



*International Conference*

# FROM SEED TO PASTA & BEYOND

**A SUSTAINABLE DURUM WHEAT CHAIN FOR  
FOOD SECURITY AND HEALTHY LIVES**

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***Posters***  
***- Abstract -***

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## LEVERAGING ENVIRONMENTAL ADAPTATION IN THE WILD WHEAT RELATIVE *TRITICUM URARTU* TO IDENTIFY GENES INVOLVED IN ADAPTATION TO CLIMATE CHANGE.

**M. Dell'Acqua<sup>1</sup>, A. Brunazzi<sup>1</sup>, A. Brandolini<sup>2</sup> and M.E. Pè<sup>1</sup>**

*1 Institute of Life Sciences, Scuola Superiore Sant'Anna, Piazza Martiri della Libertà 33, 56127 Pisa (PI), Italy*

*2 Consiglio per la Ricerca e la Sperimentazione in Agricoltura - Unità di Ricerca per la Selezione dei Cereali e la Valorizzazione delle varietà vegetali (CRA-SCV), Via Mulino 3, 26866 S. Angelo Lodigiano (LO), Italy*

Climate change urges broadening the bases of wheat diversity to withstand in a sustainable manner the new threats it poses. Natural populations of *Triticum urartu*, the donor of the A genome, represent an important reservoir of genes/alleles to be used in novel breeding strategies for both durum and bread wheat improvement. Here we adopt landscape genomics approaches and the development of a Nested Association Mapping (NAM) population on *T. urartu* with the dual objective to: i) identify the genomic loci responsible for local adaptation, and ii) fine map quantitative trait loci (QTL) of adaptive relevance. The knowledge generated will allow the transferring of useful diversity in wheat either through homologous region targeting or direct introgression. We assembled a collection of 428 natural accessions representing *T. urartu* distribution across the Fertile Crescent, and we are characterizing it following a modified genotyping-by-sequencing protocol to tackle *T. urartu* genome complexity. The collection is characterized by geographic information systems considering altitude, annual mean temperature and precipitations of the sampling area condensed in a limiting factor analysis. This will lead to the discovery of adaptive alleles through landscape genomics, whose methods include outlier loci discovery and marker-climate regression by mixed linear models. The collection, currently in a nursery field, will be phenotyped for agronomic traits in two locations allowing genome wide association studies leveraging its diversity. The development of a NAM population built by intercrossing the recurrent parent G1812, whose genome has been recently sequenced, with 10 accessions is under progress. We are producing 10 F2 families from which to derive 150 recombinant inbred lines (RIL) per family. The NAM population will also be characterized molecularly and phenotypically to side the diversity collection of 428 *T. urartu* lines in mapping traits involved in environmental adaptation of *T. urartu*.



## EFFECTS OF *THINOPYRUM PONTICUM* CHROMOSOME SEGMENTS ON YIELD TRAITS OF DURUM WHEAT GROWN UNDER RAIN-FED CONDITIONS

**Kuzmanović L., Ruggeri R., Virili M.E., Rossini F., Ceoloni C.**

To corroborate previous observations on spaced plants, revealing positive effects on yield-related traits of *Th. ponticum* 7AgL chromosomal segments transferred into durum wheat, two-year field plot trials were carried out under Mediterranean conditions of Central Italy and normal sowing density. Materials consisted of three durum wheat–*Th. ponticum* near isogenic recombinant lines (NIRLs), possessing 23%, 28% and 40% of their distal 7AL arm replaced by 7AgL chromatin, respectively. In addition to the yield and other agronomic traits of the earlier analysis, physiological and qualitative parameters were also assessed at different developmental stages, as well as duration of phenological phases. Over the two seasons, significant positive effects of defined 7AgL sub-regions on grain and tiller number, flag leaf dimensions, as well as grain and biomass yield were largely confirmed, and a positive impact on chlorophyll content and length of some phenological phases was newly detected. Overall, in spite of rather atypical weather conditions in both seasons, all three NIRLs yielded 110–660 kg ha<sup>-1</sup> more than their respective controls. Moreover, protein content was not altered in any of the NIRL by the presence of 7AgL segments, remaining higher than 15% across years. Comparative evaluation supported previous evidence indicating the NIRL with 28% 7AgL as the most promising for breeding exploitation: in its 5% most proximal 7AgL stretch, genes/QTLs enhancing numerous yield-related traits turned out to be located, including productive tiller number (+20%), flag leaf width and area (+8%), chlorophyll content at late grain filling (+15%) and at booting-to-anthesis phase (+22%). Correlation and regression analyses showed the main yield-contributing traits to be biomass, productive tiller number, grain number and flag leaf width.

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## USE OF DURUM LANDRACES AND *TRITICUM* WILD RELATIVES TO BREED ROBUST DURUM CULTIVARS FOR MEDITERRANEAN ENVIRONMENTAL CONDITIONS

**M. Miloudi Nachit<sup>1\*</sup>, Nasserhaq Nserallah<sup>2</sup>, Jihan Motowaj<sup>1</sup>, Zakaria Kehel<sup>1</sup>, Mario Pagnotta<sup>3</sup>, Linda Mondì<sup>3</sup>, Enrico Porceddu<sup>3</sup>, Filippo M. Bassi<sup>1</sup>, Ahmed Amri<sup>1</sup>**

<sup>1</sup> ICARDA, BIGM, P.O. Box: 6299, Rabat, Morocco

<sup>2</sup> INRA, Settat, Morocco

<sup>3</sup> DAFNE, Tuscia University, Via S. C. de Lellis, snc, 01100 Viterbo, Italy

To meet increasing demand for wheat in the future in the Mediterranean region, durum grain production must increase at an annual rate of 2% on an area of land that will not increase much beyond the present level. Significant advances in the understanding of the durum yield potential and stress tolerance must be achieved to increase absolute yield potential and to protect the crop from an estimated average annual loss of 25% caused by biotic (diseases and insects) and by abiotic stresses (e.g. drought, heat, cold, and salinity) that can go up to 100% loss. In our breeding, the gene-pools used to upgrade biotic and abiotic stresses are originated from the Fertile Crescent in the Middle East such as the durum landraces and the *Triticum* wild relatives. The Mediterranean durum landraces were found to possess desirable traits lacking in other materials, such as resistance to drought and cold, early growth vigor, long peduncle, and high fertile tillering. Crosses initiated in the mid-eighties at our program have generated several cultivars with high performance under environments with abiotic constraints such as drought and temperature extremes. In addition to the use of landraces, the wild ancestors of cultivated wheats were also used extensively, particularly *Aegilops speltoides*, *Ae. Longissimi*, *Ae. Biuncialis*, *Triticum urartu*, *T. monococcum*, *T. dicoccoides*, *T. timopheevi*, *T. araraticum*, *T. urartu*, *T. boeoticum*, *T. carthlicum*, and *T. dicoccum*. Durum genotypes originated from crosses with wild relatives such as *Triticum monococcum*, *dicoccoides*, *dicoccum*, and *Aegilops species* have shown significant increase of grain yield potential and yield stability in the dry and favorable environments in the Mediterranean basin.

**Keywords:** durum, genepool, landraces, *Triticum* wild relatives.

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## COMPARATIVE LEAVES MICRORNAS PROFILING DURING HEAT AND DROUGHT STRESS IN DURUM WHEAT AND BRACHYPODIUM

**Cristina Crosatti<sup>1</sup>, Lorenzo Giusti<sup>1</sup>, Primetta Faccioli<sup>1</sup>, Anna Maria De Leonardis<sup>3</sup>, Edoardo Bertolini<sup>2</sup>, Mario Enrico Pè<sup>2</sup>, Luigi Cattivelli<sup>1</sup>, Erica Mica<sup>1,2</sup>**

*1 Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria – Genomics Research Centre, Via S. Protaso, 302 - 29017 Fiorenzuola d'Arda (PC), Italy*

*2 Institute of Life Sciences - Scuola Superiore Sant'Anna, Piazza Martiri della Libertà, 33 - 56127*

*3 Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria – Cereal Research Center, SS16 Km 675, 71122, Foggia, Italy*

Plant stress response is a complex molecular process based on transcriptional and post-transcriptional regulation of several stress-related genes. microRNAs are the best-studied class of small RNAs known to play key regulatory roles in plant response to stress, besides being involved in plant development and organogenesis. Drought and heat stress are among the two environmental factors influencing crop growth, development and yield processes.

In this work, two durum wheat cultivars, Ofanto and Cappelli, were chosen for their contrasting behavior in these conditions, and exposed to stress treatments, *i.e.* heat stress (3 hours at 36°C) or drought stress (two levels with Relative Soil Water Content of 56% and 36.5%, respectively). miRNAome from the third emerging leaf was characterized following a deep sequencing approach. These results were then compared with those coming from a similar drought experiment, conducted on the third leaf of *Brachypodium distachyon*.

The availability of its small sequenced genome, its close phylogenetic relation to wheat and its drought tolerance make *Brachypodium* a candidate model species for functional analysis of relevant miRNAs. A number of candidate stress-induced microRNAs have been identified in both species and were validated through a standardized and reliable stem loop qRT-PCR procedure. In this way, by integrating small RNA expression data and *in silico* target predictions, we present new insights on the role of miRNA-mediated regulatory networks in drought stress response in cereals.

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## SCREENING OF DURUM WHEAT (*TRITICUM DURUM* DESF.) VARIETIES UNDER SALT AND DROUGHT STRESS

**Ayed Sourour<sup>1</sup>, Othmani Afef<sup>1</sup>, Rassaa Neila<sup>2</sup> and Amara Hajer<sup>3</sup>**

Durum wheat is greatly influenced by environmental stresses including water deficit and salinity mainly in arid and semi arid regions. The present study aims to determine water deficit effects of three durum wheat genotypes and to determine the relationship between some morphological and physiological traits and yield under water deficit and salinity conditions. The impact of salt stress and drought stress on three durum wheat varieties (Agili, Maali and Karim) under different salinity level (0, 3, 6, 9, 12 g/l of NaCl) and drought stress levels (0, -0.47, -1.48, -3.02, -5.11 and -7.73 bar) at the germination, in hydroponic and field conditions were studied. Final germination rate and mean daily germination were measured. Morphological studies root length, shoot length, fresh weight and dry weight of root and shoot were studied at germination and hydroponic conditions. Chlorophyll index, leaf area, plant height, own length, peduncle length, tiller number/plant, spike number/plant, grain number/spike and the relationship between some of these morphological and physiological traits and yield components were also studied for field conditions. The data showed that different level of salinity and drought significantly affected the growth. All studied traits were significantly ( $P < 0.001$ ) reduced due to salt stress and drought stress attributes by reducing root and shoot length. Our results showed significant reduction in all morpho-physiological and agronomical traits in all varieties under rainfed conditions in comparison with 50% FC and 100% FC regime water. Chlorophyll index, leaf area, peduncle length and Own length were significantly correlated to the number of spike /plant and the number of grains/spike. Therefore, the use of morpho-physiological traits as an indirect selection would be important in increase yield-based selection procedures.

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## SEEKING NOVEL ALLELES FOR TOLERANCE TO BORON TOXICITY IN A VAST COLLECTION OF DURUM WHEAT ENTRIES

**Khaoula El Hassouni<sup>1,2</sup>, Maha Attjioui<sup>3</sup>, Belkadi Bouchra<sup>1</sup>,  
Abdelkarim Filali-Maltouf<sup>1</sup>, Michel Ghanem<sup>2</sup>, Miloudi Nachit<sup>2</sup>, and Filippo M Bassi<sup>2</sup>**

*1 Mohammed V University, Rabat, Morocco*

*2 ICARDA, Cereal Breeding Platform, Rabat, Morocco*

*3 IAV University, Rabat, Morocco*

Durum wheat (*Triticum durum* Desf.) is a major cereal crop grown globally. Many biotic and abiotic constraints affect this crop. Among them, there is the sensitivity of durum wheat to toxic or deficient concentration of boron (B) in the soil. While deficiencies can be controlled with relative ease by deploying B-rich fertilizers, the same cannot be said for toxicities. The most strategic approach remains a combination of agronomical practices to reduce the toxicity levels and the deployment of B toxicity tolerant genotypes. In the present study, a worldwide collection of 1,500 durum wheat elites and landraces has been investigated for tolerance to B-toxicity. Un-replicated testing in an aerated hydroponic system was used to expose seedlings to B toxic conditions (8 mM boric acid). Only 46% of the entries had sufficient tolerance to germinate under these stressful conditions. The length of the longest seminal roots was then used as indicator of potential tolerance, and 15% of the entries extended their roots for more than 2 cm. A total of 112 entries were selected and further tested in replicated essays, comparing root length after 10 days growing in toxic and non-toxic boron (15 $\mu$ M) solutions. The ratio between the length of roots in toxic and non-toxic solution varied between 0.11 and 1.3. A total of 36 entries appeared to carry tolerance to toxic soils. 70% of these hypothetically tolerant entries were ICARDA, Australian, or Canadian elites, in addition to few landraces. The most tolerant genotypes were first an ICARDA elite known for its strong yellow pigment (T.polonicumTurkeyIG45272/6/ICAMORTA0463/5/Mra-1/4/Aus1/3/Scar/GdoVZ579//Bit) carrying various introgressions from *T. polonicum* and *T. araraticum*, the second best was the widely grown Canadian variety 'Desire', followed by the Australian cultivar 'Tjilkuri'. The two entries that grew the overall longest roots in B toxic solution were two landraces, one from Spain (IG:85015) and the other from Oman (IG: 43305), again with 'Tjilkuri' taking the 3rd place. In conclusion, novel sources of B toxicity tolerance have been identified phenotypically, but it remains to be determined whether these carry genes different than the recently cloned *Bo1* and *Bo2* genes, or if they have novel allelic combinations that can be exploited in breeding.

## ESTIMATING THE COST OF GENETIC RESISTANCE TO HESSIAN FLY IN DURUM WHEAT IN MOROCCO

**Nsarellah N., Ourinich S., Hilali A., Nachit M. and S. Lhaloui**

Durum wheat is an important crop in Morocco; it is annually planted on nearly one million hectares. More than half of the durum wheat areas in this country are encountered in unfavorable dryland suffering heavily from Hessian fly. Resistance genes (H5, H25 as well as genes from *Triticum araraticum*) were incorporated to adapted durum wheat varieties recently. Although the new varieties are solving a major problem, most of the newly created material possessing genetic resistance has been carrying poor traits. Further, adaptation to drought needs to be seconded by modified cropping techniques for the new varieties. The objective of this work is to test for any secondary effect of the presence of resistance genes and to quantify the effect of the interaction between resistance to Hessian fly and the cropping technique or the environment. One set of 220 recombinant inbred lines derived from the cross 'Cando cross-H25/bzaiz-AHF' carrying several resistance genes has been planted in two locations and under three situations. The first situation was under drought only, the second situation was under drought and Hessian fly and the third situation was under favorable water status and Hessian fly free. Comparison were made among these situations were made in order to evaluate the effect of genetic resistance to Hessian fly on yield potential, yield and yield components as well as on phenological traits. The effect of the interaction between genetic resistance and the technical as well as the natural environments was also studied.

## MAPPING OF UNIQUE AND BROAD SPECTRUM RESISTANCE GENES TO *ZYMOSEPTORIA TRITICI* IN DURUM WHEAT

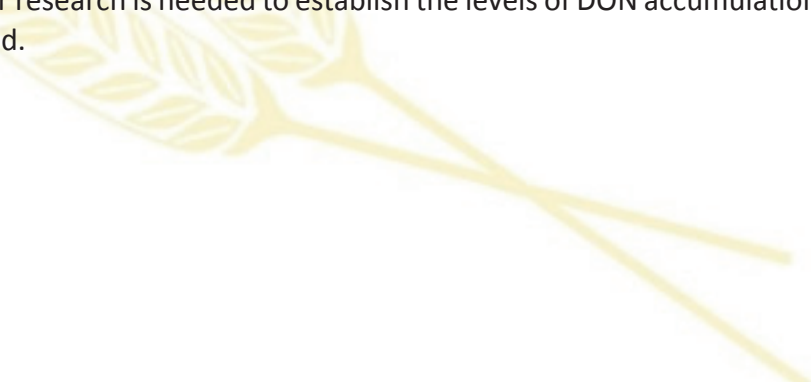
**Aouini L., Maccaferri M., Tuberosa R., Prodi A., Stefanelli S.,  
Hamza S. and Kema G.H.J.**

Septoria tritici blotch caused by *Zymoseptoria tritici* (*Z. tritici*) is a major threat for durum and bread wheat production. On bread wheat, considerable efforts were made to encounter this disease by in depth studies of host resistance. However, very little progress was made in the understanding of the genetic basis of resistance in durum wheat. In our study, we explored the durum wheat resistance to *Z. tritici* in three recombinant mapping populations developed in Italy and Tunisia. All seedling experiments were performed under controlled conditions at Wageningen University, while adult test were either performed in Italy for Kofa/Svevo and Simeto/Levante, or in Tunisia for Khiar/Agili 39. In all populations we identified major QTLs conferring either partial or complete resistance to *Z. tritici*. New locations were identified on chromosome 4B in Kofa/Svevo conferring partial resistance in the adult stage (Tuberosa *et al.*, unpublished data), and on chromosome 6B for partial seedling resistance in Simeto/Levante. Other QTLs reported in bread wheat were also identified in both populations. Interestingly, we also identified a major QTL in Khiar/Agili 39 on chromosome 2B, which was effective in the adult stage in field tests across F6-F9 generations (Ferjaoui *et al.*, unpublished data) as well as against eight *Z. tritici* isolates from various origins in the seedling stage. Another unknown major QTL was mapped on chromosome 1A, which provided resistance to three *Z. tritici* isolates in the seedling stage. Hence, our study showed the existence of novel and wide spectrum resistance alleles to *Z. tritici* in durum wheat that can be used to mitigate the impact of *Z. tritici* in this important food crop.

