Triticeae genomics for the advancement of essential European crops

"TritiGen"

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A. ABSTRACT and KEYWORDS

A.1. Abstract

Europe faces the challenge of delivering safe, high-quality, and health-promoting food and feed as well as bio-products in an economical, environmentally sensitive, and sustainable manner across environments that face climatic change and increasing abiotic and biotic stresses. Triticeae cereals (wheat, barley, rye) are essential in human and domestic animal nutrition and are arguably the most important crops for European agriculture. Existing germplasm resources and current breeding methods alone are insufficient for understanding the mechanisms underlying important traits and for catalysing a quantum leap in yield, sustainability and quality improvement. Major advances in crops will require a broad suite of direct genomics approaches, built on relevant data from model plants (rice, *Brachypodium*). Such a strategy is massively complex and can only be carried out efficiently at the international level. We propose a COST Action to coordinate, focus and strengthen national and pan-European Triticeae genomics and to address infrastructure disparities. The four Working Groups will arrange workshops, Short Term Scientific Missions, a website, and joint databases and publications.

A.2. Keywords

1. Harvest Quality and Yield Stability
2. Genetic Resources and Diversity
3. Structural, Comparative, and Functional Genomics
4. Biotic and Abiotic Stress Resistance
5. Molecular Breeding Tools

B. BACKGROUND

B.1. State of Knowledge

Cereals constitute over 50% of crop production worldwide (http://www.fao.org/); their seeds are among the most important renewable resources for food, feed and industrial raw materials. The Triticeae (wheat, barley, rye, some fodder grasses) play a major role in human and domestic animal nutrition and are the most important European crops. In 2005, the EU 25 planted 41.5 Mha, or 41% of all cultivated land, and harvested 188 MMT of wheat, barley and rye. Existing germplasm and breeding methods are insufficient to provide the quantum leap improvement needed in yield, quality and minimising environmental impact for Triticeae crops. These advances will require a broad suite of genomics and post-genomics technologies building on data from model species (particularly rice and *Brachypodium*) where useful.

Scientific capabilities and resources are now converging for these species in the development of the genomics toolkit needed for achieving the key breeding goals of the 21st century i.e improved quality and functionality, adaptation to novel end-uses, sustainability and abiotic (climatic) stress and disease resistance. Current resources include whole-genome and chromosome-specific BAC libraries, extensive EST collections, DNA chips, transient and stable transformation systems as well as large wild germplasm and mutant collections.

The toolbox will include a set of integrated approaches for: recombinational and physical mapping as well as sequence-ready map assembly; map-based cloning; highly parallel (*e.g.*, microarray) analysis of transcript, protein and metabolite pools; reverse genetics methods including TILLING (Targeted Induced Local Lesions IN Genomes); efficient transformation for functional genomics; efficient haplotype analysis and markers for breeding by genotype-based selection; the bioinformatics and computational framework to integrate all of the other approaches. These tools will allow us to exploit, efficiently and cost effectively, the knowledge developed and the resources produced over the past decades to understand the genetics of agronomically important traits for barley and wheat improvement.

Breeding programmes, whether they use classical methods accessing landraces and genes from elite lines or whether they also incorporate genes introduced through transgenesis, require extensive phenotyping selection and evaluation programmes to turn breeders' line into varieties. The development of low-cost, high-throughput molecular marker methods for association genetics and progeny selection is already important though only partially realised, and remains a target for further development. The extensive EST collections for wheat and barley (> 1.5 million), assembled as community projects, are yielding SNPs for recombinational mapping of the genes represented by the ESTs. These are also being physically mapped to barley and wheat chromosomes. The EST collections also have led to the development of Affymetrix expression arrays for both wheat and barley, developed as community projects (http://harvest.ucr.edu/). New approaches, such as expression mapping and single-feature polymorphism mapping, are being developed based on the highly reproducible array data. Proteome and metabolome analyses are only now beginning, but will be important for the cereals because their functionality depends on stored proteins and glucans.

BAC libraries of barley and wheat are being fingerprinted and end-sequenced so that physical maps anchored to the genetic maps can be established for whole genome (barley) or target chromosomes (wheat). The ultimate goal is to assemble a contiguous library of BAC clones covering the Triticeae chromosomes, enabling rapid access to the numerous genes of agronomic interest that have been only identified on genetic maps so far. The physical maps will also be the foundation for future sequencing that will ultimately enable access to the entire gene repertoire in these species. Reverse genetics methods are undergoing rapid implementation in barley and wheat. Lines from mutagenised TILLING populations have been prepared by several Action partners and are being screened for phenotypes ranging from disease resistance to altered plant architecture. Transformation efficiencies have recently improved, and together with RNAi and VIGS (virus-induced gene silencing), transformation is serving as a testbed for verification of gene function.

Through considerable effort, these tools have enabled the isolation of the first agriculturally important genes from barley and wheat by positional cloning, thus demonstrating the feasibility of forging the link between genotype and phenotype in crops having large genomes. However, in order to move from these first breakthroughs to efficient and routine gene isolation and understand the regulatory mechanisms controlling key agronomic traits, a substantial, focused effort is now needed to develop the full breadth of genomic tools and technologies that will unlock these genomes and accelerate crop improvement. This COST action will provide the framework to achieving the establishment of the tools and platforms needed to reach these goals.

