



International Conference

FROM SEED TO PASTA & BEYOND

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

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Invited Speakers & Oral Presentations
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DURUM WHEAT BREEDING: AN HISTORICAL PERSPECTIVE

Antonio Blanco and Enrico Porceddu

Durum wheat is widely cultivated in the Mediterranean Basin and north Africa, and in various regions of the world including Canada, USA, Mexico, Russia and India. With about 17 million hectares under cultivation, the world annual grain yield exceeds 35 million tons with wide variation caused mainly by drought and heat stresses. Although the intense breeding activity conducted over the last century had led to the constant release of leading cultivars, durum grain yield per hectare is lower than bread wheat. The ability of durum breeders to use existing genetic variation and to identify agronomically important genes will open new approaches for improving quantity and quality of durum wheat. The recent advances and integration of molecular markers, plant genomics and biotechnology with classical breeding methodology has provided the basis for molecular durum breeding. Cereal genomics and technologies advances for genome-wide analysis are contributing to the acceleration of gene discovery and implementation in practical durum breeding programs. The objective of this article is to briefly review historical development of durum breeding and factors influencing the applications of molecular plant breeding in durum improvement programs. Information is reviewed on exploiting wild and cultivated germplasm resources, induced novel genetic variation by TILLING, molecular dissection of quantitative traits and QTL studies to identify causal genes, genome sequencing and gene cloning efforts. Co-ordinated efforts between researchers engaged in crop biology and wheat improvement will be required to design an efficient wheat ideotype for a sustainable and productive agriculture.

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INDUSTRIAL PERSPECTIVES OF PASTA PRODUCTION WHEAT BREEDING: AN HISTORICAL PERSPECTIVE

Marco Silvestri

Agronomy Research - Barilla G.e R. Fratelli Società per Azioni

Barilla is an Italian food production group, leader in the markets for pasta worldwide, for ready-made sauces in Europe, for bakery products in Italy and for crisp breads in Scandinavia. The Group employs over 8,000 people and owns 30 production sites and 13 brands: Barilla, Mulino Bianco, Voiello, Pavesi, Gran Cereale, Pan di Stelle, Wasa, Harry's (France and Russia), Academia Barilla, Misko (Greece), Filiz (Turkey), Yemina and Vesta (Mexico).

Every year Barilla processes about 1.4 million of ton of durum wheat equivalent for its pasta production. Since pasta is a very simple product, made only of water and semolina, the quality of the raw material is crucial to get consistently the expected quality of the product. Pasta quality is a complex concept that evolve over years, adding new aspects, with consumer expectation and needs. On an industrial perspective is essential to understand in advance trends and expectation on pasta and translate them in raw material parameters correlated with the pasta performances and therefore Barilla, as market leader, is since the very beginning focused on durum wheat characteristics and quality aspects. Starting from the late 80s, to the simple commercial parameters as test weight, moisture, kernel defects ecc., new parameters, as protein content, gluten quality and color, linked with pasta firmness and cooking value became important. This gave a step change also in durum wheat breeding and variety development adding new target to the genotypes selection focused mainly on yield. With the new century, European Union starts working hardly on food safety regulation adding a new complex targets to the "quality concept". New contaminants, in particular heavy metals and mycotoxins, are continuously under discussion and agronomy and breeding are essential to develop tools and tolerant varieties improve durum wheat and pasta food safety.

New quality parameters that, more recently, are becoming more and more relevant are linked with health/nutrition and sustainable agriculture. Celiac disease as well as popular trends against carbohydrates and gluten in diet are becoming more and more relevant and need to be investigate to give sound scientific answers.

On the other hand the need to feed a growing planet ask for a more efficient production of cereals as wheat that are at the basis of the human diet. Developing new cropping systems that take advantage of new IT technology for a more precision farming is important to increase the production but reducing the input. Breeding for high yielding and high quality varieties is also essential to face this new challenge and to increase the crop resilience in a climate changing perspective.

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WHAT KIND OF PASTA FOR A HEALTHY GUT MICROBIOME?

Patrizia Brigidi, Silvia Turrone, Marco Candela

Dipartimento di Farmacia e Biotecnologie, Via Belmeloro 6, Bologna, Italy

The community of microorganisms living in our gut, known as intestinal microbiota, has been increasingly recognized as a strategic partner to maintain our energetic and immunological homeostasis. Capable of adjusting its compositional and functional layout in response to dietary changes, the gut microbial ecosystem provides us with the necessary metabolic flexibility to deal with dietary changes, allowing the optimization of the host metabolic performances in response to the different dietary substrates such as type of carbohydrates as well as to the intake of proteins and fat. In particular, recent findings have highlighted the impact of different types of grains in our diet on the compositional and functional structure of the human gut microbiome. Interestingly, whole-grain pasta have recently been reported to induce statistically significant variation in the gut microbiota composition, favoring the increase in several health-promoting butyrate producers, such as *Roseburia*, *Blautia* and *Enterococcus rectale*. This suggests the potential of whole-grain pasta to modulate the gut microbiota composition toward a healthy mutualistic profile, which, by providing the host with key microbial metabolites, can preserve a well-balanced nutritional state.

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WHEAT GENOMICS & ITS APPLICATIONS

Peter Langridge

Broadly speaking genomics tools offer knowledge and information about single genes, pathways or gene networks, and genome structure and behaviour. This knowledge and information can be deployed in several ways. Where individual genes controlling the trait of interest are known, the gene knowledge can be used to identify, discover and tag individual alleles and to develop and deploy molecular markers to track the desired alleles in breeding programs. Armed with gene knowledge, novel alleles can be sought in diverse germplasm pools, including wild relatives, expression variation can be studied and new alleles, both structural and expression, can be created either through genetic engineering or through the new genome editing techniques.

At the genome structure level, genomics and whole genome analysis can help breeders design optimal recombination strategies and deploy some new breeding techniques, such as genomic selection.

The areas where genomics tools are being applied are where the genetic control of the target trait is clearly defined and, consequently simple, or where information on individual genes is not required and genome structural and predictive models can be used. The area where genomics has struggled to have an impact is also the area where genomics was thought to offer the greatest potential, namely in describing and defining complex traits where genotype x environment interactions exert a major effect, such as drought tolerance. The limitation may not lie with the genomics technologies but rather with our understanding of the genetic basis of target traits and the interactions with an environment that is in constant flux.

A broad approach to using genomics techniques to tackle abiotic stress tolerance in wheat will be presented with some specific examples of how these results can influence crop improvement.

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APPLICATION OF PHENOMICS IN PRE-BREEDING: TRAITS, QTL, AND STRATEGIC CROSSING

M. Reynolds, G. Molero, M. Tattaris, CM Cossani, S. Sukumaran

International Maize and Wheat Improvement Centre (CIMMYT)

The application of phenomics in breeding has resulted in a new generation of wheat germplasm based on strategic crossing of complementary physiological traits. New lines achieve highly significant genetic gains under a range of environments in international trials. This ongoing effort has involved broadening the genetic base of conventional wheat gene pools through use of landraces as well as products of inter-specific hybridization between AB and D genome donors to produce synthetic wheat.

Successful application of phenomics in breeding requires reliable screening tools and platforms that can measure expression of physiological traits in realistic field environments in a repeatable way. For example, genetic gains associated with selection for canopy temperature and spectral water indices have shown that such remotely sensed traits can serve as proxies that reliably estimate water relations characteristics impacting on yield. The first aerial remote sensing platforms for large scale genetic resource screening was developed at CIMMYT in Mexico and more than half of the accessions of the World Wheat Collection have already been screened.

These high throughput field phenotyping tools have application in gene discovery, with QTL identified on 4 different chromosomes of the Seri/Babax RILs population showing a common genetic basis for adaptation to heat and drought stress. Further work showed these QTL to be associated with the response of root growth to the presence of soil moisture. Very recently, a durum x dicoccum population has been used to identify QTL associated with spike photosynthesis a trait which contributes significantly to yield under potential as well as stress conditions.

To define the best constellation of traits for application in breeding -and determine priorities for genetic understanding- it is necessary to develop conceptual models of adaptive traits that highlight wheat's genetic limitations, while pre-breeding serves as a practical tool to test alternate models.

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GENOMICS PLATFORMS FOR DURUM WHEAT

**J. Dubcovsky^{1,2}, Ksenia Krasileva^{1,3}, H. Vasquez-Gross¹, F. Paraiso¹, T. Howell¹,
X. Wang¹, P.C. Bailey³, S. Ayling³, and C. Uauy⁴**

1 Department of Plant Science, University of California, Davis CA 95616

2 Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA

3 The Genome Analysis Centre, Norwich Research Park, Norwich NR4 7UH, UK

4 John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK

We have developed an exome capture platform for wheat including 82,511 genes selected from previously published transcriptome analysis in the tetraploid variety Kronos. The capture includes 286,800 exons (76% padded with 30 bp of genomic sequence). The Initial design was optimized based on 43 captures and a β design of 84 Mb was designed. On average, we capture and map 112 Mb with roughly 70% of the mapped reads on target. We are using this exon capture platform to sequence 1500 tetraploid Kronos EMS mutants. From the analysis of the first 1000 mutant lines we detected on average ~2400 mutations per line at 95% confidence. Based on this data we expect to have a total ~3.8 million mutations at 95% confidence in the coding regions of durum wheat. A database searchable by BLAST has been created and will be publicly available after publication. Currently this database can be accessed on an individual basis with a simple request. Sequencing has been completed and we expect to complete the bioinformatics analysis by the summer of 2015. This resource will facilitate the functional characterization of most wheat genes. As examples, I will describe our mutants for the starch branching enzyme genes *SBEIIa* and *SBEIIb*, which have resulted in a 700-900% increase in resistant starch in tetraploid wheat.

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THE *PH1* GENE OF WHEAT AND ITS APPLICATION IN DURUM IMPROVEMENT

Kulvinder S. Gill, Ramanjot Bhullar, and Kanwardeep Singh

Washington State University, Department of Crop and Soil Sciences, P.O. Box 646420, Pullman, WA 99164, USA

Most of the higher plants, including some of the most important crop plants, are polyploids or ancient polyploids. Unlike in diploids, polyploids like wheat have more than two identical (homologs) or similar (homoeologs) chromosomes available for meiotic pairing thus complicating the homology search mechanism. Despite of this complexity wheat behaves as a diploid during meiosis due to the presence of chromosome pairing control gene, *Ph1* (Pairing homoeologous 1) that distinguishes the homologous from non-homologous/homoeologous chromosomes. Absence of the gene results in multivalent formation leading to sterility. Additionally, the gene also prevents wheat from breeding with related ancestors that contain a vast array of useful traits. Although the gene was identified in 1958 but was so far not been cloned. We have now identified a gene (*C-Ph1*) silencing of which results in a phenotype similar to that of the *Ph1* gene mutations including homoeologous chromosome pairing, multivalent formation, and disrupted chromosome alignment on the metaphase I plate. Two in durum and three in the bread wheat, the homoeologous copies of the *C-Ph1* gene underwent polyploid specific changes both at the structural as well as expression level, resulting in the evolution of a novel function for the *5B* copy to regulate homoeologous chromosome pairing. The *5B* copy of the gene has a novel 60bp insertion resulting in two alternate splice variants that are very different from that of the *5D* or the *5A* copies. The *5A* copy has a large 366bp deletion relative to *5D* copy possibly rendering the corresponding protein less effective. The *5B* copy of the gene evolved a very unique metaphase I (MI) specific expression: 39-fold increase between late prophase I and (MI) followed by a drop of 34-fold during the Anaphase I, thus coinciding with the expected stage for the *Ph1* gene function. Thus, the *5B*'s novel function in regulating homoeologous pairing is due to polyploid-specific insertions, alternate splicing, and/or highly specific expression during MI stage. Presence of the *C-Ph1* gene orthologs in diploid species including rice, maize, barley, and *Brachypodium* suggest that the gene is functional in diploids as well although its actual function in these species is not known. Stable RNAi silencing of the gene in *Arabidopsis* showed multivalents and centromere clustering suggesting that the gene may be performing a function in diploids similar to that observed in wheat. Cloning of the *Ph1* gene from durum wheat and the previous studies have conclusively shown that the gene is equally functional in tetraploid wheat in prohibiting homoeologous chromosome pairing and thus alien gene transfers. Currently we are in the process of optimizing a novel and targeted alien introgression strategy for tetraploid and hexaploid wheat by transiently silencing the gene to induce homoeologous chromosome pairing and alien gene transfers. It will enable us to accomplish a precise and targeted alien gene transfer in a non-GMO manner without the unwanted alien chromatin.

