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Evaluation of wheat yield and drought resistance indices across water regimes

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Drought is a wide-spread problem seriously influencing wheat (*Triticum aestivum* L.) production and quality, but development of resistant cultivars is hampered by the lack of effective selection criteria. Wheat grain yield was evaluated in a 3-year field experiment under drought, irrigated and rainfed conditions to study the relationship between grain yield under favorable and stressed conditions and to evaluate the efficiency of several resistance indices. Nine selection indices including stress susceptibility index (SSI), stress tolerance index (STI), tolerance (TOL), regression coefficient of cultivar yield on environmental index (b), yield index (YI), yield stability index (YSI), mean productivity (MP), geometric mean productivity (GMP), and superiority measure (P) were calculated based on grain yield under drought-stressed and irrigated conditions. The results showed that grain yield under irrigated condition was adversely correlated with non-irrigated condition indicating that selection in a favorable environment does not necessarily result in improved yield under stress. MP, GMP and STI were more effective in identifying high yielding cultivars in both drought-stressed and irrigated conditions (group A cultivars). Under severe stress, none of the indices used were able to identify group A cultivars, although regression coefficient (b) and SSI were found to be more useful in discriminating resistant cultivars. It is concluded that the effectiveness of selection indices in differentiating resistant cultivars varies with the stress severity.

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The genetics of drought and heat tolerance in bread wheat

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Drought can have numerous effects on a wheat plant including reduced photosynthesis, reduced kernel sink potential and/or increased pollen sterility, amongst others. However, traits such as higher stem carbohydrate accumulation and remobilization, carbon isotope discrimination, osmotic adjustment and leaf glaucousness may assist crop production under drought stress. Progress within traditional breeding programs for tolerance to abiotic stress such as drought and heat are slow, due to its complex genetic basis and the large influence of environmental variation. To date, relatively few studies have been conducted in wheat to genetically dissect tolerance to drought or heat. We crossed Kukri, a locally developed bread wheat cultivar intolerant of drought and heat, with RAC875, a locally adapted breeding line, tolerant of the two stresses. A doubled haploid population was subsequently produced, with 368 lines. In 2007, the population was grown at 5 sites in southern Australia and this poster outlines data sets collected for key morpho-physiological traits. Some results will be presented here, as well as key QTL identified for yield components, harvest index and flag leaf dimensions. Kukri and RAC875 will initially be studied for heat tolerance to identify any morpho-physiological differences such as spike fertility or grain size. Future work aims to investigate heat tolerance within the population. To this end, a growth room heat assay is being developed aiming to replicate higher temperature stress conditions in a reproducible test that produces maximum variation between adult lines and minimizes experimental error. A subset of the population has also been grown under

irrigated, high temperature conditions at CIMMYT, Mexico and some preliminary results from data collected are presented.

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Genome-wide monitoring expression changes of wild emmer wheat exposed to shock-drought stress

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Abiotic stresses are major constraints on crop productivity. Water stress is the main reason for yield loss in wheat production (Barnabas *et al.*, 2008, *PCE*, 31:11-38; Rampino *et al.*, 2006, *PCE*, 29:2143-2152). The increase in human population and decrease in water availability for agricultural use necessitate engineering of cultivated wheat for better adaptation to water scarcity without or minimum yield loss (Tuberosa and Salvi, 2006, *TIPS*, 11:405-411; Valliyodan and Nguyen, *Curr Opin Plant Biol*, 2006, 9:189-195). More than ten thousand years of domestication caused the loss of genetic background for environmental stress tolerance of hexaploid wheat (Araus *et al.*, 2007, *JXB*, 58:131-145; Mohammadi *et al.*, 2007, *PCE*, 30:630-645). The Fertile Crescent is characterized by its cold winters and drought summers, i.e. wild relatives of cultivated crops originated from this area are promising candidates for bioengineering to cope with environmental stresses (Dubcovsky and Dvorak, 2007, *Science*, 316:1862-1866; Peleg *et al.*, 2005, *PCE*, 28:176-191). Twenty-eight wild emmer wheat genotypes (*Triticum turgidum* L. subsp. *diccocoides*) specific to Turkey were analyzed for their physiological and phenological characteristics under slow drying conditions. A tolerant and a sensitive genotype, in comparison to cultivated durum wheat were chosen for further studies. Selected tolerant and sensitive wild emmer wheats were grown in hydroponics and shock-drought stressed to induce differentially regulated transcripts. As drought stress causes changes in a wide range of physiological and biochemical processes (Shinozaki and Yamaguchi-Shinozaki, 2007, *JXB*, 58:221-227), hybridization to Affymetrix GeneChip® Wheat Genome Array was selected as a method to profile genome-wide expression changes. The preliminary results show unique responses of wild emmer wheat with different tolerance to water scarcity, especially in timing of signal transduction and gives insight to understand the reason of better tolerance and adaptation to drought environment.

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QTL for canopy temperature response related to yield in both heat and drought environments

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The effects of drought due to insufficient soil water supply frequently occur concurrently with high air temperature. These combinations of environmental conditions are likely to increase in frequency if climate change predictions are realised. While there have been studies of the effects of heat shocks and high temperatures at various stages of wheat reproductive growth, there are no published reports on genomic regions associated with responses to heat and drought conditions in the field. Experiments in north-west Mexico were grown as managed droughts (i.e. growing into full moisture profile) or were planted three months after the normal planting time and irrigated. Maximum daily temperatures in the heat trials were 7 to 10°C higher (> 30°C) than in the drought trials. Yields of a set of 167 recombinant inbred lines from the Seri-Babax cross were highly correlated (>0.59) among the experiments (two of each treatment, averaging 303 and 253 gm⁻² under drought and heat, respectively). Using composite interval mapping, cooler canopy temperatures and higher grain yields were associated with the Babax

allele on linkage groups 1B, 3B and 4A under both drought and heat conditions. Cool canopy effects were also detected due to the Seri allele on linkage group 2B, but with no effect on yield. The effects of these QTL were to decrease canopy temperatures by 0.2 to 0.5°C in heat trials and by almost 1.5°C in one of the drought trials. The associated positive effects on grain yield ranged from 15 to 50 g m⁻² and included a positive effect due to the presence of the 1BS chromosome segment of Babax instead of the 1RS rye segment present in Seri.

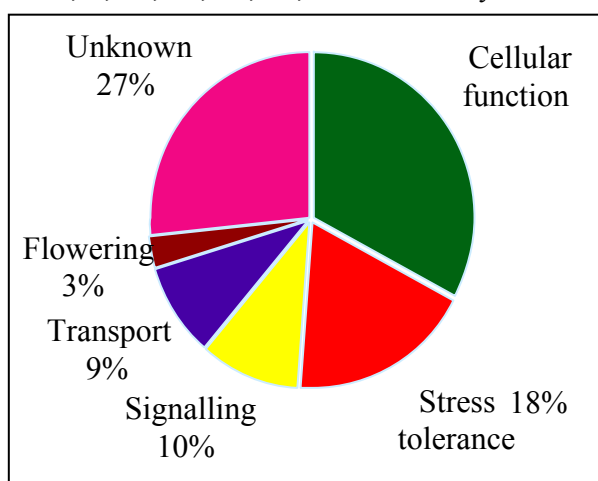
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cDNA-AFLP profiling of low-temperature-induced transcripts in wheat

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The objective of this study was to determine temporal expression profiles of transcripts during cold-acclimation of wheat plants using cDNA-AFLP. A winter hardy cultivar, Norstar, a spring cultivar, Manitou and two sets of near-isogenic lines (NILs) were used based on the *Vrn-A1* (spring) and *vrn-A1* (winter) alleles in each of the Norstar and Manitou genetic backgrounds, respectively (winter Norstar-spring Norstar, winter Manitou-spring Manitou). Plants were grown at 20°C to the 3-leaves and then acclimated at 6°C. Leaves were collected at 0, 2, 14, 21, 35, 42, 56 and 70 days of cold acclimation. Total RNA was extracted and mRNA was isolated and reverse transcribed prior to making double stranded cDNA. cDNA was digested with *EcoRI* and *MseI*, and after ligation of *EcoRI* and *MseI* adaptors, pre-amplification was performed. After selective amplification, PCR products were resolved on a PAGE gel. Differentially expressed transcripts were excised, re-amplified and sub-cloned for sequencing. After screening 64 primer combinations, 548 transcript-derived fragments (TDFs) were identified. One hundred and seventy-two of the TDFs have been sequenced and annotated. BLASTX search results indicated that 27% of the TDFs coded for unknown proteins.



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Validation of QTL for resistance to pre harvest sprouting

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Pre harvest sprouting (PHS) is a serious problem in many wheat growing regions of the world, particularly in north eastern Australia. The condition results from grain germinating before harvest, and occurs when humid conditions or rain events occur after grain maturity. PHS results in downgrading of grain quality and can cause total crop loss in extreme years. Resistance to PHS has proved to be difficult to breed for in white wheats as part of a non-dedicated selection program, and there are no current Australian varieties with an acceptable level of resistance. Screening for resistance to PHS is labour intensive and is strongly influenced by environmental conditions. It would be useful to be able to use molecular markers to enrich early generations for resistance to pre-harvest sprouting, to significantly increase the percentage of lines showing good resistance in phenotypic tests

undertaken in later generations. One major source of resistance to PHS has been identified in the line AUS1408. Several studies have mapped QTL for PHS from this source. In this study, fine mapping has been undertaken in four populations derived from this source, in the region identified on 4A as containing a QTL for resistance to PHS, to identify user-friendly markers and analysis undertaken to determine the best strategy for application of markers for this QTL. The best strategy for selection at this locus was found to be the application of two flanking markers, gpw2279 and barc170. Diversity Array Technology (DArT) has been applied to two of these populations to develop frame-work maps, to be used for QTL analysis for resistance to PHS. In each case two years of phenotypic data was used. This was provided by Dr Daryl Mares. In addition to the QTL on 4AL a major QTL was identified in one of these populations, close to the centromere, on chromosome 3B. This region had previously been identified by Mares et al. A less significant QTL was found on chromosome 4B. Only the major QTL on 4AL was evident in the second population. The expression of the QTL on 3B may be influenced by genetic background. The major QTL reported on chromosome 5BL in a study by Tan et al was not detected in this study. The affect on population enrichment using markers for the QTL on 4A and 3B was assessed.

