C tables are representing traits correlation in ExV-5 for CT1, CT2 and CD respectively. While, D, E and F representing ExV-9 for CT, CD1 and CD2 and G, H and I representing ExV-12 for CT, CD1 and CD2 respectively

A

ExV5_CT1	TSW	TG	NS	Abn	R	RS	T50	T10
TG	0.523**							
NS	0.045	0.488**						
Abn	0.492**	0.534**	-0.465**					
Rto	0.232*	-0.097	-0.725**	0.601**				
RSto	0.016	-0.106	-0.205*	0.090	-0.299**			
T50	-0.307**	-0.738**	-0.444**	-0.111	0.285**	-0.013		
T10	-0.375**	-0.747**	-0.425**	-0.326**	0.278**	-0.054	0.878**	
AUC	0.412**	0.815**	0.536**	0.310**	-0.278**	-0.030	-0.967**	-0.820**

B

ExV5_CT2	TSW	TG	NS	Abn	R	RS	T50	T10
TG	0.256**							
NS	-0.342**	0.304**						
Abn	0.329**	0.971**	0.124					
Rto	-0.036	0.309**	0.248**	0.258**				
RSto	0.368**	-0.060	-0.455**	0.051	-0.672**			
T50	0.189	-0.564**	-0.322**	-0.500**	-0.371**	0.353**		
T10	-0.151	-0.555**	-0.307**	-0.512**	-0.452**	0.325**	0.647**	
AUC	0.133	0.729**	0.400**	0.676**	0.475**	-0.336**	-0.766**	-0.772**

C

ExV5_CD	TSW	TG	NS	Abn	R	RS	T50	T10
TG	0.175							
NS	-0.246**	0.305**						
Abn	0.237**	0.978**	0.141					
Rto	0.197*	0.501**	0.140	0.496**				
RSto	0.026	-0.420**	-0.392**	-0.360**	-0.885**			
T50	0.081	-0.483**	-0.186	-0.465**	-0.420**	0.403**		
T10	-0.198*	-0.508**	-0.268**	-0.490**	-0.491**	0.477**	0.536**	
AUC	0.189*	0.728**	0.320**	0.705**	0.556**	-0.506**	-0.746**	-0.783**

Cont. Table S1

D

ExV9_CT	TSW	TG	NS	Abn	R	RS	T50	T10
TG	0.316**							
NS	0.133	0.423**						
Abn	-0.096	-0.274**	-0.981**					
Rto	0.036	0.042	-0.306**	0.343**				
RSto	-0.086	-0.291**	-0.962**	0.972**	0.269**			
T50	0.047	-0.086	-0.238**	0.238**	0.124	0.235**		
T10	-0.011	-0.096	-0.177	0.171	0.117	0.171	0.906**	
AUC	0.111	0.457**	0.473**	-0.423**	-0.113	-0.439**	-0.852**	-0.888**

E

ExV9_CD1	TSW	TG	NS	Abn	R	RS	T50	T10
TG	0.156							
NS	-0.078	0.710**						
Abn	0.227*	-0.193*	-0.821**					
Rto	0.146	-0.405**	-0.824**	0.838**				
RSto	0.058	-0.604**	-0.837**	0.679**	0.517**			
T50	0.095	-0.546**	-0.750**	0.656**	0.550**	0.792**		
T10	-0.020	-0.581**	-0.620**	0.437**	0.457**	0.738**	0.666**	
AUC	-0.010	0.673**	0.893**	-0.716**	-0.692**	-0.836**	-0.841**	-0.759**

F

ExV9_CD2	TSW	TG	NS	Abn	R	RS	T50	T10
TG	0.144							
NS	-0.168	0.493**						
Abn	0.309**	0.108	-0.794**					
Rto	0.099	-0.099	-0.713**	0.746**				
RSto	0.177	-0.502**	-0.707**	0.460**	0.130			
T50	0.139	-0.558**	-0.803**	0.552**	0.308**	0.840**		
T10	0.106	-0.455**	-0.729**	0.506**	0.228*	0.861**	0.903**	
AUC	-0.072	0.650**	0.873**	-0.540**	-0.396**	-0.845**	-0.950**	-0.901**

Cont. Table S1

G

ExV12_CT	TSW	TG	NS	Abn	R	RS	T50	T10
TG	-0.132							
NS	-0.142	0.777**						
Abn	0.157	-0.437**	-0.869**					
Rto	-0.020	-0.288**	-0.643**	0.744**				
RSto	0.247**	-0.515**	-0.714**	0.706**	0.322**			
T50	0.027	-0.680**	-0.753**	0.590**	0.356**	0.562**		
T10	0.049	-0.376**	-0.555**	0.491**	0.407**	0.484**	0.749**	
AUC	-0.104	0.912**	0.843**	-0.562**	-0.390**	-0.593**	-0.852**	-0.644**

H

ExV12_CD1	TSW	TG	NS	Abn	R	RS	T50	T10
TG	-0.049							
NS	0.085	0.907**						
Abn	-0.261**	0.007	-0.357**					
Rto	-0.231*	-0.438**	-0.604**	0.521**				
RSto	-0.071	-0.432**	-0.570**	0.447**	0.144			
T50	-0.132	-0.513**	-0.587**	0.395**	0.415**	0.541**		
T10	0.093	-0.622**	-0.636**	0.148	0.378**	0.549**	0.485**	
AUC	0.044	0.948**	0.953**	-0.189*	-0.522**	-0.550**	-0.638**	-0.667**

I

ExV12_CD2	TSW	TG	NS	Abn	R	RS	T50	T10
TG	0.035							
NS	0.096	0.864**						
Abn	-0.092	0.436**	-0.036					
Rto	-0.099	-0.195*	-0.392**	0.312**				
RSto	0.095	-0.448**	-0.555**	0.099	-0.130			
T50	-0.235*	-0.456**	-0.633**	0.439**	0.335**	0.693**		
T10	-0.125	-0.656**	-0.648**	-0.109	-0.020	0.659**	0.681**	
AUC	0.152	0.900**	0.945**	0.109	-0.263**	-0.569**	-0.727**	-0.754**

Table S2: Comparison of seed longevity QTL investigated in ExV population with puplished QTL studies on oil content, seed weight and plant nutrition effects in oilseed rape

Express x V8 " Seed longevity in oilseed rape				Oil content (OC), Fatty acid composition (FA) & Lipid orthologous genes (LOG)			
Year	**position**	**Trait name**	**chr**	**Population (trait)**	**Marker name (Interval position in cM)**	**Candidate genes detected in this region**	**Reference**
ExV-5	36,80	R_CD	A1	TN-Tr.cv. (OC)	CNU142 (34.0)		Zou J. et al., 2010
ExV-5	76,90	T50_CT2_5	A1	SG (LOG)	ZAAS165 (76.9)	AT3G13062	Zhao J. et al., 2012
ExV-5	0,00	TG_CT1_5	A3	DY4 (OC)	Bras002b (0.00), G03.960 (3.0), V09.2100 (9.3)		Delourme R. et al., 2006
ExV-5				TN-Tr.cv. (OC)	BRMS008 (0.0)		Zou J. et al., 2010
ExV-5				SG (LOG)	Na10G10b (0.0), ZAAS259 (5.1)	AT5G03290	Zhao J. et al., 2012
ExV-5	42,80	R_CT1_5	A5	SG (LOG)	SBE2.1 (37.7)	AT3G20440	Zhao J. et al., 2012
ExV-5	44,00	Abn_CT1_5	A5	SG (LOG)	ZAAS601 (43.1)	AT1G04710	Zhao J. et al., 2012
ExV-5	47,70	Abn_CT1_5	A5	SG (LOG)	MR119, ZAAS245 (43.8), BRAS002a (44.2)	AT2G33150	Zhao J. et al., 2012
ExV-5	51,50	NS_CT1_5	A5	SG (LOG)	ZAASRF11(52.4) - Ra3H10 (75.3)	AT3G22330, AT3G109390, AT3G13920, AT3G12810, AT3G12500	Zhao J. et al., 2012
ExV-5	88,20	AUC_CT1_5	A6	RNSL6 (OC)	Na12G11 (76.2), SS1949 (86.9)		Delourme R. et al., 2006
				SG (LOG)	ZAAS137 (79.5), IRONT (85.6)	AT5G63440, AT5G24380	Zhao J. et al., 2012
				TNDH (FA)	P7M1-280 (84.0)		Qui D. et al., 2006
ExV-5	0,00	TG_CT2_5	A7	SG (LOG)	ZAAS854 (0.0)	AT2G19690	Zhao J. et al., 2012
ExV-5	0,00	Abn_CT2_5	A7	SolloxXGoayou (FA)	HMR 300c-MR133.2 (2.0), MR133.2-MD20 (5.0)		Zhao J. et al., 2008
ExV-5	0,00	Abn_CD_5	A7	SG (LOG)	APOC (15.6)	AT5G66530	Zhao J. et al., 2012
ExV-5	25,10	TG_CT2_5	A7	SG (LOG)	ZAAS12a (28.5)	AT3G67650	Zhao J. et al., 2012
ExV-9	0,00	NS_CT_9	A8	SolloxXGoayou (FAC)	HMR388-HMR577 (0.0 -2.0)		Zhao J. et al., 2008
ExV-9	0,00	Abn_CT_9	A8	SG (LOG)	sN26/3(0.0), ZAAS296 (2.6)	AT1G06550	
ExV-9	10,80	Abn_CT_9	A8	SG (LOG)	ZAAS149 (11.1)	AT1G10180	Zhao J. et al., 2012
				SG (LOG)	ZAAS449 (13.2)	AT1G11090	Zhao J. et al., 2012
ExV-9	16,90	RS_CT_9	A8	TNDH (OC & FA)	sR7178(18.0), sS1702 (21.0), RA2E12 (23.0)		Qui D. et al., 2006
ExV-9				KNDH (OC)	FITO131 (17.8), o6m28-337 (20.3)		Wang X. et al., 2013
CAM	31,50	T50_CAM	A8	KNDH (OC)	H004105-1 (28.9), BnGMS312 (30.5)		Wang X. et al., 2013
ExV-5	34,50	AUC_CT2_5	A8				
ExV-5	36,90	AUC_CT2_5	A8				
CAM	36,90	T10	A8	RNSL8 (OC)	SR6068, AgCan49a (39.3), SR7178 (41.5), AgCan50 (44.7)		Delourme R. et al., 2006

ExV-5	41,10	AUC_CT2_5	A8				
ExV-5	42,10	AUC_CT2_5	A8				
ExV-5	44,60	AUC_CT2_5	A8	SG (LOG)	ZAAS781 (44.3)	AT1G30120	Zhao J. et al., 2012
ExV-5	45,40	AUC_CT2_5	A8				
				SolloxXGoayou (FAC)	HMR613-HMR595 (45.2 - 47.2)		Zhao J. et al., 2008
ExV-12	0,00	Abn_CD1_12	A9	SolloxXGoayou (FAC)	HMR612a-HMR595 (1.1 - 7.1 -)		Zhao J. et al., 2008
CAM	0,00	TSW	A9	SG (LOG)	E6M21 (0.0)		Zhao J. et al., 2012
ExV-12	13,20	Abn_CD1_12	A9	**SolloxXGoayou (FAC)**	**HMR612a-HMR595 (13.1)**		**Zhao J. et al., 2008**
ExV-12	13,20	T50_CD1_12	A9	**SolloxXGoayou (FAC)**	**HMR612a-HMR595 (19.1)**		**Zhao J. et al., 2008**
ExV-5	17,90	NS_CT1_5	A9	**KNDH (OC)**	**ZAAS352 (15.7), BnGMS281 (19.6), CB10311 (20.6), CB10103 (23.0), B069A23 (24.6)**		**Wang X. et al., 2013**
ExV-5	17,90	AUC_CT2_5	A9	**RNSL9 (OC)**	**AgCan45a, Ol12F02a, Na10A08a (25.7)**		**Delourme R. et al., 2006**
ExV-5	30,40	R_CT2_5	A9	KNDH (OC)	o8m13.248 (36.0)		Wang X. et al., 2013
ExV-5	30,40	RS_CT2_5	A9	SG (LOG)	ZAAS1032 (37.3)	AT5G47670	Zhao J. et al., 2012
ExV-5	53,40	TG_CD_5	A9	SolloxXGoayou (FAC)	HMR363a-HMR311b (54.8)		Zhao J. et al., 2008
ExV-5	56,70	TG_CD_5	A9	SG (LOG)	ZAAS643 (52.3)	AT2G38670	Zhao J. et al., 2012
ExV-5	56,70	Abn_CD_5	A9				
ExV-12	69,10	Abn_CD1_12	A9	SG (LOG)	miRNA31 (59.4), ZAAS264 (60.8)	AT4G36130	Zhao J. et al., 2012
CAM	94,60	R_CAM	A9	SG (LOG)	ZAAS248 (76.9)	AT3G57290	Zhao J. et al., 2012
ExV-12	94,60	R_CD2_12	A9	SG (LOG)	CYTAM (93.9)	AT2G24200	Zhao J. et al., 2012
CAM	97,60	R_CAM	A9	SG (LOG)	ZAAS674 (99.5), CN79a (102.5)	AT1G17340	Zhao J. et al., 2012
ExV-12	0,00	AUC_CD1_12	A10	SG (LOG)	ZAAS381 (0.0)	AT1G02390	Zhao J. et al., 2012
ExV-12	9,30	AUC_CD1_12	A10	SG (LOG)	CYPRT (18.3)	AT3G45310	Zhao J. et al., 2012
ExV-12	9,30	AUC_CD2_12	A10	KNDH (OC)	BnGMS625 (17.1)		Wang X. et al., 2013
ExV-12	17,60	TG_CT_12	A10	KNDH (OC)	CN10 (19.9)		Wang X. et al., 2013
ExV-12	17,60	NS_CT_12	A10	TNDH (OC & FA)	PW117 (20.0), Na12D11 (27.0)		Qui D. et al., 2006
ExV-9	50,40	NS_CD2_9	A10	SG (LOG)	ZAAS273 (49.4)	AT5G10740	Zhao J. et al., 2012
ExV-9	59,90	R_CD1_9	A10				
ExV-9	59,90	TSW_9	A10	SG (LOG)	miRNA6 (59.9)	AT5G08717	Zhao J. et al., 2012
ExV-9	72,20	TSW_9	A10	SG (LOG)	ZAAS653 (73.4)	AT2G26840	Zhao J. et al., 2012
ExV-5	58,80	AUC_CT2_5	C2	SG (LOG)	ZAAS671 (65.2), CN15 (68.8)	AT1G70670	Zhao J. et al., 2012
ExV-5	67,90	R_CD_5	C2				
ExV-9	0,00	NS_CD1_9	C3	SG (LOG)	ZAAS697 (0.0)	AT5G04040	Zhao J. et al., 2012
ExV-9	0,00	NS_CD1_9	C3	SG (LOG)	FERRITN (0.7)	AT5G01600	Zhao J. et al., 2012
CAM	6,30	NS	C3	SG (LOG)	SUC1 (19.7)	AT1G71880	Zhao J. et al., 2012
ExV-9	80,60	T10_CT_9	C3	TNDH (OC)	IGF1141f (87.0), E7HM31-450 (90.0)		Qui D. et al., 2006
CAM	80,60	T10	C3	SG (LOG)	Na12E02b (88.5)		Zhao J. et al., 2012
ExV-9	82,90	T10_CT_9	C3				
CAM	82,90	T10	C3	TNDH (FA)	E10HM32-140 (106.0)		Qui D. et al., 2006

ExV-12	0,00	NS_CD1_12	C4		SolloxXGoayou	HMR416-HMR363 (2.0)		Zhao J. et al., 2008
					SG (LOG)	ZAAS468/470	AT3G29510	Zhao J. et al., 2012
ExV-12	7,50	NS_CD1_12	C4		SG (LOG)	CN52 (9.9)		Zhao J. et al., 2012
ExV-5	1,90	T50_CT1_5	C5		SolloxXGoayou	HMR416-HMR363 (2.0)		Zhao J. et al., 2008
ExV-9	3,50	TG_CT_9	C5		SG (LOG)	ZAAS468/470	AT3G29510	Zhao J. et al., 2012
ExV-9	20,90	TG_CT_9	C5		SG (LOG)	CN52 (9.9)		Zhao J. et al., 2012
ExV-9	64,50	T10_CD1_9	C6		KNDH (OC)	BnGMS205 (64.2)		Wang X. et al., 2013
ExV-5	39,90	NS_CD_5	C7		SG (LOG)	ZAAS187 (37.9)	AT5G46290	Zhao G. et al., 2011
ExV-5	90,00	RS_CT2_5	C7					
ExV-5	90,00	NS_CD_5	C7		SG (LOG)	Na12A10 (90.4)		Zhao G. et al., 2011
ExV-12	103,00	T50_CD2_12	C7					

Seed weight under different locations and environments

Year	position	Trait name	chr		Seed weight traits	Interval	Candidate genes detected in this region	Reference
ExV-5	36,80	R_CD	A1				(34.7) AT4G18465	
ExV-5	76,90	T50_CT2_5	A1			(64.9-84.7)	(70.2) AT4G28250	Fan et al 2010
ExV-5	4,80	TG_CT1_5	A3		SW-LP	5.0-11.6	(5.4) AT5G10470	Ding et al., 2010
ExV-5	6,30	TG_CT1_5	A3					
ExV-5	12,20	TG_CT1_5	A3		SW		(10.8) AT5G08580	Cai et al 2012
ExV-5	45,90	Abn_CT1_5	A5				(46.1) AT3G18140	Cai et al, 2012
ExV-5	51,50	NS_CT1_5	A5		SW	52.9 (BrGMS832B), 56.0 (BoGMS1199), 58.7(BnEMS92), 60.6 **(Na12-E01)**		Fan et al 2010
ExV-5	51,50	Abn_CT1_5	A5			BnGMS293, BnEMS1072, CNU_SS029(57.9), BnEMS33(58.2), BnEMS92(59.3), Na12-E01 (61.2)	AT2G47240	Cai et al, 2012
ExV-9	60,60	R_CD2_9	A5				AT3G21650	
ExV-9	71,50	R_CD2_9	A5			CB10051(55.6), MD21(60.8)		Radoev M et al 2008
ExV-5	0,00	TG_CT2_5	A7			(0.0)		Cai et al, 2012
ExV-5	0,00	Abn_CT2_5	A7		SW	qSW.A7-2 (2.4 - 13.0)		Shi et al 2009
ExV-5	0,00	Abn_CD_5	A7		SW, SS	MR153b(5.2), MD20a(15.5), Ra2G08(21.2)		Radoev M et al 2008
ExV-5	16,40	TG_CT2_5	A7					
ExV-5	16,40	Abn_CT2_5	A7		SW, SL	EA02MC05-200 (10.5), EA02MC11-70 (14,5)		Zhang L.et al ,2011
					SW	BoGM5715 - FITO035 (6.7 -14.6)		Fan et al 2010
								Cai D 2014
								Basunanda 2010
ExV-9	0,00	NS_CT_9	A8		SL, SS	EA02MG05-210 (0.00) - CB10026b (7.0)	(0.0) AT4G38520	Zhang L.et al ,2011
ExV-9	0,00	Abn_CT_9	A8				(1.4) AT4G38550	