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IMPROVING THE HEALTH VALUE OF DURUM WHEAT

Lafiandra D., Sestili F., Botticella E., Palombieri S., Mantovani P., Massi A.

The growing diffusion of diet related diseases represents a major health issue both in developing and developed countries. A large number of epidemiological studies have associate the consumption of whole grain cereals against the onset of chronic diseases, thanks to presence of dietary fiber and bioactive compounds. Durum and bread wheat, due to their widespread use in foods, can represent an important vehicle to deliver health benefits to the human beings. This is resulting in several breeding efforts aimed to obtain varieties with increased content of dietary fiber and phytochemicals compounds and develop novel food products. Additional health benefits have also been associated with starch and in particular with the fraction termed resistant starch, defined as the starch and products of starch digestion that are not absorbed in the small intestine of healthy individuals. Resistant starch escapes digestion in the small intestine and reaches the large bowel where is fermented, in a similar way to dietary fibre, with positive effects in the protection against several chronic diseases, such as colon cancer, type 2 diabetes, obesity and cardiovascular disorders. As a close correlation has been demonstrated between the amount of resistant starch and amylose content, different approaches have been used in order to develop high-amylose wheat varieties. In particular, amylose content can be increased through the silencing of the genes encoding starch branching enzymes of class II (SBEIIa) and starch synthases II (SSII or SGP-1). Use of natural mutants and reverse genetics approaches, such as TILLING (Targeting Induced Local Lesions IN Genomes), represent powerful tools to manipulate the expression of the above genes, their use will be described in this presentation.

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DURUM WHEAT AS A BRIDGE BETWEEN WILD EMMER WHEAT GENETIC RESOURCES AND BREAD WHEAT

**Tzion Fahima¹, Huang Lin¹, Elitsur Yaniv^{1,2}, Dina Raats¹, Hanan Sela¹,
Tamar Kis-papo¹, Alan H. Schulman², Jorge Dubcovsky³, Abraham Korol¹**

1 Institute of Evolution, University of Haifa, Mt. Carmel, Haifa 31905, Israel.

2 LUKE Natural Resources Institute and University of Helsinki, Finland.

3 Department of Plant Sciences, University of California, Davis, CA 95616, USA.

Wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides* is the tetraploid ancestor of domesticated durum and bread wheats. It is fully compatible with durum wheat and can be crossed with bread wheat. Natural wild emmer populations, representing a wide range of habitats in Israel and vicinity, harbor a rich allelic repertoire of agronomic traits. Our studies aim to unravel the molecular genetic basis of agronomic traits derived from wild emmer wheat. Segregating mapping populations, developed by crossing of selected *T. dicoccoides* genotypes with *T. durum* cultivars, revealed numerous loci associated with disease resistance, drought tolerance, high grain protein content, and yield. Some of them have shown a consistent effect when introgressed into durum wheat and were also validated in the background of bread wheat. Wild emmer is a promising source of resistance to stripe rust. For example, *Yr15* and *YrH52* are dominant genes that confer particularly high resistance, while *Yr36* confers slow rusting quantitative resistance. Using comparative genomics approaches, we have developed high resolution maps for *Yr15* and *YrH52*, and we have cloned *Yr36*. *Yr36* has a unique architecture with a kinase and a START lipid-binding domains, designated *WKS* hereafter. The distribution and sequence conservation of *WKS* R-genes were compared with those of NBS-LRR R-genes (e.g. *Lr10* and *Pm3*) among wild emmer natural populations. The sequence diversity of *WKS1* was much lower than that of *Lr10* and *Pm3*, indicating that these R-genes, representing different resistance mechanisms, are shaped by different evolutionary processes. Further work is underway to clone *Yr15* and *YrH52* located on chromosome arm 1BS. The complete 1BS physical map, constructed by our group, is being utilized for positional cloning of *Yr15* and *YrH52*. These studies demonstrate the potential of wild emmer wheat gene pool for improvement of durum and bread wheats by exploitation of genes that were lost during domestication.

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MAPPING AND CLONING VALUABLE QTLS IN DURUM WHEAT

Marco Maccaferri, Silvio Salvi, Marta Graziani, Riccardo Bovina, Giuseppe Sciarra, Roberto Tuberosa

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

Progress in genomics and sequencing provides increasing opportunities to identify and clone loci relevant for genetic improvement even in species with complex genomes like wheat. As compared to hexaploid wheat, tetraploid wheat has a lower level of ploidy while encompassing a broad range of genetic diversity with its non-adapted germplasm (*T. dicoccum*, *T. dicoccoides* and related tetraploids), making it more desirable for cloning agronomically important genes. The molecular basis of genes involved in wheat adaptation including *Ppd-A1* and the *VRN* gene series has been elucidated with a main contribution from tetraploid (for the A and B genomes), particularly in the exploration of the allelic diversity variants. The loci controlling grain protein content (*GPC1*) and resistance to stripe rust *Yr36* were directly identified and cloned in *T. dicoccoides*. As to the sodium exclusion loci *Nax1* and *Nax2*, the causal genes from *T. monococcum* (A^m genome), were transferred to and evaluated in tetraploid wheat. In the case of *Bo1* conferring boron tolerance) and first cloned in hexaploid wheat, an important allelic variant was traced in a durum landrace. Additional loci currently being targeted for positional cloning include cadmium uptake, solid-stem, resistance to wheat stem sawfly and orange wheat blossom midge, spike fertility and grain weight loci. In all cases, conserved synteny across the *Triticeae* and the sequenced genome of grass species, including *T. monococcum* and barley are being exploited. At DiPSA, both linkage mapping and association mapping are being deployed to map and clone major QTLs for resistance to wheat diseases and grain yield. Fine mapping is underway in durum wheat for (i) a major QTL on chr. 3B that influences yield independently from phenology and (ii) a major QTL for resistance to *Soil-Borne Wheat Mosaic Virus* (SBCMV). QTL cloning will increasingly shed light on the molecular basis of variation in grain yield potential and stability while contributing to identify the main physiological constraints to an increase of yield potential.

While QTL analysis and cloning addressing natural variation will shed light on the functional basis of yield potential and the mechanisms of adaptation to abiotic and biotic constraints, increasing emphasis on approaches integrating resequencing, candidate gene identification, omics platforms and reverse genetics (e.g. TILLING, VIGS, etc.) will accelerate the pace of QTL cloning.

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CHROMOSOME ENGINEERING: ITS CURRENT POTENTIAL TO CAPITALIZE ON ALIEN TRAITS FOR SUSTAINABLE IMPROVEMENT OF DURUM WHEAT PRODUCTION

Carla Ceoloni, Ljiljana Kuzmanovic, Maria Elena Virili, Paola Forte, Andrea Gennaro, Alessandra Bitti

In the severely challenged agricultural perspective of this millennium, to cope with the full suite of changes and sustainably meet the requirements for food security and safety of major staple crops such as wheat, a promising avenue is offered by targeted exploitation of alien germplasm, including relatively distant gene pools from those of crop species, through chromosome engineering (CE). In fact, recent progress in molecular genetics, cytogenetics and genomics is offering unprecedented opportunities for CE to significantly impact on wheat improvement strategies. The new genotyping and phenotyping technologies are also facilitating inclusion of complex traits in the array of alien features accessible to CE, as well as gene pyramiding from various alien sources. Such 'smart' CE procedures have been profitably applied also to durum wheat, more 'sensitive' than bread wheat toward genome manipulations. One demonstrative example concerns the use of *Thinopyrum* species as donors of many useful traits. From a group 7 *Th. ponticum* chromosome arm, called 7e₁L or 7AgL, differently sized segments containing the *Lr19+Yp+Sr25* genes were firstly transferred onto 7AL of durum wheat recombinant lines. Comparative analyses of some of them, both in field trials and under controlled conditions, not only revealed positive effects on several yield-contributing traits, affecting aerial plant parts and also the root system, to be 7e₁L-linked, but also allowed assignment of underlying genetic determinants to defined 7e₁L distal sub-regions. The gene content of the same 7e₁L segments is then being enriched with genes/QTL, originating from the same *Th. ponticum* and from the closely related *Th. elongatum*, conferring resistance to Fusarium head blight. These and additional successful outcomes will be illustrated, all substantiating the validity of CE in providing a novel, breeding-responsive makeup of the durum wheat genome.

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MARKER-ASSISTED BREEDING IN DURUM WHEAT: PROGRESS AND PERSPECTIVES

Curtis J. Pozniak¹, Krysta Wiebe¹, Jemanesh Haile¹, John Clarke¹, Andrew Sharpe², Yufeng Ruan³, Fran Clarke³, Ron Knox³, and Karim Ammar⁴

1 Crop Development Centre, Department of Plant Sciences, University of Saskatchewan, CANADA

2 National Research Council of Canada, Saskatoon, CANADA

3 Agriculture and Agri-Food Canada, Semiarid Prairie Agriculture Research Centre, CANADA

4 CIMMYT, MEXICO

Marker assisted selection (MAS) has been used effectively to select for desirable alleles important to agronomic performance, disease resistance and end-use quality of durum wheat. Indeed several cultivars that express low cadmium content in grain, low lipoxygenase activity, and improved insect and rust resistance are now in commercial production, and these were developed in part using MAS. In most durum wheat breeding programs, “stacking” of multiple disease resistance genes is a focus of MAS, with the aim to develop cultivars with resistance less prone to break down. Recent advances in genomic technologies including next-generation sequencing technologies, exome capture assays, and the availability of high density single nucleotide polymorphism (SNP) arrays, have resulted in an explosion of DNA markers available for genetic analysis of durum wheat. We have used these technologies to improve resolution of quantitative trait loci (QTL) in well phenotyped bi-parental and association mapping panels, to aid cloning of genes for cadmium uptake and insect resistance, and to develop robust, high through-put markers for implementation in our breeding programs. Although genomic selection using whole genome profiling shows promise as a tool to improve complex traits in durum wheat, there is still a lack of empirical evidence to support its large-scale adoption. Here we report on our efforts to develop robust, high throughput DNA markers for MAS of disease resistance and end-use quality traits in durum wheat and report on our work to develop and validate genomic selection models using historical phenotypic data collected from our breeding programs. We anticipate that MAB will continue to be a useful strategy to improve the efficiency of genetic improvement of durum wheat, leading to improved cultivars with higher yield, enhanced abiotic and biotic stress tolerance and superior nutritional quality.