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Field selection of winter wheat (*Triticum aestivum* L.) to water shortages by a mobile automatic rain shelter (MARS)

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In Hungary abiotic stresses are the most limiting factors in wheat production. Drought is the most important abiotic stressor which causes significant yield losses. The improvement of the yields under stress conditions therefore must combine the high yield potential and specific factors, which are able to protect the crop against reductions due to different stresses. Using the automatic rain shelter which was installed in the year 2006, advanced lines of winter wheat could be tested for drought tolerance under irrigated (control treatment) and under dry conditions. 85 genotypes could be tested on two-row plots in three replications in the 2006/2007 wheat year. The automatic rain shelter covers a 720 square meter field. Rain sensors set the closing mechanism which completely covers the field plots by a convertible plastic tunnel. Drain ditches prevent the side-wetting from the neighbouring soil profile. Drought can be traced by two automatic meteorological stations which continuously measure the rainfall, sun radiation dew point, soil moisture, soil temperature, air temperature, wind direction and speed. Withdrawal of water started at early spring (10th of March), and symptoms of drought became visible from the beginning of May. The effects of drought were evaluated by the relative water content of excised leaves, depression of the yield components and grain yield and the difference between the canopy temperature of stressed / control plots of the different genotypes. Withdrawal of water caused significant effects on the genotypes tested. Based on these testing methods we have developed a novel breeding system by which we can routinely select for drought resistance. The methods applied can easily be incorporated into our working pedigree breeding system. The successfulness of our research work is best reflected in the new drought tolerant wheat varieties released. GK Hunyad, GK Békés and GK Csillag varieties were registered in 2005 and they have been successfully grown in Hungary and in foreign countries as well.

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Spring wheat lines contrasting in water soluble carbohydrate concentration. I. Growth, nitrogen absorption and partitioning

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Because of its capacity to buffer grain filling, high levels of stem water soluble carbohydrates (WSC) constitute a desirable trait to incorporate in breeding programs that target regions with high frequency of terminal water stress. In the context of selection for this trait, it is important to determine if differences between lines high and low in WSC exist under a range of environmental conditions. Another issue to consider is if there are any trade-offs associated, particularly in high yielding environments. In this study we examined the dynamics of biomass, nitrogen and WSC accumulation and water use in high (2) and low WSC (2) recombinant inbred lines (RILs) from the Seri x Babax population, in environments ranging from moderate water stress to fully irrigated. The RILs had shown transgressive segregation for WSC accumulation but otherwise similar anthesis date and height. Early sown irrigated trials offered a more consistent environment to characterise lines for their differences in WSC concentration or amount throughout the cycle. In the range of environments tested (4-8 t/ha), high WSC lines yielded up to 18% more than low WSC lines, with a phenotype of consistently lower grain number per unit area, due to a lower spike number per plant, but significantly higher thousand grain weight. The grain number per unit area was negatively correlated to the stem WSC concentration at anthesis; also, the stem WSC concentration at anthesis was negatively correlated with the stem number per unit area at anthesis. High WSC lines attained a higher average individual grain weight when the potential WSC contribution during grain filling exceeded 4-5 mg grain⁻¹. Based on the above results, we propose that the negative relationship between grain number and WSC concentration is not necessarily competitive but. Also, the lack of response of grain weight to potential WSC availability per grain in the low WSC lines fuels the hypothesis that high WSC lines may have a higher potential grain weight per se. The results are discussed in terms of impact for adaptation to drought and breeding.

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Identification of candidate CBF genes for the frost tolerance locus *Fr-A^m2* in *Triticum monococcum*

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A cluster of eleven CBF genes was recently mapped to the *Frost resistance-2* (*Fr-A^m2*) locus on chromosome 5 of *Triticum monococcum* using a cross between frost tolerant accession G3116 and frost sensitive DV92. The *Fr-A^m2* locus was mapped at the peak of two overlapping quantitative trait loci, one for frost survival and the other for differential expression of the cold regulated gene *COR14b*. Seven lines with recombination events within the CBF cluster were used to identify CBF candidate genes for these QTL. The lines carrying the critical recombination events were tested for whole plant frost survival and for differential transcript levels of cold induced *COR14b* and *DHN5* genes. The strongest effect for these traits was associated to the linked *TmCBF12*, *TmCBF14* and *TmCBF15* genes, with the G3116 allele conferring improved frost tolerance and higher levels of *COR14b* and

DHN5 transcript at 12°C than the DV92 allele. Comparison of CBF protein sequences revealed that the DV92 *TmCBF12* protein contains a deletion of five amino acids in the AP2 DNA binding domain. Electrophoretic Mobility Shift Assays confirmed that the protein encoded by this allele cannot bind to the CRT/DRE (C-repeat/dehydration-responsive element) motif present in the promoters of several cold induced genes. A smaller effect on frost tolerance was mapped to the distal group of *CBF* genes including *TmCBF16*. Transcript levels of *TmCBF16*, as well as those of *TmCBF12* and *TmCBF15* were up-regulated at mild cold temperatures in G3116 but not in DV92. Higher threshold induction temperatures can result in earlier initiation of the cold acclimation process and better resistance to subsequent freezing temperatures. The non-functional *TmCBF12* allele in DV92 can also contribute to its lower frost tolerance.

P178

Identification of a QTL on chromosome 7AS for sodium exclusion in bread wheat

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The purpose of this study was to define the location of QTLs associated with sodium exclusion in bread wheat. Two F₁-derived doubled haploid mapping populations made from crosses between Cranbrook x Halberd, 160 lines and Kukri x Excalibur, 233 lines, were used in this study. Shoot sodium accumulation was measured in both supported hydroponics and in field trials at Roseworthy, SA. The hydroponics experiments were carried out twice and the field trial once for both populations.

A QTL located on chromosome 7AS was present in both environments (hydroponics and field trials) and both populations. This QTL was suggestive (LOD = 2.9 and 3.0) and accounted for approximately 7% of the total phenotypic variation in both populations, with the favourable (sodium exclusion) allele coming from Cranbrook and Excalibur. The QTL links to the related interval on rice chromosome 8 and candidate genes are currently under investigation.

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Low temperature adaptation of heading wheat in northern Australia

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Spring radiant frost is a problem in northern Australia for wheat crops at heading. Paradoxically, crops grown in warm sub-tropical climates are at greater risk than those in temperate regions due to faster development and rapid progression through to the susceptible reproductive stages. In sub-tropical northern Australia, spring radiant frosts occur frequently during the optimal flowering time. Planting is routinely delayed so crops are not heading during the maximum frost risk period. However, this results in a considerable loss of yield potential. Small increases in post head-emergence frost resistance would allow crops to flower earlier, significantly increasing yield potential, while maintaining an acceptable frost risk. Frost damage of advanced crops is also important in Mediterranean and temperate growing regions of Australia, North America and Europe. For example in the USA, winter wheat can be damaged when warm conditions in early spring are followed by late frosts (2007). Research and screening for resistance to spring radiant frost has been undertaken in northern Australia for several decades. Our screening method allows frost resistance to be accurately assessed in isolation from frost escape mechanisms. The current trials have been conducted over 7 seasons at 2 sites in southern Queensland. A number of wheat genotypes with putative frost adaptation mechanisms exhibited no detectable advantage over standard Australian cultivars. Many

current 'freezing' chambers are not suitable for screening in-head frost resistance. We are continuing field screening to identify sources of in-head frost resistance. Current research methods and results will be discussed.

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Molecular diversity at QTLs for pre-harvest sprouting resistance in spring wheat using microsatellite markers

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Preharvest sprouting (PHS) resistance is an important wheat breeding objective, particularly in areas where high rain fall occurs during grain ripening and harvest. We have characterized 32 wheat accessions using 33 microsatellite markers flanking PHS quantitative trait loci previously identified on chromosomes of group 3, 4, 5, and 6 of hexaploid wheat. A total of 229 alleles, with an average of 6.94 alleles per marker, were observed among the 32 wheat lines. The polymorphic information content was estimated and ranged between 0.25 and 0.90, with an average of 0.67. A cluster analysis revealed 3 main clusters and three singlet wheat lines, which agreed closely with pedigree-based relationships, seed coat colour and accession origin. Canadian wheat accessions could be subdivided into four sub-clusters based on pedigree and enduse classification. Grouping of pre-harvest sprouting germplasm into clusters was consistent with cluster type-specific allele diversity observed in the PHS resistant lines AUS1408, Red-RL4137, White-RL4137 and Kenya 321.