B.2. Reasons for Cooperation and Need for a COST Action

Coordinated action at the European level is critically needed if we are to meet the consumer needs and environmental challenges expected over the next decades. Currently, genomics is undergoing rapid development to meet this challenge, but this is taking place often in a non-uniform manner in Europe and in the absence of a mechanism of coordination. Several robust national genomic programs had already been established for wheat and barley in COST countries. Various international efforts, such as the International Triticeae Mapping Initiative

(ITMI; http://wheat.pw.usda.gov/ITMI/) and the ambitious International Wheat Genome Sequencing Consortium (IWGSC, http://www.wheatgenome.org), in which some European partners participate had been established. However, these do not provide a broad European framework. In contrast, the ERA-PG networks, in order to achieve coherent development of policies and the structures, organisations in some COST countries that fund plant genomics but it does not focus on researcher-to-researcher coordination of specific topics. Other national and international research networks, such as BarleyGenomeNet (http://www.barleynet.org), aim to improve and make accessible resources and technologies for cereal genome analysis, but are limited in their membership. Several European laboratories are leading or participating in these initiatives. Pilot Triticeae genomics studies had been initiated in individual European laboratories, supported by national programs or through limited trans-national collaborations, and a few EC Framework program projects have begun (e.g., BIOEXPLOIT, DEVELONUTRI).

The research agenda described above have characteristics that make their development and application within an Action essential. First, development costs are too high for any one group or country to take on the entirety for a given crop. Second, the tools together form an integrated whole, a "toolbox," that is best deployed on a trans-national, collaborative basis. Furthermore, because the approaches are *generic* in that they can be applied across species and research problems, a network increases the tools' efficient deployment across Europe. Finally, because development of these approaches is now in a critical, rapid phase, coordination is extremely timely and will yield benefits for years to come. The ultimate aim is to create a networking infrastructure, which does not exist currently in Europe, through which research objectives can be defined, tools collaboratively developed and applied, and the benefits shared in a pre-competitive manner across the COST nations.

B.3. Complementarity with Other European Initiatives

The proposed Action is complementary with several ongoing projects and initiatives and provides a link between them. An FP6 Integrated Project, BioExploit, Contract FOOD-CT-2005-513959, aims to foster a breakthrough by developing efficient and rational breeding strategies using genomics and post-genomics tools to exploit natural host plant disease resistance. It focuses on wheat and potato. The FP6 Coordination of Research ResistVir (FOOD-CT-2005-006961) has the goal of improving co-ordination of research on genetic resistance of European crops to plant pathogenic viruses/vectors. It is focused on viruses and virology rather than the fungi of BioExploit, and is very broad regarding both fields of research (genetics, pathology, IP and patenting, transgenesis). The FP6 DeveloNutri project, presently in the negotiation phase, aims at establishing a robust metabolomics platform to optimise the nutritional value of crops, including wheat. The TritiGen Action has synergy with ResistVir regarding the use of genomic tools in studying response and resistance to viruses. In addition, there were several applications during 2006 to the EUROCORES Scheme of the ESF and to the 1st call of the ERA-PG involving one or more of the partners in the TritiGen Action.

B.4. Why COST is the Best Framework

A COST Action provides the best framework for this proposal because of the opportunity to create a network with a broad geographical base, including Central and Eastern European partners, to develop the non-competitive and pre-competitive foundations of genomics to solve a wide variety of specific problems. The specific research goals being addressed by both national and European research in the frameworks of FP6, ERA-PG, and EUROCORES projects provide the information and achievements that require the coordinating function offered by a COST Action. Genomics research is inherently generic and expensive, necessitating a transnational approach to coordinate and synergize efforts and maximise value for money, identify centres of excellence that can offer training to other COST partners thereby improving the transfer of knowledge and skills among the different EU countries and support the training of young scientists in new genomics technologies and new breeding methods. Given the differences in genomics infrastructure development across the COST countries, the proposed Action will also support the dissemination of both know-how and infrastructure development for all partners.

C. OBJECTIVES AND BENEFITS

C.1. Objectives

The main objective of this Action is to develop the technology platforms and coordinating projects that will provide efficient tools to identify and exploit qualitative and QTL alleles for improving wheat, barley and rye. Loci that confer resistance against fungal diseases (e.g., *Fusarium*, rusts, net blotch and powdery mildew and several viral diseases) are among the targets. Others, equally important, are loci that promote tolerance to abiotic stresses, such as growth on marginal land and tolerance to drought and cold, in order to cope with climate changes effects on crop yield and quality. Further target loci control quality (e.g. nutritional value, celiac disease and antioxidant (e.g. carotenoid) content in wheat, and malting quality and fibre and phytate content in barley), yield stability under low input (nitrogen
-use efficiency) and efficient biomass conversion (starch synthesis, cell wall composition for biofuels and bioproducts). Limiting the area under cultivation is an important objective for environmentally sustainable production. To this end, increases in cereal yields can be achieved by manipulation of plant architecture. Target agronomic traits include plant stature, tillering, grain, root and inflorescence architecture and leaf arrangement.

The secondary objectives are:

1. Survey and database of existing and planned Triticeae genomics research, platforms and applications in Europe; production of a list and review publication of recommended developments
2. Development of new tools and platforms for “omic” and bioinformatic analyses, portal for those recommended.
3. Publications and database regarding new and efficient methods for linkage mapping and molecular breeding
4. Collaborative development of comparative genomics for cereals and grasses; joint publications and a database.
5. Coordinated development and collaborative application of high-resolution mapping populations in the Triticeae; a publication and database of existing and planned populations.
6. Coordinated transfer of know-how and tools needed to manage, maintain, and exploit natural genetic diversity; production of a handbook on state-of-the-art methods.
7. Coordination on high-throughput phenotyping facilities for effective association genetics
8. Development of a framework for Triticeae physical mapping.
9. Triticeae Genomics edited book [under separately applied ESF funding]

C.2. Benefits

The deployment of new, environmentally sensitive crop varieties, which are capable of maintaining yield and quality across increasingly precarious environments in the face of climatic change and increasing stresses from pests and pathogens, will promote the well-being, competitiveness and prosperity of Europe. Furthermore, because it targets staple crops, outputs of the Action will be of strategic importance in securing World food supply. Meeting these challenges through timely deployment of new varieties requires increasingly rapid and sophisticated breeding. Varieties that have a high market value and fulfill demands for higher quality and lower environmental impact are needed. The tools that will be developed within the collaborative projects among this Action's partners will further reach these goals. The major increases in genomic information deriving from the coordinated COST network will allow the breeders to query gene banks (germplasm collections) in order to uncover and harness the allelic diversity needed for crop improvement. Tools developed in TritiGen will furthermore provide solid support for the implementation of marker-assisted selection in breeding. This topic is now attracting attention from leading Biotech companies (see http://www.seedquest.com/forum/ p/PickCharles/ august06.htm). Thus, the TritiGen Action aims at supporting the development of a platform to efficiently identify and select traits of agronomic importance that will benefit European breeders and in turn farmers and consumers. The tools developed by the COST partners also will improve the preservation and utilisation of biodiversity in European *in situ* and *ex situ* gene banks. We expect that the improvements gained will allow the utilization of cereals for non-food products and cereal straw for bioenergy to supplement other energy sources. The tools and and benefits of research coordinated within TritiGen are diagrammed in Figure 1.

