FINAL PUBLISHABLE SUMMARY REPORT

Project title: TriticeaeGenome (Genomics for Triticeae improvement)

Period covered: from 1 June 2008 to 31 May 2012

Project website address: www.triticeaegenome.eu

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1. Executive summary

TriticeaeGenome has been developed as a main contribution to the international consortia efforts in constructing physical maps of barley and hexaploid wheat for improving plant breeding, accelerating gene and QTL (Quantitative Trait Locus) isolation and setting up the foundation for future genome sequencing. It aimed at delivering novel information and tools to breeders and scientists based on a better understanding of the Triticeae genomes organization, evolution, and function. TriticeaeGenome (TG) was organised in 7 workpackages which major achievements are as follows:

- **WP1**: Publicly available BAC (Bacterial Artificial Chromosome) libraries have been constructed for the wheat chromosomes 3B, 3DS, 3DL, 1AS, 1AL, 1BS, and 1BL. Using FPC (FingerPrinted Contig) and the newly developed LTC (Linear Topological Contig) algorithms, BAC contigs were build using HIC (High Information Content) Fingerprints and MTP (Minimal Tiling Path) were designed for each chromosome. BAC end sequences were produced for each MTP.
- **WP2:** Integrated physical maps of wheat chromosomes 1AS, 1AL, 1BS, 1BL, 3B, 3DS, and 3DL and barley chromosomes 1H and 3H have been produced by screening 5,400 BAC contigs with ~140,000 molecular markers (SSR, STS, COS, genes, ISBPs). In total, 4301 contigs covering more than 75% of the target wheat chromosomes have been anchored whereas 560 contigs and 668 contigs were anchored on barley chromosomes 1H and 3H, respectively. High resolution mapping populations of 2600 and 4455 RILs (Recombinant Inbred Line) were obtained for fine mapping and anchoring in wheat and barley, respectively.
- **WP3:** Fine mapping (< 1 cM) and isolation of candidate genes using the physical maps produced in WP1/2 has been achieved for two Mendelian loci (YrH52, cul4) and three QTLs underlying fungal disease resistance, yield and quality traits (PV-QTL, QYld-idw, QSng.sfr) located on wheat and barley chromosomes 1BS, 1BL, 3BS and 3H,respectively. Functional validation and allele mining have been initiated for a few candidate genes.
- **WP4**: A new panel composed of 376 winter wheat varieties from France, the UK, and Germany maximizing the diversity of the European Union germplasm was created. After evaluation for yield and development related traits at 3 locations over 2 years and genotyping with various markers, Genome-wide association analyses were conducted for heading date, grain yield and plant height. Known and new chromosomal locations underlying these traits were identified.
- **WP5**: Bioinformatics tools and resources have been developed to support the TG project. Two interactive databases have been established at the INRA-URGI for wheat and HGMU-MIPS for barley. WP5 also developed LTC, a new algorithm for physical map assembly and a comparative viewer called CrowsNest to enable the visualisation of physical and genetic maps of single plant species and their comparisons with other genomes. Finally, TriAnnot, a pipeline for the automated annotation of Triticeae genome sequences has been completed.
- **WP6**: Nine training courses in emerging technological approaches have been organized for project members and external scientists and students. Results from the TG project

were disseminated at more than 250 events to a global audience size of ~ 85 000. Since May 2010, the public website has received 11 000 visits from all over the world.

- **WP7**: The efficient management of the project permitted to submit deliverables and periodic reports on time as well as organize the project meetings as scheduled.

2. Summary description of the project context and objectives

The domestication and large-scale cultivation of bread wheat and barley in the Fertile Crescent approximately 10,000 years ago is thought to have provided the first foundations for large-scale settlement and the steady rise of cities and nation states in the Near East and Europe. This linkage remains today as the environment and economies are linked interdependently on a global scale. Several factors, such as global climate change, increased population pressures, increased cereal consumption for food and fuel, salinization, and long-term drought in major wheat growing regions, all conspire to create a unique set of challenges for global food supply. Our capacity to respond to and meet these challenges is of major political concern at the international level. In Europe, a comprehensive response is being planned through the development of a knowledge based bio-economy that aims to secure global food supplies in an environmentally sustainable manner. The EU economy relies heavily on agriculture (17 million farms and 8% of EU25 workforce employed in the agricultural sector) and the EU seed market with 8.4 billion € annually represents the largest regional market (30% of the global market). Wheat (87 Mt vs 64 in USA) and barley (42 Mt vs 12Mt in Canada) are the most important cereal crops grown in Europe (Figure 1) and Europe has a long history of breeding and improvement of these essential species.

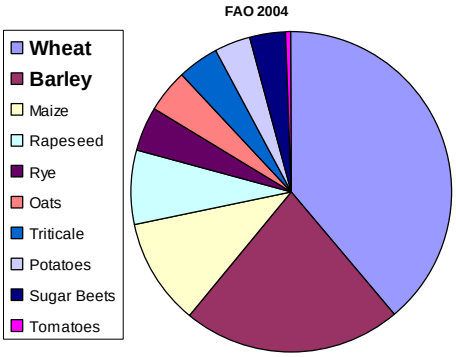

Figure 1: Harvest area (Ha) for the main crops in Europe (FAOstat)

However, the existing Triticeae varieties and the current breeding methods will not enable the advancements needed for the quantum leap in yield, quality, and biomass conversion efficiency necessary to ensure the competitiveness and independence of European agriculture. In rice and maize, large genomics programs have been developed either at the international level or in the US in the past 15 years. They have resulted in very efficient marker-assisted selection programs, gene cloning, and transformation pipelines that are illustrative clearly of the significant impact of genomics on rice and maize crop

improvement. In contrast, wheat and barley genomics have suffered from a chronic lack of long term investment that has resulted, in comparison, in less efficient improvement of the Triticeae (barley, wheat and rye) species despite the high value of these crops. Moreover, comparative genomics studies have shown the limits of rice as a model to support gene isolation in wheat and barley, especially for disease resistance and quality related traits. In 2008, when the TG project was launched there were no large physical maps established for the wheat and barley genomes and despite the fact that hundreds of genes and QTL have been located on barley, wheat and rye genetic maps, less than 20 genes were cloned and each only after 8 or 10 years of complex and uncertain map based cloning efforts. Although the density of genetic maps has been improved through joint efforts in the Triticeae community there was still a need for more saturated maps and for transferring new technologies for high throughput mapping in wheat, in particular, to ensure efficient map-based cloning, marker-assisted selection, and molecular breeding of the varieties of tomorrow's agriculture.

One of the major obstacles for developing genomics resources for the wheat and barley genome is their complexity in terms of size (17 Gb for the bread wheat genome, i.e., 5 times the human genome and 40 times the rice genome; 5Gb for barley), repeat content (>80%), and ploidy level (e.g. hexaploid wheat). Often considered too challenging and costly Triticeae genomics and its application to crop improvement had lagged behind advances in other cereal crops for many years. At the beginning of the 2000's, however, the convergence of several technology developments has changed this situation and provided a basis for establishing genomic programs in these species. New and more efficient scientific capabilities and resources such as whole-genome [1-5] and chromosome-specific BAC libraries [6, 7], extensive marker collections (see graingenes database at http://wheat.pw.usda.gov/GG2), transformation systems, wild germplasm and mutant collections became available for the Triticeae. The development of commercially available microarray platforms also has permitted the first genome wide functional studies in barley and wheat [8]. These resources enabled the map-based cloning of the first genes of agronomic interest in hexaploid wheat [9, 10] and barley [11, 12]. The sequencing of BAC clones at different target loci in barley and wheat homoeologous genomes during these map-based cloning projects also provided access for the first time to large genomic sequences of the Triticeae. This provided the first insights into the organisation, composition and through comparative genomics, into the mechanisms that have shaped their genomes during evolution ([13]). Despite these breakthroughs, however, positional cloning in the Triticeae remained laborious and time consuming, largely due to difficulty in establishing physical contigs because repetitive DNA limits success in chromosome walking. Moreover, new genetic materials (high recombinant populations, diversity panels) and genetic markers at high density were needed still to develop association genetics and efficient marker-assisted selection for breeding. Thus, a major effort was needed in both species to develop genomics resources and promote their application for Triticeae improvement. International projects, such as the International Wheat Genome Sequencing Consortium (IWGSC, www.wheatgenome.org) and the International Barley Sequencing Consortium (IBSC, barleygenome.org) were launched in 2005 and 2006, respectively, to address these needs. The objective was to bundle synergistic resources and expertise into concerted efforts to construct physical maps and sequence the hexaploid wheat and barley genomes. Their first important mid-term goals were to develop anchored physical maps

of the 7 chromosomes of barley cv Morex, a reference cultivar for barley genomics resource development, and of the 21 chromosomes of the hexaploid wheat cultivar 'Chinese Spring' (cv. CS), a genotype with a wealth of cytogenetic stocks that are pivotal to mapping of the wheat genome. In 2006, the IWGSC roadmap aimed at completing the physical map of the D genome of the wild diploid *Ae. tauschii* (NSF project led by J. Dvorak), as a framework for the construction of the physical maps for the 7 chromosomes of the D genome of hexaploid wheat and to construct the physical maps of the homoeologous A and B chromosomes of group 1, 3, 5 and 7. The D genome project was initiated in 2001 (http://wheat.pw.usda.gov/PhysicalMapping/) and had established efficient protocols and softwares to perform BAC fingerprinting and contig assembly that were then used for the hexaploid wheat genome project. A whole-genome approach was not seen to be practical or cost-effective as this would have required fingerprinting and specific assembly of homoeologous BAC contigs comprising over 2 million BAC clones. There was a further difficulty in anchoring specifically the homoeologous BAC contigs from such a large and complex set of contigs to their chromosomal location. Thus, the strategy developed for the construction of physical maps in hexaploid wheat was based on a chromosome by chromosome analysis. This chromosome specific strategy was pioneered in Europe by IEB (Czech Rep.) and INRA (France). It relied on the recent improvement of chromosome sorting and BAC library construction technologies that allowed for the construction of individual chromosome-specific BAC libraries [14-16]. The first wheat chromosome BAC library was built in 2004 [7] and has been used successfully to launch a pilot project to establish a chromosome landing ready physical map of chromosome 3B, the largest wheat chromosome (2 x the rice genome). The physical map of chromosome 3B was published in 2008 after the start of the TG project [17] and it provided the proof of concept for this approach at the international level. The expertise gained during the Ae. tauschii and 3B project have been instrumental for the TriticeaeGenome project and the other IWGSC projects.

Given the economic importance of wheat and barley in the EU and the current scientific excellence of its public and private research institutes in Triticeae genetics and genomics, Europe was in a unique position to take the lead in this field. Thus, the goal of the TriticeaeGenome project was to develop and use new resources to achieve significant progress in Triticeae genomics, achieve a better understanding of the Triticeae genome organization, evolution, and function to support efficient gene isolation, optimized exploitation of genetic resources and breeding of new varieties for European agriculture. To achieve this goal, TriticeaeGenome wanted to integrate an ambitious combination of approaches, including plant genomics & genetics, crop breeding and agronomy using modern techniques, including marker- assisted selection and genetic engineering, as well as bioinformatics. The concept was also to develop barley and wheat projects together in a single project to synergize the efforts and extend the long tradition of collaboration between the two communities since the creation of the International Triticeae Mapping Initiative (ITMI, http://wheat.pw.usda.gov/ITMI/) in 1989. In a coordinated and collaborative manner, it aimed at leveraging efforts and resources developed at the international level in other grasses (rice, sorghum maize, Brachypodium) and contribute significantly to international genome sequencing projects in wheat and barley. Beyond its scientific and economic objectives, the project had the objective to reinforce the leadership of Europe in Triticeae genomics and produce a

competitive advantage for EU farmers and the food and feed industry in the global market.

TriticeaeGenome had 7 major objectives that served as the foundation for workpackages:

(1) Construct and anchor to the genetic maps, physical maps of the Triticeae group 1 and 3 chromosomes that carry a large number of important agronomic traits (e.g. disease resistance, yield and quality).
(2) Isolate five genes and QTLs underlying disease resistance, yield and quality traits in wheat and barley. Target loci include fungal disease resistance genes in bread wheat and wild wheat T. dicoccoides, a QTL for grain yield in durum wheat, a pentosan viscosity QTL for a highly relevant quality trait in bread wheat and a gene involved in the determination of plant architecture (tillering) with a potential impact on yield in barley.
(3) Identify and exploit new alleles for the isolated genes through the use of natural and mutant populations as well as wild germplasm.
(4) Support the development of new varieties that meet farmer, processor and consumer needs through molecular breeding using the new genomic resources developed in the project to perform association mapping for yield traits on a new panel of UK, French and German elite wheat varieties.
(5) Develop new bioinformatics tools to structure, relate and comprehensively analyse the large scale genomics data gathered within the project. The objectives were to establish new tools for genomic sequence annotation, a new algorithm for contig assembly and an integrative database for the display of genetic and physical maps linked to markers, traits and phenotypes and other grass genomes.
(6) Provide training in emerging technological approaches, disseminate the results and transfer technology to industry.
(7) Provide a professional management of the project to coordinate and integrate Triticeae genomics research and facilitate interactions with other on-going projects at the national, European and international levels, to avoid overlaps and to enhance the efficiency of the project.