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Visual monitoring of water deficit stress using infra-red thermography in wheat

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Although drought occurrence is sporadic, it is one of the significant abiotic stresses decreasing wheat yields in the dry areas of West Asia and North Africa. Genetic improvement of drought tolerance by using synthetic hexaploid wheat resources has been the subject of recent studies that incorporate alien genes from wild relatives. Drought stress occurs mainly through increasing the soil water deficit during plant growth, and the breeding methodology requires a physiological parameter to instantly monitor increasing water stress. In this study, infra-red thermography was used to instantly monitor changes in the leaf temperature of wheat plants grown under increasing water deficit in controlled conditions, and to examine the effect of soil water stress on canopy temperature under changing solar radiation in field conditions. Canopy temperatures of 12 wheat genotypes were also compared after foliar treatment with pyraclostrobin fungicide. Leaf temperature responded rapidly to the daily change in soil water content. Large differences in canopy temperature were generated by water deficit stress in the field, and were enough to instantly indicate the soil water stress. There was a slight reduction in canopy temperature after fungicide treatment, which may indicate an acceleration of transpiration contributing to the translocation of assimilates to grain. Synthetic wheat genotypes with higher grain yield under dry soil conditions showed lower leaf temperatures and higher root water uptake abilities than those of parental genotypes. The results suggested that leaf or canopy temperature was a reliable indicator for detecting the thermal changes of wheat foliage under water deficit and chemical treatment. Infra-red thermography can be a visual tool to instantly monitor leaf or canopy temperature under increasing soil water deficit in the field. Use of infra-red thermography in screening wheat genotypes for drought stress was also discussed.

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Evaluation of wheat drought tolerance under four water regimes based on a dual isotope ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) conceptual model

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Measurement of natural variations in the relative abundances of plant stable carbon isotopes ^{12}C and ^{13}C , by means of a carbon isotope discrimination ($\Delta^{13}\text{C}$) technique, has been widely used in C_3 plant species to select and evaluate plant cultivars that can withstand drought. A plant cultivar, which is resistant to water scarcity should display less depletion in ^{13}C compared with a susceptible cultivar. Interpretation of $\Delta^{13}\text{C}$ may not be straightforward since variation may occur through a coherent change in stomatal restriction of CO_2 diffusion and down-regulation of photosynthetic capacity, which may result in similar $\Delta^{13}\text{C}$ values. Recently, a dual isotope approach based on ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) of bulk leaf tissue has been suggested to differentiate between sources of variation in $\Delta^{13}\text{C}$. This model gives insight into the relationship between stomatal conductance (g_s) and photosynthetic capacity (A_{max}) caused by different environmental constraints and plant-internal factors. Reports on using this model in cereal plants are very scarce, especially when water is the main environmental constraint. We used this model to explain relationships between leaf gas exchange parameters and growth and yield responses of six winter wheat (*Triticum aestivum*) cultivars with similar crop phenology. In addition, we assessed the validity of ^{18}O as a useful physiological trait under suboptimal water available conditions in a pot experiment with four different soil water contents under a rain protecting shelter with natural ventilation conditions in the faculty of Bioscience Engineering, Ghent, Belgium. All leaf gas exchange parameters were measured on mature flag leaf blades at post anthesis stage. Isotope analysis ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) were done on bulk flag leaf dry matters collected after gas exchange analysis. We found significant negative correlations between g_s and $\delta^{18}\text{O}$ and g_s and $\delta^{13}\text{C}$. Our results reveal that this model is capable to predict drought tolerant wheat cultivars and to interpret post anthesis variations in (g_s) and (A_{max}) of wheat cultivars while drought stress levels are increasing.

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Microarray analysis of salt-responsive genes in common wheat

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We have developed 22k and 11k oligo-DNA microarrays of common wheat. They have been designed to cover approximately 32,000 unique genes represented by a large number of expressed sequence tags (ESTs). In order to characterize salt-responsive genes in common wheat, expression profiles of transcripts in response to salt-treatment were simultaneously obtained using the microarrays. Two-week-old seedlings of Chinese Spring wheat were treated with 150 mM NaCl for 1, 6 and 24 hours, and their roots and shoots were separately subjected to hybridizations of microarrays. Consequently, 5,996 genes showed changes in expression of more than two-fold, and were classified into 12 groups based on their expression patterns. These salt-responsive genes were assigned their functions with the Gene Ontology (GO) terms. Genes assigned to transcription factor, transcription-regulator activity and DNA binding functions were preferentially classified into early response groups. On the other hand, those assigned transferase and transporter activity were classified into late response groups. These data suggest that multiple signal transduction pathways in response to salt treatment exist in wheat. Transcription factors (TFs), namely AP2/EREBP, MYB, NAC and WRKY, which have been reported as participants in salt-tolerant pathway, changed their expression levels in response to salt-treatment. Among them, only a few TFs showed high sequence similarity to genes in rice. Furthermore,

comparing the microarray data for wheat and rice, a small number of genes were up- or down-regulated in common in response to salt treatment. These investigations suggest that salt-responsive genes distinct from rice might be uniquely present in wheat, and wheat genes identified here are candidates for salt-stress tolerance related. The signal data of microarrays discussed in this study have been deposited in the Gene Expression Omnibus (GEO) of NCBI.

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Characterization of ABA sensitivity mutants altered abiotic stress tolerance in common wheat

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Abcisic acid (ABA) regulates plant growth and development, including response to environmental stress such as drought, high-salinity and cold/freezing stresses. To study roles of ABA in cold acclimation and development of freezing tolerance in wheat, expression profiles of abiotic stress-responsive *Cor/Lea* genes and their transcription factor genes were studied using ABA sensitivity mutants of common wheat. A mutant line 'EH47-1' was less sensitive to exogenous ABA than the original line 'Kitakei-1354' as judged by the magnitude of ABA inhibition of seedling growth. 'ABA27' was an ABA-hypersensitive mutant line and derived from EMS-treated seeds of cv. 'Chihoku-komugi' (Chihoku). Although there were no significant differences in expression levels of *Cor/Lea* and transcription factor genes between the mutant and parental lines during low temperature treatment, the levels of freezing tolerance in these mutant lines were improved comparing with the parental lines. Especially, the mutant lines were significantly higher freezing tolerant than the parental lines without cold acclimation. Therefore, ABA sensitivity seems to be associated with determination of freezing tolerance level in wheat, however the molecular mechanism in the improvement of freezing tolerance remains unknown. To obtain further information on the role of ABA in abiotic stress responses, other ABA sensitivity mutant lines were characterized. ABA31, ABA59 and ABA90 were derived from Chihoku as well as ABA27, and ABA122 and ABA126 were from cv. 'Horoshiri-komugi'. Bioassay showed that ABA31 and ABA126 were ABA-hypersensitive mutants and that ABA59 and ABA122 were less sensitive to ABA than parental lines, whereas no significant alteration was observed in the ABA inhibition of seedling growth in ABA90 compared with Chihoku.

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Comparison of global gene expression between wild emmer wheat genotypes contrasting in drought resistance

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Plants adapt to drought at the molecular, cellular and whole plant levels by a range of physiological and biochemical mechanisms, controlled by a network of genes which could be activated or repressed in response to drought stress. Wild emmer wheat (*Triticum dicoccoides*), the progenitor of cultivated wheat, is a promising source for improvement of drought resistance. The aim of this study was to identify candidate genes for drought resistance derived from wild emmer wheat. We describe here a

comparison of global gene expression between drought resistant and drought susceptible genotypes of wild emmer wheat, under normal irrigation vs. drought stress conditions, using Affymetrix GeneChip® technology. ANOVA analysis revealed 1,091 differentially expressed transcripts ($p < 0.0001$) in at least one of the four genotype/treatment combinations. Further analysis showed that a group of 60 genotype-specific transcripts were highly expressed under drought stress in the resistant genotype. Gene annotation showed that 70% of these transcripts are functional proteins, 9% are regulatory proteins and 21% were not identified. The identified functional proteins are involved in important biological pathways, such as: membrane structure; metal ion, hormone and water transport; carbohydrate and protein metabolism; and senescence processes. Furthermore, some of these proteins are known to be involved in drought tolerance in other plant species. Therefore, these transcripts are considered as potential candidate genes for drought resistance. We show here that the wild emmer wheat gene pool is a promising source for improvement of drought resistance in cultivated wheat.

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Comparative proteomic analysis of heat stress on the metabolic seed protein fraction in the Italian durum wheat cultivar Svevo

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In Central and Southern Italy, where durum wheat is mostly grown and represents one of the most important crops, grain filling occurs between April and May, when sudden increases in temperature may take place. High temperature during grain filling has already been recognized to cause a deviation of expected properties and quality characteristics of bread wheat doughs. Wheat grain proteins are typically classified according to their solubility properties into albumins (water soluble), globulins (salt soluble) and prolamins (gliadins and glutenins). These latter make up the gluten, and are mostly responsible for rheological properties of wheat doughs. Non-prolamin fractions include proteins with metabolic activity or structural function. In order to verify the consequences of heat stress on endosperm protein accumulation in durum wheat, we submitted the cultivar Svevo to two thermal regimes (heat stress vs. control). Two-dimensional electrophoresis (IEF/SDS-PAGE) was carried out on the metabolic fraction, in order to identify differentially expressed polypeptides. This analysis revealed 132 differentially expressed polypeptides (both up- and down regulated). They were collected and their identification performed by MALDI TOF and MALDI-TOF-TOF. Approximately 50% of the picked spots revealed by gel analysis as being differentially regulated were identified by NCBI nr and TIGR wheat protein databases search. The identified proteins were functionally diverse and included: HSP, proteins involved in ATP synthesis, in glycolysis, in carbohydrate metabolism, and in signal transduction.