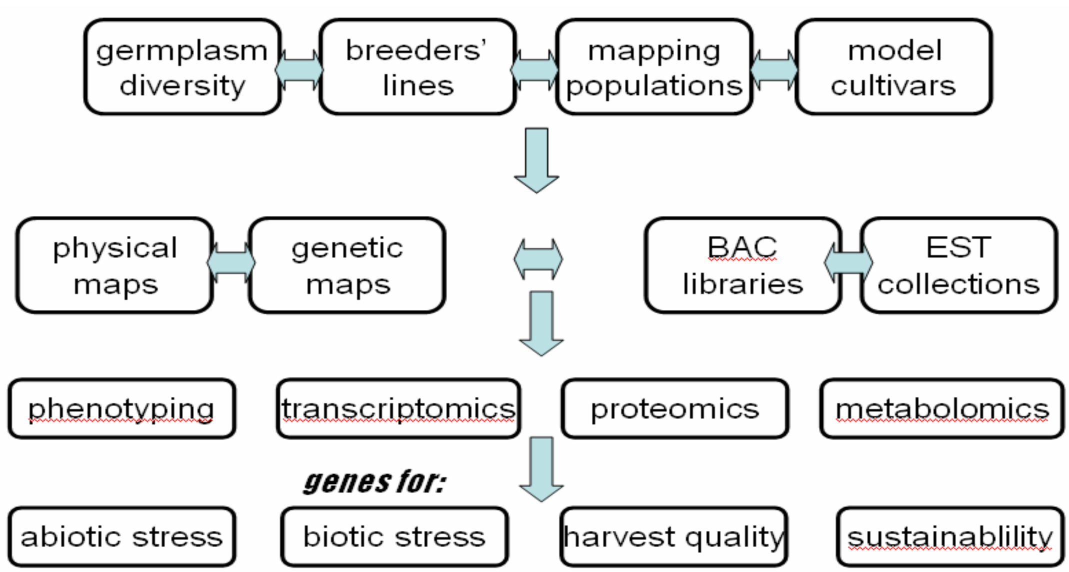


Figure 1. Objectives and Benefits of TritiGen

Lastly, the COST action will stimulate not only applications, but also basic research in this area, promoting scientific excellence in genomics and the plant sciences generally. As we move beyond the current model systems (Arabidopsis, rice) to crop plants important to Europe, insights into biological phenomena not relevant to these two model plants will also emerge. Furthermore, comparative genomics will give insight into genome evolution and evolutionary selective pressures. This only becomes possible when large-scale sequencing in different species is carried out. Europe has taken the lead in some of the research areas for wheat and barley genomics in the past two years and the support of a COST action will greatly help to strengthen this position and allow more EU partners to be involved in international initiatives. The development of the genomics tools and knowledge that are planned in this COST action should accelerate map based cloning of a number of genes and QTL that are involved in key traits for the EU agriculture. This includes:

Biotic stress resistance

Biotic stress resistance generally, and durable disease resistance in particular, is an important but often elusive goal in breeding programmes in most countries. Climate change, furthermore, makes disease resistance a moving target because pathogen profiles shift as the climate does. As the major staple crops of Europe and the COST countries, wheat and barley are subject to a number of fungal diseases in particular that can have devastating impact on harvest stability and yields. The research undertaken and coordinated in this COST Action will have direct impact on the discovery and application of novel disease resistances genes.

Abiotic stress resistance

Drought, low temperature and salinity are the most important abiotic stress factors limiting crop productivity. Abiotic stress will increase with global warming due to increases in drought, storms, and soil salinity. These will be important throughout Europe, but will also have relevance to the developing world and especially to agriculture in marginal areas. In addition to map based cloning, systems-based analyses spanning transcription, translation, and metabolite –omics will be particularly important for identifying the genetic basis of abiotic stresse resistance

Harvest quality (incl. starch, protein, fibre, carotenoids, phytate, gluten allergenicity)

High-quality and health-promoting food produced in a sustainable and efficient manner is a key to affluence in the COST countries. Arguably, it also represents the most efficient path to well-being because it goes hand in hand with preventative public health strategies for aging European populations. Hence, quality represents the most valuable property of germplasm to improve, and also represents an alternative to expanding the area under cultivation. For example, improved grain digestibility leads to a higher grain to meat conversion efficiency, decreasing the yield needed to sustain livestock production. Starch quality, particularly the position and extent of amylopectin branching and the amount of amylose, affects starch crystallinity, retrogradation, and properties ranging from bread staling and beer caloric content to hypoglycaemic index in foods and ethanol yield for bio-fuels. Protein content and quality in wheat and barley has direct implications for human and animal nutrition as well as malt production. Phytate content and availability in grain affects both human and animal nutrition as well as phosphorous in soil and hence groundwater quality. Fibre content in grains is an essential component of barley and wheat quality because of its relevance to cancer, diabetes and intestinal function. Carotenoids are antioxidant pigments that give the typical yellow pigmentation to wheat (e.g., durum wheat) endosperm. The research planned by the partners of this Action addresses these key quality traits of Triticeae grains. Coeliac disease is a common hereditary food-related intestinal disorders, caused by T-cell responses to peptides derived from gliadin and glutenin proteins of wheat, barley and rye. Furthermore, non-heritable IgE-mediated allergic responses to Triticeae grain proteins are very widespread. Reduction of these problems with the aid of genomics is an important goal of TritiGen partners.

