# Wheat and barley Legacy for Breeding Improvement

## Final Report Summary - WHEALBI (Wheat and barley Legacy for Breeding Improvement)

**Executive Summary:**
WHEALBI's aims were to develop and implement tools, methods and procedures to facilitate the characterisation of wild relatives and local varieties of wheat and barley as sources of genes for use in crop improvement. WHEALBI was organised in 7 workpackages which major achievements are:

- **WP1:** A panel of 512 wheat and 512 barley genetic resources was selected from European Genebanks for deep genotypic and phenotypic characterisation in subsequent WPs of the project. All 1024 wheat and barley accessions are available as multiplied seed stocks from INRA (wheat) and from IPK-GGR (barley) and can be accessed through URGI's portal (https://wheat-urgi.versailles.inra.fr/Projects/WHEALBI).

- **WP2:** Using state of the art genomic technologies, over 600,000 and 500,000 robust genic based SNPs were identified in an assembled worldwide collection of 500 wheat and barley, respectively. Using this data, the genetic impact of domestication, selection and adaptation in both wheat and barley were explored. Importantly, this legacy dataset will provide a rich source of genetic diversity, within the broader research and breeding community to further our understanding and improve diverse traits, from environmental adaptation to disease resistance and nutrient use efficiency. Two manuscript describing exome variation and highlighting its consequences on adaptive traits have been written and submitted to peer review journals.

- **WP3:** This workpackage has completed all phenotyping analyses carried out to describe: the basic adaptive traits of the whole WHEALBI collections, the response to drought of spring wheat evaluated both in the field and in high throughput phenotyping platform; the relationship between canopy development and yield in barley; the response to Septoria, leaf rust and powdery mildew in wheat and to powdery mildew and P. teres in barley. The phenotyping activities for diseases resistance have identified many known and novel sources of resistance.
- WP4: The WHEALBI wheat and barley germplasms (WP1 data) were integrated in the information system. The evaluation of multi-trait and multi-environment models on WHEALBI data was carried out. Exome sequencing and phenotyping of large wheat and barley collections poses a number of challenges concerning storing, access and application to biological questions. WP4 developed databases and data exploration tools, allowing the scientific community to access the data generated by the WHEALBI consortium. WP4 provided a number of recommendations about imputation methods and statistical methodologies for genomic prediction.

- WP5: The promoters of 4 genes SPA, MCB1, MYBS3 and SHP were annotated using a tool developed by INRA and work focused on the SHP promoter for the yeast-one-hybrid experiments. The molecular diversity of agronomically important genes in barley and wheat germplasm across Europe has been studied. This has resulted in a comprehensive overview of the sequence diversity of genes involved in grain quality, frost tolerance, as well as resistance genes against several wheat and barley diseases. Whereas some loci revealed a large allelic diversity in the studied germplasm, other genes were identical or highly similar.

- WP6: The overall objective of this workpackage is to serve as inspiration for future use of the WHEALBI legacy. Modelling how best to use different approaches will be valuable in future pre-breeding projects, both academic and industrial. The objective of WP6 was to carry out and evaluate six different approaches to pre-breeding aimed at delivering novel genetic diversity and knowledge to commercial plant breeders. Lines have been selected for further evaluation as well as exploitation in commercial breeding programmes.

Project Context and Objectives:

To satisfy the demand of an expanding population, agriculture faces the challenge of delivering safer, high quality, and health-promoting food and feed in an economic, environmentally sensitive, and sustainable manner. A sustained effort is thus required to generate crops with higher and more stable yields across diverse and changing environments. Wheat and barley are key renewable resources and among the most important crops worldwide, particularly in Europe. Indeed EU (27) is the first for production and a major provider of world market for wheat, and by far first producer and exporter for barley, both for animal feed and malting purposes.

Wheat yields have been stagnating in the European Union (EU) since the mid-1990s (FAOSTAT2010). In a recent study in France, Brisson et al (2010) showed that this stagnation was mostly due to climate, i.e. increased frequency and severity of negative factors such as drought or high temperatures. These effects of climate are more severe in Southern European and are not expected to improve, whatever the climate scenario to occur (Ray et al 2012, Moore and Lobell 2015).

In addition, intensification of agricultural practices is associated with increased crop damages caused by pests and diseases. Chemical control has been increasingly used and Europe is currently the number one user of pesticides in the world, with cereal cultivation accounting for 40% of this consumption. This situation leads to high production costs, emergence of resistance to pesticides, and environmental and human health concerns. The most sustainable alternative to pesticides is the use of crop varieties that are genetically resistant to pathogens. Thus, genetics is one of the principal leverage for addressing the effects of both climate change, and societal demands for environmental sustainability and healthy products.

In order to flourish, breeding programmes in either large companies or small and medium size enterprises (SMEs) will need to select new varieties that are both adapted to changing environments and to more sustainable cropping systems while maintaining or improving yield. This implies more resilience to biotic and abiotic stresses, increased water and nitrogen use efficiency and higher stability of yield and grain quality. Agronomists and crop (eco)-physiologists must help define optimal combinations of traits, or so-called ideotypes. Crop management systems must also evolve to fit the improved properties of these new ideotypes. They will have to consider issues such as yield stability in adverse conditions, lower dependence on chemicals (fertilizers and pesticides), and maintenance of desirable grain composition under sub-optimal plant nutrition. Eco-physiology and gene (network) modelling will help define crop ideotypes for the future that are aligned to new crop management practices.

WHEALBI aims were to develop and implement tools, methods and procedures to facilitate the characterisation of wild relatives and local varieties of wheat and barley as sources of genes for use in crop improvement. It will explore the application of modern molecular, computational and analytical tools to provide understanding of the evolutionary processes that have shaped the current diversity in the gene pool and to predict exploitable value from unadapted germplasm. The project also aimed to develop innovative methods to optimise the use of these resources in pre-breeding and breeding programmes. New ideotypes were evaluated in innovative cropping systems under several climatic conditions. Particular attention being paid to the usefulness of the developed tools and knowledge and their transfer to stakeholders, particularly SMEs, breeders as well as present and future farmers. The WHEALBI project used a multidisciplinary approach, including genetics, genomics, ecophysiology, bioinformatics, biostatistics, agronomy and socio-economy to
improve European production of wheat and barley, through increasing productivity, robustness and adaptation to changing environmental conditions. The project challenge was to improve the efficiency of wheat and barley breeding programmes and design sustainable crop management systems adapted to new plant ideotypes.

Project Results:

WHEALBI was divided into seven research and technological workpackages, one workpackage for the dissemination and transfer of results and a workpackage dedicated to project management.

WP1 – Identifying circa 1000 germplasm accessions representing genetic diversity in European agriculture

WP1 objectives were to identify core subsets of wheat and barley germplasm, that provide 1) a fair balance of worldwide geographic and historical origins provide to allow a study of cereal evolution to be performed and 2) give priority to germplasm more suited to EU growing conditions, particularly by overrepresenting winter habit material. WP1 aimed to provide 1) high quality DNA of #500 barley and 500 wheat accessions for exome sequencing and repository; and 2) seeds of #500 barley and #500 wheat accessions for agronomic evaluation of key adaptive traits and to provide guidelines and protocols for optimal conservation and utilization of genetic resources. This workpackage was organized in 3 tasks and involved 11 partners. All five deliverables associated with WP1 were submitted.

Task 1.1: Provision of CORE and PARENT genetic resources

The targets and objectives of the task were to sample accessions in genetic resources collections and available varieties to achieve the best compromise between 1) representativeness of the world genetic diversity, 2) minimum adaptation to European growing conditions to carry out relevant field evaluation and 3) be suited to both evolutionary studies (i.e. good temporal/geographical representation) and pre-breeding objectives for adaptive traits: the panel of 512 barley and 512 wheat genotypes were compiled and passport data made available on the project webpage of URGI.

Task 1.2: Assemble and distribute approximately 1000 WHEALBI accessions, isolate DNA and establish biorepositories

Heuristic strategies were used to when identifying accessions representative of the worldwide diversity while providing sufficient adapted material for relevant evaluation in European condition. WHEALBI samples were largely taken from existing core collections at INRA and IPK and completed by partner's proposal with key varieties or breeding lines, parents of studied populations (such as MAGIC/NAM in WP6). Overall, seeds for DNA extraction and downstream activities were provided largely on time and DNA samples were extracted and were also provided to downstream WPs. Passport data for all accessions were imported into URGI/WHEALBI database at INRA. Legal issues for MTAs to be used for seed distribution were solved except for about 40 wheat and barley accessions. Passport data of all 512 WHEALBI wheat accessions were submitted into GnpIS database (SIREGAL).

Within WP3, all material was multiplied and stored at INRA GDEC genebank (Wheat) and IPK group GGR (belonging to Genbank 6 department), respectively. Passport information of all material has been forwarded and stored at URGI.