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Genetic analysis of wheat nitrogen use efficiency: coincidence between QTL for agronomical and physiological traits

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Nitrogen (N) fertilizer represents one of the main inputs of the winter wheat crop in terms of operational cost for the farmer but also in terms of energetic cost for its production and application. Moreover, it increases pollution risk through leaching and volatilisation. Our objective is to develop knowledge and tools to breed varieties exhibiting a higher N use efficiency (NUE). To achieve this, we developed a QTL approach using two wheat mapping populations that were grown in seven locations under high and low N fertilization regimes. Environments were characterized using a N stress index based on probe genotypes. QTL were detected for grain yield, grain protein yield and their components as well as sensitivity to lowered N fertilization. To better describe the functioning processes involved in NUE, we characterized a sub-sample of 120 lines of one of the populations at low N fertilization under controlled conditions at an early growth stage using a conceptual model of carbon/nitrogen functioning. QTL detection was performed on root architecture traits and model efficiencies. The same population was characterized in the field for activities of marker enzymes of assimilation and recycling (nitrate reductase, glutamine synthetase, glutamate dehydrogenase). QTL were detected on the whole genome with some regions being specific to a N fertilization level. The dwarfing gene (Rht-B1), the photoperiod sensitivity gene (Ppd-D1) and the awn inhibitor gene (B1) coincided with the highest numbers of QTL and showed a significant interaction with the N level. Under controlled conditions, QTL were detected for root architecture, for model efficiencies and for integrative traits. Coincidences between QTL detected in the field for agronomical and physiological traits and under controlled conditions are presented.

P188

Mapping QTLs related to yield and yield components under drought in bread wheat

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So far only few studies targeted quantitative trait loci (QTLs) related to yield and yield components under drought stress in wheat because of the complexity of the trait, difficulties to provide appropriate stress environments for field evaluations and lack of proper mapping populations. Two parents contrasting in agronomic and morphological traits of interest, a typical Iranian drought tolerant landrace, *Tabassi*, and a highly bred and non-drought tolerant European wheat variety, *Taifun*, were crossed to produce 118 F2:7 recombinant inbred lines (RILs). A linkage map, based on 204 polymorphic SSR markers, as well as three morphological traits, *awnedness*, *spike pubescence* and *flag leaf waxiness*, providing 217 loci in total, was constructed covering 2795 cM. Phenotypic data for grain yield and grain number per ten spikes, 1000-kernel weight, spike length, spikelet per spike, plant height, and ear emergence time were collected under two non-drought stress and two drought stress conditions. In addition a post anthesis drought stress was induced by Potassium Iodide (KI). QTL

analysis was done by composite interval mapping (CIM). Out of a total of 146 putative QTLs having clear peaks and a minimum LOD of 3, 39 were identified as major QTLs ($R^2 \geq 10\%$) with an average of 5.6 QTLs per trait. In most cases, presence of major QTLs was confirmed by minor QTLs localized on the same chromosome but identified in different environments. With 11 major QTLs, chromosome 4D seems to be the richest one. For 5 traits, 8 QTLs were found under drought stress conditions. On chromosomes 7A and 7B 3 major yield QTLs were found in the KI experiment, in Iran and under non-stress condition in Austria.

P189

Generation of drought-resistant transgenic cereals using transcription factors isolated from wheat grain

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Transcription factors have been shown to control the activity of multiple stress response genes in a coordinated manner and therefore represent attractive targets for application in molecular plant breeding. Several families of transcription factors, such as DREB/CBF, ERF, MYK, MYB, AREB/ABF, NAC and HDZip class I and II, have been shown to be involved in the regulation of drought response in plants. The aim of our research is to investigate the transcriptional regulation of drought response at the reproductive stage of wheat development with a view to increase drought tolerance. Using the yeast one-hybrid system and the Drought Responsive promoter Element (DRE) as a bait, we have identified and cloned cDNAs of two novel DREB transcription factors from unstressed developing wheat grain. These factors, designated *TaDREB2* and *TaDREB3*, were over-expressed in wheat and barley transgenic plants under constitutive (double 35S) and drought inducible (maize Rab17) promoters. Barley plants (cv. Golden Promise) were transformed using *Agrobacterium* mediated transformation while biolistic transformation was used to produce transgenic wheat (cv. Bobwhite). The presence and expression of the transgenes in T_0 plants has been confirmed by PCR, Southern and Northern blot hybridization. T_1 transgenic plants with constitutive over-expression of *TaDREB2* showed a delay in germination and flowering; they were also slower growing and darker than control plants. *TaDREB3* showed a similar phenotype, except they were about 1/3 smaller than wild type plants and produced twice as many tillers. T_1 transgenic plants showed remarkable drought tolerance on the seedling stage of development. The drought tolerant phenotype correlated with the strength of expression of the transgenes. Statistically significant increase of water use efficiency was detected for *TaDREB3* transgenic plants, but not for *TaDREB2* transgenic plants. This suggests different mechanisms of drought tolerance conferred by these transcription factors. Detailed analysis of drought, cold and salt tolerance in barley and wheat transgenic plants are currently in progress. This work includes assessment of grain yield in the absence of stress and under mild stress at the reproductive stage of plant development, analysis of phenotypes of transgenic plants with over-expression of DREB factors under drought inducible promoters and field trials.

P190

QTLs for yield and adaptation to reduced water and nitrogen inputs in durum wheat (*Triticum durum* Desf.)

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A recombinant inbred line population of 184 RILs was generated by Produttori Sementi Bologna Spa, Italy, from the cross between the two Italian semi-dwarf durum wheat cultivars Meridiano and Claudio. The two parents are both high yielding, medium- to early-heading, elite cultivars widely adapted to the

Mediterranean temperate durum wheat growing areas. In 2007, the lines were evaluated at three sites for a total of five field trials: northern Italy in Cadriano, Bologna, under optimal water and nitrogen conditions, southern Italy in Lucera, Foggia (rainfed Mediterranean environment) and Obregon, Mexico, the CIMMYT's primary yield testing location, under optimal, water-stressed and nitrogen-stressed conditions. A linkage map is under construction using both SSR and DArT markers. In a preliminary analysis, 125 evenly spaced SSRs were used to investigate the association (single-marker linear regression) with heading date, plant height, grain yield, grain weight, density of fertile tillers, test weight and grain protein content. The results pointed out that two chromosome regions were primarily involved in the genetic control of most of the considered traits, including grain yield (QTL clusters), on chrs. 4B and 5A. Favourable alleles were inherited from the parental cultivar Claudio at the 4B QTL region and from Meridiano at the 5A QTL. Additional markers with highly significant effects on grain yield were found on chrs. 2B, 3B, 5B and 7B, with favourable alleles contributed by both parents. This accounted for the wide transgressive segregation observed among the lines and for the presence of lines that significantly out-yielded both parents. Grain yield of the RILs was highly predictable based on the SSR alleles present at four key-chromosome regions.

P191

Association mapping in durum wheat grown in a broad range of water regimes

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In major crops, panels of germplasm accessions are actively being assembled for allele mining at targeted candidate genes and for whole-genome association mapping studies. A germplasm collection of 189 cultivated durum wheat accessions was selected from several Mediterranean countries as well as from the CIMMYT and ICARDA breeding programs. Sixteen trials (rainfed and irrigated) were carried out in 2004 and 2005 in Italy, Spain, Morocco, Tunisia, Syria and Lebanon. Across the environments, the materials showed an average yield ranging from 0.9 to 8.3 t/ha. Yield, yield components, agronomic and physiological traits were recorded and tested for significant association with the allelic profile of 180 SSR markers distributed over the 14 linkage groups. SSR markers were previously tested for polymorphism and most of them showed from two to four frequent alleles in the collections. Population structure was evaluated and the clustering data were used as covariates in the association test. SSR markers showing significant associations in four to six up to 8/10 environments were identified for the traits with the highest heritability values, i.e. plant height, heading date, peduncle length and kernel weight, with R^2 values ranging from 5 to 10%. As to yield and yield components, the majority of the associations could be identified only for two to four environments, with an average R^2 value lower than 5%. The detailed results, including the co-location of significant SSRs for the different traits and the allelic effect of some of the most interesting markers will be presented. The results of this study will be compared to those reported in other QTL studies in wheat.

P192

Halt irrigation effects on drum wheats at Iranian semi-arid environment

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In Iranian wheat farms, we can see a lot of winter wheat genotypes same as bread or drum wheats that planting as irrigated systems. These farmers must be save the water for more outputs and halt or stop irrigation is the new scenario for solving the part of this national water limitation. In order to study the effects of halt irrigation on yield components and seed yield of new four genotypes of drum wheat this field experiment was conducted at 2006 in semi arid environment of Iran. The experiment design was split plot with 4 replications that the main plots were 3 irrigation regimes included non stop irrigation at all growth and development stages of drum plants, halt irrigation at heading stage and halt irrigation at grain filling stage. Drum wheat genotypes were the subplot arrangement variable Yavarous, WD791982, WD791582, WD792282. The results showed that the halt irrigation effect on grain yield and its components was significant at 5% meaning level. The halt of irrigation at heading stage was the main factor for reduce grain production factor in all drum genotypes. The interaction effects between halt of irrigation and genotypes were significant at 1% meaning level. The resistance ability of WD791982 to halt irrigation and low water stress at heading stage was more of others by 5675Kg per hectare grain production. The lowest grain yield was for sensitive drum genotype WD791982 by 50% about WD791982.