ExV-9	9,50	NS_CT_9	A8	SL, SS	SA10TC11-100 (9.3), SA03TC09-370 (10.1), SA03TC09-650 (10.4)	17406238_247 (Br)	Zhang L.et al ,2011
ExV-9	9,50	Abn_CT_9	A8				Cai et al, 2012
ExV-9	12,80	NS_CT_9	A8			17950629_142(Br)	Cai et al, 2012
ExV-9				SS		(17.2) AT1G19250	Cai et al, 2012
ExV-9	18,70	NS_CT_9	A8			SHK1	Cai et al, 2012
ExV-9	18,70	Abn_CT_9	A8			(19.6) AT1G15500	Cai et al, 2012
ExV-12	20,80	Abn_CT_12	A8		SA06TC04-270 (19.7), S77096 (27.0)		Zhang L.et al , 2011
CAM	36,90	T10	A8			(37.5) AT4G01030	Cai et al, 2012
ExV-5	30,40	R_CT2_5	A9			(31.5) AT3G63130	Cai et al, 2012
ExV-5	30,40	RS_CT2_5	A9			(32.1) AT3G63250	Cai et al, 2012
ExV-12	30,40	Abn_CD1_12	A9			(32.4) AT3G62030	Cai et al, 2012
ExV-12	69,10	Abn_CD1_12	A9	SW	BrGMS1115 (62.0) - BnEMS696b (77.0)		Yang P. et al, 2012
ExV-12	0,00	AUC_CD1_12	A10			(0.0) AT5G16850	
ExV-12	9,30	TG_CT_12	A10			(9) AT5G19750	
ExV-12	17,60	AUC_CD1_12	A10	SW	BnEMS731(19.9), BnEMS1185(21.0), BRMS-017B(22.9)	(19.9)AT5G59140	Cai et al, 2012
ExV-12	17,60	AUC_CD2_12	A10			(21,0) AT5G58375	
ExV-9	50,40	NS_CD2_9	A10	SW-LP	51.0 -60.7		Ding G etal.,2012
ExV-9	59,90	TSW_9	A10	SW-NP	52.1 - 60.8		Ding G etal.,2012
ExV-12	19,10	TG_CT_12	C2				
ExV-12	33,60	TG_CT_12	C2	SL	CB10026a (48.1), BnGMS633 (54.3)	(33,2) AT5G17690	Zhang L.et al , 2011
ExV-12	37,00	TG_CT_12	C2	SW	BrGMS627(40.8), BoGMS1274(43.7), BnEMS1017(44.8), Na12A01(46.2), BrGMS407B(50.1)	AT5G21960	Cai et al, 2012
ExV-12	42,00	TG_CT_12	C2			AT5G18550	
ExV-12	43,40	TG_CT_12	C2		CB10316, Na12E04b (34.2 - 36.4), Na12A01b (37.4 - 37.7)		Radoev M et al 2008
ExV-9	0,00	NS_CD1_9	C3	SW, SL, SS	EA02MC05-160 (00.0), Ol10-D03 (1.9), EA13MC01-160 (6.0)	AT5G43695 (0)	Zhang L.et al , 2011
CAM	2,80	R_CAM	C3			AT5G27885 (3,1)	Cai et al, 2012
CAM	4,30	R_CAM	C3			AT5G16210 (5)	Cai et al, 2012
CAM	79,80	T10	C3	SL	BnEMS757 (82.0), BoGMS510 (83.0)		Yang P. et al, 2012
ExV-12	0,00	NS_CD1_12	C4		pX106gE (0 - 5.6)		Udall et al, 2006
ExV-9	86,20	TG_CD1_9	C6	**SW**	**E32M48_412E (85.0), CB10234 (88.1)**		**Radoev M et al 2008**

Nutrition accumulation in mother plant and seeds

Year	position	Trait name	chr	Phosphorus & boron defeciency	Interval	Candidate genes detected in this region	Reference
ExV-5	36,80	R_CD	A1	Mg-LP	37.1 - 45.0		Ding et al., 2010
CAM	36,80	T50	A1		30.1 - 38.2	PHR1	Shi T. et al., 2013
ExV-5	39,60	R_CD	A1	Ca-LP	39.1 - 43.8		Ding et al., 2010
ExV-5	76,90	T50_CT2_5	A1	P-LP, Mg-LP	74.9-79.0		Ding et al., 2010
ExV-5	77,90	T50_CT2_5	A1	SBA-LB	70.3-72.7		Shi L., 2012
CAM	80,50	T50	A1	SBA-LB	72.7-80.8	AT5G25430.1	Shi L., 2012
ExV-5	0,00	TG_CT1_5	A3	Fe-NP	0.0 - 5.0		Ding et al., 2010
CAM	34,00	R_CAM	A5	SW-LP	30.9 - 37.7	PHT1;4	Shi T. et al., 2013
CAM	36,70	R_CAMj	A5	SW-LP	30.9 - 37.7	PHT1;4	Shi T. et al., 2013
ExV-5	43,70	NS_CT1_5	A5	Fe-NP	20 - 41.1		Ding et al., 2010
ExV-5	49,90	NS_CT1_5	A5	P-NP	44.1 - 56.1		Ding et al., 2010
ExV-5	49,90	Abn_CT1_5	A5	SL-LB	44.6-48.5		Shi L., 2012
ExV-5	51,50	R_CT1_5	A5	Ca-NP	49.6 - 54.5		Ding et al., 2010
ExV-5	51,50	NS_CT1_5	A5	Mg-NP	51.9 - 62.1		
				SNLP	87.2 -102.0 (88.2)		Ding G etal.,2012
ExV-5	16,40	TG_CT2_5	A7	Ca-NP	12.8 - 15.3		Ding et al., 2010
ExV-5	16,40	Abn_CT2_5	A7	Mg-NP	13.2 - 16.8		Ding et al., 2010
ExV-5	25,10	TG_CT2_5	A7	SDW-Ad	27.4-31.9		Shi L. et al., 2012
ExV-9	9,50	NS_CT_9	A8	P-NP	6.7 - 8.7		Ding et al., 2010
ExV-9	10,80	Abn_CT_9	A8	Zn-NP	11.2 - 18.0		Ding et al., 2010
ExV-5	34,50	AUC_CT2_5	A8	SN-LP	27.0 -37.6 (30.3)		Ding G etal.,2012
ExV-9	59,40	T50_CD2_9	A8	SY-LP	58.9 - 66.6		Ding G etal.,2012
ExV-12	13,20	Abn_CD1_12	A9	P-LP	8.1 - 15.9		Ding et al., 2010
ExV-12	13,20	T50_CD1_12	A9	Cu-LP	6.5 - 13.9		Ding et al., 2010
ExV-5	53,40	TG_CD_5	A9	PH-OP	55.1 - 63.1	LPR1	Shi T. et al., 2013
ExV-5	56,70	TG_CD_5	A9	Fe-LP	58.5 - 63.1		Ding et al., 2010
ExV-5	56,70	Abn_CD_5	A9				
ExV-12	69,10	Abn_CD1_12	A9	PH-LP	66.0-71.5	LPR1	Shi T. et al., 2013
CAM	100,20	R_CAM	A9	Mn-NP	75.3 - 79.5		Ding et al., 2010
ExV-9	102,60	TSW_9	A9	Ca-LP	98.3 - 108.2		Ding et al., 2010
ExV-12	9,30	TG_CT_12	A10	Mn-NP	6.8 - 12.8		Ding et al., 2010
ExV-12	9,30	NS_CT_12	A10	Mn-LP	5.2 - 9.3		Ding et al., 2010
ExV-12	9,30	AUC_CT_12	A10				
ExV-12	9,30	TG_CD1_12	A10	Mn-NP	17.4 - 19.4		Ding et al., 2010
ExV-12	9,30	AUC_CD1_12	A10	Zn-NP	15.0 - 17.3		Ding et al., 2010

ExV-12	17,60	AUC_CD1_12	A10		Cu-NP	19.3 - 21.8		Ding et al., 2010
ExV-12	17,60	AUC_CD2_12	A10		Cu-LP	18.6 - 25.9		Ding et al., 2010
ExV-9	48,00	Abn_CD1_9	A10		Fe-NP	46.3 - 60.0		Ding et al., 2010
ExV-9	72,20	TSW_9	A10		SW-LP	72.8 - 87.3	SQD2	
ExV-12	33,60	TG_CT_12	C2		P-LP	38.5 - 55.7		Ding et al., 2010
ExV-12	37,00	TG_CT_12	C2		PDW, PU (HP), RL(HP&LP)	41.0 - 43.3		Yang M. et al., 2010
ExV-12	37,00	TG_CT_12	C2		Ca-LP , Ca-LP	(38.0 - 44.8) - (46.9 - 53.0)		Ding et al., 2010
ExV-5	58,80	AUC_CT2_5	C2		RL-Ad	57.3-64.2		Shi L et al., 2012
ExV-5	67,90	R_CD_5	C2					
ExV-9	0,00	NS_CD1_9	C3		RBA-LB	0.0.-1.1		Shi L et al., 2012
ExV9	2,80	NS_CD2_9	C3		SDW-LB	3.0-7.0		Shi L et al., 2012
CAM	2,80	NS	C3		P-LP	4.2 - 12.3		Ding et al., 2010
ExV-9	4,30	NS_CD2_9	C3		DW, PU, PL (LP)	9.5-11.4		Yang M. et al 2010
CAM	6,30	NS	C3		Zn-LP	16.8 - 23.2		Ding et al., 2010
CAM	11,20	NS	C3		Mg-LP	24.5 - 33.0		Ding et al., 2010
CAM	82,90	T10	C3		SDW-LB	106.9-111.4		Shi L et al., 2012
ExV-12	104,20	AUC_CD2_12	C3		SDW-LB	106.9-111.4		Shi L., 2012
ExV-12	45,90	TSW_12	C4		RDW-LB	34.0-42.6	AT5G05080.1	Shi L., 2012
ExV-9	3,50	TG_CT_9	C5		SL-NB	48.1-49.8		Shi L., 2012
ExV-5	36,50	NS_CD_5	C7		FBH-LP	19.1 - 38.5	RNS1 gene	Shi T. et al., 2013
ExV-5	83,90	NS_CD_5	C7		Zn-NP	47.6 - 53.5		Ding et al., 2010
ExV-5	90,00	RS_CT2_5	C7					
ExV-5	90,00	NS_CD_5	C7		Cu-NP - Fe-NP	48.8 - 52.9		Ding et al., 2010
ExV-12	103,00	T50_CD2_12	C7					
ExV-12	99,50	T50_CD2_12	C9		SDW-NB	85.0-99.8		Shi L., 2012

Table S3: The coefficient ofcorrelation among seed longevity related traits in ExV-9 represented in, thousand seed weight (TSW), total germination(TG), normal seedlings (NS), total abnormality(Ab), root abnormality(R), root and hypocotyle (shoot) abnormality (RS), area under the curve after 150 hours of germination (AUC), time till 50% of germination (T50) and time till 10% of germination (T10) and the oil, protein and fatty acid composition measurements directly after harvest. A: shows the correlation in control and B &C: shows the correlation after CD1 and CD2 respectively. Table A. show the correlation in ExV-5 and B. in ExV-9 and C. in ExV-12.

A: ExV-5	TGW	TG	NS	Ab	R	RS	AUC	T50	T10	Oil	Protein	GSL	S	C18:1	C18:3	C22:1
TSW	1,00	.304**	.150	-.073	.001	-.094	.151	.015	.006	-.056	.027	.144	.148	-.049	.110	-.106
TG		1,00	.408**	-.148	-.023	-.187*	.520**	.008	-.031	.110	-.173	.048	.040	-.089	.065	-.123
NS			1,00	-.963**	-.411**	-.962**	.554**	-.296**	-.223*	-.004	-.141	-.213*	-.169	-.203*	.208*	-.259**
Ab				1,00	.439**	.987**	-.447**	.323**	.233**	.037	.101	.245**	.195*	.193*	-.206*	.244**
R					1,00	.295**	-.170	.161	.147	-.012	.132	.100	.141	.145	-.244**	.110
RS						1,00	.223*	.316**	.223*	.038	.088	.240**	.178*	.183*	-.181*	.245**
AUC							1,00	-.838**	-.829**	.158	-.147	.079	.078	.004	-.006	-.061
T50								1,00	.978**	-.127	.065	-.091	-.091	-.070	.057	-.018
T10									1,00	-.131	.068	-.100	-.087	-.070	.061	-.025
Oil										1,00	-.632**	.003	-.353**	.630**	-.638**	.640**
Protein											1,00	.071	.365**	.074	.185*	.085
GSL												1,00	.881**	.236**	-.070	.189*
S													1,00	.021	.164	-.059
C18:1														1,00	-.815**	.934**
C18:3															1,00	-.729**
C22:1																1,00

Cont. Table S3

B: ExV-9	TGW	TG	NS	Ab	Rto	Rsto	AUC	T50	T10	Oil	Protein	GSL	S	C18:1	C18:3	C22:1
TGW	1,00	.159	-.083	.234**	.151	.046	-.006	.114	-.002	-.056	.027	.144	.148	-.049	.110	-.106
TG		1,00	.691**	-.184*	-.373**	-.598**	.736**	-.586**	-.559**	.288**	-.193*	-.155	-.298**	.088	.040	.140
NS			1,00	-.837**	-.817**	-.818**	.924**	-.777**	-.640**	.221*	-.067	-.197*	-.253**	.135	-.060	.161
Ab				1,00	.831**	.661**	-.703**	.669**	.452**	-.090	-.050	.155	.126	-.118	.114	-.118
R					1,00	.521**	-.677**	.550**	.463**	-.072	-.016	.143	.135	-.040	-.009	-.065
RS						1,00	-.834**	.778**	.766**	-.261**	.061	.172	.222*	-.213*	.134	-.239**
AUC							1,00	-.889**	-.796**	.359**	-.094	-.143	-.263**	.294**	-.196*	.319**
T50								1,00	.781**	-.363**	.119	.110	.242**	-.334**	.298**	-.339**
T10									1,00	-.314**	-.024	.049	.157	-.370**	.260**	-.408**
Oil										1,00	-.632**	.003	-.353**	.630**	-.638**	.640**
Protein											1,00	.071	.365**	.074	.185*	.085
GSL												1,00	.881**	.236**	-.070	.189*
S													1,00	.021	.164	-.059
C18:1														1,00	-.815**	.934**
C18:3															1,00	-.729**
C22:1																1,00

Cont. Table S3

C: ExV-12	TGW	TG	NS	Ab	Rto	Rsto	AUC	T50	T10	Oil	Protein	GSL	S	C18:1	C18:3	C22:1
TGW	1,00	.142	-.169	.302**	.122	.198*	-.063	.126	.088	-.056	.027	.144	.148	-.049	.110	-.106
TG		1,00	.559**	.020	-.127	-.537**	.722**	-.671**	-.537**	.328**	-.237**	-.096	-.236**	.118	.017	.124
NS			1,00	-.818**	-.727**	-.755**	.874**	-.804**	-.748**	.122	-.015	-.203*	-.215*	.083	-.021	.087
Ab				1,00	.788**	.537**	-.552**	.565**	.529**	.080	-.146	.177	.094	-.019	.037	-.020
R					1,00	.170	-.378**	.301**	.236**	.131	-.033	.206*	.125	.168	-.118	.198*
RS						1,00	-.844**	.854**	.839**	-.259**	.078	.128	.191*	-.236**	.158	-.255**
AUC							1,00	-.957**	-.912**	.333**	-.085	-.140	-.236**	.272**	-.166	.290**
T50								1,00	.913**	-.308**	.094	.146	.248**	-.247**	.189*	-.280**
T10									1,00	-.322**	.057	.136	.214*	-.297**	-.237**	-.320**
Oil										1,00	-.632**	.003	-.353**	.630**	-.638**	.640**
Protein											1,00	.071	.365**	.074	.185*	.085
GSL												1,00	.881**	.236**	-.070	.189*
S													1,00	.021	.164	-.059
C18:1														1,00	-.815**	.934**
C18:3															1,00	-.729**
C22:1																1,00

Table S4:. Eleven SSR markers which identified by putative candidate genes (detected in previously published comparative maps of oilseed rape depending on the physical positions of Arabidopsis pseudochromosomes and their closest anchored markers, these positions were detected using the *in-silico* mapping approach) were liked to seed longevity traits which estimated in ExV population, 3 seed sets, (ExV-5, ExV-9 and ExV-12) on the linkage groups A5, A7, A8, A9, A10 and C4. The Area under the curve (AUC), Normal seedlings (NS), total germination (TG) root abnormality (R), total abnormality (Abn), thousand seed weight (TSW) and time required till 50% of germination (T50) which were investigated after control germination (_CT_) and/or experimental ageing (_CD_) could be controlled functionally by these group of genes

Year	LG	Locus	Traits	Repeated in	At. hits	Function	Reference
ExV-5	**A5**	Na12E01b	NS_CT1_5, Abn_CT1_5		*AT4G19633.1*	Heat shock factor related protein.	Qiu et al. (2006a) Bancroft et al (2007a)
	A5	Na10E02	NS_CT1_5	CAM-QTL(Abn), ExV-9 (Abn_CD2)	*AT1G10155.1*	Phloem protein 2-A10 (PP2-A10), match 2-A9 protein in *A. thanlina* (AT1G31200.1).	Lowe et al (2004a)
	A7	Ra2G08	TG_CT1_5, Abn_CT1_5, TG_CT2_5, Abn_CT2_5, T50_CD_5	CAM-QTL (Abn)	*AT3G28070.1*	Nodulin "MtN21-like": transporter family protein.	Piquemal et al (2005a)
	A9	Na10B11	AUC_CDT_5		*AT1G28290.1*	AGP31: Play a role in vascular tissue function during defense and development.	Piquemal et al (2005a) Bancroft et al (2007a)
	A9	BRAS020	R_CT2_5		*AT1G12220.1*	Resistance gene, mediates resistance against the bacterial pathogen Pseudomonas syringae.	Delourme et al (2006a)
	A9	Na14C12	TG_CD_5		*AT4G34180.1*	Matching cyclase family protein, located in cell wall and involved in response to salt stress.	Piquemal et al (2005a)
ExV-9	**A8**	Ra1F06	AUC_CD1_9, R_CD2_9, RS_CD2_9, T50_CD2_9		*AT1G15120.1*	Ubiquinol-cytochrome C reductase hinge protein: mitochondrial electron transport.	Piquemal et al (2005a)
	A10	Ol10B11	TSW_9, Abn_CD1_9, AUC_CD2_9, NS_CD2_9,		*AT3G24630.1*	TON1 RECRUITING MOTIF 34	Piquemal et al (2005a)
	A10	Na12H04	TSW_9		*AT2G45660.1*	Control flowering and it is required for CO to promote flowering.	Lowe et al (2004a) Piquemal et al (2005a)
ExV-12	**C4**	Ol12D02	RS_CT_12		*AT3G62980.1*	An auxin receptor that mediates auxin-regulated transcription.	Piquemal et al (2005a)
	C4	Na10C01a (c)	TSW_12		*AT5G31905.1*	Transposable element gene: copia-like retrotransposon family.	Piquemal et al (2005a) Qiu et al. (2006a) Bancroft et al (2007a)
CAM-QTL	**C3**	Na10C01a (c)	AUC	ExV-12 (RS)	*AT5G31905.1*	Transposable element gene: copia-like retrotransposon family.	Piquemal et al (2005a) Qiu et al. (2006a) Bancroft et al (2007a)

Table S5: A diverse set of 215 inbred lines of oilseed rape "*Brassica napus* L." belonged to ERANET-ASSYST project (ASSYST). According to the release year there are four groups coloured with green, blue, yellow and gray from older to recent years respectively. Additionally three subgroups according to seed quality are listed.

