transPLANT milestone report

MS20 (work package 7): 20 reference genomes incorporated in transPLANT hub and submitted to comparative analysis

EMBL-EBI is providing access to many of the genomes described in the registry available for interactive and programmatic analysis through the Ensembl Plants (http://plants.ensembl.org) site. Ensembl, originally developed in the course of the Human Genome Project but subsequently applied to other domains, is a powerful tool suite for the analysis and display of genome scale data, and Ensembl Plants is the EBI's primary user interface for accessing plant data. We have used transPLANT funding to increase our capacity to include additional reference genomes incorporated in Ensembl Plants. In the third year of the grant, we have made four releases of Ensembl Plants, and incorporated fourteen following additional genomes: Amborella trichopoda (a tropical shrub), Brassica oleracea (whose cultivars include cabbage, Brussels sprouts, broccoli, kohl, kale and cauliflower), Leersia perrieri (a wild grass), Ostreococcus lucimarinus (a unicellular picoplankton), seven new rice genomes (Oryza barthii, Oryza glumaepatula, Oryza meridionalis, Oryza nivara, Oryza punctata, and Oryza rufipogon), Prunus persica (peach), Theobroma cacao (the cultivated chocolate tree), and Triticum aestivum (the bread wheat genome). The representation of the wheat genome specifically resulted from collaboration with the transPLANT partners at TGAC, INRA and HGMU: TGAC generated the sequence data and genome assembly and HGMU generated the primary genome annotation, and we have worked together to ensure that a consistent set of reference sequence and annotation is available in the nucleotide sequence archives (maintained at EMBL-EBI) and at all sites operating in the transPLANT project (Ensembl Plants, MIPS Plants DB, and the International Wheat Genome Sequencing Consortium's site, which is operated by INRA).

These genomes have been analysed comparatively using the Ensembl Compara functional genomics pipeline, which has 2 elements: a protein-based analysis, which infers evolutionary relationships after clustering and alignment (and which are performed over the domain of all plants) and a pairwise DNA-based analysis, performed using the alignment tools blastZ and lastZ. We have aligned the reference japonica cultivar of rice against 32 other genomes and the model plant Arabidopsis thaliana against 22 (not every monocot is aligned against Arabidopsis); grape is additionally used as a reference against 9 other dicot species. In addition, we have further alignments within selected groups of closely related species, e.g. rice species, grasses within the Pooideae and Panicoideae sub-families. The precise comparisons available are shown in Figure 1; a total of 84 inter-species comparisons are now available, compared with 26 at the time of the last annual report. In addition, internal comparisons are also available between the three homoeologous genomes of the polyploid required the redevelopment of the pipeline to ensure that 1:m bread wheat, which relationships (resulting from genome-specific gene expansion/loss) are captured in both directions. In addition, the large size and fragmented nature of bread wheat additionally required optimization in both the pipeline code and the supporting database infrastructure to improve speed and reduce the required use of compute resources, and changes in the visualization interface to support visualization intra-species (as well as inter-species) relationships, and to better link the genomic analysis with the protein-based analysis (see below) for the purpose of exploring polyploid genomes.

Figure 1 Pairwise genomic comparisons available in Ensembl Plants.

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Arabidopsis thaliana : Brassica rapa	LASTZ_NET
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Arabidopsis thaliana : Oryza brachyantha	LASTZ_NET
Arabidopsis thaliana : Oryza glaberrima	BLASTZ_NET
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Setaria italica : Oryza sativa Japonica	LASTZ_NET
Setaria italica : Sorghum bicolor	LASTZ_NET
Setaria Italica : Zea mays	LASTZ_NET
Solanum lycopersicum	4 genome alignments
Solanum lycopersicum : Arabidopsis thaliana	LASTZ_NET
Solanum lycopersicum : Oryza sativa Japonica	LASTZ_NET
Solanum lycopersicum : Solanum tuberosum	LASTZ_NET
Solanum lycopersicum : Vitis vinifera	LASTZ_NET
Solanum tuberosum	3 genome alignments
Solanum tuberosum : Oryza sativa Japonica	LASIZ_NET
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For particularly closely related genomes, these detailed analyses are also used to support assertions of synteny (which can also be visualised in the user interface). A full list of species for which syntenic data is available is provided in Figure 2.





Legend: Light blue square: synteny data available. Pink/white square: synteny data not available.

The protein-centric analysis has (as of July 2014) placed 1,232,576 proteins from 38 plant genomes and selected outlying eukaryotic species (*Homo sapiens*, *Drosophila melanogaster*, *Caenorhabditis elegans*, *Ciona intestinalis* and *Saccharomyces cerevisiae*) into 56,134 clusters. A particularly interesting feature is the large collection of orthologues identified from the 21 cereal genomes (counting the 3 individual bread wheat genomes individually) that are now available in the resource. Based on a calculation of probable evolutionary history based on fitting gene trees to the species tree, we have placed 690,172 cereal genes into 39,216 groups of implied orthology. A total of 7,203 clusters (containing 260,004 genes) cover all 21 genomes, while 18,433 orthologous clusters cover 2-21 species with a single representative from each genome in the group. This data set helps identify annotation errors and sites of biological diversity (due to recent gene duplication/deletion etc.), and (differential) rates of selective/non-selective evolution can also be calculated when the

species distance is relatively close. An example of a "perfect" orthologous set over all 21 genomes is shown in figure 3, as visualized in the Ensembl Plants user interface.

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BRADI1G66240, Brachypodium distachyon	
CS03G0280700, Oryza sativa Japonica	
ORUF 03G13380, Oryza rulipogon	
BGIOSGA012352, Oryza sativa Indica	
ONIVA03G13980. Oryza nivara	
ORGLA03G0122800, Oryza glabernima	
OBABT03G12710. Orvza bathii	
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GFMZM2G049322, Zea mays	

In addition, nine of these genomes: *Amborella trichopoda, Arabidopsis thaliana, Chlamydomonas reinhardtii, Cyanidioschyzon merolae, Oryza sativa, Physomitrella patens, Selaginella moellendorffii, Solanum lycopersicum* and *Vitis vinifera* have been included in a broad range taxonomic analysis aimed at identification and presentation of large protein families from across the taxonomy.

The data are available through the Ensembl Plants user interface, and are searchable through the integrated search facility available through the transPLANT portal (see work package 6 report).