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IMPROVING FUSARIUM HEAD BLIGHT RESISTANCE IN DURUM WHEAT – CHALLENGES AND CHANCES

Hermann Buerstmayr, Noemie Prat, Maria Buerstmayr, Barbara Steiner

BOKU - University of Natural Resources and Life Sciences, Vienna. Department for Agrobiotechnology Tulln. Konrad Lorenz Str. 20, 3430 Tulln, Austria

e-mail: hermann.buerstmayr@boku.ac.at

Fusarium head blight (FHB) is a serious threat worldwide due to its dramatic consequences and effects on small grain cereal production such as yield and quality losses and most importantly mycotoxin contamination. Durum wheat (*Triticum durum* Desf.) is particularly susceptible to FHB. Enhancing resistance has proven difficult due to the narrow genetic variation for this trait in the durum wheat gene pool. Broadening the genetic basis by incorporating resistance alleles from wild and cultivated relatives is a promising approach for durum resistance breeding. This review summarizes the current information on sources available for FHB resistance improvement in durum wheat which include wild and cultivated tetraploid wheat, hexaploid wheat and alien species. The genetic basis of FHB resistance of a few tetraploid sources in the *T. durum* background has been dissected by QTL mapping. So far, thirteen QTL with small to moderate effects have repeatedly been detected on 11 chromosomes with alleles improving FHB resistance deriving from relatives and durum wheat itself. Notably, the QTL found in tetraploid wheat populations largely overlap with the QTL identified in hexaploid wheat suggesting a common genetic basis of FHB resistance. Own ongoing research on FHB resistance improvement will be presented. Recent results indicate that allele introgression from cultivated and wild relatives into *T. durum* wheat appears promising, and QTL pyramiding seems a practicable strategy for FHB resistance breeding.

Further reading:

Prat N, Buerstmayr M, Steiner B, Robert O, Buerstmayr H (2014) Current knowledge on resistance to Fusarium head blight in tetraploid wheat. *Mol Breed* 34:1689-1699

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IMPROVING CANADIAN DURUM WHEAT QUALITY: OBJECTIVES FOR GENETIC ENHANCEMENT AND SCIENCE-BASED GRADE STANDARDS

B.X. Fu¹ and C.J. Pozniak²

1 Grain Research Laboratory, Canadian Grain Commission, 1404 – 303 Main Street, Winnipeg, MB, Canada;

2 Crop Development Centre, University of Saskatchewan, Saskatoon, SK, Canada

Canada Western Amber Durum (CWAD) wheat is well recognized for its safety, consistency, and superior processing performance. Genetic improvement and a science-based grading system are the cornerstones for the success of CWAD as the supplier of choice for premium quality in the global markets. The current quality objectives for releasing new varieties in the CWAD class focus on protein content, milling performance, pigment concentration and pigment loss, gluten strength, and cadmium level. Quality objectives are intended as guidelines for wheat breeders when screening early generations and determining the appropriate advanced lines to submit for testing over the trial period. Candidate lines are tested and compared against approved check varieties for the trial and should aim to meet the established objectives. Recent modifications to the objectives include streamlining the gluten strength requirement and its evaluation and removing the cap on yellow pigment content. The quality data of the major varieties released in the last two decades were drawn from Durum Cooperative Trials to show the improvement in quality traits. Their impact on the market is clearly demonstrated by the end-use quality of export cargoes monitored by the Canadian Grain Commission (CGC). Changing market demands have been satisfied by new durum wheat varieties with the required end use quality characteristics. The Canadian wheat quality assurance system allows customers to select a class and grade of Canadian wheat with confidence that it will meet their requirements and perform as expected. Based on defined quality models for each wheat class, the CGC designates the class of wheat for which new varieties are eligible. In Canada, wheat is graded for physical condition according to grade standards established by the CGC. These standards are based on results of scientific research on the effects of grading factors on end-use quality. Recent research on the effects of HVK, smudge, and sprout damage on durum wheat quality will be used as examples to illustrate the scientific basis for setting tolerances of grading factors in CWAD.

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PHENOTYPING FOR SINK-SOURCE RELATIONSHIPS IN WHEAT

Araus J.L., Sanchez-Bragado R., Elazab A., Vergara O., Kefauver S.C., Serret M.D. and Bort J.

Durum wheat is among the most cultivated herbaceous crops in the Mediterranean basin where drought, together with other abiotic and biotic stresses, limits grain yield. Whereas crop breeding may improve the performance of durum wheat under stress conditions, the development of effective field-based high-throughput phenotyping platforms remains a bottleneck for future breeding advances. Progress in sensors, aeronautics, and high-performance computing are paving the way. The most successful traits for field phenotyping integrate in time (throughout the crop cycle) and space (at the canopy level) the performance of the crop in terms of capturing resources (e.g. radiation, water, nutrients) and how efficiently these resources are used. Different methodological approaches have been proposed to evaluate these traits in the field. They can be summarized into two categories: (i) proximal (remote) sensing and imaging, (ii) laboratory analyses, including near-infrared reflectance spectroscopy (NIRS) analyses, in the harvestable parts of the crop.

Vegetation indices and canopy temperature are the most usual remote-sensing traits to assess wheat performance under Mediterranean conditions. However wide differences still exist in the costs of the different equipment deployed. Moreover the use of conventional RGB imagery is raising interest not just due to its low implicit cost, in terms of sensors and software needed, but also because the wide spectrum of opportunities for field phenotyping it may fulfil. On the other hand laboratory analyses of key plant parts may complement direct phenotyping under field conditions. Within this category, the analysis of carbon isotope signatures is among the most consolidated, but other isotopes are being investigated.

This communication will present different examples of phenotyping techniques, traits and their application in durum wheat.

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ADAPTATION TO CLIMATE CHANGE: CHALLENGES AND OPPORTUNITIES

Rizza F.¹, Badeck F-W.¹, Marè C.¹, Mazzucotelli E.¹, Bagnaresi P., Reggiani F.¹, Alberici R.¹, Baronchelli M.¹, Fares C.², Menga V., Diretto G.⁴, Falcone G.⁴, Sulli M.⁴, Frusciante S.⁴, Giuliano G.⁴, Zaldei A.³, Miglietta F.³, Cattivelli L.¹

1 Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia agraria (CRA), Genomics Research Centre, Via San Protaso 302, 29017- Fiorenzuola D'Arda, Italy

2 Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia agraria (CRA), Cereal Research Centre, S.S. 16- km 675, 71122- Foggia, Italy

3 Consiglio Nazionale delle Ricerche (CNR), Istituto di Biometeorologia Via G. Caproni, 8, 50145 Firenze Italy, Italy

4 Agenzia nazionale per le nuove tecnologie, l'energia e lo sviluppo economico sostenibile (ENEA) Centro Ricerca della Casaccia, Via Anguillarese 301, 00123 Roma, Italy

Climate changes will strongly impact on plant growth, yield and quality. Increase in average temperature and reduced water availability in some regions of the world are the main consequences of the increased atmospheric CO₂ concentration. Nevertheless, the expected atmospheric CO₂ increases will have per se an effect on plant growth with the potential to lead to increased carbon assimilation by C3 photosynthesis. The global CO₂ concentration was 396 ppm in 2013 and it is expected to increase leading to estimated concentrations around 550 ppm by year 2050. To evaluate the effects of CO₂ increase on growth, yield and grain and pasta quality in durum wheat, a two-year field experiment in a FACE (Free Air Carbon dioxide Enrichment) facility was carried out at the experimental farm of the CRA-Genomics Research Centre in Fiorenzuola d'Arda (Italy). The results have demonstrated a general yield increase associated to a negative trend for protein content. A significant genetic diversity in plant responses to elevated CO₂ for both yield and quality parameters was observed. Transcriptomic and metabolomic analyses have been used to describe the impact of elevated CO₂ on leaf and seed metabolism in plant grown in open field conditions. An up-regulation of genes related to sucrose synthesis and a modulation of genes involved in nitrogen and lipid metabolism was detected.

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DURUM WHEAT AS A STAPLE CROP FOR FOOD SECURITY AND POVERTY ALLEVIATION

Michael Baum^{1*}, Yigezu Yigezu¹, Nazari Kumarse¹, Ahmed Amri¹, F.M Bassi¹

1 International Center for Agricultural Research in the Dry Areas (ICARDA), Biodiversity and Integrated Gene Management Program (BIGM), P.O. Box: 6299, Rabat, Morocco

Durum wheat (*Triticum turgidum* subsp.*durum* L.) is the second most important triticum species worldwide covering an area between 17 and 18 million hectares. The durum market for 2013/2014 has seen a usage of 35.8 million tons (MT) against a production of just 35.1 MT, and this unbalance assures high trading prices for the durum grain. The European Union is both the largest producer and largest processor of durum wheat, and Canada is the second-largest. However, India and Turkey are rapidly increasing the surface cultivated with this crop. The countries of the Middle-East and North Africa remain among the largest producers, and now Ethiopia is also re-instating the importance of this crop. In this complex trading scenario, it is critical to understand the two layers of durum cultivation, one being its reality as international cash crop, the other is its use as historical tradition of many societies. ICARDA operates in this scenario trying to both use the market price as a strategy for poverty alleviation, but also targets small holder farmers as a traditional source for food security in the traditional durum growing countries around the Mediterranean sea such as Turkey, Syria, Lebanon, Morocco, Tunisia and Algeria. Here, these considerations are presented in the global picture and in contrast with the global risks associated with damaging diseases and pests. Further, ICARDA is involved in defining new production areas for this crop and the preliminary results of testing durum wheat along the Senegal River seem to suggest that new competitors might soon rise on the international market.

Keywords: *Triticum, global trade, Mediterranean, Mauritania, Senegal.*

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ENVIRONMENTAL AND GENETIC FACTORS AFFECTING YIELD FORMATION IN WHEAT

Conxita Royo, Dolors Villegas, Karim Ammar & Fanny Àlvaro

The full expression of the yield potential of wheat is frequently constrained by environmental factors, particularly in areas where abiotic stresses occur regularly. Due to the genotype x environment (GE) interaction wheat cultivars perform differently in contrasting environments. As a consequence of adaptive mechanisms the yield formation strategies of landraces grown for centuries on specific regions were determined by the prevalent environmental conditions. Maximizing wheat yield in a given environment requires optimizing the use of resources while avoiding negative effects of stresses during the growing period. This can only be achieved by growing cultivars with a flowering time fitting properly in the target environment. Although time to flowering is genetically controlled by three gene systems, it is largely determined by crop responses to temperature and photoperiod. Experiments conducted in durum wheat in contrasting latitudes revealed the influence exerted by the combination of temperature, daylength and radiation on yield formation and their effect on the determination of the yield component mostly limiting yield on each environment. Studies on the influence of *Ppd-1* genes, regulating photoperiod response, showed that their effect on flowering time affected dry matter production and allocation in the plant and the relative contribution of photosynthesis and remobilization of pre-anthesis assimilates to the growing grains during grain filling. Results showing the role of growth habit and the *Rht-B1b* dwarfing allele on the pattern of wheat adaptation and grain yield formation strategy will be presented. Experiments conducted in bread and durum wheats demonstrated that the number of grains per spike is consistently the yield component with the largest genetic effect and the lowest environmental influence. Evidences supporting a large effect of kernel weight on yield formation in cooler environments, and the predominant influence of the number of spikes per unit area in the warmer ones will be presented.

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FROM SOWING TO HARVESTING. ADDRESSING SPATIAL-TEMPORAL VARIABILITY IN DURUM WHEAT CULTIVATION FOR IMPROVING RESOURCE USE EFFICIENCY AND SUSTAINABILITY

Francesco Morari

DAFNAE, University of Padova, francesco.morari@unipd.it

Precision Agriculture represents a major breakthrough as it allows to manage the spatial-temporal variability in agricultural production in a more economic and environmentally efficient approach. Site-specific applications of agronomic input on wheat embrace the entire crop cycle from sowing to harvesting. Particularly, they offer new potentialities to meet production and grain quality standards. On the one hand, nitrogen (N) variable rate application (VRA) could play a pivotal role to drive a quality-oriented fertilisation and, on the other hand, precision harvesting could be an alternative method to maximise the tonnage of higher quality grades, allowing to separate the grain according to its quality. Site-specific techniques are fostered by the diffusion of on-the-go sensing technology, such as crop reflectance sensors and grain quality sensors.