No	BnAssyst-Nr.	Germplasm Type (OSR: oilseed rape)	Country	Seed Quality Subgroup of Winter OSR	Release Period	Q
1	1	Winter OSR	France	00	2000-2007	Q1
2	2	Winter OSR	France	00	2000-2007	UNG
3	3	Winter OSR	France	00	2000-2007	Q2
4	4	Winter OSR	United_Kingdom	00	1990-1999	Q2
5	5	Winter OSR	France	00	1990-1999	Q1
6	6	Winter OSR	United_Kingdom	00	1990-1999	Q2
7	7	Winter OSR	France	00	2000-2007	Q2
8	8	Winter OSR	Germany	00	2000-2007	Q2
9	9	Winter OSR	United_Kingdom	00	2000-2007	Q2
10	10	Winter OSR	United_Kingdom	00	1990-1999	Q2
11	11	Winter OSR	United_Kingdom	00	2000-2007	Q2
12	12	Winter OSR	Germany	00	1990-1999	Q2
13	13	Winter OSR	France	00	2000-2007	Q1
14	14	Winter OSR	France	00	2000-2007	Q2
15	16	Winter OSR	France	00	-	Q2
16	17	Winter OSR	France	00	-	Q2
17	18	Winter OSR	France	00	2000-2007	Q2
18	19	-	-	-	-	Q2
19	20	Winter OSR	France	00	2000-2007	Q1
20	21	Winter OSR	France	00	2000-2007	Q2
21	22	Winter OSR	France	00	2000-2007	Q2
22	23	Winter OSR	France	00	2000-2007	Q2
23	24	Winter OSR	France	00	2000-2007	Q2
24	25	Winter OSR	France	00	2000-2007	Q2
25	26	Winter OSR	France	00	2000-2007	Q3
26	27	Winter fodder	Germany	-	-	Q2
27	28	Winter OSR	France	00	1990-1999	Q2
28	29	Winter OSR	France	00	1990-1999	Q1
29	30	Winter OSR	Germany	00	1990-1999	Q2
30	31	-	-	-	-	Q1
31	32	Winter OSR	Germany	00	1980-1989	Q2
32	33	Winter OSR	Germany	00	1990-1999	Q1
33	34	Winter OSR	Germany	00	-	Q1
34	35	Winter OSR	Germany	00	-	Q2
35	36	Winter OSR	Germany	00	1990-1999	Q2
36	37	Winter OSR	France	00	-	Q2
37	38	Winter OSR	Germany	00	2000-2007	Q1

No	BnAssyst-Nr.	Germplasm Type (OSR: oilseed rape)	Country	Seed Quality Subgroup of Winter OSR	Release Period	Q
38	39	Winter OSR	Germany	00	2000-2007	Q2
39	40	Winter OSR	Germany	00	1990-1999	Q2
40	41	Winter OSR	unknown	00	-	Q2
41	42	Winter OSR	Sweden	00	1990-1999	Q2
42	43	Winter OSR	Germany	00	1990-1999	Q2
43	44	Winter OSR	France	00	1990-1999	Q2
44	45	Winter OSR	unknown	00	2000-2007	Q2
45	46	Winter OSR	Germany	00	1990-1999	Q2
46	47	Winter OSR	unknown	00	2000-2007	Q2
47	48	Winter OSR	France	00	2000-2007	Q2
48	49	Winter OSR	France	00	2000-2007	UNG
49	50	Winter OSR	unknown	00	2000-2007	Q2
50	51	Winter OSR	unknown	00	2000-2007	Q2
51	52	Winter OSR	unknown	00	2000-2007	Q2
52	53	Winter OSR	unknown	00	2000-2007	Q2
53	54	Winter OSR	unknown	00	1990-1999	Q2
54	55	Winter OSR	unknown	00	2000-2007	Q2
55	56	Winter OSR	unknown	00	-	Q2
56	57	Winter OSR	unknown	00	-	Q2
57	58	Winter OSR	unknown	00	-	Q2
58	59	Winter OSR	unknown	00	2000-2007	Q1
59	60	Winter OSR	Germany	00	2000-2007	Q2
60	61	Winter OSR	Germany	00	2000-2007	Q2
61	62	Winter OSR	Germany	00	2000-2007	Q2
62	63	Winter OSR	Germany	00	2000-2007	Q2
63	64	Winter OSR	Germany	00	-	Q2
64	65	Winter OSR	Germany	00	-	Q2
65	66	Winter OSR	Germany	00	-	Q2
66	67	Spring OSR	Canada	-	-	Q1
67	68	Winter OSR	Germany	00	-	Q2
68	69	Winter OSR	Germany	00	-	Q2
69	70	Winter OSR	Germany	00	-	Q2
70	71	Winter OSR	Germany	00	-	Q2
71	72	Winter OSR	Germany	00	2000-2007	Q2
72	73	Winter OSR	Germany	00	-	Q2
73	74	Winter OSR	unknown	00	-	Q2
74	75	Winter fodder	Germany	-	-	Q3
75	76	Winter fodder	Germany	-	-	Q1
76	77	Winter OSR	Germany	00	-	Q2
77	78	Winter OSR	Germany	00	1990-1999	Q2
78	79	Winter OSR	unknown	00	-	UNG

No	BnAssyst-Nr.	Germplasm Type (OSR: oilseed rape)	Country	Seed Quality Subgroup of Winter OSR	Release Period	Q
79	80	Winter OSR	Former_GDR	00	-	Q1
80	81	Winter OSR	unknown	00	1990-1999	Q2
81	82	Winter OSR	Germany	00	2000-2007	Q2
82	83	Winter OSR	Germany	00	1990-1999	Q2
83	84	Winter OSR	France	00	2000-2007	Q2
84	85	Winter OSR	France	00	2000-2007	Q2
85	86	Winter OSR	Germany	00	2000-2007	Q2
86	87	Winter OSR	United_Kingdom	00	2000-2007	Q1
87	88	Winter OSR	United_Kingdom	00	2000-2007	Q1
88	89	Winter OSR	United_Kingdom	-	-	Q2
89	90	Winter OSR	United_Kingdom	-	-	UNG
90	91	Winter OSR	France	+0/0+	-	Q1
91	93	Winter OSR	United_Kingdom	-	-	Q2
92	96	Winter OSR	United_Kingdom	-	-	Q2
93	97	Winter OSR	United_Kingdom	-	-	Q2
94	98	Winter OSR	France	+0/0+	-	Q2
95	99	Winter OSR	France	00	-	Q1
96	101	Winter OSR	France	00	-	UNG
97	102	Winter OSR	Germany	+0/0+	1954-1979	Q2
98	103	Winter OSR	France	+0/0+	-	Q1
99	105	Winter OSR	Germany	-	-	Q2
100	106	Winter OSR	United_Kingdom	-	-	Q2
101	107	Winter OSR	Austria	++	-	Q1
102	108	Winter OSR	Germany	++	-	Q1
103	109	Winter OSR	Japan	++	-	Q1
104	110	Winter OSR	Italy	-	-	Q3
105	111	Winter OSR	China	-	-	Q3
106	112	Winter OSR	Germany	++	-	UNG
107	113	Winter OSR	France	00	1980-1989	Q2
108	114	Winter OSR	Former_GDR	++	1954-1979	Q1
109	115	-	-	-	-	UNG
110	116	Winter OSR	Germany	+0/0+	1990-1999	Q2
111	117	Winter OSR	Germany	+0/0+	1990-1999	Q1
112	118	Winter OSR	Germany	++	1990-1999	Q1
113	119	Winter OSR	Germany	00	1980-1989	Q1
114	120	Winter OSR	France	00	1980-1989	Q2
115	121	Winter OSR	France	+0/0+	1954-1979	Q2
116	122	Winter OSR	Germany	00	1980-1989	Q2
117	123	Winter OSR	Germany	00	1980-1989	Q2
118	124	Winter OSR	United_Kingdom	00	1990-1999	Q2
119	125	Winter OSR	unknown	00	-	Q2

No	BnAssyst-Nr.	Germplasm Type (OSR: oilseed rape)	Country	Seed Quality Subgroup of Winter OSR	Release Period	Q
120	126	Winter OSR	unknown	00	1990-1999	Q1
121	127	Winter OSR	unknown	00	-	Q2
122	128	Winter OSR	Germany	00	1990-1999	Q2
123	129	Winter OSR	unknown	-	-	UNG
124	130	Winter OSR	unknown	-	-	Q1
125	131	Winter OSR	unknown	-	-	Q3
126	132	Winter OSR	Germany	-	-	UNG
127	133	Winter OSR	Poland	-	1954-1979	Q1
128	134	Winter OSR	Poland	00	1980-1989	UNG
129	135	Winter OSR	Sweden	-	-	Q1
130	136	Winter OSR	Germany	00	1980-1989	Q2
131	137	Winter OSR	Germany	++	-	Q2
132	138	Winter OSR	Germany	-	1954-1979	Q1
133	139	Winter OSR	Germany	-	-	Q1
134	140	Winter OSR	Germany	++	1980-1989	Q2
135	141	Winter OSR	Germany	-	-	Q2
136	142	Winter OSR	Germany	-	1954-1979	UNG
137	143	Winter OSR	Germany	++	-	Q1
138	144	Winter OSR	Germany	++	-	Q1
139	145	Winter OSR	Japan	-	-	Q3
140	146	Winter OSR	Poland	+0/0+	-	UNG
141	147	Winter OSR	Poland	00	-	UNG
142	148	Winter OSR	Sweden	+0/0+	-	Q1
143	149	Winter OSR	Germany	-	-	UNG
144	150	Winter OSR	Czech_Rep	-	1954-1979	Q3
145	151	Winter OSR	Germany	00	1980-1989	Q2
146	152	Winter OSR	Germany	00	1980-1989	UNG
147	153	Winter OSR	Germany	-	-	Q2
148	154	Winter OSR	Germany	00	-	Q1
149	155	Winter OSR	Germany	-	-	UNG
150	156	Winter OSR	Germany	+0/0+	1980-1989	Q1
151	157	Winter OSR	Former_GDR	00	1990-1999	Q2
152	158	Winter OSR	France	-	-	Q1
153	159	Winter OSR	France	-	-	Q1
154	160	Winter OSR	Sweden	00	-	Q1
155	161	Winter OSR	Russia	-	1954-1979	Q3
156	162	Winter OSR	Moldova	-	-	Q2
157	163	Winter OSR	Russia	-	1980-1989	Q1
158	164	Winter OSR	Ukraine	-	-	Q1
159	165	Winter OSR	Sweden	00	-	Q1
160	166	Winter OSR	Sweden	++	1954-1979	Q1

No	BnAssyst-Nr.	Germplasm Type (OSR: oilseed rape)	Country	Seed Quality Subgroup of Winter OSR	Release Period	Q
161	167	Winter OSR	Former_GDR	++	-	Q1
162	168	Winter OSR	France	++	1954-1979	Q1
163	169	Winter OSR	France	++	-	Q1
164	170	Winter OSR	Poland	-	-	Q1
165	171	Winter OSR	Russia	-	1980-1989	Q1
166	172	Winter OSR	Czech_Rep	-	-	Q1
167	173	Winter OSR	Poland	-	-	Q1
168	174	Winter OSR	Germany	++	-	Q1
169	175	Winter OSR	Poland	+0/0+	-	UNG
170	176	Winter OSR	Czech_Rep	-	-	Q1
171	177	Winter OSR	Sweden	-	-	Q1
172	178	Winter OSR	Russia	-	1980-1989	Q1
173	179	Winter OSR	Poland	++	-	Q3
174	180	Winter OSR	unknown	-	-	Q1
175	181	Winter OSR	unknown	-	-	Q2
176	182	Winter OSR	unknown	-	-	Q2
177	183	Winter OSR	unknown	-	-	Q1
178	184	Winter OSR	unknown	++	-	Q3
179	185	Winter fodder	United_Kingdom	-	-	Q3
180	186	Winter fodder	New_Zealand	-	-	Q3
181	187	Winter fodder	Netherlands	-	-	Q3
182	188	Winter fodder	Ireland	-	-	Q1
183	189	Winter fodder	Sweden	-	-	Q3
184	190	Winter fodder	New_Zealand	-	-	Q3
185	191	Winter fodder	Norway	-	-	Q1
186	192	Winter fodder	Netherlands	-	-	Q1
187	193	Winter fodder	United_Kingdom	-	-	Q3
188	194	Winter fodder	United_Kingdom	-	-	Q3
189	195	Winter fodder	Germany	-	-	Q2
190	196	Winter fodder	Japan	-	-	Q2
191	197	Winter fodder	United_Kingdom	-	-	Q1
192	198	Winter fodder	Italy	-	-	Q3
193	199	Winter fodder	France	-	-	Q3
194	200	Winter fodder	Sweden	-	-	Q3
195	201	Winter fodder	Sweden	-	-	Q1
196	202	-	-	-	-	UNG
197	203	Vegetable	Japan	-	-	Q3
198	204	Unspecified	unknown	-	-	Q3
199	206	-	-	-	-	Q1
200	207	Vegetable	Zimbabwe	-	-	Q1
201	208	Vegetable	Portugal	-	-	Q3

No	BnAssyst-Nr.	Germplasm Type (OSR: oilseed rape)	Country	Seed Quality Subgroup of Winter OSR	Release Period	Q
202	209	Vegetable	United_Kingdom	-	-	Q3
203	210	Vegetable	unknown	-	-	Q3
204	211	Vegetable	Netherlands	-	-	Q3
205	212	Winter OSR	Czech_Rep	-	1954-1979	Q1
206	213	Winter OSR	Japan	-	-	Q1
207	214	Unspecified	India	-	-	Q3
208	216	Unspecified	Mongolia	-	-	Q2
209	217	Spring OSR	Russia	-	-	Q3
210	218	Vegetable	Netherlands	-	-	Q2
211	219	Vegetable	Germany	-	-	Q3
212	221	Vegetable	USA	-	-	Q3
213	222	-	-	-	-	Q3
214	223	Unspecified	Taiwan	-	-	Q3
215	224	Unspecified	Algeria	-	-	Q3

Table S6: Phenotypic variations (R^2) of genome wide association study for ASSYST population: Thousand seed weight (TSW) together with eleven traits were examined in control (CT) and after controlled deterioration (CD) (Traits abbreviations are explained in Fig. 10)**.**

Marker	Chr	Position	TSW	CT											CD										
				TG	NS	Abn	R	RS	RL	SHL	RSH	T10	T50	AUC	TG	NS	Abn	R	RS	RL	SHL	RSH	T10	T50	AUC
Bn-Scaffold000051-p149827	A01	545303					0,06																		
Bn-ctg7180014764929-p6706	A01	2845492															0,04								
Bn-ctg7180014738315-p2017	A01	4049117					0,06																		
Bn-Scaffold000005-p1968757	A03	1595070														0,05									
Bn-ctg7180014774289-p2882	A03	3483561							0,06		0,06														
Bn-Scaffold000039-p674225	A03	6775781														0,05								0,06	
Bn-Scaffold000039-p989874	A03	7081732							0,05																
Bn-Scaffold000001-p8844866	A03	1,7E+07									0,05														
Bn-Scaffold000001-p9283934	A03	1,7E+07																		0,05					
Bn-ctg7180014742416-p2432	A03	1,9E+07										0,05													
Bn-ctg7180014727104-p3259	A03	2,1E+07																	0,05						
Bn-Scaffold000027-p1338617	A03	2,4E+07							0,06																
Bn-Scaffold000016-p141266	A04	16958		0,05	0,06																				
Bn-Scaffold000016-p1309884	A04	1265282		0,05	0,06																				
Bn-ctg7180014707487-p1878	A04	1E+07													0,05										
Bn-ctg7180014732811-p14904	A04	1,2E+07													0,06										
Bn-ctg7180014749049-p1676	A04	1,3E+07							0,05																
Bn-ctg7180014758040-p17442	A04	1,7E+07																				0,06			

