

# European Cereals Genetics Co-operative Newsletter 2012

Proceedings of the 15th International EWAC Conference  
7 – 11 November 2011  
Novi Sad, Serbia



[www.ewac.eu](http://www.ewac.eu)

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## Results and Discussion

Concerning the methodological questions the image analysis showed that the software was able to handle the low contrast and was furthermore adapted specifically to the root system characteristics of a monocotyledonous plant during this experiment.

The time period for the analysis of this barley subset was four weeks; then plants hit the bottom of the rhizotrones. During this time water stress was not severe enough to significantly reduce growth rates, but it was still possible to detect small differences in growth rates between the varieties (see Figure 2). This effect was only quantitative, not qualitative, which means that plants react to changing environmental parameters in the same manner, but on different scales.

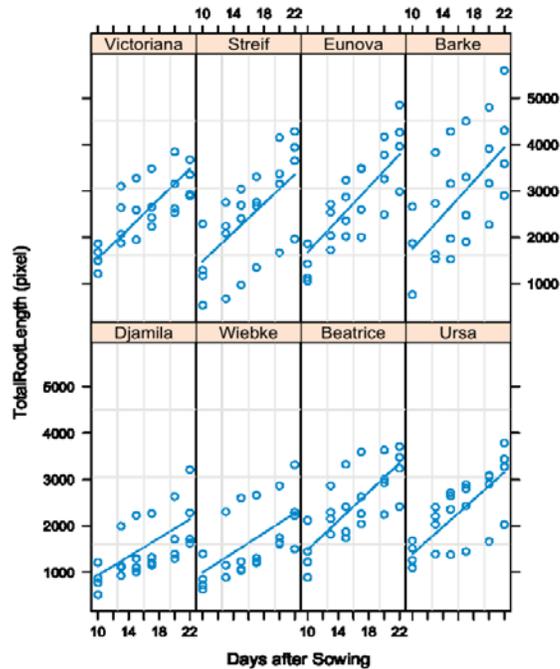


Fig. 4: Fitting a linear mixed model with random effects for both the intercept and the slope showed significant differences in total root length between the varieties

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## The International Wheat Genome Sequencing Consortium (IWGSC): Building the foundation for a paradigm shift in wheat breeding

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As the staple food for 35% of the world's population and the most widely produced crop, wheat is one of the most important crop species. Genomics offers powerful tools for

understanding the molecular basis of phenotypic variation, accelerating gene cloning and marker-assisted selection, and for improving the efficiency of exploiting genetic diversity. Genomics is leading to a new revolution in plant breeding as it enables the direct study of the relationship between the genotype and phenotype for a significant number of traits and the direct study of genes underlying those traits (Tester and Langridge, 2010; Feuillet et al., 2011). With a genome sequence in hand, breeders can have access to complete, ordered gene catalogue and an almost unlimited number of molecular markers that can be used for marker-assisted selection and precision breeding approaches (Collard and Mackill, 2008; Tester and Langridge, 2010; Prohens, 2011; Choulet et al., 2012).

Despite the socio-economic importance of bread wheat and the recognition of the power that a genome sequence brings to breeding programs, bread wheat is one of the last major crops without a high quality, reference genome sequence. Bread wheat has been slow in building genomic resources particularly because of the very nature of its genome: an allohexaploid ( $2n=6x=42$ ) that is extremely large in size (17Gb, more than 6 times the size of the maize genome) and has a very high (>90%) repetitive content of the genome thereby complicating genome assembly (Paux et al., 2008; Feuillet et al., 2011). These factors have made it more difficult and more costly to sequence the bread wheat genome than any other major crop.

Recently, however, significant technological advances have occurred that have made sequencing the wheat genome tractable at a reasonable cost. After a US Department of Agriculture-National Science Foundation funded workshop that confirmed the need for sequencing the wheat genome and assessed different strategies and objectives (Gill et al., 2004), it became clear that the bread wheat genome could be sequenced as a result of these technological advancements. With a mission of rectifying the paltry state of genomic resources for sequencing the wheat genome, a group of growers, breeders, and scientists launched the International Wheat Genome Sequencing Consortium (IWGSC; [www.wheatgenome.org](http://www.wheatgenome.org)) in 2005. The underlying goal of the consortium is to accelerate wheat improvement by obtaining a high quality, manually annotated, reference genome sequence of bread wheat that is anchored to the genetic and phenotypic maps. Using a milestone-based, adaptable strategy, the IWGSC provides breeders an increasing array of tools and resources while working towards obtaining a reference genome sequence (Feuillet and Eversole, 2007).

As an organization led by growers, breeders, and scientists rather than sequencing experts, the consortium is focused on building a foundation for wheat improvement and on facilitating rapid application of the results from IWGSC supported projects. Obtaining a “genome sequence” without any view towards the ultimate utilization of the sequence by breeders has never been a consortium goal. A major consideration in designing the strategy to obtain a reference quality sequence, thus, was to understand exactly for what the sequence would be used. We did not merely want to have a tool for comparing the wheat genome with other genomes. Instead, we wanted a genome sequence of sufficient quality to enable gene isolation, functional analyses, new allele discovery for pre-breeding, epigenetic modifications, polymorphism discovery for marker-assisted selection, and an increased understanding of the impact of transposable elements on gene regulation (Feuillet et al., 2011). To provide these capabilities, an integrated and ordered wheat genome sequence is essential.