Task 1.3: Development of guidelines and protocols for Next Generation Valorization and Utilization of PGRFA collections

The results of the task were drafted into a detailed deliverable report, which was substantiated by developing case studies for the individual aspects covered in the report. As an example, one case study addressed the aspect of how existing datasets (passport) data of genebank accessions can be data-mined and connected to other public datasets (e.g. climate data) and then linked to project specific phenotypic and genotypic datasets.

WP2 – Identify the nature and extent of gene sequence diversity in the wheat and barley genepool

WP2 objectives were to provide raw sequence data (NGS) of #500 barley and 500 wheat for storing in WHEALBI database and to provide an extensive catalogue of exome-DNA polymorphisms of #500 barley and #500 wheat accessions. This workpackage was organised in 3 tasks and involved 6 partners. All three deliverables associated with WP2 were submitted.

Task 2.1: Exome capture and resequencing of approximately 1000 wheat and barley accessions selected in WP1

Exome captures for both barley and wheat were conducted at PTP and Earlham Institute (formally TGAC) respectively. All 512 accessions chosen in WP1, for both wheat and barley were successfully captured, sequenced and variants called, providing data for
WHEALBI and legacy data for future use.

Exome sequencing for barley and wheat accessions generated an average of 40 million and 37 million read pairs / sample, respectively.

Task 2.2: QC sequences, deconvolute and establish robust pipelines for calling sequence variants

Pipelines for both crops were developed within this task and used to filter, map and identify SNPs.

Barley: Following QC correctly paired reads longer than 50 bp were used for further processing, this resulted in a total of 24 million reads mapped to the reference genome and BAM files generated for variant analysis from 510 accession (2 accessions with less than 50000 reads were removed). All BAM files were processed together using GATK, from 510 accessions, 67 accessions with an excess of duplicates were removed from the variant calling due to technical issues. Genotype calls were validated in collaboration with IPK and JHI using genotyping by sequencing (GbS) a custom 384 SNP chip (BeadExpress GoldenGate assay, Illumina), respectively. From both of these we were able to verify 403 accessions (40 accessions failed quality). From these at total of 64,523,315 variants were extracted. A set of filters were then applied to the data and resulted in 449,237 robust SNPs. A dedicated exchange server has been setup at PTP for downloading the raw and filtered variants. The server can be accessed from any SFTP client (e.g. FileZilla is a good option). Instructions for all partners to access the data were posted on the collaborative platform.

A greater number of robust SNPs were identified. Original filtering was too stringent and many important SNPs were lost (449,237 SNPs reported Month 30). The improved filtering has resulted in over 2 million robust SNPs.

Wheat: Following QC, from the original 512 accessions, 10 failed sequencing and capture with low read counts. The total number of reads mapped to the reference was over 20.5 million post trimming. Variants were called using FreeBayes software v0.9.20 (https://github.com/ekg/freebayes) rather than GATK as used with the barley data because of hexaploid nature of wheat. SNPs were annotated using IWGSC v2.2 assembly with 99,386 genes. Validation was carried out using Axiom 820K and iSelect 80K SNP arrays (details from CerealsDB, http://www.cerealsdb.uk.net/) although only a small number of accessions were in common between these and the exome capture (38 accessions). Despite this, HMGU were able to determine a false discovery rate of only 3.29%. The data is accessible for partners via ftp maintained at HMGU. Overall, from the 43,932 captured gene models, 6,800,653 SNPs have been identified for the 502 successfully sequenced genotypes.

The new variant calls were imputed to reduce the amount of missing values and then post-processed through the same filtering steps established in the previous runs, yielding a final variant set of 620k SNPs and small InDels. The quality of this set was evaluated by comparison of genotype information to publicly available results from SNP array-based genotyping experiments. This evaluation suggested a very low false discovery rate (FDR) below 2%. The variation data was complemented with presence/absence analyses. The detected SNPs and Indels were then used to analyse the phylogenetic relationship between samples and to assess the spatio-temporal change in diversity between wheat accessions. The final variation dataset has been further analysed in close cooperation with the group of Jerome Salse (INRA)(Task 2.3).

Task 2.3: Genome wide analysis of molecular variation

Barley: From these various analyses we were able to identify regions of reduced diversity and recombination along barley chromosomes. Genetic structuring of populations and differentiation between groups were observed using various population genetics approaches. By examining the relationship between inter-individual genetic identities of pairs of accessions and geographic distances a significant overall reduction in genetic identity with geographic distance was observed. We developed methods that revealed associations between genetic diversity and environmental variables in our dataset using bioclimatic variables from WorldClim database. We applied haplotype analysis to specific genes (flowering-related genes) and strong geographical structuring of haplotypes.

Diversity analysis in CORE barley germplasm 2,125,873 SNPs were identified and removing minor alleles (frequency less than 5%) resulted in a working set of 449,237 SNPs, distributed across each of the 7 chromosomes. Diversity was examined at several levels, overall chromosomes, within chromosomes and across all accessions, between wild and cultivated, within cultivated and between row types.

1. Overall the diversity across chromosomes was similar, with a mean value ranging from 0.272(6H) to 0.286 (3H).
2. Diversity, calculated using a rolling average, varied along each chromosome. Different chromosomes exhibit different patterns, with increased diversity towards the telomeric ends of the chromosomes.
3. The genetic relationship between individual accessions was explored using a pairwise similarity and cluster analysis and a subset of randomly chosen SNPs (1,000 SNPs from each of the 7 chromosomes). The resultant dendrogram highlights the distinctiveness of the
Exploring local adaptation using CORE germplasm collection and flowering time data from spring sown common garden experiments.

As part of Task 2.3 we have attempted to use the data generated to test whether landraces are actually adapted to their local production systems. This issue is particularly important for designing crop breeding strategies that respond appropriately to anthropogenic climate change. We collected geographical, environmental and phenotypic data for 170 accessions, of spring and winter barley that were included in six WHEALBI field trials undertaken at four locations (MTA-ATK, Hungary, spring and winter planting; CREA, Italy, winter planting; CU, Turkey, winter planting; JHI, Scotland, spring and winter planting). We represented the data graphically by plotting heading date against latitudinal distance for each trial site. The hypothesis is that the ideotype for each site is at zero and deviations from this are associated with geographical distance i.e. less well adapted. As expected for spring landraces for which we had geographical co-ordinates, evidence of local adaptation for the spring trials was observed, with those from the similar geography showing optimal heading i.e. zero and those non-adapted accessions at greater latitudinal distances heading earlier.

In the case of formally bred cultivars, using mid-point locations, linear regression of heading date against latitudinal distances for each of the three groups of barley accessions (spring-type landraces, spring-type formally-bred cultivars and winter-type formally bred-cultivars) was performed. There was an indication of adaptation for the spring landraces in the spring trials, but in the case of spring-type formally-bred cultivars in spring-planted trials, evidence for adaptation based on latitudinal proximity appeared very weak or absent. In the case of the formally-bred winter material in the winter-planted trials, there was no indication of adaption by latitudinal proximity. Our analysis supports the conventional (but frequently unproven) wisdom of local adaption in landrace material, and that this adaptation is greater than that found in formally-bred material. Our data therefore provide support for the utility of landrace material in responding to anthropogenic climate change challenges.

Besides the report, 371 cultivated barley were used to write a manuscript about the genetic basis of adaptation in barley. This manuscript uses exome capture on the crop to allow more detailed study of adaptation. This work involved sequencing of a wide collection of carefully chosen barley materials that included an extensive range of cultivated germplasm, coupled with multi-environment (location and season) field phenotyping. This research represents the first for which both detailed exome and multi-site, cross-season phenotypic data for several basic traits, including flowering time, 1,000 grain weight, plant height and awn length, has become available for a broad range of cultivated barley types and therefore provides new opportunities for analysis. In our analysis we included both landraces and formally bred (i.e. breeder-developed) cultivar materials, were of 2- and 6-row barley types, and were of spring and winter growth habits. The spring and winter habits of different barley varieties, with their different responses to cold, heat and light, make barley a particularly interesting crop for exploring environmentally-adaptive responses to climate, and this has provided data to explore issues of crop adaptation and provided an in-depth case study of the multi-genic control of flowering in domesticated barley.

Specific outcomes:
- Exome sequences reveal substantial variation in the barley collection and significant sub-population structure in barley corresponds with origins, histories and growth habit
- Chromosome-level genetic differentiation and linkage disequilibrium analysis reveal strong differentiation by barley category and highly variable linkage decay.
- Field trial data and adaptation analysis reveal significant adaptation-based phenotypic variation in barley, especially in landraces
- Multi-environment genome wide association scans support the power of barley germplasm panels and cross-site field phenotyping for trait genetic analysis
- Dissecting adaptation and the genetic control of days to heading confirms the importance of known flowering-associated genes in barley
- Adaptation and latitudinal distribution of haplotypes at key flowering-associated genes indicate the importance of sampling contexts and barley category in understanding adaptive responses

Wheat: As with the barley data, methods and software for genome wide evolutionary analyses has been tested using published wheat data from INRA. As the data has been distributed for wheat, these approaches are now being used by INRA and HMGU. Diversity in wheat has been examined at the ploidy, genome and gene level. Using the approaches developed at INRA.
(i) At the polyploidy level, difference in SNP density and frequency has been characterized between 15 diploids, 49 tetraploids, 439 hexaploids.