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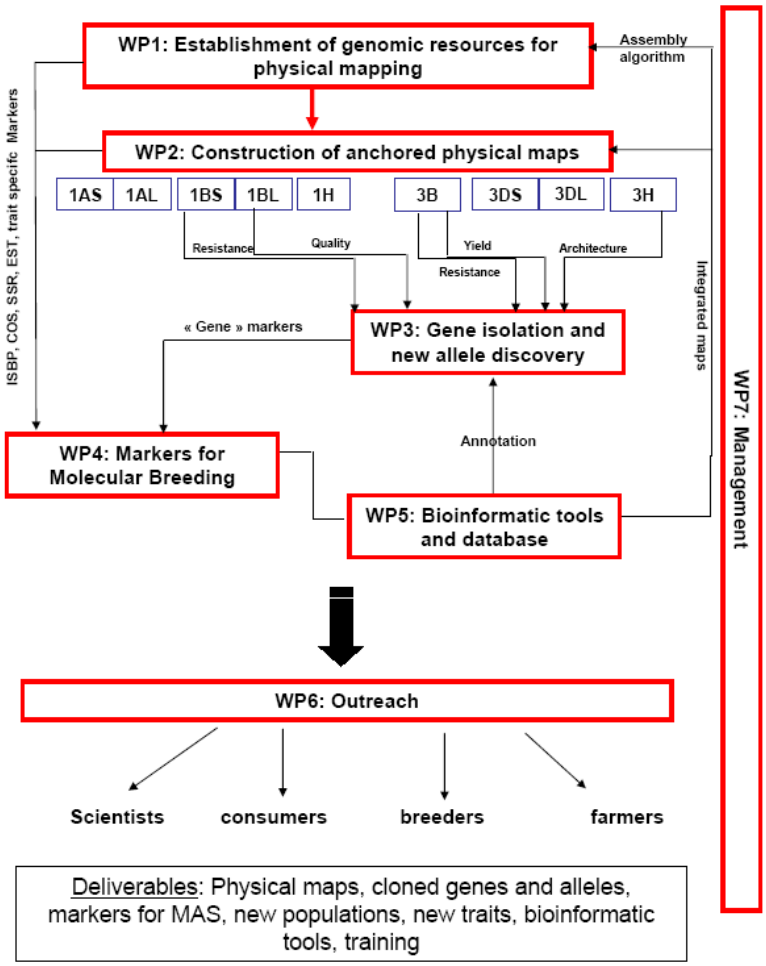
3. Description of the main S&T results/foregrounds

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



WP1 - Establishment of genomics resources for physical mapping of wheat group 1 and 3 chromosomes

WP1 aimed at developing the genomic resources (BAC libraries, BAC fingerprinting, contig assemblies, BAC pools, BAC end sequences) needed to construct anchored physical maps of the targeted chromosomes of the project (3B, 1A, 1B and 3D) in WP2 and, provide a substrate for map-based cloning in WP3 and for future sequencing of these chromosomes. It was organized in 5 tasks and involved 7 partners. Seven deliverables were associated to WP1 and have all been achieved.

Task 1.1: Wheat chromosome (arm) BAC libraries construction

Task 1.1 has been achieved by IEB and resulted in the construction of BAC libraries for chromosome arms 1AS, 1AL, 1BS, 1BL, 3DS, and 3DL as well as a second library for chromosome 3B. All libraries are now available for the TG partners and the international community as an output of the project (D 1.1) under a MTA (more information on the INRA-CNRGV website http://cnrgv.toulouse.inra.fr/en/Library/Wheat). The average insert size ranges from 103kb (1AL) to 126kb (3Bv2). Considering the molecular size of chromosomes and the extent of contamination by other chromosomes, the libraries represent 9.11x (3Bv2) to 15.7x (1BS and 1AL) chromosome coverage. A publication referring to the TG project and

describing these resources has been published in Cytogenetic and Genome Research (Safar et al, 2010).

Chromosome / arm	Library code	Number of clones	Average insert size (kb)	Chrom. coverage
1AS	TaaCsp1AShA	31,104	111	11.8 x
1AL	TaaCsp1ALhA	49,536	103	8.0 x
1AL	TaaCsp1ALhB	43,008	109	7.7 x
1BS	TaaCsp1BShA	55,296	113	15.7 x
1BL	TaaCsp1BLhA	92,160	114	15.4 x
3B	TaaCsp3BFhA	67 968	103	6.2 x
3B	TaaCsp3BFhB	82,176	126	9.1 x
3DS	TaaCsp3DShA	36,864	110	11.0 x
3DL	TaaCsp3DLhA	64,512	105	12.2 x

In addition to the original working plan, IEB has been producing amplified DNA from wheat chromosome 3B and chromosome arms 1AS, 1AL, 1BS, 1BL, 3DS, 3DL as well as from barley chromosome arms 3HS and 3HL (funded by BMBF project GABI barlex 0314000). The amplified DNA was supplied to project partners (HU, IPK, INRA, UZH, JIC, MTT) for physical mapping and Next Generation Shotgun sequencing to identify genic sequences, facilitate comparative genome analysis and develop DNA markers to anchored the physical maps in WP2.

Task 1.2: Fingerprint of the wheat BAC libraries

BAC DNA extraction and fingerprinting have been performed by IGA for all partners. In total, 463'395 BAC clones have been fingerprinted and processed at IGA using the GeneMapper, FPB, GenoProfiler and programs to monitor the fingerprints quality. The average performance was of about 80% which is good considering the number of FPC gel entries with high quality fingerprints generated.

Chr.	Fingerprinted BACs	High quality FP	FP Performance	Useful HICFs
1AS	31104	25918	83%	25918
1AL	92544	72724	76%	70537
1BS	55296	50620	89%	49412
1BL	92160	65413	61%*	65413
3Bv2	89916	70195	85%	131792**
3DS	36863	28033	76%	27880
3DL	65512	55780	86%	55780
Total	**463395**	**368683**	**79.5%**	

* Problem with sequencer
** Total of 3Bv1 and v2 maps

In addition to the original plan, we explored the feasibility of using new fingerprinting technologies for physical mapping in wheat INRA performed a pilot project with Keygene for assessing the performance of their new physical mapping technique called Whole Genome profiling that is based on the sequencing of the ends of restriction endonuclease fragments after digestion of BAC pools. Using a subset of 30% of the 3B project, we showed that compared to the SNaPshot technology that was used until now, WGP provides more accurate physical maps with a similar length but with less mis-assembled BACs and chimeric

contigs while providing sequence tag information that can be used in supporting sequence pooling strategies. This proof of concept opened the way for other project within the IWGSC and currently 5 additional maps are performed using WGP. The results were published in BMC Genomics (Philippe et al, 2012) in the final reporting period.

Task 1.3: Assembly of BAC contigs

Contigs were assembled by each partner in charge using the FPC program for all the target chromosomes using the same guideline that was produced by a WP1 taskforce. This guaranteed homogeneity and quality of these first assemblies and ensure the design of a reliable MTP for the next steps. This guideline has been adopted by the IWGSC as a standard and has been published in the Annual Wheat Newsletter (Appels et al 2011). This guideline is also available on the TG public website (see http://www.triticeaegenome.eu/page.php?optim=Deliverables). The assemblies covered between 86% and up to 99% of the chromosome and the N50 ranged between 326 Kb and more than 2 Mb. A minimal Tiling Path was designed for all chromosome/arms and sent to INRA CNRGV for rearraying to prepare for screening and anchoring in WP2. In addition, some groups used a new assembly algorithm developed by HU in WP5 to perform assemblies and compared it to the FPC assembly. In fact, the high level of repeated DNA of the Triticeae genomes requires the application of very stringent criteria to ensure a reliable assembly with the FPC software, which often results in short contig lengths as well as an unreliable assembly in some difficult regions. To address these problems, HU developed the Linear Topology Contig (LTC) program which reduces the rate of false connections and Q-clones by systematically exploring the topological contig structure and performing iterative clone clustering and ordering, so that highly reliable and longer contigs than in FPC are recovered. The results showed that LTC maps are longer and more reliable than the FPC maps. LTC is now recommended for all the IWGSC physical maps. These assemblies were then improved in WP2 after anchoring and manual assembly.

Chr.	Useful HICFs	Contigs (e -45)	N50	Total size (% coverage)	MTP
1AS	25918	804 (FPC)	326 kb	272 Mb (85%)	3414
		715 (LTC)	486 kb	297 Mb (93%)	-
1AL	70537	2551	481 kb	587 Mb (86%)	7470
1BL	65413	1277 (FPC)	576 kb	695 Mb (103%)	8253
		616 (LTC)	1128 kb	498 Mb (93%)	5550
1BS	49412	1793 (FPC)	1033kb	270 Mb (86%)	3647
		1075 (LTC)	2429Kb	280 Mb (87%)	6477
3B	131792	1205	679 kb	986 Mb (99%)	9216
3DS	27880	945	388 kb	310 Mb (97%)	3823
3DL	55780	1000	573 kb	416 Mb (99%)	5826

Task 1.4: BAC libraries pooling from the MTP and rearraying for BES

A list of clones representing the MTPs of all chromosomes was established based on the assembly performed by each partner in Task 1.3. Each BAC library has been rearrayed by INRA CNRGV based on the MTP list and three-dimensional pools were constructed from each MTP. The MTPs clones were then sent to IGA for BAC end sequencing (Task 1.5) and

the MTP pools were sent to each partner for screening and anchoring (WP2). To provide more information about the pooling design to all partners and ensure their use in the most efficient way for screening in WP2, a file summarizing the characteristics of each pool and the PCR screening conditions has been established by INRA-CNRGV. This protocol is available on the TG public website (see http://www.triticeaegenome.eu/page.php?optim=Deliverables).

Task 1.5: BAC end sequencing for marker development and genome analysis in wheat

IGA received glycerol stock clones rearranged from the INRA-CNRGV for all chromosomes and performed BAC DNA extraction in 384-well format for BAC end sequencing using Big-Dye Terminator Sanger. In total 83812 BAC clones (64,2 Mb) have been end-sequenced (forward and reverse) by IGA. The percentage of good quality sequences (>300 bp) ranged from 77% to about 88% with an average read length between 550-700bp. These BES have been useful for marker development as well as anchoring activities performed in WP2.

Conclusions: WP1 has been highly successful in performing all activities planned originally and delivering results on time. In many aspects the work performed in WP1 established standards and protocols for the whole wheat community and had a strong impact at the international level. Additional activities have been performed to evaluate new technologies and exploit new opportunities linked to the development of Next Generation Sequencing technologies. Two publications specifically referring to results of the WP1 have already been published, a manuscript describing the new 3B physical map has also been published (Rustenholz et al, 2011) and manuscripts are underway for each of the 6 other chromosome 1AS, 1AL, 1BS, 1BL, 3DS and 3DL physical maps.

WP2 – Construction of anchored physical maps

The general objective of WP2 was to construct anchored physical maps of the group 1 and 3 chromosomes in wheat and barley by aligning and ordering the BAC contigs assembled in WP1 and associated projects (i.e. while genome barley physical map) to their chromosomal location on genetic maps. To achieve that WP2 was organized in 4 tasks the specific objectives of which were to: (1) provide high resolution mapping populations of wheat and barley, (2) generate new genetic markers at high density for wheat and barley group 1 and 3 chromosomes from novel resources and (3) use the markers to screen BAC library pools from MTP-derived contigs (from WP1 an associated project) for anchoring the physical map to the genetic maps and for the map based cloning (WP3) and molecular breeding (WP4) of traits of agronomic interest on these chromosomes. Moreover, WP2 aimed at providing a platform for comparative genomics of the barley and wheat homoeologous chromosomes at different scales (from maps to large sequenced regions), evolutionary studies of duplications in the grass genomes and synteny based cloning of genes of interest present on chromosomes of group 1 and 3 in wheat and barley. 11 partners were involved in WP2 and 4 deliverables that were all achieved on time were planned.

Task 2.1: Marker development for anchoring

The objectives of this task were (1) to collect as comprehensive as possible information about sequence-based markers from public databanks and (2) develop new markers for high-density anchoring and mapping. During the course of the project, public gene-

(sequence-) based marker information was collected and integrated into the HMGU-MIPS and INRA-URGI databases for barley and wheat, respectively, providing mirrored access to the data.

(1) Integration of sequence based wheat and barley markers into the MIPS and URGI databases

For barley, 8,616 barley markers from three different genetic maps have been submitted to the MIPS PlantsDB (HMGU) (http://mips.helmholtz-muenchen.de/plant/genomes.jsp). All barley marker data are presented to the scientific community in the context of additional barley genomic data. This circumvents the integration of the barley markers into the barley genome zipper, a virtual ordered gene map of barley (http://mips.helmholtz-muenchen.de/plant/barley/gz/index.jsp), and visualization with various tools as well as tools for comparative Triticeae genomics. The central access point for Triticeae data at MIPS PlantsDB is http://mips.helmholtz-muenchen.de/plant/triticeae/index.jsp, whereas the barley instance can be accessed directly at http://mips.helmholtz-muenchen.de/plant/barley/index.jsp.

For wheat, URGI collected and integrated markers into their global information system (GNPiS) (http://urgi.versailles.inra.fr/GnpMap/mapping/welcome.do). Currently, the data stored at URGI comprise 24 wheat genetic maps, including the 3B and 1BL neighbor maps developed in the framework of the TriticeaeGenome project for physical map anchoring. These maps contain more than 17 000 markers and 700 QTLs. All wheat data (markers, physical maps etc…) including the publicly released data from the TG project are accessible at http://urgi.versailles.inra.fr/Species/Wheat/Data.

