

Meeting Report The Aaronsohn-ITMI International Conference

CATHERINE FEUILLET,^a PETER LANGRIDGE,^b AND ROBBIE WAUGH^c

^aUMR INRA-UBP 1095, Amélioration et Santé des Plantes, Domaine de Crouelle, 234 Avenue du Brézet, 63100 Clermont-Ferrand, France

^bAustralian Centre for Plant Functional Genomics, University of Adelaide, SA 5064, Australia

^cScottish Crop Research Institute, Invergowrie, DD2 5DA, UK

The Aaronsohn-ITMI international conference was held 16–20 April 2007 in Tiberias (Israel) to celebrate the 100 year anniversary of the rediscovery of wild emmer wheat by Aaron Aaronsohn in Rosh Pinna, a few kilometers away from Tiberias. The workshop, attended by more than 110 participants from 22 countries, also provided an opportunity to discuss the latest developments in Triticeae genetics and genomics.

WILD EMMER WHEAT

In the opening session of the workshop, Shaul Katz (Hebrew Univ., Israel) reported on the history of the discovery of wild emmer wheat, the wild ancestor of tetraploid wheat. This goes back to 1855 when botanists (Kotschy, Koernicke) from Austria came to Palestine and found a sample of a cereal with ancestral features (“Urweizen”) that had not been previously recorded. Later, Aaron Aaronsohn returned to Israel from Germany with the support of his “patrons” (Warburg, Schweinfurth, and Ascherson) with the mission to find Kotschy’s wheat in the wild. After a first unsuccessful mission in 1902, Aaronsohn identified a new *Triticum* species in Rosh Pinna in 1906 that was later recognized as the wild emmer wheat of Kotschy. The features of this species led Aaronsohn to propose its use in breeding programs to improve wheat, particularly in semiarid areas. This visionary idea was recognized by the USDA and Aaronsohn was sponsored to establish the first agricultural experiment station in Palestine (Atlit) in 1910. Although this did not lead to concrete results at that time, the impact of Aaronsohn’s vision is still apparent, and wild relatives of wheat are used increasingly in breeding

programs as a source of new alleles. Wild emmer wheat also represents a key material for studying the evolution of wheat genomes and the impact of polyploidization on genome structure and evolution, as exemplified by Moshe Feldman (Weizman Institute, Israel) in his lecture. There is genetic asymmetry between the A and B genomes of wild emmer wheat. For example, genes controlling inflorescence morphology, growth habit, domestication, and autogamous behavior are found preferentially on the A genome, whereas genes regulating ecological adaptation (such as disease resistance genes, stress related genes) are found on the B genome. The B genome also contains a larger amount of repetitive elements and rRNA genes and displays a higher level of polymorphism than the A genome. Moshe Feldman described two types of changes (revolutionary and evolutionary) that affect the genome after polyploidization and indicated that the donor of the B genome must have had a larger genome than *Aegilops speltoides*, the closest ancestor to the B genome of wild and cultivated polyploid wheat species.

For further information:

E-mail: saranga@agri.huji.ac.il

Archaeological data were presented by Avi Gopher (Tel Aviv Univ., Israel), who concluded that the Neolithic revolution was more a cultural rather than an economic revolution. Mordechai Kislev (Bar-Ilan Univ., Israel) presented an archaeobotanical description of *T. parvicoccum*, an extinct small-grained wheat taxon from the Bronze Age, suggested to be the ancestor of *T. durum*. Jan Dvorak (UC Davis, USA) reported on a large analysis of the structure of wild emmer wheat populations and gave evidence for when and where domestication occurred in the Fertile Crescent. Two large clusters of southern and of northern populations were identified with evidence for massive gene flow between them after domestication. While Israel-Lebanon-Syria appears to be the birthplace of wild emmer wheat, it seems that domestication occurred in Turkey (Diyarbakir), with a second site in the Levant. Interestingly, domestication of legumes seems to have followed a quite different path than the one of cereals, and Shahal Abbo (Hebrew Univ., Israel) proposed to learn lessons from the success story of cereal domestication to change the way grain legume's improvement has been performed.