## CHARACTERIZATION OF FHB RESISTANCE TO *FUSARIUM* SPECIES AND ASSOCIATED DON ACCUMULATION IN SPRING WHEAT

**S. Berraies, Y. Ruan, R. Knox, F. Clarke, R. DePauw, R. Cuthbert**

Fusarium head blight (FHB) is an important wheat disease in Canada leading to yield reduction and poor grain quality. *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae* and *M. nivaleare* the species most commonly associated with the disease. Infected grain is often contaminated with deoxynivalenol(DON), a mycotoxin mainly produced by *F. culmorum* and *F. graminearum*. The identification of the species causing FHB is a high priority when breeding for FHB resistance and low DON accumulation. The objective of this study was to identify *Fusarium* species causing FHB in wheat lines grown in the field near Swift Current, Canada. Ten genotypes under natural infection conditions were collected and *Fusarium* species identified. Eighty kernels for each genotype were plated on Potato Dextrose Agar and incubated for one week. Identification of species in *Fusarium* colonies was based on the colour of the colony first and then the morphology of the conidia. The results showed the presence of the species *F. avenaceum*, *F. culmorum* and *F. graminearum* at 58%, 24% and 16% respectively. A positive association ( $R= 0.46$ ) between infected kernels and FHB index was observed. Blackbird, Alsen and Sumai 3 showed a high percentage of infected kernels despite the absence of visible symptoms. Further research is needed to establish the levels of DON accumulation associated with the species identified.





# FROM SEED TO PASTA & BEYOND

*A Sustainable Durum Wheat Chain for Food Security and Healthy Lives*

## ADVANCE YIELD TRIAL- DURUM WHEAT REACTIONS TO STRIPE, LEAF AND STEM RUSTS

**Zafer Mert, Kadir Akan, Selami Yazar, Mehmet Emin Alyamaç, Emin Dönmez**

This study aims to determine reactions of Advance Yield Trial- Durum Wheat (AYT-DW) germplasm to rust. A total of 96 wheat genotypes were tested to rusts diseases. These genotypes were developed by The Central Research Institute for Field Crops (CRICF) Wheat Breeding Unit.

For this purpose, seedling test were conducted for stem, leaf and stripe rusts. Evaluations were carried out at the research facilities of CRICF at Yenimahalle and İcikçe locations in Ankara in the 2014 season.

For seedling test; the seedling was inoculated with *Pst* populations (virulent on *Yr2, 6, 7, 8, 9, 25, 27, Sd, Su, Avs*) and local *Pgt* (avirulent on *Sr24, Sr26, Sr27, and Sr31*) and *Pt* (avirulent on *Lr9, Lr19, Lr24, and Lr28*). Stripe, leaf and stem rusts development on each entry were scored after 14 days with 0-4 and 0-9 scale for stripe rust and leaf-stem rust respectively. For adult plant reactions; the genotypes were inoculated with local stripe rust population. Disease scores on each entry were scored using the modified Cobb scale. The susceptible check cv. Little Club had reached 80-100% infection severity in June - July 2014. Coefficients of infections were calculated and values below 20 were considered to be resistant.

Forty eight (50%) (seedling) genotypes and 57 (59%) (adult stage) genotypes were resistant to local *Pst* and 9 (9%) (seedling) were resistant to the local *Pt* and 39 (41%) (seedling) genotypes were resistant to local *Pgt*. The reactions of Durum Wheat genotypes in Advance Yield Trial to stem, leaf and stripe rust reaction were determined with this research.

*This study was financed and supported by General Directorate of Agricultural Research and Policy of the (Project no: TAGEM/TBAD/13/A12/P01/002) Ministry of Food, Agriculture and Livestock of Turkey*

# FROM SEED TO PASTA & BEYOND

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## REACTIONS OF SOME DURUM WHEAT MATERIALS OBTAINED CROSSES WITH WILD TYPES TO YELLOW, LEAF AND STEM RUSTS

**Kadir Akan, Zafer Mert, Ayten Salantur, Bayram Özdemir**

Turkey is an important center of plant gen/genes. These gene/genes are an important source of resource variations. The purpose of this study was determining of the reactions of some wild X cultivars (2) durum wheat materials to rust disease. Wild durum wheat materials were collected from different regions of Turkey. Two Cultivars were crossed with pure wild types from single spike. This study aims to determine reactions to rust diseases for wild X cultivars durum wheat crosses. A total of 37 wheat genotypes were tested against yellow, leaf and stem rusts. Evaluations were carried out at the research facilities of CRIFC at İkiççe locations in Ankara in the 2014 season.

For seedling test; the seedling was inoculated with *Pst* populations (virulent on Yr2, 6, 7, 8, 9, 25, 27, Sd, Su, Avs) and local *Pgt* (avirulent on Sr24, Sr26, Sr27, and Sr31) and *Pt* (avirulent on Lr9, Lr19, Lr24, and Lr28). Yellow, leaf and stem rusts development on each entry were scored after 14 days with 0-4 and 0-9 scale for yellow rust and leaf-stem rust respectively. For adult plant reactions; the genotypes were inoculated with local yellow rust populations developments on each entry were scored using the modified Cobb scale. The susceptible check cv. Little Club had reached 80-100S infection severity in June - July 2014. Coefficients of infections were calculated and values below 20 were considered to be resistant.

One (3%) (seedling) genotypes and 2 (5%) (adult stage) genotypes were resistant to local *Pst*, 0 (0%) (seedling) were resistant to the local *Pt* and 26 (70%) (seedling) genotypes were resistant to local *Pgt*. The resistant genotypes were determined with this research.

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## RESISTANCE OF SPRING DURUM WHEAT YELLOW RUST RESISTANCE SOURCES TO STEM, LEAF AND YELLOW RUSTS

**Kadir Akan, Zafer Mert, Lütfi Çetin**

The use of the genetic resistance to rust diseases is economic and environmental safety way. Last ten years 17 resistant genotypes to local yellow rust mainly virulent on *Yr2, 6, 7, 8+, 9, 25, Sd, Avs* resistance gene/genes in greenhouse and field conditions were developed until new yellow rust race virulent on *Yr27* detected in 2010. The aim of this study was to determine resistance of these genotypes in Sprig Durum Wheat yellow rust resistance nursery (SDW-YRRN) to new yellow rust race, local leaf and stem rust populations. Evaluations were carried out at the research facilities of CRIFC at Yenimahalle and İkiçe locations in Ankara in the 2014 season.

For adult plant reactions; the genotypes were inoculated with local *Pst* populations (virulent on *Yr2, 6, 7, 8, 9, 25, 27, Sd, Su, Avs*). Yellow rust developments on each entry were scored using the modified Cobb scale. The susceptible check cv. Little Club had reached 80-100S infection severity in June - July 2014. Coefficients of infections were calculated and values below 20 were considered to be resistant. For seedling test; the seedling was inoculated with local *Pgt* (avirulent on *Sr24, Sr26, Sr27* and *Sr31*), *Pt* (avirulent on *Lr9, Lr19, Lr24* and *Lr28*) and *Pst* populations. Stem, leaf and yellow rusts development on each entry were scored after 14 days with 0-4 and 0-9 scale for stem-leaf rust and yellow rust respectively.

Seventeen (100%) (seedling) were resistant to the local *Pt* and 8 (47%) (seedling) genotypes were resistant to local *Pgt* and 14 (82%) (seedling) genotypes and 15 (88%) (adult stage) genotypes were resistant to local *Pst*. The resistant genotypes in spring durum wheat yellow rust resistance nursery were determined with this research.

*This study was financed and supported by General Directorate of Agricultural Research and Policy of the Ministry of Food, Agriculture and Livestock of Turkey*

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## THE REACTIONS OF SOME LANDRACES DURUM WHEAT MATERIALS TO YELLOW, LEAF AND STEM RUSTS

**Kadir Akan, Zafer Mert, Kürşad Özbek, Alptekin Karagöz**

Landraces are one of the genetic resources. Landraces were collected from different regions in Turkey. The purpose of this study; determining of the reactions of some durum wheat landraces to rust disease. Materials in the form of the population were purified as a single spike.

A total of 100 wheat durum wheat landraces seedling test were conducted for yellow, leaf and stem rusts. Evaluations were carried out at the research facilities of CRIFC at Yenimahalle and İcikçe locations in Ankara in the 2014 season.

For seedling test; the seedling was inoculated with *Pst* populations (virulent on *Yr2, 6, 7, 8, 9, 25, 27, Sd, Su, Avs*) and local *Pgt* (avirulent on *Sr24, Sr26, Sr27, and Sr31*) and *Pt* (avirulent on *Lr9, Lr19, Lr24, and Lr28*). Yellow, leaf and stem rusts development on each entry were scored after 14 days with 0-4 and 0-9 scale for yellow rust and leaf-stem rust respectively. For adult plant reactions; the genotypes were inoculated with local yellow rust populations and disease developments on each entry were scored using the modified Cobb scale. The susceptible check cv. Little Club had reached 80-100S infection severity in June - July 2014. Coefficients of infections were calculated and values below 20 were considered to be resistant.

Thirty two (32%) (seedling) genotypes and 44 (44%) (adult stage) genotypes were resistant to local *Pst*, 41 (41%) (seedling) were resistant to the local *Pt* and 32 (32%) (seedling) genotypes were resistant to local *Pgt*. The resistant landraces were determined with this research.

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## POPULATION STRUCTURE, LINKAGE DISEQUILIBRIUM AND GENOME WIDE ASSOCIATION MAPPING FOR RESISTANCE TO STEM RUST IN A TETRAPLOID WHEAT COLLECTION

**Pecchioni N.\* , Laidò G.\* , Panio G.\* , Marone D.\* , Russo M.A.\* , Ficco D.B.M.\* ,  
Giovanniello V.\* , Ferragonio P.\* , Cattivelli L.\*\* , Steffenson B.\*\*\*, De Vita P.\* ,  
Mastrangelo A.M.\***

*\*) Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Cereal Research Centre, Foggia, Italy*

*\*\*\*) Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Genomics Research Centre, Fiorenzuola d'Arda (PC), Italy*

*\*\*\*) Department of Plant Pathology, University of Minnesota, USA*

A tetraploid wheat (*T. turgidum* L.,  $2n=4x=28$ ; AABB genome) collection of 230 inbred lines, including 128 durum wheat varieties plus 102 wild and domesticated accessions, has been employed in this study. The germplasm collection consists of modern cultivars together with indigenous and exotic landraces, , most materials being representative of the Italian durum wheat breeding programmes over the last 100 years. Each accession was genotyped according to 26 SSR and 970 DArT markers. Exploiting the mapping information obtained by durum wheat consensus map, the map positions of 592 DArT markers were obtained. Our results on population structure clearly show a sharp separation of durum wheat accessions from the rest of the naked and hulled tetraploid wheats. Using the dataset of 592 DArT markers with known map position, the level of LD was evaluated in the whole collection, in the durum sub-sample and in the two groups identified with population structure analysis. The wild accessions were characterized by very low levels of LD, while a higher LD value was observed for the subgroup containing the durum wheat genotypes. The tetraploid collection was evaluated in controlled conditions for resistance to stem rust Ug99 (TTKSK race). A great variability was observed for Ug99 resistance in the tetraploid wheat collection, allowing by genome-wide association mapping to identify resistance loci both within wild and domesticated accessions and in the genepool of cultivated durum wheat varieties. Forty-six QTLs for seedling resistance to the race TTKSK were identified on all chromosomes. Some QTLs from previous studies were confirmed, and others identified which were not previously reported. A search for candidate genes for stem rust resistance was carried out in the QTL regions. Many of them corresponded to NBS-LRR genes and protein kinases with LRR domains.

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## THE DURUM WHEAT LEAF RUST RESISTANCE LOCUS *Lr14a* MAPS IN A CHROMOSOME REGION ENRICHED IN GENES INVOLVED IN THE PLANT-PATHOGEN RECOGNITION AND INTERACTION PATHWAYS

**Maccaferri M.<sup>1</sup>, Terracciano I.<sup>1</sup>, Bassi F.<sup>1,2</sup>, Mantovani P.<sup>1,3</sup>, Sanguineti M.C.<sup>1</sup>, Salvi S.<sup>1</sup>, Massi A.<sup>3</sup>, Ammar K.<sup>4</sup>, Kolmer J.<sup>5</sup>, Simkova´ H.<sup>6</sup>, Dolezel J.<sup>6</sup>, Tuberosa R.<sup>1</sup>**

*1 Department of Agricultural Sciences (DipSA), University of Bologna, Bologna 40127, Italy*

*2 Durum Improvement Program, ICARDA, P.O. Box 5466, Aleppo, Syria*

*3 Societa` Produttori Sementi Bologna, Via Macero 1, Argelato, Bologna 40050, Italy*

*4 CIMMYT, Carretera Mexico-Veracruz KM. 45, 56130 Texcoco, Mexico*

*5 Cereal Disease Laboratory, Agricultural Research Service, US Department of Agriculture, St. Paul, MN 55108, USA*

*6 Institute of Experimental Botany, 77200 Olomouc, Czech Republic*

Leaf rust (*Puccinia triticina* Eriks. & Henn.) is a major disease affecting durum wheat production. The *Lr14a*-resistant gene present in the durum wheat cv. Creso and its derivative cv. Colosseo is one of the best characterized leaf-rust resistance sources deployed in durum wheat breeding. *Lr14a* has been mapped close to the simple sequence repeat markers *gwm146*, *gwm344* and *wmc10* in the distal portion of the chromosome arm 7BL, a gene-dense region. The objectives of this study were: (1) to enrich the *Lr14a* region with single nucleotide polymorphisms (SNPs) and high-resolution melting (HRM)-based markers developed from conserved ortholog set (COS) genes; (2) to further investigate the gene content and colinearity of this region with the Brachypodium and rice genomes. Ten new COS-SNP and five HRM markers were mapped within an 8.0 cM interval spanning *Lr14a*. Eight COS-SNPs were mapped in an interval of 4.1 cM including *Lr14a*, between markers *YP7B-1* and *ubw35*. This region corresponds to a well-defined syntenic interval in both *Brachypodium* chromosome 1 and rice chromosome 6, where several genes involved in plant-pathogen recognition and interaction are located. Among those, genes with known functions are: *Bradi1g29537* that has a cyclin-L1-1domain, *Bradi1g29530* (zinc knuckle domain), *Bradi1g29471* (a serine carboxypeptidase homoeolog) and *Bradi1g29450*, an NBS-LRR disease resistance protein homolog of *Yr10* and present in three replicated versions. All of these genes are potentially involved in the disease-response mechanisms. Fine mapping activities and the functional characterization of those genes will provide insights on the mechanisms underlying the involvement of this chromosome regions in wheat defense mechanism.

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## NEW RACE OF LEAF RUST IN DURUM WHEAT IN SPAIN

**I. Solis<sup>1</sup>, N. H. Soleiman<sup>1</sup>, F. Martinez<sup>1</sup>, M. H. Soliman<sup>2</sup>, J. C. Sillero<sup>3</sup>, D. Villegas<sup>4</sup>, F. Alvaro<sup>4</sup>, C. Royo<sup>4</sup>, J. Serra<sup>5</sup>, K. Ammar<sup>6</sup>**

*1 Dep. Ciencias Agroforestales, University of Seville, Ctra de Utrera km1, 41013 Seville*

*2 Department of Wheat Diseases, Agriculture Research Center, 9 Gamah Street, 12619 Giza, Egypt; Spain*

*3 IFAPA Centro Alameda del Obispo, Apdo. 3092, Córdoba Spain*

*4 IRTA, Field Crops Program, Avda Rovira Roure 191, 25198 Lleida, Spain*

*5 IRTA-Mas Badia, 17134 La Tallada d'Empordà, Girona, Spain*

*6 International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico*

Leaf rust, caused by the fungus *Puccinia triticina*, is an important disease of durum wheat in Spain, mainly in the south (Guadalquivir Valley). Resistance to this pathogen is common in modern durum germplasm but frequently based on a very widespread R-gene, *Lr14a*. This gene has been effective for more than thirty years globally and remains so in many parts of the world.