(ii) At the genome level, difference in SNP density and frequency has been characterized between A, B and D subgenome of the hexaploid bread wheat genotypes (439 lines) using various fixation and selective indices.

(iii) At the gene level, for genes driving key phenotypic traits, diversity across the genepool has been described identifying changes at candidate genes involved either in domestication or in the subsequent improvement process.

Explore trends in the frequency and location of sequence-predicted functional variants

Over 620,000 small-scale variant positions targeting 41,032 genes were identified from exome capture sequencing of 487 wheat accessions (after discarding accessions with too many missing Data). The variants comprised 56,163 Indels (9%) and 563,995 SNPs (91%). Contrasts in normalized SNP density (average number of SNP per kbp) as well as allelic diversity (He index) were observed at the subgenomic level (lowest diversity D genome, greatest for B genome) and within gene compartments (comparing introns, exons, UTR and intergenic sequences).

At the spatial/temporal levels, phylogenetic inferences show clear accession grouping according to geographical origin (America, Western Europe, Eastern Europe, Fertile Crescent and Asia), with a clear separation between accessions from the Asian and European/American gene pool.

In comparing landraces and modern varieties, we identified genomic regions showing domestication (including domestication genes such as Btr, Tg, Q genes conferring respectively a brittle rachis, tenacious glume, and a non-free-threshing character) and breeding (including Ppd, VRN, Gi, CO, CUL, Glutenin and Gliadin genes) signatures.

Phenotypic data (WP3) from four locations across Europe (UK, France, Hungary and Turkey) provided the opportunity to link the allelic variation observed at 390,657 SNPs and InDels to such phenotypes through multi-environment genome-wide association studies (GWAS) for heading date (yielding 48 major peaks including Ppd, VNR, FDL, WPCL genes) and plant height (with 40 significant associations including Rht-1 genes on chromosomes 4A, 4B and 4D). Overall, our dataset represents a comprehensive wheat structural variant resource for deciphering the genomic selection signatures of modern wheats as well as for the improvement of elite bread wheat cultivars.

The manuscript explores the impact of 10,000 years of hybridization, selection, adaptation and plant breeding that underlies the genetic makeup of modern bread wheats. Exome sequencing revealed large genetic variations at the genic, chromosomal and subgenomic levels in a worldwide panel of almost 500 accessions from all across the geographical ranges of the wheat species complex. JHI conducted genome-wide analyses of differentiation within our wheat population which unveiled its origin and expansion, and selected genes since its domestication. Data supports a reconciled model of wheat evolution and provides novel avenues for future breeding improvement. The manuscript was accepted for publication in Nature Genetics, Feb 15th, 2019).

WP3 – Phenotypic exploitation of the WHEALBI germplasm collection

WP3 objectives were to phenotype both legacy collections (#500 barley and #500 wheat accession) in a series of field trials in 6 location across an environmental gradient from Scotland to Turkey; to evaluate a range of different traits using precision phenotyping on different subsets of barley and wheat accessions including drought tolerance, canopy development and resistance to Blumeria graminis f.sp. hordei (barley powdery mildew), net blotch (P. teres) and Bipolaris sorokiniana in barley and resistance to powdery mildew, Fusarium head blight (FHB) and Staganospora nodorum leaf blotch in wheat. This workpackage was organized in 4 tasks and involved 9 partners. All four deliverables associated with WP3 were submitted.

Task 3.1: Standard phenotyping of basic adaptive traits (common garden trial)

This activity has been accomplished through the organization of a network of field trials (common garden experiments) across different latitudes (from Scotland to Turkey) and climatic conditions (from wet to extreme dry, from sea level to 1200 meters), where all the WHEALBI accessions have been tested. Overall, 10 common garden experiments have been successfully carried out for barley and 8 for wheat (Figure 9). The plants were grown in the fields according to the local environmental conditions as expected. The plants were grown in the fields according to the local environmental conditions as expected. All data were collected using a standard file format (Ephesis, prepared by INRA-URGI) that allowed storage of the data in the WHEALBI database and further analysis. The analysis of the data (adjusted means and basic statistical analysis i.e. boxplots, AMMI, mixed models) were carried out in conjunction with WP4. The phenotypic data acquired highlight the wide genetic diversity present in the WHEALBI germplasm, suggesting that different genetic factors are responsible for the adaptation of each genotype to specific environmental conditions. These data were analysed together with the molecular data generated by exome capture and resequencing in WP2 to identify the genes responsible of specific adaptive traits.
The management of a large set of germplasm, its multiplication and distribution to a large network of field trials as well as the scoring of the phenotypic data carried out by different researchers in different locations, the harvesting and threshing thousands of plots are all activities that are subject to human error (most frequent is the mix up of samples). Since these errors can significantly impact the quality of the data, the WHEALBI steering committee decided to verify the phenotypic data through a repatriation experiments carried out in the growing season 2016-2017. A sample of seed from each single plot of all WHEALBI trials were collected and sown in a single location with the same accessions form different locations grown side by side for direct comparisons. The results of the repatriation have highlighted an average of 2-3% of errors in most field trials, with the exception of two locations where the errors were above 10%. This information was used to curate the phenotypic data, and unreliable phenotypic data was removed from the analysis and subsequent publication.

Phenological traits from this network field trials have been used as proof of concept for genome wide analyse using the exome variants in the barley and wheat manuscripts described in WP2.

Task 3.2: Precision phenotyping of drought tolerance related traits

This task provided a detailed phenotypic evaluation of drought tolerance screen-house and open field condition in Israel for more than 250 accessions of wheat (Figure 10), as well as an extremely detailed phenotypic characterization in Germany of about 130 accessions exposed to drought stress in both vegetative and grain filling stage using a precision high through put phenotyping facility (Figure 11). The field evaluation of drought response was carried out in two years with a different design. Data collected and analysed included grain yield, number of fertile spikes, spike weight, grain weight per spike, number of grains per spike, thousand grain weight, fruiting efficiency, days to heading, plant height, peduncle length, peduncle extrusion, spike length, flag leaf length, width and area, specific leaf weight, osmotic potential, osmotic adjustment, leaf rolling and glaucesness. Thermal and RGB aerial photographs were used to calculate canopy temperature, crop water stress index, percentage of ground cover, green-red vegetative index. A wide phenotypic distribution was found for each of the measured traits in both experiment and treatments. Average grain yield (across all genotypes) under water-limited treatment was reduced relative to the well-watered control by ~50% and ~30%, in the in first and second years, respectively. The genotypes tested exhibited a wide range of genetic diversity and a wide range of phenotypic diversity in each of the experiments conducted. Concerning the results of the precision phenotyping experiments, vegetative drought affected and reduced plant grain yield, mainly by reduced seed set. During stress, biomass formation slowed down, stopped and led to wilting systems. Moreover, photosynthesis was negatively affected but could recover up to the level of control treatment within one week after re-watering. High broad sense heritability estimates were observed indicating the suitability of current panel for GWAS with >450,000 SNPs from exome capture. 88 QTL regions spread over 17 chromosomes were significantly associated to at least one measured physiological trait. Stage and drought specific QTL were detected for all traits, including the physiological traits. The panel of 130 spring wheat lines consists of very old landraces (1926-1951), historic cultivars (until 1950ies), more recent cultivars (1960ies to 90ies), breeding material and current varieties (year of release 2000-2010). Beside the well-known trends for plant height and harvest index, in both drought setups the current varieties outperformed the landraces in the most significant yield parameters (grain yield per plant, grain number per year).

Task 3.3: Precision phenotyping of canopy development

The detailed characterization of plant canopy development along the whole plant life cycle was completed for two growing seasons. Starting from December 2015, canopy development was characterised through several campaigns during which digital photos were taken from above and analysed for green excess, canopy spectral reflectance, height measurements and scoring of lodging and diseases. Highly significant differences between accessions for all studied traits and large spans in the trait values demonstrated the high diversity of the barley accessions tested. High between year correlations and broad sense heritabilities suggest that the data set is a valuable resource for GWAS and allele mining approaches. The data set allows for the study of the impacts of prostrate or erect growth habit in the vegetative growth phase, of lodging sensitivity and tolerance to powdery mildew. Significant between accession differences resulted for canopy cover and NDVI at the level of whole crop cycle integrals as well as instantaneously. These indices for canopy development correlated with grain yield and yield components, relationships that will be further investigated with multivariate analyses. Relationships among traits will be studied with path analyses also taking into account meteorological indices in the diverse developmental phases.

The experimental trial was visited in April 2016 by the participants of the Training Course on “Field Phenotyping” and by WHEALBI partners during the project annual meeting. In addition, frost tolerance tests were done with plants in first leaf stage grown in trays within growth cabinets, cold hardened and leaves harvested in the canopy development field experiment. The plants and leaves were exposed to freezing stress and the damage assessed with measurements of chlorophyll fluorescence.
Task 3.4: Precision phenotyping for disease response

Several protocols/technologies for disease analysis were designed. FHB/wheat. The first field experiment resulted in a total of 3,048 pictures have been taken to score for diseases after artificial infection. These were automatically analysed with new algorithms developed by INRA and collaborators to determine disease severities for each accession.