Marker	Chr	Position	TSW	CT											CD										
				TG	NS	Abn	R	RS	RL	SHL	RSH	T10	T50	AUC	TG	NS	Abn	R	RS	RL	SHL	RSH	T10	T50	AUC
Bn-Scaffold000004-p4962830	A05	413761							0,05																
Bn-Scaffold000004-p2113069	A05	2242721							0,07																
Bn-Scaffold000067-p1340022	A05	2865701										0,05													
Bn-Scaffold000004-p2925914	A05	2982867		0,06										0,05											
Bn-Scaffold000004-p5075668	A05	4896407										0,06	0,12	0,07											
Bn-Scaffold000025-p677725	A05	7006208									0,04														
Bn-ctg7180014737732-p4709	A05	1,2E+07											0,05												
Bn-Scaffold000001-p816500	A05	1,9E+07									0,04														
Bn-Scaffold000067-p840178	A05	2E+07											0,06												
Bn-Scaffold000044-p810737	A06	1,7E+07														0,05		0,05							0,05
Bn-Scaffold000047-p1114729	A06	2,1E+07											0,05												
Bn-Scaffold000018-p791182	A07	327145											0,06												
Bn-Scaffold000017-p2826265	A07	4886957							0,08		0,05														
Bn-Scaffold000019-p34260	A07	2,3E+07																0,06							
Bn-Scaffold000010-p1372478	A08	1708896																							0,06
Bn-ctg7180014738749-p1249	A08	1850766															0,04								
Bn-Scaffold000015-p2547584	A08	1875704				0,05																			
Bn-Scaffold000032-p1409221	A08	8647526																0,05							
Bn-ctg7180014759930-p6323	A08	9116789									0,04														
Bn-Scaffold000020-p568288	A08	1,6E+07														0,05		0,06							0,06

Marker	Chr	Position	TSW	CT											CD										
				TG	NS	Abn	R	RS	RL	SHL	RSH	T10	T50	AUC	TG	NS	Abn	R	RS	RL	SHL	RSH	T10	T50	AUC
Bn-ctg7180014763461-p2115	A09	2417918						0,07																	
Bn-Scaffold000057-p1076787	A09	6121910							0,05																
Bn-Scaffold000054-p520347	A09	7380876		0,06																					
Bn-Scaffold000054-p586777	A09	7442849							0,05																
Bn-Scaffold000058-p205581	A09	8308215																						0,07	0,07
Bn-Scaffold000056-p327484	A09	9739761													0,05										
Bn-Scaffold000022-p1843971	A09	1,1E+07																			0,06				
Bn-Scaffold000066-p209445	A09	1,4E+07													0,06										
Bn-Scaffold000059-p219199	A09	1,6E+07																				0,06			
Bn-Scaffold000006-p173473	A09	2,4E+07																0,07							
Bn-Scaffold000006-p3416772	A09	2,7E+07									0,05														
Bn-Scaffold000002-p4278788	A10	1798853		0,06									0,08	0,07											
Bn-Scaffold000002-p5401773	A10	9070602																		0,10					
Bn-Scaffold000002-p3340573	A10	1,1E+07							0,06																
Bn-Scaffold000008-p452324	A10	1,3E+07		0,06								0,05													
Bn-Scaffold000008-p1084171	A10	1,4E+07																							
Bn-Scaffold000008-p1935430	A10	1,5E+07			0,05																				
Bn-Scaffold000008-p3233403	A10	1,6E+07	0,05																						
Bn-Scaffold000059-p836121	Ann	1842191																		0,09					
Bn-Scaffold000063-p1183010	Ann	1,5E+07										0,05	0,05												

Marker	Chr	Position	TSW	CT											CD										
				TG	NS	Abn	R	RS	RL	SHL	RSH	T10	T50	AUC	TG	NS	Abn	R	RS	RL	SHL	RSH	T10	T50	AUC
Bn-Scaffold000002-p4747623	Ann	4,3E+07									0,05														
Bn-Scaffold000066-p859390	Ann	4,5E+07		0,05																					
Bn-Scaffold000015-p612514	Ann	4,5E+07																	0,05						
Bn-ctg7180014747340-p4257	C01	7262088											0,06												
Bn-ctg7180014743505-p5860	C01	1,1E+07							0,05																
Bn-ctg7180014758460-p1198	C01	1,7E+07																0,06							
Bn-ctg7180014727113-p1118	C01	2E+07																		0,05					
Bn-ctg7180014755552-p3167	C01	3,1E+07																	0,06						
Bn-ctg7180014743660-p3110	C01	3,1E+07						0,06							0,05				0,06						
Bn-ctg7180014761021-p1313	C02	449403											0,07												
Bn-ctg7180014768109-p5331	C02	912628					0,06																		
Bn-ctg7180014733521-p8651	C02	4057041											0,06												
Bn-ctg7180014740945-p1487	C02	7745921																		0,06					
Bn-ctg7180014741423-p2909	C02	1,1E+07							0,04																
Bn-ctg7180014738642-p2713	C02	1,2E+07								0,05															
Bn-ctg7180014750900-p1843	C02	2,3E+07																		0,07					
Bn-ctg7180014727249-p2020	C02	3,6E+07											0,05												
Bn-ctg7180014767012-p1420	C02	4,5E+07						0,09																	
Bn-ctg7180014750535-p7525	C03	1135768																0,05							
Bn-ctg7180014770381-p5282	C03	7069134		0,07										0,06											

Marker	Chr	Position	TSW	CT											CD										
				TG	NS	Abn	R	RS	RL	SHL	RSH	T10	T50	AUC	TG	NS	Abn	R	RS	RL	SHL	RSH	T10	T50	AUC
Bn-ctg7180014763132-p7226	C03	7991901																							0,05
Bn-ctg7180014759848-p17449	C03	8050930																	0,07						
Bn-ctg7180014740332-p3544	C04	2578300			0,05																				
Bn-ctg7180014744894-p12663	C04	5163438											0,05		0,05										
Bn-ctg7180014733575-p2198	C04	1E+07											0,09												
Bn-ctg7180014733601-p8356	C04	1,5E+07											0,14	0,08											
Bn-ctg7180014755210-p17484	C04	1,6E+07					0,07																		
Bn-ctg7180014735576-p352	C04	1,7E+07										0,05													
Bn-ctg7180014769499-p7274	C04	1,9E+07																	0,05						
Bn-ctg7180014768132-p5231	C04	3,7E+07									0,05														
Bn-ctg7180014745665-p455	C04	4E+07							0,05																
Bn-ctg7180014755765-p1245	C05	7160727															0,04								
Bn-ctg7180014733138-p1497	C05	3,6E+07				0,05																			
Bn-ctg7180014763023-p2277	C05	4,1E+07																					0,06		
Bn-ctg7180014776000-p4855	C06	510654																					0,05		
Bn-ctg7180014766165-p11542	C06	3E+07								0,05															
Bn-ctg7180014763849-p1235	C06	3,3E+07															0,04								
Bn-ctg7180014775195-p3592	C07	2626487														0,05									
Bn-ctg7180014756277-p14305	C07	2,8E+07																		0,05					
Bn-ctg7180014749178-p27761	C07	3,3E+07															0,05								

Marker	Chr	Position	TSW	CT											CD										
				TG	NS	Abn	R	RS	RL	SHL	RSH	T10	T50	AUC	TG	NS	Abn	R	RS	RL	SHL	RSH	T10	T50	AUC
Bn-ctg7180014731895-p3998	C07	3,4E+07					0,06																		
Bn-ctg7180014746444-p5025	C07	3,5E+07		0,05									0,08	0,05											
Bn-ctg7180014750011-p3580	C07	3,9E+07													0,05										
Bn-ctg7180014765472-p17288	C07	4E+07				0,05																			
Bn-ctg7180014766893-p5826	C08	776968																0,05							
Bn-ctg7180014738749-p3115	C08	1953569															0,05								
Bn-ctg7180014764762-p4908	C08	2,2E+07				0,06																			
Bn-ctg7180014767714-p3896	C08	2,7E+07						0,05																	
Bn-ctg7180014768503-p3926	C08	2,9E+07					0,06																		
Bn-ctg7180014758136-p17878	C08	3,3E+07													0,04										
Bn-ctg7180014769363-p513	C08	3,5E+07												0,05											
Bn-ctg7180014727337-p703	C09	3705755											0,05												
Bn-ctg7180014755223-p1470	C09	4031239					0,07																		
Bn-ctg7180014774341-p878	C09	1,5E+07																					0,05		
Bn-Scaffold000022-p1655638	C09	1,7E+07																	0,05						
Bn-ctg7180014734156-p1293	C09	3,3E+07											0,06												
Bn-ctg7180014757797-p4687	C09	4,2E+07																					0,05		
Bn-ctg7180014761389-p3731	Cnn	3,1E+07															0,05								
Bn-ctg7180014705205-p1670	UKN	0		0,06										0,05											
Bn-Scaffold000003-p378289	UKN	0																		0,06					

Marker	Chr	Position	TSW	CT											CD										
				TG	NS	Abn	R	RS	RL	SHL	RSH	T10	T50	AUC	TG	NS	Abn	R	RS	RL	SHL	RSH	T10	T50	AUC
Bn-ctg7180014761471-p7945	UKN	0											0,08												
Bn-ctg7180014744722-p621	UKN	0									0,05														
Bn-ctg7180014760347-p4698	UKN	0											0,05	0,06											
Bn-ctg7180014770133-p1816	UKN	0														0,07									0,05
Bn-ctg7180014738197-p1371	UKN	0																							0,05
Bn-Scaffold000014-p2266836	UKN	0																		0,06					
Bn-Scaffold000062-p433881	UKN	0																0,06							
Bn-Scaffold000006-p4235917	UKN	0															0,06								
Bn-ctg7180014762134-p4200	UKN	0	0,06																						
Bn-Scaffold000057-p1186692	UKN	0																0,05							
Bn-Scaffold000006-p836032	UKN	0									0,06			0,05											
Bn-Scaffold000011-p941666	UKN	0																0,05							
Bn-Scaffold000024-p432016	UKN	0		0,05	0,06																				
Bn-Scaffold000034-p871223	UKN	0												0,05											0,05
Bn-Scaffold000001-p2617977	UKN	0									0,05														
Bn-ctg7180014771470-p924	UKN	0																							0,06
Bn-ctg7180014776143-p9952	UKN	0							0,06																
Bn-Scaffold000048-p1071281	UKN	0																					0,05	0,05	
Bn-ctg7180014775927-p796	UKN	0																					0,05		
Bn-ctg7180014749194-p3989	UKN	0					0,06																		

Marker	Chr	Position	TSW	CT											CD										
				TG	NS	Abn	R	RS	RL	SHL	RSH	T10	T50	AUC	TG	NS	Abn	R	RS	RL	SHL	RSH	T10	T50	AUC
Bn-ctg7180014744734-p4489	UKN	0							0,05																
Bn-ctg7180014761742-p16321	UKN	0		0,05	0,06		0,08								0,06										
Bn-Scaffold000004-p2795066	UKN	0																	0,05						
Bn-ctg7180014733789-p3993	UKN	0											0,08												

Table S7: Gene ontology (GO) analysis (using GOstat approach) of genetically associated SNPs with thousand seed weight and eleven other traits tested in control germination (CT) and after experimental ageing (CD). Gene ontology biological process identification number (GOBPID), *P*-value given by the hypergeometric test (p<0.01), Ratio of odds that a GO term is enriched in the selected category (odds ratio), Expected number of transcripts found associated with the GO term for enrichment (Exp-Count), Real number of transcripts found associated with the GO term (count), Population size of transcripts found associated with the GO term within the analysis (size) are listed for each candidate gene.

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
TSW	1	GO:0009859	3,64E-06	133,99	0,03	3	11	pollen hydration
	2	GO:0002215	4,77E-05	355,52	0,01	2	4	defense response to nematode
	3	GO:0030968	0,0003	3,75	3,08	11	1087	endoplasmic reticulum unfolded protein response
	4	GO:0006984	0,0003	3,75	3,08	11	1087	ER-nucleus signaling pathway
	5	GO:0035967	0,0003	3,73	3,09	11	1092	cellular response to topologically incorrect protein
	6	GO:0034620	0,0003	3,73	3,09	11	1092	cellular response to unfolded protein
	7	GO:0006986	0,0003	3,71	3,11	11	1099	response to unfolded protein
	8	GO:0033345	0,0005	71,10	0,03	2	12	asparagine catabolic process via L-aspartate
	9	GO:0006516	0,0005	71,10	0,03	2	12	glycoprotein catabolic process
	10	GO:0006530	0,0005	71,10	0,03	2	12	asparagine catabolic process
	11	GO:0019406	0,0007	59,24	0,04	2	14	hexitol biosynthetic process
	12	GO:0006059	0,0007	59,24	0,04	2	14	hexitol metabolic process
	13	GO:0019593	0,0007	59,24	0,04	2	14	mannitol biosynthetic process
	14	GO:0019594	0,0007	59,24	0,04	2	14	mannitol metabolic process
	15	GO:0009065	0,0007	18,47	0,17	3	61	glutamine family amino acid catabolic process
	16	GO:0019401	0,0012	44,43	0,05	2	18	alditol biosynthetic process
	17	GO:0009445	0,0016	37,41	0,06	2	21	putrescine metabolic process
	18	GO:0009446	0,0016	37,41	0,06	2	21	putrescine biosynthetic process
	19	GO:0009862	0,0017	2,87	4,38	12	1546	systemic acquired resistance, salicylic acid mediated signaling pathway
	20	GO:0035966	0,0026	2,49	5,90	14	2081	response to topologically incorrect protein
	21	GO:0042743	0,0029	2,56	5,32	13	1876	hydrogen peroxide metabolic process
	22	GO:0009410	0,0032	5,25	0,98	5	347	response to xenobiotic stimulus
	23	GO:0006528	0,0033	25,38	0,09	2	30	asparagine metabolic process

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	24	GO:0006596	0,0034	10,50	0,30	3	105	polyamine biosynthetic process
	25	GO:0046173	0,0035	24,51	0,09	2	31	polyol biosynthetic process
	26	GO:0009100	0,0035	24,51	0,09	2	31	glycoprotein metabolic process
	27	GO:0072593	0,0040	2,46	5,53	13	1951	reactive oxygen species metabolic process
	28	GO:0045333	0,0046	4,05	1,53	6	539	cellular respiration
	29	GO:0045132	0,0049	4,76	1,08	5	382	meiotic chromosome segregation
TG-CT	1	GO:0000017	4,21E-05	76,42	0,08	3	6	alpha-glucoside transport
	2	GO:0042946	4,21E-05	76,42	0,08	3	6	glucoside transport
	3	GO:0015768	4,21E-05	76,42	0,08	3	6	maltose transport
	4	GO:0048544	0,0003	4,58	2,07	9	160	recognition of pollen
	5	GO:0008037	0,0003	4,58	2,07	9	160	cell recognition
	6	GO:0009856	0,0003	1,84	23,53	42	1816	pollination
	7	GO:0009875	0,0017	3,47	2,70	9	208	pollen-pistil interaction
	8	GO:0019676	0,0024	38,16	0,08	2	6	ammonia assimilation cycle
	9	GO:0070646	0,0024	1,98	11,91	23	919	protein modification by small protein removal
	10	GO:0015766	0,0025	7,65	0,57	4	44	disaccharide transport
	11	GO:0015772	0,0025	7,65	0,57	4	44	oligosaccharide transport
	12	GO:0050665	0,0028	2,66	4,65	12	359	hydrogen peroxide biosynthetic process
	13	GO:0016926	0,0044	2,50	4,92	12	380	protein desumoylation
TG-CD	1	GO:0019295	1,13E-05	142,33	0,05	3	5	coenzyme M biosynthetic process
	2	GO:0019296	1,13E-05	142,33	0,05	3	5	coenzyme M metabolic process
	3	GO:0006428	9,01E-05	20,00	0,24	4	23	isoleucyl-tRNA aminoacylation
	4	GO:0018342	0,0002	15,83	0,29	4	28	protein prenylation
	5	GO:0042306	0,0003	28,46	0,14	3	13	regulation of protein import into nucleus
	6	GO:0019438	0,0004	1,62	40,38	63	3856	aromatic compound biosynthetic process
	7	GO:0009108	0,0005	2,65	6,63	17	633	coenzyme biosynthetic process
	8	GO:0046166	0,0006	94,76	0,04	2	4	glyceraldehyde-3-phosphate biosynthetic process
	9	GO:0046184	0,0006	94,76	0,04	2	4	aldehyde biosynthetic process

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	10	GO:0006642	0,0006	94,76	0,04	2	4	triglyceride mobilization
	11	GO:0006450	0,0007	10,85	0,41	4	39	regulation of translational fidelity
	12	GO:0046937	0,0010	17,79	0,20	3	19	phytochelatin metabolic process
	13	GO:0046938	0,0010	17,79	0,20	3	19	phytochelatin biosynthetic process
	14	GO:0019751	0,0011	4,63	1,58	7	151	polyol metabolic process
	15	GO:0046822	0,0015	14,98	0,23	3	22	regulation of nucleocytoplasmic transport
	16	GO:0033157	0,0015	14,98	0,23	3	22	regulation of intracellular protein transport
	17	GO:0043043	0,0016	8,63	0,50	4	48	peptide biosynthetic process
	18	GO:0009809	0,0019	3,39	2,74	9	262	lignin biosynthetic process
	19	GO:0016051	0,0020	1,47	49,98	71	4773	carbohydrate biosynthetic process
	20	GO:0070201	0,0022	12,94	0,26	3	25	regulation of establishment of protein localization
	21	GO:0051223	0,0022	12,94	0,26	3	25	regulation of protein transport
	22	GO:0055068	0,0022	37,90	0,07	2	7	cobalt ion homeostasis
	23	GO:0006824	0,0022	37,90	0,07	2	7	cobalt ion transport
	24	GO:0000398	0,0023	2,87	3,95	11	377	mRNA splicing, via spliceosome
	25	GO:0006952	0,0024	1,36	87,14	113	8322	defense response
	26	GO:0032386	0,0028	11,86	0,28	3	27	regulation of intracellular transport
	27	GO:0006397	0,0028	2,35	6,54	15	625	mRNA processing
	28	GO:0006448	0,0032	7,03	0,61	4	58	regulation of translational elongation
	29	GO:0050832	0,0032	1,64	24,43	39	2333	defense response to fungus
	30	GO:0009699	0,0034	1,74	18,25	31	1743	phenylpropanoid biosynthetic process
	31	GO:0000375	0,0037	2,69	4,20	11	401	RNA splicing, via transesterification reactions
	32	GO:0000377	0,0037	2,69	4,20	11	401	RNA splicing, via transesterification reactions with bulged adenosine as nucleophile
	33	GO:0006725	0,0038	1,43	51,34	71	4903	cellular aromatic compound metabolic process
	34	GO:0009698	0,0038	1,66	22,29	36	2129	phenylpropanoid metabolic process
	35	GO:0007004	0,0047	23,69	0,10	2	10	telomere maintenance via telomerase
	36	GO:0034637	0,0047	1,45	44,11	62	4213	cellular carbohydrate biosynthetic process
	37	GO:0006828	0,0049	6,23	0,68	4	65	manganese ion transport