However, after reports of its earlier breakdown in France (2000) and Tunisia (2013), rust pustules of a high infection type were found on leaves of cultivars carrying *Lr14a* in two, far-apart locations, in Spain (Cadiz and Girona), in 2013. Samples were taken at these two locations from cultivars Gallareta (*Lr72*) and Don Jaime (*Lr14a*) and single pustule isolates were characterized for their virulence spectrum on the Thatcher differentials and on durum genotypes with known R-genes. Results clearly indicated that the 4 isolates were part of the same race, designated herein as DBB/BS, with clear and combined virulence on both *Lr14a* and *Lr72*, which differentiates it from the French race characterized in 2000.

The virulence spectrum of this new Spanish race resembled those identified from 75 isolates previously identified in a wider survey carried-out between 2009 and 2011, but with the added virulence on *Lr14a*. This indicates that DBB/BS is likely to have evolved from previously occurring Spanish races via a single step mutation.

These results are important to consider by breeders in their breeding schemes and gene deployment strategies, especially in light of the very high frequency of *Lr14a* in all germplasm groups worldwide.

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## JOINT LINKAGE AND ASSOCIATION MAPPING FOR ROOT SEMINAL ARCHITECTURE TRAITS IN DURUM WHEAT

**Walid El-Feki, Ghasemali Nazemi, Marco Maccaferri, Maria Angela Canè, Silvio Salvi, Rossella Francia, Chiara Colalongo, and Roberto Tuberosa**

*Department of Agricultural Sciences - University of Bologna, Viale Fanin, 44, 40127 - Bologna - Italy*

The study of root architectural system (RSA) traits as related to crop performance can help to identify proxy traits for enhancing adaptation to different soil properties, moisture conditions, nutrient concentration. It is currently a major research focus for durum wheat, a cereal crop adapted to the Mediterranean Basin, where durum is cultivated under environments ranging from favorable to dryland areas characterized by frequent drought episodes and high temperature stresses, mainly during grain filling. RSA features were investigated at seedling stage in two biparental recombinant inbred line populations (Colosseo × Lloyd and Meridiano × Claudio) and in a panel for association mapping including elite cultivars and breeding lines from worldwide for searching quantitative trait loci (QTLs) underlying the inherent genetic variation. The QTLs identified were compared with QTLs detected for grain yield and its component traits, plant height and peduncle length measured in a previous study where the same accessions were evaluated in 15 field trials with a broad range of soil moisture availability and productivity (Maccaferri et al. in *J Exp Bot* 62:409–438, 2011). Seminal root angle and root number appear the most promising traits for further studies on the adaptive role of RSA plasticity on field performance in environments differing for water availability. QTLs were prioritized based on (i) consistent detection in different genetic materials, (ii) concomitant effects on yield components and agronomic traits. Our results provide novel insights on the genetic control of RSA and its implications on field performance of durum wheat.



## OVEREXPRESSION OF DURUM WHEAT GENE *TdPIP2;1* DRIVEN BY ITS OWN PROMOTER ENHANCES DROUGHT TOLERANCE IN RICE

**Khaled Masmoudi<sup>1,2</sup> & Malika Ayadi<sup>1</sup>**

*1 Plant Protection and Improvement Laboratory, Center of Biotechnology, Sfax (CBS), Tunisia, University of Sfax, Route Sidi Mansour Km 6, B.P. "1177".*

*2 International Center for Biosaline Agriculture (ICBA), P.O.Box 14660, Dubai, UAE.*

Plant aquaporins are encoded by a gene family with members exhibiting both tissue specific and inducible regulation. Plasma membrane intrinsic proteins (PIP) cluster in two phylogenetic groups, PIP1 and PIP2 with different water channel activities when expressed in *Xenopus* oocytes. Two durum wheat (*Triticum turgidum* L. subsp. durum) cDNAs encoding *TdPIP1;1* and *TdPIP2;1* belonging to the PIP1 and PIP2 subfamilies, respectively were cloned. Contrary to *TdPIP1;1*, expression of *TdPIP2;1* in *Xenopus* oocytes resulted in an increase in the membrane osmotic water-permeability coefficient (Pf) compared to water injected oocytes. Confocal microscopy analysis of tobacco roots expressing *TdPIP1;1* and *TdPIP2;1* fused to the green fluorescent protein showed that the proteins were localized at the plasma membrane. *TdPIP1;1* and *TdPIP2;1* are differentially regulated in roots and leaves of the salt-tolerant wheat variety (Om Rabiaa) when challenged with salt stress and abscisic acid treatment. To elucidate the stress tolerance mechanism at the transcriptional level, we isolated and characterized the promoter region of the *TdPIP2;1* gene. Sequence analysis of pro*TdPIP2;1* revealed the presence of cis regulatory elements which could be required for abiotic stress responsiveness, tissue-specific and vascular expression. Histochemical analysis of pro*TdPIP2;1::TdPIP2;1::Gus* fusion in transgenic rice (cv. Nipponbare) plants revealed that the GUS activity was observed in leaves, stems and roots of stably transformed rice T3 plants. Histological sections prepared revealed accumulation of GUS products in phloem, xylem and in some cells adjacent to xylem. The transcripts were up-regulated by dehydration. Transgenic rice plants overexpressing pro*TdPIP2;1* in fusion with *TdPIP2;1* gene, showed enhanced drought tolerance, while wild type plants were more sensitive and exhibited symptoms of wilting and chlorosis. These findings suggest that expression of the *TdPIP2;1* gene regulated by its own promoter achieves enhanced drought tolerance in rice.

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## ASSOCIATION MAPPING FOR SEEDLING ROOT TRAITS AND AGRONOMIC PERFORMANCE IN DURUM WHEAT

**Maria Angela Canè, Marco Maccaferri, Ghasemali Nazemi, Silvio Salvi, Rossella Francia, Chiara Colalongo, Roberto Tuberosa**

*Department of Agricultural Sciences, Viale Fanin 44, 40127 Bologna, Italy*

Association mapping provides useful insights on the genetic architecture of quantitative traits, which in turn allows an informed choice of the lines to be crossed for a more accurate characterization of major QTLs in a biparental genetic background. In this study, seedlings of 183 durum wheat elite accessions were evaluated in order to identify QTLs for root system architecture (RSA). The QTLs identified were compared with QTLs detected for grain yield and its component traits, plant height and peduncle length measured in a previous study where the same accessions were evaluated in 15 field trials with a broad range of soil moisture availability and productivity (Maccaferri et al. in *J Exp Bot* 62:409–438, 2011). The following RSA features were investigated in seedlings at the four-leaf stage: seminal root angle, primary root length, total root length, average root length, root number and shoot length. Highly significant differences among accessions were detected for all traits. The highest repeatability ( $h^2 = 0.72$ ) was observed for seminal root angle. Out of the 48 QTLs detected for RSA, 15 overlapped with QTLs for agronomic traits and/or grain yield in two or more environments. The congruency of the effects of RSA traits and agronomic traits was evaluated. Seminal root angle and root number appear the most promising traits for further studies on the adaptive role of RSA plasticity on field performance in environments differing for water availability.

## SCREENING OF ELITE DURUM GERMPLASM FOR QTL ASSOCIATED TO HEAT TOLERANCE

**Ghasemali N., Bovina R., Maccaferri M., Tuberosa R., Salvi S.**

Durum wheat is primarily grown under rainfed conditions in Mediterranean areas where episodes of drought and heat stress are major factors limiting grain yield. This study aimed at the identification of QTLs for proxies for tolerance to heat stress in durum wheat, a recurrent abiotic stress. Cell membrane stability (CMS) as related to ion leakage was used as a proxy of heat tolerance in an association mapping experiment based on a panel of 183 elite accessions of durum wheat developed in Mediterranean countries, Southwestern USA and Mexico that were previously evaluated for grain yield across 15 field trials in Mediterranean environments. The heat stress was applied at the seedling stage on: i) detached leaves heat-treated in the incubator for 1 h at 45 °C (Constitutive tolerance, termed CON), ii) whole plants in pots first treated at 37 °C and then at 45 °C (5 hours), termed as acquired tolerance (AQU) iii) a combination of the two (CON\_AQU). Analysis of variance revealed highly significant differences ( $P < 0.01$ ) among genotypes for all three treatments. The experimental material indicated a wide range of variation for the three heat response traits with coefficient of variation (CV) and heritability of 4.8 and 29% for AQU, 6.1 and 67% for CON and 20.9 and 69% for CON\_AQU. Association mapping based on Illumina 90K SNP array genotyping revealed 38 QTLs significant at marker-wise level  $P < 0.01$ , including for each trait five major loci explaining approximately 40% of the total variation (40.7% for AQU, 38.2% for CON and 35.1% for CON\_AQU). The overlap between the major heat tolerance QTLs and the grain yield QTLs evidenced in the field trials is being investigated.

## MAPPING QTLs FOR FUSARIUM HEAD BLIGHT RESPONSE IN A DURUM WHEAT ELITE POPULATION

**M. Maccaferri<sup>1</sup>, S. Corneti<sup>1</sup>, A. Ricci<sup>1</sup>, S. Stefanelli<sup>1</sup>, E. Braidà<sup>2</sup>, P. Lancioni<sup>2</sup>,  
K. Ammar<sup>3</sup>, A. Prodi<sup>1</sup>, A. Pisi<sup>1</sup>, M. Pascale<sup>4</sup>, V. Lippolis<sup>4</sup>, A. Massi<sup>2</sup>, R. Tuberosa<sup>1</sup>**

*1 Department of Agricultural Science - University of Bologna, Viale Fanin 44, 40127 Bologna-Italy;*

*2 Società Produttori Sementi Bologna, Via Macero 1, 40050 Argelato - Italy;*

*3 CIMMYT, Carretera Mexico-Veracruz KM. 45, 56130 Texcoco-Mexico;*

*4 Institute of Sciences of Food Production (ISPA), National Research Council of Italy (CNR), via G. Amendola 122/O, 70126 Bari-Italy*

Durum wheat is strongly affected by Fusarium head blight (FHB). Thus, understanding the genetic basis of resistance is a major objective of breeding. In this study, a population of 249 F7:8 recombinant inbred lines (RILs) obtained from the cross Kofa (desertDurum®) x Svevo (Italian cultivar) has been evaluated for FHB response in five artificially inoculated field trials (mix suspension of *F. graminearum* and *F. culmorum* conidia) carried out in 2010 and 2011 in Italy and Mexico. Fusarium incidence and severity, grain yield, number of grains per spike, percentage of fusarium-damaged kernels, thousand kernel weight, deoxynivalenol (DON) content were recorded. Heritability values of FHB symptoms were medium to high across trials (from 35 to 62%). Both Kofa and Svevo showed intermediate responses, while the RILs showed extensive transgressive segregation, allowing us to map 14 QTL clusters for FHB responses and DON content. Only two of these QTL clusters, on chromosomes 2A and 7B, were coincident with QTLs for heading date. Two major QTLs consistently proved to affect FHB response across trials were located on chromosome arms 2BL and 3BS, in coincidence with major QTLs for plant height, peduncle length, senescence and grain yield previously identified in this population (Maccaferri et al. *Genetics* 178: 489-511). In both cases, the alleles responsible for constitutive reduced height and peduncle length, increased senescence rate and decreased grain yield were also associated to increased fusarium susceptibility. The role of these two QTLs is being further investigated with near isogenic lines, particularly to elucidate the pleiotropic vs. tight linkage relationships, in view also of the FHB escape associated to plant height. Four QTLs for DON content were also found, two of which were DON-specific and did not influence other disease symptoms. The contribution of "AGER - Agroalimentare e Ricerca", project "From Seed to Pasta" is acknowledged.

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## DE NOVO TRANSCRIPTOME ASSEMBLY, ANNOTATION AND SNP MINING IN DURUM WHEAT

**Danara Ormanbekova<sup>1</sup>, Marco Maccaferri<sup>1</sup>, Silvio Salvi<sup>1</sup>, Vera Vendramin<sup>2,3</sup>, Davide Scaglione<sup>2,3</sup>, Simone Scalabrin<sup>2,3</sup>, Michele Morgante<sup>2,3</sup>, Pier Luigi Martelli<sup>4</sup>, Rita Casadio<sup>4</sup>, Roberto Tuberosa<sup>1</sup>**

*1 Department of Agricultural Sciences, University of Bologna, Bologna, Italy*

*2 Institute of Applied Genomics, Udine, Italy*

*3 Department of Agricultural and Environmental Sciences, University of Udine, Udine, Italy*

*4 Biocomputing Group, University of Bologna, Bologna, Italy*

Although knowledge of the full wheat genome sequence is still lacking, the transcriptome provides the basis for many research and application purposes. In this work, the durum wheat (*Triticum turgidum* ssp. *durum*) transcriptome from the reference cultivar Svevo and 12 additional cultivars from worldwide were sequenced, assembled and annotated, followed by a post-assembly analysis to distinguish between A- and B-homeologous genomes and varietal SNPs. The quality of the assembly was tested against CDS from genes from *Triticum aestivum* chromosome 3B-reference sequences and a set of 16,803 *Triticum aestivum* full-length cDNAs. A novel method based on the *de novo* reconstruction of haplotypes from paired-end reads coupled with a double-reference mapping procedure aided by *T. urartu* transcripts was adopted to distinguish between homeologs. With the k-mer parameter set to 64, a total of 384 million paired-end reads were assembled in 180,108 contigs. Increasing k-mer size from 41 to 64 improved the number of genes reconstructed for both the wheat 3B chromosome and the full-length cDNA datasets. Based on comparative genomics approach, 34,797 transcripts were annotated. The identified SNPs were divided in 14,579 locus-specific SNPs and 497,934 inter plus intra-homeologous SNPs (including the varietal SNP). This work contributes towards the production of homeolog-specific assembly of the wheat transcriptome and of a representative well-annotated database of durum wheat expressed genes. Moreover, the annotated transcript sequence provides valuable information towards the characterization of expressed portions of the genome.

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## A HIGH-DENSITY, SNP-BASED CONSENSUS MAP OF TETRAPLOID WHEAT AS A BRIDGE TO INTEGRATE DURUM AND BREAD WHEAT GENOMICS AND BREEDING

**Marco Maccaferri<sup>1</sup>, Andrea Ricci<sup>1</sup>, Silvio Salvi<sup>1</sup>, Sara Giulia Milner<sup>1</sup>, Enrico Noli<sup>1</sup>, Pier Luigi Martelli<sup>2</sup>, Rita Casadio<sup>2</sup>, Eduard Akhunov<sup>3</sup>, Simone Scalabrin<sup>4</sup>, Vera Vendramin<sup>4</sup>, Karim Ammar<sup>5</sup>, Antonio Blanco<sup>6</sup>, Francesca Desiderio<sup>7</sup>, Assaf Distelfeld<sup>8</sup>, Jorge Dubcovsky<sup>9</sup>, Tzion Fahima<sup>10</sup>, Justin Faris<sup>11</sup>, Abraham Korol<sup>10</sup>, Andrea Massi<sup>12</sup>, Anna Maria Mastrangelo<sup>13</sup>, Michele Morgante<sup>4</sup>, Curtis Pozniak<sup>14</sup>, Amidou N'Diaye<sup>14</sup>, Steven Xu<sup>11</sup>, Roberto Tuberosa<sup>1</sup>**

*1 Department of Agricultural Science (DipSA), Viale Fanin 44, University of Bologna, 40127 Bologna, Italy*

*2 Biocomputing Group, Via Selmi 3, University of Bologna, 40126 Bologna, Italy*

*3 Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506*

*4 Istituto di Genomica Applicata, c/o Parco Scientifico e Tecnologico L. Danieli, 33100 Udine, Italy and University of Udine, Dipartimento di Scienze Agrarie e Ambientali, 33100 Udine, Italy*

*5 CIMMYT Carretera Mexico-Veracruz km 45, 56130 Texcoco, Mexico*

*6 Dipartimento di Biologia e Chimica Agro-forestale ed ambientale, Università di Bari, Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy*

*7 Consiglio per la ricerca e la sperimentazione in agricoltura, Genomics Research Centre, Via San Protaso 302, 29017 Fiorenzuola d'Arda, Italy*

*8 Faculty of Life Sciences, Dept. of Molecular Biology and Ecology of Plants, Tel Aviv University, 69978 Tel Aviv, Israel*

*9 Department of Plant Sciences, University of California, Davis, CA 95616, USA, and Howard Hughes Medical Institute, Chevy Chase, MD 20815*

*10 Department of Evolutionary and Environmental Biology, Institute of Evolution, Faculty of Science and Science Education, University of Haifa, Mt. Carmel, 31905 Haifa, Israel*

*11 USDA-ARS Cereal Crops Research Unit, NCSL 1605 Albrecht Blvd. N., Fargo, 58102-2765 ND, USA*

*12 Società Produttori Sementi Bologna (PSB), Via Macero 1, 40050 Argelato, Italy*

*13 Consiglio per la ricerca e la sperimentazione in agricoltura, Cereal Research Centre, S.S. 673 km 25.200, 71122 Foggia, Italy*

*14 Crop Development Centre and Department of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada*

Consensus linkage maps are important tools in crop genomics. We have assembled a high-density tetraploid wheat consensus map from 13 bi-parental populations obtained from durum wheat (*Triticum turgidum* ssp. *durum*), emmer (*T. turgidum* ssp. *dicoccum*) and wild emmer (*T. turgidum* ssp. *dicoccoides*). Component maps were recalculated from raw genotypic data using a common mapping procedure. The consensus map harbored 30,144 markers (including 26,626 SNPs and 791 SSRs), half of which were present in at least two component maps. The final map spanned 2,631 cM with complete genome coverage. Comparison with hexaploid wheat indicated the presence of fewer regions with suppressed recombination in tetraploid, making this map valuable for the A and B genomes of wheat in general. Sequence similarity analysis allowed us to relate genic-SNPs to chromosome-specific transcripts from the Chinese Spring survey sequence. Dense patterns of homeologous relationships have been established between the A- and B-genome maps and

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between non-syntenic homeologous chromosome regions tracing to ancient translocation events. This framework is valuable to infer the map location of target loci/QTLs homeologs. The consensus map is being used as a framework for association mapping and meta-QTL analyses, the results of which are presented. Because most SNP and SSR markers were previously mapped in bread wheat, the consensus will facilitate a more effective integration and exploitation of genes and QTL for wheat breeding purposes.