Brown rust/wheat. To identify resistance genes for breeding a step-wise approach was adopted to evaluate the panel. At each step, only resistant accessions were retained and tested in the next step with a broader array of isolates. 454 accessions were evaluated with 4 isolates and 318 resistant accessions were identified.

Septoria leaf blotch/wheat. 454 accessions were evaluated with the reference isolate IPO-09415 and 120 accessions showed resistance. The 120 accessions were then tested with three additional French isolates carrying different virulences and 30 accessions have already been retained for further evaluations (Figure 12). In addition, novel phenotyping methodologies have developed for septoria allowing the quantification of the pycnidia number by image analysis and of the sporulation capacity with a particle analyser.

As an alternative screening strategy, the same wheat panel was evaluated using the Septoria Mexican isolate IPO90006, which is a master differentiator as it is virulent for Stb6, Stb7 and Stb9 (the former gene is very prevalent in wheat germplasm and frequently co-occurs with other Stb genes) and the isolate IPO87016 (Uruguay, virulent on Stb4+Stb6+Stb13+Stb14).

Powdery mildew/wheat. 344 wheat accessions were found susceptible to at least one of the four isolates employed, while 27 were resistant to all isolates. More Bgt isolates were further used in order to discover additional sources of resistance.

Powdery mildew/barley. The results from inoculation with D353 showed a good phenotypic spread ranging from immune to extremely susceptible (>80% leaf area covered by powdery mildew pustules). Approximately 25% of the accessions exhibited a quantitative resistance ranging from 5% to 30% of infected leaf area. This group carrying potentially more durable forms of resistance is not specific to a particular group of accessions (wild, landraces, breeding lines, cultivars).

Yellow rust/wheat. The field phenotyping analysis has highlighted a full range of response from completely resistant to fully susceptible genotypes.

In all disease/host combinations the screening highlighted the considerable variation, identifying both resistance and susceptible phenotypes, which provided WP4 and WP5 data for GWAS. The results of this task have identified new sources of resistance against all diseases tested. Many of the associations identified have not been previously reported, thus representing novel sources of resistance. These new sources of resistance will be subject to more detailed investigation to clone the corresponding genes, which can subsequently be used in breeding programmes.

Net blotch/barley. A panel of 6-row barley accessions part of the Whealbi barley collection were screened for resistance to net blotch through artificial inoculation with a field isolate (from CREA) of Pyrenophora teres. A preliminary GWA scan using exome capture data showed significant associations on chromosomes 4HS and 5HL.

WP4 – Data integration and analysis tools

WP4 objectives were to provide a data management system for sequence and evaluation data of # 500 wheat accessions; to provide a data management system for sequence and evaluation data of # 500 barley accessions; to provide an informatics pipeline for SNP detection and missing data imputation from NGS data and to provide informatics tools for multivariate approaches and genomic prediction across multiple environments. This workpackage was organized in 3 tasks and involved 6 partners. All three deliverables associated with WP4 were submitted.

Task 4.1: Data storage and interoperability

INRA-URGI completed the integration of the WHEALBI wheat and barley germplasm and phenotyping data in the information system (GnpIS database). A web-page is available with the links to the WHEALBI data (Figure 13):
https://wheaturgi.versailles.inra.fr/Projects/Whealbi.

These data are:
- 509 wheat accessions (collaboration with INRA-GDEC), including passport data, photos, geolocation, WHEALBI identifiers.
- 511 barley accessions (collaboration with IPK).
- A trait ontology (WIPO) developed in the frame of the BreedWheat project and updated for the needs of WHEALBI. This ontology have been used to generate the phenotyping data.
- Wheat and barley phenotyping data (collaboration with CREA): 12 trials on 6 sites.
HMGU: Extending on the previously reported platform for accessing the variation data on the web, the platform has been modified to accommodate the latest wheat reference sequence and variation data. A search function now allows researchers to rapidly access variation data at regions of interest and the web-enabled genome browser provides a comprehensive view of the variation data in the context of the reference sequence and the gene annotation. Additional information tracks can be easily added by an administrator and also (temporarily) by the user. The web interface further allows the retrieval of detailed variant information for single variants, e.g. concrete genotype of the currently displayed sample, allele frequencies and nucleotide diversity across the whole panel. The data access can be restricted to prevent the dissemination of unpublished data to the public. In that case, access details can be requested from the contact given on the website https://pgsb.helmholtz-muenchen.de/whealbi. All available data has also been integrated into the URGI repository and can be accessed from there. The technological base supporting the data access platform was completely replaced by a distributable AWS S3-compatible storage tier which enables rapid access and display of large data files. This storage system was evaluated and set-up for the purpose of this platform which now allows the use of 3rd party open-source tools for displaying comprehensive genomic information and associated geographic maps. Changes at the API layer however required a full reimplementation of the data access layer to enable the use of S3-compatible storage and data had to be converted into appropriately indexed, binary formats. Further, sample meta data was also cleaned and converted into the required format and search indices were built to support the search function introduced above. Additionally, a hands-on training of variant calling was prepared and executed in Wageningen which used a sub-sampling of the data generated in Whealbi to teach an actionable work-flow of variant detection from reference and raw sequence data to variation data. The goal of the class, each attendant completing the full process of variant calling, was reached.

Task 4.2: Efficient approaches to impute and utilize sequence variation data for prediction and mapping of complex traits

In a report submitted at M54, WU explained why and in which situations imputation strategies might be a useful tool to reduce the genotyping costs, described a number of commonly used imputation methods, compared results of imputation methods, and provided recommendations about the convenience of imputation strategies. Two case studies are included; one in wheat and one in maize. The wheat example consisted of a diversity panel that was imputed with Beagle 4 and Flmpute and evaluates the effect of the composition of the set of reference individuals. The main conclusion is that methods considering the genetic similarity (sample uniformly from the genetic space) when choosing the reference set outperform methods that do not (e.g. random). The maize example considers a Nested Association Mapping population and compares Beagle and magicImpute. When markers are missing at random, Beagle performed very well as long as the missing fraction ≤0.8 and it broke down at the high missing fraction 0.95. Markers missing for genotypes characterized at low density, were poorly imputed by Beagle. In contrast, magicImpute showed a high accuracy for this scenario. This shows that Beagle might not be the best method for imputation when the aim is to reduce cost by imputing progeny that is characterized at lower density than the parents. Imputed exome SNP were used, jointly with evaluation data from WP3, to explore relationships between genome variation and trait adaptation. Figure illustrates an example of association statistics between haplotypes at key-genes controlling the circadian clock and days to heading in the WHEALBI barley collection. A manuscript will be accepted for publication in The Plant Journal, after minor revisions.

Task 4.3: Multivariate approaches for genomic prediction across multiple environments

The work was performed in collaboration with the other WPs and consisted of two reports (one describing genomic prediction models and one applying these models to a wheat data set) and one paper about multi-environment analysis of barley adaptation. Multivariate genomic prediction can have a number of applications and prediction objectives. Common applications are the prediction of multiple traits in a single environment, or the prediction of a single trait across multiple environments. Prediction objectives can consider prediction of unobserved genotypes in observed environments, prediction of observed genotypes in unobserved environments, or (more challenging) prediction of unobserved genotypes in unobserved environments. WU described different models for multivariate genomic prediction, emphasizing the differences between univariate and multivariate cases. Firstly, the report concerning D4.2 describes when multivariate prediction is expected to be advantageous, compared to univariate prediction. Then, it continues with a description of mixed models for multivariate prediction. These models can consider a fixed part for genotypic sensitivities to the environment and a random part to model the polygenic effects. Finally, the report illustrates different model classes with examples from the literature and with applications to the Whealbi wheat diversity panel. WU evaluated and developed an extensive set of genomic prediction models that include terms for QTLs, polygenic effects, covariables and combinations of these three types of model terms. These modelling approaches are formulated within a general mixed model framework for prediction of multiple traits in multiple environments where the models contain

Wheat SNPs (collaboration with HMGU) are available on the official wheat reference sequence browser (WGSC RefSeq v1.0).
networks obtained by stage gave a first list with 130 putative interactor genes belonging to 36 TF families. Twenty-nine interactors were revealed that 1,159 TF genes are expressed in the grain of T. monococcum. In the following, we focussed on 580 of these TFs. The first analysis concerning the integration of all the RNAseq data are described in a manuscript that was submitted. A blast analysis were built using the RulNet platform for network inference (http://rulnet.isima.fr). The results of the

WP5 objectives were to provide a list of validated candidate genes regulating Seed Storage Protein genes in response to nutrient supply; to provide a list of haplotypes at the key adaptive and frost and drought tolerance genes in barley; to provide a list of haplotypes at five candidate genes targeted by barley domestication and improvement; to provide a list of haplotypes constructed within the exomes. Exploration of circadian clock-associated genes related to days to heading revealed complexities in GxE effect directions, and the importance of latitudinally-based genomic context in the expression of large effect alleles. Analysis provides practical information for future crop breeding. This manuscript was accepted in The Plant Journal (5fth of March 2019), provided that minor revisions are done.