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	38	GO:0044257	0,0049	1,62	23,44	37	2239	cellular protein catabolic process
	39	GO:0006771	0,0049	9,49	0,35	3	33	riboflavin metabolic process
	40	GO:0009231	0,0049	9,49	0,35	3	33	riboflavin biosynthetic process
	41	GO:0042727	0,0049	9,49	0,35	3	33	flavin-containing compound biosynthetic process
NS-CT	1	GO:0000746	0,0007	69,47	0,04	2	7	conjugation
	2	GO:0042450	0,0007	69,47	0,04	2	7	arginine biosynthetic process via ornithine
	3	GO:0016139	0,0009	5,71	1,09	6	190	glycoside catabolic process
	4	GO:0042777	0,0012	49,62	0,05	2	9	plasma membrane ATP synthesis coupled proton transport
	5	GO:0022403	0,0012	2,04	12,74	25	2214	cell cycle phase
	6	GO:0000278	0,0027	2,29	7,19	16	1250	mitotic cell cycle
	7	GO:0080136	0,0029	28,94	0,08	2	14	priming of cellular response to stress
	8	GO:0045935	0,0029	1,96	12,13	23	2108	positive regulation of nucleobase-containing compound metabolic process
	9	GO:0051173	0,0029	1,96	12,15	23	2111	positive regulation of nitrogen compound metabolic process
	10	GO:0006464	0,0038	1,58	29,73	45	5168	cellular protein modification process
	11	GO:0006591	0,0042	23,15	0,10	2	17	ornithine metabolic process
	12	GO:0018193	0,0043	2,99	3,09	9	537	peptidyl-amino acid modification
	13	GO:0009864	0,0046	9,49	0,33	3	58	induced systemic resistance, jasmonic acid mediated signaling pathway
	14	GO:0051254	0,0050	1,90	11,95	22	2077	positive regulation of RNA metabolic process
	15	GO:0045893	0,0050	1,90	11,95	22	2077	positive regulation of transcription, DNA-templated
NS-CD	1	GO:0048523	4,75E-05	1,77	36,69	62	3729	negative regulation of cellular process
	2	GO:0046087	5,12E-05	60,65	0,08	3	8	cytidine metabolic process
	3	GO:0009972	5,12E-05	60,65	0,08	3	8	cytidine deamination
	4	GO:0006216	5,12E-05	60,65	0,08	3	8	cytidine catabolic process
	5	GO:0042592	0,0002	1,91	21,69	40	2205	homeostatic process
	6	GO:0010080	0,0003	27,57	0,14	3	14	regulation of floral meristem growth
	7	GO:0031935	0,0004	25,27	0,15	3	15	regulation of chromatin silencing
	8	GO:0072507	0,0004	3,87	2,69	10	273	divalent inorganic cation homeostasis
	9	GO:0055074	0,0005	5,38	1,37	7	139	calcium ion homeostasis

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	10	GO:0046133	0,0005	23,32	0,16	3	16	pyrimidine ribonucleoside catabolic process
	11	GO:0046135	0,0005	23,32	0,16	3	16	pyrimidine nucleoside catabolic process
	12	GO:0060968	0,0008	18,95	0,19	3	19	regulation of gene silencing
	13	GO:0010451	0,0010	17,83	0,20	3	20	floral meristem growth
	14	GO:0048878	0,0010	2,00	13,91	27	1414	chemical homeostasis
	15	GO:0051253	0,0011	1,87	17,66	32	1795	negative regulation of RNA metabolic process
	16	GO:0045892	0,0011	1,87	17,66	32	1795	negative regulation of transcription, DNA-templated
	17	GO:2000113	0,0011	1,85	18,42	33	1872	negative regulation of cellular macromolecule biosynthetic process
	18	GO:0010558	0,0011	1,85	18,42	33	1872	negative regulation of macromolecule biosynthetic process
	19	GO:0009695	0,0012	2,42	7,23	17	735	jasmonic acid biosynthetic process
	20	GO:0055065	0,0014	3,29	3,14	10	319	metal ion homeostasis
	21	GO:0009890	0,0014	1,82	18,69	33	1900	negative regulation of biosynthetic process
	22	GO:0031327	0,0014	1,82	18,69	33	1900	negative regulation of cellular biosynthetic process
	23	GO:0010219	0,0014	15,16	0,23	3	23	regulation of vernalization response
	24	GO:0021700	0,0016	1,82	18,09	32	1839	developmental maturation
	25	GO:0045934	0,0017	1,81	18,19	32	1849	negative regulation of nucleobase-containing compound metabolic process
	26	GO:0031408	0,0018	2,32	7,54	17	766	oxylipin biosynthetic process
	27	GO:0051172	0,0020	1,80	18,33	32	1863	negative regulation of nitrogen compound metabolic process
	28	GO:0031324	0,0020	1,76	19,85	34	2018	negative regulation of cellular metabolic process
	29	GO:0055080	0,0020	2,05	11,02	22	1120	cation homeostasis
	30	GO:0065008	0,0023	1,45	54,59	76	5549	regulation of biological quality
	31	GO:0007059	0,0026	2,38	6,47	15	658	chromosome segregation
	32	GO:0072529	0,0026	12,13	0,28	3	28	pyrimidine-containing compound catabolic process
	33	GO:0006342	0,0027	2,03	10,60	21	1077	chromatin silencing
	34	GO:0050801	0,0028	1,96	12,06	23	1226	ion homeostasis
	35	GO:0045814	0,0028	2,03	10,64	21	1081	negative regulation of gene expression, epigenetic
	36	GO:0008272	0,0033	6,98	0,61	4	62	sulfate transport
	37	GO:0010094	0,0033	28,84	0,09	2	9	specification of carpel identity

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	38	GO:0010221	0,0033	28,84	0,09	2	9	negative regulation of vernalization response
	39	GO:0060771	0,0033	28,84	0,09	2	9	phyllotactic patterning
	40	GO:0055082	0,0034	2,03	10,09	20	1026	cellular chemical homeostasis
	41	GO:0048764	0,0035	1,76	17,50	30	1779	trichoblast maturation
	42	GO:0048765	0,0035	1,76	17,50	30	1779	root hair cell differentiation
	43	GO:0048469	0,0035	1,76	17,51	30	1780	cell maturation
	44	GO:0010260	0,0035	2,38	6,04	14	614	organ senescence
	45	GO:0042454	0,0038	10,45	0,31	3	32	ribonucleoside catabolic process
	46	GO:0009164	0,0038	10,45	0,31	3	32	nucleoside catabolic process
	47	GO:0031407	0,0038	2,05	9,50	19	966	oxylipin metabolic process
	48	GO:0010054	0,0039	1,73	18,43	31	1873	trichoblast differentiation
	49	GO:0048519	0,0040	1,41	57,53	78	5848	negative regulation of biological process
	50	GO:0009909	0,0041	1,75	16,95	29	1723	regulation of flower development
	51	GO:0009992	0,0045	9,78	0,33	3	34	cellular water homeostasis
	52	GO:0044248	0,0046	1,35	77,37	100	7864	cellular catabolic process
Abn-CT	1	GO:0080028	6,12E-08	34,46	0,21	6	40	nitrile biosynthetic process
	2	GO:0050898	6,12E-08	34,46	0,21	6	40	nitrile metabolic process
	3	GO:0048513	6,59E-06	1,81	49,22	80	9505	organ development
	4	GO:0048731	7,24E-06	1,81	49,34	80	9529	system development
	5	GO:0019748	7,51E-06	2,13	23,83	47	4603	secondary metabolic process
	6	GO:0016139	7,66E-06	8,61	0,98	8	190	glycoside catabolic process
	7	GO:0042221	8,76E-06	1,63	92,73	130	17909	response to chemical
	8	GO:0044249	1,00E-05	1,58	135,84	176	26235	cellular biosynthetic process
	9	GO:0010033	1,19E-05	1,70	65,11	98	12574	response to organic substance
	10	GO:0019762	1,47E-05	9,57	0,78	7	150	glucosinolate catabolic process
	11	GO:0019759	1,47E-05	9,57	0,78	7	150	glycosinolate catabolic process
	12	GO:0016145	1,47E-05	9,57	0,78	7	150	S-glycoside catabolic process
	13	GO:0009725	1,62E-05	1,87	37,52	64	7247	response to hormone

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	14	GO:0009719	1,66E-05	1,86	37,56	64	7254	response to endogenous stimulus
	15	GO:0009058	2,36E-05	1,55	141,47	180	27322	biosynthetic process
	16	GO:0009733	2,51E-05	2,57	11,10	27	2144	response to auxin
	17	GO:0009753	2,73E-05	2,32	15,11	33	2918	response to jasmonic acid
	18	GO:0050789	4,88E-05	1,53	116,31	152	22462	regulation of biological process
	19	GO:0009755	5,18E-05	2,30	14,25	31	2752	hormone-mediated signaling pathway
	20	GO:0032870	5,32E-05	2,30	14,27	31	2756	cellular response to hormone stimulus
	21	GO:0071495	5,32E-05	2,30	14,27	31	2756	cellular response to endogenous stimulus
	22	GO:0019222	5,77E-05	1,61	70,31	101	13578	regulation of metabolic process
	23	GO:0044273	6,86E-05	7,43	0,99	7	191	sulfur compound catabolic process
	24	GO:0048856	7,31E-05	1,59	72,48	103	13998	anatomical structure development
	25	GO:0065007	0,0001	1,50	127,50	162	24624	biological regulation
	26	GO:0051171	0,0001	1,65	50,97	77	9843	regulation of nitrogen compound metabolic process
	27	GO:0031326	0,0001	1,64	53,61	80	10353	regulation of cellular biosynthetic process
	28	GO:0060255	0,0001	1,62	57,03	84	11014	regulation of macromolecule metabolic process
	29	GO:0009889	0,0001	1,63	53,74	80	10378	regulation of biosynthetic process
	30	GO:0009698	0,0001	2,38	11,02	25	2129	phenylpropanoid metabolic process
	31	GO:0080027	0,0002	10,69	0,50	5	96	response to herbivore
	32	GO:0019219	0,0002	1,65	50,50	76	9753	regulation of nucleobase-containing compound metabolic process
	33	GO:0031323	0,0002	1,59	61,69	89	11913	regulation of cellular metabolic process
	34	GO:0009059	0,0002	1,53	83,95	114	16213	macromolecule biosynthetic process
	35	GO:2000112	0,0002	1,63	50,08	75	9671	regulation of cellular macromolecule biosynthetic process
	36	GO:0010556	0,0002	1,63	50,08	75	9671	regulation of macromolecule biosynthetic process
	37	GO:0080090	0,0002	1,59	58,65	85	11326	regulation of primary metabolic process
	38	GO:0009620	0,0002	2,06	16,93	33	3269	response to fungus
	39	GO:0034645	0,0003	1,51	82,21	111	15876	cellular macromolecule biosynthetic process
	40	GO:0033036	0,0003	1,71	36,48	58	7045	macromolecule localization
	41	GO:0006605	0,0003	1,87	23,90	42	4616	protein targeting

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	42	GO:0044237	0,0003	1,46	201,60	234	38934	cellular metabolic process
	43	GO:0046068	0,0003	25,26	0,13	3	26	cGMP metabolic process
	44	GO:0006182	0,0003	25,26	0,13	3	26	cGMP biosynthetic process
	45	GO:0009190	0,0003	25,26	0,13	3	26	cyclic nucleotide biosynthetic process
	46	GO:0009187	0,0003	25,26	0,13	3	26	cyclic nucleotide metabolic process
	47	GO:0006950	0,0003	1,47	101,86	132	19672	response to stress
	48	GO:0008104	0,0004	1,75	30,53	50	5897	protein localization
	49	GO:0015031	0,0004	1,76	28,97	48	5594	protein transport
	50	GO:0045184	0,0004	1,76	28,97	48	5594	establishment of protein localization
	51	GO:0033554	0,0004	1,66	38,76	60	7486	cellular response to stress
	52	GO:0009611	0,0005	2,28	10,54	23	2035	response to wounding
	53	GO:0006807	0,0005	1,44	117,70	148	22731	nitrogen compound metabolic process
	54	GO:0010363	0,0005	2,19	11,96	25	2309	regulation of plant-type hypersensitive response
	55	GO:0006979	0,0005	2,08	14,11	28	2725	response to oxidative stress
	56	GO:0034641	0,0005	1,44	116,02	146	22406	cellular nitrogen compound metabolic process
	57	GO:2001141	0,0005	1,59	47,50	70	9174	regulation of RNA biosynthetic process
	58	GO:0006355	0,0005	1,59	47,50	70	9174	regulation of transcription, DNA-templated
	59	GO:0080135	0,0006	2,16	12,09	25	2334	regulation of cellular response to stress
	60	GO:0043900	0,0006	3,23	3,85	12	743	regulation of multi-organism process
	61	GO:0007155	0,0006	4,03	2,31	9	446	cell adhesion
	62	GO:0022610	0,0006	4,03	2,31	9	446	biological adhesion
	63	GO:0051252	0,0006	1,58	47,70	70	9212	regulation of RNA metabolic process
	64	GO:0044275	0,0006	2,03	15,00	29	2897	cellular carbohydrate catabolic process
	65	GO:0008219	0,0007	1,97	16,50	31	3187	cell death
	66	GO:0016265	0,0007	1,97	16,50	31	3187	death
	67	GO:0010200	0,0007	2,06	13,73	27	2651	response to chitin
	68	GO:0016052	0,0008	1,98	15,91	30	3073	carbohydrate catabolic process
	69	GO:0007275	0,0008	1,46	80,85	107	15614	multicellular organismal development

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	70	GO:0000302	0,0008	2,32	8,97	20	1733	response to reactive oxygen species
	71	GO:0009791	0,0009	1,59	43,18	64	8339	post-embryonic development
	72	GO:0050896	0,0010	1,40	151,38	181	29236	response to stimulus
	73	GO:0006351	0,0010	1,54	50,27	72	9709	transcription, DNA-templated
	74	GO:0010468	0,0010	1,53	52,88	75	10212	regulation of gene expression
	75	GO:0032774	0,0010	1,54	50,36	72	9725	RNA biosynthetic process
	76	GO:0044262	0,0010	1,62	36,82	56	7111	cellular carbohydrate metabolic process
	77	GO:0090304	0,0011	1,44	84,28	110	16277	nucleic acid metabolic process
	78	GO:0002831	0,0011	3,17	3,58	11	692	regulation of response to biotic stimulus
	79	GO:0042180	0,0011	1,56	45,39	66	8766	cellular ketone metabolic process
	80	GO:0009743	0,0012	1,80	21,67	37	4185	response to carbohydrate
	81	GO:0080134	0,0012	1,88	17,87	32	3451	regulation of response to stress
	82	GO:0008152	0,0012	1,43	230,34	258	44485	metabolic process
	83	GO:0009626	0,0012	2,01	13,47	26	2602	plant-type hypersensitive response
	84	GO:0045088	0,0012	2,01	13,49	26	2605	regulation of innate immune response
	85	GO:0034050	0,0013	2,01	13,50	26	2608	host programmed cell death induced by symbiont
	86	GO:0006612	0,0013	2,07	12,09	24	2335	protein targeting to membrane
	87	GO:0031537	0,0013	6,52	0,80	5	154	regulation of anthocyanin metabolic process
	88	GO:0046394	0,0014	1,69	28,21	45	5449	carboxylic acid biosynthetic process
	89	GO:0016053	0,0014	1,69	28,21	45	5449	organic acid biosynthetic process
	90	GO:0050776	0,0014	1,99	13,59	26	2624	regulation of immune response
	91	GO:0019752	0,0014	1,55	44,92	65	8675	carboxylic acid metabolic process
	92	GO:0043436	0,0014	1,55	44,92	65	8675	oxoacid metabolic process
	93	GO:0032787	0,0014	1,65	31,53	49	6090	monocarboxylic acid metabolic process
	94	GO:0002682	0,0014	1,99	13,63	26	2632	regulation of immune system process
	95	GO:0006082	0,0014	1,55	44,94	65	8680	organic acid metabolic process
	96	GO:0072593	0,0015	2,16	10,10	21	1951	reactive oxygen species metabolic process
	97	GO:0050794	0,0015	1,41	97,92	124	18911	regulation of cellular process

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	98	GO:0009867	0,0015	2,25	8,76	19	1692	jasmonic acid mediated signaling pathway
	99	GO:0071395	0,0015	2,25	8,76	19	1692	cellular response to jasmonic acid stimulus
	100	GO:0016070	0,0015	1,45	72,32	96	13967	RNA metabolic process
	101	GO:0044260	0,0015	1,38	129,30	157	24971	cellular macromolecule metabolic process
	102	GO:0010260	0,0015	3,24	3,18	10	614	organ senescence
	103	GO:0031347	0,0017	1,85	17,50	31	3380	regulation of defense response
	104	GO:0070727	0,0017	1,67	27,74	44	5358	cellular macromolecule localization
	105	GO:0010438	0,0018	13,51	0,24	3	46	cellular response to sulfur starvation
	106	GO:0043067	0,0018	1,98	13,12	25	2534	regulation of programmed cell death
	107	GO:0006139	0,0019	1,40	99,45	125	19206	nucleobase-containing compound metabolic process
	108	GO:0032501	0,0019	1,41	84,74	109	16365	multicellular organismal process
	109	GO:0044283	0,0020	1,60	33,72	51	6512	small molecule biosynthetic process
	110	GO:0034613	0,0020	1,67	27,18	43	5249	cellular protein localization
	111	GO:0010941	0,0021	1,96	13,28	25	2564	regulation of cell death
	112	GO:0032502	0,0021	1,41	87,68	112	16933	developmental process
	113	GO:0010035	0,0021	1,62	31,33	48	6051	response to inorganic substance
	114	GO:0044281	0,0022	1,41	86,83	111	16769	small molecule metabolic process
	115	GO:0051649	0,0022	1,57	37,22	55	7189	establishment of localization in cell
	116	GO:0046907	0,0022	1,59	34,73	52	6708	intracellular transport
	117	GO:0048767	0,0023	2,51	5,76	14	1113	root hair elongation
	118	GO:0033198	0,0023	32,19	0,07	2	14	response to ATP
	119	GO:0051641	0,0023	1,55	39,02	57	7535	cellular localization
	120	GO:0010015	0,0025	1,99	12,00	23	2317	root morphogenesis
	121	GO:0009812	0,0025	2,31	7,14	16	1379	flavonoid metabolic process
	122	GO:0006886	0,0026	1,65	26,72	42	5161	intracellular protein transport
	123	GO:0010053	0,0026	2,09	9,88	20	1909	root epidermal cell differentiation
	124	GO:0050793	0,0026	1,75	20,35	34	3930	regulation of developmental process
	125	GO:0000162	0,0027	7,25	0,57	4	111	tryptophan biosynthetic process