Agronomic sustainability

Increasingly intense agriculture has resulted in the simplification of rural landscapes through the expansion of agricultural land, enlargement of field size and removal of non-crop habitat. These changes are considered to be an important cause of the rapid decline in farmland biodiversity, with the remaining biodiversity concentrated in field edges and non-crop habitats. The programme of TritiGen will bring two benefits in this regard: First, environmental sustainability will greatly benefit from improved yields and more rational land use, because diversified landscapes hold the most potential for biodiversity conservation. Yield increases can be obtained by manipulating plant architecture and physiology. Second, the current effects and pressures on rural biodiversity can be tracked effectively by the marker methods under development in this Action.

Specific benefits of the proposed Action will include:

1. Effective assessment, management and exploitation of natural genetic diversity
2. Coordination and development of new and relevant high-resolution mapping populations
3. Establishment of new methods and tools for genetic analysis and molecular breeding
4. High throughput gene isolation through the establishment of physical maps and the sequencing of target regions of agronomic interest, use of comparative genomics to optimise gene discovery
5. Coordination of high-throughput phenotyping facilities for association genetics
6. Enhancement of Triticeae transcriptomics, proteomics and metabolomics platforms
7. Efficient comparative genomics with other cereals and with forage grasses
8. Development of new tools and platforms for genetic and bioinformatic analyses

D. SCIENTIFIC PROGRAMME

Tools to be used by in TritiGen are shown in Figure 2.

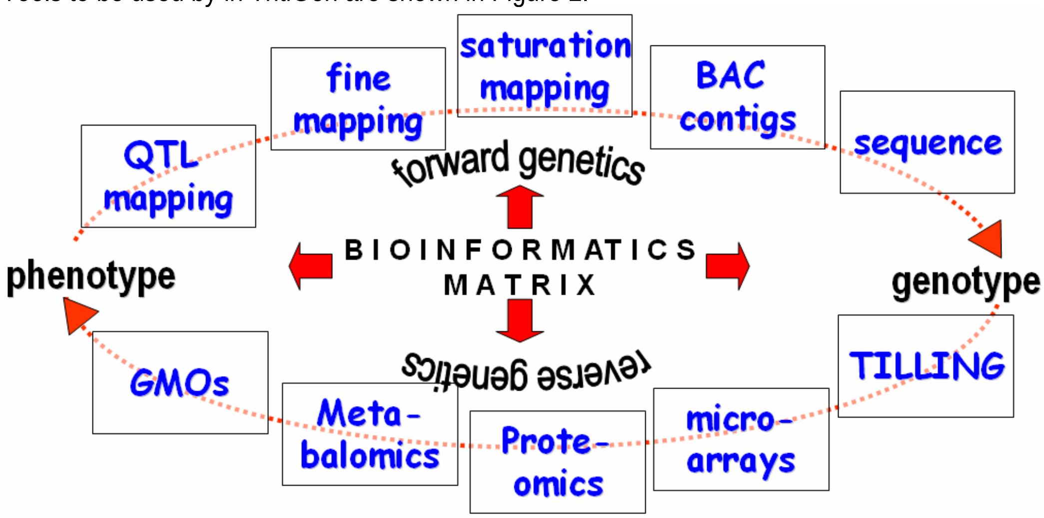


Figure 2. Tools and approaches in the TritiGen scientific programme

Tools for Assessing, Harvesting and Applying Genetic Diversity (WG1)

Development and deployment of more rapid and robust, less expensive, and denser molecular markers is a continuing area of development and attracts great interest both for basic research, pre-competitive but applied work, and in practical breeding and germplasm management. There will be a continuing need both to sample the genetic diversity of the genome and have the ability to recognize specific allelic forms. The most commonly used methods currently are microsatellites, AFLP, retrotransposons and single nucleotide polymorphism (SNP). The area undergoing perhaps the most rapid development is SNPS. Action partners are currently mining wheat and barley SNPs from EST databases, testing and mapping them in crosses and linking them to physical maps. In order to minimise both segregation with genes of interest and linkage disequilibrium not linked to the trait itself, the ultimate goal is to develop one SNP per 50 kb, or roughly 100 000 SNPs for the basic seven-chromosomes of the Triticeae. Partners in TritiGen will apply markers to assess germplasm diversity, phylogeographic patterns of diversity and haplotype, marker-assisted selection, association genetics and genome evolution studies.

Accessing the Physical Genome for Sustainability and Quality (WG2)

Physical Map. Through much effort, the genomic tools developed recently for the Triticeae have enabled the isolation of the first agriculturally important genes from barley and wheat by positional cloning. The next steps towards unlocking the Triticeae genomes include developing contig-based physical maps for barley and hexaploid wheat genomes that will serve as the basic resources for high-throughput gene isolation and large-scale sequencing. This will require linkage of contiguous physical sequences (in the 1000s) to the recombinational maps by very high density placement of genes and markers. The maps of barley and wheat will be anchored by common markers to facilitate comparative genomics. Mapped anchor ESTs will be used for integrating the genetic and physical maps of both species, maximizing the efficiency of exploiting genome colinearity to target and isolate genes for traits. These achievements will make possible a la carte sequences of high-priority regions.