WP5 – Allele and pathway mining for adaptive traits and grain quality

A tool dedicated to in-silico annotation of cis-regulatory motifs in promoters was developed. The in silico footprinting made in this preliminary work proved to be useful to select the most relevant cismotifs for functional validation. Results of the transient expression in wheat developing endosperm experiments suggested that ROC8 and TaHdZip transcription factors might repress the SHP promoter activity. These genes are thus good targets for allele mining of key players of the storage protein regulatory network.

The experimental work was laborious and despite our efforts, the list of identified regulatory proteins remained short. To detect more candidates, we developed a strategy based on a regulatory network derived from RNAseq data of grains at different developmental stages of a wheat model species (the diploid einkorn, Triticum monococcum), grown with different N and S supply. All the networks were built using the RulNet platform for network inference (http://rulnet.isima.fr). The results of the first analysis concerning the integration of all the RNAseq data are described in a manuscript that was submitted. A blast analysis revealed that 1,159 TF genes are expressed in the grain of T. monococum. In the following, we focussed on 580 of these TFs. The networks obtained by stage gave a first list with 130 putative interactor genes belonging to 36 TF families. Twenty-nine interactors were
gene Pm2 is not functional in cv. Chinese Spring. There is no complete gene in the reference genome annotation and the gene was also chromosome 1A in the Pm3 gene. 61 accessions show variants to known Pm3 alleles at 56 positions. The powdery mildew resistance (Snn1) and an ortholog of a rice durable resistance gene (Xa21- like) was performed. 122 variants in the vcf le are reported on.

At UZH, allele mining for the wheat accessions at two powdery mildew resistance loci (Pm2, Pm3), a Stagonospora resistance gene haplotypes per gene. As a trend, minor haplotypes were correlated with enhanced resistance. All SNPs per candidate gene exhibiting a –log(10) p value of >3 were used to calculate gene haplotypes. This resulted in up to 5 QTL containing 1 or more significantly associated candidate genes were identified. This resulted in 27 candidate genes (Figure 18).

Associations were found for all traits. Race-nonspecific association peaks were located on chromosomes 2H, 3H, 4H and 5H. A total of Associations were calculated in R by using a mixed linear model (Trait + Subpopulation +SNP +Kinship). Several significant associations were found for all traits. Race-nonspecific association peaks were located on chromosomes 2H, 3H, 4H and 5H. A total of 10 QTL containing 1 or more significantly associated candidate genes were identified. This resulted in 27 candidate genes (Figure 18). All SNPs per candidate gene exhibiting a −log(10) p value of >3 were used to calculate gene haplotypes. This resulted in up to 5 haplotypes per gene. As a trend, minor haplotypes were correlated with enhanced resistance.

At UZH, allele mining for the wheat accessions at two powdery mildew resistance loci (Pm2, Pm3), a Stagonospora resistance gene (Sn1) and an ortholog of a rice durable resistance gene (Xa21- like) was performed. 122 variants in the vcf file are reported on chromosome 1A in the Pm3 gene. 61 accessions show variants to known Pm3 alleles at 56 positions. The powdery mildew resistance gene Pm2 is not functional in cv. Chinese Spring. There is no complete gene in the reference genome annotation and the gene was also

Eighteen candidate TFs were associated at P value<0.001 with at least one trait concerning SSP composition. However, associations found have to be considered with caution and need to be confirmed using larger samples. Still, some of them were found in several locations and for several related traits. This suggests a certain robustness. In addition, Y1H (Yeast one-hybrids) and statistical approaches indicated that TFs of the C3H family likely play a role in the expression of SSP.

Task 5.2: Allele mining at key genes for adaptive traits

At the barley frost tolerance locus, 41 alleles, carrying deletions from 35bp to 6.4kb were identified. This information was exploited to select a core collection of about 80 barley accessions representing the total genetic diversity of CBFs and Vrn-H1 unveiled in the WheatBI panel. Candidate genes involved in several traits for mining of novel alleles in bread wheat and wild emmer wheat were selected. The resulted candidate LD-islands were compared with positions of genes showing different expression in wet and dry conditions.

For drought stress resistance in wheat, in silico analysis enabled determining "macro-haplotypes" of SNP highly associated with traits related to drought resistance (Figure 17). Three 1Mbp segments (on 3A, 4B and 6A) were identified as a primary target for future genetic dissection. A secondary target set for future genetic dissection included five 1Mbp intervals (on 1A, 3B, 4D, 5B and 7D). By combining GWAS with information on QTL and differential transcription between drought vs. well-watered conditions three 1Mbp segments (on 3A, 4B and 6A) were revealed as a primary target for future genetic dissection based on focused mining of novel alleles in a T. dicoccoides collection that displayed high variation for drought tolerance. The suggested secondary target set includes five 1Mbp intervals (on 1A, 3B, 4D, 5B and 7D).

In barley, the Frost resistance-H1 (Fr-H1) and Frost resistance-H2 (Fr-H2) loci explain most of the phenotypic variation for frost tolerance, and the responsible candidate genes are known. A total of 27 alleles were detected at the HvBM5a gene for Fr-H1/Vrn-H1, carrying deletions from 25 bp to 8.9 Kb in the regulatory regions of promoter or first intron. With respect to plants carrying a true winter allele, some of the new alleles detected showed a reduced vernalization requirement, with an anticipation of heading after two weeks only of low vernalizing temperatures. Concerning the cluster of CBF genes mapping at Fr-H2, 57 SNPs were identified on six CBF genes, with preliminary results suggesting a significant association between a haplotype at HvCBF14 and the frost resistance phenotype (measured through the physiological parameter Fv/Fm). On the contrary, no differences were observed for genotypes carrying different paralogs/paralog combinations at HvCBF2, identified by looking at heterozygous calls from exome data. Finally, copy number variation at the tandemly duplicated genes HvCBF2A-HvCBF4B was observed by counting the number of sequencing reads mapping at the locus, with lines showing up to a 10X increase of read depth with respect to the reference Morex.

Also in barley, five gene regions were mined using the exome capture data, including brittle rachis, row type, grain characteristics (hulless) and photoperiod genes, with varying degrees of success, depending on annotation and whether the gene was present on the original 2012 genome assembly (IBGSC, 2012 Nature 491:711-716) and the subsequent capture array (Mascher et al., 2013 Plant Journal 76: 494-505). For the brittle rachis gene that was recently identified after the exome capture was designed, the actual gene was not present on the capture, although genes in the region that may be the correct one are used as examples in this study. In contrast, the photoperiod gene on 2H is well represented on the capture array. After filtering for missing data SNPs, haplotypes were constructed for each gene and ranged from 4 to 26.

Task 5.3: Allele-mining for biotic stress resistance genes

At IPK, the phenotypic data of powdery mildew infection from task 3.4 were used for a GWAS analysis in barley, using 449,585 high confidence SNP provided by WHEALBI. GWAS was calculated for three traits:

a) Infection (% of diseased leaf surface) by Bgh isolate D35/3
b) Infection (% of diseased leaf surface) by Bgh isolate Ri III
c) Infection (% of diseased leaf surface) by either Bgh isolate producing higher values

Associations were calculated in R by using a mixed linear model (Trait + Subpopulation +SNP +Kinship). Several significant associations were found for all traits. Race-nonspecific association peaks were located on chromosomes 2H, 3H, 4H and 5H. A total of 10 QTL containing 1 or more significantly associated candidate genes were identified. This resulted in 27 candidate genes (Figure 18). All SNPs per candidate gene exhibiting a −log(10) p value of >3 were used to calculate gene haplotypes. This resulted in up to 5 haplotypes per gene. As a trend, minor haplotypes were correlated with enhanced resistance.

At UZH, allele mining for the wheat accessions at two powdery mildew resistance loci (Pm2, Pm3), a Stagonospora resistance gene (Sn1) and an ortholog of a rice durable resistance gene (Xa21- like) was performed. 122 variants in the vcf file are reported on chromosome 1A in the Pm3 gene. 61 accessions show variants to known Pm3 alleles at 56 positions. The powdery mildew resistance gene Pm2 is not functional in cv. Chinese Spring. There is no complete gene in the reference genome annotation and the gene was also...
not complete on the sequence capture array. Therefore, we considered only a truncated version of the Pm2 gene. 442 accessions with the D sub-genome were considered. In total 66 variants were found in the Pm2 gene for those accessions. For example, 258 accessions gained a stop codon at position 43408812 (177 accessions have missing data). In total, we identified 52 accessions that probably carry Pm2. Two accessions could carry new Pm2 alleles, both are from A. tauschii (WW-247 and WW-248). The remaining 50 accessions do not show further variations compared to resistant Pm2 and can therefore not be considered as new Pm2 alleles. Snn1 is located on chr1B. 447 accessions with the B sub-genome were considered. In total 4 variants (one intron variant and three synonymous variants) can be found in the Snn1 region for those accessions. According to the literature, there are two homologs of the rice resistance gene Xa21 in wheat, Xa21-like1 and Xa21-like2. For Xa21-like2, there were no variants reported in the WHEALBI vcf. 442 accessions with the D sub-genome were considered. In total 2 variants (a synonymous variant in accessions WW-097 and WW-307 and a non-synonymous variant in accession WW-242) can be found in the Xa21-like1 region for those accessions.