Experiments on N variable rate application on durum wheat in North Italy demonstrated that if no other stresses than N availability occur, sensor-based N recommendation matches crop requirements. Only unpredictable weather conditions result in low N use efficiency with risk for the environment, partially mitigated by VRA. Results evidenced the possibility to apply on durum wheat sensor-based input correction, extending it to a wide area in Southern Europe. Encouraging results were obtained also applying precision harvesting whose economic viability resulted influenced by the field size and shape, protein variability and the protein premium payment.

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TOWARD A HEALTHIER PASTA

Mike Sissons

Pasta is already a healthy food in terms of its composition and calorie content but with new trends in functional foods, more health conscious consumers, a rise in chronic diseases and new wheat types, there is now the possibility to further enhance the health value of pasta. This paper will review current trends providing data from studies and our own work on how to make pasta even more attractive to the consumer interested in healthier foods.



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NOVEL FOODS AS AN ALTERNATIVE TO A GLUTEN-FREE DIET

Mauro Rossi

CNR, Italy

Gluten intolerance is now better defined as ‘gluten-related disorders’, encompassing three disease entities: coeliac gluten sensitivity (classically coeliac disease), non-coeliac gluten sensitivity and wheat allergy. The latter is a quite rare condition. Coeliac disease (CD) is instead observed in genetically predisposed subjects, influencing 1% of population in developed countries. On the other hand, the prevalence of nonceliac gluten sensitivity (NCGS) in the general population is supposed to be many times higher than that of CD. This may help to explain the amplitude of the gluten-free market, whose consumers are not only CD patients but also undiagnosed NCGS people, who find that living gluten-free makes them feel better. Currently, a series of strategies have been explored to evaluate bio-technological approaches aimed at detoxifying gluten. In particular, the use of prolyl endopeptidases (PEPs) to preventively treat wheat flour has been considered because of the high content of proline residues in gluten that protects against digestive proteolysis. Another approach explored the activity of food-grade microbial transglutaminase (mTG). In particular, the transamidation of gliadin by treatment of wheat flour with mTG and lysine alkyl esters was shown to cause a dramatic down-regulation of inflammation induced by intestinal lymphocytes in CD patients. Both these strategies introduce food ingredients that have not been used for human consumption to a significant degree in the EU before 15 May 1997. For this reason, they are considered novel foods. Under the Novel Foods Regulation such foods are then subject to a pre-market safety assessment before a decision is made on EU-wide authorisation.

In conclusion, the application of strategies aimed at reintroduction of (modified) wheat products appears very promising in the perspective to ameliorate the condition of gluten intolerance in CD and NCGS patients. Nevertheless, novel food solutions remain to be exhaustively evaluated in dietary intervention studies before to obtain EU approval for marketing.

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DURUM WHEAT AND PASTA MARKET: PRICE EVOLUTION, VOLATILITY ISSUES AND PERSPECTIVES

Enrica Gentile, Mauro Bruni

Durum wheat production presently represents around 4,6% of the overall production of wheat, mostly concentrated in two main areas in the world, North America and the Mediterranean Basin. The highest portion of this production is covered by a very limited number of countries, among which Canada (4,8 mio tons), the EU (7,0 mio tons among Italy – 3,7 mio -, France, Spain and Greece), and the North African countries (5,1), followed by Siria and Turkey (2,9), Mexico (2,3), Kazakhstan (2,1), USA (1,6) and Australia (0,5). During the last five years the world stocks of durum wheat have been progressively reducing, due to consumption levels almost always higher than production, while the overall trade flows have been increasing by around 12% during the same period. Canada is by far the largest world exporter while US and the EU, used to be exporters until some years ago, have now permanently become net importers. The limited dimension of the market, together with the concentration of the supply in well limited areas in the world, make the durum wheat market a very volatile one, due to its high susceptibility to fluctuation in production and stock reductions.

Looking at the market of pasta, consumption levels continue to be more or less flat in the “traditional” consuming countries (Italy, UE, US and others), some of which are still performing a slightly increasing demand, but on the other side result to be extremely interesting in a number of “new” consuming countries, where pasta has just entered the population’s diet and it’s now achieving larger and larger parts of the market. Among countries with the highest growth rate in pasta consumption levels, it is possible to cite Venezuela (+74,9%), Peru (+64%), China (+48%) and Senegal (+46%), followed by a number of countries where growth rates are a bit lower but the overall dimension of the market largely compensate the lower increasing rate, in terms of opportunities. Among them Brazil (+27,5%), Russia (+26,8%) and India (+12%).

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INNOVATION IN PASTA PRODUCTION

Andrea Cardinali

Rustichella d'Abruzzo S.p.A., Italy

Rustichella d'Abruzzo, founded by Gaetano Sergiacomo in 1924, is a pasta factory in Abruzzo that still produces pasta by following the techniques of craft with the dies strictly bronze and long drying at low temperature. Quality and innovation have always been key elements of Rustichella D'Abruzzo that supported the presence in over 70 countries worldwide. Over the years, in collaboration with research centers and institutes University allowed to follow two main lines of research; the first focused on developing products based on new formulations through the use of functional substances, supplements and bio active compounds; the second it based on the development of new process technologies in order to obtain an improvement of the organoleptic characteristics of the products and to increase the added value.

Specifically, in collaboration with a team of athletes and nutritionists, it born the line of products Pasta Young Endurance, three references dedicated to food / dietary supplement for athletes. Among these are a pasta enriched with 5% of maltodextrins for use before competitions, one pasta enriched with a 4% branched amino acids BCAA in free form to ensure the growth and muscle development and another hyper protein pasta with 50% of proteins of high biological value designed to ensure an appropriate muscle recovery after an intense sport. In the same line of research, a collaboration established with the ministerial project PASS-MI_138 WORLD 2015, which involved 3 universities, 3 research centers and 3 Enterprises of agro food, was born a functional pasta enriched in barley Prebiotics and in Probiotics. The innovative product, associates to the benefits of a balanced diet rich in fiber (β -Glucans) and phenolic compounds, the presence of germinating spores of lactobacillus Coagulans that as a result of stress promoted by the cooking process, establish a guest in the intestine flora comparable to that related to antidismicrobic medicine.

From the second line of research is born a new kind of Spaghetti characterized by a 90 second instant cooking obtained with the aid of a special extrusion technology patented by Rustichella D'Abruzzo (PS2014A000003). This matrix gives to the toroidal structure open of the product a mechanical characteristics comparable to classics spaghetti, knocking down drastically, however, the cooking time, thus making the product usable even for rapid preparations at the base of the concept of fast food. An extraordinary added value so far unthinkable starting from dry products of durum wheat semolina.

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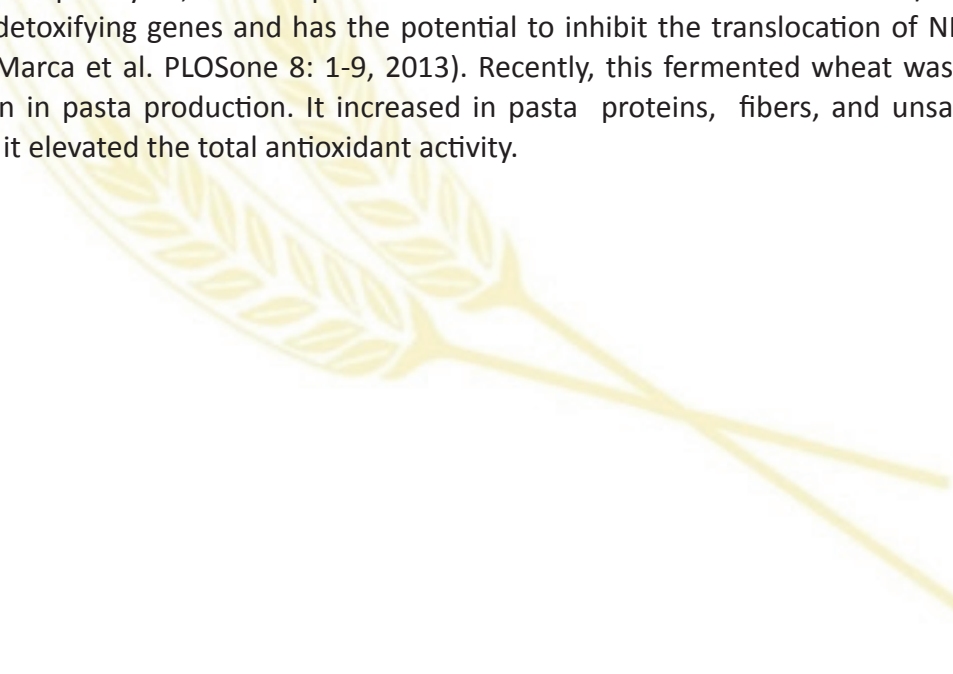
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NUTRACEUTICAL PROPERTIES OF FERMENTED WHEAT AND ITS USE IN PASTA PRODUCTION

Vincenzo Longo

IBBA-CNR, Italy

In recent years, several studies have shown that plant and food extracts play a protective role in the etiology of various diseases. We have performed studies on Lisosan G a fermented of *Triticum Sativum*. *In vivo*, Lisosan G protects against the cisplatin induced toxicity (Longo et al. Food Chem Tox 49: 233–237, 2011), and a recent paper showed that Lisosan G helps prevent microcirculatory dysfunction (Lubrano et al., Indian J Med Res 136: 82-88, 2012). We also showed, by using primary cultures of rat hepatocytes, that this powder of wheat is an effective inducer of ARE/Nrf2-regulated antioxidant/detoxifying genes and has the potential to inhibit the translocation of NF-κB into the nucleus (La Marca et al. PLOSone 8: 1-9, 2013). Recently, this fermented wheat was used at low concentration in pasta production. It increased in pasta proteins, fibers, and unsaturated fatty acids; in add it elevated the total antioxidant activity.



CANDIDATE GENE AND GENOMIC APPROACHES TO IMPROVE WHEAT QUALITY TRAITS

Gadaleta A., Giancaspro A., Giove S.I., Nigro D., Colasuonno P., Marcotuli I., Incerti O., Blanco A.

The nutritional quality of cereals is an important component of the human diet as the cereals represent the largest component of world food supplies. Most quality traits are complicated quantitative traits. Quantitative trait loci (QTLs) analysis has provided an effective approach to dissect complicated traits into component loci to study their relative effects on a specific trait. In a particular genetic background, QTL analysis allows the presence of QTLs to be identified, thereby providing breeders with targets for marker-assisted variety improvement.

Indeed the development of high-density genetic linkage maps is particularly important for bridging information between genome sequence efforts and breeding programs.

Among quality traits of great importance are grain protein quantity and quality, carotenoid and dietary fibre content. In durum wheat, seed storage proteins are important not only from the nutritional standpoint, but they have even greater significance for pasta-making quality. The carotenoid pigments have an enormous importance for the nutritional value and determine the flour color. Indeed the antioxidant activity of carotenoid, together to the protein, increases the nutritional and technologic characters of flour. Dietary fibre have been demonstrated to have many health benefit including immunomodulatory activity, cholesterol lower activity, fecal bulking effect, enhanced absorption of certain mineral, prebiotic effects. One of the most important portion of the fibre soluble fraction with beneficial effects for human health are β -glucans (BG) a component of grasses cell walls. In the present work quality traits in wheat were studied by quantitative trait locus (QTL) analysis in different recombinant inbred line (RIL) populations and in a durum wheat collection identifying major QTL and candidate genes for this three important characters.

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METABOLOMICS IN DURUM WHEAT: WHAT ELSE?