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	126	GO:0009595	0,0028	2,81	4,03	11	779	detection of biotic stimulus
	127	GO:0048522	0,0028	1,74	20,47	34	3954	positive regulation of cellular process
	128	GO:0007030	0,0028	2,44	5,91	14	1141	Golgi organization
	129	GO:0046219	0,0029	7,12	0,59	4	113	indolalkylamine biosynthetic process
	130	GO:0006725	0,0031	1,65	25,39	40	4903	cellular aromatic compound metabolic process
	131	GO:0009759	0,0031	10,96	0,29	3	56	indole glucosinolate biosynthetic process
	132	GO:0009913	0,0035	1,90	13,09	24	2528	epidermal cell differentiation
	133	GO:0000271	0,0035	1,88	13,84	25	2672	polysaccharide biosynthetic process
	134	GO:0034599	0,0036	3,08	3,00	9	580	cellular response to oxidative stress
	135	GO:0009409	0,0036	1,77	17,66	30	3410	response to cold
	136	GO:0008544	0,0036	1,90	13,11	24	2532	epidermis development
	137	GO:0048518	0,0037	1,67	23,22	37	4484	positive regulation of biological process
	138	GO:0012501	0,0037	1,82	15,40	27	2975	programmed cell death
	139	GO:0007568	0,0040	2,67	4,24	11	819	aging
	140	GO:0044271	0,0040	1,63	25,78	40	4978	cellular nitrogen compound biosynthetic process
	141	GO:0009073	0,0040	3,64	1,98	7	382	aromatic amino acid family biosynthetic process
	142	GO:0009723	0,0040	2,01	10,30	20	1989	response to ethylene
	143	GO:0046417	0,0044	3,58	2,01	7	388	chorismate metabolic process
	144	GO:0070887	0,0045	1,59	27,61	42	5332	cellular response to chemical stimulus
	145	GO:0010054	0,0046	2,02	9,70	19	1873	trichoblast differentiation
	146	GO:0044238	0,0046	1,33	197,10	222	38065	primary metabolic process
	147	GO:0042743	0,0047	2,02	9,71	19	1876	hydrogen peroxide metabolic process
Abn-CD	1	GO:0052548	1,29E-07	12,56	0,81	9	80	regulation of endopeptidase activity
	2	GO:0010951	1,29E-07	12,56	0,81	9	80	negative regulation of endopeptidase activity
	3	GO:0010466	1,33E-06	9,28	1,06	9	105	negative regulation of peptidase activity
	4	GO:0052547	1,33E-06	9,28	1,06	9	105	regulation of peptidase activity
	5	GO:0051346	1,69E-06	9,00	1,09	9	108	negative regulation of hydrolase activity
	6	GO:0051169	0,0006	2,26	10,03	22	992	nuclear transport

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	7	GO:0006913	0,0006	2,26	10,03	22	992	nucleocytoplasmic transport
	8	GO:0051336	0,0008	3,56	2,91	10	288	regulation of hydrolase activity
	9	GO:0070574	0,0010	17,35	0,20	3	20	cadmium ion transmembrane transport
	10	GO:0046037	0,0021	39,27	0,07	2	7	GMP metabolic process
	11	GO:0006177	0,0021	39,27	0,07	2	7	GMP biosynthetic process
	12	GO:0006405	0,0023	3,07	3,36	10	332	RNA export from nucleus
	13	GO:0051236	0,0023	3,07	3,36	10	332	establishment of RNA localization
	14	GO:0050657	0,0023	3,07	3,36	10	332	nucleic acid transport
	15	GO:0050658	0,0023	3,07	3,36	10	332	RNA transport
	16	GO:0006403	0,0023	3,06	3,37	10	333	RNA localization
	17	GO:0051168	0,0039	2,84	3,62	10	358	nuclear export
	18	GO:0051028	0,0044	2,98	3,10	9	307	mRNA transport
	19	GO:0006406	0,0044	2,98	3,10	9	307	mRNA export from nucleus
R-CT	1	GO:0009401	0,0002	234,47	0,03	2	3	phosphoenolpyruvate-dependent sugar phosphotransferase system
	2	GO:0007059	0,0006	2,77	5,58	15	658	chromosome segregation
	3	GO:0046486	0,0012	2,49	6,60	16	778	glycerolipid metabolic process
	4	GO:0006879	0,0012	6,69	0,79	5	93	cellular iron ion homeostasis
	5	GO:0006650	0,0014	2,53	6,09	15	718	glycerophospholipid metabolic process
	6	GO:0046474	0,0016	2,60	5,53	14	652	glycerophospholipid biosynthetic process
	7	GO:0016556	0,0021	3,35	2,77	9	326	mRNA modification
	8	GO:0042440	0,0021	1,92	13,96	26	1645	pigment metabolic process
	9	GO:0019408	0,0022	7,84	0,54	4	64	dolichol biosynthetic process
	10	GO:0016093	0,0022	7,84	0,54	4	64	polyprenol metabolic process
	11	GO:0016094	0,0022	7,84	0,54	4	64	polyprenol biosynthetic process
	12	GO:0019348	0,0022	7,84	0,54	4	64	dolichol metabolic process
	13	GO:0006828	0,0023	7,71	0,55	4	65	manganese ion transport
	14	GO:0045017	0,0025	2,48	5,80	14	684	glycerolipid biosynthetic process
	15	GO:0046148	0,0026	2,01	11,26	22	1327	pigment biosynthetic process

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	16	GO:0019684	0,0028	2,07	9,92	20	1169	photosynthesis, light reaction
	17	GO:0007062	0,0029	2,64	4,67	12	550	sister chromatid cohesion
	18	GO:0048585	0,0034	1,78	16,74	29	1973	negative regulation of response to stimulus
	19	GO:0048255	0,0038	26,05	0,09	2	11	mRNA stabilization
	20	GO:0043487	0,0038	26,05	0,09	2	11	regulation of RNA stability
	21	GO:0043488	0,0038	26,05	0,09	2	11	regulation of mRNA stability
	22	GO:0043489	0,0038	26,05	0,09	2	11	RNA stabilization
	23	GO:0009653	0,0039	1,37	70,88	93	8352	anatomical structure morphogenesis
	24	GO:0046339	0,0044	9,78	0,33	3	39	diacylglycerol metabolic process
R-CD	1	GO:0008272	1,96E-05	9,44	0,83	7	62	sulfate transport
	2	GO:0010618	0,0003	14,79	0,32	4	24	aerenchyma formation
	3	GO:0046068	0,0004	13,45	0,35	4	26	cGMP metabolic process
	4	GO:0006182	0,0004	13,45	0,35	4	26	cGMP biosynthetic process
	5	GO:0009190	0,0004	13,45	0,35	4	26	cyclic nucleotide biosynthetic process
	6	GO:0009187	0,0004	13,45	0,35	4	26	cyclic nucleotide metabolic process
	7	GO:0048445	0,0004	6,84	0,95	6	71	carpel morphogenesis
	8	GO:0006649	0,0010	18,48	0,20	3	15	phospholipid transfer to membrane
	9	GO:0008643	0,0014	2,75	4,87	13	364	carbohydrate transport
	10	GO:0030493	0,0017	49,22	0,07	2	5	bacteriochlorophyll metabolic process
	11	GO:0030494	0,0017	49,22	0,07	2	5	bacteriochlorophyll biosynthetic process
	12	GO:0015781	0,0020	13,86	0,25	3	19	pyrimidine nucleotide-sugar transport
	13	GO:0015783	0,0020	13,86	0,25	3	19	GDP-fucose transport
	14	GO:0015786	0,0020	13,86	0,25	3	19	UDP-glucose transport
	15	GO:0005983	0,0020	4,88	1,30	6	97	starch catabolic process
	16	GO:0044247	0,0021	4,15	1,77	7	132	cellular polysaccharide catabolic process
	17	GO:0009251	0,0030	4,49	1,41	6	105	glucan catabolic process
	18	GO:0010483	0,0035	11,08	0,31	3	23	pollen tube reception
	19	GO:0051703	0,0035	11,08	0,31	3	23	intraspecies interaction between organisms

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	20	GO:0006833	0,0047	1,87	12,59	23	940	water transport
	21	GO:0042044	0,0047	1,87	12,59	23	940	fluid transport
	22	GO:0036079	0,0049	9,64	0,35	3	26	purine nucleotide-sugar transport
	23	GO:0071702	0,0050	1,49	35,70	52	2666	organic substance transport
RS-CT	1	GO:0008360	0,0007	61,82	0,04	2	11	regulation of cell shape
	2	GO:0042539	0,0010	50,58	0,05	2	13	hypotonic salinity response
	3	GO:0006971	0,0010	50,58	0,05	2	13	hypotonic response
	4	GO:0009059	0,0019	1,51	58,53	79	16213	macromolecule biosynthetic process
	5	GO:0010197	0,0021	7,83	0,53	4	147	polar nucleus fusion
	6	GO:0009411	0,0022	2,76	4,52	12	1251	response to UV
	7	GO:0009559	0,0024	7,51	0,55	4	153	embryo sac central cell differentiation
	8	GO:0034645	0,0025	1,50	57,32	77	15876	cellular macromolecule biosynthetic process
	9	GO:0010224	0,0031	3,82	1,89	7	523	response to UV-B
	10	GO:0009661	0,0036	Inf	0,00	1	1	chromoplast organization
	11	GO:0009786	0,0037	24,18	0,09	2	25	regulation of asymmetric cell division
	12	GO:0046283	0,0039	4,19	1,48	6	409	anthocyanin-containing compound metabolic process
	13	GO:0016045	0,0047	6,18	0,67	4	185	detection of bacterium
RS-CD	1	GO:0043620	3,44E-05	61,13	0,07	3	12	regulation of DNA-templated transcription in response to stress
	2	GO:0043618	3,44E-05	61,13	0,07	3	12	regulation of transcription from RNA polymerase II promoter in response to stress
	3	GO:0043619	3,44E-05	61,13	0,07	3	12	regulation of transcription from RNA polymerase II promoter in response to oxidative stress
	4	GO:0006357	0,0002	10,12	0,52	5	96	regulation of transcription from RNA polymerase II promoter
	5	GO:0051173	0,0003	2,26	11,54	25	2111	positive regulation of nitrogen compound metabolic process
	6	GO:0051254	0,0006	2,20	11,35	24	2077	positive regulation of RNA metabolic process
	7	GO:0045893	0,0006	2,20	11,35	24	2077	positive regulation of transcription, DNA-templated
	8	GO:0048522	0,0006	1,85	21,61	38	3954	positive regulation of cellular process
	9	GO:0010628	0,0006	2,18	11,46	24	2096	positive regulation of gene expression
	10	GO:0045935	0,0007	2,17	11,52	24	2108	positive regulation of nucleobase-containing compound metabolic process
	11	GO:0009891	0,0008	1,98	15,26	29	2792	positive regulation of biosynthetic process

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	12	GO:0031328	0,0008	1,98	15,26	29	2792	positive regulation of cellular biosynthetic process
	13	GO:0010557	0,0010	2,11	11,84	24	2166	positive regulation of macromolecule biosynthetic process
	14	GO:0031325	0,0012	1,94	15,63	29	2859	positive regulation of cellular metabolic process
	15	GO:0009893	0,0013	1,92	15,73	29	2878	positive regulation of metabolic process
	16	GO:0010604	0,0013	2,06	12,11	24	2215	positive regulation of macromolecule metabolic process
	17	GO:0080028	0,0014	14,86	0,22	3	40	nitrile biosynthetic process
	18	GO:0050898	0,0014	14,86	0,22	3	40	nitrile metabolic process
	19	GO:0007338	0,0016	40,64	0,06	2	11	single fertilization
	20	GO:2000762	0,0018	2,82	4,39	12	803	regulation of phenylpropanoid metabolic process
	21	GO:0042538	0,0019	2,67	5,02	13	919	hyperosmotic salinity response
	22	GO:0006470	0,0019	36,58	0,07	2	12	protein dephosphorylation
	23	GO:0070940	0,0019	36,58	0,07	2	12	dephosphorylation of RNA polymerase II C-terminal domain
	24	GO:0015824	0,0020	3,69	2,24	8	409	proline transport
	25	GO:0009620	0,0023	1,81	17,87	31	3269	response to fungus
	26	GO:0010150	0,0025	3,57	2,31	8	422	leaf senescence
	27	GO:0048518	0,0030	1,66	24,51	39	4484	positive regulation of biological process
	28	GO:0009838	0,0031	5,32	0,97	5	178	abscission
	29	GO:0043455	0,0038	2,55	4,83	12	884	regulation of secondary metabolic process
	30	GO:0031326	0,0042	1,43	56,59	76	10353	regulation of cellular biosynthetic process
	31	GO:0015804	0,0043	3,25	2,53	8	463	neutral amino acid transport
	32	GO:0009889	0,0044	1,43	56,72	76	10378	regulation of biosynthetic process
RL-CT	1	GO:0002229	6,18E-05	5,72	1,74	9	81	defense response to oomycetes
	2	GO:0002239	9,92E-05	4,35	2,73	11	127	response to oomycetes
	3	GO:0051254	0,0005	1,57	44,60	68	2077	positive regulation of RNA metabolic process
	4	GO:0045893	0,0005	1,57	44,60	68	2077	positive regulation of transcription, DNA-templated
	5	GO:0010557	0,0006	1,55	46,51	70	2166	positive regulation of macromolecule biosynthetic process
	6	GO:0010628	0,0006	1,55	45,01	68	2096	positive regulation of gene expression
	7	GO:0009746	0,0006	1,85	19,99	36	931	response to hexose

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	8	GO:0034284	0,0006	1,85	19,99	36	931	response to monosaccharide
	9	GO:0045935	0,0007	1,54	45,27	68	2108	positive regulation of nucleobase-containing compound metabolic process
	10	GO:0051173	0,0007	1,54	45,33	68	2111	positive regulation of nitrogen compound metabolic process
	11	GO:0042537	0,0008	6,10	1,10	6	51	benzene-containing compound metabolic process
	12	GO:0018874	0,0008	6,10	1,10	6	51	benzoate metabolic process
	13	GO:0009750	0,0009	1,87	18,15	33	845	response to fructose
	14	GO:0006388	0,0009	10,75	0,45	4	21	tRNA splicing, via endonucleolytic cleavage and ligation
	15	GO:0010604	0,0010	1,51	47,57	70	2215	positive regulation of macromolecule metabolic process
	16	GO:0016578	0,0014	91,26	0,06	2	3	histone deubiquitination
	17	GO:0006744	0,0017	3,52	2,71	9	126	ubiquinone biosynthetic process
	18	GO:0006022	0,0018	5,08	1,29	6	60	aminoglycan metabolic process
	19	GO:0070328	0,0027	45,63	0,09	2	4	triglyceride homeostasis
	20	GO:0010479	0,0027	45,63	0,09	2	4	stele development
	21	GO:0033388	0,0027	45,63	0,09	2	4	putrescine biosynthetic process from arginine
	22	GO:0055091	0,0027	45,63	0,09	2	4	phospholipid homeostasis
	23	GO:0055088	0,0027	45,63	0,09	2	4	lipid homeostasis
	24	GO:0055089	0,0027	45,63	0,09	2	4	fatty acid homeostasis
	25	GO:0010244	0,0029	7,61	0,60	4	28	response to low fluence blue light stimulus by blue low-fluence system
	26	GO:0010220	0,0030	12,45	0,30	3	14	positive regulation of vernalization response
	27	GO:0006743	0,0031	3,19	2,96	9	138	ubiquinone metabolic process
	28	GO:0034052	0,0033	5,44	1,01	5	47	positive regulation of plant-type hypersensitive response
	29	GO:0043462	0,0037	11,41	0,32	3	15	regulation of ATPase activity
	30	GO:0031325	0,0038	1,38	61,39	83	2859	positive regulation of cellular metabolic process
	31	GO:0046352	0,0042	6,77	0,67	4	31	disaccharide catabolic process
	32	GO:0009891	0,0043	1,38	59,96	81	2792	positive regulation of biosynthetic process
	33	GO:0031328	0,0043	1,38	59,96	81	2792	positive regulation of cellular biosynthetic process
	34	GO:0031329	0,0044	2,52	4,94	12	230	regulation of cellular catabolic process
	35	GO:0005993	0,0044	30,42	0,11	2	5	trehalose catabolic process

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	36	GO:0009893	0,0045	1,37	61,80	83	2878	positive regulation of metabolic process
	37	GO:0007131	0,0046	1,98	10,39	20	484	reciprocal meiotic recombination
	38	GO:0035825	0,0046	1,98	10,39	20	484	reciprocal DNA recombination
	39	GO:0048518	0,0046	1,30	96,29	122	4484	positive regulation of biological process
	40	GO:0048584	0,0049	2,10	8,33	17	388	positive regulation of response to stimulus
RL-CD	1	GO:0046160	6,50E-08	180,51	0,04	4	8	heme a metabolic process
	2	GO:0006784	6,50E-08	180,51	0,04	4	8	heme a biosynthetic process
	3	GO:0048598	6,59E-07	22,20	0,31	6	55	embryonic morphogenesis
	4	GO:0048658	8,12E-06	20,55	0,27	5	49	anther wall tapetum development
	5	GO:0000103	1,54E-05	12,36	0,52	6	94	sulfate assimilation
	6	GO:0010086	3,63E-05	60,01	0,07	3	12	embryonic root morphogenesis
	7	GO:0010198	3,63E-05	60,01	0,07	3	12	synergid death
	8	GO:0048466	4,59E-05	3,56	4,39	15	789	androecium development
	9	GO:0048443	4,59E-05	3,56	4,39	15	789	stamen development
	10	GO:0048653	7,06E-05	4,86	2,14	10	385	anther development
	11	GO:0007231	9,08E-05	41,54	0,09	3	16	osmosensory signaling pathway
	12	GO:0033500	9,08E-05	41,54	0,09	3	16	carbohydrate homeostasis
	13	GO:0071470	9,08E-05	41,54	0,09	3	16	cellular response to osmotic stress
	14	GO:0010029	0,0003	4,51	2,07	9	372	regulation of seed germination
	15	GO:0010483	0,0003	27,00	0,13	3	23	pollen tube reception
	16	GO:0051703	0,0003	27,00	0,13	3	23	intraspecies interaction between organisms
	17	GO:0007108	0,0003	119,71	0,03	2	5	cytokinesis, initiation of separation
	18	GO:0000920	0,0003	119,71	0,03	2	5	cytokinetic cell separation
	19	GO:0050918	0,0005	11,64	0,37	4	66	positive chemotaxis
	20	GO:0010183	0,0005	11,64	0,37	4	66	pollen tube guidance
	21	GO:0006997	0,0005	3,74	2,77	10	497	nucleus organization
	22	GO:0010623	0,0006	20,77	0,16	3	29	developmental programmed cell death
	23	GO:0042330	0,0007	10,77	0,40	4	71	taxis