Sustainability and quality. The tools to be developed in WG1 and WG2 will open several key avenues to sustainability and quality. The overall *synteny* (parallel sets of chromosomes) and *colinearity* (genes in the same order) of the Triticeae genomes means that having parallel physical and genetic maps in the each species aids in finding new genes and alleles in all. For example, disease resistance genes are under relatively rapid evolution, but tend to be clustered in common areas of the chromosomes. Localising a cluster of resistance genes in wheat opens access to the syntenic cluster in barley and rye, where the pathogen pressures and consequent evolutionary changes have likely been different. Furthermore, the genes controlling fundamental aspects of plant architecture (grain development, root and shoot morphology) are likely to be ancient. Exploitation of the physical map and extensive sequences from syntenic regions carrying such genes will provide a path into analysis of variation in their function. Moreover, access to the physical genome will help to increase the genetic input into breeding programs.

Finally, colinearity between the Triticeae genomes and the sequenced genomes of rice and *Brachypodium* will be exploited wherever possible to aid the development of structural and functional Triticeae genomics. Despite a lack of whole genome colinearity, rice has proven useful in providing additional markers for fine mapping. A sequencing project as well as the construction of a physical map and the generation of functional resources is underway for *Brachypodium* (JGI/DOE). These resources will also be useful in providing markers for anchoring the barley and wheat physical maps.

Implementation of Genomics Approaches for Understanding Cereal Traits (WG3)

Many or most traits of interest in the Triticeae that control the sustainability and value of the crop, ranging from biotic and abiotic stress resistance to grain quality and plant architecture, are derived from the action of multiple, interacting genes. Understanding and improving these traits necessarily requires genomics tools. Technologies to identify differentially expressed genes and to analyse gene expression levels on a genomic scale are being applied by a number of TritiGen partners. In particular, Community-wide efforts have led to the development of common barley and wheat microarrays on the Affymetrix platform. Some partners have cDNA array platforms; these are useful for Triticeae species that do not hybridise well to the Affymetrix arrays. The new availability of high-throughput sequencers is leading some partners to introduce massively parallel signature sequencing (MPSS). Such “open” approaches to gene expression will complement the "closed" microarray approaches and also lead to improvement of the microarray oligonucleotide sets. TritiGen will coordinate use of, and data analysis from, these platforms.

Proteomics tools are relatively underdeveloped among the TritiGen partners, compared with the transcriptome techniques. Partners have two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) setups as well as access to more sophisticated tools. These include tandem mass spectrometry (MS-MS), two-dimensional liquid chromatography (2D-LC), also coupled to MS-MS, and automated MALDI-TOF systems. Likewise, analysis of the metabolome remains a major development target for such important systems as developing Triticeae endosperm. Development and application of metabolomics will be most efficient in a few centres of excellence, supported by widespread collaborations within the TritiGen framework.

Implementation of these approaches is at the core of the TritiGen research plan. For example, for biotic resistance traits, microarray analysis for the identification of resistance gene candidates as well as genes in the defence response pathways will be extremely important. Application of resistance genes in variety development will rely on efficient marker-assisted selection (MAS), which is dependent on efficient molecular markers linked to the genes being introgressed. Abiotic stresses, particularly drought, low temperature and salinity, place major limits on cereal productivity. Crop species belonging to the tribe Triticeae represent, world-wide, the main foodstuff sources for men and animals; they are cultivated from the Arctic to the Sahara margins. Such a great geographic range already suggests that the Triticeae genomes should contain genes for wide environmental adaptability and good stress resistance. The identification of the genetic components of stress tolerance is, therefore, a requirement to ensure further breeding progress since the traditional selection process has met only limited success due to genotype × environment interactions.

Complex traits such as grain quality or plant architecture is comprised of many factors, among them starch structure, protein content and the presence of epitopes for allergic response, phytate and mineral content and availability, fibre content, and carotenoid content. The research during this Action will explore strategies aimed at identifying candidate genes controlling these traits, positionally cloning them, verifying their function, and developing closely linked markers for the deployment of the best alleles through MAS. This strategy represents a major goal for the next decade. These traits may be conferred by multiple genes whose products cause major changes in cell physiology.

Functional Genomics for Testing and Validation of Candidate Genes (WG4)

Combining the structural resources (WG1,2) with emerging functional genomics tools will facilitate the characterisation of key traits and the validation of candidate genes for streamlined marker-assisted selection, genetic engineering and further application-oriented research. A set of functional genomics approaches are being developed by TritiGen partners, including RNAi strategies to assess gene function, transient and stable transformation, TILLING populations for reverse genetics, and SNP association mapping. The functional genomics tools, displayed on the lower, "reverse genetics" side of Fig. 2, enable one to associate particular candidate genes to traits. The approach represents, for the genome as a whole, a major goal due to the large number of genes and their interaction.

One central strategy to assess the role of gene is to remove it or suppress it. Because "knock out" of gene function by homologous recombination is not available in higher plants, TritiGen partners are taking a number of other approaches. Transformation is being used to introduce antisense constructs or to achieve co-suppression by over-expression, both of which reduce the level of endogenous transcripts of the target gene. This is now being complemented by the post-transcriptional gene silencing approaches of RNAi and VIGs, which are being developed by partners for the Triticeae. Advances in Triticeae transformation by partners in TritiGen is also making possible insertional mutagenesis strategies applying T-DNA and transposons. TritiGen partners are actively developing TILLING populations, which contain mutagen-induced point mutations, intended for collaborative exploitation. The transformation, and mutagenesis approaches will necessarily be combined with transcriptome, proteome, and metabolome analyses as described above for WG3.

Bioinformatics (WG1 and others)

The genomic analyses described above will generate huge amounts of data of different kinds that will need to be integrated with phenotype and pedigree information. This represents a major challenge within TritiGen. Many of the Tritigen partners have bioinformatics units or researchers working on particular types of datasets. The TritiGen Action will seek to bring them together in new collaborations so that data can be mined using common platforms or interfaces.