(2) Development of new markers

The original plan was to essentially use BAC end sequences from the MTP produced in WP1 to develop markers and anchor the physical maps to genetic maps. However, during the 4 years of the project, the revolution in high throughput sequencing and genotyping technologies provided us with new opportunities to develop a much larger number of markers and perform anchoring at high resolution. It became possible to shotgun sequence individual chromosomes (see WP1) or BAC pools from the MTPs and use these sequences to develop thousands of markers from genes or transposable elements for each chromosomes and develop high throughput genotyping platforms.

In barley, all 7 chromosomes were shotgun sequenced and a virtual gene order was established (Mayer et al. 2011, The Plant Cell 23: 1249–1263) in the framework of projects developed in parallel to TG. The resource was made available to the TG partners and provided an opportunity of designing PCR primers for marker development in an automated fashion for many hundred of genes per chromosome. Almost 4000 primer pairs were designed for chromosome 1H and 3H and can be utilized for marker development. This information is going to be included into the MIPS data repository in the very near future. In addition, gene based microarrays were developed and used for anchoring. In particular, SCRI developed Agilent custom microarrays representing 15K and 42K barley expressed sequence tag (EST) contig sequences that were used to screen the MTP pools of wheat chromosome 3B in a pilot study and of the whole barley genome, respectively. This type of platform enables the anchoring of thousands of genes in a single experiment and was also developed specifically for wheat (see below).

In wheat, markers were developed from a large variety of sources: BAC end sequences, BAC pools was sequenced by Illumina HiSeq2000, Illumina or 454 shotgun sequences of sorted chromosomes, Unigene microarrays and Conserved Orthologous Sets (COS):

- 454 and Illumina shotgun sequences were obtained for the group 1 chromosomes and for chromosome 3D and used to design gene and ISBP markers.
- About 170'000 BAC end sequences generated for each MTP was used to develop markers or anchor markers by sequence similarity
- A NimbleGen array of 40K wheat unigene was designed by INRA and used for anchoring the MTPs of chromosome 3B, 1A and 1B
- A NimbleGen array of 17K 1BL specific ISBP markers was designed by INRA and used to anchor the 1BL physical map as a pilot study
- After a revision of the strategy for marker development in WP4 (see below), it was decided to develop SNP markers from 5000 genome-wide COS primer pairs obtained after synteny analyses between the wheat/rice/Brachypodium/sorghum and maize genomes. A set of 8538 high confidence COS-SNPs were obtained from 6 hexaploid wheat cultivars including some of the WP4 panel and tetraploid wheat genotypes of interest for the TG partners.

Task 2.2: Generating resources for high resolution genetic mapping in wheat and barley

The objectives of this task were to develop new mapping populations to support high-resolution genetic anchoring of physical maps in wheat and barley. 2000 RILs (F8) and 4000 RILs (F8) were planned for wheat and barley, respectively. Seed stocks were multiplied as much as possible in the final round and are available.

The Morex x Barke high resolution barley RIL population was established by single seed descent and comprises 4455 individuals. DNA has been extracted from 2409 and 2046 F8 individuals and 2407 individuals were genotyped by using a Vera Code multiplex for 384 barley SNP marker to generate a basic genetic map. 368 F8 RILs were analysed with a 9K iSelect barley SNP and a SNP map comprising >3900 loci was established. At the end of the project larger quantities of F9 family seeds and DNA aliquots are available to project partners in summer 2012.

A 2600 Renan x Chinese Spring wheat RIL population was established and multiplied until the F8 generation. During multiplication, three phenotypic traits have been recorded: heading date, plant height and presence of awns. At the same time, DNA from a set of randomly selected 400 F7 lines (bulk of 5 plants = F6 generation) has been extracted for SNP genotyping. A subset of 381 F2 lines, from which F8 progenies are available in the final population, were used to establish the 1BL genetic map at INRA (84 markers) and to start up the 1AL genetic map at SU.

Wheat and barley high resolution mapping populations are available to the community (contact Pierre Sourdille pierre.sourdille@clermont.inra.fr from INRA for wheat and Viktor Korzun viktor.korzun@kws.com from KWL for barley).

Task 2.3: Anchoring BAC contigs to chromosomes by high throughput PCR-based screening methods

Physical maps and MTPs were delivered by WP1 during the third reporting phase for the 1AS, 1AL, 1BS, 1BL, 3B, 3DS and 3DL wheat chromosomes and the physical maps of the

homoeologous barley chromosomes 1H and 3H that were produced in the framework of the whole genome physical mapping by the IBSC (International Barley Sequencing Consortium). Using both publicly available collected in the TG databases and newly developed markers, the partners anchored as many molecular markers as possible (see above) on their physical contigs. A large range of original methods were used by the TG partners providing useful strategies to the rest of the wheat community. This included a coordinated strategy of utilizing COS markers and BAC pool screening with a 44K microarray of wheat unigenes. At the current stage the anchored physical maps in wheat and barley cover on average 75% or more of the physical length of the chromosomes (arms).

Chrom.	contigs	MTP BACs	MTP pools	SSRs	ISBPs	PCR-based ESTs	COSs	Hyb-based ESTs	SNPs	Other markers	Synteny-based anchors	anchored contigs	Coverage (% of arm size)
1AS	458	3414	49	20				647			749	160	97 Mb (35.3%)
1AL	1175	7470	60	21	178	10	156	1222			430	772	287.4 Mb (55%)
1BS	385	6447	57		68			1074			253	272	259.7 Mb (82.5%)
1BL	616	6976	63	70	4233		21	1161		4	350	317	396 Mb (74%)
3B	1205	9314	65	291	711	294		2836		147	1122	812	749 Mb (75%)
3DS	945	3827	50	39					213		185	451	(49%)
3DL	1000	5826	55				619	2558	18		628	289	295Mb (65%)
1H	_	7005	59	_	_	277	_	_	_	50,867	_	560	560 Mb (75%)
3H	_	8863	64	_	_	296	_	_	_	76,156	_	668	668 Mb (75%)

Table 1: Summary of the physical map anchoring

For wheat a total of more than 5,400 contigs were obtained and screened with approximately 140,000 molecular markers. This anchored 4301 contigs, covering more than 75% of the chromosomes. For barley, a total of 560 contigs were anchored and ordered along chromosome 1H whereas 668 contigs were ordered along chromosome 3H which also equals about 75 % of the length of these chromosomes. The maps will be improved beyond TG using chromosome shotgun sequence assemblies of each chromosomes developed by IWGSC and sequence tags derived from high-throughput “Genotyping by Sequencing” approaches. These anchored physical maps provide the wheat and barley communities with very useful tools for map-based cloning of genes of agronomic importance and pave the way to the sequencing of the wheat and barley genome.

Task 2.4: Comparative genetic and physical mapping with other grass genomes

Thanks to the high throughput development of gene-based markers in the TG project, it became possible to compare the gene content of complete homoeologous Triticeae linkage groups. This novel information represents a highly innovative achievement that was not planned at the beginning of the project. It helped reaching a better understanding of genome colinearity in the *Triticeae*. In addition, it provided unique opportunities for generating new marker resources as well as strategies for high-density map anchoring.

Based on the experience of shotgun sequencing of flow-sorted barley chromosomes a collaboration within the TG project was initiated for comparative survey sequencing of homoeologous group 1 chromosomes between wheat and barley. The results showed that

the Triticeae genomes have accumulated nonsyntenic genes frequently since their divergence from the other grass genomes (Wicker et al. 2011). The comparative studies on group 1 and 3 chromosomes identified a significant number of wheat genic sequences that did not fall into conserved gene order between homoeologous chromosomes or if compared to syntenic regions of sequenced grass genomes. Some of these may be mobilised gene fragments or pseudogenes, but a significant proportion may be bona fide wheat genes.

Previous work at INRA defined a macrosyntenic framework linking the genome sequences of the sorghum, rice and Brachypodium genomes. This information was integrated with wheat bin-mapped ESTs and barley high- resolution EST-based genetic maps. It showed that Brachypodium chromosome 2 is syntenic with Triticeae group 1 and group 3 chromosomes, with the group 1 region nested in group 3. This analysis identified 21,045 orthologous relationships defining syntenic alignments providing the basis for conserved orthologous sequence (COS) markers design.

One of the important conclusion from Task 2.4 was that syntenic ordering of wheat genes and physical maps provide a useful initial framework, but it cannot be relied on to provide a durable long-range order of genes. For this, wheat gene assemblies need to be aligned to BAC contigs and high- resolution genetic markers and BAC sequences aligned to long range physical and genetic maps.

Conclusions: All milestones and deliverables of WP2 were achieved and submitted on time. Large datasets of public markers are available and ready for queries from project databases. Novel marker resources have been established and are currently exploited for anchoring and mapping. Repeatedly TG project partners have proven that they can integrate efficiently new and innovative approaches into the original working plan thereby achieving more than originally planned and providing new strategies to the entire wheat and barley communities. The achievements of WP2 contribute significantly to the success of the entire TG project and set new standards in Triticeae genome analysis and Triticeae-centric comparative grass genomics.

WP3 – Gene isolation and new alleles discovery

WP3 aimed at fine mapping and identification of candidate genes (CGs) for two Mendelian loci and three QTLs underlying fungal disease resistance, yield and quality traits and located on chromosomes group 1 or 3 in wheat or barley. The work was organized in four tasks and involved 5 partners that were each responsible for a target locus as follows:

- *YrH52* stripe rust resistance gene from *T. dicoccoides*. Chromosome 1BS (HU partner)
- *cul4* barley tillering gene. Chromosome 3HL (UMIL partner)
- *PV-QTL*, a pentosan viscosity QTL in bread wheat. Chromosome 1BL (INRA partner)
- *QYld-idw*, a QTL for grain yield from *T. durum*. Chromosome 3BS (UNIBO partner)
- *QSng.sfr*, a QTL for resistance to glume blotch in bread wheat. Chromosome 3BS (UZH partner)

All these genes represent agronomically relevant traits and serve as a test case for improvements in gene mapping based on physical knowledge of the wheat and barley genomes deriving from WP1 and 2. Five deliverables were planned and submitted on time.

Task 3.1: High-resolution mapping of the genes underlying the target traits

The objectives of Task 3.1 were to build high density genetic maps and establish physical contigs at the 5 targeted loci on chromosomes 1B, 3B and 3H using the resources developed in WP1/2. The strategy was to (i) increase marker density and genetic map resolution, (ii) improve the precision of phenotyping and (iii) identify closely linked markers and physical contigs associated with the traits. At the beginning of the project, the genetic intervals spanning the traits ranged from 4 to 16 cM (centimorgan, unit for measuring genetic linkage). Using a large variety of strategies, it was possible to identify additional markers and reduce the confidence intervals to less than 1 cM for each trait (see Table 2). Physical contigs were identified for all loci with single or only a few candidate gene(s) in some cases (see Task 3.2).

Traits - Species	**Closest markers distance (progress at each year of the project)**					**Physical map and candidate genes**
	M0	**M12**	**M24**	**M36**	**M48**	
Tillering (Mendelian locus) - Barley	5.5 cM	4.3 cM	1 cM	0.23 cM	0.02 cM	1 BAC spanning cul4 (ca. 170 kb) CG identified and validated.
Stripe rust resistance (Mendelian locus) - Wild emmer wheat	21.4 cM	1.3 cM	0.8 cM	0.6 cM	0.2 cM	2 non-overlapping contigs (total of 3.7 Mb with estimated gap of 0.4Mb)
Glume blotch resistance (QTL) - Bread wheat	11 cM	11 cM	11 cM	11 cM	high density map of the region + 6 recombination events	3 contigs (1.58Mb)
Fiber content (QTL) - Bread wheat	41.7 cM	9.26 cM	7.63 cM	2 cM	0.8 cM	1 contig (645 kb) achored to co-segregating CG
Yield (QTL) - Durum wheat	15 cM	11 cM	11 cM	1 contig	0.22 cM	Region < 200 kb in 3B physical map 4 CGs

Table 2: Summary of the progress of positional cloning projects

***YrH52*:** Using a F_2 population of 2828 plants, and comparative genomics approach, the genetic map of *YrH52* was refined to a 1.1 cM interval on chromosome 1BS, with closest markers 0.2 cM proximal and 0.9 cM distal, respectively. Flanking markers matched two non-overlapping contigs from the 1BS physical map, spanning a 3.3 Mb region with an intervening gap estimated to be 0.4 Mb in size. The ratio between physical and genetic distance in this genomic region suggests that the *YrH52* gene is located within the proximal contig covered by 12 MTP BAC clones.

***PV-QTL-1B*:** Using a combination of association mapping and synteny based mapping with 176 COS markers, the *PV-QTL-1B* was located in a 1.9 cM interval. Genetic mapping in biparental populations resulted in 5 recombinant lines within a <1 cM *PV-QTL-1B* interval for HIF construction. Orthologous regions were used as source of candidate genes in rice (Os5, 9 genes), sorghum (Sb9, 11 genes) and *Brachypodium* (Bd2, 9 genes). Using the 1B

physical map, 4 contigs and 1 BAC were identified within the interval covering the *QTL*. Among these contigs one is considered as best candidate.