Beleggia Romina

Among the “omics” approaches, metabolomics, although relatively young, and still very much in development, it is now being widely applied: in fact the changes in the levels of metabolites represent the response of the biological systems to genetic and environmental factors.

Several studies, in which metabolomics was used, will be presented: for example in the evaluation of the effects of domestication and breeding in tetraploid wheat kernels, in the study of the effect of genotype, environment and GxE interaction in the metabolites composition of durum wheat.

In addition this approach was used in the evaluation of quality of durum wheat and pasta describing the differences and the modifications undergone during the processes required to transform semolina into pasta. During the pasta making, the constitutive components of semolina are subjected to different modifications that impact upon, or define, the taste, the nutritional quality and the flavor of the end product. Also the effect of cooking on metabolites composition of different kinds of pasta was presented.

The approach used in these works shown the high potentiality of metabolomics for plant and food investigations in regard to quality, process transformation, safety and nutrition.

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FROM SEED TO PASTA: INCREASING THE POTENTIAL NUTRITIONAL VALUE OF PASTA THROUGH INNOVATIVE TECHNOLOGICAL PROCESSES

R. Ciccoritti, A. Cammerata, G. Terracciano, D. Sgrulletta, L. Gazza

Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia agraria (CRA-QCE), Via Cassia 176, 00191 Rome, Italy.

The health promoting properties of whole grain cereals are linked to the presence of a large spectrum of bioactive compounds, localized in the outermost layers of the kernel and in the germ, which are removed during the traditional milling process. Considering the importance of durum wheat in the Mediterranean diet and of the health promoting effects of these bioactive compounds, the present research was focused on increasing the potential nutritional value of pasta through innovative technological processes. Following the objective, the characterization of the bioactive compounds of three durum wheat cultivars from seed throughout the pasta production by a novel grinding system was performed. The innovative milling involved a mild debranning followed by a micronization process which allows to produce very fine whole flours. The obtained wholemeals were then fractioned by air classification obtaining two principal fractions: fine (F) and coarse (G). In the successive steps, durum wheat semolina were mixed with G or F fractions and the obtained integrated flours were subjected to a sieving step before the pasta-making process.

Innovative enriched pasta samples were characterized for their Total Dietary Fibre (TDF), Arabinoxilan (AX), 5-n alkylresorcinol (AR), Protein, Total and Resistant Starch (RS) contents and were compared to the pasta obtained by the traditional milling process. The enriched pasta (spaghetti shape) presented an increase of TDF, AX and AR content with respect to the traditional one (4.8 ± 0.3 vs $3.0 \pm 0.5\%$ d.m; 2.70 ± 0.26 vs $1.80 \pm 0.30\%$ d.m and 192.7 ± 15.7 vs 88.7 ± 5.3 ug/g d.m., respectively).

The sensorial evaluation of pasta highlighted a good global quality of the enriched products compared to the traditional one. In conclusion, the innovative technological system, recently patented, allowed to improve the potential nutritional and health value of durum wheat flour and related end-products without neglecting the sensorial aspects.

NANOENCAPSULATION OF SC-CO₂ EXTRACTED WHEAT BRAN OIL BY α -CYCLODEXTRINS: A STABILITY STUDY

Miriana Durante¹, Pier Paolo Marrese², Marcello Salvatore Lenucci², Giovanni Mita¹

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

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

Supercritical carbon dioxide (SC-CO₂) technology has proven effective for the extraction of solvent-free, food-grade oils from several by-products of the cereal milling industry. SC-CO₂ extracted durum wheat bran oil is a rich source of bioactive compounds, mostly polyunsaturated fatty acids, tocopherols and carotenoids [1]. It is potentially useful as high-quality ingredient in the preparation of innovative functional foods (including fresh and dried pasta) with improved nutritional and antioxidant properties, provided that its long-term storage stability is enhanced. We previously demonstrated that sodium alginate beads encapsulation extends the stability of such oil over time [2]. However, the preparation is not suitable for making pasta or bakery products, due to the macroscopic size of the beads. Here we describe the nanoencapsulation of wheat bran oil in α -cyclodextrins, natural bottomless bowl-shaped glucose based molecules recently approved in Europe as soluble dietary fiber and novel food ingredient [3], to obtain a freeze-dried water soluble ready-to-mix powder. The stability of tocopherols and carotenoids, compared to the unencapsulated oil, was also monitored in different combinations of storage conditions (25°C, dark; 25°C, light; 4°C, dark).

Regardless of light, storage at 25°C determined a rapid decrease in tocopherol and carotenoid concentrations in the unencapsulated oil (control), while encapsulation in α -cyclodextrins resulted in a greater protective effect of the nutritionally important compounds. Storage at 4°C synergize with encapsulation in preventing the oxidative degradation of both tocopherols and carotenoids for over 40 days.

These findings encourages further studies in order to scale up the process for possible industrial production of high quality bioactive ingredients from wheat bran.

[1] Durante et al., 2012. J. Agr. Food Chem. 60, 10689-10695.

[2] Durante et al., 2012. Phytochem Rev. 11, 255-262.

[3] OJ L 146/12, 05.06.2008.

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GENETIC ARCHITECTURE OF KERNELS QUALITY TRAITS IN AN EVOLUTIONARY PREBREEDING POPULATION OF DURUM WHEAT

Jacques David, Yan Holtz, Muriel Tavaud, Vincent Ranwez, Pierre Roumet, Frédéric Compan, Aline Rocher.

Recent advances in genotyping and phenotyping technologies can now be applied in durum wheat and permit to address a genome wide approach to disclose the genetic architecture of kernel quality traits. DNA chips can allow the genotyping of thousands of SNP markers on several hundreds of lines while NIRS (Near Infra Red spectroscopy) allow the prediction of relevant traits in durum wheat quality, e.g., protein content, yellow pigment content and semolina yield. Genome Wide Association Studies (GWAS) have proven their potential in detecting chromosomal areas implied in the variation of traits. Good panels should have a diversity level, a low structuration and reduced level of linkage disequilibrium. Assembling panels from lines of diverse origin is usually the rule in many crops but may lead to a substantial structuration effect and to spurious associations. We propose here to use lines drawn from an original composite cross population, originally dedicated to create a pre breeding resource.

The Evolutionary Prebreeding pOpulation (EPO) is continuously grown since 1997 with a light selection and a 10% outcrossing rate. We report the first results of a GWAS study on 300 hundred lines drawn from the 2009 harvest of EPO. Using a set of 62 000 polymorphic SNP drawn along the genome, we report the associations found between this set of markers and a set of traits related to the kernel quality predicted by NIRS.

First, the characteristics of the EPO population will be illustrated to examine its power to detect association. We will report the fine genetic structuration and the fine scale Linkage disequilibrium on the 3B chromosome on which the SNP can be physically located. Then kernels quality traits will be dissected by GWAS to detect the most important chromosomal regions involved in the variation. Finally, each trait will be predicted as the whole level to discuss the potential of Genomic Selection (GS) in the improvement of quality in the durum kernel.

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A Sustainable Durum Wheat Chain for Food Security and Healthy Lives

GWAS AND CANDIDATE GENES FOR ARABINOXYLAN IN WHEATS

Ilaria Marcotuli, Rachel Burton, Geoffry Fincher, Antonio Blanco, Agata Gadaleta

Non-starch polysaccharides (NSPs) are also known as dietary fibre and have been shown to have many health benefits. Arabinoxylans (AX) is the main component of NSPs in wheat and is involved in the bread-making mechanism and in starch extraction. Furthermore, arabinoxylans are soluble dietary fibre with potential health-promoting effects in human nutrition. Despite their high value for human health, few studies have been carried out on the genetics of AX content in durum wheat. The genetic variability of arabinoxylan content was investigated in a set of 104 tetraploid wheat genotypes through a genome wide association study. The amount of arabinoxylan, expressed as percentage of w/w on the dry weight of the kernel, ranged from 1.8% to 5.5% with a mean value of 4.0%. Genotyping by SNP markers revealed a total of 37 marker trait associations (MTAs), identifying 19 QTL regions associated with AX content. The highest number of MTAs were identified on chromosome 5A (7) identifying 3 QTL regions involved in the control of this important trait, while the lowest number of MTAs was detected on chromosomes 2B and 4B, where only 1 MTA identified a single locus. Synteny analysis of SNP markers associated sequences against the annotated genes and proteins in *Brachypodium distachyon*, *Oryza Sativa* and *Sorghum bicolor* allowed the identification of 9 QTLs coincident with candidate genes including glycosyl hydrolase *GH35*, which encodes for *Gal7*, and a glucosyltransferase *GT31* on the chromosome 1A; a cluster of *GT1* on chromosome 2B which included *TaUGT1* and *cisZog1* genes; a glycosyl hydrolase which encodes for *CelC* gene on 3A; *Ugt12887* and *TaUGT1* genes on chromosome 5A; the 1,3- β -D-glucan synthase (*Gsl12*) and a glucosyl hydrolase (*Cel8*) on chromosome 7A.

This study provides significant MTAs for arabinoxylan grain content in tetraploid wheat genotypes that can be used, after validation, in molecular breeding for developing superior varieties with higher fibre content.

QUANTITATIVE TRAIT LOCI MAPPING AND CORRELATION ANALYSIS ON GRAIN MULTIPLE NUTRITION CONTENTS IN DURUM WHEAT × WILD EMMER WHEAT RIL POPULATION

Yan Jun, Fan Di, Xue Wen-Tao, Fahima Tzion, Cheng Jian-Ping*, Zhao Gang*

The nutrients of wheat grain are essential and important nutrition sources for humans health. Wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides*, the direct progenitor of wheat, offers a valuable genetic resource for wheat improvement. In the current study, the genetic and physiological basis of grain total soluble protein (TSP), total flavonoids (TF), total phenolics (TP), phytate (PHY) and yellow pigment concentration (YPC) were studied in tetraploid wheat population of 152 recombinant inbred lines (RILs), derived from a cross between durum wheat (cv. Langdon) and wild emmer (accession G18-16) planted at Guizhou Province in China. Only the trait of TP in five tested traits was not normal distributed. Wide genetic variation and transgressive segregation were found among RILs for the five grain trait contents. A total of six significant QTLs were mapped for the five trait contents with LOD scores ranging between 2.1–3.5, and explaining 7.6–12.4 % of phenotypic variation, respectively. Six QTLs had been detected on chromosome 2A, 3A, 4A, 6B and 7A. Higher grain nutrients were conferred by G18-16 allele at 4 loci (2A, 3A, 6B, 7A) and Langdon allele at 2 loci (4A2). The identified QTLs may facilitate the use of wild alleles for improvement of the multiple grain traits nutritional quality in elite wheat cultivars. Correlation network analysis exhibited that all the grain nutrient-related traits displayed negative correlations with eight agronomic traits and only YPC significantly isolated. PCA results also proved TP and YPC diverse from other nutrition traits, and the two traits mainly contributed the diversity of RILs on PCA axis, indicating the possible unique mechanisms for grain TP and YPC. Meanwhile, for the purpose of backcross and fine mapping, PCA also provided two groups of lines with large variation on all grain nutrient-related traits, each group contains four lines, respectively.

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IMPROVEMENT OF DURUM QUALITY AND CREATION OF NEW ALLELIC VARIATION FOR GLUTENIN IN EMS—MUTANT DURUM WHEAT POPULATION (*TRITICUM TURGIDUM* L. SUB SP. *DURUM* (DESF.))