E. ORGANISATION

The proposed COST Action, TritiGen, will be organised into four interactive Working Groups (WGs), which are listed below. They will serve to coordinate research by the COST Action participants on the topics included under the WG. Their pattern of interaction and synergy is shown in Figure 3, where they are labelled as well by their acronyms. The Working Groups are organised "horizontally" by the types of tools they apply and by the types of data and analyses carried under the research projects and programmes of the participants. The groups are also organized "vertically" by the main practical objectives of the participants: biotic stress resistance; abiotic stress resistance; grain and harvest quality; agronomic sustainability. These are described in more detail in Section C.2, Benefits, above. The third dimension is the particular Triticeae species (e.g., wheat, barley, rye). For maximum synergy, these are not separated within the working groups or into their own working groups.

1. <u>Tools for Assessing and Harvesting Genetic Diversity (DivGen)</u>

   Topics include: molecular marker methods and their high-throughput implementation and applications; Triticeae genetic diversity and phylo-geography; statistics & bioinformatics of diversity data; molecular cytogenetic approaches for probing evolutionary dynamics; statistics and bioinformatics of diversity data

2. <u>Accessing the Physical Genome for Sustainability and Quality (PhysGen)</u>

   Topics include: physical maps and contigs; large-scale sequencing; bioinformatics for assembly and annotation; exploitation of synteny

3. <u>Implementation of Genomics Approaches for Understanding Cereal Traits (TraitGen)</u>

   Topics include: transcriptomics platforms; proteomics; metabolomics; systems approaches to data integration. Traits include: abiotic and biotic stress resistance; harvest and grain quality; sustainability; plant architecture and development

4. <u>Functional Genomics for Testing and Validation of Candidate Genes (FuncGen)</u>

Topics include transient and stable transformation; VIGS; RNAi; T-DNA tagging; TILLING and SNP association genetics; forward and reverse genetics tools.

The leaders of each WG will be approved at the kick-off meeting. There have already been exchanges among those preparing the Action's proposal on this subject and a consensus is emerging. Each WG leader will be tasked with coordinating the activities within the WG, ensuring that the objectives of the WG are met in a timely fashion and preparing reports for WG and Management Committee (MC) meetings. They will also be expected to direct the organisation of WG meetings and sessions as well as to oversee the interaction with the other WGs as well as with other relevant Actions and European projects.

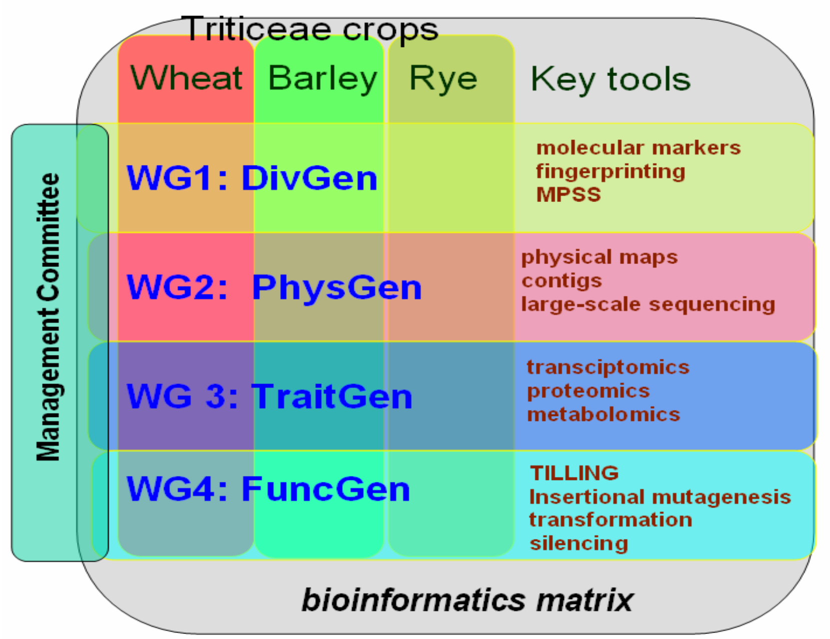


Figure 3. TritiGen Working groups

Overall management of the TritiGen action will be carried out by the Management Committee (MC). The MC Chairperson and the WG leaders will together prepare meeting formats, agenda, and minutes. The MC Chairperson will also liaise with COST office. In addition to the WG leaders, a person in charge of coordinating the Short Term Scientific Missions (STSM) will be selected at the first meeting. The STSM coordinator will be a member of the MC. A person will also be selected as Webmaster to oversee maintenance and development of the website. He or she will be from the MC. Another person will be selected as Dissemination Coordinator from among MC members.

Coordination will be achieved by several means. The MC itself carries out its internal coordination via email and by two annual meetings held in conjunction with WG meetings. Coordination of research carried out by members of the Action, the key objective of TritiGen will be accomplished through two annual workshops, two larger conferences during the course of the Action, STSMs, and the member area of the website (discussed in more detail below). In addition, *ad hoc* small group meetings (SGMs) will be arranged for intensive workshop-style progress on such issues as database compatibility and software mining tools, map construction, or coordination of genome sequencing tasks. Results from SGMs will be presented in the WG meetings and conferences as well as on the Action website.

The WG meetings will form the core interactive forums of TritiGen. Two will be held annually. At each meeting, alternating WGs will be paired in series, e.g.: 1+4, 2+3, 1+3, 2+4, in order to optimise between frequency of meeting, intimacy and manageability of meeting size, and maximal cross-interactions. In addition, all four WGs will meet, besides at the kick-off meeting, at a larger mid-term (second year) and final (fourth year) conference. Industrial participants will be invited to the WG meetings and conferences as will leading experts from outside the COST countries with whom large-scale research collaborations in Triticeae genomics are underway. Such collaborations are in place with partners in Australia, Japan, and the USA. Participants from the COST Near Neighbour countries, many of whom have already been identified during preparation of this Action, will likewise be invited to participate in the WG meetings and in the conferences.