GENES INVOLVED IN DOMESTICATION

Over the last years, the isolation and characterization of a number genes involved in modification of spike traits has led to a better understanding of the genetic basis of domestication. This was exemplified by Bikram Gill (Kansas State Univ., USA), who presented the genetic analysis of the *Br1* (Brittle spike), *Tg* (Tough glume), and *Q* (spike shape, square head) loci. In 1995, the finding that some domestication traits are found at syntenic locations in different cereal genomes led to the idea that these traits may have resulted from convergent evolution in cereals. However, this is not the case for shattering genes such as *Br1* and *2* in wheat, *qSH1* in rice, and *tb1* in maize, which are not found at orthologous positions. This suggests that shattering resistance in cereals has evolved through independent and different pathways. This was also found in comparative mapping studies of the *Tg* loci located at the distal ends of chromosomes 2A and 2D but do not correspond to the same locus. The *Q* locus, which affects, among other traits, head shape, has been cloned recently, and B. Gill presented data on the functional analysis of the *AP2*-like gene that is responsible for this character. A single nucleotide change at position 329 accounts for the differential expression of the *Q* and *q* alleles, and only the *Q* protein is able to form homodimers that autoregulate *Q* gene expression.

GENOME STRUCTURE

Over the last two years, international consortia have

emerged under the umbrella of ITMI to develop resources and methodologies for sequencing the wheat and barley genomes. Catherine Feuillet (INRA, France) presented a chromosome-based approach that has been chosen by the IWGSC to tackle the hexaploid wheat genome cv. Chinese Spring. She showed results of structural and evolutionary studies on chromosome 3B, which are used in a pilot study to refine the strategies for the whole wheat genome. A chromosome landing ready physical map of 3B anchored to the cytogenetic and genetic maps is now in its final phase of assembly and a Minimum Tilling Path (MTP) of 7,500 clones covering 80% of the chromosome has been established. The MTP is now being used for different applications such as genome structure and evolution analyses through BAC end sequencing, map-based cloning, gene space characterization, functional studies, and large-scale sequencing projects.

Construction of a barley physical map is also underway and Andreas Graner (IPK, Germany) presented the different milestones (physical map, anchoring, sequencing, annotation, colinearity studies) that will be undertaken in the framework of the International Barley Sequencing Consortium (IBSC). High Information content Fingerprinting (HICF) of 300,000 clones from Morex BAC libraries and of 83,000 gene-containing BACs has started, and anchoring on high-density arrays, is being tested. The aim is to have a 15× coverage map anchored with 5,000 to 6,000 ESTs. New sequencing technologies have been evaluated to determine their value in capturing with high efficiency and at reasonable cost the gene content of BAC contigs.

Defining the tools most adapted to Triticeae Genome analysis is essential for the success of these initiatives. Abraham Korol (Univ. Haifa, Israel) presented a new approach to improving the building of physical maps by taking into account specificities of the Triticeae genomes such as the high repeat content and the fact that fragments generated by HICF are not equally distributed. He is testing new algorithms to improve several parameters such as the distance function, the clustering, and the stringency contig assembly. He is now applying these methods to the fingerprints generated by the group of Catherine Feuillet on chromosome 3B.

Both structural genomics and functional genomics studies are required to understand the genetic basis of diversity. Robbie Waugh (SCRI, UK) is studying whether the pattern of differential gene expression that can be observed between barley varieties is common, is genetically determined, and whether the causes of any observed allelic imbalance are the result of *cis*- or *trans*-acting regulatory factors. He presented data showing that thousands of genes are differentially expressed

between Steptoe and Morex and are stably inherited in their offspring as “expression QTL”. Their data allowed over 5,000 genes to be mapped on the barley genetic map, providing a powerful bridge between the rice genome sequence and the location of barley phenotypic traits.

Intraspecific comparative analysis can also provide valuable information about the mechanism of genome evolution. This was exemplified by Boulos Chalhoub (INRA, France), who presented results from a project aimed at comparing 13 loci across the A, B, and D genomes in different “ploidy” contexts. The analysis of 7 loci (70 BACs) allowed the annotation of 100 novel transposable elements (TE) and showed that only the genes are conserved between the A, B, and D genomes. This was also the case when BACs from the B and S genomes were compared. In contrast, comparison of diploid and polyploid D genomes showed higher conservation, with 80% of TEs found at orthologous positions. A comparative transcriptomic analysis between species with the A, AB, D, and ABD genomes led to the identification of 7,000 genes differentially expressed between at least two genotypes, including 3,640 genes that are silenced in at least one species compared to the others.