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## MULTI-POPULATIONS GENTIC MAP AS A TOOL FOR THE APPLICATION OF GENOMIC SELECTION IN DURUM WHEAT

**Hafssa Kabbaj<sup>1,2</sup>, Ayed Al-Abdallat<sup>3</sup>, Abdelkarim Filali-Maltouf<sup>1</sup>, Bouchra Belkadi<sup>1</sup>, Jesse Poland<sup>4</sup>, M. Miloudi Nachit<sup>2</sup>, and Filippo M. Bassi<sup>2</sup>**

*1 Mohammed V University, Rabat, Morocco*

*2 ICARDA, Cereal Breeding Platform, Rabat, Morocco*

*3 ICARDA, Biotechnology Platform, Cairo, Egypt*

*4 Kansans State University, Manhattan, USA*

Durum wheat is an important crop for the human diet and it is consumed largely in the form of traditional dishes such as pasta, couscous, unraised breads, bourghul, frike, etc. Worldwide, the consumption of durum wheat is gaining popularity because of its nutritional properties. In order to ensure that durum wheat production maintains the pace with the increase in demand, it is necessary an increase in production of approximately 1.5% per year. Genomic selection (GS) appears to be the only possible tool to deliver this level of gains per year. Here we present the first step toward the validation of GS in durum wheat. Four RILs populations: Jennah Khetifa/Cham1 /3/ Omrabi5/T.dicoccoides600545//Omrabi5 developed for pyramiding drought tolerance; SW-Algia/Gidara1/Cham1 for Septoria tritici resistance; Younes/ Gidara2 for combining drought tolerance and yield potential; and Icamor/ Gidara2 for combining Hessian fly resistance with high yield potential. A total of 576 individuals were sown at three locations in Morocco and Lebanon for collection of yield, yield components, and disease/insect data. The same individuals where genotyped using the 'genotyping by sequencing' approach developed at KSU, which uses two restriction enzymes for genome complexity reduction and 96-multiplex sequencing by bar coding. The four populations are nested in pairs of two, with two parents in common (i.e. Cham1 and Gidara2). Using the shared pedigree, two independent maps were constructed and were then merged into a single consensus map. On the basis of the markers ordering, it is now possible to perform allele imputation for those markers with large sets of missing data, a common problem when deploying GbS. The genotyping results, as well as preliminary studies of markers-phenotype association will be presented.



## GENETIC DIVERSITY AND ASSOCIATION STUDIES IN A COLLECTION OF JORDANIAN DURUM WHEAT LANDRACES

**Moneer Mansor, Nasab Rawashdeh, Maen Hasan, Abdul Qader Jighly, Amer Karadsheh, Nasri Haddad, Ayed Al Abdallat**

The domestication of durum wheat (*Triticum turgidum subsp. durum*) from its progenitor emmer wheat (*Triticum turgidum subsp. dicoccoides*) took place prior to 7000 B.C in the “Fertile Crescent” region that includes parts of Jordan. In this study, an active collection of Jordanian durum wheat landraces (86 accessions) were collected from farmers’ fields at different locations across Jordan. In addition, 149 Jordanian durum wheat accessions from ICARDA Gene bank representing a historical set that was collected before 1980 from Jordanian farmers’ fields at different locations across Jordan, a small set of 45 landraces from 11 different countries (international set) and 7 released Jordanian varieties were included. The durum wheat accessions were genotyped using the Illumina Infinium iSelect 90K SNP wheat array. The durum wheat accessions were grouped into three sub-populations, where the first sub-group contained 114 accessions; the second sub-group contained 100 accessions; the third sub-group contained 63 landrace. The diversity analysis indicates that the active collection includes landraces with high similarity to the historical accessions indicating the continuous preference of them by the farmers and their resilience to climate change associated conditions. A genome wide association mapping panel consisting of 156 landrace (43 from the international set, 49 accessions from the historical set, 57 accessions from the active set and 7 released varieties) was assembled. The panel was grown to evaluate different phenological traits in two locations in Jordan under rainfed and irrigated conditions. Variations in morphological, developmental and yield related-traits including heading date, maturity date, plant height, awn’s color, spike length, grain number per spike, biological yield and 1000 kernel weight were observed. For GWAS, a mixed linear model considering the kinship and structure was used to identify SNP markers associated with selected traits in the tested panel.

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## GENETIC VARIABILITY FOR SEED-RELATED TRAITS AMONG DURUM WHEAT POPULATIONS DERIVED FROM DIFFERENT RELATIVE AND LANDRACE BASED ON TWO HIGH RESOLUTION MAPS

**Guerra Davide<sup>1</sup>, Desiderio Francesca<sup>1</sup>, Marè Caterina<sup>1</sup>, Zarei Leila<sup>2</sup>, Farshadfar Ezatollah<sup>2</sup>, Kianoosh Cheghamirza<sup>2</sup>, Piarulli Luciana<sup>3</sup>, Simeone Rosanna<sup>3</sup>, Anzalone Stefania<sup>4</sup>, Palumbo Massimo<sup>4</sup>, Bagnaresi Paolo<sup>1</sup>, Cattivelli Luigi<sup>1</sup>, Mazzucotelli Elisabetta<sup>1</sup>**

*1 Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria – Genomics Research Centre, Via S. Protaso, 302 - 29017 Fiorenzuola d'Arda (PC), Italy*

*2 Agronomy and Plant Breeding Department, Razi University, Kermanshah, Iran, Biotechnology Research Department for Drought Resistance, Razi University, Kermanshah, Iran.*

*4 Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Research Center for Citrus and Mediterranean Crops, C.so Savoia 190, I-95024, Acireale (CT), Italy.*

*3 Department of Soil, Plant and Food Sciences - Sect. Genetics and Plant Breeding, University of Bari Aldo Moro, Via Amendola 165/A, 70126 Bari (Italy).*

Modern durum wheat varieties, as a consequence of domestication and breeding aimed at increasing kernel yield and weight, exhibit larger kernel size and, in particular grain width. This, has led to current varieties displaying a rather uniform seed size. In contrast, wheat relatives and landraces show a much greater variability for kernel size and shape and represent a useful resource for the understanding of the genetic component controlling the dimension of the seeds.

This work relies on two RIL populations: the first one derived from the cross between the Italian cultivar Latino and the emmer accession MG5323 (*T. turgidum* ssp. *dicoccum*), while the second one resulted from the cross between the Iranian cultivar Zardak and the Iranian landrace accession 249. Both populations have been genotyped with the 90K iSelect (Illumina) and the corresponding high resolution marker map has been produced.

The two RIL populations have been phenotyped for seed morphology traits (e.g. length, width, and width/length ratio), kernel weight and yield in different environments. QTL analysis have revealed different genomic regions associated to each trait.

## TOWARDS THE CLONING OF *QYld.idw-3B*, A MAJOR GRAIN YIELD QTL IN DURUM WHEAT

**Graziani M.<sup>1</sup>, Maccaferri M.<sup>1</sup>, Salvi S.<sup>1</sup>, Sanguineti M.C.<sup>1</sup>, Paux E.<sup>2</sup>, Feuillet C.<sup>2</sup>, Simková H.<sup>3</sup>, Dolezel J.<sup>3</sup>, Massi A.<sup>4</sup>, Tuberosa R.<sup>1</sup>**

*1 DiSTA, University of Bologna, Viale Fanin 44, 40127 Bologna, Italy;*

*2 INRA GDEC, Clermont-Ferrand, Clermont-Theix, 63122 Saint-Genes-Champanelle, France;*

*3 Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Sokolovská 6, CZ-77200 Olomouc, Czech Republic,*

*4 Società Produttori Sementi Bologna, Via Macero 1, 40050 Argelato, Bologna, Italy*

*durum wheat, grain yield, positional cloning*

A major QTL (*QYld.idw-3B*) for grain yield was detected on the chromosome 3BS in a mapping population (Kofa x Svevo) of durum wheat (Maccaferri et al. 2008, *Genetics* 178:489-511). *QYld.idw-3B* influences also other important agronomic traits such as plant height, peduncle length, leaf greenness and thousand kernel weight. The fine mapping of *QYld.idw-3B* was performed within the FP7-funded TriticeaeGenome project. Three NIL pairs were crossed to obtain a large mapping population of 7,500 F2 that were screened to identify recombinants within the QTL interval. In 2011, 233 F4 segmental isolines were phenotypically characterized for plant height and peduncle length, two main traits influenced by *QYld.idw-3B*. To confirm the effect of the QTL on plant height, peduncle length, grain yield and related traits, a replicated field experiment was carried out in 2012 with the 61 most informative segmental isolines. The results confirm the co-localization of all traits in the same region of 0.22 cM. All markers added in the region were anchored to the physical map of chr. 3B of cv Chinese Spring (Paux et al. 2011, *Science* 322:101-104) and this allowed us to identify the most likely candidate genes in the target region. The functional characterization of these genes is underway by expression analysis. Additionally, to confirm and/or identify new candidate genes for *Qld.idw-3B* the BAC library of *Triticum turgidum* LDN65 (Cenci et al. 2004, *Genome* 47: 911–917), characterized by a haplotype in the target region similar to cv. Kofa, was screened. The BAC clones identified are being sequenced.

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## HIGH-THROUGHPUT PHENOTYPING OF DURUM WHEAT NEAR-ISOGENIC LINES UNDER WATER DEFICIT CONDITIONS

**Sciara G.\* , Salvi S.\* , Cané M.A.\* , Bovina R.\* , Welcker C.\*\* , Cabrera L.\*\* , Grau A.\*\* ,  
Tardieu F.\*\* & Tuberosa R.\***

*\*) Department of Agricultural Sciences – University of Bologna, Viale Fanin, 44, 40127 Bologna, Italy*

*\*\*\*) Laboratory of Plant Ecophysiological Responses to Environmental Stresses – INRA, Place P. Viala  
3, 34060 Montpellier, France*

Four pairs of durum wheat near-isogenic lines (NILs) for a major yield QTL (*Qyld.idw-3B*; Maccaferri et al., 2008) were tested (8 reps; 1 pot/rep) in the high-throughput phenotyping platform PhenoArch (INRA, Montpellier), a greenhouse platform for large-scale automated imagery and evapotranspiration measurements. Plants were grown till the milk stage of the kernel. Through image analysis and using suitable models, it has been possible to estimate biomass accumulation for each plant. Each pot within the design has been weighted at least once per day allowing for the accurate estimation of daily evapo-transpiration. The wheat NILs were subjected to three levels of drought stress: no stress (soil water potential > -1 Bar), mild stress (soil water potential of -5/-8 Bar) and severe stress (soil water potential ~ -13 Bar). The analysis of the results is in progress. Preliminary results confirm the effect of *Qyld.idw-3B* on total plant height, flag leaf insertion height and peduncle length. Nevertheless, no major allelic effect has been observed as regard of WUE and biomass accumulation in the three water regimes. These results suggest that the major effect of the QTL on yield appears to be due to physiological processes occurring in the later stages of grain filling, most likely to an effect of the QTL on the accumulation and/or translocation of water soluble carbohydrate.

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## ALLELIC VARIATION AT LOCI CONTROLLING HEADING TIME AND GRAIN YIELD COMPONENTS IN DURUM WHEAT

**Ricci A., Maccaferri M., Stefanelli S., Corneti S., Salvi S. Sanguineti M.C., Tuberosa R.**

*DipSA - University of Bologna, Viale Fanin 44, 40127 Bologna (Italy)*

Flowering time is key to the reproductive success in plants and plays a pivotal role to ensure reproductive success. Because of its importance, flowering is under the control of a complex genetic network that integrates both environmental and endogenous signals, such as photoperiod and temperature (vernalization requirement). In cereals, major genes have been identified in the vernalization- and photoperiod-sensitivity pathways (*VRN1*, *VRN2*, *VRN3* and *PPD*) forming a regulatory loop to control flowering time. Here, we characterize further genetic variation relevant for elite durum wheat adaptation in Southern Europe. In this study, a population of 176 recombinant inbred lines (RILs) developed by crossing Colosseo (C) and Lloyd (L) cvs. was characterized for heading date (HD) and kernel weight (TKW) in the Po Valley (Italy) and QTL analysis was carried out. Under the growing conditions of Southern Europe (autumn sowing), both C and L parents are medium- to late-heading and maturing. Phenotypic traits were collected over four field trials conducted in 2006, 2007, 2009 and 2010. The CxL linkage map was based on SSR (321), DaRT (372) and the Illumina 90k SNP assays (6.163). Major QTLs for HD were mapped on chromosome 2B ( $R^2 = 44.3\%$ ) and 7A ( $R^2 = 31\%$ ), respectively, in coincidence with known *PPD-1* and *VRN-3* genes. A third QTL for HD was found on pericentromeric region of chromosome 5A which corresponds to the colinear region in *B. distachyon* genome flanked by *Bradi4g03680.1* and *Bradi3g36397.1* genes, respectively, and possibly controlling earliness per se ( $R^2 = 4.7\%$ ). For this QTL we cannot exclude the coincidence with the homeolog of *VRN4-D4*. QTLs for TKW were mapped on chromosome 2B ( $R^2 = 9.2\%$ ), 5AS ( $R^2 = 9.8\%$ ) and 5AL ( $R^2 = 6.9\%$ ). The QTL on 2B was not coincident with *PPD-B1* while the QTL on 5AL, based on the syntenic analysis in the QTL confidence interval, mapped away from the *TmQ* (*Bradi1g03880.1*) thus excluding any relevant role of this gene in determining TKW. Genetic analysis at major candidate genes revealed that Colosseo carried alleles for earliness at both *Ppd-B1* and *Vrn-3A* and for lateness at *Qhd.ubo-5A*, whereas the Loyd alleles are in opposite phase. In Colosseo and Lloyd, *Ppd-B1* and *Vrn-3A* gene sequences showed unique deletions in the upstream of 5'-UTR region. In fact, in *Vrn-3A* Colosseo allele a 1.475 bp deletion at the putative gene promoter region has been detected, whereas in *Ppd-B1* Colosseo allele a deletion of 10 bp has been detected 1.234 bp upstream of the 5'-UTR region. Co-dominant *PPD-B1* and *VRN-3A* specific markers were developed and mapped on CxL RILs populations and a further QTL analysis was carried out. *PPD-B1* and *VRN-3A* specific markers were always found to map within the confidence interval of the QTL peaks for heading date. These results indicate that *Ppd-B1* and *Vrn-3A* Colosseo alleles might be useful for fine tuning heading time and vernalization requirement in tetraploid wheat under the growing conditions of Southern Europe.

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## ALLELIC VARIATION IN MAJOR GENES (*Rht-B1*, *Ppd-A1* AND *Vrn-A1*) AND ITS EFFECT ON PLANT HEIGHT, PEDUNCLE LENGTH AND FLOWERING/ HEADING DATE IN A DURUM WHEAT COLLECTION UNDER FIELD CONDITIONS

Roncallo P.F.<sup>1,2</sup>, Larsen A.O.<sup>3</sup>, Zaffora B.<sup>1</sup>, Monticelli N.A.<sup>1</sup>, Diaz Godoy P.<sup>1</sup>, Rochón M.<sup>1</sup>, Cuestas J.M.<sup>1</sup>, Gonzalez L.<sup>4</sup>, Jensen C.<sup>3</sup>, Campos P.<sup>5</sup>, Miranda R.<sup>6</sup>, Dreisigacker S.<sup>6</sup>, Echenique V.C.<sup>1,2</sup>

*1* Depto. de Agronomía, Universidad Nacional del Sur, Bahía Blanca, Argentina;

*2* CERZOS-CONICET, Bahía Blanca;

*3* INTA CEI Barrow, Tres Arroyos;

*4* Buck Semillas, Necochea;

*5* INTA Bordenave, Bordenave;

*6* Asociación de Coopetativas Argentinas (ACA), Bahía Blanca;

*7* International Maize and Wheat Improvement Center (CIMMYT), D.F., México.