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P194

Quantifying the value to grain yield of QTL for adaptation and tolerance to abiotic stress in bread wheat

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To improve the productivity and yield stability of wheat in rainfed environments the importance of traits associated with tolerance to abiotic stress needs to be quantified. QTL analysis in a doubled-haploid population derived from a cross between Berkut and Krichauff was used to do this. Berkut is a broadly-adapted CIMMYT wheat whilst Krichauff is adapted to the winter rainfall, alkaline soils of southern Australia. The population was grown in rainfed trials in Australia, Morocco and Kazakhstan and under irrigation in Mexico, India and Pakistan. Rainfed grain yields ranged from 416 kg/ha to 1919 kg/ha. Three QTL on chromosomes 4A (near *wmc048b*), 6A (near *wPt-7063*) and 6B (near *wPt-4924*) were associated with yield at multiple sites. Together they accounted for between 4% and 21% of the genotypic variation in yield, with effects on yield of individual QTL at specific sites ranging from 8 to 18%. At a low yielding site (< 600 kg/ha) *wPt-4924* was associated with head tipping, with higher yields associated with greater tipping. Under limited irrigation in Mexico, yield QTL were associated with pre-anthesis canopy temperature (CT) and leaf waxiness. Low CT and greater waxiness improved yield. While yield QTL were detected, the physiological basis of these has not

been described. However, under severely stressed conditions in southern Australia, a degree of grain abortion appears to confer a yield advantage.

P195

Providing a biological interpretation of Genotype x Environment interactions in bread wheat

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Many soils in the Australian wheat belt have chemical and physical properties that restrict root growth, limit crop water use and grain yield. Faced with this array of constraints, plant breeders need to prioritise their breeding objectives, targeting those traits that will lead to the greatest yield gain. Analysis of Genotype x Environment (GE) interactions provides some guide but generally past analyses have been based solely on grain yield. Interpretation of GE analyses could be enhanced if other characteristics of the varieties were included. The aim of this work is to incorporate phenotypic data on varietal responses to important nutritional constraints, tolerance to root disease, physiological and developmental characteristics and genetic information into a large GE analysis. The data set consists of grain yields of 52 genotypes grown in 234 trials conducted over 68 locations in the Australian wheat belt between 1994 and 2005. An initial two stage analysis was performed. The first stage estimated Genotype means for each environment (Year x Site combination). Mean yields were then used to fit a 2-Factor Analytic model to describe the GE interaction. As expected, marked differences in yield and yield stability were evident. The varieties Axe, Yitpi, Stylet, and Wyalkatchem yield consistently well across all environments, while Hartog, Baxter and Sunstate have consistently low yields. Westonia, Aroona and Schomburgk show relatively good adaptation to WA and poor yields in NSW and Victoria, while Ventura, Ruby and Matong yield well in NSW and have consistent yields in WA, SA and Victoria. The next step in this analysis is to use information on tolerance to soil stresses, root disease and the physiological characteristics of the genotypes to provide a biological interpretation of this GE interaction.

P196

Genetic mechanisms involved in late maturity alpha-amylase in wheat

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Late maturity alpha-amylase (LMA) is a genetic disorder associated with premature synthesis of high pI alpha-amylase isozymes during the middle to later stages of grain development under normal growing conditions or, more commonly, when exposed to a cool temperature shock. For the majority of LMA-prone genotypes, exposure to a significant temperature differential (i.e., a cool temperature shock), rather than cool temperature alone appears to be important for consistent and maximum expression of LMA. High alpha-amylase activity is retained in the grain at harvest ripeness, resulting in low falling number and consequently a failure to meet receival standards. Expression of LMA in wheat is dependent on QTL on chromosomes 7B and 3B, however, the level of expression is affected by a range of factors that include: genotype, environment and agronomy. This study compares the levels of LMA expression in a range of *Triticum aestivum* isolines involving different GA insensitive genes, 1B/1R and/or 7Ag/7D translocation; *Triticum durum* isolines; primary synthetics and derived synthetics. The results appeared to confirm that GA insensitive/ semi-dwarfing genes not only reduce the level of expression but also introduce the requirement for a cool temperature shock. 1B/1R

enhanced whilst 7Ag/7D slightly reduced expression respectively. Synthetics showed extreme levels of LMA, presenting a limitation on their widespread use in breeding programs.

P197

Mapping quantitative trait loci associated with salinity tolerance in synthetic derived backcrossed bread lines

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Soil salinity is one of the major environmental problems affecting agricultural production in most parts of the world, both in irrigated and dryland conditions. Improving salinity tolerance of wheat is a key target for many wheat breeding programs worldwide. Amongst the synthetic hexaploid wheats (SHWs) ($2n=6x=42$, AABBDD) derived from crosses between *Triticum turgidum* L. var. durum, ($2n=4x=28$, genome AABB) and *Aegilops tauschii* (syn *Ae. squarrose*, *T. tauschii*; $2n=2x=14$, genome DD), significant variation was observed for salinity tolerance. To investigate natural allelic variants contributing to quantitative variation for salinity tolerance in bread wheat, we used a quantitative trait mapping approach to analyse two populations of BC₁F₆ synthetic backcross lines (SBLs) derived from crossing SHWs to common bread wheat. The SBLs were screened for salinity tolerance based on the sodium exclusion mechanism. From preliminary analysis using simple interval mapping, several quantitative trait loci (QTLs) that confer salinity tolerance were identified. The QTLs are located on chromosomes 2B, 2D, 3D, 4B, 4D, 6D, 7A and 7D. Of these, only chromosome 4D region has previously been reported as contributing to tolerance. The SBLs proved to be a promising tool to identify, characterize and introgress different salinity tolerance genes into adapted wheat genetic backgrounds.

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Drought adaptation attributes and associated molecular markers in the Seri/Babax hexaploid wheat (*Triticum aestivum*, L.) population

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Agronomic and physiological traits associated with drought adaptation were assessed in Mexico and South Australia within the Seri/Babax recombinant inbred line (RIL) population, the progeny of which are similar in height and maturity but divergent in their sensitivity to drought. Under drought, canopy temperature (CT) was the trait best associated with performance ($R^2= 0.71$, $p<0.0001$) and highly heritable ($h^2= 0.65$, $p<0.0001$). CT reflects a mechanism of dehydration avoidance expressed throughout the growing season and across latitudes, which can be utilised as a selection criteria to identify yield performance under drought. Early vigour under drought, suggested by a high association of CT with estimates of biomass at booting ($r= -0.44$, $p<0.0001$), leaf chlorophyll ($r= -0.22$, $p<0.0001$) and plant height ($r= -0.64$, $p<0.0001$), contrasted with the small relationships with anthesis,

maturity and osmotic potential. Hence, results suggest that the ability to extract water from the soil under increasing soil water deficit is a major attribute of drought adaptation. Bulked segregant analysis (BSA) of CT and of associated secondary traits under drought was performed with a molecular database of 127 PCR-based and AFLP markers. One-way analysis of variance indicated significant associations of loci explaining phenotypic variance under drought and rainfed conditions, of 20-70% in Mexico and 20-45% in Australia ($F \geq 5.00$, $p < 0.05$). While the value of such associations needs to be further investigated in other genetic backgrounds and environments, results suggest a viability in the efficient tailoring of markers to improve yields in regions and latitudes with different rainfall patterns and drought environments. Further genomic and transcriptomic studies should be conducted in the Seri/Babax RILs to dissect the basis of the dehydration avoidance mechanism epitomised by CT and for unravelling the complex relationship between drought adaptation and performance under drought.

P200

Ethiopian durum wheat germplasm and hitchhiking mapping for drought QTL

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Nowadays, that several genetic maps of wheat are available, it is possible to select several loci along a chromosome in order to discover their variability distribution and determine if selective sweeps are present. In fact, if a locus is under selection pressure its variability is reduced, due to the fixation of single positive allele, and so do also the loci nearby the selected ones, due to their linkage with it. The neutral variants that are linked to the beneficial mutations are also affected by selective sweeps. A durum wheat collection of 234 genotypes from nine populations of three Ethiopian regions: (Tigray, Gonder and Shewa) with contrasting environments, was analyzed with 25 SSR loci localized along chromosome 4 and used to identify the hitchhiking mapping of this chromosome. Ethiopian durum wheat germplasm possesses peculiar characteristics when compared with those from other countries, due to a wide range of geographical divergence, climatic and altitude and can provide many beneficial traits, including disease resistance and tolerance to environmental stresses, such as drought and low temperature. Chromosome 4 was chosen since it is one of the chromosomes where QTLs for drought tolerance have been detected. The primers were selected to have, ideally, a locus every 5 cM. The results indicate the presence of 2 selective sweeps in the regions with drier environments. One, as expected, is located in a telomeric position of the short arm, in a position similar to the one where previous QTL for drought tolerance was found, the other is localized in a new position on the 4AL. These data indicate the existing of high selective pressure in the particular climatic conditions of Ethiopian territory, which is from always a natural laboratory for the study of the genetic variability and for the selection of genotypes with particular climatic adaptations.

P201

Genomic dissection of whole-plant responses to water deficit in durum wheat × wild emmer wheat RIL population

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Drought is the major environmental factor limiting wheat productivity and yield stability worldwide. Developing drought-resistant cultivars is an important target in wheat breeding. Wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides*, gene pool harbors a rich allelic repertoire for various morpho-physiological traits conferring drought resistance. A mapping population (152 RILs), derived from a cross between durum wheat (cv. Langdon) and wild emmer (acc. G18-16), was evaluated in the field under contrasting irrigation regimes. Wide genetic variation and transgressive segregation were found among RILs for productivity and drought related morpho-physiological traits. A genetic map with 307 SSR and DArT markers accounted for a total length of 2,317.1 cM, with an average density of one marker per 7.5 cM. A total of 102 QTLs for 10 traits were identified. A number of QTLs showed environmental specificity, accounting for productivity and related physiological traits under water-limited or well-watered conditions. Major genomic regions controlling productivity and related physiological traits were identified on chromosomes 2B, 4A, 5A and 7B, highlighting different adaptations mechanisms. Associations between QTLs for various traits suggest that several mechanisms are involved in adaptation to drought stress. The identified QTLs may facilitate the dissection of drought adaptive complexes in wheat and the improvement of drought resistance in elite wheat cultivars by marker assisted breeding.