Yr15 is a wheat gene against the fungal pathogen stripe rust: The screening of the 461 genotypes by co-dominant gene-specific FMM markers revealed that all 461 samples from the WHEALBI collection contained the wtk1 non-functional allele. Due to the release of the new Chinese Spring (CS) reference genome IWGSCv1.0 a new .vcf file with the SNP calling was provided for the exome capture data. Consequently, allele mining for the wheat accessions was repeated at two powdery mildew resistance loci (Pm2, Pm3), a Stagonospora resistance gene (Sn1) and an ortholog of a rice durable resistance gene (Xa21-like). Work in task 5.3 also included functional studies towards the end of the project. The new Pm2 allele, tentatively name Pm2j was molecularly characterized and the functional validation of a candidate gene conferring race-specific resistance to powdery mildew derived from the results delivered in WP3.4 was initiated. This potentially novel powdery mildew (Pm) resistance gene has been identified through GWAS in the WHEALBI population. It was confirmed that ~ 35 genotypes, resistant at phenotypic level against the diagnostic isolate, had the exact same sequence showing the identical three amino acid changes compared to the haplotype in cultivar Chinese Spring.

Furthermore, 15 randomly chosen susceptible lines had the Chinese Spring version of the gene. Three different approaches for the identification of possible candidate genes involved in Fusarium head blight resistance allowed the identification of a set of 270 genes potentially involved in wheat resistance or susceptibility against FHB. Using a BLAST search against the RefSeqv1.0 the physical positions of these genes were determined. SNP present in these genes were identified within the wheat exome data. A total of 264 SNP were identified from 49 genes. None of these SNPs were shown to be significantly associated with FHB related traits in WP2 GWAS data. A final list of 33 candidate genes form barley against Blumeria graminis f. sp. hordei (Bgh) was determined based on the performed genome-wide association studies for three traits. The four most promising candidates were selected after confirmation of the annotated gene models and different PCR based amplification tests. For the functional validation the selected genes were tested in Bgh attacked leaves via particle bombardment by transient gene silencing and transient allele complementation. This overexpression assay form 3 candidates lead to the identification of one allele which causes increased Bgh susceptibility in the tested resistant genotype.

WP6 – Genome-assisted pre-breeding and breeding methods

WP6 objectives were to provide a list of # 30 barley and wheat lines adapted to European conditions all with novel, wide allelic diversity for yield per se, yield stability, and yield related trait; to provide >10 barley lines with incorporated genetic diversity for useful traits in regions otherwise showing no or very little diversity in elite European spring barley malting varieties; to provide >10 wheat lines with introgressed QTLs for yield and yield related traits originating from wild emmer wheat, accompanied with associated molecular markers for further use in breeding; to provide >5 wheat lines with translocated barley chromosome segments for further exploitation in breeding and to provide statistical model(s) for efficient introgression of QTL(s) into breeding programmes. This workpackage was organized in 3 tasks and involved 8 partners. All five deliverables associated with WP6 were submitted.

Task 6.1: Introggression of wide allelic diversity into elite barley and wheat varieties

The successful inclusion of SIS into the task for additional phenotyping was a major achievement and added value to the whole work package. 64 elite pre-breeding lines from a KWS NAM (Nested Association Mapping) population were tested in replicated yield trial in the UK. Best line was 9% higher yielding than the recurrent parent, showing that high-yielding and locally adapted lines can be generated from pre-breeding. On average, the 64 lines carried 11.8% exotic genetic diversity. Six pre-breeding lines were selected by the commercial breeding team for use in their crossing programme. KWS has established a pre-breeding pipelines which exploits results from the WHEALBI project (Figure 19).

48 other lines from the INRA NAM population, selected based on a combination of previous years field performance and genetic characterization were yield tested in four locations in France by INRA and Italy by SIS partner. Five lines were identified showing
superior performance across environments, showing that selection for broad adaptability is possible. Further work on exploiting the genetic data is expected post-project. Genotyping of 380DH lines from the barley MAGIC population with the 50K SNP chip was completed, resulting in 18K informative genetic markers. The phenotyping for post-harvest traits was also completed. A few lines outperforming modern varieties across environments were identified. Combined analysis of phenotypic and genotypic data revealed a QTL for yield on 7H, and more association mapping is on-going.

Task 6.2: Introgression of targeted genomic regions into elite barley and wheat varieties

This task involved inter- and intra-specific crossing which is always associated with certain level of risk of failure. All three main partners (HU, KWS, and ATK) have all worked towards their objectives. The first round of tetraploid-by-hexaploid crosses carried out at HU did not produce viable pentaploid plants hence a new round of crossing has been carried out (Figure 20). Unfortunately it is impossible to predict the potential physiological disorders in these types of crosses but it is expected that eventually HU will succeed in producing viable pentaploid hybrids. Similarly, the translocation work performed at ATK is a numbers game with low percentage of successful and fertile wheat/barley lines expected. The fact that already six fertile 7H and 4H translocation lines have been generated is a success. The work at KWS does not have the same chromosomal challenges as the other two projects. The crossing and backcrossing to 80 barley landraces is a challenge in itself and once the exome capture data is available this number can be reduced based on available diversity in the target regions. This task has produced some successful translocation and, as expected, difficulties with interspecific crossing.

We identified and selected promising GPC-QTL (Grain Protein Content) for introgression into bread wheat cultivars: on chromosome 4BS, 7AL and 5BS. Three KASP markers were developed for each, used for transferring the QTL to BC3F3. During the last 18 months we screened 344 BC2F1, 707 BC3F1 and 537 BC3F2 plants. BC3F3 will be grown in 2018 for protein content and yield validation and will include sisters with and without the QTL, as well as recombinants in the QTL regions. Combined, this procedure resulted in: 129, 30, 3, 28 and 30 lines based on the cultivars Ruta, Gedera, Galil, Barnir, Yuval, and Zahir respectively. We also evaluated the results of the field experiment conducted in Israel in 2017 (obtained from Y. Saranga, WP3), and their GPC values obtained from Maria Megyeri in MTA ATK. Based on these, we chose four European lines that have high yield but differ in GPC in order to test them together with our introgression lines in 2018.

In order to better inform the introgression targets at B1 from each of the 29 landrace families 20 individuals were selfed and seed sent to New Zealand for multiplication. The resulting B1F3 generation was grown in a yield trial in Cambridgeshire, UK replicated in 2016 and 2017. This 384-plot trial was assessed for yield, yield components, agronomic traits and phenology traits. After spatial adjustment of the data (using the array of check genotypes) association analysis was carried out for each target haplotype. Exome capture data has enabled us to identify informative SNPs in the target haplotype regions (in silico) and to design KASP marker assays for the efficient selection of each target. This has been done (with a very low fail rate) and employed in the selection of a first batch of B2 individuals carrying candidate introgressions which have been backcrossed and are now at B3F2. Currently B3F2 individuals are being marker selected in order to identify homozygotes for the target introgressions. Selfed seed at B3F2 will be available in March 2018 completing the introgression phase of the programme. Seed will be delivered to the KWS line breeder for inclusion in ongoing breeding programs in March 2018 and also multiplied to B3F4. Beyond the WHEALBI project the actual performance of the introgression material will be validated in yield trials by KWS.

The genotype carrying 3HS.3BL compensating translocation was transferred into a modern Martonvásár wheat cultivar, Mv Bodri. The introgression of the 3HS.3BL translocation significantly reduced the plant height due to the incorporation of the dwarfing allele RhtD1b. The presence of the 3HS.3BL translocation in the Mv Bodri wheat background improved tillering and seeds per plant productivity in field experiments carried out in Martonvásár, Hungary. In general, the selected lines had good spike fertility and the phenology matched Mv Bodri.

Taking advantage of the breakage-fusion mechanism of univalent chromosomes at meiosis, the ‘Rannaya’ winter wheat 7B monosomic line was used as female partner to the 7H addition line male, leading to the development of a compensating wheat/barley Robertsonian translocation line (7BS.7HL centric fusion, 2n = 42) which exhibited higher salt tolerance and elevated grain β-glucan content.

Three translocation lines were developed from a cross between Rannaja 6A or 6B monosomic and Asakaze/Manas 6H disomic addition lines. Initial tests indicated that the 6BS.6HL introgression in the wheat background had better yield than the Asakaze wheat parental line. The plants carrying the 6HS.6BL disomic centric fusion have low fertility. Monosomic 6A.6HL translocation lines have been developed but not yet tested.