Where appropriate, common meetings and cross-invitations will be directed to participants of other COST Actions, especially those in Actions 853, Agricultural Bio-Markers for Array Technology (until 06/03/2007), and 860, Sustainable low-input cereal production: required varietal characteristics and crop diversity (until 10/05/2008), as well as in other Actions that are still pending, such as 872, Exploiting genomics to understand plant–nematode interactions (until 01/09/2010), and others not yet approved but which may start during the period of TritiGen (this includes a current proteomics proposal which has clear synergy with TritiGen). Furthermore, joint meetings and other forms of collaboration will be sought with EU projects (such as BIOEXPLOIT and DEVELONUTRI) and others in EUROCORES and ERA-PG relevant to TritiGen.

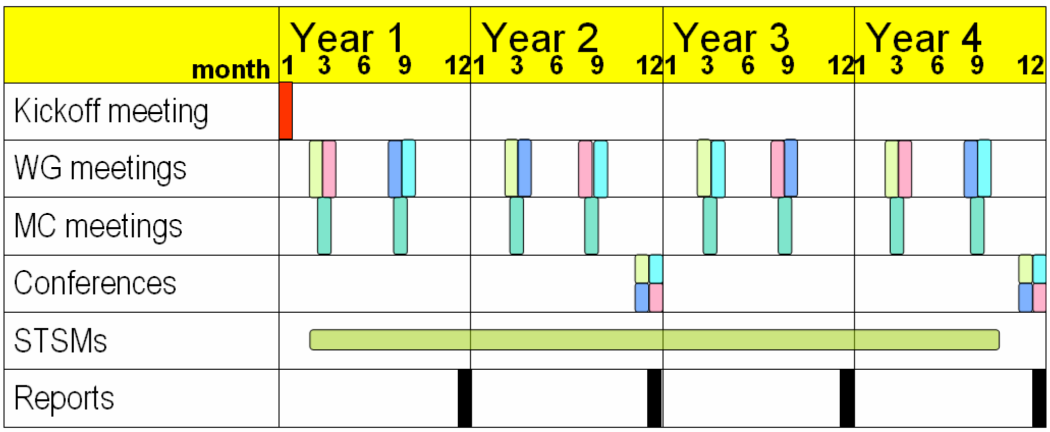
A STSM programme will be initiated to allow exchange of scientists. This will be an effective way to start and energise collaborations, to make new equipment and methods familiar to the Action participants, and to standardise experimental and analytical approaches so that large-scale tasks can be effectively organised. The STMS programme will apply to scientists in all four WGs, and will be seen in particular as a way of promoting the involvement of young and promising scientists in international collaborative projects and in mobility for advanced training.

At the outset of the Action, a website will be set up that will serve several purposes: explaining the Action to the general public and interested scientists; familiarising potential, but unaffiliated participants; disseminating Action deliverables to interested parties; coordinating the Action and research undertaken by its participants. These objectives will be met by segmenting the website into several sections. The homepage will provide general information, as well as links to other pages listing the participants and contacts, upcoming meetings, and working groups. There will be information on how to join the Action linked to the homepage as well. Databases, publications, public software and other outcomes of the Action will be available through pages that require registration but that will otherwise be open. This will enable the Action to make contact with interested parties. A blog-style newsletter will be present in this section of the website. The third layer of the website will comprise a Members' Area, which will be a portal that is password protected. The portal will provide means of distributing both administrative tools such as travel claim forms and deliverable reports and documents and news relating to the research coordinated under the TritiGen Action. It will have separate areas for each WG. It will also provide a means of rapidly contacting members of specific WGs or the entire Action. The participants will have the right to deposit information and to modify files via the portal so as keep the website updated. The Webmaster will ensure that files intended for the public areas will be kept updated.

F. TIMETABLE

The TritiGen Action is planned for four years. This length of time is justified by the scale of the Triticeae genomics effort and the coordination plans for the Action. The position of each activity relative to the start date is shown in Table I. The colours correspond to those of the WGs in Figure 3. At the kick-off meeting, in addition to partners formally acceding to the Action, the WG coordinators, the Webmaster and the Dissemination Coordinator will be nominated and selected. The first WG meeting is at month 3 in order to permit time for organisation following the kick-off meeting, and will be held every six months. MC meetings will be planned to coincide with the WGs, and are envisaged as taking place twice a year. The two larger conferences will be held at the middle and end of the Action.

Table I. Timetable for TritiGen COST Action



G. ECONOMIC DIMENSION

The following 27 COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Iceland, Israel, Italy, Latvia, Lithuania, Netherlands, Norway, Poland, Portugal, Serbia and Montenegro, Spain, Sweden, Switzerland, Turkey, United Kingdom. On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at 16.9 M€ for the total duration of the Action. This estimate is valid under the assumption that all the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

H. DISSEMINATION PLAN

The TritiGen COST Action goals, activities, and outcomes will be disseminated to the various target groups and societal sectors as is outlined in Table II. The central source for updated information will be the Action website, which will provide paths to the other channels of dissemination. The databases and common protocols that are deliverables of the Action will be linked to the homepage after, or in lieu of, conventional publication. The secure web portal will serve as a workspace for Action partners to build these resources, as well as a common notice board and blog-style newsletter. At the outset of the TritiGen Action, we will prepare a colourful PDF brochure or flyer that will be distributed via email lists of partners and of organisations such as EPSO (European Plant Science Organisation), as well as made available at the website. The email list will be expanded via registration at the website, and will be used to distribute information about WG meetings and Conferences, as well as about new documents and outcomes available at the website. The semi-annual WG meetings will, nevertheless, serve as the most important tool for dissemination and networking within the Action. The larger Conferences will be held twice during the Action, and will bring together participants, from a wide range of sectors and disciplines, who had earlier become aware of TritiGen through other dissemination means. A uniquely European aspect of this Action is that European breeders of barley and wheat are generally small companies, even family-run businesses. They are dependent on networking to become aware of new developments because their size permits no more than breeding and marketing. Our dissemination efforts will, through seed and breeding associations, be aimed also to reach them.