Wheat and barley genomes are characterized by having the bulk of their genomes comprised of transposable elements. Alan Schulman (MTT, Finland) presented data on the retrotransposon life cycle and the causes and consequences of movement of autonomous and non-autonomous transposable elements (TEs). He provided evidence that non-autonomous elements could parasitize autonomous TEs to gain mobility—just as defective interfering particles do in RNA viruses. Describing the differences between classes of elements, he described how the Triticeae genome could be considered a complex dynamic where the mobility and impact of TEs is influenced by external factors such as drought and heat, and can be under selection at an organismal level. His message was supported and enhanced by Alexander Balyayev (Univ. Haifa), who observed significant temporal fluctuations in the copy number of transposable elements in wild populations of *Aegilops speltoides* over several generations. He suggested that even at a population level, TE action may be the most important driver of genotypic population change as a result of their ability to create mutations, modify patterns of gene expression, or promote chromosomal aberrations. Eduard Akhunov (UC Davis) provided further evidence for the role of TEs in genome evolution. He described a potential mechanism and impact of inter-chromosomal gene duplication by analysis of the ALP gene family in wheat. Their data indicated that the principle mechanism ALP

gene family evolution involved a series of as-yet-uncharacterized transposon-mediated events. The frequency of these events was estimated to be three times higher than the average duplication rate of single copy genes. During this process, upstream elements have been lost, and levels and patterns of expression of the duplicated genes significantly changed. Taihachi Kawahara (Kyoto Univ., Japan) analyzed the intra- and interspecific variation in chloroplast DNA in diploid *Aegilops-Triticum* species, with implications on the relationships within and among *Aegilops-Triticum* species. Elena Salina (IC&G, Russia) presented a comparative analysis of the distribution of *Aegilops* macrosatellite families (Spelt1 and Spelt2), transferability of microsatellite loci, and the data on chromosome mapping for *Triticum* and *Aegilops* genome. Alberto Cenci (INRA, France) analyzed the phylogenetic position of *Aegilops speltoides* in the *Aegilops/Triticum* species complex, suggesting that *Ae. speltoides* occupies an external position in the phylogenetic tree of *Aegilops/Triticum* complex.

GENE ISOLATION

Gene isolation is still laborious in wheat, and the use of model genomes can be helpful to accelerate map-based cloning. Beat Keller (Univ. Zurich, Switzerland) provided examples of the use of rice and *Brachypodium* to support the isolation of the durable leaf rust R gene, Lr34. Comparative sequence analysis between rice-*Brachypodium*, and wheat BACs at this locus indicated an inversion in rice compared to wheat and *Brachypodium*, thereby suggesting fewer rearrangements between these two species than with rice. A number of genes were not conserved between wheat and *Brachypodium*, indicating that *Brachypodium* will be useful for comparative analysis, for reannotation of the rice genome, and as a source of markers for wheat genomics, but that it cannot replace large-scale sequence analysis in wheat and barley. Beat Keller also presented a detailed comparison of 112 NBS LRR types of disease resistance genes in grasses and a model for the evolutionary events that occur at R gene clusters. The results indicate that illegitimate recombination provides the seed of unequal crossing over, the driving force for disease R gene evolution.

A further example of positional cloning was presented by Geoff Fincher (ACPF, Australia). Malting quality is an essential trait in barley, and it is linked with the presence of β -1,3;1,4-glucans in the endosperm. Different approaches were undertaken to identify the β -1,3;1,4-glucan synthase gene family. A first candidate gene approach based on structural similarities with the cellulose synthase did not identify any correlations be-

tween the presence of β -1,3;1,4-glucans and *Csl* genes, and a QTL approach was undertaken. Six candidate genes that belong to the *Csl* gene family were identified using syntenic relationships with rice. Validation was undertaken through *Arabidopsis thaliana* transformation and biochemical studies of enzymatic activity to prove the functional identity of the gene in hydrolyzing specifically β -1,3;1,4-glucans.