P202

***Cbf* gene regulation in wheat in response to varying cold acclimation induction temperatures**

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In wheat, *Cbf* genes are considered master switches regulating response to cold and are candidate genes for low-temperature (LT) tolerance QTL localized at Fr-A2. *Cbf* expression is generally greater in cold hardy compared to tender wheat genotypes. Most *Cbf* expression studies to date have focused on characterizing *Cbf* expression induced after cold exposure, but have not quantified differences in expression associated with the rate of response to LT exposure. In this research, we examined the expression of *TaCbf* genes in winter wheat cultivar “Norstar” at 0, 2, 4, 8, and 48 hr after exposure to four induction temperatures (6, 10, 16, 18°C) in a 16h day. At 0 and 48 hr, plants were sampled minutes after full exposure to light. Orthologous *Cbfs* were also studied in the cold sensitive winter barley “Kold” and cold-hardy winter rye “Puma” to allow cross-species comparisons. Regardless of species, the expression patterns of *Cbfs* were variable, with a complex *Cbf* gene x time x induction temperature interaction. In wheat, *TaCbfIII-5* was expressed only at 2- and 4 hr after exposure to 6°C and transcripts were not detected at higher temperatures. In contrast, *TaCbfIVc-B14* was expressed at all temperatures. In barley, *HvCbfI* was expressed at 2 and 4 hr after exposure to 6°C, but was only expressed after 48 hr at 18°C. For those *Cbfs* expressed at higher induction temperatures, expression was strongly repressed at 48 hours, just as plants came out of the dark. Downstream *Cor* gene transcripts were also absent at 48 hr at 15 and 18°C with similar patterns observed in wheat, barley and rye. These observations indicate that in cereals, *Cbf* expression is regulated not only by induction temperature, but also by light regulated circadian rhythm. This work suggests that sample timing, induction temperature, and light related factors must be considered in future studies involving functional characterization of *Cbf* genes in wheat.

P203

Characterisation of durum germplasm for aluminium resistance using nutrient solution culture

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Durum wheat (*Triticum turgidum* L ssp *durum*) is the most sensitive member of the Triticeae. In the literature, very limited genetic variability for aluminium tolerance has been reported in tetraploids. We evaluated 408 genotypes of the subspecies *durum*, *dicoccon* and *carthlicum* wheats for Al resistance, procured from the Australian Winter Cereal Collection, Tamworth, Australia. The screen was conducted using two replicates of each accession, with each replicate split between three 40-litre tubs. Each tub held 4 strips of 21 accessions, each represented by seven seeds. An incomplete block design for strips nested within tubs was employed and spatially optimised using DiGger allowing for row and column effects and positive correlations between locations within tubs. Seedlings were initially grown in nutrient solution in dark for 48 h. Control and Al treated (10 µM) were grown subsequently for further 48 hrs. The longest seminal root from each seedling was then measured and per cent relative root growth was measured. We used a new measure 'Incremental crop tolerance (ICT)' that reflect the incremental root regrowth between genotypes associated with Al resistance, over and above difference in underlying root vigour. Ten per cent of the genotypes having higher ICT indices were further evaluated for aluminium resistance using a nutrient solution containing 20 µM of Al. Statistical analysis indicated that three accessions were Al-resistant. The genetic identity (AABB) of these genotypes was confirmed using D genome specific markers Dgas44, *TaALMT1*, QSSR (domestication gene based marker) and gamma gliadin. Identification of Al-resistant genotypes will allow us to develop improved germplasm for Al resistance, suitable for cultivation on acidic soils.

P204

A high throughput method of EcoTILLING for detecting haplotype diversity within the aluminium resistance gene (*TaALMT1*) of wheat

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TaALMT1 (Aluminium activated Malate Transporter of *Triticum aestivum* L), the gene controlling aluminium resistance via an Al-activated malate efflux mechanism, has recently been cloned, characterised and mapped on the long arm of chromosome 4D of wheat. The identification of novel alleles associated for Al resistance is required for further continual improvement. Besides, SNP and CEL 1 restriction enzyme based methods for EcoTILLING, gene specific markers based on INDELS and SSR could be utilised for preliminary genotyping and subsequently as a result of allele binning, unique haplotypes can be verified by sequencing. In order to validate this approach, we determined allelic diversity at the *TaALMT1* locus in a wide range of 1425 germplasm collections that included wheat cultivars, landraces, *T. aestivum* ssp *spelta*, ssp. *macha*, ssp. *sphaerococcum*, ssp. *compactum* and ssp. *vavilovii*, and D genome containing accessions of *Aegilops tauschii*, *A. cylindrica* and tetraploid and hexaploid *A. crassa*, *A. ventricosa*, *A. vavilovii* and *A. juvenale*. *TaALMT1* gene specific markers targeting repetitive INDELS within intron 3 region and an upstream region were employed sequentially using pooling approach. The repetitive INDEL marker detected 9 alleles. A subset of

germplasm with different alleles was genotyped with the long and short fragment markers targeting the upstream sequence of the *TaALMT1* gene. Alleles were binned and the data were confirmed by sequencing the unique haplotypes. The haplotype data were compared on a subset of genetically diverse wheat collections previously identified utilising DArT marker loci. The presence of *TaALMT1* alleles in *A tauschii* and the absence of *TaALMT1* alleles within ‘non-D’ genome species reconfirmed that *A tauschii* is a donor source of Al tolerance in modern wheats. Al-activated malate efflux measurements indicated that Al-resistance is indeed conditioned by the *TaALMT1* gene.

P205

Utility of derived synthetic wheats to enhance adaptive traits for grain yield in the north Australian region

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Interspecific crosses at The International Maize and Wheat Improvement Centre (CIMMYT) to produce ‘synthetic’ hexaploid (bread) wheats have increased genetic diversity in hexaploid wheat. Whilst conventional hexaploid CIMMYT germplasm is generally adapted to the north Australian region, the worth of derived synthetic (SYN) wheats is largely unknown, particularly for traits in addition to yield. Assessment of a total of 495 SYN lines across one to four environments in 2004/05/06 revealed the top 20% had high grain yield and grain weight, and lower percent screenings (grains passing through a 2mm slotted screen) compared to commercial checks. We assessed 92 selected SYN lines across four to 13 environments in 2006/07, with SYN lines generally having higher grain weight, lower percent screenings, greater canopy temperature depression (ctd), and enhanced “stay green” (independent of time to anthesis) compared to commercial checks. SYN lines generally had lower grain yield, although the relative yield performance of SYN lines improved in low mean yield environments (~1-2 t/ha). Enhanced genetic performance for maintenance of grain weight, ctd and stay green may be driving adaptation of SYN lines to drought stressed environments, and SYN wheats offer the northern Australian region exploitable genetic variation for these adaptive traits. Due to their generally lower yield *per se*, backcrossing and top crossing to adapted material is being undertaken to develop high yielding progeny that are enriched for adaptive traits grain weight, ctd and stay green. A subset of these SYN lines has been genotyped using DArT markers. While the number of synthetic-specific markers retained vary in these SYN lines, markers in some chromosomal regions from the primary synthetic appear to have been preferentially retained; further DArT marker data collection and analyses are underway.

P206

Genetic and physiological dissection of transpiration efficiency in wheat

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Crops reliant on rainfall for growth commonly experience water deficits that reduce grain yield and quality. Selection for genotypic adaptation to water-limited environments is challenging and progress is slow. Many factors contribute to slow breeding progress in dry, rainfed environments: (1) strict requirements for improved grain quality and disease resistance reduce genetic variance for performance under drought; (2) drought is a dynamic entity changing in timing and severity from one year/site to another; and (3) both the above contribute to large genotype × environment interaction to reduce repeatability of genotype performance and confidence in selection. The opportunity exists to compliment existing selection for yield with selection for novel trait variation to improve performance.

Water-use efficiency (WUE as biomass ÷ water used) is genetically correlated with improved biomass and yield under drought. Transpiration efficiency (TE), the ratio of net photosynthesis to water transpired, is an important component of WUE in environments where stored soil water accounts for a major portion of crop water use. Carbon isotope discrimination (Δ), through its negative relationship with transpiration efficiency and ease of measurement, has been used in selection of higher wheat yields in breeding for dry, rainfed environments. Mapping studies have shown Δ to be genetically complex. In wheat, chromosomal regions for Δ collocate across populations, while some regions (e.g. 2BS, 3BS, 4AS and 7AS) have been retained in phenotypic selection for Δ from the donor cultivar Quarrion, indicating high breeding value. Detailed studies aimed at physiological dissection of Δ indicate some Δ genomic regions are associated with changes in leaf conductance and/or canopy temperature, and others with regions varying for possible surrogates (e.g. leaf thickness and N content) of photosynthetic capacity. This information is leading toward development of quick, less-expensive protocols for screening breeding populations in early generations for improved performance under water-limited conditions without compromising performance in the absence of water deficit.