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	24	GO:0006935	0,0007	10,77	0,40	4	71	chemotaxis
	25	GO:0070271	0,0009	2,00	14,60	28	2623	protein complex biogenesis
	26	GO:0006461	0,0009	2,00	14,60	28	2623	protein complex assembly
	27	GO:0071822	0,0014	1,94	15,05	28	2704	protein complex subunit organization
	28	GO:0065003	0,0019	1,87	16,11	29	2894	macromolecular complex assembly
	29	GO:0043623	0,0019	1,94	13,90	26	2497	cellular protein complex assembly
	30	GO:0040011	0,0022	7,75	0,54	4	97	locomotion
	31	GO:0048367	0,0022	1,89	14,82	27	2662	shoot system development
	32	GO:0043933	0,0029	1,81	16,63	29	2988	macromolecular complex subunit organization
	33	GO:0072351	0,0035	25,65	0,09	2	16	tricarboxylic acid biosynthetic process
	34	GO:0030417	0,0035	25,65	0,09	2	16	nicotianamine metabolic process
	35	GO:0030418	0,0035	25,65	0,09	2	16	nicotianamine biosynthetic process
	36	GO:0010188	0,0036	10,38	0,31	3	55	response to microbial phytotoxin
	37	GO:0034622	0,0038	1,82	15,41	27	2768	cellular macromolecular complex assembly
	38	GO:0010204	0,0040	6,49	0,64	4	115	defense response signaling pathway, resistance gene-independent
	39	GO:0006783	0,0041	6,44	0,65	4	116	heme biosynthetic process
	40	GO:0018106	0,0042	9,81	0,32	3	58	peptidyl-histidine phosphorylation
	41	GO:0019538	0,0044	1,39	72,84	94	13085	protein metabolic process
	42	GO:0010187	0,0045	6,27	0,66	4	119	negative regulation of seed germination
	43	GO:0051241	0,0045	6,27	0,66	4	119	negative regulation of multicellular organismal process
	44	GO:0070407	0,0050	21,12	0,11	2	19	oxidation-dependent protein catabolic process
SHL-CT	1	GO:0042538	3,16E-05	7,16	1,22	8	919	hyperosmotic salinity response
	2	GO:0080127	7,74E-05	192,85	0,01	2	10	fruit septum development
	3	GO:0080052	9,45E-05	171,42	0,01	2	11	response to histidine
	4	GO:0080053	9,45E-05	171,42	0,01	2	11	response to phenylalanine
	5	GO:0043201	9,45E-05	171,42	0,01	2	11	response to leucine
	6	GO:0010467	0,0002	2,25	19,77	35	14941	gene expression
	7	GO:0080125	0,0003	85,70	0,03	2	20	multicellular structure septum development

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	8	GO:0016070	0,0007	2,12	18,48	32	13967	RNA metabolic process
	9	GO:0042938	0,0008	53,18	0,04	2	31	dipeptide transport
	10	GO:0006972	0,0009	4,34	1,98	8	1500	hyperosmotic response
	11	GO:0031086	0,0010	46,73	0,05	2	35	nuclear-transcribed mRNA catabolic process, deadenylation-independent decay
	12	GO:0031087	0,0010	46,73	0,05	2	35	deadenylation-independent decapping of nuclear-transcribed mRNA
	13	GO:0001101	0,0011	45,36	0,05	2	36	response to acid chemical
	14	GO:0014075	0,0011	45,36	0,05	2	36	response to amine
	15	GO:0043200	0,0011	45,36	0,05	2	36	response to amino acid
	16	GO:0010243	0,0012	42,84	0,05	2	38	response to organonitrogen compound
	17	GO:0032544	0,0012	42,84	0,05	2	38	plastid translation
	18	GO:0010557	0,0023	3,38	2,87	9	2166	positive regulation of macromolecule biosynthetic process
	19	GO:0042939	0,0025	28,55	0,07	2	56	tripeptide transport
	20	GO:0006412	0,0026	3,09	3,50	10	2645	translation
	21	GO:0010604	0,0027	3,30	2,93	9	2215	positive regulation of macromolecule metabolic process
	22	GO:0022613	0,0033	3,86	1,93	7	1457	ribonucleoprotein complex biogenesis
	23	GO:0009641	0,0038	23,01	0,09	2	69	shade avoidance
	24	GO:0010582	0,0043	21,71	0,10	2	73	floral meristem determinacy
	25	GO:0010022	0,0048	20,55	0,10	2	77	meristem determinacy
	26	GO:0090304	0,0048	1,83	21,54	33	16277	nucleic acid metabolic process
SHL-CD	1	GO:0051176	9,90E-05	184,98	0,02	2	7	positive regulation of sulfur metabolic process
	2	GO:0015977	0,0005	20,83	0,15	3	70	carbon fixation
	3	GO:0019253	0,0005	20,83	0,15	3	70	reductive pentose-phosphate cycle
	4	GO:0019685	0,0005	20,22	0,16	3	72	photosynthesis, dark reaction
	5	GO:0080001	0,0017	35,56	0,06	2	28	mucilage extrusion from seed coat
RSH-CT	1	GO:0009691	0,0005	4,20	2,25	9	171	cytokinin biosynthetic process
	2	GO:0043462	0,0009	18,83	0,20	3	15	regulation of ATPase activity
	3	GO:0006457	0,0015	1,83	17,96	32	1366	protein folding
	4	GO:0000041	0,0029	1,75	18,77	32	1428	transition metal ion transport

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	5	GO:0032781	0,0035	30,09	0,09	2	7	positive regulation of ATPase activity
	6	GO:0009871	0,0042	10,27	0,33	3	25	jasmonic acid and ethylene-dependent systemic resistance, ethylene mediated signaling pathway
	7	GO:0048227	0,0046	25,07	0,11	2	8	plasma membrane to endosome transport
	8	GO:0045962	0,0047	9,82	0,34	3	26	positive regulation of development, heterochronic
RSH-CD	1	GO:0051182	4,35E-05	372,77	0,01	2	4	coenzyme transport
	2	GO:0072348	4,35E-05	372,77	0,01	2	4	sulfur compound transport
	3	GO:0015860	4,35E-05	372,77	0,01	2	4	purine nucleoside transmembrane transport
	4	GO:0015805	4,35E-05	372,77	0,01	2	4	S-adenosyl-L-methionine transport
	5	GO:0051181	0,0002	32,11	0,10	3	38	cofactor transport
	6	GO:0048466	0,0003	4,42	2,13	9	789	androecium development
	7	GO:0048443	0,0003	4,42	2,13	9	789	stamen development
	8	GO:0009641	0,0009	17,02	0,19	3	69	shade avoidance
	9	GO:0015858	0,0013	41,41	0,05	2	20	nucleoside transport
	10	GO:0009611	0,0014	2,68	5,50	14	2035	response to wounding
	11	GO:0031146	0,0026	28,66	0,08	2	28	SCF-dependent proteasomal ubiquitin-dependent protein catabolic process
	12	GO:0006865	0,0027	2,84	4,04	11	1494	amino acid transport
	13	GO:0072337	0,0028	27,60	0,08	2	29	modified amino acid transport
	14	GO:0048438	0,0034	2,42	6,08	14	2249	floral whorl development
	15	GO:0015837	0,0038	2,71	4,23	11	1566	amine transport
	16	GO:0046942	0,0042	2,68	4,29	11	1585	carboxylic acid transport
	17	GO:0015849	0,0045	2,64	4,34	11	1604	organic acid transport
T50-CT	1	GO:0010154	0,0002	1,78	28,91	50	1285	fruit development
	2	GO:0009960	0,0004	3,61	3,24	11	144	endosperm development
	3	GO:0010218	0,0009	2,19	10,37	22	461	response to far red light
	4	GO:0010541	0,0009	4,92	1,55	7	69	acropetal auxin transport
	5	GO:0060918	0,0012	2,15	10,57	22	470	auxin transport
	6	GO:0019482	0,0012	18,66	0,22	3	10	beta-alanine metabolic process
	7	GO:0019484	0,0012	18,66	0,22	3	10	beta-alanine catabolic process

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	8	GO:0009401	0,0015	87,02	0,07	2	3	phosphoenolpyruvate-dependent sugar phosphotransferase system
	9	GO:0009926	0,0015	2,15	10,10	21	449	auxin polar transport
	10	GO:0016337	0,0016	9,17	0,52	4	23	single organismal cell-cell adhesion
	11	GO:0009914	0,0017	2,08	10,89	22	484	hormone transport
	12	GO:0048316	0,0018	1,65	26,68	43	1186	seed development
	13	GO:0072523	0,0019	1,65	26,77	43	1190	purine-containing compound catabolic process
	14	GO:0006200	0,0020	1,96	13,16	25	585	ATP catabolic process
	15	GO:0034655	0,0026	1,62	27,22	43	1210	nucleobase-containing compound catabolic process
	16	GO:0006540	0,0027	13,06	0,29	3	13	glutamate decarboxylation to succinate
	17	GO:0006538	0,0027	13,06	0,29	3	13	glutamate catabolic process
	18	GO:0009450	0,0027	13,06	0,29	3	13	gamma-aminobutyric acid catabolic process
	19	GO:0009301	0,0029	43,51	0,09	2	4	snRNA transcription
	20	GO:0010315	0,0031	3,91	1,91	7	85	auxin efflux
	21	GO:0019408	0,0032	4,51	1,44	6	64	dolichol biosynthetic process
	22	GO:0016093	0,0032	4,51	1,44	6	64	polyprenol metabolic process
	23	GO:0016094	0,0032	4,51	1,44	6	64	polyprenol biosynthetic process
	24	GO:0019348	0,0032	4,51	1,44	6	64	dolichol metabolic process
	25	GO:0052386	0,0032	2,95	3,55	10	158	cell wall thickening
	26	GO:0051180	0,0034	11,87	0,31	3	14	vitamin transport
	27	GO:0015884	0,0034	11,87	0,31	3	14	folic acid transport
	28	GO:0006195	0,0038	1,60	26,23	41	1166	purine nucleotide catabolic process
	29	GO:0009166	0,0038	1,60	26,23	41	1166	nucleotide catabolic process
	30	GO:0009958	0,0042	3,04	3,10	9	138	positive gravitropism
	31	GO:0003006	0,0045	1,23	174,61	208	7762	developmental process involved in reproduction
	32	GO:0010395	0,0048	29,00	0,11	2	5	rhamnogalacturonan I metabolic process
	33	GO:0010400	0,0048	29,00	0,11	2	5	rhamnogalacturonan I side chain metabolic process
T50-CD	1	GO:0010025	3,87E-07	38,21	0,14	5	117	wax biosynthetic process
	2	GO:0010166	5,81E-07	35,07	0,16	5	127	wax metabolic process

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	3	GO:0046218	3,80E-05	10,51	0,62	6	498	indolalkylamine catabolic process
	4	GO:0006569	3,80E-05	10,51	0,62	6	498	tryptophan catabolic process
	5	GO:0044270	4,13E-05	4,91	2,51	11	2026	cellular nitrogen compound catabolic process
	6	GO:0046700	4,20E-05	4,90	2,51	11	2030	heterocycle catabolic process
	7	GO:0009074	4,77E-05	10,08	0,64	6	519	aromatic amino acid family catabolic process
	8	GO:0019344	5,87E-05	6,57	1,33	8	1076	cysteine biosynthetic process
	9	GO:0042436	5,94E-05	9,68	0,67	6	540	indole-containing compound catabolic process
	10	GO:0006534	6,02E-05	6,54	1,34	8	1080	cysteine metabolic process
	11	GO:0006788	6,76E-05	206,64	0,01	2	10	heme oxidation
	12	GO:0009070	7,28E-05	6,36	1,37	8	1110	serine family amino acid biosynthetic process
	13	GO:0006568	8,94E-05	8,97	0,72	6	582	tryptophan metabolic process
	14	GO:0006586	8,94E-05	8,97	0,72	6	582	indolalkylamine metabolic process
	15	GO:0042742	9,15E-05	4,16	3,24	12	2618	defense response to bacterium
	16	GO:0046394	0,0001	3,12	6,74	18	5449	carboxylic acid biosynthetic process
	17	GO:0016053	0,0001	3,12	6,74	18	5449	organic acid biosynthetic process
	18	GO:0019439	0,0001	8,42	0,77	6	619	aromatic compound catabolic process
	19	GO:0010024	0,0002	127,15	0,02	2	15	phytochromobilin biosynthetic process
	20	GO:0051202	0,0002	127,15	0,02	2	15	phytochromobilin metabolic process
	21	GO:0009063	0,0002	6,56	1,15	7	933	cellular amino acid catabolic process
	22	GO:0009684	0,0002	7,94	0,81	6	656	indoleacetic acid biosynthetic process
	23	GO:0009683	0,0002	7,89	0,82	6	660	indoleacetic acid metabolic process
	24	GO:0070417	0,0002	28,16	0,11	3	92	cellular response to cold
	25	GO:0009069	0,0002	5,31	1,64	8	1323	serine family amino acid metabolic process
	26	GO:0009851	0,0003	7,18	0,90	6	724	auxin biosynthetic process
	27	GO:0042402	0,0003	7,06	0,91	6	736	cellular biogenic amine catabolic process
	28	GO:0044283	0,0003	2,75	8,06	19	6512	small molecule biosynthetic process
	29	GO:0009617	0,0004	3,37	4,32	13	3496	response to bacterium
	30	GO:0000097	0,0005	4,79	1,81	8	1463	sulfur amino acid biosynthetic process

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	31	GO:0042435	0,0005	6,43	1,00	6	806	indole-containing compound biosynthetic process
	32	GO:0010019	0,0005	66,11	0,03	2	27	chloroplast-nucleus signaling pathway
	33	GO:0042168	0,0005	20,37	0,16	3	126	heme metabolic process
	34	GO:0009850	0,0006	6,24	1,03	6	831	auxin metabolic process
	35	GO:0009310	0,0006	5,24	1,44	7	1161	amine catabolic process
	36	GO:0042430	0,0008	5,88	1,09	6	880	indole-containing compound metabolic process
	37	GO:0000096	0,0010	4,25	2,03	8	1644	sulfur amino acid metabolic process
	38	GO:0048544	0,0011	15,95	0,20	3	160	recognition of pollen
	39	GO:0008037	0,0011	15,95	0,20	3	160	cell recognition
	40	GO:0034754	0,0012	5,46	1,17	6	947	cellular hormone metabolic process
	41	GO:0016114	0,0012	4,64	1,62	7	1309	terpenoid biosynthetic process
	42	GO:0010325	0,0014	39,34	0,05	2	44	raffinose family oligosaccharide biosynthetic process
	43	GO:0019761	0,0017	5,04	1,27	6	1024	glucosinolate biosynthetic process
	44	GO:0019758	0,0017	5,04	1,27	6	1024	glycosinolate biosynthetic process
	45	GO:0016144	0,0017	5,04	1,27	6	1024	S-glycoside biosynthetic process
	46	GO:0006576	0,0018	5,01	1,28	6	1031	cellular biogenic amine metabolic process
	47	GO:0016051	0,0021	2,64	5,90	14	4773	carbohydrate biosynthetic process
	48	GO:0006633	0,0022	4,16	1,80	7	1456	fatty acid biosynthetic process
	49	GO:0009875	0,0023	12,21	0,26	3	208	pollen-pistil interaction
	50	GO:0006721	0,0023	4,15	1,80	7	1459	terpenoid metabolic process
	51	GO:0051704	0,0024	2,14	12,56	23	10153	multi-organism process
	52	GO:0042446	0,0030	3,93	1,90	7	1538	hormone biosynthetic process
	53	GO:0032787	0,0030	2,38	7,53	16	6090	monocarboxylic acid metabolic process
	54	GO:0018130	0,0032	3,01	3,61	10	2917	heterocycle biosynthetic process
	55	GO:0016138	0,0035	4,35	1,46	6	1182	glycoside biosynthetic process
	56	GO:0009233	0,0037	408,45	0,00	1	3	menaquinone metabolic process
	57	GO:0009234	0,0037	408,45	0,00	1	3	menaquinone biosynthetic process
	58	GO:0046395	0,0041	3,36	2,56	8	2067	carboxylic acid catabolic process