Labhilili Mustapha¹, Elyadini Meryem^{1,2}, Bentata Fatiha¹, Azeqour Mohamed², Taghouti Mouna¹, Kahama Issa^{1,2,3}, Martin Perry⁴, Gaboune Fatima¹, Nasserlah Nessrelah⁵, Nachit Miloud⁶ and Filippo Bassi⁶

1 Biotechnology Research Unit, Rabat Regional Agronomic Research Center, INRA, Avenue Mohamed Belarhi Alaoui, B. P. 6356 Rabat-Institute, Rabat 10101, Morocco

2 Faculty of Sciences and Technology, Université Hassan II Mohammedia, B. P. 146, Mohammedia 20800, Morocco

3 Faculty of Sciences and Technology, Semlalia, B. P. 2390, Marrakech-Maroc 40 000, Morocco

4 Rothamsted Research Institute, Harpenden, Herts AL5 2JQ, UK

5 Settat Regional Agronomic Research Center, INRA, B. P. 589, Settat 26000, Morocco

6 ICARDA, P. O. Box 6299, Rabat-Institut, Rabat 10101, Morocco

Durum Wheat is the many cereal crop cultivated and adapted in Mediterranean regions. It's used in pasta, couscous, bourghoul and bread. The High Molecular Weight HMW-GS and Low-Molecular-Weight LMW-GS Glutenin Subunits are major determinants of wheat dough processing qualities. For improving quality of durum and creation of new genotypes, Tilling populations was generated by EMS mutagenesis from Cham1 durum variety. Protein quality was investigated by SDS sedimentation, protein content, gluten content and thousand kernel weights. Electrophoresis SDS-Page was used for the investigation of new allelic variations in the HMW-GS and Low-Molecular-Weight LMW-GS Glutenin Subunits. Lines with high expression or with new allele were selected from 1500 lines of tilling population depending on the variations of their electrophoresis profiles. The analysis of Glu-B1 HMW-GS has showed two types of profiles: lines deficient on Bx7 and lines with expression of new protein between (Bx7—By8) allelic pair combination. The majority of these mutant lines have showed quality compounds associated with bad technological characteristics like the parent Cham1, with the exception of two mutant lines expressing new protein that have significantly stronger gluten properties and one mutant deficient on Bx7 HMW-GS that developed high value of SDS. For Glu-B3 LMW-GS coding for LMW glutenin type 1 and 2, new mutant lines showing expression of new protein pattern were selected. The mutant showing over-expression of bands on gel SDS-PAGE for LMW type1 like Cham1, have produced the highest protein and gluten content, while the new mutant lines showing new profile have showed high value of Gluten and SDS.

Key words: *HMW-GS, LMW-GS, Triticum durum, tilling population, quality.*

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A Sustainable Durum Wheat Chain for Food Security and Healthy Lives

AN SNP-BASED CONSENSUS MAP FOR CHARACTERIZING AND EXPLOITING GENETIC DIVERSITY IN DURUM WHEAT

Maccaferri M.¹, Ricci A.¹, Salvi S.¹, Milner S.G.¹, Noli E.¹, Martelli P.L.², Casadio R.², Akhunov E.³, Scalabrin S.^{4,5}, Vendramin V.^{4,5}, Ammar K.⁶, Blanco A.⁷, Desiderio F.⁸, Distelfeld A.⁹, Dubcovsky J.^{10,11}, Fahima T.¹², Faris J.¹³, Korol A.¹², Massi A.¹⁴, Mastrangelo A.M.¹⁵, Morgante M.^{4,5}, Pozniak C.¹⁶, N'Diaye A.¹⁶, Xu S.¹³, Tuberosa R.¹

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

2 Biocomputing Group, University of Bologna, Bologna, Italy

3 Department of Plant Pathology, Kansas State University, Manhattan, KS, USA

4 Istituto di Genomica Applicata, Udine, Italy

5 Dipartimento di Scienze Agrarie e Ambientali, University of Udine, Udine, Italy

6 CIMMYT Carretera Mexico, Texcoco, Mexico

7 Dipartimento di Biologia e Chimica Agro-forestale ed ambientale, Università di Bari, Aldo Moro, Bari, Italy

8 Consiglio per la ricerca e la sperimentazione in agricoltura, Genomics Research Centre, Fiorenzuola d'Arda, Italy

9 Faculty of Life Sciences, Department of Molecular Biology and Ecology of Plants, Tel Aviv University, Tel Aviv, Israel

10 Department of Plant Sciences, University of California, Davis, CA, USA

11 Howard Hughes Medical Institute, Chevy Chase, MD, USA

12 Department of Evolutionary and Environmental Biology, Institute of Evolution, Faculty of Science and Science Education, University of Haifa, Haifa, Israel

13 USDA-ARS Cereal Crops Research Unit, Fargo, ND, USA

14 Società Produttori Sementi Bologna (PSB), Argelato, Italy

15 Consiglio per la ricerca e la sperimentazione in agricoltura, Cereal Research Centre, Foggia, Italy

16 Crop Development Centre and Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada

Consensus linkage maps are important tools in crop genomics for cross-referencing markers, genes and QTLs. A high-density tetraploid wheat consensus map has been assembled by integrating 13 data sets from biparental populations involving durum wheat cultivars (*Triticum turgidum* ssp. durum), cultivated emmer (*T. turgidum* ssp. dicoccum) and their ancestor (wild emmer, *T. turgidum* ssp. dicoccoides). The consensus map harboured 30 144 markers (including 26 626 SNPs from wheat transcripts and 791 SSRs), half of which were present in at least two component maps. The final map spanned 2,631 cM of all 14 durum wheat chromosomes and, differently from the individual component maps, all markers fell within the 14 linkage groups. Comparisons with bread wheat indicated fewer regions with recombination suppression, making this consensus map a valuable tool for mapping in the A and B genomes for both durum and bread wheat. Sequence similarity analysis allowed us to relate mapped gene-derived SNPs to chromosome-specific transcripts. Dense patterns of homeologous relationships have been established between the A- and B-genome maps and between nonsyntenic homeologous chromosome regions as well, the latter tracing to ancient translocation events. The gene-based homeologous relationships are valuable to infer the map location of homeologs of target loci/QTLs. The consensus is being used as a gene-based framework to complement information from single map QTL analysis and association mapping panels. Because most SNP and SSR markers were previously mapped in bread wheat, this consensus map will also facilitate a more effective integration and exploitation of genes and QTL for wheat breeding purposes.

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GENOMICS-BASED APPROACHES FOR MAPPING AGRONOMIC TRAITS IN DURUM WHEAT: FROM CONSENSUS REGIONS TO CANDIDATE GENES

Anna Maria Mastrangelo

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

Linkage and association mapping are well established approaches to study the genetic architecture of agronomic traits in crops. The recent and fast progresses in the techniques of genotype analysis has allowed the rapid scoring of huge numbers of individuals at cheap costs. 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 developed, together with recombinant inbred line populations which segregate for specific traits. The tetraploid collection and the segregating populations have been genotyped with the wheat 90k iSelect Infinium SNP assay and phenotypically evaluated for traits related to the environmental sustainability (resistance to pathogens and interaction with microorganisms at level of root apparatus) and seed quality. The availability of genetic maps that are very dense in gene-related molecular markers offers a valuable tool for the identification of candidate genes for the QTL for traits of interest. The genetic position within or very near the confidence interval of a QTL, and the putative function of the gene itself are two criteria which define a candidate gene. A number of QTL have been identified for the traits of interest, and the analysis with SNP markers revealed interesting correspondences between chromosome regions controlling different traits and candidate genes, which will serve for the understanding of molecular mechanisms controlling the phenotypic features and for the marker assisted selection of improved durum wheat lines.

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DEVELOPMENT OF GENETIC AND GENOMIC RESOURCES FOR TARGET BREEDING IN DURUM WHEAT

Steven S. Xu, Shiaoman Chao, Justin D. Faris, Timothy L. Friesen, Zhaohui Liu, Yue Jin, Matthew N. Rouse, and Elias M. Elias

Durum wheat (*Triticum durum*) is an important commodity crop in the Northern Great Plains where 32% and 62% of durum wheat acreage in the United States in 2014 was planted in Montana and North Dakota, respectively. In recent years, durum wheat production in North Dakota has suffered serious losses from outbreaks of Fusarium head blight (FHB), and it has also been threatened by other fungal diseases such as tan spot, Septoria nodorum blotch (SNB), and stem rust. In addition, wheat stem sawfly is a serious problem for durum production in Montana and western North Dakota. To assist the durum breeding programs in developing new durum cultivars with resistance to these diseases, we have extensively evaluated tetraploid wheat collections maintained at the USDA National Small Grains Collection and identified a number of durum and other tetraploid wheat (*T. carthlicum*, *T. dicoccum*, *T. dicoccoides*, and *T. turgidum*) accessions with high levels of resistance to FHB, tan spot, SNB, and stem rust. Based on the evaluation results, we assembled two association mapping panels and developed 10 mapping populations comprised of over 2,000 doubled haploids or recombinant inbred lines. By using these panels and populations, we identified a number of unique genes or QTLs and their linked markers for resistance to FHB, tan spot, SNB, and stem rust through association and linkage analysis. Several genes or QTLs for resistance to FHB and stem rust have been transferred into the durum cultivars. For developing elite durum germplasm for resistance to sawfly, we have transferred a gene controlling super-solid stem from a durum landrace (Golden Ball) into six durum cultivars recently developed in North Dakota. The elite durum germplasm lines and molecular markers developed from this study are currently being used to develop new durum cultivars with resistance to FHB, stem rust, and sawfly.

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BREEDING FOR SUSTAINABLE DURUM WHEAT PRODUCTION

Filippo M. Bassi^{1*}, Miloudi M. Nachit¹, Ayed Al-Abdallat¹, Nazari Kumarse¹, Ahmed Amri¹

1 ICARDA, BIGM, P.O. Box: 6299, Rabat, Morocco

The durum wheat (*Triticum durum* Desf.) program of ICARDA has released 93 varieties in 16 countries since its initial inception in 1982. Most of the varieties have been targeted to the dry areas of CWANA and are mostly cultivated by smallholder farmers. Our team has always focused on those farmers that can not afford fungicide or pesticide applications, and that need stable yields under a severely changing and harshening climate. This was achieved by a relentless use of landraces and wild relatives in our crossing program. The outcome is presented here, with wide crosses yielding nearly 2 tons/ha above the commercial checks, while maintaining excellent quality characteristics and good response to biotic and abiotic stresses. Therefore, the ICARDA germplasm packages a wide array of resistance and tolerance traits that we are only now starting to better comprehend. A large collection of 1,500 entries represented by 700 elite and released varieties from 20 countries, and 800 landraces selected by FIGs from 32 countries, have been evaluated for two seasons under various constraints. The subsetting of this large panel yielded 384 unique entries that segregate for all the traits we have used in our investigations thus far. These include: response to pests (Hessian fly and saw fly), resistance to diseases (leaf, yellow, stem rusts, and tan spot), yield under heat stress, and tolerance to toxic levels of boron. The results of these studies will be presented and connected to the molecular information available, searching for novel alleles that can be exploited by durum breeders Worldwide.

Keywords: *Triticum, landraces, elites, boron toxicity, association mapping, ICARDA.*

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HYBRID BREEDING IN DURUM: WHAT CAN WE LEARN FROM BREAD WHEAT?

Friedrich Longin and Alisa-Naomi Sieber

State Plant Breeding Institute, University of Hohenheim; e-mail: friedrich.longin@uni-hohenheim.de;

In bread wheat, recent large experimental studies confirmed a high commercial heterosis for hybrid wheat. Across all quality classes, a commercial heterosis larger than 1 ton per hectare was identified. Furthermore, yield stability of hybrids was larger than for line varieties. For durum, a small study comprising 16 durum lines and their 40 factorial hybrids tested at four field locations in Germany confirmed this high commercial heterosis also for durum wheat. 20 out of the 40 hybrids had a higher grain yield than the best parental line, which was the German variety Duramar. However, for protein content, sedimentation volume and yellow colour measured as b-value, a negative heterosis was reported. Nevertheless, high yielding hybrids with good quality could be identified.