P207

Mobilization of dry matter and its relations with drought stress in wheat genotypes

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Wheat crops grown under post anthesis drought stress condition may need high level of stem reserve for grain filling. For understanding the genotypic variation of stem reserves differences in wheat under drought stress conditions, 20 winter wheat genotypes were evaluated under both post-anthesis drought stress and normal conditions in Ardabil Agricultural research station in two successive growing seasons 2005-2007 using Randomized Complete Block Design with three replications. The results showed that there were significant differences between genotypes in stem reserve under both normal and post anthesis drought stress conditions. Post-anthesis drought stress did not affect kernel numbers per spike. The rate of dry matter accumulation by kernels was considerably decreased by water deficit. Dry weight of vegetative organs decreased in grain filling period under stress and normal conditions, by contrast, in anthesis stage. But, the rate of translocated dry matter was much higher in genotypes No 14, NO 15, No 16, No18, No 19 and NO. 20 under drought stress condition. 1000GW and weight of kernels per spike were more severely reduced by water deficit. The positive correlation of grain yield with grain weight per spike, 1000GW, remobilization of dry matter, harvest index and stress tolerance index (STI) and significant negative correlation of grain yield with drought susceptibility index (SSI) revealed that selection must be exercised for high harvest index, grain weight per spike, 1000GW, remobilization of dry matter and STI in stress condition. The negative correlation of 'STI' with 'SSI' indicated the efficiency of 'STI' as a selection criterion for identifying the drought tolerant with high yield potential in winter wheat genotypes.

P208

Structural changes of the photosynthetic apparatus, morphological and cultivation responses in different wheat genotypes under drought stress condition

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This project was carried out with 64 different wheat genotypes under both irrigated and drought stress conditions, using simple lattice experimental design with two replications in research area of agricultural research station of Ardabil and research area and laboratory of genetic resources institute of Azerbaijan during two grown years from 2005 to 2007. Results showed that all characters studied of wheat genotypes had different responses under both irrigated and drought stress conditions. There were significant differences ($p < 0.01$) between studied wheat genotypes in all characters examined. Drought was reduced all characters studied, however, amount of reductions were different in genotypes examined. For example highly drought tolerant wheat genotypes showed higher amount of leaf area index, amount of water per dry weight of leaf and amount of water per wet weight of leaf than other genotypes studied. Drought was reduced wheat grain yield, by 61.9% and stress intensity (SI) for wheat grain yield was 0.62. Genotypes studied of wheat were classified in to three groups such as tolerant, moderate tolerance and susceptible by genotypic responses of wheat genotypes under both irrigated and drought stress conditions and on the basis of values of SSI and STI indices. Results of germination capability of wheat genotypes evaluated under 16 atmosphere drought stress of PEG and control (water) treatments showed same results as field experiments on the basis of the classifications of wheat genotypes for three classes such as tolerant, moderate tolerance and susceptible. Results of changes of amounts of chlorophyll also showed significant differences ($p < 0.01$) between genotypes examined on the basis of amount of chl a, chl b, chl a+b and chl a/b under 20 atmosphere drought stress of PEG and control (water) treatments. Drought on average reduced amount of chl a, chl b and chl a+b, by 2.5, 6.6 and 2.5% respectively. However the amounts of reduction in chl a, chl b and chl a+b were different between genotypes studied, so that the amounts of chl a, chl b and chl a+b were increased in drought tolerant wheat genotypes. According to the depression degrees of chl. a+b the classifications of wheat genotypes were also same as the classifications of field experiments and germination capability under 16 atmosphere drought stress of PEG and control (water) treatments. The amount of chlorophyll was positively correlated ($r = 0.36^{**}$) with grain yield and with leaf area index ($r = 0.15^{ns}$) of wheat genotypes. Drought was reduced genotypic variation and heritability of wheat genotypes in most of examined characters. However heritability of grain yield and leaf area index was increased in drought stress condition. Finally according to the studied characters the genotypes No. 6, 30, 37, 42, 43 and 44 were found as tolerant genotypes.

P209

Stem reserve and its contribution to grain yield of wheat (*Triticum aestivum* L.) genotypes under drought stress conditions

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Two experiments were conducted on 11, diverse wheat genotypes, which were grown under well watered, and drought stress conditions in field experiments. Genotypic variation for internodes characteristics and contribution of pre-anthesis assimilates to grain yield was measured at 10 days intervals in wheat (*Triticum aestivum* L.) genotypes during 2006-2007 year. A mechanistic model was

used to analyze the daily rates of dry matter mobilization from stem internodes to kernels, and its contribution to kernel weight. The main effect of irrigations, genotypes, and dates of harvest, and genotype \times date of harvest interaction were significant in all studied characters ($p < 0.01$). Internodes length, weight and specific weight with exception of stem specific weight were reduced under drought stress condition. Drought was decreased vegetative organs (above ground dry matter) weight at anthesis and maturity, grain yield per spike, grain No. per spike and 1000 grain weight by 5.7, 24.5, 21.2, 15.7 and 6.4 %, respectively. Translocation of dry matter from vegetative organs (above ground dry matter) to developing kernels and mobilization efficiency were considerably increased under drought stress condition, by 60.1 and 74 %, respectively. Contribution of pre anthesis assimilate was also highly increased under drought stress condition. Translocation of dry matter from peduncle, penultimate, and the lower internodes ranged from 51.2 to 76.9, 106.8 to 182.3 and from 100 to 208.6 mg under well watered and drought stress conditions, respectively. Mobilization of dry matter was higher in drought stress condition than in well watered for peduncle 50.2 %, penultimate internode 70.7 and for lower internodes 111.6 % respectively. Drought was increased the contribution of pre-anthesis assimilates to grain yield by 81.5 % in peduncle, 108.1 % in penultimate internode and by 153.8 % in lower-internodes respectively. Drought was also increased the translocation efficiency by 84, 91.3 and 111.6 % for peduncle, penultimate internode and lower internodes, respectively. Vegetative organ (above ground dry matter) at anthesis was correlated with grain weight per spike ($r = 0.68^*$), translocation of dry matter ($r = 0.34$) under drought stress condition. Translocation of dry matter was correlated with lower internodes, penultimate and peduncle maximum weight under drought stress condition by $r = 0.55$, $r = 0.56$ and 0.34 , respectively.

P210

Selection criteria for drought tolerance in spring wheat (*Triticum aestivum* L.)

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Twenty spring wheat genotypes were evaluated both under moisture stress (E_1) and non stress (E_2) environments for yield and yield contributing traits, grain weight/spike and biological yield in RBD with 3 replications. Drought susceptibility index (S) and index of drought resistance (IDR) at 2-leaf stage were worked out. Sufficient genetic variability was in existence for all the characters studied. The combined analysis of variance over environments indicated presence of variability among the genotypes, differences in environments and differential response of genotypes over environments as indicated from their significant mean square values except for harvest index and tillers/plant. Grain yield and biological yield showed maximum sensitivity as affected by E_1 and grain weight showed the least. The significant positive correlation of grain yield with biological yield and harvest index in E_1 ; significant negative correlation of grain yield with 'S' under E_1 and significant positive correlation of grains/spike and grain weight with grain weight/spike under both the environments revealed that selection must be exercised for high biomass, grain weight/spike and harvest index for yield improvement under dry land conditions. The negative association of IDR with 'S' indicated the inherent importance of IDR as a selection criterion for assessing the drought tolerance at seedling stage which is an easy, inexpensive and rapid method of screening large germplasm to characterize drought tolerant genotypes. Path coefficient analysis revealed that biological yield and harvest index exhibited high positive direct effects on grain yield under both the environments. Tillers/plant and grain weight/spike had mainly indirect effects on grain yield via biological yield under E_1 .

P211

The Dof transcription factor family in wheat

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Wheat is considered one of the world's most important crops. As improvements in harvest index fast approach a theoretical maximum, future gains in yield may depend on increases in crop biomass while maintaining harvest index. Utilization of transcription factors is a promising approach for generation of crops with superior characteristics as a single transcription factor can regulate coordinated expression of multiple genes in pathways. Plant specific Dof (DNA binding with One Finger) proteins play a diverse range of roles in the areas of plant growth and development, from regulation of the C4PEPC gene promoter to the regulation of seed storage proteins. This family is a potential target for improving wheat crop biomass. However, at present very little is known about the Dof family in wheat with only two proteins having been identified to date. This study has identified all Dof family members in wheat from available sequence data. Organ specificity and responses to stresses such as drought on Dof gene expression were analysed using quantitative RT-PCR to identify potentially growth related members. From this analysis two Dof proteins were selected with a potential role in growth and the full length sequences have been isolated and over-expressed in wheat.

P212

Efficiency of stress-adaptive traits Chlorophyll fluorescence and Membrane thermo-stability in wheat under high temperature

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Heat stress affect at least 15 million ha of spring wheat alone in developing world and over 7 million ha are grown in continual heat stress, mainly environment with mean daily temperature of greater than 17.5°C in the coolest month (Fischer and Byerlee, 1991). The demand for the wheat is expected to grow by approximately 1.6 percent per year worldwide and by two percent per year in developing countries by the year 2020. The most important step is to recombine elite genotypes, introgression of genetically diverse source to incorporate *Vrn*, *Ppd*, and *Eps* genes, increase partitioning of photo-assimilates, stem reserves, stay green, improve radiation use efficiency through out grain filling. An experiment conducted for the measurement of membrane thermo stability and chlorophyll fluorescence in six crosses in parents and F1s at post anthesis stage during year 2006-07. Acquired thermo tolerance showed significant variation in late sown for parents and F1s. It was inferred that genotype (EIGN1 and PBW 435) and F1s (PBW 343 x PBW 435 and EIGN1 x Raj 3765) conferred less relative injury and greater thermo tolerance positively through maintaining cellular membrane integrity under high temperature. Data based on chlorophyll fluorescence revealed reduction in all genotypes and F1s for Fv/m, variable fluorescence in late sown under high temperature compared to normal sown. The genotypes and F1s that showed high Fv/m value under both environment displayed good tolerance to high temperature. Based on the findings suggested that large and diverse genotypes with more number of replication be included and data should be recorded at different dates for the precise estimation and determining the stability of traits under high temperature conditions.