**Keywords:** *Durum wheat, molecular markers, breeding.*

Heading date is an important trait in wheat breeding as it affects adaptation and yield potential. Selection for height and the introduction of semi-dwarfing alleles at the *Rht* loci have been proven to be effective for wheat yield improvement. The aim of this work was to analyze the allelic variation in three major genes (*Rht-B1*, *Ppd-A1* and *Vrn-A1*) in a durum wheat collection (*Triticum turgidum* L. var *durum*) and its phenotypic effects. Field trials were conducted during 2011 (133 entries, 2 environments), 2013 (168 entries, 2 environments) and 2014 (168 entries, 5 environments) using an alpha-lattice statistical design, in 5 assays. The genotyping was performed using the KASP™ genotyping technology and four single nucleotide polymorphisms (SNP) were detected in the 168 durum wheat genotypes. The effect of the allelic variation in these loci on the phenotype was assessed by using the ANOVA test, considering different number of field evaluations for each trait: peduncle length (4), plant height (5), spikes/m<sup>2</sup> (5) heading date (8) and flowering time (1).

The semi-dwarfing allele (*Rht-B1b*) was carried by 85.5% of the analyzed materials, showing a significant effect in all the environments with an average reduction in plant height (-23.6%) and peduncle length (-33.3%), but not showing a significant effect on the number of spikes/m<sup>2</sup>. Regarding to the heading date/flowering time traits, a relative high frequency of the spring allele *Vrn-A1b* (11.3%) at the *Vrn-A1* locus (Marquis allele) and three alleles at the photoperiod *A1* locus (*Ppd-A1*) (the deleted types `GS-100`, `GS-105` and the intact allele) were detected. The combination of *Vrn-A1b*/intact sequence alleles (11.3%) for the haplotype *Vrn-A1/Ppd-A1* showed, in average, a delay of 9.5 days in heading date respect to the genotypes carrying the `GS-100` photoperiod insensitive allele. These are interesting results to be used for MAS in national durum breeding programs.

## GLUTAMINE SYNTHETASE GENE EXPRESSION AND ENZYME ASSAY TO STUDY NITROGEN USE EFFICIENCY IN WHEAT.

**Nigro D., Fortunato S., Giove S.L., Paradiso A., Anderson O.D., Blanco A., De Pinto M.C., Gadaleta A.**

Nitrogen uptake is an essential element in crop improvement and cereals breeding. Plants response to nitrogen fertilization is estimated with the “Nitrogen Use Efficiency” (NUE), an indicator of the amount of useful product (yield of grain) per unit of nitrogen applied. Recently, there has been a considerable interest in identifying the processes involved in N uptake and metabolism. Glutamine synthetase (GS) is a key enzyme for nitrogen assimilation in plants, which catalyses the ATP-dependent condensation of ammonium and glutamate into glutamine, the principal precursor for the synthesis of most nitrogenous cellular compounds.

In order to determine the involvement of this gene in the control of mechanisms related to NUE, ten durum wheat genotypes, previously evaluated for grain yield components and GPC in different years and environments, were grown in a replicated field trial in three different nitrogen regimes (0, 60 and 140 units of N/h) supplied at seedlings, flowering and filling phases. Ten days after each nitrogen supplement, leaves samples were collected and both GS enzyme activity and gene expression studies were carried out. Results underlined high variability in GS expression and enzyme activity among wheat genotypes. Significant differences were found among different phenological phases in the same genotype, as a demonstration of the complexity of nitrogen metabolism processes and of the GS regulating system. These results were confirmed through western blotting analysis with a GS global antibody on each extract previously used for enzyme activity. This study demonstrated that nitrogen nutrition affects GS expression and activity throughout all developmental stages suggesting that the amounts of nitrogen uptaken and utilized can be manipulated by breeding and agronomy practice to optimize NUE without affecting production or grain protein content.

## ASPARAGINE SYNTHETASE (*AsnS*) GENES IN DURUM WHEAT

**Pasquale L. Curci<sup>1</sup>, William Marande<sup>2</sup>, H el ene Berg es<sup>2</sup>, Marco Maccaferri<sup>3</sup>,  
Roberto Tuberosa<sup>3</sup>, Gabriella Sonnante<sup>1</sup>**

*1 Institute of Biosciences and Bioresources, National Research Council, Bari, Italy;*

*2 French Plant Genomic Resource Centre, INRA, Castanet Tolosan, France*

*3 Department of Agricultural Science (DipSA) University of Bologna, Bologna, Italy*

One of the hardest challenges in the field of plant breeding at this moment is to improve the Nitrogen Use Efficiency (NUE). Plants require large amounts of nitrogen for their growth and survival. At present, the increase in investment in agriculture is mainly due to the use of N-fertilizer because it directly affects yield. Moreover, plants can only use approximately 30-40% of the applied N, and more than 40% of the N fertilizer is lost in the surrounding environment, representing a serious pollution problem.

Glutamine-dependent asparagine synthetase (*AsnS*) is one of the main enzymes responsible for N assimilation forming asparagine, an ideal amino acid for the N transport and storage due to its high N:C ratio. In most plant species, *AsnS* seems to be encoded by a small gene family, in which the *AsnS* gene number ranges between one and four.

Here, we isolated and sequenced four different *AsnS* genes by hybridization of a durum wheat BAC-library macroarray. Structural and phylogenetic analyses showed that these genes belong to the first of the three major *AsnS* gene classes. Within the first class, two subclasses were observed, as for other monocots. Two *AsnS* clustered with the *AsnS1* clade, whereas the remaining two *AsnS* belonged to the *AsnS2* group.

Quantitative PCR in durum wheat plants grown in nitrogen stress condition highlighted a big difference between *AsnS1* and *AsnS2* genes, being the former ones significantly more expressed than the latter ones. Both the *AsnS1* genes showed to be significantly down-regulated in nitrogen stress condition, especially in leaves.

In order to locate *AsnS* genes in durum wheat genome, three approaches were followed: cross-species comparisons within other monocots, analysis of the wheat sequence repository, and *in silico* anchoring to SNP-based maps.

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## INTEGRATED MOLECULAR AND METABOLITE PROFILING FOR DURUM WHEAT PLANTS GROWN UNDER ELEVATED CO<sub>2</sub>: RESULTS OF A FREE AIR CARBON DIOXIDE ENRICHMENT (FACE) EXPERIMENT

**Marè C.<sup>1</sup>, Diretto G.<sup>3</sup>, Bagnaresi P.<sup>1</sup>, Falcone G.<sup>3</sup>, Frusciante S.<sup>3</sup>, Sulli M.<sup>3</sup>, Zechini A.<sup>1</sup>, Mazzucotelli E.<sup>1</sup>, Rizza F.<sup>1</sup>, Zaldei A.<sup>2</sup>, Giuliano G.<sup>3</sup>, Miglietta F.<sup>2</sup>, Cattivelli L.<sup>1</sup>, Badeck F.W.<sup>1</sup>**

*1 Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria – Genomics Research Centre, Via S. Protaso, 302 - 29017 Fiorenzuola d'Arda (PC), Italy*

*2 CNR-IBIMET, Istituto di Biometeorologia, Firenze, Italy*

*3 ENEA, Centro Ricerche Casaccia, S. Maria di Galeria, Roma, Italy*

The atmospheric CO<sub>2</sub> is rising due to the release of CO<sub>2</sub> from burning of fuels from industrial processes and to the anthropic land use effects. The global CO<sub>2</sub> concentration was 396 ppm in 2013 and it is expected to increase leading to estimated concentrations around 550 ppm for the year 2050. The rise in atmospheric CO<sub>2</sub> leads to increased carbon assimilation by C3 photosynthesis. The studies on the effects of elevated CO<sub>2</sub> on C3 plant have demonstrated a stimulation of photosynthetic production and, subsequently, growth. However, the changes in demand/supply of carbohydrates and nitrogen in plants growing under elevated CO<sub>2</sub> lead to modifications in the chemical biomass composition and in the quality of harvested plant organs.

The effects of CO<sub>2</sub> rising (550 ppm) on growth, yield as well grain and pasta quality were evaluated on 12 genotypes of *durum wheat* in a field experiment in FACE facility (Free Air Carbon dioxide Enrichment) at CRA-GPG for two years (2011/12-2012/13). An increase in crop yield with a reduction in grain protein content were generally observed.

To understand the molecular response to elevated CO<sub>2</sub>, an integrated transcriptomic and metabolomic analysis was carried out on flag leaves collected at flowering in May 2012. The work was focused on four genotypes (Cappelli, Ofanto, Simeto and Claudio) showing different values of photosynthetic activity on the flag leaf before the collection. An up-regulation of genes related to sucrose synthesis as well a modulation of genes involved in nitrogen and lipid metabolism was detected. A significant variation in metabolite quantity was observed in the same leaf samples as well a decrease in the amount of amino acids and polar lipids and, limited to some cultivars, higher sugar/polyol and flavonoid contents.

The results highlighted that a diversity in the molecular response to elevated CO<sub>2</sub> in terms of both gene modulation and metabolite accumulation.

## CHROMOSOMAL LOCATION OF AWN AND GLUME COLOUR IN DURUM WHEAT.

**J. Clarke, F. Clarke, R. Knox, A. N'Diaye, K. Wiebe, J. Ens, C. Pozniak**

Durum wheat shows diversity for morphological characteristics such as awn and glume colour. Black awns are common, while black and bronze glume colour are less common in modern durum breeding programs. Bronze glumes are generally culled from breeding materials because of a linkage with low gluten strength. Black awns can be a nuisance in final purification of breeder seed of new cultivars because the expression of awn colour may be indistinct in some environmental conditions. Therefore, DNA markers for the colour traits would be useful to facilitate seed purification. A Gallareta/Demetra doubled haploid population, with Gallareta having black awns and white glumes and Demetra having bronze awns and glumes, was screened with the 90K Infinium iSelect array. QTL analysis was performed with QGene. Phenotypic segregation ratios fit the expectation of single gene control of black awn and bronze glumes, as previously published. Phenotypic classes were white glume/white awn, white glume/black awn, bronze glume/black awn, and bronze glume/bronze awn. There was no bronze glume/white awn class, consistent with literature indicating epistatic effect of the bronze glume colour locus on awn colour. Awn colour was localized to the distal end of chromosome 1A based on the published durum consensus SNP map, with Gallareta carrying the black allele and Demetra the white. Ten SNP markers located at approximately 0 cM were strongly associated with awn colour, but did not co-segregate perfectly, probably due to phenotyping errors caused by indistinct expression of the black colour. The markers did co-segregate perfectly with awn colour in a validation population of purified breeding lines. Bronze glume/awn phenotype co-segregated with 15 SNP markers in the region of 35 cM on chromosome 1B. Markers developed from these SNPs should be useful in final purification of seed of new cultivars, and selection against bronze glume colour.

## OPTIMIZING SOMATIC EMBRYOGENESIS AND AGROBACTERIUM MEDIATED TRANSFORMATION IN DURUM WHEAT

**Ahansal Khadija, Aadel Hanane, Udupa M. Sripada, Gaboun Fatima, Abdelwahd Rabha, Ibriz Mohammed and Iraqi Driss**

The first step in the application of biotechnology in durum wheat is the establishment of an efficient plant regeneration protocol. We used mature and immature embryos, as explants from four durum wheat varieties (Amria, Chaoui, Marouane and Tomouh) to study the effects of 2,4-D and picloram at concentrations of 2 mg.l<sup>-1</sup>, and their combination on induction of embryogenic and plantlets regeneration. We considered callus weight (after 4 weeks); percentage of callus induction, relative fresh weight growth rate (RFWGR), plantlets regeneration, number of plantlets regenerating per callus and the number of plantlets regenerating per callus regenerated for the evaluation. A significant effect of variety, auxin and variety × auxin interaction were observed for RFWGR of callus and regeneration from both immature and mature embryo explants. The auxin used for callus induction had a significant effect on plantlets regeneration ( $p < 0.001$ ). Picloram showed higher plantlets regenerations rate from immature embryos (40.86%) followed by 2,4-D (31.96%), from mature embryos picloram was the best for plantlets regeneration rates (19.8%). A comparison of the responses of the two explants used indicated that the immature embryos were the most useful explant for plantlets regeneration in durum wheat. Thereby the induction medium containing picloram is used for genetic transformation experiments. The transformation was initiated from the mature whole embryos, half mature embryos and mature embryos scraped into small pieces of the four varieties of durum wheat using *Agrobacterium* techniques and *pBY520(HVA1)* plasmid containing the gene for tolerance to drought and the resistance to *Basta*. The percentage of callus after 40 days of genetic transformation was high for all varieties derived from whole embryo. After transfer to glasshouse, evaluation of the expression of the bar gene in leaves by basta panting and molecular analysis confirmed integration of the transgene.

## MUTATION DISCOVERY PIPELINE FOR A TETRAPLOID WHEAT TILLING POPULATION

**Fruzangohar M.<sup>1</sup>, Incerti O.<sup>2</sup>, Tricker P.J.<sup>1</sup>, Watson-Haigh N.S.<sup>1</sup>, Fleury D.<sup>1</sup>, Akhunov E.D.<sup>3</sup>, Gadaleta A.<sup>2</sup>, Blanco A.<sup>2</sup>, Baumann U.<sup>1\*</sup>**

Targeted resequencing from a large genome like durum wheat (13 Gbp) provides the possibility to interrogate more samples with less effort. Exome-capture technology efficiently reduces the complexity of a large genome by capturing just the exonic regions. However, detecting putative mutations in captured sequences when a reference genome is not available is challenging. Multiplexing samples in each capture assay can increase the throughput and reduce the cost of exome sequencing. Ideally multiplexing should not compromise mutation discovery; the balance between multiplexing and reliable mutations calling in tetraploid wheat has yet to be determined empirically.

In this study we examined the effect of multiplexing and of pre-reduction of samples to their unmethylated fraction prior to exome capture on mutation detection. We addressed the difficulties in the mutation discovery process in the absence of a reference genome. This was followed by the development of bioinformatics tools and statistical tests to detect different types of mutations. We applied our methods to the unmutagenized control type cv 'Aureo' and five EMS-mutagenized lines. For each mutant two samples were prepared, one of un-treated gDNA and the other of gDNA reduced to the unmethylated fraction.

We compared our results to previous exome-sequencing of tetraploid wheat and found very similar mutation rates. Multiplexing did not affect capture efficiency or normalized coverage and may have had unexpected benefits in equalizing the distribution of reads. Pre-reduction of DNA samples to their unmethylated fraction significantly improved coverage and capture efficiency. We were able to assign a probability estimate to each putative mutation and detect mutation types without prior assumptions. We further proposed a statistical method to infer the zygosity of each detected mutation. Finally, we selected a subset of predicted mutations of different mutation type and zygosity for verification.

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## A TBP-BASED APPROACH FOR SIMPLE GENETIC PROFILING AND FAST SNPS IDENTIFICATION IN WHEAT GENOTYPES

**Silletti S., Morello L., Gavazzi F., Braglia L., Gualdi V., Strozzi F and Breviario D.**

The issue of food authenticity and traceability is of increasing importance also in the wheat sector, where ancient genotypes (spelta, emmer wheat) or particular cultivars (i.e. kamut) are regarded as healthier alternatives to bread wheat and have reached high commercial value.

TBP ( $\beta$ -tubulin based polymorphisms) is a PCR-based, multilocus, co-dominant marker useful for fast fingerprinting of any higher plant species. Based on intron length polymorphism of members of the  $\beta$ -tubulin gene family, it is able to provide a unique, specie-specific genetic profile (barcode) using two universal primer pairs. This approach has been successfully applied to the authentication of the composition of mixed feed and food matrices. We applied TBP analysis, followed by amplicon separation by Capillary Electrophoresis, to commercially relevant wheat genotypes (emmer, spelta, einkorn) and to 32 different durum wheat accessions. Specific profiles were clearly obtained for the different species, while few intron length polymorphisms were present at the variety level.

Hence, we wondered if the limited but highly variable intronic region amplified by TBP primers (5-10 kbp/haploid genome) was suitable to uncover enough allelic sequence polymorphisms at the variety level. We followed a NGS approach on the Miseq platform to sequence TBP amplicons of 47 wheat samples (4 exaploid, 32 tetraploid and 11 diploid wheat relatives or ancestors) in order to provide an initial TBP sequence database and to identify SNPs among genotypes. Sequence data were processed as a *de novo* amplicon assembly and all the contigs from *T. aestivum* of the cultivar Bilancia were manually refined and used as reference for sequence re-assembly. The limited region analyzed makes the bioinformatics analysis extremely handy suggesting the possibility of an application of this technique as a very simple tool to evaluate genetic diversity in wheat. Application to phylogenetic analysis is also under evaluation.