The gametocidal system was applied to produce new wheat-barley translocation lines from the wheat/barley addition lines.
We have collaborated to a barley multi-model study conducted as part of the FACCE-JPI MACSUR knowledge hub aiming to for strategy for ideotype research has been presented by A. Dambreville at the International Crop Modelling symposium in Berlin in March.

A strategy for in silico ideotype mining has been developed and tested in three contrasted European environments (Figure 21). The results of this study have been accepted for publication in Journal of Experimental Botany. The WP succeeded in dening a list of traits and measures including weather, soil characteristics and crop traits to calibrate the process-based model SiriusQuality2.

The sensitivity analysis of SiriusQuality2 identied several traits (e.g. number of leaves produced after oral initiation, phyllochron, neutral genetic introgressions. Analyzing pre-breeding populations which have undergone heavy selection under eld conditions requires a different strategy than analysing populations with a balanced family structure. Combined analysis of the genotypic and SNP data has also been made available to WU who will attempt different methods of analysis for identifying positive, negative and neutral genetic introgressions. Further models are possible in which alleles of peripheral parents are combined in classes. This this population, data were prepared for use in later QTL mapping and genomic prediction.

Meetings with WU has taken place to explain the structure of the pre-breeding programme at KWS and discuss how to use modelling to evaluate the pre-breeding scheme. Data on selection intensities from BC1F2 generation through to BC1F5 generation were provided for analysis. The aim is to assess population size and selection intensity and compare with performance and genetic diversity. The 35K SNP data has also been made available to WU who will attempt different methods of analysis for identifying positive, negative and neutral genetic introgressions. Analyzing pre-breeding populations which have undergone heavy selection under field conditions requires a different strategy than analysing populations with a balanced family structure. Combined analysis of the genotypic and phenotypic data from KWS' wheat pre-breeding populations identied exotic genomic regions contributing positively towards yield, and also identied the pre-breeding crosses which had the potentially highest pre-breeding value. Preliminary results about QTL detection models were presented at the nal Whealbi meeting.

Candidate QTL mapping and genomic prediction models were developed for application to the KWS pre-breeding data. WU formulated a general mixed model framework for prediction of trait k for genotype i that contains terms for QTLs (Q) and polygenic terms (M). Multi-trait QTL detection in the backcross nested association mapping from KWS requires some modications to the general model to account for the population structure in combination with multiple traits. Fixed intercept terms need to be added for the means of the individual (sub) populations. Allele substitution effects can be deined in various ways. Once by contrasting the central parent with all peripheral parents, another time by a unique substitution effect per population.

Further models are possible in which alleles of peripheral parents are combined in classes. This this population, data were prepared for use in later QTL mapping and genomic prediction.

WP7 – Innovative crop management practices to identify wheat and barley ideotypes with enhanced performance

WP7 objectives were to provide a list of candidate traits and ideotypes for improving wheat adaptation and stability in target populations of European environments from crop model simulations; to provide phenotypic datasets and report for performance of # 20 barley and wheat lines under conservation management; to provide phenotypic datasets and report for performance of # 20 barley and wheat lines under organic husbandry and to provide a report on the economic value of different management practices. This workpackage was organized in 4 tasks and involved 6 partners. All four deliverables associated with WP7 were submitted.

Task 7.1: Simulation mining of adaptive & constitutive traits to improve grain yield & quality stability

The sensitivity analysis of SiriusQuality2 identied several traits (e.g. number of leaves produced after floral initiation, phyllochron, potential duration of grain filling, light use eiciency) associated with high and stable yield and protein concentration in a range of environments. The results of this study have been accepted for publication in Journal of Experimental Botany. The WP succeeded in defining a list of traits and measures including weather, soil characteristics and crop traits to calibrate the process-based model SiriusQuality2.

A strategy for in silico ideotype mining has been developed and tested in three contrasted European environments (Figure 21). The strategy for ideotype research has been presented by A. Dambreville at the International Crop Modelling symposium in Berlin in March 2016. We have collabored to a barley multi-model study conducted as part of the FACCE-JPI MACSUR knowledge hub aiming to for
designing future barley ideotypes for Boreal and Mediterranean climatic zones in Europe and a paper has been submitted for publication. Three additional scientific papers are expected to be submitted by the end of Task 7.1 on i) the model of leaf area development of SiriusQuality2; ii) the identification of genotypic parameters related to this model and there genetic variability in elite cultivars; and, iii) the innovative strategy for wheat ideotype research.

The work carried out in this task has led to the setup of an innovative approach for ideotype research based on resource use optimisations. Using this approach, we identified a set of important parameters needed to optimize the efficiencies under different water and N scenarios within Europe. We also highlighted a strong significance of anthesis date within the optimized population. This Deliverable has led to two scientific publications with a further publication in preparation. Two papers based on the work carried out in the previous period on the genetic variability of model parameters and ideotyping mining have been drafted.

Task 7.2: Conservation management

Field experimental platforms were established under conservation management practices at all partner sites. Completed an initial selection of ~30 winter barley and ~60 winter wheat accessions selected and sown in nursery plots for seed multiplication and initial phenotypic observations.

The nursery trials at NIAB for seed increase and initial phenotypic assessments were harvested in August 2015. Accessions were evaluated for disease, crop height, lodging and maturity date to better enable the selection of the core accessions for the field platform experiments at partner sites. Seed was collected and processed (weighed, cleaned and dressed where necessary) and dispatched to WP7 partners in September 2015 ready for sowing.

A core set of 10 wheat and 10 barley accessions were chosen to ensure a wide range of diverse genetic material including material from old cultivars, landraces and adapted elite lines. Field experiments were conducted over two growing seasons (2015-16 and 2016-17) at three experimental sites in the UK (NIAB), Italy (SIS) and Hungary (ATK). The treatments were three soil tillage methods (ploughing, deep non-inversion and shallow non-inversion) and two N fertilisation rates (Figure 22). The experimental design was a split-split plot, with tillage as the main plot, N level as sub plots, and genotypes as sub-sub plots.

Using grain yield as the main indicator of performance, the results showed that landrace accessions of wheat and barley yielded less than the commercial cultivars, regardless of fertilisation level and tillage practice. In general, Old cultivars were competitive with the newer elite cultivar performance. The general impact of tillage practices on genotype performance, across all sites and years, did not have a consistently significant impact on yield either in wheat or barley. The lack of significant genotype x N fertilisation interactions suggested that the hypothesis is not supported, that older cvs or landraces may harbour beneficial alleles not present in elite germplasm which would give them an advantage under non-inversion tillage or reduced N inputs. Indeed, N use and efficiency was driven by differences in grain yield, so the higher-yielding elite (or recently introduced) cvs showed greater NUE than the lower yielding landraces.

Task 7.3: Organic husbandry

Field experimental platforms were established under organic management practices at partner site. Additional selection of spring cereal accessions to compliment the initial selection of ~30 barley and ~60 wheat accessions to ensure a wide range of diverse genetic material.

Two years of data were analysed on 20 winter wheat (10 WHEALBI set plus 10 British entries, from landraces to Elite Breeding Lines, selected by ORC in the previous phases of the project) and 10 barley entries in ploughed and shallow-non-inversion systems. The experiment was set up as a split-plot Randomised Complete Block, with Tillage as a main factor and Variety as a subfactor. All data were analysed through a mixed model as in R/lme4.

The variety factor was split in a set of contrasts to test specific hypotheses related to differences between varietal classes. In particular, we focused on (1) differences between UK and non-UK varieties; (2) within UK varieties, differences between landraces and proper varieties (historic, modern); (3) within UK proper varieties, differences between historic and modern varieties; (4) within UK modern varieties, differences between commercial and those selected as elite breeding lines; (5-7) the same as (2-4) for the non-UK varieties. The contrast matrix was completed by comparisons between individual varieties within single classes, thus resulting in 19 orthogonal linear contrasts.

The effect of shallow non-inversion tillage appeared to be evident and consistent in a slightly reduced performance in wheat, in terms of establishment, early ground cover, N uptake, weed abundance and biomass, ears density and, last, yield. Yield performance of different genetic classes (Landraces, Historic, Modern, Elite), showing that the advantage of progressive steps in wheat breeding might be diluted or lost in organic systems, especially in reduced tillage conditions.

Across the two years of our trial, no “best cultivar” could be identified. Yield advantage of modern cultivars and elite breeding lines over
heritage cultivars across both years was inconsistent. Moreover, when looking in detail at relationships among different morphological traits, yield did not appear to be linked to some of the key features of modern wheat, particularly short straw and high harvest index (grain/total biomass). The most consistent yield driver seemed to be ground canopy cover at the onset of stem extension, probably because of its relationship with resource capture and timely competition against weeds.