The presentation of Masahiro Yano (NIAS, Japan) clearly illustrated the power of having access to a genome sequence for the efficient detection and cloning of QTL underlying agronomically-important traits (flowering time, disease resistance, and seed dormancy) in rice. Jorge Dubcovsky (UC Davis) also presented the success stories of his group on the cloning and characterization of genes involved in vernalization and photoperiod in barley and wheat. The isolation of the *Vrn1*, 2, and 3 genes has allowed RNAi, yeast 2-hybrid, and allelic polymorphism studies that have shed light on the complex network of interactions that regulates flowering and development in response to the photoperiod in barley and wheat. He presented evidence for an epistatic interaction between *Vrn1* and *Vrn2* and a model that compiles all the knowledge accumulated to date on the regulation of flowering in barley and hexaploid wheat. In collaboration with the group of Tzion Fahima (Univ. Haifa), his group has also recently cloned a gene (*NAC*) involved in grain protein content. Assaf Distelfeld (UC Davis) presented the map-based cloning of this transcription factor, which also affects mineral content and appears to interfere with the remobilization of nutrients to the grain during senescence.

GENE EXPRESSION

Full length (FL) cDNAs are essential for genome annotation in barley and wheat. Projects are underway in Japan to produce FLcDNA collections as reported by Yasunari Ogihara (Yokohama City Univ.), who presented the MUGEST project for wheat. Methylation plays a central role in the regulation of both gene and transposable element expression. Khalil Kashkush (Ben-Gurion Univ., Israel) performed a genome-wide analysis of cytosine methylation and transcription of RT in rice, with a particular focus on the structural and functional characterization of the non-autonomous element Dasheng. He showed evidence that the LTR of such elements can act as promoters of the transcription of adjacent genes and that methylation affects the regulation of Dasheng in rice. Polymorphism of the Dasheng insertion between two rice subspecies was shown to be associated with differences in gene expression and methylation. Hikmet Budak (Sabanci Univ., Turkey)

studied gene expression of durum wheat upon exposure to cadmium (Cd). Two genes, namely NADH dehydrogenase subunit 1 (EC907725), which is known to be involved in stress mechanisms in plant systems, and the *PsaC* gene encoding a photosystem I (PSI) 9 kDa subunit (EC907731), were identified as being heavily induced upon Cd treatment.

MOLECULAR BREEDING AND UTILIZATION OF WILD GERMLASM

The difficulties in understanding and dealing with complex, interconnected regulatory pathways in relation to improving tolerance to general abiotic and, specifically, drought stress were introduced by Peter Langridge (ACPFPG, Australia), Roberto Tuberosa (Bologna, Italy), and Yehoshua Saranga (Hebrew Univ.). They reminded us that one of Aaron Aaronsohn's early recommendations was to use drought-tolerant emmer wheat in breeding to produce drought-resistant races. By combining QTL and association genetic mapping with appropriate genetic resources and the modern tools of plant genomics, the conference participants were reassured that components of stress perception, transduction, and response were being identified and that progress, through both contemporary breeding and GM, is possible and indeed happening in different research and development programs. However, we were reminded that such improvements are not sought in isolation. Evans Laguda (CSIRO, Australia) described his group's recent work in characterizing the multi-pathogenic adult plant leaf rust resistance locus *Lr46/Yr29/Ltn2*. He described the use of irradiation mutants and of *Brachypodium* as a genomic model to assist in gene identification. His current results seem to suggest that this complex resistance is controlled by a single gene as opposed to a cluster of R-genes. Ismail Cakmak (Sabanci Univ., Turkey) presented an analysis of Zn and protein concentrations in grain of wild wheat and cultivated wheat, suggesting that wild wheats represent a valuable source of genetic diversity for increasing Zn concentration and improving Zn deficiency tolerance of cultivated wheats.

After a fascinating introductory talk by Dani Zamir (Hebrew Univ.) that summarized progress made by introducing exotic alleles into tomato, three talks described current work in the characterization and utilization of biodiverse genetic material in wheat and barley. Susanne Dreisigacker (CYMMIT, Mexico) described the application of SSR and DArT technology to quantify and partition genetic diversity in the CIMMYT Elite Spring Wheat Yield Trials and to exploit LD-mapping to identify associations between markers and disease resistance, maturity, and yield traits. A key message was that

LD-mapping is likely to work on this type of material (though resolution varies across the genome) and will supplement traditional bi-parental mapping approaches. This overall principle was extended to a world bread wheat core collection of 372 lines (Francois Balfourier, INRA). Much closer to home (i.e., Israel), Karl Schmidt (IPK, Germany) described the concept of “natural selection mapping” through a description of DNA sequence polymorphism analysis of wild barley from “Evolution Canyon”, a location we were all to experience personally 24 hours later.