QYld-idw-3BS: Fine mapping of the *QYld.idw-3B* QTL on 3BS was performed with 61 informative segmental isolines derived from approx 7,500 F2 plants. Based on the results of the molecular and phenotypic data, and on the reference Chinese Spring 3B physical map, the QTL position was narrowed down to a region of approximately 200 kb. Grain yield data expected for July 2012 will be used to confirm this result. Sequence information available from chromosome 3B of Chinese Spring provided by INRA for this region indicates the presence of 4 candidate genes.

QSng.sfr: Fine mapping for the QSng.sfr*BS* was performed using a high-resolution mapping population of 1,320 NILs. Phenotypic data from field infection tests performed on 192 recombinant lines in summer 2011 were analyzed. Single marker analysis allowed us to determine a region on the genetic map which is highly associated with the trait, corresponding to 6 recombination events. According to the information from the recently sequenced physical contigs of chromosome 3B (INRA), the region of highest interest consists of three sequenced contigs and has a size of 1.58 Mb (in wheat). Preliminary annotation suggests that these contigs contain 24 genes (based on *Brachypodium* CDS).

cul4: Screening of a fine mapping population (4949 F3 plants) identified 179 recombinants for the cul4 region on chromosome 3H. These were phenotyped and genotyped with 8 new markers developed from the barley genome zipper information (available from IPK): a candidate gene cosegregating with cul4 was identified as well as two flanking SNPs defining a 0.23 cM interval. Using information from the barley physical map new flanking markers were identified confining the genetic position of the locus to 0.09 cM interval, corresponding to a single BAC clone. The CG was confirmed to co-segregate with the phenotype.

Task 3.2: Identification of candidate genes

Task 3.2 aimed at identifying candidates for the 5 target genes. Progress was achieved for all traits as follows:

- **cul4:** A BAC clone was identified that contains a CG cosegregating with the *cul4* locus. Other genes annotated from the same BAC clone showed recombination with the phenotype, thus confirming the possible role of the CG.

- ***PV-QTL-1B***: Expression data from wheat regarding the grain development and five low-viscosity *vs*. five high-viscosity lines were mapped on the final QTL micro-synteny regions. Overall, among the genes included in the QTL interval (9 genes in rice, 9 in *Brachypodium* and 11 in sorghum), two genes (of unknown functions) corresponding to two COS markers (COS 116 and COS 140) are differentially expressed during grain development in wheat and thus are good candidates for the viscosity trait.

- ***QYld-idw-3BS***: The phenotyping of the segmental isolines with recombination events in the target area allowed for a substantial progress in the genetic resolution of the target region and in identifying the most likely candidates for *Qyld.idw-3B*. Among the 41 genes, 8 pseudogenes and 4 gene fragments present on the 3.16 Mb contig (ctg 954) harboring *Qyld.idw-3B*, four appear particularly promising and will be advanced to down- and up-regulation to validate their role.

- ***YrH52:*** Wheat ESTs closely linked to the *YrH52* gene region were used as anchors to the *Brachypodium*, rice and sorghum colinear regions (Bd2 101 kb, Os5 59 kb and Sb9 91 kb). In total, annotations of 17 syntenic genes (7 from Bd2, 5 from Os5 and 5 from Sb9) in the *YrH52* gene region were extracted as a potential CGs. Genetic mapping of these genes in the *YrH52* genetic map is underway.

- *QSng.sfr*: The genetic interval of highest interest and significant association with the target trait corresponds to three physical contigs with a total length of 1.58 Mb. Physical BAC contigs of cultivar Chinese Spring were sequenced by INRA partners (WP1), which allowed for the identification of 24 genes based on *Brachypodium distachyon* CDS database.

Task 3.3: Functional validation of candidate genes

Task 3.3 aimed at the functional validation of candidate genes (CGs) for four WP3 target loci (UMIL, HU, INRA and UZH). Various complementary approaches were considered including:

- Allelic comparisons in different genetic materials, including mutagenised populations.
- Analyses of gene expression.
- Stable transformation.

The choice of the most suitable validation approach depends on the inheritance of the trait (eg Mendelian or quantitative), the genetic materials available, the number of CGs under evaluation and the mode of action of the gene(s) of interest. For example, stable transformation by biolistic or *Agrobacterium tumefaciens* often provides conclusive evidence for the involvement on the CG in the relevant trait. However, because of the regeneration and life cycle time (about 6 months) this kind of experiment remains laborious in the Triticeae species. Therefore, it is generally undertaken after preliminary validation of the CG by other approaches.

As detailed in Tasks 3.1 and 3.2 sections, progress in fine mapping and identification of CGs varies for the different target loci. Thus, different validation approaches were undertaken:

- In the case of cul4, a single CG was identified based on genetic/physical mapping (see Tasks 3.1 and 3.2) and validated by resequencing of available *cul4* and wild-type stocks: three independent mutated alleles were identified and phenotypic severity was linked to the type of mutation and supported by expression analyses showing lack of the CG transcript in a deletion mutant. As a complementary validation approach, construction of RNAi and overexpression cassettes was initiated to carry out stable transformation experiments in collaboration with IPK.

- For PV-QTL, microarray experiments identified genes that are differentially expressed between 200 and 400 degree days (physiological phase of the arabinoxylan biosynthesis in wheat endosperm) as well as between five high-viscosity and five low-viscosity lines within the target mapping population. Combination of this information with mapping results points to two candidate genes (of unknown functions) with differential expression during wheat grain development (referenced as COS number 116/140).

- In two other projects which target disease resistance genes (YrH52 and QSng.sfr), focus is still on identification of a limited number of candidate genes for functional tests. Novel materials and methods have been developed for future validation as for such traits the R gene is often not present in the reference cultivar Chinese Spring and the resistant allele needs to be isolated from the resistant parent. A novel EMS-mutagenized population and a

protocol for biolistic transformation of *T. dicoccoides* were developed by HU and INRA, respectively for the YrH52 gene. While identification of CGs is still preliminary in the case of QSng.sfr, previous experience of this group with VIGS will be valuable for future validation.

Task 3.4: New alleles discovery

Task 3.4 started at M37 and aimed at analyzing allelic diversity of candidate genes for four WP3 target loci (UMIL, UNIBO, INRA and UZH) using available genetic materials and gene banks. As described in Tasks 3.1, 3.2 and 3.3 sections, progress in fine mapping, identification and functional characterization of candidate genes varies for the different target loci and therefore allele discovery was not implemented for all genes.

***cul4,* 3H.** While allelic diversity still needs to be examined in natural populations, the CG was resequenced in 5 barley cultivars. In addition, in collaboration with UNIBO a search for new alleles was initiated by screening 25 lines from a TILLING population and 24 additional lines previously identified for tillering defects are available. Close colinearity between *cul4* and the wheat *tin3* tillering locus indicates that the CG identified for *cul4* may be involved in control of plant architecture in wheat as well. To validate this hypothesis, partial genomic sequences were also obtained for related homoeologues from bread wheat, *T. urartu* (A genome), *Ae. speltoides* (S genome related to the B genome of bread wheat), and *Ae. tauschii* (D genome), providing a starting point for the functional characterization of the gene in wheat.

***PV-QTL,* 1BL.** At this stage, allelic diversity was not yet approached for CGs for this QTL. However, a diversity panel of 150 lines already phenotyped is available for future analyses.

***QYld-idw,* 3BS**. Because a single candidate gene for *QYld.idw-3B* has not yet been identified, and considering the extensive (> 5 cM) linkage disequilibrium usually detected in elite durum wheat, an allelic diversity study was conducted via haplotype analysis by means of SSR profiling. A core set of 11 durum wheat cultivars, including the parental cultivars Kofa and Svevo and other parents of cross populations, was investigated with 45 SSR markers spanning ca. 13 cM. Based on the four markers closest to the peak of *QYld.idw-3B*, four different haplotypes, including insertion/deletion polymorphisms, were identified. The haplotypes of Kofa and Svevo were not unique, and were shared by one and four other cultivars, respectively. A larger set of durum wheat cultivars (approximately 200) is being investigated with a 90-K wheat SNP array. In connection with the extensive LD typically present in durum wheat, this analysis will provide a more comprehensive overview of haplotype diversity at *QYld.idw-3B*.

***QSng.sfr,* 3BS.** Because identification of CGs is still preliminary, allelic diversity was not analysed yet, but a panel of 44 wheat cultivars evaluated for resistance to *Stagonospora nodorum* glume blotch and used earlier in association mapping study is available for future allelic discovery.

Conclusions: All WP3 milestones and deliverables were achieved on time. Given the complexity of map based cloning in wheat and barley, the achievements of WP3 within the four years of the TG project are remarkable. For the 5 loci, genetic intervals of less than 1 cM and physical contigs carrying candidate genes for at least 3 loci have been identified already. This is to compare to the 8-10 years efforts that it took for every reported cloning in these species. All projects will be completed by the partners using the TG project resources and publications reporting the cloning are expected within the next 2 years.

WP4 – Molecular markers for breeding

WP4 aimed at the development of markers and plant material for identifying traits of agronomic interest and supporting the creation of new varieties by marker-assisted selection. The WP involved 3 partners including two private breeding companies. It was developed through 2 tasks that provided 3 deliverables.

Task 4.1: Development of trait specific markers

The strategy planned originally was to develop a maximal set of markers targeting loci of interest (QTL intervals) on wheat chromosome 1 and 3 using markers developed in WP2 for the physical map and to detect among these sequences putative gene of interest for QTL present on chromosomes 1 and 3. To enhance the pace of marker discovery in wheat, a pool resource from the entire Chinese Spring BAC library was planned. The entire strategy has been revised after a year to adjust to new technologies and new capabilities and eventually generate whole genome markers rather than being restricted to the group 1 and 3 chromosomes targeted in the project. This enabled association analyses planned on the TG panel in task 4.2 to be much more efficient and relevant for breeders. Thus, instead of using BAC end sequences only, the partners agreed to use genome wide available markers and develop SNPs from 5000 COS markers that cover the whole wheat genome. SNP-COS marker development was performed with financial support from WP4 in the WP2 (for a detailed description see WP2). The pilot work initiated in year 1 on genic BES of chromosome 3B led to the development of 219 amplicons that were used for deep sequencing in 8 different varieties for SNP development. In total, 123 SNP were identified and delivered to the TG database at URGI along with validation information for 67 of them, tested with the KASPar technology. Two-dimensional pools of the entire CS BAC library (average insert sizes 130kb, 9x coverage) were produced in the first year of the project to ease marker screening. With this scheme, only 216 PCR reactions are needed to screen 4X of the CS BAC library and identify positive BAC pools. This resource has been since then extensively used by the wheat community for map based cloning projects.

Task 4.2: Association genetics

The aim of Task 4.2 was to create a new diversity panel of 376 European winter wheat lines suitable for association mapping and use this panel to initiate analyses. The WP4 partners first identified a primary panel of 743 varieties and performed small observation trials. NIAB and BGA worked then together to reduce the panel to 376 varieties based on (1) general phenotypic levels of adaptation to field conditions in UK, France and Germany, (2) phenotypic homogeneity and (3) genotypic diversity based on 14 SSR markers. An observation score protocol was agreed by consortium partners that encompassed winter damage, flowering time, height, straw strength and time to harvest ripeness. In each environment, plant breeders excluded varieties that were too tall (more than 130cm), too late (more than 10 days later than mid-point flowering), too early (more than 10 days earlier than mid-point flowering) or had very weak straw. In addition, based on plant rows observations at NIAB, varieties that were too heterogeneous were also rejected. SSR data was then used to identify genotypic duplicates and phylogenetic analysis deployed to identify the most genetically diverse subset of 376 varieties. Seeds were multiplied (6 kg of seeds for each

variety) to supply for full-scale observation trials that were performed during the last 2 years of the project in three locations in the UK, Germany and France. Moreover, the panel was genotyped with DArT markers, setting the first step for Genome-wide association mapping. Genotyping the panel with markers associated with major genes (Ppd-D1, Rht-B1, Rht-D1…) also allowed a first comparison with phenotypic data between the two years of field trials.

The success of association mapping i.e. the capability to relate a trait and markers accurately along the genome relies on a good knowledge of the structure of the panel to avoid spurious associations only linked to the relatedness of the individuals. Principal coordinates analysis (PCO) indicated that the geographical origin of the varieties strongly influences population sub-structure in the TG panel, consistent with the way the panel was assembled. While performing this analysis, it became obvious that the choice of markers to use was crucial, as using the complete marker dataset introduced a bias towards the 1RS-1BL rye translocation. A selection of markers evenly distributed across the genome suppressed this bias.