P213

Genetic analysis of leaf rolling in wheat

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There is evidence that leaf rolling may be associated with improved grain size under drought. But studies investigating the amount and nature of genotypic variation in leaf rolling of wheat are rare. The aim of this work was to investigate the extent of genotypic variation and the genetic system governing leaf rolling in wheat. Two separate approaches were taken to understanding the genetics of leaf rolling: one used analysis of six generation means in 2 crosses involving unrelated leaf-rolling line donors, B403D and K648R, and a non-rolling cultivar, Silverstar; the other, by means of a full 15×15 diallel-mating design, the 15 genotypes representing a range of germplasm from Australia and other countries. Two methods used to assess the leaf-rolling propensity were compared: 1 – by measuring leaf-rolling score (LRS) at anthesis on intact leaves in the field; and 2 – by determining mathematically the degree of curvature of the cross-sectional shape (mean curvature, κ) of these leaves at equilibrium in a range of PEG solutions varying in osmotic potential. Mean curvature was found to be an objective measurement, which can be used to differentiate rolling and non-rolling lines without the need to impose a soil water deficit. It is therefore independent of the confounding factors encountered in the field. Leaf-rolling genotypes were associated with positive κ (*i.e.* they were already curved ‘inwards’) in the absence of water stress and responded quickly to variation in water status, whereas non-rolling lines had negative κ (*i.e.* they were curved ‘outwards’) in the absence of water stress and had much slower response time to imposition of water stress. Broad- and narrow-sense heritabilities were high for both LRS and κ , suggesting that fixation of desirable alleles would be readily achieved in selection during early stages of inbreeding. The two genetic approaches indicated that LRS and κ were under strong additive genetic control with a small amount of dominance. The diallel-mating design also identified new lines with alleles that could be used for improving the propensity for leaf rolling in breeding programs. Contrary to popular belief, it was found that mechanical constraints resulting from negative curvature – not bulliform cells – were responsible for the delay in the inwards rolling response to water stress in non-rolling germplasm.

P214

TRITIMED, a multidisciplinary project to improve drought adaptation in durum wheat

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Durum wheat is one of the most widely cultivated crops in the Mediterranean basin. It is mainly grown under rainfed conditions characterized by drought and thus water is a major determinant of growth and final yield. Molecular genetics and genomic tools offer new opportunities to identify allelic variation, study candidate genes and select loci defining responses and yield adaptation to drought. The EUFVI TRITIMED project <http://www.rothamsted.bbsrc.ac.uk/cpi/mers/dh.html> integrates quantitative genetics, crop physiology, transcriptome and biochemical network studies to identify loci controlling wheat responses and growth under drought. A mapping population of durum wheat from cv. Lahn (high yield potential) x cv. Cham1 (drought adaptation) was studied in different field environments in Syria, Tunisia, Morocco, Spain and Italy. Quantitative trait loci for grain yield, yield components,

plant development, photosynthetic and physiological traits have been identified from over 30 field trials. GxE interactions will be explored to screen for loci specific to each environment and to identify relevant ideotypes. Individuals showing stability of yield under drought were selected for transcriptome studies under controlled environment and field conditions. Pathway and network analysis of global gene expression using ONDEX is uncovering metabolic pathways involved in early and late responses to water stress in top performing genoplasm. Our results demonstrate that genetic variability in regulation of stomatal conductance and osmotic adjustment play a major role in determining yield stability of durum wheat under drought conditions in the Mediterranean. The financial contribution of the FP6 EU (project INCO-CT-2004-509136) is gratefully acknowledged.

P215

Two major QTLs on chr. 2BL and 3BS influence grain yield and related traits in durum wheat across a broad range of water regimes

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Resistance to drought stress, especially in terms of yield stability under the various stress conditions that frequently occur in the drought-prone areas of the Mediterranean basin, is a main objective for durum wheat improvement. A total of 249 RILs (Kofa x Svevo) were evaluated in 16 trials conducted in 2004-2005 in Italy, Spain, Morocco, Tunisia, Syria and Lebanon, under a broad range of water regimes (rainfed and irrigated) and yield potential (from 0.5 to 5.8 t/ha). Two major, epistatic QTLs on chr. 2BL and 3BS influenced yield and related physiological traits, but not heading date, in a broad range of environments. In both cases, coincidence between the QTLs for grain yield and those for plant height, peduncle length, SPAD, NDVI index and kernel weight was observed. Epistasis favored the parental genotypes and negatively affected the performance of the recombinant genotypes. On a mean basis, the R^2 values for grain yield of the 2BL and 3BS QTLs were equal to 21.5 and 13.8%, respectively. The effects of these two QTLs were fully validated in a set of 11 trials conducted in 2006. In view of the relevance and consistency of their effects on grain yield and other agronomically valuable traits, the 2BL and 3BS QTLs are being isogenized in order to proceed with their fine mapping and, on the basis of their effects on peduncle length, their positional cloning (EU project TriticeaeGenomics).

P216

Genotypic variation in the expression levels of antioxidative enzyme genes in *Triticum aestivum*

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An elevated level of reactive oxygen species (ROS), such as superoxide, H₂O₂ and hydroxyl radical, is often produced in plants under abiotic stress conditions, including drought stress and high light intensity. These ROS molecules at high concentrations are harmful to cells and can inhibit carbon assimilation and cell growth. Plants possess a number of antioxidative systems to prevent ROS accumulation. In this study, we have examined genotypic differences in expression levels of some antioxidative enzyme genes in several recombinant inbred lines from a cross between Seri and Babax

(SB). Our study revealed considerable genotypic variation in the mRNA levels of a number of antioxidative genes among SB lines, contrasting in anthesis and maturity biomass production, grown in Northern Australia, which is prone to drought stress and high light intensity injury. The mRNA levels of several antioxidative genes were generally higher in the SB line with high anthesis and maturity biomass than the line with low biomass. The potential association of ROS-scavenging genes with biomass production will be further investigated.

P217

Isolation and characterization of a plasma membrane Na⁺/H⁺ antiporter gene *TaSOS1* from wheat

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In plants, Na⁺/H⁺ antiporters in the membrane, by removing toxic Na⁺ from the cytoplasm and out of the cell, are critical for growth in high salinity. We have cloned from *Triticum aestivum* L., by means of RACE-PCR, the *TaSOS1* gene which encodes a plasma membrane Na⁺/H⁺ antiporter. *TaSOS1* encodes a polypeptide of 1,142 amino acid residues with a theoretical molecular mass of 126.4 kDa. It is predicted to contain 12 putative transmembrane domains. Conserved domain analysis also shows that there is a Na⁺/H⁺ exchanger conserved domain in the N terminal region and a consensus cyclic nucleotide-binding motif in the middle. The TaSOS1-GFP fusion protein was clearly targeted to the plasma membrane in bombardment transformed onion cells, indicating that the TaSOS1 protein is indeed a plasma membrane-bound protein. To test functional activity, TaSOS1 and another putative vacuolar Na⁺/H⁺ antiporter, TaNHX2, were overexpressed in yeast salt sensitive mutants. *TaSOS1* expression enhanced Na⁺ and Li⁺ tolerance. TaNHX2 could also confer Na⁺ tolerance, but less than TaSOS1 and there was no Li⁺ tolerance. Using RT-PCR analysis, *TaSOS1* transcripts were detected in all investigated tissues, including roots, shoots, spikes and anthers. *TaSOS1* was up-regulated by both ABA and salt, but the increases were less than in *AtSOS1* in *Arabidopsis* where there is significant upregulation by salt stress but not ABA. Those results indicate *TaSOS1* play an important role in salt stress response in common wheat.

P218

System mutations revealed in National collection of Chernobyl` mutants of common wheat

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Chernobyl disaster caused in April 1986 (Ukraine) was one of the most dramatic technogenic catastrophe of XX century which had powerful influence on the nature. In that time at the Chernobyl fields in the year of accident four varieties of common wheats were planted: Bilotserkivska 47, Poliska 70, Myronivska 808 and Kyianka. 239 accessions of common wheat which during two years in plantations (1986) and in self-sowing (1987) grew near the Chernobyl Reactor were picked up by academician D.M. Grodzinsky and kindly provided for station in Bila Tserkva for further investigations and analysis. During 1988-2007 at the research station in Bila Tserkva it was analysed the genetic changes in winter wheat occurred due to the ionizing radiation which appeared as the result of Chernobyl Nuclear Power Plant Accident. In M₄-M₁₂ it was selected about 2000 mutant lines. Among mutants in different generations mainly appeared the plants with different kinds of

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abnormality in structure. However the all possible mutations we were divided in four types. I. Mutation of ears (spelt, squire head, compactum and *T. vavilovi* types); II. Mutation of stem (from 25 cm up to 116 cm and more); III Physiological mutations of plant development (date of heading and flowering, dysfunction of hormonal system, sterile flowers) IV Mutation of resistance for biotic and abiotic stresses. It was performed the genetic analysis of mutants possesses the type of ears such as *T.spelta*, *T.compactum* and *T.vavilovii*. Obtained results might explain the mutant origin of those wheats. More over the results of planting of common wheat varieties in the territory of stable chronic influence of ionizing radiation stimulated appearance of mutations, which were identified not ever in other varieties but the other species of wheat. This assumption need further support from molecular genetic data.