Due to their polyploid nature, tetraploid and hexaploid wheats are well known to tolerate chromosomal or sub-chromosomal perturbations, including introgression from wild or related species. This offers the possibility of utilizing desirable traits from outside the primary gene pools in crop improvement strategies. Three presentations described how some of the barriers to interspecific hybridization have been overcome, and introduced strategies and successes in mobilizing traits from wild relatives to crops. Carla Ceoloni (Univ. Tuscia, Italy) gave examples of the utilization of resistance genes from *Thinopyrum ponticum* and from *Ae. longissima* into durum wheat, and Eitan Millet (Tel Aviv Univ.), progress on mobilizing yellow rust resistance genes from *Ae. sharonensis* into bread wheat. Interestingly, preliminary analysis of the resistant progenies from their populations appeared also to be resistant to the potentially devastating stem rust race Ug99. Herman Buerstmayr (Univ. Tüln, Austria) provided an update on their attempt to understand and exploit *Fusarium* head blight resistance from wild emmer wheat in durum.

TRITICEAE IN THE WILD

In preparation for field visits to several sites where wild relatives of the Triticeae crops grow in abundance, a report on the Israel national park and conservation system was provided by Didi Kaplan (Nature and Park Protection Authority, Israel). Nature reserves cover around 12% of Israel. In northern Israel parks range from as small as 0.1 ha to around 10,000 ha. Only around 22% or just over 20,000 ha consist of grasslands, but these contain stands of *Hordeum*, *Avena*, *Triticum*, and *Aegilops* species. Despite the significance of the grasslands, it appears that no nature reserves were established specifically to protect the native grasses. Therefore the occurrence of the magnificent stands of grasses is fortuitous rather than planned. Nevertheless, current management practices are helping to sustain the grasslands. For example, around 60% of the Yahudia Nature Reserve is grazed by cattle to decrease the impact of fires. Studies

have found that the grazing has favored species diversity, and 487 different species have been identified in this reserve of 6620 ha.

The explanation of geology and climate conditions across northern Israel emphasized the significance of the region as a source for diversity. Not only was there a huge variation in altitude across a small distance, from –200 m to over 1100 m asl, but also a concomitant variation in temperature and rainfall and a diversity of soil types.

The use of the nature parks to study diversity was nicely demonstrated through the work presented by Yehoshua Anikster (Tel Aviv Univ.). Anikster described a study of disease incidence at the Ammiad site over a ten-year period. Using 250 permanent sampling points, they scored incidence of the three rusts, mildew, *Septoria*, take-all, and *Ascochyte*. All diseases were present, but at only a low density. However, when transferred to nurseries, all accession showed susceptibility although some variation was found in susceptibility of *Septoria*, *Ascochyte* and mildew. Many plants also showed resistance to many European isolates of the diseases. These results suggest that it is the environment rather than genetic factors that leads to low disease incidence.

The work reported by Zvi Peleg (Hebrew Univ.) described diversity in wild emmers across an aridity gradient. The aim of the work was to identify environmental factors that affect distribution and diversity. The results showed that rainfall was the major factor affecting diversity between populations, with peaks in diversity at intermediate habitats. This was probably due to year-to-year variations, which show shifts in selective pressures at the intermediate sites. The most diverse sites were in the 350 to 550 mm annual rainfall range. The study indicated that the highest priority should be given to conservation at the intermediate sites.

The scientific presentations were concluded by a tour-de-force of evolutionary genetics by Eviatar Nevo (Univ. Haifa). Describing the tremendous amount of genetic diversity between even adjacent populations—such as those across a 400-meter transect at “Evolution Canyon” on which much of the work he presented was conducted—his thesis was that the evolution of molecular and organismal diversity is nonrandom, and in this location at least, driven by abiotic and biotic environmental heterogeneity and stress.

The workshop closed with a lecture by Ran Aaronsohn (Hebrew Univ.), who provided the historical context of his granduncle’s life and visionary scientific ideas. This was an ideal conclusion to a very successful meeting, giving a perfect combination of scientific and historical perspectives on the genetics and evolution of wheat and barley.