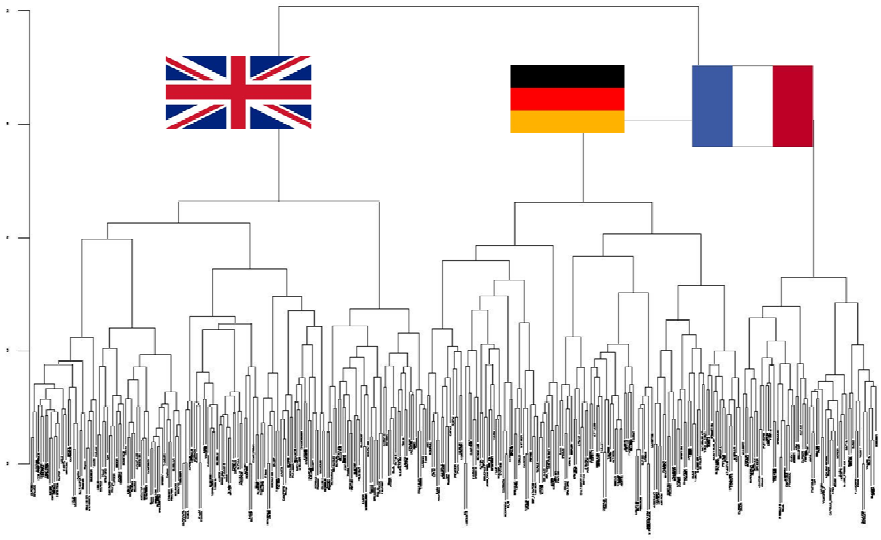


Figure 2: Dendrogram of the 376 TG panel varieties based on Rogers' genetic distance using UPGMA. The genetic distance was computed using 332 SNP. Information from geographical origin showed its influence within the branching of the tree

A multi-environment trials (MET) analysis was conducted, first by analyzing the data from each site separately, then using the site means across sites. The results showed that for Grain Yield (GY) heritability was low (0.265), as expected, with a large genotype x environment interaction. This emphasized the importance of carrying out association on variety yields from each site separately in addition to an average yield across site. For Plant Height (PH), heritability was of 0.73 and for Heading Date (HD), it was of 0.633. These values are lower than expected, largely due to within trial error term, as heritabilities varied a lot between trials. Correlations between sites were rather good. The dataset thus assembled is of much interest to characterize the European germplasm for these traits. The power analysis of the panel showed that there is high power to detect QTL with modest contribution to trait heritability (i.e. with the target locus itself). For unlinked markers, the average detection probability was very low. Finally, the power to detect the target declines quickly

with genetic distance. This emphasizes the need for high resolution genotyping of this panel to make the most out of it.

Genome-Wide Association Studies were conducted for the three traits (GY, PH and HD) resulting in the identification of chromosomal regions associated with the traits: regions on chromosomes 1B, 2A, 2B, 3D, 4B, 5B, 6B, 6D and 7A were found associated with HD, 1A, 1B, 1D, 2A, 2B, 2D, 3B, 4A, 4B, 4D, 5A, 6A and 7A for PH and 1B, 2A, 2D, 3B, 4A, 4B, 4D, 5B, 5D, 6B and 7A for GY. Strong associations were also found for major genes like Ppd-D1 or Rht2.

Finally, a set of 407 COS-SNP markers developed in WP2 was genotyped on the panel. Analyses are underway

Thus, T4.2 allowed the characterization of the power of the TG panel, showing it held good promises for association identification. It also allowed the identification of regions involved in three adaptive traits, both consistent with the literature, but also new regions. These results, as well as the phenotypic characterization of the panel will be disseminated in the form of two articles, currently in preparation for submission. Moreover, the TG panel became a source of interest to external collaborators and requests have been made to use it for other projects worldwide. At the 3rd annual meeting held in Prague, the partners agreed to provide other groups with the panel pending that phenotyping data obtained in other programs are provided back to the TG partners. This will be handled in the TG database at URGI. The TG panel has been multiplied for distribution to partners and is available to the community under a MTA (contact Nick Gosman nick.gosman@niab.com from NIAB). The panel has been already distributed to collaborators in India and Argentina.

Conclusions: All deliverables and milestones including a new one with the development of the COS markers in WP2 were achieved. “Breeders were happy” was one of the conclusions of WP4 at the final TG meeting which illustrates the achievement of this WP. The TG panel has proven to be a new and interesting resource to perform association mapping in wheat and it will be used beyond the TG project by NIAB and Biogemma as well as other breeding companies that showed interest in accessing it. Combined with the high density of markers available now in wheat and the anchored physical maps this should led to the identification of new regions and the facilitated isolation of genes associated with yield and other important traits for the EU farmers.

WP5 - Bioinformatics

To efficiently exploit the genomics data generated within TriticeaeGenome, WP5 aimed to develop bioinformatics tools to support the construction of the wheat and barley physical maps (for WP2), assist in contig assembly and anchoring (for WP2), analyse and annotate Triticeae genomic sequences (for WP3) and integrate data and tools in a platform for Triticeae genomics. Deliverables of WP5 included databases, management and dissemination, tools for genomic sequence analysis and contig assembly, resources for data integration and analysis and, standards for sequence data representation and processing. Five partners were involved in WP5 and 5 deliverables were planned and were all delivered on time.

Task 5.1: Development of a web interface integrating genetics and physical maps and for project data management

In this task, INRA-URGI and HMGU-MIPS established dedicated databases for wheat and barley respectively, using common format to facilitate the exchange of data between the two sites. To provide and disseminate this information to the wider community, a database infrastructure for storing all genome associated information – genetic and physical maps as well as sequence data – has been developed. The databases comprise complex interfaces embracing complex search and browse opportunities both between different types of maps as well as between different Triticeae genomes and different types of marker information. Work in the WP has been a continuous process over the past years focussed towards three steps needed in the process to build up the resource:

All along the project, the databases have integrated markers, genetic maps and physical maps generated by the partners. The current status of the TG databases is as follows:

- For wheat: the URGI Information System comprises

  - 24 genetic maps including 2 new genetic maps delivered during the TG project (3B and 1BL neighbor maps)
  - 17098 markers including 1112 delivered during the TG project in addition of the 1039 COS (Conserved Ortholog Set)
  - Physical maps: the wheat physical map viewer was updated regularly and is now in a version 4 that integrates 7 maps from the TG project: 3B v1, 3B v2 (final version), 1BL v1, 1BL v2 (final version), 1AS v1, 1BS v1, 3DS v1, 3DL v1. http://urgi.versailles.inra.fr/gb2/gbrowse/wheat_phys_pub/

- For barley: The HGMU PlantsDB database comprises

  - 8,616 barley markers from three different maps. Within the plant genome database framework of MIPS PlantsDB (http://mips.helmholtz-muenchen.de/plant/genomes.jsp), all marker data is presented to the scientific community in the context of additional barley genomic data. This circumvents the integration of the barley markers into the barley genome zipper, a virtual ordered gene map of barley (http://mips.helmholtz-muenchen.de/plant/barley/gz/index.jsp), and visualisation with various tools as well as tools for comparative triticeae genomics.
  - information about the barley whole genome physical map: http://seacow.helmholtz-muenchen.de/cgi-bin/gb2/gbrowse/Barley_PhysMap

All barley marker data is exchanged regularly with URGI Versailles (INRA), vice versa marker data from wheat is loaded into PlantsDB (MIPS) from the reference repository at URGI Versailles. Besides marker data from both barley and wheat, BAC clone sequences, BAC end sequences and FPC contigs from barley are hosted. All data mentioned are made available from dedicated MIPS PlantsDB web interfaces/portals as well as via download. To allow users to explore the data available in a comparative way and make use of knowledge transfer between model and crop systems a number of dedicated tools were developed. A

summary of the project data with links to the tool available at URGI and MIPS is maintained at the URGI website: http://urgi.versailles.inra.fr/Projects/TriticeaeGenome

These resources are hosted in two institutes that will continue to implement them and will guarantee their sustainability beyond the TG project. Strong links established during the TG project between the two groups will also enable the continuous interaction between wheat and barley databases.

Task 5.2: Bioinformatic re-sampling approach for physical mapping based on restriction fingerprinting

The aim of this task was to develop a new algorithm for contig assembly that takes into account the specific features of the wheat and barley genomes *i..e*. high repetitivity and redundancy that affect the capacity to build accurate physical contigs. The efforts focused on solving two major problems in physical mapping in complex genomes: (i) clustering of clones, and (ii) ordering the clones. The goal was to develop and implement a tool that results in contigs as large as possible and with the highest reliability in the ordering of clones within the contigs.

HU achieved the development of a novel assembly algorithm called Linear Topology Contig (LTC). LTC essentially (i) reduces the rate of false connections and Q-clones by using a new cutoff calculation method; (ii) obtains reliable clusters robust to the exclusion of single clone or clone overlap; (iii) explores the topological contig structure by considering contigs as networks of clones connected by significant overlaps; (iv) performs iterative clone clustering combined with ordering and order verification using re-sampling methods; and (v) uses global optimization methods for clone ordering and Band Map construction. It was tested first for assembly and MTP selection of wheat chromosome 1BS. In addition, LTC was used for quality control of contigs and MTPs obtained by FPC for chromosome 3B and some others data sets. By systematically exploring the topological contig structure and performing iterative clone clustering and ordering LTC helped to detect chimeric contigs, wrong ordering, and problematic places in the MTPs, so that highly reliable and longer contigs than in FPC were recovered. LTC dramatically improved the TG maps (see Table 1 WP1) and is now recommended by the IWGSC for all the wheat physical maps construction. LTC has been published in 2011 by Frenkel et al (HU, INRA) in BMC bioinformatics. The LTC package can be exported to other labs (this has been done in several TG labs) and can be used by people with some informatics skills. HU is exploring the possibility to develop a user friendly “commercial type” of LTC package.

Task 5.3: Comparative genome informatics tools

The aim of T5.3 was to develop a platform for the integration of the different datasets (physical maps, BAC ends, COS markers, contigs sequences etc…) generated in TG on wheat and barley into a grass comparative framework to link the information with other grass sequences. To achieve this, HGMU-MIPS developed the Genome Zipper approach (Mayer et al, 2009; 2011) in barley (http://mips.helmholtz-muenchen.de/plant/barley/gz/index.jsp). In this approach, genetic, cytogenetic and physical mapping data are integrated with shotgun sequences from individual chromosomes for generating a specific gene index for an entire

barley chromosome. The Gene index is then integrated with data from other grass genomes to deliver a comprehensive virtual linear gene order model. This approach has been used in the TG project for the wheat chromosomes and is integrated into the WP2 anchoring information.

Finding and analyzing syntenic regions both within and between species is a common task in comparative genome analysis. To support this HGMU-MIPS developed CrowsNest, a Comparative Map Viewer that can be accessed at: http://mips.helmholtz-muenchen.de/plant/crowsNest/.CrowsNest uses a dynamic graphical interface to visualize and investigate genome-wide chromosome organization as well as genome-wide synteny between two or more plant genomes. CrowsNest provides four different visualization levels, from macro-synteny down to micro-synteny views and was initialized with pre-computed data from Brachypodium, Rice and Sorghum. It visualizes both physical and genetic maps of single plant species and compare them among each other. The viewer illustrates syntenic relationships between two or more related grass species based on pre-calculated results. It integrates Triticeae (and Triticeae Genome) data with genomic data from finished and published grass genomic data.

Task 5.4: Bioinformatic tools for developing a semi-automated annotation pipeline

Genome annotation is generally a long and recursive process, the difficulty of which increases with the size and complexity of the genome. It relies on a successive combination of software, algorithms, and methods, as well as the availability of accurate and updated sequence databanks. To manage the large amount of data generated by >1Gb genome size sequencing projects, sequence annotation needs to be automated i.e. performed through a pipeline that combines all different programs and minimizes subsequent manual curation which is long and laborious. With this in mind and the perspective of large sequence data sets that were expected in the WP2 and 3 of the TG project as well as in other projects within the IWGSC and ISBC consortia, INRA developed a pipeline called TriAnnot. The objective was to have a versatile, easy-to-use online automated tool for annotation to provide easy access to markers and candidate genes as well as perform structural and evolutionary genomics studies in the Triticeae. TriAnnot allows for the annotation and masking of transposable elements, the structural and functional annotation of protein-coding genes with an evidence-based quality indexing, and the identification of conserved non-coding sequences and molecular markers. It is parallelized on a 712 CPU computing cluster that can run a 1 Gb sequence annotation in less than one day. It is accessible through a web interface for small scale analyses (http://urgi.versailles.inra.fr/Tools/TriAnnot-pipeline) or through a server for large scale annotations. The performance of TriAnnot was evaluated in terms of sensitivity, specificity, and general fitness using curated reference sequence sets from rice and wheat. In less than 8 hours, TriAnnot was able to predict more than 83% of the 3,748 CDS from rice chromosome 1 with a fitness of 67.4%. On a set of 12 reference Mb-sized contigs from wheat chromosome 3B, TriAnnot predicted and annotated 93.3% of the genes among which 54% were perfectly identified in accordance with the reference annotation. It also allowed the curation of 12 genes based on new biological evidences, increasing the percentage of perfect gene prediction to 63%. TriAnnot systematically showed a higher fitness than other annotation pipelines that are not improved for wheat. As it is easily adaptable to the annotation of other plant genomes, TriAnnot should become a useful

resource for the annotation of large and complex genomes in the future. A publication describing TriAnnot has been produced (Leroy et al, 2012)

Conclusions: WP5 has greatly contributed to advance genomics analyses in the Triticeae. First, it supported the establishment of two databases that are now the reference at the international level for wheat and barley genomics. Thanks to the leadership of the TG project in this field, INRA-URGI (in Versailles, France) is now hosting the IWGSC sequence repository for the physical maps, survey sequence and reference sequences of bread wheat while HMGU-MIPS (in Neuherberg, Germany) is the dedicated database for all the IBSC data. Moreover, the bioinformatic tools that were developed within WP5 for physical map assembly, comparative genomics and genome annotation became references at the international level.