Task 7.4: Socio-economic assessment of innovative crop management practices

One of the conclusions is that reduced tillage could be implemented on multiple kinds of farms. In most of the cases, reducing the mechanization and the labour cost, results in a benefit on the gross margin, even if the crop yield is slightly reduced. Within this task we have highlighted the role of European and national policies, which play a major role on the development of conservation agriculture and reduced tillage in Europe. With the CAP 2014-2020, very few measures were engaged in the soil conservation, even if there was some ambition at the commission level before adoption of specific "greening" at the member state level. We can expect or hope that the next reform of the CAP in 2020 could include soil protection and conservation in the mandatory measures.

Potential Impact:
1.4.1 Potential impact

The WHEALBI project main outputs are:
• A publicly accessible collection of 1024 geo-referenced inbred lines of wheat and barley chosen from across the geographic range, to broadly represent the whole species and focusing on germplasm more adapted to European conditions.;
• Deep sequence (5-20X) of the exomes of the same wheat and barley accessions than enabled extensive study of genetic variation and evolution across geographical and historical ranges.;
• Life history trait and phenotypic data from all wheat and barley accessions grown in multiple environments along a wide climatic gradient in Europe, from Scotland to Israel;
• More detailed phenotypic data of selected subsets of wheat and barley accessions from high-throughput/precision phenotyping platforms, e.g. canopy development, disease scores, frost and drought tolerance;
• A data repository and management system containing all of the above data with a unique access portal;
• An interpretation of the observed patterns of diversity in relation to geography and environment, published in high impact journals;
• A list of candidate genes and alleles involved in key traits such as grain quality, frost and drought tolerance and resistance against fungal diseases;
• Pre-breeding pipelines using up-to-date statistical approaches and tools to integrate new useful variation into applied breeding programmes, including those from old varieties and wild relatives;
• Identification of new sustainable crop management systems and their economic evaluation at both farm and EU levels;
• Identification of best ideotypes suited for innovative sustainable cropping systems, with reduced environmental impact (in terms of pollution, energy use, greenhouse gas emissions);
• Advice to policy makers at EU level on project related impacts (e.g. in relation to support agriculture, agro-environment and other CAP - Common Agricultural Policy - related issues).

As consequence of the WHEALBI activities, a number of downstream effects will be promoted.

Development of breeding tools to support the breeding sector
The tools that have been developed in WHEALBI and the proof of concept made on the exome sequences will place EU research and industry in an ideal position to valorise future developments in genomics. This will help maintain EU scientists and breeders in the first circle of top world achievements in cereal genomics.

Development of new varieties with increased genetic variation and improved agronomic, processing and nutritional characteristics to support the farming sector
Succeeding in filling the "yield gap" of European wheat and barley production will help to maintain the gross value of major cereals production. With a reasonable figure of 30 kg/ha/year on average, across all EU countries, this would lead to about 50 more million tons in the decade following WHEALBI, i.e. a gross value of around 10 billion euros, and is certainly crucial for the maintenance of EU export capacity.

Widening the range of available adapted cereal genotypes
The efficient exploitation of untapped biodiversity of small grain cereal genetic resources, including wild relatives and landraces,
through genome based methods will ensure the potential of long-term genetic progress, particularly for specific adaptive traits to crop management systems which have been little considered up to now.

Environmental impact of new varieties grown in improved and/or novel management practices
Development of new varieties with durable resistance to diseases will enable farmers to reduce the use of fungicides, saving around 500 million euros per annum while maintaining yield and safer production of grains. This output will enable farmers to comply with the IPM requirements.

Adopting tilling conservation on at least 50% of the EU cereals growing areas will stop the degradation and even improve the soil organic matter content. In addition to the positive impact on soil erosion and fertility, this will lead to the sequestration of substantial quantities of carbon.

Contribution to food security through more productive, diversified and resilient European cereal production
WHEALBI outcomes will enable EU agriculture to maintain its cereal production at a high level with more limited year to year variations. This will have positive impact on world production stability, limiting the volatility of cereal prices which has dramatic consequences on farmer incomes and consumers well-being, particularly in developing countries, but also on poor people in developed ones.

1.4.2 Main dissemination activities
WP8 facilitated the dissemination of all activities within WHEALBI. Thus, general public, students, young researchers, stakeholders were informed and participated all along the project through trainings, the final Stakeholder forum and scientific conference, videos (15 released), newsletters (5 released) as well as many other tangible communication actions.

Over the course of the entire project, more than 120 dissemination and communication events took place in 20 different countries. The presentations (talks, posters, open days, college project, trainings, etc) reached a total audience estimated as more than 50 000 people.

The College project, which included five colleges from five participating countries, was a success. Colleges that participated were: Vetagro Sup (France), Easton&Otley College (UK), Anhalt University of Applied Science (Germany), Instituto Tecnico Agrario e Chimico Scarabelli (Italy) and Georgikon Faculty (Hungary). Results were excellent as regards to interest of the students, dissemination and students access to the project.

Seven trainings on genomics, phenomics, bioinformatics and data mining, applied breeding and agronomy were organised for scientists in the frame of the project.

Breeders, farmers and cooperatives were targeted via NIAB Innovation Farm, a knowledge exchange and research demonstration facility. The final Stakeholder forum was organised in December 2018 at the European Seed Association, in Brussels. Besides the European Commission, ETP Plant For The Future, European Seed Association and National plant breeders associations attended.

WHEALBI's public website (http://www.WHEALBI.eu/) was developed to allow the wide dissemination of project information to different stakeholder groups (scientists, consumers, farmers, breeding and seed industry).

WHEALBI has been presented to several large congresses over the four reporting periods: PAG (USA, January 2014), European Seed Association Annual Meeting (May 2014), ITMI-EUCARPIA (Denmark, July 2014), National Organic Cereals (UK, 2014), Organic Producer Conference (UK, 2014) International Wheat Conference (Australia, September 2015), EUCARPIA (Switzerland, August 2017), Genotype to Phenotype Modelling of Plant Adaptation (The Netherlands, November 2017).

A scientific conference open to non-WHEALBI partners was organised in Edinburgh during the final meeting. As many as 21 short presentations were given, across all WPs.

During the course of the project, WHEALBI interacted with other projects and initiatives (Annex 1 and 2). The consortium gave permission to a PhD Student at JHI to share exome SNP data of 9 barley lines with the breeding society SECobra (not a partner in WHEALBI). For the other demands of access to the whole SNP datasets, the reply was that they should be publically released after publication, expected before June, 2019.

According to the External Advisory Board of WHEALBI composed of Kellye Eversole (IWGSC), Hélène Lucas (Wheat Initiative until 2017), Pierre Devaux (Florimond Desprez), Silvia Travella (Plant for the Future ETP), Mike Bevan (JIC), Frank Ordon (JKI, wheat Initiative since 2018), Benjamin Kilian () and Scott Chapman (CSIRO), WHEALBI has been an ambitious and interesting programme that they would rank as excellent. 4 EAB members attended the final meeting and wrote a report that highlighted key points.

Wheat and barley accessions have been extensively characterized at the molecular level using exome capture and re-sequencing. This
yielded a wealth of data that have been used throughout WHEALBI. In parallel, these lines have been phenotypically evaluated for a range of life history traits under field conditions. Subsets were phenotyped for abiotic and biotic stress-related traits, many of which were challenging to assess such as drought tolerance which required bespoke precision phenotyping solutions. Exchange of seeds and import regulations proved challenging, and the barley collection did not pass phytosanitary requirements and proposed activities had to be revised.

The EAB observed strong collaboration between the wheat and barley groups, referring specifically to the study of gene orthologs to assess signatures of domestication and breeding history of these two important crops. As to (pre-)breeding, the EAB was interested to see that not only landraces could be resistant to all major strains of pathogens but also some recent cultivars in the case of Septoria leaf blotch. Through successive crosses carried out by the companies involved, original introgressed lines (pre-breeding material) have been constructed and evaluated under further evaluation. The EAB is confident that all stakeholders will benefit from newly developed material. Although the project has ended, the pipelines developed and analyses for association scans will continue to be used by the consortium. In addition, with respect to resistance screening validation of identified and novel resistance under field conditions will provide valuable genes for future breeding.

The EAB has motivated the Executive committee, to actively share the success stories of this WHEALBI initiative widely. First exciting manuscripts were submitted to high impact journals and many more will follow.

1.4.3 Exploitation of results

Six lines developed from the wheat pre-breeding project have entered the commercial wheat breeding programme. These were all crossed to commercial varieties in early 2019 and the progeny will enter the product development pipeline. If we have been successful in introducing new genetics for increased performance, these will indirectly be selected for during the selection process and end up in commercial varieties grown initially by UK farmers. As the European wheat breeding industry is closely interconnected, over time these genes will spread into other part of European wheat communities. For barley, the pre-breeding lines have been handed over to the commercial spring barley breeding in France who will evaluate the lines and select promising material for crossing into commercial spring barley lines, which is successful have a pan-European market.

List of Websites:
Address of the project public website: [http://www.WHEALBI.eu](http://www.WHEALBI.eu)

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Related documents

[final1-whealbi-final-report.pdf](final1-whealbi-final-report.pdf)

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