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	59	GO:0016054	0,0041	3,36	2,56	8	2067	organic acid catabolic process
	60	GO:0019760	0,0044	4,15	1,53	6	1239	glucosinolate metabolic process
	61	GO:0019757	0,0044	4,15	1,53	6	1239	glycosinolate metabolic process
	62	GO:0016143	0,0044	4,15	1,53	6	1239	S-glycoside metabolic process
	63	GO:0009072	0,0046	4,12	1,54	6	1246	aromatic amino acid family metabolic process
T10-CT	1	GO:0046451	4,27E-06	16,07	0,43	6	49	diaminopimelate metabolic process
	2	GO:0009085	4,27E-06	16,07	0,43	6	49	lysine biosynthetic process
	3	GO:0009089	4,27E-06	16,07	0,43	6	49	lysine biosynthetic process via diaminopimelate
	4	GO:0006553	5,42E-06	15,36	0,44	6	51	lysine metabolic process
	5	GO:0016126	0,0003	2,78	6,32	17	727	sterol biosynthetic process
	6	GO:0016125	0,0004	2,70	6,51	17	749	sterol metabolic process
	7	GO:0071554	0,0005	1,62	38,62	60	4445	cell wall organization or biogenesis
	8	GO:0042546	0,0006	2,03	14,22	28	1637	cell wall biogenesis
	9	GO:0007043	0,0008	19,11	0,18	3	21	cell-cell junction assembly
	10	GO:0009769	0,0009	18,10	0,19	3	22	photosynthesis, light harvesting in photosystem II
	11	GO:0034329	0,0015	14,95	0,23	3	26	cell junction assembly
	12	GO:0070592	0,0018	2,32	7,52	17	866	cell wall polysaccharide biosynthetic process
	13	GO:0006305	0,0019	2,55	5,64	14	649	DNA alkylation
	14	GO:0006306	0,0019	2,55	5,64	14	649	DNA methylation
	15	GO:0070589	0,0019	2,31	7,57	17	871	cellular component macromolecule biosynthetic process
	16	GO:0044038	0,0019	2,31	7,57	17	871	cell wall macromolecule biosynthetic process
	17	GO:0006304	0,0020	2,54	5,67	14	653	DNA modification
	18	GO:0006694	0,0020	2,13	9,63	20	1109	steroid biosynthetic process
	19	GO:0015727	0,0020	38,16	0,07	2	8	lactate transport
	20	GO:0034330	0,0029	11,46	0,29	3	33	cell junction organization
	21	GO:0045216	0,0029	11,46	0,29	3	33	cell-cell junction organization
	22	GO:0008283	0,0030	2,06	9,97	20	1148	cell proliferation
	23	GO:0008202	0,0035	1,99	10,85	21	1249	steroid metabolic process

Trait	No	GOBPID	P-value	Odds Ratio	Exp-Count	Count	Size	Term
	24	GO:0010413	0,0036	2,22	7,38	16	849	glucuronoxylan metabolic process
	25	GO:0045492	0,0037	2,21	7,41	16	853	xylan biosynthetic process
	26	GO:0034051	0,0039	25,44	0,10	2	11	negative regulation of plant-type hypersensitive response
	27	GO:0002098	0,0039	25,44	0,10	2	11	tRNA wobble uridine modification
	28	GO:0016131	0,0044	2,31	6,20	14	714	brassinosteroid metabolic process
	29	GO:0016128	0,0045	2,31	6,22	14	716	phytosteroid metabolic process
	30	GO:0016132	0,0046	2,38	5,59	13	643	brassinosteroid biosynthetic process
	31	GO:0090066	0,0047	2,22	6,92	15	796	regulation of anatomical structure size
	32	GO:0032535	0,0047	2,22	6,92	15	796	regulation of cellular component size
	33	GO:0016129	0,0048	2,38	5,60	13	645	phytosteroid biosynthetic process
T10-CD	1	GO:0046937	0,0004	24,97	0,14	3	19	phytochelatin metabolic process
	2	GO:0046938	0,0004	24,97	0,14	3	19	phytochelatin biosynthetic process
	3	GO:0043043	0,0005	12,13	0,36	4	48	peptide biosynthetic process
	4	GO:0051085	0,0011	16,65	0,20	3	27	chaperone mediated protein folding requiring cofactor
	5	GO:0061077	0,0011	16,65	0,20	3	27	chaperone-mediated protein folding
	6	GO:0051084	0,0019	13,32	0,25	3	33	'de novo' posttranslational protein folding
	7	GO:0006458	0,0019	13,32	0,25	3	33	'de novo' protein folding
	8	GO:0006842	0,0029	29,54	0,08	2	11	tricarboxylic acid transport
	9	GO:0015746	0,0029	29,54	0,08	2	11	citrate transport
	10	GO:0080119	0,0044	9,74	0,33	3	44	ER body organization
	11	GO:0042026	0,0047	9,51	0,34	3	45	protein refolding

Curriculum Vitae

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9/2004-9/2010: Research Assistant in National Research Center (NRC), Cairo, Egypt at Plant Biotechnology department, Genetic Engeneering and Biotechnology Division.

11/2003-8/2004: Research Assistant in Cairo University, Faculty of Agriculture, Genetic engeneering and Biotechnology lab, Egypt

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Doctoral research about "Molecular Mapping of Loci Determining Seed Longevity in "*Brassica napus*"

2007-2010: **PhD student at Institute of Genetic Engeneering and Biotechnology, Monofia University, Egypt**
Doctoral research about "Production of transgenic canola more tolerant to drought and with low phytic acid content"

2004-2007: **M.Sc. Faculty of Agriculture, Cairo University, Egypt**
(Biotechnology- Molecular characterization and genetic transformation)
Master research study about "Studies of Somatic embryogenesis of some Date Plam Cultivars"

1999-2003: **B.Sc Faculty of Agriculture, Cairo University, Egypt**
Main specialization: Pomology
Minor specialization: Vegetables production

Work Experience

1- Seed germination and evaluation
2- Artificial ageing and seedling evaluation
3- Classical QTL mapping using softwares QTL Cartographer, PlabQTL and Genestat
4- Genome Wide Association Mapping using softwares Tassel and Genestat
5- Phenotypic data analysis using softwares Sigma plot, SPSS and PLABSTAT
6- Genotypic data analysis using softwares Structure (for population structure), Tassel (for PCA and Kinship analysis), Phylip and SplitsTree4 (for drawing phylogenetic tree)
7- Tissue culture of Rice, Barley, Tomato, Potato, Canola and Several medicinal plants
8- Agrobacterium mediated-transformation in tomato, rice, potato and canola
9- Biolistic mediated transformation of Barley, Date palm and Canola.
10- Basic techniques of molecular biology, DNA manipulation, PCR, DNA fingerprinting, protein and isozymes, cloning, southern, northern and western hybridization.

International training and fellowships

[8-12] Sep 2014: **Genetic diversity-Practical course.** 42 hours practical course on DNA sequencing, sequence analysis, Nucleotide and haplotype diversity and statistical tests for selection, DNA divergence between populations, Nutrality test, gene flow and genetic differentiation,, linkage disequilibrium, DNA alignment, building trees from sequence data, genome wide association mapping. Softwares used during training: DNASP5, MEGA5, Splits Tree4, Tassel 2.1 and Tassel 4. The training held at IPK institute, Gatersleben, Germany.

[18] Nov 2013: **Graduate school symposium** in Hohenheim university. "Future challenges of plant breeding 'Preparing the next generation" Workshop attendance.

[1-30] April 2009: **Center of Biotechnology (CBS), Sfax, Tunisia.** Training period on Agrobacterium and biolistic-mediated transformation of Barley and Canola.

[3-14] Nov 2008: **ICGEB New Delhi, India.** Training course on Molecular Aspects of Salt and Drought Tolerance in Crop Plants.

[1-30] Oct 2008: **ICGEB, New Delhi, India.** Training period in Plant Transformation group on canola transformation and some cloning applications.

[3-7] Dec 2005: **COMSATS international training course:** Plant Biotechnology: A New Promise for Sustainable Development, Fac. of Agriculture, Cairo Uni., Egypt.

[1-30] May 2007: **Pavia University, Italy.** Training mission in Dept. of Genetics and Microbiology, Pavia Univ. Italy, on Agrobacterium-mediated transformation of rice and canola.

Conferences:

Oral presentation

[24-25] Aug 2015: **[6th] Annual International Arab Workshop on Biotechnology.** Entiteled: Plant Genetic Resources: Conservation, Documentation & Better Utilization. National Research Center (NRC), Cairo, Egypt. Abstract title: **"**Linkage and association genetic studies in oilseed rape (*Brassica napus*) displayed common QTL for seed deterioration and longevity".

[5-8]Jul 2015: **International Scociety for Seed Science (ISSS). Seed longevity workshop.** Entiteled: Seeds for future generations–Determinants of longevity. Wernigerode, Germany. Abstract title: "Genetic studies in oilseed rape (*Brassica napus*) revealed QTL for seed longevity related to seed weight, oil content and nutrition accumulation in seeds**"**.

[4-7] Jun 2012: **8th Plant Science Student Conference (PSSC) at IPK, Gatersleben, Germany.** Abstract title: "Different seed viability after natural and expermintal ageing suggesting genetic control of seed longevity".

[14-17] Jun 2011: **7th Plant Science Student Conference (PSSC) at Leibniz inistitute of Plant Biochemistry (IPB), Halle (Salle)**, **Germany**. Abstract title: "Seed survival in genebanks – the unset of a study in oilseed rape".

[8-9] Mai 2011: **Arbeitstagung der Arbeitsgemeinschaft Saatgut und Sortenwesen. Saatguterhaltung und Nutzbarmachung von Kulturpflanzen und heimischen Wildraten. Osnabrück University, Germany.** Ankundigung. Abstract title: "Genetic Studies on seed longevity of oilseed rape *Brassica napus* L. stored under different conditions".

[3-5] Nov 2009: **3rd International Conference of Genetic Engeneering & Biotechnology Research Division: Biotechnology for Better Life. Session 11: Plant Biotechnology III.** Abstract title: Establishment of regeneration and transformation system for semi-dry Egyptian date palm cultivar "Swei".

[3-5] Nov 2009: **3rd International Conference of Genetic Engeneering & Biotechnology Research Division: Biotechnology for Better Life. Session 22: Plant Biotechnology V.** Abstract title: "Establishment of regeneration and transformation system for some North African barley cultivars".

[11-14] Dec 2006: **1st International Egyptian-Jordanian Conference on Biotechnology and Sustainable Development: Current Status and Future Scenarios. National Research Center, Cairo, Egypt.** Abstract title: " RAPD analysis of semi dry Egyptian date palm during somatic embryogenesis".

Posters

[9-12] Jul 2013: **International Scociety for Seed Science (ISSS). 4th workshop on the molecular aspects of seed dormancy and germination. UPMC Surbonne University Paris, France.** Abstract titele: "QTL mapping of seed longevity in the oil seed rape population 'Express xV8". **Mai Allam**, Manuela Nage1, Rod Snowdon, Wolfgang Friedt and Andreas Börner.

[28-31] Mai 2013: **9th Plant Science Student Conference (PSSC) at at Leibniz inistitute of Plant Biochemistry (IPB), Halle (Salle)**, **Germany**. Abstract title: "Molecular mapping of loci affecting seed longevity in *Brassica napus* L." **Mai Allam**, Manuela Nage1, Rod Snowdon, Wolfgang Friedt Christian Möllar and Andreas Börner.

[21-24] May 2012: **19th EUCARPIA General Congress. Budapest, Hungary.** Abstract titele: "Seed Longevity in oilseed rap *Brassica napus* L.- Genetic variation afer long term storage and experminatal ageing" **Mai Allam**, Manuela Nage1, Rod Snowdon, Wolfgang Friedt and Andreas Börner.

[28]Feb-[1]Mar 2012: German Socity for Plant Breeding (GBZ) Conference, Justus Liebig University, Gissen, Germany - Breeding crops for susstainable agriculture production. Abstract titele: "Genetic linkage studies in seed longevity". **Mai Allam**, Manuela Nage1, Rod Snowdon, Wolfgang Friedt and Andreas Börner.

[4-5] Oct. 2011: **IPK institute's day. IPK-Gatersleben, Germany.** Abstract title**:** "Viability of seed collections in genebanks-crucial aspects of seed longevity". Manuela Nagel, Mian A. Rehman Arif, **Mai Allam**, Isaac O. Daniel,Mathias Gäbler, Raj K. Pasam, Benjamin Kilian, Nils Stein & Andreas Börner.

Shared research presented by co-authers

[3-5] Nov 2009: **3rd International Conference of Genetic Engeneering & Biotechnology Research Division: Biotechnology for Better Life. Session 22: Plant Biotechnology V. NRC, Cairo,** Egypt. Abstract title: "In vitro Proliferation and Genetic Characterization of *Silene leucophylla*". Shered research by M. Saker, M. Eldemerdash and **M. Allam**. presented by second auther.

[26-30] Oct 2008: **5th Congress of Scientific Research Outlook in the Arab World Scientific Innovation and Sustained Development. Fez, Morocco.** " A System for In Vitro Propagation of Date Palm Via Somatic Embryogenesis Using Suspension Culture". **Mai A. Allam**, Saker, M.M., Abd El Zaher, M. H. And Amina, H.G. Presented by second auther and published as extended Abstract (Code No.1679BIOC).

Attendance and organizing team

[24-25] Aug 2015: 6th Annual International Arab Workshop on Biotechnology. Entiteled: Plant Genetic Resources: Conservation, Documentation & Better Utilization. National Research Center (NRC), Cairo, Egypt. (**Scientific committee and organizing team**)

[29]Jun-[4]Jul 2014: EUCARPIA Cereal Section & ITMI Conference, Wernigerode, Germany. Conference title: "Cereals for Food, Feed and Fuel- Challenge for Global Improvement". (**Attendance and organization**)

[4-7] Jun 2012: 8th Plant Science Student Conference (PSSC) at IPK, Gatersleben, Germany. (**Attendance and organization**)

[3-5]Mar 2009: 4th International Conference of Pharmaceutical and Drug Industries research division. National Research Center (NRC), Cairo, Egypt. (**Attendance**)

[11-14] Dec 2006: 1st International Egyptian-Jordanian Conference on Biotechnology and Sustainable Development: Current Status and Future Scenarios. National Research Center, Cairo, Egypt. (**Attendance and organization**)

[14-17] Nov 2006: 2nd International Conference of Genetic Engineering and its Application. Sharm El-Sheik, City South Sinai, Egypt. (**Attendance**)

Projects:
I have been shared in achievement of some research projects in the following topics:

Member of the USA-Egyptian project entitled: development of molecular markers linked to pest resistance genes in barley population, (2004-2006), funded by USA-Egypt partnership program, in collaboration with Kansas state university (KSU).

Member of Egyptian team and coordinator of multinational (Egypt, Algeria and Tunisia) NEPAD project entitled: Genetic improvement of nutritional quality and drought and salt tolerance of North Africa barely germplasm (2008-2010).

Member of project entitled: towards production of transgenic date palm resistant to notorious pests, (2003-2006), funded by international center for genetic engineering and biotechnology (ICGEB), UN.

Member of project entitled: *In vitro* propagation for some rare edicinal Plant species. (2005-2007)

List of Publications

Mai Allam, Manuela Nagel, Rod Snowdon, Wolfgang Friedt, Andreas Maurer, Klaus Pillen, Andreas Börner. QTL mapping for seed longevity in oilseed rape (*Brassica napus* L.). *Submitted to Theoretical and Applied Genetics (TAAG-D-15-00490).*

Börner A. S. Landjeva, M. Nagel, M.A. Rehman Arif, **M. Allam**, M. Agacka, T. Doroszewska, U.Lohwasser (2014) Plant genetic resources for food and agriculture (PGRFA)-Maintainance and research. Genetic and Plant Physiology, Volume 4: 13-21. Special issue (Part1)- Conference "Plant physiology and genetics-Achievments and challenges 24-26 September 2014, Sofia, Bulgaria

Jörg Schatzki, **Mai Allam**, Coretta Klöppel, Manuela Nagel, Andreas Börner and Christian Möllers (2013). Genetic variation for secondary seed dormancy and seed 1 longevity in a set of current European winter oilseed rape cultivars. Plant Breeding 132, 174–179

Mai Allam, Manuela Nagel, Rod Snowdon, Wolfgang Friedt & Andreas Börner (2012) Genetic studies on seed longevity of oilseed rape (*Brassica napus* L.) stored under different conditions Kurzfassungen der Beiträge zur Tagung der Arbeitsgemeinschaft Saatgut und Sortenwesen der Gesellschaft für Pflanzenbauwissenschaften und der Gesellschaft für Pflanzenzüchtung am 8. und 9. Mai 2012 in Osnabrück. Berichte Ges. Pflanzenbauwiss. 6, 43–47

Börner, Manuela Nagel, Nian Abdur Rehman Arif, **Mai Allam** and Urlike Lohwasser (2012). *Ex-situ* genebank collections –Important tools for plant genetics and breeding Andreas Plant breeding for future generations-19th EUCARPIA general congress. Budapest, Hungary ,2012.

M. Saker, M. El-Demerdash, **Mai A. Allam** (2011). *In vitro* propagation and genetic characterization as effective tools for conservation of Silene leucophylla, grown in St. Katherine Protected Area, Sinai, Egypt **Journal of Genetic Engineering and Biotechnology 9, 21–27.**

Mahmoud M. Saker, **Mai A. Allam**, Amina H. Goma and Abd El-Zaher M. H. (2007). Development of Suspension Culture System for In Vitro Propagation of Date Palm. J. Genetic Engineering & Biotechnology 5(1, 2): 51-56.

Mahmoud M. Saker, **Mai A. Allam**, Amina H. Goma and Abd El-Zaher, M.H (2007). Optimization of Some Factors Affecting Genetic Transformation of Semi-Dry Egyptian Date Palm Cultivar (Sewi) Using Particle Bombardment. J. Genetic Engineering & Biotechnology 5(1, 2): 57-62.

Saker, M.M., **Mai A. Allam**, Abd El Zaher, M. H. And Amina, H.G (2007). RAPD analysis of semi-dray Egyptian date palm during somatic embryogenesis. **1st Int. Egyptian-Jordanian conference on Biotechnology and Sustainable Development**: Current Status and Future Scenarios. Conference book, Plant Biotechnology:6, Pp. 92: 103.

Affirmation/ eidesstattliche Erklärung

I hereby declare that the submitted thesis has been completed by me, the undersigned, and that I have not used any other than permitted reference sources or materials or engaged any plagiarism. All the references and the other sources used in the presented work have been appropriately acknowledged in the work. I further declare that the work has not been previously submitted for the purpose of academic examination, either in its original or similar form, anywhere else.

I hereby declare that I have no criminal record and that no preliminary investigations are pending against me.

Hiermit erkläre ich, dass ich diese eingereichte Dissertation selbständig angefertigt und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Die den benutzten Hilfsmitteln wörtlich oder inhaltlich entnommenen Stellen habe ich unter Quellenangaben kenntlich gemacht. Die vorliegende Arbeit wurde in gleicher oder ähnlicher Form noch keiner anderen Institution oder Prüfungsbehörde vorgelegt.

Des Weiteren erkläre ich, dass ich weder vorbestraft bin noch dass gegen mich Ermittlungsverfahren anhängig sind.

Gatersleben, den .7.2015

[ ]
Mai A. Allam