4. Potential impact, main dissemination activities and exploitation of results

The ambition of the TriticeaeGenome project was to have several far-reaching impacts: (1) establish a strategy for genomics in two of the most challenging genomes yet to be mapped, (2) nucleate international-scale collaborative projects to complete genome analysis and provide the foundation for future sequencing of these genomes, (3) provide the foundation for revolutionizing plant breeding and genetic engineering in wheat and barley, and (4) contribute to the training and education of a new generation of Triticeae scientists and breeders. Two workpackages, WP6 and 7 were dedicated to reach these objectives by ensuring proper dissemination of the results of TG to the broad scientific community and the different stakeholders, establishing a network of external collaborators (TGN) and proposing training activities in key technological and methodological areas.

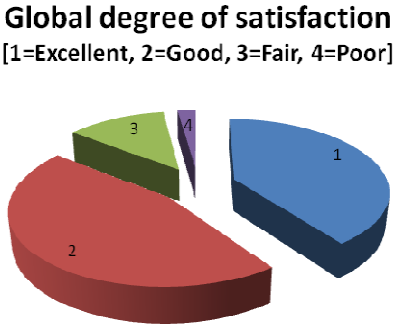
Training of European scientists

The objective of the task was to organize training workshops during the 4 years of the project to serve as a Technology Transfer Program for scientists and students in universities, public institutions, and industry within and outside the TriticeaeGenome project, to ensure optimal access and understanding of the knowledge generated within the project and increase its exploitation. During the entire project, TriticeaeGenome organized nine training courses in seven countries. A total of 168 participants including 90 external collaborators (including members of the External Advisory Board and Triticeae Genomics Network of TG project) attended the 9 courses. The courses, taught by leading experts, provided the participants with state-of-the-art practical experience in methods and technologies that are part of the Triticeae Genome toolbox. More specifically, training was provided in:

- BAC fingerprinting (IGA, Italy) in January 2009;
- Chromosome sorting and analysis (IEB, Czech Republic) in June 2009;
- Physical map assembly and anchoring (INRA, France) in September 2009;
- High through-put genotyping approaches (UMIL, Italy) in October 2009;
- Marker development (IPK, Germany) in March 2010;
- Multilocus genetic maps; consensus mapping and QTL analysis (HU, Israel) in October 2010;

- Applied bioinformatics in Plant Sciences (MTT, in Greece) in December 2010;
- Genetic analysis using genomics scale tools (SCRI= JHI, UK) in January 2012;
- Map based cloning (UZH and UNIBO, in Italy) in April 2012.

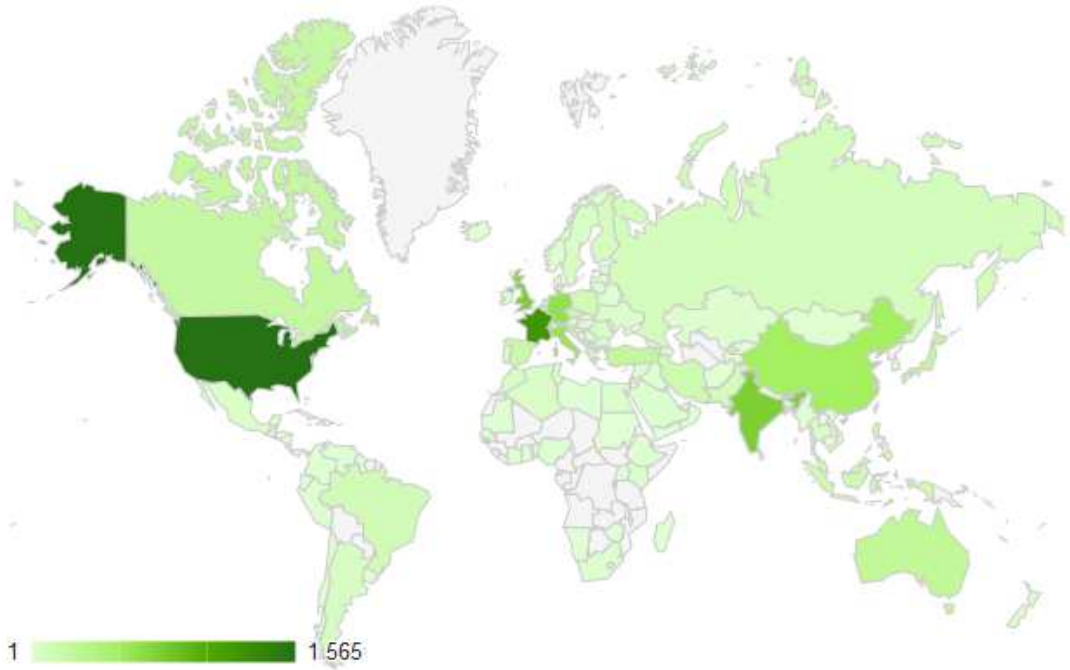
A report was produced at the end of each training course in the form of a Milestone. A training evaluation form was also sent to the participants at the end of each training course to collect their opinions on different aspects such as the training course objectives and organization. According to the 120 forms received from 168 participants, most of the participants' satisfaction was "good to excellent". The main satisfaction of the participants was that the selection of topics and speakers was of high quality. Moreover, many budding collaborations and networks of development were established among the course participants and organizers, which will bear fruit beyond the end of the project.



Communication through the project public website

The TriticeaeGenome public website (www.triticeaegenome.eu) was developed to allow the wide dissemination of project information to different stakeholder groups (scientists, consumers, farmers, breeding and seed industry). The TG website has been prepared and continuously updated by INRA Transfert with the collaboration of all partners. In addition to a homepage and a main presentation of the project, the website offered pages describing project activities, WPs objectives and results, events, media releases, management structure, external collaborators, links to associated projects, initiatives, and platforms, as well as pages for job opportunities and contact information.

In order to evaluate the number of the TG website visits, a counter was included via Google Analytics on each website page. The TG website received 11 000 hits since May 2010 for 26 000 pages views from 123 different countries (US, France, India, UK, Germany, Italy, China, Australia, etc), with an average number of pages view of 2.5 per visit. Among the 11 000 visitors, 77.78% were new visitors and 22.22% returning visitors. The website served as a portal for both partners, external collaborators, and the general public.



Overview of the worldwide TG website visits (from 1 May 2010 to 31 May 2012)

Dissemination of project results at scientific and technological events

The objectives of this task which involved all 17 organizations participating in the TG project were to carry out dissemination to the research community and stakeholders, through seminars, posters, media publications, invited presentations and talks, publications and appearances.

Over the course of the entire project, a total of 250 dissemination events took place in 30 countries worldwide. The presentations (talks and posters) reached a total audience estimated as 85000.

The TriticeaeGenome project poster which was updated regularly with the latest results obtained in the different WPs has been presented to several large congresses over the four reporting periods: Plant and animal Genome XVII conference (USA, January 2009), 19th ITMI-3rd COST Tritigen meeting (France, September 2009), 8th Plant GEM congress (Portugal, October 2009), 9th IPMB congress (USA, October 2009), Plant and animal Genome XVIII conference (USA, January 2010), International Symposium on Genomics of Plant Genetic Resources (Italy, April 2010), Healthgrain Symposium (Sweden, May 2010), Plant Breeding and Management for Human Nutrition conference (Finland, June 2010), Plant and animal Genome XIX conference (USA, January 2011), 9th Plant GEM congress (Turkey, May 2011), Plant and animal Genome XX conference (USA, January 2012). The TG project leaflet, TG project newsletter as well as the 2 articles published in the "International Innovation" reports from 2010 and 2012 were disseminated with the poster in these events.

During the course of the project, 20 articles referring to the TG project were published (see table A.1). The abstracts of these publications are available on the TG pubic website (http://www.triticeaegenome.eu/page.php?optim=Publications). In addition, two articles on the TG project objectives and main results, have been published in the annual "International

Innovation" Reports in 2010 and 2012, targeting decision-makers, administrators, and stakeholders (also available on the TG public website http://www.triticeaegenome.eu/page.php?optim=Press-releases). The article from the 2010 report was part of a series of reports covering emerging research and development being carried out within sustainable development as well as food and agricultural safety and quality. The 2012 report focused on successful FP7 European projects. Both of the reports were distributed to over 38'000 stakeholders across all countries in Europe, North America and the INCO countries at every level in the government, policy, research and private sector community all related to food and agriculture.

During the 1st TG project annual meeting held at IPK Gatersleben (Germany, April 2009), a joint workshop was organised with the COST Tritigen Action FA0604. In addition to the 42 TG participants, over 45 external participants attended this workshop. The workshop was organised in 2 sessions of plenary lectures, the first one on physical mapping in wheat and barley (Tritigen WG2 PhysGen) and the second one on the use of model genomes and bioinformatics resources for the Triticeae (Tritigen WG3 TraitGen and WG2A Bioinformatics).

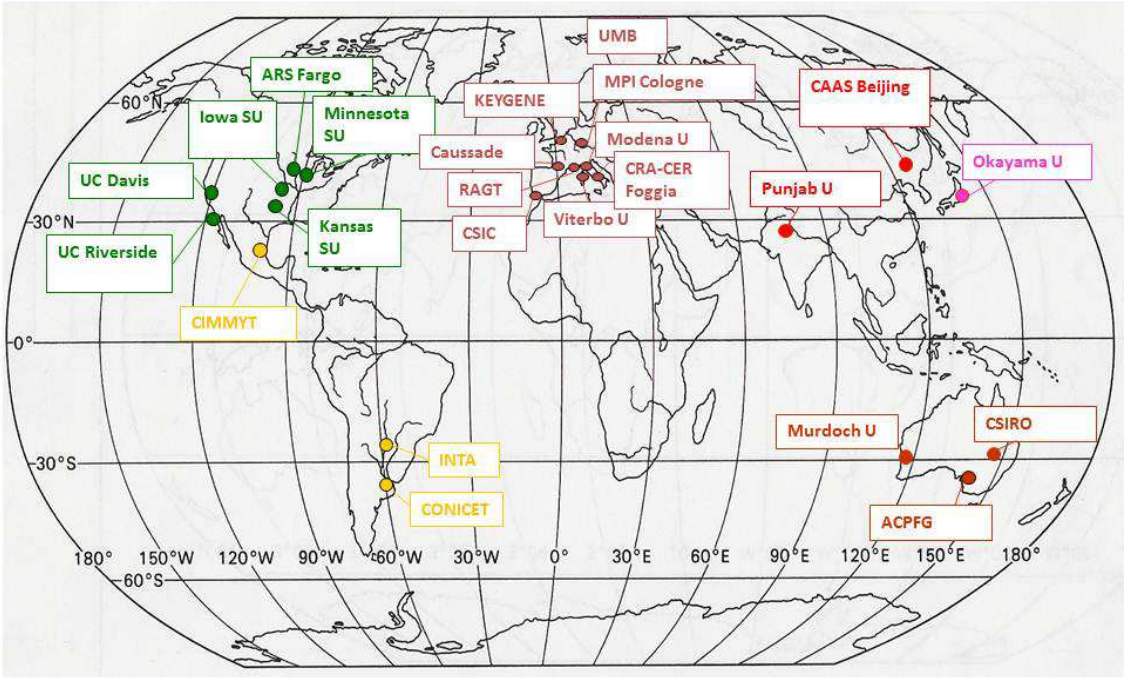
During the TG project final meeting held at INRA Versailles (France, Mai 2012), an open day was organized to disseminate broadly the final results of the project. This was well attended with 69 registered participants including 20 external persons from several breeding companies (e.g. Syngenta, Florimond Desprez, Bayer) and other public research laboratories. Three speakers were specifically invited to illustrate the impact of the TG project on other projects and initiatives. Rudi Apples from Murdoch University in Australia provided an overview on how the TG project impacted the wheat projects in Australia directly through access to results as well as from an example of a well organized large collaborative project. Marcelo Helguera from INTA in Argentina provided examples of the many collaborative projects that were established between his WHEATBIOTECH project and the TG project as a follow up of the twinning activity that was started in 2009. Finally, Kellye Eversole, the Executive director of the IWGSC provided an overview of the direct impact that the TG project had on the IWGSC projects both in terms of contributing largely to advance the IWGSC physical maps and providing a leadership in methods and technologies for the wheat genome sequencing activities worldwide. Most of the presentations and posters presented during the final meeting are available on the TG public website of a wider dissemination for the project results (http://www.triticeaegenome.eu/news.php?optimurl=TriticeaeGenome-final-meeting-in-Versailles--France).

Interactions of TG project with other projects and initiatives

During the establishment of the TG project, an external group called the Triticeae Genomics Network (TGN) was initiated with laboratories that have expressed their intention to collaborate with the project. From an initial list of 16 members, the TGN reached 24 members at the end of the project thereby indicating the interest from the community to collaborate with the TG partners and have pre publication access to the results of the project. The interactions with the TGN were well developed throughout the project duration and selected results of the TG project were exchanged regularly.

A table reporting the interactions with the TGN members and TG partners has been produced (available on the TG public website http://www.triticeaegenome.eu/page.php?optim=External-collaborators and Annex 1) and a

form was sent to the different TGN members so that they can describe the use they make of the data/results received.