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## **PASTA, BREAD AND FANTASY: INNOVATION IN THE PRODUCTION OF FUNCTIONAL FOODS FOR CONSUMERS' WELLBEING.**

**Pier Paolo Marrese, Monica De Caroli, Andrea Iurlaro, Mariarosaria De Pascali, Patrizia Rampino, Giuseppe Dalessandro, Luigi De Bellis, Carla Perrotta, Gabriella Piro, Marcello Salvatore Lenucci**

*Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali (Di.S.Te.B.A.), Università del Salento, Via Prov.le Lecce-Monteroni, 73100 Lecce (Italy)*

Durum wheat based products, including bread and pasta, are essential pillars of the Mediterranean diet, but their nutritional value is generally low due to the use of refined semolina in the dough. Bread and pasta are eaten daily and meet the customer's favor for simplicity of use in relation to handling, storage and cooking. This makes them an optimal starting material to develop innovative fortified functional foods. Grain milling novel technologies allow the recovery of different fractions enriched in specific bioactives suitable to reconstitute the whole grain phytocomplex. Besides, other ready-to-mix plant derived matrices can be properly blended to improve the nutritional value of the dough with specific combinations of molecules, meeting the demand for custom-tailored foods designed to satisfy specific needs or preferences (nutritional, physiological, cultural, sensorial, etc.). Here we describe the use of a freeze-dried tomato matrix to prepare dried spaghetti pasta and fresh bread fortified with lycopene, a red carotenoid pigment with powerful antioxidant activity, thought to aid in preventing some degenerative pathologies such as cancer and cardiovascular diseases. The addition of the matrix to the semolina in a 1.5% w/w ratio allowed the preparation of intensely red colored pasta and bread showing only minor differences in macro- and micro-structure. Between 30 to 40% lycopene loss was observed during the production processes; however an average portion (70 g) of either pasta or bread fully fulfill the Recommended Daily Allowance (RDA) for lycopene (~7 mg/die). Simultaneously, the total antioxidant activity was doubled with respect to the traditional products. A rapid decrease of lycopene content (-47% after 13 days, -27% after 30 days, respectively) was observed during pasta and bread storage (~25°C in the dark, with air exposure) stressing the importance for the use of proper packaging systems or other strategies to improve their stability over time.

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## GENETIC VARIABILITY AND ANTI-INFLAMMATORY PROPERTIES OF PHENOLIC ACIDS IN DURUM WHEAT

**Barbara Laddomada<sup>1</sup>, Miriana Durante<sup>1</sup>, Fiorenza Minervini<sup>2</sup>, Antonella Garbetta<sup>2</sup>, Angela Cardinali<sup>2</sup>, Isabella D'Antuono<sup>2</sup>, Sofia Caretto<sup>1</sup>, Antonio Blanco<sup>3</sup>, Giovanni Mita<sup>1</sup>**

*1 C.N.R., Institute of Sciences of Food Production (ISPA), Lecce, Via Monteroni, 73100, Lecce (Italy)*

*2 C.N.R., Institute of Sciences of Food Production (ISPA), Via Amendola 122/O, 70124, Bari (Italy)*

*3 Department of Soil, Plant, and Food Sciences, Plant Breeding Section, University of Bari "Aldo Moro", Via Amendola, 165/a, 70124, Bari (Italy)*

Whole wheat foods are significant source of compounds exhibiting health-promoting properties. One of the most abundant class of phytochemicals in the wheat grain is represented by phenolic acids that are typically localized in the bran and germ portions.

The objective of this study was to estimate the extent of genetic variation for phenolic acids in durum wheat (*T. turgidum* L. ssp. *durum*). In addition, this study aimed at evaluating the anti-inflammatory activity of phenolic acids contained in whole-meal flour extracts.

Phenolic acids were recovered from the whole meal flours of 65 durum cultivars and subsequently identified and quantified by HPLC-DAD analysis. Then, the anti-inflammatory activity of phenolic acids extracts was evaluated on LPS-stimulated HT-29 human colon cells by measuring the levels of interleukin 8 (IL-8) and transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1).

A large variation for the content of phenolic acids was observed among genotypes and, on average, it accounted for 830  $\mu$ g/g dry weight. Whole meal flour extract significantly inhibited the secretion of the pro-inflammatory IL-8 mediator at 66  $\mu$ g/mL of phenolic acids. Conversely, the secretion of the anti-inflammatory mediator TGF- $\beta$ 1 was not modified by addition of phenolic acids to HT-29 cells.

Results showed that durum cultivars have different contents of phenolic acids, suggesting that a number of elite varieties could be used for breeding purposes. Moreover, results provide further insight into the health-related benefits of durum wheat foods as depending on the anti-inflammatory activity of phenolic acids.

## USE OF DEBRANNING PRODUCTS FOR THE PRODUCTION OF PASTA WITH HIGH CONTENT OF ANTIOXIDANT COMPOUNDS

**Martini D., Ciccoritti R., Taddei F., Nicoletti I., Corradini D., D'Egidio M.G.**

One of the main challenges for modern cereal technologies is to find innovative solutions for the production of cereal-based foods jointly rich in bioactive compounds and with high technological and sensory quality, in order to help consumers in increasing the consumption of less refined foods. Debranning process seems to be a valuable approach to achieve these goals.

In the present studies the content of antioxidant compounds, including phenolic acids (PAs), total phenolic compounds (TPC) and yellow coloured pigments (YCP) as well as the total antioxidant capacity (TAC) of two pasta samples made by using debranning products, was investigated. In detail, two different durum wheat products were made: i) a pasta produced by enriching semolina with selected debranning fractions (DF) obtained by sequential debranning (pasta 1); ii) a pasta produced by whole milled debranned kernels (DK) (pasta 2). These pasta samples were compared to traditional pasta (pasta 3), considered as a reference.

Results showed that pasta 1 and 2 had significant higher content of PAs and TPC in comparison with traditional pasta. Therefore, the use of whole milled DK or semolina enriched with DF for pasta-making process allows to obtain pasta samples with a high content of phenolic compounds.

The impact of the different processes (extrusion, drying and cooking) on the occurrence of PAs and TPC was different among the pasta samples under study. Moreover, the 3 PA and TPC forms (soluble free, soluble conjugated and insoluble bound) were differently influenced by the processes. The statistical analysis revealed high correlation between PAs and TPC, while a lower but significant correlation was observed with YCP.

In conclusion, the present study demonstrated that the occurrence of phenolic compounds increased significantly when DF and DK are used in pasta-making. Therefore this approach could be useful for the production of cereal-based foods with high nutritional and healthy potential.



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## MICRONUTRIENT CONCENTRATION IN CARYOPSIS OF EINKORN WHEAT ACCESSIONS (*TRITICUM MONOCOCCUM* L.), ANCIENT LANDRACES AND IMPROVED CULTIVARS OF DURUM WHEAT (*TRITICUM TURGIDUM* L. SUBSP. *DURUM*)

**Sciacca F.\* , Allegra M.\* , Brambilla M.\*\* , Leonardi A.\* , Licciardello S.\* , Pesce A.\* , Puleo A.\* , Rocuzzo G.\* , Romano E.\*\* , Torrisi B.\* , Virzì N.\* , Palumbo M.\***

*Consiglio per la ricerca e l'analisi dell'economia agraria (CRA)*

*(\*) Centro di Ricerca per l'Agricoltura e le Colture Mediterranee (CRA-ACM), Research Centre for Citrus and Mediterranean Crops. Corso Savoia, 190 - 95024 Acireale (Ct), Italy*

*(\*\*) Unità di Ricerca per l'Ingegneria Agraria (CRA-ING) - Laboratorio di Ricerca di Treviglio - Via Milano, 43 - 24047 Treviglio (BG), Italy*

Micronutrient deficiencies in human organisms, in particular zinc (Zn) and iron (Fe), are widespread especially in developing world and in general affects more than two million people. About 800,000 child deaths worldwide per year are attributable to Zn deficiency in underdeveloped countries. Wheat-based food has great importance in human nutrition and differences between ancient and modern genotypes concern not only the yields but also the caryopsis composition that greatly affects derived products nutritional properties. Several authors have reported a large genetic variability for micronutrients in kernels of different cereal species and the existence of this variability is essential for successful breeding programs aiming to development of micronutrient-rich new genotypes. In this work, caryopsis micronutrient concentration of three Einkorn wheat accessions (*Triticum monococcum* L.), three ancient Sicilian landraces and three improved varieties of durum wheat (*Triticum turgidum* L. subsp. *durum*) was investigated, with the ultimate aim of providing useful information to increase the nutritional value of wheat-derived products. All genotypes were grown at identical environmental conditions, in the CRA experimental farm placed in Sicily (Lat. 37°32' N; Lon. 14°34' E). At full ripening, caryopses underwent chemical characterization measuring macro-(N, P, K and Ca) and micro-elements (Fe, Zn, Mn, Cu, Li and Sr) concentrations by means of ICP-OES (Optima 2000 DV, Perkin Elmer, Italia). Univariate and multivariate data processing showed significant differences among the three groups of genotypes and the results revealed higher micronutrient concentration in *Triticum monococcum* and in Sicilian landraces than in modern durum wheat varieties.

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## MICROBIOLOGICAL QUALITY OF WHEAT FLOUR CONSUMED IN MOROCCO

**Jihane Ennadir, Rachida Hassikou, Farida Ohmani, Jamila Hammamouchi, Fatima Bouazza, Aicha Qasmaoui, Zakaria Mennane, Amina Ouazzani Touhami, Reda Charof et Khadija Khedid**

Cereal products (soft and hard wheat) are a basic staple food in the Moroccan diet. A total of 60 samples of two types of wheat flours used for human consumption were collected; 30 samples among this collection were obtained from various households using Moroccan varieties of wheat produced in traditional flour mills. The rest of the samples were purchased from retail wheat flour sources in the Rabat and Sale city markets. Standard plate counts (SPC), total and faecal coliforms, Clostridium, Salmonella spp., Shigella spp., Staphylococcus aureus, Listeria monocytogenes, yeast, lactic acid bacteria, and molds, were carried out to assess the microbiological quality of wheat flour. Microbiological interpretation of the criteria was performed according to standards implemented by the Codex Alimentarius Commission. Most frequent counts, in traditional and industrial wheat flour, were total aerobic mesophilic bacteria with an average  $4 \times 10^4$  and  $2.5 \times 10^4$  cfu/g, respectively. The results showed higher coliform and fungi counts in house than in commercial samples. Pathogenic flora as Salmonella spp., Shigella spp., S. aureus, L. monocytogenes, and Clostridium were not detected in all investigated samples. Bacterial strains isolated from both flours belong to the following genera: Enterobacter spp., Serratia spp., Klebsiella spp., Pantoea spp., Leclercia spp., Proteus spp. The most frequent genus of the investigated isolates was Aspergillus (81%). Microbial counts were lower than the limit laid down in the Codex Alimentarius, attributing to these flours a satisfactory microbiological quality.

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## CD CONCENTRATIONS IN GRAINS OF DURUM WHEAT CULTIVARS

**Marzia Vergine, Alessio Aprile, Erika Sabella, Eliana Nutricati, Carla Perrotta, Luigi De Bellis**

Durum wheat (*Triticum turgidum* L. var. durum) accumulates cadmium (Cd) in grains when grown in Cd contaminated soils. Cd is a non-essential element that affects negatively plant growth and development. It is released into the environment by power stations, heating systems, metal-working industries and urban traffic. It is widely used in electroplating, pigments, plastic stabilizers and nickel-cadmium batteries. Cadmium is recognized as an extremely significant pollutant due to its high toxicity and large solubility in water (Benavides *et al.* 2005). Elevated levels of Cd in humans can cause kidney damage and renal dysfunction. Other diseases associated with Cd exposure are pulmonary emphysema and the Itai–Itai (“ouch–ouch”) disease (Gupta *et al.* 2014). Since cadmium accumulation in foods represents a risk for the consumer health, the international trade organizations limits the acceptable concentration of Cd in edible crops.

To characterize durum wheat cultivars in response to Cd contaminated soils, 12 durum wheat cultivars were grown in a hydroponic systems that allowed to finely control the nutrient concentrations. Treated plants were grown in the presence of 0.5  $\mu\text{M}$  Cd, that mimic low contaminated soils. Leaves, roots and grains were collected for Cd analysis.

Preliminary data indicate that durum wheat young plants grown in the presence of Cd show different Cd concentrations between roots and shoots: about the 80% of the total Cd found in the whole plants is stored in roots. The 12 cultivars showed similar concentrations in root tissues. On the contrary, differences in Cd accumulation were found in leaves as well as in grains.

These data suggest that different accumulation of Cd in grains is related to genotype.

To avoid commercial restrictions, durum wheat cultivar with low cadmium accumulations in grains should be preferred by farmers.

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## DIVERSITY AMONG *TRITICUM DURUM* CULTIVARS AND BREEDING LINES FOR HIGH VERSUS LOW CADMIUM CONTENT IN SEEDS ASSESSED WITH THE CAPS MARKER *USW47*

Simone Zimmerl<sup>1</sup>, Julia Lafferty<sup>2</sup>, Hermann Buerstmayr<sup>1</sup>

*1 BOKU - University of Natural Resources and Life Sciences Vienna, Department IFA-Tulln, Institute for Biotechnology in Plant Production, Tulln, Austria*

*2 Saatzucht Donau GesmbH & CoKG, Probstdorf, Austria*

Cadmium (Cd) is a toxic heavy metal that occurs naturally in soils. Durum wheat is known to accumulate generally more Cd than other cereal crops. The uptake of Cd in durum wheat is governed by the gene *Cdu1*, which co-segregates with several DNA markers, such as the co-dominant marker *usw47* and the dominant marker *ScOPC20*. A panel of 314 durum wheat cultivars or lines originating from 16 countries or regions were assessed with *usw47*. The plant material was mainly comprised of cultivars and modern breeding lines. From this set, 165 durum wheat lines were classified as low-Cd accumulators, 144 high-Cd accumulators and five were heterogeneous. A smaller subset of 16 cultivars had previously been evaluated for Cd accumulation in replicated field trials. Lines with the high-Cd allele showed a 2.4-fold higher Cd content in the seeds than lines with the low-Cd allele. We also compared the utility of markers *usw47* and *ScOPC20* as selection tools. Marker-assisted selection appears as a robust and practicable tool for breeding durum cultivars with low-Cd content.

## VALIDATION OF MOLECULAR MARKERS FOR PASTA-COOKING QUALITY TRAITS IN DURUM WHEAT

**Amallah Lamiae<sup>1,2</sup>, Taghouti Mouna<sup>2</sup>, Rhrib Keltoum<sup>2</sup>, Gaboun Fatima<sup>2</sup>, Hassikou Rachida<sup>1</sup>, Diria Ghizlane<sup>2</sup>**

*1 Laboratory of Botanic, Mycology and Environment, Biology Department, Faculty of Sciences, Rabat, Morocco.*

*2 Regional Center of Agronomic Research (CRRRA) Rabat, Morocco*

Over recent years, pasta-cooking quality has become an important commercial issue for durum wheat breeders. Modern breeding methods are most efficient for producing and supplying the best quality raw materials to the pasta industry. Marker-assisted selection (MAS) can improve plant breeding by saving time and labor in breeding programs. The objective of this study was to assess the effectiveness of molecular MAS for quality traits. For that purposes, DNA and biochemical markers were jointly used for the analysis of quality traits, affecting pasta-cooking quality in durum wheat genotypes. A total of 137 durum wheat (*Triticum turgidum ssp. durum*) Mediterranean landraces, international lines and Moroccan cultivars were analyzed for gluten strength, yellow pigment and protein content. Additionally, eighteen simple sequence repeat (SSR) markers covering a wide genome area were used, respectively: 10 GWM, 3 WMC, 2 CFA, 1 UHW, 1 GPW and 1 DupW. Of these, 14 (78%) were polymorphic and four monomorphic. Fifty-five bands were scored across accessions. Good concordance between quality traits and SSRs were detected. The present study showed the efficiency of SSR technology, which holds promise for a wide range of applications in marker-aided wheat breeding programs.

## CHARACTERIZATION OF DURUM WHEAT HMW-GS Bx20 AND By20 SEQUENCES BY A MOLECULAR AND PROTEOMIC APPROACH

**Santagati V.D.<sup>1</sup>, Sestili F.<sup>1</sup>, Lafiandra D.<sup>1</sup>, D'Ovidio R.<sup>1</sup>, Rogneaux H.<sup>2</sup>, Masci S.<sup>1</sup>**

*1 Department of Agriculture, Forestry, Nature and Energy (DAFNE), University of Tuscia, Via S. Camillo de Lellis s.n.c., 01100 Viterbo, Italy*

*2 INRA UR1268 BIA, Rue de la Géraudière, BP 71627, 44316 Nantes, France*

High molecular weight glutenin subunits variation is in general important because of its great influence on the glutenin polymers structure, that is related to wheat dough technological properties. Among the different subunits, the pair Bx20 and By20 is known to have generally a negative effect on quality, but the reasons are not clear: Bx20 is known to have only two cysteine residues, that theoretically make this subunit a chain extender of the glutenin polymer, just like the other Bx subunits, that show the typical four cysteine residues, two of which should be involved in intra-molecular disulfide bonds. By20 has never been characterized so far. Here we report the nucleotide sequences of Bx20 and By20 genes isolated from the durum wheat cultivar Lira biotype 45 and the validation of the corresponding deduced amino acid sequences by using Mass Spectrometry analyses. Three amino acid substitutions, caused by as many SNPs, have been identified in the Bx20 subunit with respect to the deduced sequence present in NCBI, whereas the By20 nucleotide and amino acid sequences, revealed a great similarity to By15, both at gene and protein levels, showing only two amino acid differences, caused by as many SNPs.