The major drawback, however, for hybrid wheat in general and especially for durum wheat seems the high cleistogamy and the lack of an efficient sterility system. Without large research efforts on these topics, hybrid durum will never be profitable. Finally, heterotic groups have to be identified or created artificially via reciprocal recurrent selection. These three tasks require large experimental studies comprising hundreds of durum lines suggesting joint projects across countries. The German "Zuchtwert"-project can be seen as model for it, where all German wheat breeders work together in order to establish heterotic groups and find better pollinators in bread wheat.

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SINGLE SEED DESCENT (SSD) A TOOL TO EXPLOIT THE NATURAL DIVERSITY TO INCREASE DURUM WHEAT SUSTAINABILITY AND ITS ADAPTATION TO CLIMATE CHANGES

Janni M., De Paola D., Rapanà N., Carella G., Pignone D.

A large wealth of genetic diversity is present in the diversity centers of durum wheat. However, modern breeding selection methods are mostly based on improvement of elite lines and this limits the genetic pool which breeders can exploit for the production of new varieties ready to face the predicted climate changes and the demand for reduced inputs.

In terms of durum wheat sustainability, the main goal of modern breeding is the production of new varieties carrying new adaptation traits to both abiotic and biotic stress as well as an improved nitrogen use efficiency (NUE) in order to reduce lands pollution.

The desired traits are often found in landraces still cultivated *in situ* or stored *ex situ* in germplasm collections. The Eurisco database totals over 17,000 *Triticum durum* or synonyms accessions, some 6,800 of which are reported to be traditional varieties and/or landraces. The usefulness of these resources is incontestable, but, at the same time, their great number hampers the possibility to explore in depth their genepool. The challenge is to reduce the number of genotypes to be screened for new useful traits, while maintaining a good representation of the genetic background.

A possible approach to reduce the number of genotypes to be assessed is the constitution of a working *durum* wheat collection assembled by mean of a single seed descent (SSD) approach.

In the present contribution we report on the constitution and characterization of a *durum* wheat SSD working collection worth of a handy number of genotypes. Moreover, the results of a phenotyping experiment of the SSD collection growth under different regimes of nitrogen fertilization will be presented.

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UNEXPLORED DIVERSITY MAY BRING BETTER TRAITS IN DURUM WHEAT: A GENOME WIDE ASSOCIATION STUDY ON ETHIOPIAN LANDRACES MATERIAL

**Dell'acqua M.¹, Mengistu K.D.^{1,2}, Kidane Y.^{1,3}, Catellani M.¹, Mancini C.¹, Fadda C.⁴,
Pè M.E.¹**

1 Institute of Life Sciences, Scuola Superiore Sant'Anna, Pisa, Italy.

2 Dept. of Dryland Crop and Horticultural Sciences, Makalè, Ethiopia

3 Sirinka Agricultural Research Center, Sirinka, Ethiopia

4 Bioversity International, Nairobi, Kenya

Durum wheat (*Triticum turgidum* spp. *durum*) is a strategic crop for contributing to food security and livelihood improvement of many populations across the world. Several studies witnessed and reported the uniqueness of the Ethiopian tetraploid wheat germplasm for different useful traits, disclosing the possibility to exploit it in breeding. We characterized for 10 agronomic traits a set of 307 Ethiopian durum wheat landraces and 28 Ethiopian improved lines, representing Ethiopian durum wheat diversity. Lines were grown for two years in two different agro-ecological zones of northern Ethiopia. We found extensive phenotypic variation within the panel; notably, several landraces outperformed improved varieties for several traits. This collection was genotyped using the Wheat 90K SNP chip yielding 48,762 polymorphic markers, confirming that the panel harnesses great genetic diversity. A principal component analysis and neighbour-joining phylogeny reported wide, unordered diversity within the landraces subset, and marked its complete separation from the improved lines. A Bayesian clustering analysis reported 10 cryptic clusters with poor correspondence with spatial structure of sampling areas, confirming high gene flow and admixture among landraces. Separate diversity analyses for AA and BB genome ruled out divergent evolution. Linkage disequilibrium decay was comparable on the two genomes, and dropped below r^2 0.1 within 10 to 15 cM. We performed genome-wide association (GWA) mapping with a mixed linear model considering random effects of kinship. We performed model selection for each trait by quantile-quantile plots quality check. We identified strong associations for a number of traits, including three major peaks for flowering time and several signals for structural and yield traits. The diversity of this collection was harnessed in a nested association mapping (NAM) population built by crossing 50 diverging landraces with a recurrent improved durum wheat line. This population, now composed by little less than 10,000 recombinant inbred lines at F5, collects genomic mosaics of the original founders in an ordered way. Its diversity and accumulation of recombination events will allow high power in fine mapping of durum wheat complex traits.

GENETIC DIVERSITY IN A DIVERSE WINTER DURUM WHEAT (TRITICUM DURUM) PANEL

Alisa-Naomi Sieber, C. Friedrich H. Longin, and Tobias Würschum

Durum (*Triticum durum*) is the main source for the production of pasta. Growing areas for durum production are expanding and regions with an autumn-sowing combined with harsh winters become more and more interesting. Winter hardiness and frost tolerance are therefore of great economic importance to maintain and even increase yield potential of durum. In order to support breeding of winter durum and to facilitate genomic approaches, the aim of this study was to characterize the population structure and genetic diversity of winter durum germplasm. In particular, our objectives were to (i) evaluate the distribution of the used markers among the two genomes of durum, (ii) describe genetic relationships and population structure among winter durum, (iii) determine the extent and patterns of LD across the durum genome, and (iv) discuss consequences for durum wheat breeding. We used a highly diverse and unique set of winter durum genotypes, and a genotyping-by-sequencing approach (DArTseq) yielding 30,611 genome-wide distributed PAV and SNP markers. The PCoA explained more than 30 % of the total genetic variation by the first and second principal coordinate. The diversity panel clustered clearly corresponding to the country of origin of the lines and to the breeding history of winter durum. The genome-wide analysis of PIC showed no variability among and along chromosomes. The A and B genomes showed a similar extent of linkage disequilibrium (LD) for closely linked markers and a decay of LD within approximately 2-5 cM. In addition, we observed strong variability of LD along the chromosomes. Taken together, high-density marker data can be a valuable tool for genomic approaches in durum wheat. It could be shown that a family structure exists in winter types. This information can assist scientists and breeders alike to improve the understanding of complex traits like frost tolerance and to facilitate knowledge-based breeding in durum wheat.

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NEW RESISTANCE GENES FOR LEAF RUST AND POWDERY MILDEW DERIVED FROM *T. TURGIDUM* SSP. *DICOCCUM*

Francesca Desiderio¹, Martina Cavallo¹, Francesca Mangione¹, Luciana Piarulli², Davide Guerra¹, Diego Rubiales³, Marina Pasquini¹, Giampiero Valè⁵, Rosanna Simeone², Antonio Blanco², Elisabetta Mazzucotelli¹, Luigi Cattivelli¹

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

3 Institute for Sustainable Agriculture (CSIC), Av. Menéndez Pidal s/n, Campus Alameda del Obispo, Apartado 4084, 14080 Córdoba, Spain.

4 Council for Agricultural Research and Economics (CRA), Cereal Quality Research Unit, Via Cassia, 176, 00191 Roma, Italy.

5 Council for Agricultural Research and Economics (CRA), Rice Research Unit, S.S. 11 to Torino, Km 2,5, I- 13100, Vercelli, Italy.

The tetraploid wheat relative *Triticum turgidum* ssp. *dicoccum* shows particular promises as a donor of useful genetic variation for several traits including disease resistances to be introgressed in cultivated wheat. The accession MG5323 of ssp. *dicoccum*, which showed useful level of resistance to leaf rust and powdery mildew diseases, was crossed with the susceptible durum wheat cultivar Latino. A total of 110 recombinant inbred lines (RILs) were produced and a high resolution marker map was developed based on the 90K Infinium (Illumina). The parents and RIL population were phenotyped with two *Puccinia triticina* (VMC03 and 12766) and one *Blumeria graminis* (O2) isolates. Quantitative trait loci (QTL) analysis allowed the identification of one major resistance gene conferring resistance to leaf rust on the short arm of chromosome 1B, explaining a total phenotypic variation ranging from 41.37 to 49.51%. Two additional minor resistance genes located on chromosome 7B explained a phenotypic variation ranging between 17.77 and 25.81%. For both QTLs the resistant allele was provided by MG5323. A significant positive epistatic effect was detected between QTLs, indicating that different QTLs contribute different degrees of resistance. Moreover, analysis of the leaf rust responses of the RILs demonstrated complementary actions between genes on chromosome 1B and 7B. Analysis of powdery mildew resistance identified a single dominant gene on the short arm of chromosome 2B explaining 67.90% of total phenotypic variation. MG5323 provided the resistant allele at the QTL. A fine mapping approach of the major genes for both diseases was undertaken by developing a large F₂-based high resolution mapping population and the flanking and peak markers were used to select a number of recombinant lines that are currently under phenotypic evaluation. The closest linked markers have been converted into PCR-based markers and are suitable for marker-assisted selection (MAS) in resistance breeding.

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QTL MAPPING AND CANDIDATE GENES FOR *FUSARIUM* HEAD BLIGHT RESISTANCE IN DURUM WHEAT

Giancaspro A., Giove S.I., Zito D., Lionetti V., Ravaglia S., Bellincampi D., Blanco A., Gadaleta A.

Fusarium graminearum, one of the causal agents of Fusarium Head Blight (FHB), leads to severe losses in grain yield and quality due to the production of mycotoxins harmful to human and livestock. Different sources for FHB resistance were identified in common wheat (*Triticum aestivum* L.) while efficient FHB resistance genes in durum wheat (*Triticum turgidum* ssp. *durum*), one of the cereals most susceptible to *F. graminearum* infection, have not been found. New evidence indicate that content and composition of cell wall polymers can play a role in the outcome of host-pathogen interactions.

The aim of this work was to study FHB resistance in a newly-developed durum wheat segregant Recombined Inbred Lines (RIL) population obtained by crossing the bread wheat line 02-5B-318 and the durum cv. Saragolla, respectively FHB resistant and susceptible. The RILs were evaluated for FHB resistance and bio-agronomic traits in replicated field trials carried out in Valenzano (BA) and Bologna for two years, recording incidence and disease severity after infection.

A genetic linkage map consisting of about 5,000 SNP markers from a 90K Illumina Infinium array, was developed in order to perform a QTL analysis to identify regions involved in the control of this trait. All the SNPs were searched for putative function and the pectin methylesterase gene (*wheat-pme1*) was found involved in FHB resistance. The role of *wheat-pme1* as a candidate gene for *Fusarium* resistance was validated in RT-qPCR expression studies on 02-5B-318 and Saragolla. Expression of *wheat-pme1* was induced by *Fusarium* infection only in the susceptible plants, whereas a down-regulation of the gene was observed in the resistant plants. These results suggested that the more pronounced de-methylesterification of cell wall pectin observed during *Fusarium* infection in the susceptible Saragolla could be associated to the activity of this enzyme, and indicated *wheat-pme1* as a possible susceptibility factor involved in *wheat-Fusarium* pathosystem.