Overview of the international composition of the Triticeae Genomics Network (TGN)

All TG partners originally members of the European Triticeae Genomics Initiative (www.etgi.org) continued to actively participate to networking activities that were until May 2010 supported by the COST action: FA0604 Tritigen. Most of them were also key partners of the International consortia for sequencing the wheat (IWGSC, www.wheatgenome.org) and barley (IBSC, www.barleygenome.org) genomes. All these parallel consortia ensured interactions and coordination with international projects that have similar goals as the TG project.

For example, seven members of the IWGSC attended the 3rd TG annual meeting held in Prague (Czech Republic, April 2011). This provided them with the opportunity to see all the project results and discuss with the TG partners about their transferability to the wheat community. Reciprocally, a Coordinating Committee meeting of the IWGSC dealing with the wheat survey sequencing initiative and physical mapping has been organised just after the TG meeting thereby permitting 28 members of the TG project to learn about new tools and be involved in the discussions of the wheat sequencing consortium.

The TriticeaeGenome project developed interactions at least with 5 international (e.g. ITMI, International SNP initiative, Barley genome zipper), 4 European (e.g. ETGI, Tritigen FA0604, BARCODE) and 22 national (e.g. Breedwheat-France, 3B SEQ-France, BARLEX-Germany, TRITEX-Germany, BBSRC phenotyping LOLAs-UK, AGER-Italy) projects/initiatives (see Annex 2).

Impact on the scientific community

According to the External Advisory Board of TriticeaeGenome project composed of Kellye Eversole (IWGSC, USA), Peter Langridge (ACPFG, Australia), Pierre Devaux (European Seed Association, France) and Silvia Travella (European Technology Platform Plants for the

Future, Belgium), the TriticeaeGenome has been an outstanding success and represents an example to be followed for future collaborative, multidisciplinary, public-private projects supported by the European Commission. Already, many national and international wheat projects are attempting to imitate the breadth and success of the TG project. The EAB estimates that "as a result of the TG project, there has been a worldwide paradigm shift in triticeae research from structural genomics to rapid application of results, emphasizing the strength of public-private partnerships to face the complex challenges of Triticeae production. The TG project has pioneered many methods, processes, standards, resources and tools for triticeae genomics, some of which have been adopted by many organizations including the International Wheat Genome Sequencing Consortium (IWGSC) and the International Barley Sequencing Consortium (IBSC)". A few of these pioneering results include:

- ✓ Annotation standards and the TriAnnot Pipeline;
- ✓ Refined standards and methods for efficient and accurate physical mapping of individual bread wheat chromosomes;
- ✓ A new algorithm for assembling physical maps in complex genomes;
- ✓ Gene validation tools and methods for association studies;
- ✓ Bioinformatics platforms for comparative genomics;
- ✓ The creation of the IWGSC physical map and sequence repository at the INRA-URGI;
- ✓ Refined whole genome profiling technology for application to wheat chromosomes;
- ✓ Pioneering survey sequencing of individual barley and wheat chromosomes;
- ✓ Creating high resolution mapping populations in wheat and barley and a new panel for association genetics studies in wheat
- ✓ Executing state-of-the-art training platforms that spanned the full scope of the project, from fundamental to applied genomics;
- ✓ Effectively harnessing the triple helix of innovation by leveraging academia, industry, and governmental expertise for advancing the overall field of triticeae genomics;
- ✓ Exploiting the various multi-disciplinary, multi-institutional skills and cohered these to achieve an impressive level of project success.

The aspiration of TriticeaeGenome was to play a major role in the international efforts to create the next generation of wheat and barley varieties and enable these essential species to contribute to the new Green Revolution for the sustainable production of one of mankind most important foods while preserving the agricultural habitat. The success of the project has certainly contributed to these objectives.

Among the 31 project/initiatives with which the TriticeaeGenome project developed interactions, 4 of them were built on the basis of the TG project results: the French Breedwheat project (using 1B chromosome physical map), the French 3B SEQ project (using MTP from 3B chromosome), The German 3H SEQ project (using MTP from 3H chromosome) and the German TRITEX project (using 1H and 3H physical maps information). In addition, 7 wheat genes and 5 barley genes are currently under cloning using the TG project resources (physical maps, markers, bioinformatics tools and databases).

In addition, based on the results and interactions established in the TG project, new projects are under construction at the EU level for the final round of the FP7 program. These projects involving several TG partners will be proposed in the Cooperation and People categories.

Several TG partners will be involved in answering to the call KBBE.2013.1.2-03 *"Integrated approach towards small grain cereal production and diversification in Europe"*. Up to two projects may be funded on this topic; INRA (with the support of JHI, previously SCRI, and KWL) will be the coordinator of a 'major small grain cereal crops' project and IPK (with the support of KWL) the coordinator of a 'minor small grain cereal crops' project. Both projects will be built in close collaboration with the help of INRA Transfert. In addition, an ITN coordinated by the TG coordinator is currently under construction with 8 partners from the TG project.

Impact on the breeding community

One of the objectives of TriticeaeGenome was to provide the foundation for accelerated gene isolation and perfect marker development for breeding. The construction of 3 new physical maps in wheat and the contribution to the physical mapping effort in barley, the development of thousands of new molecular markers anchored to the physical maps, the development of large recombinant populations in barley and wheat for high resolution mapping and the integration of all of these data in public databases contributed greatly to this objective.

Moreover, WP4 was specifically designed to implement molecular breeding. It was led by breeders and enabled to reinforce the relationship between public and private breeders and scientists. One of the main objectives was to create a new panel of European wheat varieties to perform association genetics studies with the markers developed in the TG project. The ultimate goal is to identify new regions of the genome underlying key agronomic traits such as yield and stress tolerance. The TG wheat panel was established by NIAB, Biogemma/Limagrain, which are two of the key EU breeding entities in the UK and France. First association studies were performed on 3 traits and the panel will continue to be used by the TG breeding partners.

Phenotype (yield, yield components, agronomic characters) and genotype data (DArT, SNPs and pleiotropic gene markers) is currently data-based at NIAB. At NIAB, the association panel is being used to fine map and characterize the yield and flowering time effects of five earliness per se (Eps) QTL as part of on-going research into the importance of these loci in efforts to optimize flowering time for specific environments without the yield penalty often associated with Ppd-D1. Biogemma is using the TriticeaeGenome data to cross validate loci already identified in internal programs and to fine map the most promising and robust yield related loci.

Arrangements are currently being made with the Germplasm Resources Unit (GRU) of the John Innes Centre in Norwich, UK to provide long-term storage of pure seed stocks for use by interested organizations beyond the end of the project. The panel is available to other EU breeders through the TG project and has been also identified as promising by other breeding programs worldwide. Seeds have been distributed already in India and Argentina. The phenotypic and genotypic data generated in the project can be available only upon request and under such conditions: If the partner agrees to contribute to increase the panel's global knowledge.

In addition, the Morex x Barke high resolution barley RIL and Renan x Chinese Spring wheat RIL populations developed in WP2 (by INRA/BGA and IPK/KWL) are highly valuable genomic resources and these material will be used not only by the broad scientific society by also by private breeding companies. This would permit to speed up the process of cloning

genes and QTLs in both species and enhance the procedure of an identification of "diagnostic markers" for the traits of agronomic importance. This would finally result in the best yielding and sustainable varieties in the farmer fields.

Taken together, these resources (data and seeds) provide public and private research institutes and breeding companies with the opportunity to develop markers for traits of interest within their breeding programmes.

5. Relevant contact details

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Annex 1: Interactions between TG partners and the Triticeae Genomics Network (TGN)

Organisation	Country	Team contact	TG contact	Explanation of the collaboration
Consejo Superior de Investigaciones Cientificas (CSIC)	Spain	P. Hernandez	Catherine Feuillet (INRA) Etienne Paux (INRA) Jaroslav Dolezel (IEB) Miroslav Valarik (IEB)	Collaboration on the transferability of Molecular markers from wheat to *H. chilense*. A student from her group visited the INRA Clermont-Ferrand in the framework, of a short term scientific mission of the COST action Tritigen FA0604. Pilar Hernandez is one of the Working group leaders of the COST action Tritigen an she is also leading the Spanish effort within the IWGSC in particular through the coordination of the 4A chromosome 454 survey sequencing with J. Dolezel. Collaborations with INRA Clermont-Ferrand on the development of ISBP markers on the 4A chromosome survey.
Università di Modena e Reggio Emilia (UMRE)	Italy	N. Pecchioni	Nils Stein (IPK)	Collaboration on contig construction across a CBF gene cluster on barley chromosome 5H. Collaboration for 454 shotgun sequencing of 96 5H BAC clones, in Modena, financed by the CariCarpi Bank Foundation (Project GENOMORE), with library preparation at IPK. In 2010, a PostDoc of UNIMORE visited IPK for 6 months; with the same aim, the same PD visits IPK for one month in 2011.
CRA Genomics Research Centre	Italy	L. Cattivelli	Catherine Feuillet (INRA)	Collaboration on the construction of the physical map of chromosome 5A within the framework of the IWGSC. Several members of the group of L. Cattivelli visited the INRA Clermont-Ferrand to be trained in physical mapping (TG training workshop) and marker development (6 months postdoctoral stay).
ARS/UC Davis	USA	J. Dvorak	Catherine Feuillet (INRA)	Co-Chair of the IWGSC. Collaboration on the isolation and characterization of genes involved in chromosome pairing in wheat and *Ae. speltoides* on chromosome group 3. Interactions on physical mapping projects within the IWGSC.
Kansas State University (KSU)	USA	B. Gill	Catherine Feuillet (INRA)	Co-chair of the IWGSC. KSU is leading the construction of the 3A physical map within the IWGSC. Collaborations are established between KSU and INRA Clermont-Ferrand on comparisons between homoeologous regions of

				chromosome 3B and 3A. The interaction with TG will enable to perform comparative genomics studies between 3A, 3B, and 3D.
Australian Centre for Plant Functional Genomics (ACPFG)	Australia	P. Langridge	Nils Stein (IPK)/ Catherine Feuillet (INRA)	Member of the EAB of TG. Collaborations between ACPFG and INRA Clermont-Ferrand on the cloning of a QTL for drought resistance on 3BL (Phd in cotutelle, J. Bonneau). P. Langridge is part of the Australian project for the physical mapping and sequencing of the wheat chromosome 7A within the IWGSC framework.
University Murdoch (UM)	Australia	R. Appels	Catherine Feuillet (INRA)	Co-chair of the IWGSC. Collaborations with INRA Clermont-Ferrand on the first physical mapping effort and on the sequencing of MB sized BAC contigs from chromosome 3B as well as on the cloning of SR2 on 3BS.
University of California – Riverside (UC)	USA	T. Close	Nils Stein (IPK)	Provided a list of 83,831 gene-positive barley BACs to IBSC partners (Stein, Waugh), compiled from work supported from a NSF project and other information received from IPK and numerous other barley researchers. Designed a specialized Illumina 1536-SNP assay for IPK. Provided the ACE file of barley EST assembly #35 to several IBSC partners (Sato, Schulman, Stein, Waugh); assembly was developed at UC Riverside by S. Wanamaker and T. Close from EST sequences provided by numerous barley researchers. Provided a preview of information from UC Riverside anchoring Illumina OPA markers to barley BACs in a physical map composed of gene-bearing BACs. Provided online access to a physical map developed from HICF fingerprinting by MingCheng Luo of gene-bearing BACs of Morex barley (http://phymap.ucdavis.edu/barley/). Provided online access to a series of draft barley genome assemblies (www.harvest-blast.org). Provided online access to a barley transcriptome assembly from deep sequencing (www.harvest-blast.org).
Australia's Commonwealth Scientific and Industrial Research	Australia	Wolfgang Spielmeyer	Catherine Feuillet, Hélène Bergès (INRA)	Collaborations on the cloning of disease R genes on chromosome 3BS: Sr2 (with R. Appels) and SV2 with the colleagues from the WHEATBIOTECH project, also member of the TGN (physical map of

Organisation (CSIRO)				3B, marker design, BAC library construction).
Okayama University (OU)	Japan	K. Sato	Nils Stein (IPK)	Prepublication access to markers from Okayama transcript map for Physical map anchoring.
University of Minnesota (UM)	USA	G. Muehlbauer	Laura Rossini (UMIL)	Co-director of the USDA-CSREES-funded Triticeae Coordinated Agricultural Project (Triticeae CAP). Collaboration established in 2007 following writing of the TG proposal when a mutual interest in the genetic control of tillering in barley emerged between the groups of Gary Muehlbauer (UM) and Laura Rossini (UMIL). Specifically, the cul4 mutant has been targeted for positional cloning within TG and GM has provided to LR F2 populations deriving from 5 different cul4 x wt crosses as well as an initial set of 8 SNP markers previously located near the target locus. These materials, along with materials developed by LR were used for fine mapping of the gene. The groups have also been exchanging information and ideas on other tillering mutants. Following the identification of the cul4 CG by UMIL, the two groups have exchanged information on the gene, as well as molecular and phenotypic data on mutant alleles they have characterized. Interactions are ongoing to exchange results and execute additional experiments in preparation for a joint publication on characterization and positional cloning of cul4.
Institute of Crop Germplasm Resources-CAAS Beijing (ICGRB)	China	J. Jizeng	Catherine Feuillet (INRA)	Collaborations with INRA Clermont-Ferrand on the analysis of the LD along chromosome 3B and the sequencing of centromeric regions of chromosome 3B.
International maize and wheat improvement center (CIMMYT)	Mexico	J. Crouch	Catherine Feuillet (INRA)	Collaboration established early on to have access to marker that will be developed within the TG project. No further exchange so far. INRA Clermont-Ferrand will organize a meeting with CIMMYT in autumn in France and use the opportunity to reactivate the collaboration.
WHEATBIOTECH (Mercosur) project	Argentina	M. Helguera (INTA) V Echenique (CONICET)	Catherine Feuillet (INRA) Roberto Tuberosa	During 2010 in the 2nd Twinning Workshop between EC and Argentina at Athens, a new collaboration was established to support the survey sequencing of chromosome 4D by researchers from