Moreover, no post-translational modification has been identified.

## DETERMINATION OF ALLELIC VARIATION AT HMW-GS AND LMW-GS LOCI IN MOROCCAN DURUM WHEAT CULTIVARS USING GENE-SPECIFIC PCR MARKERS

**Fatima Henkrar<sup>1,2,3,4</sup>, Jamal Elhaddoury<sup>3</sup>, Hassan Ouabbou<sup>3</sup>, Driss Iraqi<sup>1</sup>, Najib Bendaou<sup>4</sup>, Sripada M. Udupa<sup>1,2</sup>**

Wheat seed storage proteins play an important role in dough properties and in bread making quality in various wheat varieties. In Morocco, few studies were realized on the allelic variation in prolamin protein especially in durum wheat. The objectives of the present study were to determine the allelic variation of HMW-GS and LMW-GS in 26 of durum wheat using gene-specific PCR markers. The cultivars provided from National Gene Bank of Morocco, INRA, Settat, Morocco were used to characterize the glutenin alleles at *GluA1*, *GluB1*, *GluA3* and *GluB3*. Durum wheat varieties were planted under greenhouse, and total genomic DNA was extracted from fresh leaves of a single plant using a CTAB protocol. PCR-Amplification was carried out using specific primers of glutenin. Allele profiles were used to calculate the number of alleles per locus, their frequency, allelic variation, genetic distance and relationships (UPGMA Cluster analysis) among the varieties. We can notice from this study that most of the Moroccan wheat cultivars showed a polymorphism in the glutenin loci and carried good baking quality alleles. However, some alleles should be avoided in future durum wheat breeding program.

**Key words:** *Durum wheat, Moroccan wheat, glutenin, HMW, LMW, allelic variation.*

## DISULFIDE BOND FORMATION IN THE ASSEMBLY OF GLUTENIN SUBUNITS

**Federica Savazzini, Donald D. Kasarda and Aldo Ceriotti**

The unique functional properties of wheat flours are largely determined by the characteristics of storage proteins, which are the main components of gluten. The polymeric fraction of gluten proteins is assembled starting from low-molecular-weight subunits (LMW-GS), high-molecular-weight subunits (HMW-GS), and “aggregated” gliadins. While it is well established that interchain disulfide bonds play a crucial role in this process, the organisation of the different subunits in the final polymers remains unclear. We have used heterologous expression of glutenin subunits in tobacco protoplasts to get insight into the rules governing polymer formation. Using this approach, we have previously shown that homopolymeric assembly of the B11-33 LMW-GS occurs via the formation of disulfide bonds involving all possible combinations of two specific cysteine residues present in the monomeric protein. We now extend this observation to other classes of LMW-GS, in which one of the two cysteine residues implicated in assembly is located at a different position. Our results indicate that, also in the case of these proteins, assembly invariably involves all possible combinations of the two crucial cysteine residues. Using the same approach, we have also analyzed the homopolymeric assembly of a HMW-GS, and the interaction between LMW-GS and HMW-GS. Our findings are consistent with the view that assembly of glutenin subunits can produce a large number of distinct polymers, which are stabilized by disulfide bonds involving different combinations of the cysteine residues that remain available for interchain disulfide bond formation after monomer folding.



## 2-DE COMPARATIVE GLUTEN ANALYSIS BETWEEN ONE MODERN AND ONE OLD DURUM WHEAT GENOTYPE

**M.A. De Santis, M.M. Giuliani, L. Giuzio, P. De Vita, Z. Flagella**

In this study gliadin and glutenin composition of one old and one modern durum wheat genotype (*Triticum turgidum* L. spp. *durum*) grown under Mediterranean environment, was investigated by two dimensional electrophoresis (2-DE). The two genotypes, Cappelli and Simeto, were chosen both on the basis of the year of release (1915 and 1988, respectively) and of their different gluten quality, being higher for Simeto than Cappelli. The genotypes were grown for two crop seasons (2012/13 and 2013/14) on a clay-loam soil at Foggia, Southern Italy (41° 28' N, 15° 32' E). At maturity from kernels, gliadins and glutenins were extracted and separated by 2-DE. Image analysis was performed using ImageMaster 2D Platinum 6.0 software (GE Healthcare Bio-Sciences AB). Glutenin gels were subdivided in three gel regions: >60kDa (mainly HMW-GS), 60-37kDa (mainly LMW-GS c-type) and 37kDa (mainly LMW-GS b-type). Gliadins were subdivided in three gel regions: 75-50kDa (mainly  $\omega$ -gliadins), 50-37kDa (mainly  $\omega,\gamma$ -gliadins) and 37-25kDa (mainly  $\alpha,\beta$  and  $\gamma$ - gliadins). T-test and analysis of variance (ANOVA) has been performed by JMP software. The first year was characterized by lower rainfall in the grain filling period. Differences between genotypes and years were more evident for the glutenin fraction than gliadin one. In particular, in the second year Cappelli showed LMW-GS-c type region higher than Simeto, which instead showed higher values of HMW-GS and LMW-GS b-type region. In the first more stressed year Cappelli showed an increase in H region and a decrease in LMW-GS b-type region; on the contrary Simeto showed an increase of LMW-GS-b type and a decrease of LMW-GS c-type region. The difference in gluten composition between the old and the modern durum wheat genotype will be discussed in the light of the differences in technological quality.

# FROM SEED TO PASTA & BEYOND

*A Sustainable Durum Wheat Chain for Food Security and Healthy Lives*

## SOFT KERNEL DURUM WHEAT – A BETTER WHEAT FOR GLOBAL PASTA PRODUCTION

**Marco C. Simeone<sup>1</sup>, Domenico Lafiandra<sup>1</sup>, Craig F. Morris<sup>2</sup>**

*1 DAFNE, Università della Tuscia – 01100 Viterbo (Italy)*

*2 USDA-ARS, WWQL, Washington State University, Pullman, WA (USA)*

Kernel texture is one of the most important factors governing the milling performance, flour quality, processing, and food uses of all types of wheat. We successfully transferred the genes responsible for kernel softness from bread wheat into durum wheat by translocating a short chromosome fragment using non-GMO means. The obtained soft-kernel durum wheat has never existed before, and certainly has not been at the disposal of plant breeders and food technologists. As such this development has the potential for an expansive and profound effect on the way that durum grain is milled and on the products that are made from it. For instance, our “soft” durum wheat grain can be efficiently milled with considerable energy, time and additional machinery (typically needed to reduce semolina particle size) savings; bread wheat mills will efficiently deal with the soft textured durum grain. In addition, the promoted lower starch damage and the finer particle size of the soft durum flour contribute to reduce water absorption during dough formation, a generally desirable attribute for both economy and environmental sustainability. The chromosome translocation does not alter any gluten property, and therefore dough strength and extensibility are similar to a normal durum wheat. The soft durum flour hydrates more evenly, and the produced pasta dries at similar rates compared to pasta made from the commercial semolina. We also observed a higher dried breaking strength, an equal to slightly longer cooking time, and a similar to slightly higher cooked firmness. We are currently collaborating with the North Dakota University and CIMMYT to move this new trait into elite breeding lines. This work will ensure that Soft durum germplasm with competitive yield, disease resistance, and superior agronomics will be available to farmers, millers, and food processors. Pre-commercial trials are underway in central Italy and the U.S. Pacific Northwest.

# FROM SEED TO PASTA & BEYOND

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## DEVELOPMENT OF DURUM WHEAT WITH VARYING B-TYPE STARCH GRANULE CONTENT

**Gururaj Kadkol and Mike Sissons**

*NSW Department of Primary Industries, Calala, NSW 2340*

We have created populations of durum wheat with varying proportion of B-type starch granules to alter pasta quality and possibly milling yield. Starch is the main carbohydrate in the endosperm of wheat grain. In mature grain, starch is deposited as small B-type (3–9  $\mu\text{m}$ ) and larger A-type granules (13–16  $\mu\text{m}$ ). The former have a greater surface to volume ratio and this affects flour water absorption and starch gluten separation. Differences in starch granule size distributions have been identified within tetraploid wheat species in previous studies (B-granules, 17–47%). In pasta, improved firmness was obtained with elevated B-granule content (32–44%) with possible effects on starch digestibility. Work in hard wheat varieties has shown a good relationship between flour yield and the starch granule size distribution, suggesting that developing varieties with specific granule distributions could improve commercial flour yield.

We have made crosses with *Triticum dicoccum* lines to develop durum with higher and lower B granule content than the normal range (~27-34%) and identified lines with 41% and 18% B-granule in F2 derived F4 lines. We have evaluated these lines for agronomic traits including yield. The prospects for developing high yielding durums with high and low B granule content will be discussed in view of this data.

Future work will aim to continue to develop these populations in an elite background of acceptable technological quality and yield. Pasta making quality and milling yield potential will be assessed in the extreme variants.

## DETERMINATION OF RESISTANT STARCH CONTENT IN FLOURS OF THIRTY DURUM WHEAT CULTIVARS

**Sabella Erika, Aprile Alessio, Vergine Marzia, Negro Carmine, De Bellis Luigi**

A high proportion of starches in foods are digested into monosaccharides by the human digestive system and absorbed through the small intestinal villi; however, a fraction of the material (defined as Resistant Starch, RS) resists digestion and passes through to the large intestine where it acts like dietary fiber, which may have health benefits. RS intake seems to increase satiety and reduce fat storage, decrease postprandial glycemic and insulinemic responses, lower plasma cholesterol and triglyceride concentrations, improve whole body insulin sensitivity. These properties make RS an attractive dietary target for the prevention of diseases associated with dyslipidemia and insulin resistance as well as the development of weight loss diets and dietary therapies for the treatment of Type 2 diabetes and coronary heart disease.

Most of the starch consumed in the human diet includes wheat starch.

The two polymers of starch are amylose and amylopectin; resistant starch is characterized from a low amylose and high amylopectin content that determines high elasticity of the flour mixtures (for example, for Japanese noodles production) and a greater shelf-life of baked products; therefore the ratio of amylose and amylopectin supplies nutritional and technological properties for the final products. We investigated the Resistant starch content in flour of thirty different durum wheat cultivars grown in two experimental soils located in two different sites: Foggia and Potenza (Italy). Levels of Resistant Starch were determined using the Megazyme RS assay kit (K-RSTAR 08/11; AOAC Method 2002.02, AACC Method 32-40.01). Data were obtained from three replicates for each cultivar from the two experimental field. Preliminary data show a good variability among the thirty genotypes.

Wheat is the dominant crop in temperate countries being used for bread, pasta and other food products and the increasing awareness of the important role of wheat-based products in a healthy diet is a current important target for scientific research. Results supply important information for durum wheat cultivar selection, both for industrial uses and human health.

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## EVALUATION OF NUTRITIONAL AND RHEOLOGICAL PROPERTIES OF HIGH AMYLOSE DURUM WHEAT LINES

**Ermelinda Botticella, Francesco Sestili, Paola Mantovani, Alessandro Cammerata, Maria Grazia D'Egidio, Andrea Massi, Domenico Lafiandra**

Starch represents a major nutrient in the human diet providing essentially a source of energy; more recently the modification of its composition has resulted to be associated to new functionalities both at nutritional and technological level. The possibility to increase the amylose content in the wheat kernel has aroused a more intense interest as this polymer is correlated with the amount of resistant starch (RS) in food. RS is not digested in the stomach and plays a role similar to the dietary fiber inside the intestine with beneficial effects for human health.

The suppression of the gene coding SSIIa (starch synthase IIa), a major enzyme involved in amylopectin synthesis, has led to the production of high amylose genotypes in cereals and has been widely associated with several pleiotropic effects negatively affecting agronomic traits.

In the present study we have characterized major traits, such as seed weight, total starch,  $\beta$ -glucan, protein and amylose content in a set of mutant families derived from the introgression of the SSIIa *null* trait in Svevo, an Italian durum wheat elite cultivar. A large degree of variability was detected and used to select wheat lines with either improved quality or agronomic traits. The SSIIa null genotypes resulted enriched in total dietary fiber both for the increase in resistant starch and  $\beta$ -glucan content. Rheological properties of semolina produced from the selected mutant lines were investigated and compared with the control. In order to gain the best compromise between higher fiber content and an acceptable dough quality, rheological properties of semolina blends were also investigated and data will be presented.

## ANALISI QTL PER CONTENUTO DI PIGMENTO GIALLO NELLA GRANELLA IN FRUMENTO DURO

**Marco Maccaferri<sup>1</sup>, Simona Corneti<sup>1</sup>, Maria A. Canè<sup>1</sup>, Silvio Salvi<sup>1</sup>,  
Maria C. Sanguineti<sup>1</sup>, Chiara Colalongo<sup>1</sup>, Andrea Massi<sup>2</sup>, Roberto Tuberosa<sup>1</sup>**

*1 Dipartimento di Scienze Agrarie, Viale Fanin 44, Università di Bologna, 40127 Bologna*

*2 Società Produttori Sementi Bologna, Via Macero 1, 40050 Argelato (BO)*

Il contenuto di pigmento giallo (luteine) nella granella è un importante carattere qualitativo per il frumento duro. Sebbene questo carattere abbia un controllo genetico di tipo quantitativo, è caratterizzato da livelli medio-elevati di ereditabilità. Questo aspetto, insieme alla presenza di elevata variabilità genetica nel germoplasma, lo rende un carattere valido per il miglioramento genetico. In questo studio il carattere è stato oggetto di analisi genetica per la mappatura di loci per caratteri quantitativi (QTL) utilizzando tre popolazioni di linee inbred ricombinanti (RIL) e un panel di 189 varietà e linee avanzate, utilizzato per mappatura di QTL per associazione genetica (Durum Panel; Maccaferri et al. 2005 *Molecular Breeding* 15: 271-289). Le popolazioni ricombinanti sono state ottenute dagli incroci: Kofa × Svevo (KS; 249 RIL), Colosseo × Lloyd (CL; 176 RIL), Meridiano × Claudio (MC; 181 RIL). I QTL principali riscontrati nelle tre popolazioni RIL e nel panel di germoplasma elite sono stati mappati nei seguenti bracci cromosomici: 1AS (pop. CL e Durum Panel), 4AL (pop. KS), 4BL (pop. KS e CL e Durum Panel), 5BL (pop. KS e CL), 6A-centromerico (pop. KS, CL e Durum Panel), 7AL (Durum Panel), 7BS (pop. KS, CL e MC e Durum Panel) e 7BL (Durum Panel). I QTL evidenziati nei gruppi 4 e 7 erano già noti, essendo stati riscontrati in precedenti lavori di mappatura. Sono stati identificati anche molti QTL con effetti di entità minore, la maggior parte dei quali popolazione-specifici. Questi risultati sono utili al fine di ottenere un quadro generale sulla localizzazione e rilevanza dei QTL per contenuto di pigmento giallo in frumento duro, per un utilizzo più efficiente delle risorse genetiche nel miglioramento assistito da marcatori molecolari.

Si ringrazia “AGER - Agroalimentare e Ricerca” per il supporto di questa ricerca nell’ambito del progetto “From Seed to Pasta”.

# FROM SEED TO PASTA & BEYOND

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## AGRONOMICAL PERFORMANCES AND QUALITY CHARACTERISTICS OF WIDE CROSSES OF DURUM WHEAT

**Meryem Zaïm<sup>1,2</sup>, Khaoula El Hassouni<sup>1,2</sup>, Sourour Ayed<sup>3</sup>, Abdelkarim Filali-Maltouf<sup>1</sup>, Miloudi Nachit<sup>2</sup>, and Filippo M Bassi<sup>2</sup>**

*1 Mohammed V University, Rabat, Morocco*

*2 ICARDA, Cereal Breeding Platform, Rabat, Morocco*

*3 Regional Research Development Office of Agriculture in Semi Arid North West of Kef, Boulifa 7100-Kef, Tunisia*

Durum is cultivated on over 17 million hectares Worldwide, where it is faced with various biotic and abiotic constrains. The products derived from semolina and durum flour have a characteristic cooking firmness and inviting yellow color that make them a consumer's favorite. Therefore, durum wheat breeding programs need to maintain these quality characteristics, while improving the agronomical performances of the varieties. A stratagem has been the use of interspecific crosses with wild relatives. This approach has often resulted in superior cultivars in terms of resistance to biotic and abiotic stresses, but with less than ideal quality characteristics. In this study, we investigated the correctness of this statement by comparing 19 elites and 8 commercial cultivars against 20 wide-crosses made between elites and wild relatives. The wide-crosses were all top crosses to *T. dicoccum*, *T. dicocoides*, *T araraticum*, and *A. speltoides*. The elites were derived from the 37<sup>th</sup> ICARDA Durum Yield Trials, while the commercial cultivars were: Om Rabi 5, Azeghar 2, Korifla, Waha, Miki 3, Tomouh, Faraj, and Louiza. The material was grown in three environments in Morocco. Wide-cross and elites both out yielded the best commercial checks, while the wide-crosses carried consistently better disease resistances than the elites or the commercial checks. Finally, significant differences could be identified among all entries for gluten strength, yellow pigment and grain protein content. Protein characterization of all entries revealed five possible high molecular weight glutenin subunits. For Glu-B1, the alleles producing protein bands 20+9 and 7+8 were the most common. For the low molecular weight glutenin subunits, the population studied was classified into two groups, with the elites having mainly pattern LMW-1 and the wide-crosses LMW-2. In the elites, the strongest gluten was found for the combinations 20+9/LM1, 6+8/LM2 and 7+8/LM2, while in the wide-crosses the strongest gluten was by 6+8/LM2. In conclusion, it was possible to identify wide-crosses that yielded the same as elites, matched commercial checks for end-product quality, and were superior to both for disease resistance.