One means of spreading familiarity with TritiGen will be a collective poster to be used by participants travelling to scientific meetings. The poster will be updated as necessary during the Action and distributed to the partners as a PDF file for local printing. In addition, research collaborations by TritiGen partners will be encouraged to prepare scientific posters mentioning the Action for presentation at conferences. An Action-wide competition for a TritiGen logo will be held at the Action outset to increase our recognition. The multitude of research collaborations and projects within the Action will lead to joint scientific papers, which will also cite TritiGen. Finally, partners will be encouraged to prepare news releases in their national languages for distribution to the media, based on the summary and technical annex as well as emerging results. To coordinate these dissemination activities and to report on them to the MC and WG leaders, a Dissemination Coordinator will be selected at the kick-off meeting.

Table II. Means of Dissemination and Target Groups

Dissemination event	Quantity	Target
Website	1	Public, Industry, Academia, Governments, EC, EP
Databases & Protocols	Multiple	Academia, Industry
Website partners' portal	1	Partners
PDF brochure	100s	Academia, Industry, Governments, EC, EP
Email list	100s	Academia, Industry, Governments, EC, EP
WG meetings	16	Partners, Academia, Industry
Action Conferences	2	Partners, Academia, Industry, Government
Collective Action posters	1 – 4	Academia, Industry
Scientific posters at conferences	4 – 10	Academia, Industry
Collaborative scientific papers	Multiple	Partners, Academia, Industry, Government
News releases and popular media	Multiple	Public, Industry, Government

TritiGen

Part II

Additional Information

A. History of the proposal

Discussion of launching a COST proposal date bact to the establishment of the European Triticeae Genomics Initiative (ETGI, http://www.etgi.org), which was launched in 2005. ETGI collected a list of European scientists from both the public and private research sectors. Two exploratory workshops were held at international meetings attended by subsets of the interested groups. Members of ETGI drafted two research White Papers, for wheat and barley, and circulated them among interested parties in the EC. However, ETGI is a bootstrap initiative and has no funding for research, coordination, meetings, or other activities. This COST proposal represents an outgrowth of ETGI with the objective of providing the means to coordinate and concentrate the various new European Triticeae genomics efforts in universities, research institutes and companies across Europe to develop a platform that will efficiently support the development of a competitive and sustainable agriculture in the coming decades. Active discussions with partners in France, Germany, Denmark, Norway, Italy and the UK in particular led to the nucleating of ideas and the drafting of the Prleiminary Proposal. The network as it exists presently was substantially in place by May 2006.

B. List of experts involved in preparation of this proposal

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C. Recent Publications of the partners of the proposal

The five most recent and relevant publications of each partner is listed. Partners from whom no lists were received at the time of filing are shown in small case.

1. Austria

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CRAW

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8. Estonia

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9. Finland

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D. Companies interested in this Action

Company	Business area	Contact Person	Country, www page	WG interest
Agroservice ISEA	Seed company	Dr. Tassinari, Dr. Langella	rlangella@iseasementi.com http://www.sementi.it/associate/cereali.html	WG1 WG4
Lochow - Petkus GmbH	Breeding of cereals	Dr. V. Korzun	www.lochow-petkus.de	WG1; WG2; WG3; WG4
Keygene N.V.	Plant molecular genetics	Anker P Sørensen	The Netherlands www.keygene.com	WG1, WG4
Metapontum Agrobios	Plant Biothechnology	Dr.Francesco Cellini	Italy www.agrobios.it	WG4
Apsovsementi *(ETGI supporting industry)*	Wheat breeding	Dr. Carlo Invernizzi R & D Manager c.invernizzi@apsovsementi.it	Italy, www.apsovsementi.it	WG1 WG3
Saaten-Union Resistenzlabor GmbH	Molecular marker, Mapping, Cereal Plant Breeding, Tissue Culture (Mapping populations), Genetic Transformation	Dr. Jens Weyen	Germany, www.saaten-union-labor.de	WG1 WG4
Nordsaat Saatzucht	Plant Breeding	Dr. L. Kuntze	http://www.saaten-union.de	WG1, WG4
Saatzucht Breun	Plant Breeding	Mr. Breun	http://www.breun.de	WG1, WG4
IDna Genetics Ltd	DNA based Analytical Services	Peter Isaac	United Kingdom, www.idnagenetics.com	WG1
British Wheat Breeders	Plant Breeding		United Kingdom	WG1
Sejet Plant Breeding	Wheat and barley	Birger Eriksen	http://www.sejet.com/	1,2,3,4
Pajberg Foundation	Wheat and barley	Hans Haldrup	http://www.pajbjergfonden.dk/	1
Abed Foundation	Wheat and barley	Morten Helt Poulsen	.....	1
Boreal Plant Breeding	Barley	Eero Nissila	http://www.boreal.fi/MIT.php?MITkieli=eng&sivu=english	1,3
Saatzucht-Donau GmbH&CoKG	Cereal breeding	DI. Johann Birschitzky	www.saatzucht-donau.at	
Agricultural Research Institute Kromeriz, Ltd.	Research, Breeding, Consultancy	Jaroslav Spunar	http://www.vukrom.cz/www/ english/index.htm	1
Graminor	Cereal breeding	Magne Gullord	http://www.graminor.no/	1,2,3,4
Institute of Field and	Plant breeding and seeds	Dr Borislav Kobiljski	www.ifvcns.co.yu	WG1, WG2,

vegetable Crops, Novi Sad, Serbia	selling			WG3, WG4
Sejet Planteforædling	Plant Breeding	Lars Eriksen	Denmark www.sejet.dk	1,2,4
BIOGEN SrL	Contract sequencing	Dr. Luigi Ciocca	Italy, Biogen1@faswebnet.it	WG2
Semillas Batlle	Breeding	Francisco Batlle	Spain, www.semillasbatlle.com	WG1
La Moravia, Grupo Damm	Malting house	Agustí Rubio	Spain, www.damm.es	WG1
Svalöf Weibull	Plant Breeding	Stine Tuvesson	http://www.swseed.com	1