		G. Tranquilli (INTA) Norma Paniego (CONICET-INTA)	(UNIBO) Sébastien Faure (BGA) Miroslav Valarik (IEB)	Argentina within the IWGSC Initiative. In 2010 4D chromosome DNA was provided by Jaroslav Dolezel group and 454 sequenced in Argentina with the sponsorship of CONICET and INTA. During 2011, in the IWGSC Meeting at Prague a collaboration with Mario Caccamo (TACG, UK) and Klause Mayer (HMGU-MIPS, Germany) was established to cooperate in 4D survey sequencing analysis. Visit of one researcher in 2011 at TACG to learn about NGS data assembly. During 2011 through the support of WheatBiotech researchers the official support of Argentina to the G20 International Wheat Research Initiative was obtained. During 2011 significant progress in the assembly of 454 sequence from 4D chromosome was done. This information was also used in the development of ISBP and SSR markers which are being validated. in march 2012 additional DNA from 4D chromosome was used for paired end sequencing (3Kb) to further improve the assembly and building of contigs. Seed from the TriticeaeGenome 372 association panel is already in Agentina and it will be planted in two locations during 2012 for multiplication and further yield components mapping. FSacco and M Dieguez has build a BAC library from Sinvalocho which will be used for positional cloning of the SV2 slow rust resistance gene previously mapped in 3BS chromosome in cooperation with Catherine Feuillet's group.
Norwegian University of Life Sciences (UMB)	Norway	Odd-Arne Olsen	Catherine Feuillet (INRA)	Collaboration established within the framework of the TG and IWGSC to support the physical mapping and sequencing project led by Norway on chromosome 7B. Members of the Group of O-A Olsen will come to the INRA Clermont-Ferrand to be trained in physical mapping and marker development and gain experience from the 3B project.
Northern Crop Science Laboratory, USDA-ARS, Fargo	USA	Justin D Faris	Tzion Fahima (HU)	Dr. Justin Faris is working on positional cloning of a toxin sensitivity gene on 1BS. This gene is present in the cultivar Chinese Spring, the donor line of the BAC libraries that were constructed by the TG consortium. Dr Faris has

				assembled and sequenced a 650 kb contig of the target region with Langdon durum BACs, but he was not been able to verify that the contig spans the toxin locus. The group in Haifa University is collaborating with Dr. Faris in the identification of a Chinese Spring BAC contig that is spanning the target gene. The group in Haifa is providing Dr. Faris access to the 1BS physical contigs assembled in Haifa. The group in Haifa is screening the 1BS MTP pools with pairs of PCR primers closely linked to the toxin sensitivity gene in order to identify BAC contigs that are spanning the target region
KEYGENE N.V.	The Netherlands	Edwin van der Vossen	Catherine Feuillet (INRA)	INRA Clermont-Ferrand is collaborating with Keygene to evaluate the feasibility of physical mapping in wheat using the WGP technology developed by Keygene. Data from the 3B project are used in a pilot experiment and a publication is expected with acknowledgment of the TR support.
Punjab Agricultural University (PAU)	India	Kuldeep Singh	Catherine Feuillet (INRA), Nick Gosman (NIAB)	Transfer of the Triticeaegenome association panel developed in WP4.

Annex 2: Interactions between TG and relevant National, European and International projects

1) Expected information flows between TriticeaeGenome and other projects

Projects acronym, funding, dates	Connections with TriticeaeGenome	Involved partners and countries
International projects and initiatives		
ITMI	→ Potentially SNPs platform for anchoring the physical maps of TriticeaeGenome ← Provide potential platform for map based cloning of agricultural important genes and markers development	All partners working on Triticeae
IWGSC	← Give the access to the 4 TriticeaeGenome physical maps	All partners working on wheat
IBSC	→ provides strategic framework for sequencing the barley genome ← utilizes anchoring info of 1H and 3H for MTP design for sequencing these chromosomes → Brings markers for anchoring in barley ← provides physical/genetic linkage data to aid sequencing assembly	IPK (Germany), SCRI (UK), MTT (Finland)
International SNP initiative (IWGSC, ITMI)	→ Potentially SNPs platform for anchoring the physical maps of TriticeaeGenome ← Provide a list of COS SNPs markers generated from TriticeaeGenome	INRA (France), BGA (France), KWL (Germany)
Barley genome Zipper	As a template for IWGSC wheat sequencing. Used as supporting evidence for a BBSRC barley genome sequencing project in the UK	HMGU (Germany), SCRI (UK), IPK (Germany)
European projects and initiatives		
ETGI	→ Network of Triticeae stakeholders at EU level beyond the limits of a FP7 project ← Provide potential platform for map based cloning of agricultural important genes and markers development	All partners working on Triticeae
DIGITAL	→ provide whole genome ISBP-derived SNP markers as well as their position on genetic maps ← potentially provide location of markers onto	INRA (France), KWL (Germany)

Projects acronym, funding, dates	Connections with TriticeaeGenome	Involved partners and countries
	physical maps of 4 chromosomes	
Tritigen FA0604 (COST)	→ brings in markers, interactions with supporting projects in physical and functional genomics, connections to stakeholders ← provides expertise on physical mapping, map assembly, latest results on Triticeae map assembly	MTT (Finland), INRA (France), BGA (France), UNIBO (Italy)
BARCODE (ERA-PG) 11/2007-11/2010	→ provides 560,000 BACend sequences for marker development ←will use 1H and 3H anchored contigs for gene cloning	IPK (Germany), SCRI (UK), IGA (Italy)
transPLANT (EU FP7 project) 09/2011-09/2015	Integration, interconnection and maintenance of triticeae genomic resources developed during TriticeaeGenome in the broader framework of transPLANT.	INRA (France), HMGU (Germany), IPK (Germany) and many others
National projects and initiatives		
Breedwheat (Investissement d'avenir) 09/2011-09/2020	→ Will improve the anchoring of the physical maps of TriticeaeGenome ← Will provide access to newly identified genes and regulatory sequences to be used in BREEDWHEAT as well as to physical maps ← → Associations results on yield (under constraint or not) will be compared on 2 different panels (European vs French varieties)	INRA and BGA (France)
3B SEQ (ANR) 2010-2013	→ Potentially Provide a unlimited amount of markers on 3B to improve the anchoring of the 3B map in TriticeaeGenome ← Will use the physical and MTP achieved in TriticeaeGenome for 3B	INRA (France)
GAINSPEED (ANR) 2010-2013	→ Will provide large amount of markers developed from the 3B sequence for association studies done in TriticeaeGenome ←Precise location of genetic factors that will speed up the fine mapping planned in GainSpeed	INRA and BGA (France)
RYE SELECT (BMBF application) 2011-2014	→ Potentially SNPs platform for anchoring the physical maps of rye ← Provide the information about colinearity between Triticeae genomes	KWL and IPK (Germany)

Projects acronym, funding, dates	Connections with TriticeaeGenome	Involved partners and countries
CROPsRESIST (Plant KBBE application)	→ Potentially provide the information about localisation of important resistance genes on 3B ← Provide a unlimited amount of markers on 3B	KWL (Germany)
BARLEX (BMBF, Germany) 2007-2011	→ provides genome wide FPC map, anchoring and sequencing information ← Will use the anchoring info of 1H and 3H for physmap improvement; will use improved contigs fro gene isolation	IPK and HMGU (Germany)
3H_SEQ (WGL, Germany) 2010-2012	→ will deliver important information in comparative genomics between barley and wheat and other grasses ← will use MTP for 3H	IPK (Germany)
CIRC/BBSRC (applied for)	→ Potentially use association mapping panel to replicate associations for yield and quality identified in elite MAGIC population ← Replicate MAGIC associations in TG panel	NIAB (UK)
Statutory trials in UK, France, Germany	→ Use trials data from TG panel to anchor re-analyses of historical NL/RL trials series in studies of genetic and environmental trends over time. ← Historical data can be used to augment phenotypic data in the TG panel	NIAB (UK) and others
BBSRC phenotyping LOLAs	→ Potentially use genotype data from TG panel for association studies with phenotype data to come from high-throughput phenotyping studies ← Refine phenotypes for associations identified from field data.	NIAB (UK)
Italian 5A project	Provide the physical map of 5AS and 5AL chromosome arms ← Information on COS markers for chromosome 5A; CS x Renan F2 segregating population (DNA samples); software for prediction of ISBP markers in 454 sequencing data (ISBP finder) and training of people on ISBP markers The information/data obtained from INRA were used to develop markers for the physical map of the chromosome 5A.	IGA and CRA (Italy)
AGER, Italian	→ Use intron sequences of the COS in Chinese	UNIBO and IGA

Projects acronym, funding, dates	Connections with TriticeaeGenome	Involved partners and countries
project	Spring to extend the capture to 13 more genotypes of durum wheat	(Italy)
PAE37108 (WheatBiotech) (2009-2012) Association Mapping	← → Future use of genotype data from a T. durum panel provided by R. Tuberosa under the frame of TriticeaeGenome and PAE37108 twinning activities for association studies with phenotype data to come from phenotyping studies in Argentina. ← → A similar analysis will be performed with the TG association panel which is already in Argentina and will planted in two location in 2012.	MINCYT, INTA, and CONICET (Argentina)
PAE37108 (WheatBiotech) (2009-2012) 4D chromosome Survey Sequencing	← Deliver high quality 4D chromosome DNA (IEB Czech Rep.) in the frame of the IWGSC and TG projects. → Provide 4D sequence raw data (454 shotgun and 3Kb paired end data) and assemblies. Use of Genome zipper and triannot pipelines for sequences annotation, gene content and synteny studies. → Deliver ISBP and usats markers specific for 4D chromosome validated and anchored in a genetic map.	MINCYT, INTA, and CONICET (Argentina)
PAE37108 (WheatBiotech) (2009-2012) Positional Cloning of SV2	→ Provide phenotypic and genotypic data from SV2 high density maps. ← Provide the physical map of chromosome 3B and deliver new marker based on 3B chromosome sequence of cv Chinese Spring for high density mapping at the SV2 region. ← Provide de construction of a BAC library chromosome 3B from cv Sinvalocho MA, to generate the physical map of the region harbouring the SV gene.	MINCYT, INTA, and CONICET (Argentina)
TriAnnot pipeline V3.5	→ As a resource for automatic structural and functional annotation for: - The IWGSC wheat sequencing project - The ANR 3BSEQ project - The Breedwheat project → Collaboration on going for BAC sequence annotations of: - Barley – N. Stein, IPK (Germany) - Saccharum – M-A Van Sluys, University of Sao Paulo, (Brasil)	INRA (France)

Projects acronym, funding, dates	Connections with TriticeaeGenome	Involved partners and countries
	- Passiflora – L. Carneiro, University of SP/Piracicaba (Brazil) - Oak, C. Plomion, INRA Bordeaux (France)	
AGROGEN (AGL2010-17316) 2011-2013	← → Development of ISBP markers based on 4A survey sequencing	IAS-CSIC (Spain) Interactions with INRA (France) and IEB (Czech Republic)
TRITEX(BMBF, Germany) 2011-2014	→ Provides advanced genome wide FPC map with sequencing-based anchoring information ← Will use further anchoring info of 1H and 3H for physmap improvement; will use barley and wheat data for integration into comparative genomics platform	HMGU-MIPS (Germany), IPK (Germany)
BBSRC Barley sequencing 2012-2015	→ Framework maps from TG & GenomeZipper will be utilised as basis for building complete genome sequence	JHI, TGAC, EBI (UK)
BBSRC CIRC barley processability 2012-2016	→ Framework maps from TG & GenomeZipper will assist candidate gene isolation	JHI (UK)
BBSRC CIRC barley grain skinning 2012-2016	→ Framework maps from TG & GenomeZipper will assist candidate gene isolation	JHI, SAC (UK)
ExpResBar (BMBF) 2010-13	→ Use of high resolution mapping population Barke x Morex RILs for fine mapping of the target regions	KWL, IPK JKI (Gemany)

2) **Projects built on the base of TG project results** (the projects listed below must be detailed in the table above)

- Breedwheat
- 3B SEQ
- 3H SEQ
- TRITEX

3) Genes currently under cloning using TG project resources

Wheat:

- SV2 (INTA)
- Sr2 (CSIRO)
- Yr (UC davis)
- QTL yield/drought (ACPFG)
- Tin (INRA/CSIRO)

- Tsn (USDA Fargo)
- LrForno_1BS (UZH)

Barley:

- mat-a, mat-c, mat-I (SCRI)
- VRS3 (SCRI)
- Zeocriton1 (SCRI)
- Trd1 (SCRI)
- Cul4 clones and CGs identified for the other target loci (UMIL)