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## ON-FARM CONSERVATION AND VALORIZATION OF TUNISIAN LOCAL DURUM WHEAT AND BARLEY LANDRACES.

**Amine Slim, Mohamed Ali Dridi, Sripada Udupa**

Tunisia is known as a center of diversity and producer of cereals especially for Durum wheat (DW) and barley (B) that are very important crops with a multitude of uses. Tunisian farmers are mostly cultivating improved DW and B varieties while their yields are closely dependant of the weather and fluctuating due to biotic and abiotic stresses in addition to the effects of climate changes. On the other hand, small farmers in the marginal areas are still cultivating local landraces known for their adaptation to difficult conditions, their stable yields and better technological quality.

Therefore, the National Gene Bank of Tunisia organized collecting missions covering 8 governorates (40 sites). The local landraces collected were multiplied and distributed to farmers for on-farm conservation in addition to the active and base collections conserved ex-situ in the gene bank.

The on-farm conservation activity started in 2012-2013 cropping season by giving 4860 kg of 9 DW local landraces to 30 farmers (2 women farmers) area of 40.5 hectares in 10 governorates.

In 2014-2015 cropping season, about 9000 kg of 21 local DW landraces and 7 local B landraces were distributed to 56 farmers (14 women farmers) covering about 80 hectares in 14 governorates from the north to the south of Tunisia. Some of these farmers give an add-value to this local landraces by growing in organic farming and by processing end product such as Couscous, Borghoul, Mhammes, Bsissa based on the women local knowledge.



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## CHALLENGES AND PROSPECTS OF DURUM WHEAT BREEDING IN IRAN

**Sadeghzadeh B.<sup>1</sup>, Mohammadi R.<sup>1</sup>, Karimizadeh R.<sup>1</sup>, Nachit M.<sup>2</sup>**

*1 Dryland Agricultural Research Institute (DARI), P.O.Box 119, Maragheh, Iran.*

*2 International Centre for Agriculture Research in the Dry Area (ICARDA), P.O.Box 5466, Aleppo, Syria*

Although durum wheat is not cultivated as widely as bread wheat in Iran, it occupies about 300,000 ha mainly in warm and semi-warm areas of the country. In spite of the importance of durum for Iranian rural economies, the country has not all succeeded in its research and development efforts to substantially improve durum productivity. The combinations of increasing demand for durum and durum products, and relatively low durum productivity made the country to import half of its demand (about 300,000 tons per year).

The country is generally known of arid and semi-arid climate. Hence, about 4.0 million hectares (or 60 percent) of wheat cultivation area is totally reliant on rainfall. Durum is generally sown in marginal environments subject to great climatic fluctuations during the growing season. Abiotic (cold, drought, heat, salinity) and biotic (e.g. yellow rust, leaf rust, septoria, fusarium) stresses are among the major limiting factors for durum production and they are common all over the country. In addition to these stresses, lack of good agronomy is a major limiting factor to achievement of the genetic potential of improved cultivars. Under rainfed condition, average yields of durum is low (e.g. 600 kg/ha in 2011-12), where the country average was 2.5 t/ha. Over 80% of Iranian durum is produced in the four South-West provinces (Ilam, Khuzestan, Kohkiluyeh-Boyerahmad, and Fars) with warm and semi-warm climates. There is also some potential for durum production in the cold highlands (the West and North-West) depending on future varietal adaptation.

Frost damage along with drought and heat stresses is one of the most common adverse environmental factors affecting autumn sown durum productivity in cold regions. The main factors causing winterkill can be summarized as: (i) inadequate hardening, due to late emergence in autumn or a sudden drop in temperature; (ii) long periods of cold-induced desiccation; (iii) prolonged periods of low sub-zero temperatures (below -15 °C); (iv) alternate freezing and thawing. Whenever winter conditions differentiate genotypes for winter survival, evaluation of winter hardiness in the field is desirable. Field evaluation allows large-scale, inexpensive characterization of breeding materials against the full range of factors affecting winter survival, whereas controlled freeze tests measure only low temperature tolerance. For this reason, most breeding programs, regardless of available resources, favor field testing to measure winter survival, despite of its disadvantages such as great variability within a field due to non-uniform snow cover, soil preparation, planting depth, soil and plant moisture, etc. With the identification of genes that control frost resistance and the development of markers, it is likely that some of the problems related to field testing and/or controlled environment screening will be overcome. However, field testing will remain for some time to come the final measure of the winter hardiness of a wheat cultivar.

To overcome all of these constraints, small durum breeding program was established at Seed and Plant Improvement Institute (SPII). Then, the program was relocated to Dryland Agricultural Research Institute (DARI) in 1990s where it remains today as the lead agency of the national program especially for cold and rainfed highlands. DARI in collaboration with ICARDA and CIMMYT could release some durum varieties (e.g. Seimareh, Dehdasht, Saji), however, accelerating of the process of releasing of new cultivars with high yield and good quality should be considered especially for cold regions. Hence, a strong collaboration with the durum program at ICARDA needs to be reinforced based on exchange of germplasm with cold tolerance; and to have DARI more involved in the ongoing breeding in Turkey and the high plateaus in the Atlas Mountains in Algeria and Morocco.

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## DURUM WHEAT IMPROVEMENT IN INDIA-EMPHASIZING YIELD AND QUALITY TRAITS

**Bhudeva Singh Tyagi, Gyanendra Singh, SK Singh, Sewa Ram, V Tiwari, Indu Sharma**

Wheat is the staple food of above 40% human population across the globe. India recorded the highest wheat production of 96 million tons during 2014. *Triticum durum* is the second most important wheat species under cultivation in the world and India, as well. The durum wheat production in India is estimated around 2.5 million tons which is consumed domestically. The due emphasis on durum research has not been given in India and the productivity levels are lower in durums as compared to bread wheat as a result the area under durum cultivation is declining. Due to changes in food habits now the durum transformation industries are developing in the country. But modern fast food based on durum requires higher quality like higher test weight, beta-carotene, protein and milling recovery. Many varieties have recently been developed with bolder and lustrous grains and with high protein content which can produce very good pasta products. The semolina recovery is also high with maximum beta –carotene. The yellow berry incidence is very low, free from Karnal bunt and suitable for export.

Thus in view of the above the present study was planned to find out the variability among the available genotypes for yield contributing as well that of quality traits. For incorporation of desired traits into adapted genetic background, the crosses were attempted to study the heritability, character association and the combining abilities in hybrids. The data pertaining to plant height, flag leaf area, days to 50% flowering, number of effective tillers per plant, days to maturity, spike length, number of grains per spike, 1000-grain weight, biological yield per plant, harvest index and grain yield per plant were recorded. HI 8498, PBW 34, HI 8638 and PDW 291 were found to be good combiners and hence these have been utilized in the wheat breeding for the improvement of yield components. While for quality traits the genotypes PDW 233, DDW 12, PDW 314 were used and found desirable crosses under high yielding conditions.

The cross combination PDW 291 x DDW 12 showed significant SCA effects for harvest index; the cross NIDW 291 x HI 8498 showed significant SCA effects for 1000-grain weight and biological yield per plant; the cross HI 8498 x PDW 233 showed significant SCA effects for beta carotene content. The above crosses are expected to give a number of useful promising progenies, which are also being evaluated under artificial black rust epidemics. The efforts made on the above in the form of varietal development, release and notification is given as under:

Out of 90 durum genotypes evaluated here the durum wheat genotypes MPO 1215, MPO 1255, MPO 1262, HD 4672, HI 8498, HI 8627 and HI 8663 also showed resistance to the stem rust race Ug99 and its variants during screening in Kenya.

This multipronged strategy of durum improvement in India has resulted into the development of high yielding genotypes with resistance and high quality parameters.

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## **DURUM WHEAT INITIATIVES IN PAKISTAN: NEED FOR CONCERTED EFFORTS BETWEEN ALL CONTRIBUTORS TO THE VALUE CHAIN**

**Joshi, K.D., Abrar, M., Ahmad, J., Ahmad, G., Afridi, K., Hussain, M., Ullah, G., Saleem, T., Ali, A., Taran, S.A., Ammar, K. & Imtiaz, M.**

Despite the inception of durum wheat breeding since 1975 and release of two varieties nearly three decades ago, Pakistani farmers do not grow this crop today due to lack of awareness of its potential, demand, and marketing for durum wheat in the country. In the last 10 years food habits changed substantially with some demand for durum derived products, which calls for focused durum wheat R4D in Pakistan. Analysis of available data from various durum wheat nurseries and trials conducted in Pakistan for last 3-4 years identified promising genotypes with grain yield potential of more than 5 t ha<sup>-1</sup> and yield stability allowing them to fit into existing cropping patterns.

Nearly 300 durum wheat lines analyzed for nutrition, processing and end-use quality revealed great genetic diversity for protein, yellowness and sedimentation values. The average protein content was nearly 15% while yellowness and sedimentation values were in the acceptable range in general. Durum wheat lines grown in Nowshersa and Quetta showed good combination of all the three quality traits. Lines grown in Bhawalpur and Sakrand also had good combination of protein and sedimentation values; however, they were low in yellowness values. Lines grown in Faisalabad were low in protein and yellowness. Interestingly, all the durum wheat lines were high in sedimentation value. Ten lines with a right combination of high yields, protein, yellowness and sedimentation values are being evaluated in durum wheat National Uniformity Yield Trial across nine locations during 2014-15 cropping season. International Maize and Wheat Improvement Centre (CIMMYT) is facilitating durum wheat initiatives in Pakistan by partnering with all major national partners from the identification and release of most promising durum wheat genotypes, accelerating seed production, fast tracking their deployment through to facilitating and supporting business communities for durum wheat milling, processing and market chain development.

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## DURUM WHEAT VALUE CHAIN IN ETHIOPIA

**T. Chiari<sup>1</sup>, Genene G.<sup>1</sup>, Aliye H.<sup>2</sup>, Tafa J.<sup>2</sup>, Tesfaye L.<sup>3</sup>, and Fekadu A.<sup>4</sup>**

*1 Istituto Agronomico per l'Oltremare (IAO), Via A. Cocchi, 4 - 50131 Florence, Italy*

*2 Oromia Agricultural Research Institute (OARI), Addis Ababa, Ethiopia*

*3 Sinana Agricultural research Center (SARC), Sinana, Bale, Oromia Region, Ethiopia*

*4 Oromia Bureau of Finance and Economic Development (BoFED), Addis Ababa, Ethiopia*

Durum wheat has been cultivated in Ethiopia for thousands of years, whilst now bread wheat has quite completely substituted it. In the meantime, local demand for long and short pasta is astonishingly growing as response to fast urbanization, change of life styles and consumption habits. Data on pasta importation are witnessing the above fact.

The Ethio-Italian Development Cooperation Project “Agricultural Value Chains in Oromia” addressed five potential districts of Bale zone, and it demonstrated that the increasing demand for huge batches of durum wheat by national pasta making factories could be fulfilled by local production via capacitating farmers’ cooperatives.

Activities started in the 2011/12 cropping season, after industries agreed with cooperatives to purchase their produce accordingly to a supply contract which establishes an incentive price (*premium*) for every increment of protein content over a 10.5 % threshold. SARC, as a no-partisan body, determined the quality of marketed batches. First year qualitative and economic results were greatly appreciated by contracting parties, hence durum wheat dramatically expanded in Bale and neighboring zones. In 2014/15, production reached about 15,000 tons (a 30-time fold increase after three years).

Nationally registered varieties that were selected over years and multiplied by SARC, have been the foundation of the value chain. Besides Bakalcha and Ejersa varieties, some others are currently included in the program. SARC is jointly following-up the establishment of a quality-declared farmer-based seed system.

Specialized Ethiopian institutions and stakeholders, as per their contributing role in agriculture and rural development, research, trade and market, cooperative promotion and industrial development, are accompanying the value chain development. BoFED has the overall project responsibility, with the technical assistance of IAO, which is furthermore involving potential Italian research institutions and enterprises.

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## RICE SEED DELIVERY SYSTEM IN BANGLADESH: A SUCCESS STORY

**M. Khalequzzaman<sup>1</sup>, M.A. Siddique<sup>2</sup>, ESMH Rashid<sup>2</sup>**

Seed is the most important input for crop production and no harvest will be achieved without seed. Quality seed is mandatory for getting a good harvest and 8 to 10 per cent yield increases by using quality seed alone resulted from farmer's participatory experiments in Bangladesh and Philippines. Timely access of seed to poor farmers with proper genetic, physiological and phytosanitary quality plays an important role for move forward to a sound seed sector which ultimately ensures the food security and rural economic development of country like Bangladesh.

Rice seed delivery system and the process developed by the Bangladesh Rice Research Institute with the collaboration of GO-NGO-Private Sector (PS) seed producing organizations are discussed and the emergence of sustainable rice seed network is reviewed in this article. For the availability of quality seed to the resource poor farmer's, GO, NGO and PSs must work together to make a level playing field for all seed entrepreneurs. For this reason, the 'Sustainable Rice Seed Network' had to be established, addressing all large, medium and small-scale seed entrepreneurs for making the seed business competitive in the country as well as to ensure the quality of seed at farmer's level.

In this network, the number of the partner organizations involved 3 in 1998 which increased to 412 in 2008, 750 in 2012 and 900 in 2014. Similarly, the supply of formal seed is increased from 5% to about 42% which is an ultimate effect of network functioning. This rice seed network serves the farmers with five key roles; adequate supply of quality seeds of modern varieties at affordable prices in the right time. There is a very congenial atmosphere already created in the country in working jointly by the GO-NGO-PS through rice seed network which would help in more production, eventually would attain food security.

Acceleration in the management of the seed system through rice seed net with the involvement of truly pluralistic approach will help to enhance the supply of quality seed at a faster rate and thereby the use of quality seed will increase. Increased use of quality seed will help to increase productivity and income, which will help reducing poverty in Bangladesh.

## WHEAT GENETIC RESOURCES AND EXPLOTATION IN BREEDING IN TURKEY

**Alptekin Karagöz, İrfan Özberk**

Having hosted 11.707 plant taxon, Turkey is in an extraordinary position for plant genetic resources. Around 31,82% of the plants (3.649) are endemic. Turkey is center of origin and / or diversity of several cultivated plant species including wheat.

Wheat originates from the area called “Fertile Crescent”. Wild relatives of wheat are rather widespread in Turkey especially in South East Anatolia. It is agreed that diploid wheat has been first domesticated and cultivated than dispersed to the other parts of the world from Karacadağ which is located in South East of Turkey. *Triticum* L. and *Aegilops* L. genera have species in three ploidy level namely diploid ( $2n = 14$ ), tetraploid ( $2n = 28$ ) and hexaploid ( $2n = 42$ ). Total number of wild wheat species are 27 in the world, 20 of them exist in Turkey.

Three genomes play a significant role in wheat evolution. Origin of A genome is *Triticum urartu* and D genome is *Ae. tauschii*. B genome is known to be related with *Ae. speltoides*. All of the tetraploid and hexaploid cultivated wheat species are of wild emmer (*T. turgidum* L. subsp. *dicoccoides*) origin which is a result of hybridization between *Ae. speltoides* and *T. urartu* species. Wild emmer develops gradually into cultivated emmer (*T. turgidum* L. subsp. *dicoccon.*) first and than into durum wheat (*T. turgidum*) in time. Bread wheat (*T. aestivum*) is the result of hybridization between *T. dicoccoides* and *Aegilops tauschii*. At the initial stage of variety development in Turkey, pure lines were selected from land race populations. Then those pure line varieties were crossed by semi dwarf foreign origin material and high yielding varieties were developed. The desirable characteristics of land races have been identified through molecular markers recently. Genetic selection methods utilized in variety development made the procedure even shorten. Utilization from the genetic resources in resistance breeding has yet to spend great efforts in Turkey.

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