Combined strategies are being deployed by the consortium to achieve a reference genome sequence of the hexaploid, bread wheat genome (*Triticum aestivum* L.), cultivar Chinese Spring. These include physical mapping of Chinese Spring and *Aegilops tauschii* (the D-genome progenitor of bread wheat), as well as survey sequencing and BAC-based (i.e., the minimum tiling path of the physical map) reference sequencing of Chinese Spring. The

physical map of *Aegilops tauschii* was completed in 2011 and publication of the results is expected in 2012 (*Personal communication, J. Dvorak*). The IWGSC follows a chromosome-specific approach for physical mapping, survey and high quality sequencing of Chinese Spring. The chromosome-based approach, made possible through technological advancements in flow-sorting of Chinese Spring chromosomes by the group of Jaroslav Doležel (Doležel et al., 2007), reduces the complexity of the bread wheat genome by physical mapping and sequencing individual chromosomes or chromosome arms the size of which ranges from 224-800Mb. Further, the chromosome-based approach facilitates international collaboration and divides the costs of obtaining a reference sequence into manageable pieces. The chromosome-based approach will deliver a complete, finished reference genome sequence that, in addition to genic sequences, will provide critical information on non-coding, intergenic sequences that underlie many biological functions (Feuillet et al., 2011).

To construct the physical maps, chromosome specific BAC libraries are created for each of the 21 chromosomes of bread wheat, *cv.* Chinese Spring. As of October 2011, more than 2.2 million BAC clones had been developed for the BAC libraries of all but 4 chromosomes of bread wheat (<http://olomouc.ueb.cas.cz/dna-libraries/cereals>). Using these BAC-libraries, physical maps are then developed. The information produced during the construction of these maps is useful for gene cloning and marker development as well as increasing our understanding of the gene space organization and regulation. Further, the physical map itself serves as the substrate for sequencing. The completion of the physical map of the largest wheat chromosome (3B, ~ 1Gb) in 2008 confirmed the feasibility of this approach (Paux et al., 2008). Physical mapping of the remaining chromosomes is underway. By November 2011, the IWGSC reached the milestone of securing funding for the development of physical maps for all 21 bread wheat chromosomes. The next physical mapping milestone to be achieved is the completion of all of the chromosome-based physical maps. With all of the funding in place, this is expected by 2013.

To facilitate anchoring, marker development, and to gain a first insight into the gene space composition, survey sequences were completed with construction of the physical maps. As survey sequences were available for only half of the chromosomes by 2010, an internationally coordinated initiative was launched to provide survey sequences and the virtual gene order for all 21 chromosomes. By October 2011, survey sequence coverage had been achieved for all 21 chromosomes. Survey sequences of 30-50 fold coverage, based on Illumina technology, and assemblies of sequences for 29 chromosome arms were completed by the fall of 2011. Illumina survey sequences will be completed and assembled for the remaining chromosome arms by early 2012. Using the assemblies, a virtual gene order is derived by exploiting the conserved synteny among grasses through a process called the “GenomeZipper” (Mayer et al., 2009). This draft, virtual gene order in the individual chromosomes/chromosome arms is expected to be completed by early 2012. The survey sequences provide only partial information on the order and orientation of the contiguous sequences (contigs) and do not represent a complete, reference genome. Rather, they enable *in silico* mapping, facilitate annotation of genes within contigs, and support localized synteny studies. The chromosome-based survey sequence initiative is expected to be completed by mid-2012.

The next step towards obtaining a high quality reference sequence of the bread wheat genome is to sequence the minimal tiling path (MTP) of the individual chromosomes/chromosome arms. Using clones selected from a physical map containing fragments making up the genome with minimal overlap, a MTP of clones is delineated to provide the substrate for sequencing (Choulet et al., 2012). Sequencing the MTP of chromosome 3B began in 2010 and of chromosome 7B in 2011. By September 2011, the IWGSC reached two more milestones by completing the production of sequence for chromosome 3B and securing funding for the MTP

sequencing of 8 additional Chinese Spring chromosomes. The next milestone is to finalize the funding for MTP sequencing of all of the 21 bread wheat chromosomes by 2013, which, if successful, would result in the completion of sequence production by 2014.

Information on the various IWGSC projects as well as the availability of data can be found on the consortium website: <http://www.wheatgenome.org>. An IWGSC sequence repository has been established by INRA-URGI (<http://urgi.versailles.inra.fr/Species/Wheat/Sequence-Repository>). Pre-publication access to IWGSC data is publicly available for BLAST as long as the user agrees to respect the right of the IWGSC to produce the first chromosomal and global analysis of the data. Raw sequence data will be deposited in short read archive and download of sequence assemblies will be available upon publication.

The IWGSC made significant progress in 2011 and it is anticipated that even more milestones will be achieved in 2012. Membership in the consortium is open to anyone who supports the goal of obtaining a high quality, reference sequence of the bread wheat genome.

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## Genomic selection strategies for wheat improvement

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## Introduction

Plant breeding strategies are driven by new methods and technologies. Crop improvement began with the hunter-gatherers who practiced mass selection and domesticated the species