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MAPPING YIELD AND YIELD-COMPONENT QTL IN A DURUM WHEAT (*TRITICUM TURGIDUM* SSP. *DURUM*) MULTI-PARENTAL CROSS POPULATION

Sara G. Milner¹, Marco Maccaferri¹, Bevan E. Huang², Elisabetta Frascaroli¹, Paola Mantovani³, Andrea Massi³, Roberto Tuberosa¹, Silvio Salvi¹

1 DipSA - University of Bologna, Bologna, Italy

2 CSIRO, Food Futures Flagship and CMIS, Brisbane, Australia

3 Società Produttori Sementi Bologna, Argelato, Italy

Multiparental cross designs for mapping quantitative trait loci (QTLs) in crops are efficient alternatives to conventional biparental crosses because they exploit a broader genetic basis and are characterized by higher mapping resolution. We assembled a recombinant inbred population (338 F_{7:8} RILs) in durum wheat (*Triticum durum* Desf.) from a balanced four-way cross of four elite cultivars (Neodur, Claudio, Colosseo and Rascon/Tarro. NCCR). NCCR was phenotyped in four-environment field trials for 18 traits, including yield, yield-components, morpho-physiological and quality traits. A linkage map spanning 2,663 cM and including 7,594 single nucleotide polymorphisms (SNPs) was produced by genotyping 338 RILs with a wheat-dedicated 90K SNP array. QTL analysis enabled us to detect 62 QTLs. Two QTLs for grain yield were mapped across environments and 23 QTLs for grain yield components. A novel major QTL for number of grain per spikelet/ear was mapped on chr 2A and shown to control up to 39% of phenotypic variance. Despite the four founders, an average of only 2.1 different functional haplotypes were estimated per QTL. More generally, our QTL results confirmed the importance of relatively minor and environment-specific QTL in controlling grain yield in the elite durum wheat germplasm.

EXPLOITING THE GENETIC VARIABILITY OF *TDDRF1* GENE: A NOVEL FUNCTIONAL MARKER ASSOCIATED WITH YIELD IN DROUGHT CONDITIONS

Galeffi P. *, Cantale C. *, Di Bianco D. */**, Thiyagarajan K. * and Ammar K. **

* ENEA, Italy;

** CIMMYT, Mexico;

In the present study, polymorphisms of the *Triticum durum* dehydration responsive factor 1 (*TdDRF1*) gene were used to develop a functional marker.

The transcription factors are considered among the best candidate genes for developing functional markers, since they are components of the signal transduction pathways that coordinate the expression of several downstream genes.

TdDRF1 is a drought related gene belonging to the DREB gene family codifying for transcription factors. It was isolated in durum wheat and fully characterized. The characterization included the expression of its transcripts, the evaluation of polymorphisms in a large number of durum wheat varieties, phylogenetic studies involving ancestors and related species, a possible relationship between an identified transposon encompassing the coding sequence and the gene evolution.

During the marker characterization, the two copies of the gene in the two A and B chromosomes were isolated and sequenced, thus obtaining a panel of selective primers able to distinguish the two gene copies. Furthermore, based on IWGSC Sequence Repository at URGI, the gene was assigned to the long arm of Chromosome 1 in both genomes.

SNPs in the gene sequence were used for developing a new functional marker related to agronomical traits. Agronomic data from a recombinant inbred line population of durum wheat grown during two seasons, under different environmental conditions, were used for analysis. A significant association between a specific polymorphism in the *TdDRF1* gene and the grain yields in drought conditions was observed.

Thus, a novel functional molecular marker –drought and yield related- was identified. After the required validation, it could be used in future breeding programmes aimed at improving yields in drought conditions.

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A Sustainable Durum Wheat Chain for Food Security and Healthy Lives

CHALLENGES AND OPPORTUNITIES FOR SUSTAINABLE PRODUCTION OF DURUM WHEAT IN THE MEDITERRANEAN BASIN.

Pierluigi Meriggi

Horta Srl, Piacenza

In agriculture, a cropping system is sustainable when it guarantees the needs of the present without compromising the ability of future generations to meet their own needs. Therefore, the exploitation of natural resources (soil fertility, genetic resources, mineral fertilizers, water, etc.) have to be economically feasible, socially acceptable, as well as compatible with the maintenance of ecological processes and ecosystems.

Nowadays many are the instruments available to move from a conventional to a sustainable agriculture; they range from the application of innovative agriculture practices, the use of Information and Communication Technology, new approaches of integrated Farm/Crop/Pest Management and the implementation of tools evaluating cropping sustainability performances. Their integration will guarantee resources's conservation, trade competitiveness and it will enhance the quality of life for farmers and society over the long term.

The latter instruments are not a recommendation for the future, but a commitment for the present. Actually, the development of this specific recipe for ensuring sustainability is regulated by a European comprehensive legislation framework. Since the '90s, the Regulation concerning the placing of plant protection products on the market (Regulation 1107/2009/EC), the Directive about the machinery for pesticide application (Directive 127/2009/EC), the Regulation concerning statistics on pesticides (Regulation 1185/2009/EC), the Directive for community action in the field of water policy (Directive 60/2000/CE), the Nitrates Directive (Directive 676/1991/EC), the Directive establishing a framework for Community action to achieve the sustainable use of pesticides (Directive 128/2009/EC), the Directive concerning the environmental impact assessment (Directive 42/2001/CE) and the Common Agriculture Policies of 2007-2013 and 2014-2020 have headed toward the contemporary sustainability principles.

This holistic approach, based on the strong interconnection between the three pillars of sustainability, is the challenge of up-to-date agriculture. Some national and international authorities and corporations, to pursue sustainability, have implemented protocols and frameworks to monitor sustainability and measuring progresses.

Horta's proposal for durum wheat is based on implementation of web-based Decision Support Systems (DSSs) for strategic and tactical decisions and the evaluation of sustainability by indicators-based holistic frameworks.

A DSS helps farmers and agricultural advisors on decision-making for cultivation during crop season according to the complexity of the environment (soil, pathogens, weeds, nutrients and insects) and the weather patterns, following the principles of sustainable agriculture. Indeed, a DSS integrate environmental data, crop growth, and mathematical models to provide the users with useful information for crop management and protection.

The DSS granoduro.net® is an example of interactive support system, allowing a more rational use of external inputs (varieties, fertilizers, pesticides, etc.) in terms of both quality and quantity, in such a way to save natural resources and increase economic and social returns for farmers and community.

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This DSS was developed and field validated with a project ensuring the quality of this innovative approaches to sustainable cultivation of durum wheat in Italy.

The method represents an example how to put the theoretical paradigms of sustainability into effect by transforming them into practical recommendations for implementing more sustainable agricultural practices.

To assess sustainability performance, this DSS was connected with a economic and environmental footprint calculator assessing Carbon Footprint, Water Footprint, Ecological Footprint, Net Income, and Nitrogen Efficiency. The footprint calculator will be upgraded into 2015/2016 with other indicators regarding six ecosystem compartments: health, air, soil, biodiversity, energy, and water. This will ensure a holistic perspective of environmental sustainability progresses.



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PASTA QUALITY FACES THE EU'S SUSTAINABLE AGRICULTURE CHALLENGE

Marie-Hélène Morel

UMR « IATE- Ingénierie des polymères et Technologie Emergentes », INRA, Montpellier, France

Durum wheat is a traditional cereal crop of the Mediterranean countries. Compared to common wheat its distinguishing feature is almost exclusively used for human consumption in the form of semolina, pasta and couscous. In this context, demands of the durum wheat processing industries are of utmost importance and drive the agricultural practices. Industries target on fully vitreous grains of yellow-amber colour with high protein content. For the farmers, reaching durum grain quality standards mainly rely on appropriate crop management techniques and especially on fertilization inputs. Durum wheat production is thus demanding and potentially could have high environmental impact in case of nitrogen leaching from soils. Submitted to several abiotic stresses, durum yield is in average low to medium compared to other crops. Until a couple of years ago, this was not a problem for the farmers from south of France. Indeed in the years 2000's durum was marketed at a high price and farmers especially those from the south of France received financial supports from the EU's common agricultural policy (CAP). Compared to common wheat, the market price was so high that durum growing increase in central France, traditionally more focused on common wheat production. Because of milder climatic conditions higher yield were achieved compared to south France, while grain quality was secured by increasing nitrogen supplies. Since that time the landscape of durum production in France has profoundly changed. Starting from 2003 the automatic price support from CAP was cancelled and since 2008 emphasis has been placed on environmental concerns with an increasingly regulated use of nitrogen fertilizers and pesticides. A direct consequence of these changes has been the progressive decline in durum-wheat growing lands by 20% in EU-27 over the past 10 years. In France between 2012 and 2014, the durum wheat land occupancy dropped by 33% while production fell by 40%. In order to reverse this trend before it threatened the economic position the French durum wheat sector, French public agronomic research institutes launched the "Plateforme Blé Dur" a brainstorming group gathering all the actors of the durum sector. During this presentation, the key strategic orientations adopted by the "Plateforme Blé Dur" and the recently launched research program "Dur-Dur" funded by the French National Research Agency in 2014 will be detailed. The objective of Dur-Dur is to deliver sustainable and innovative agronomic and technological solutions towards securing the French durum wheat sector, from the farmers to the end-users.

MYCOTOXINS IN DURUM WHEAT AND STRATEGIES FOR THEIR REDUCTION

Antonio Moretti and Antonio Logrieco

Durum wheat is a crop that can be frequently attacked at global level by several fungal species able to produce a wide range of toxic metabolites, mycotoxins, harmful toward plant, animal and humans. The mycotoxins can be produced *in planta* since the first stages of fungal colonization. However, the most worrisome aspect of their production is the accumulation of mycotoxins in the kernels at the maturity. The occurrence of many mycotoxins in the durum wheat kernels is regulated at European level and, for durum wheat, the most important and spread are the mycotoxins produced by species of the genus *Fusarium* that are the main casual agents of the complex disease called Fusarium Head Blight (FHB). Several species of *Fusarium* are involved in this disease causing mainly the accumulation in the kernels of deoxynivalenol (DON), the most common mycotoxin detected in the durum wheat in Italy, and several other *Fusarium* mycotoxins such as nivalenol and T-2 and HT-2 toxins. Since each of these species has its own mycotoxin profile, it is crucial to correctly identify them in order to evaluate the risks associated to their occurrence on wheat. Moreover, several factors can influence the development of FHB on wheat: environmental parameters, geographic areas, year of harvest, and agronomic choice (e.g. use of tillage, crop rotation, and variety selection). An appropriate management of the agronomic factors can dramatically contribute to a significant reduction of mycotoxin risk in the durum wheat. In the presentation, the main pathways for a correct management of durum wheat crop aimed at the reduction of mycotoxin risk in pre-harvest will be presented by emphasizing the main agronomic choices addressed to minimizing the occurrence of *Fusarium* mycotoxins in the kernels.

RELATIONSHIP BETWEEN GRAIN PROTEIN CONTENT, GRAIN YIELD AND NITROGEN USE EFFICIENCY ASSESSED IN TUNISIAN DURUM WHEAT GENOTYPES

Ayadi S.¹, Karmous C.^{1,2}, Chamekh Z.¹, Hammami Z.¹, Rezgui S.¹ and Trifa Y.¹

1 National Institute of Agriculture of Tunis, 43, Avenue Charles Nicole, 1082 Tunis, Tunisia.

2 Department of Plant Sciences, Agricultural High School of Mateur, Tunisia.

In Tunisian durum wheat (*Triticum turgidum* ssp *durum*), grain protein content (GPC) is among the important factors influencing pasta-making quality. Semolina with high protein content produces pasta with increased tolerance to overcooking and greater cooked firmness. Therefore, GPC is influenced by several factors including nitrogen remobilization from vegetative organs and direct post-anthesis nitrogen uptake (Nup) from the soil. Understanding the relationship between GPC and nitrogen use efficiency (NUE), will enable durum wheat breeders to develop cultivars that not only produce high grain yield (GY) and high GPC, but also exhibit better NUE. A study was carried out at the experimental site Mateur in the sub-humid area of Tunisia during two cropping season 2009/10 and 2010/11. Four wheat genotypes were evaluated during these cropping seasons for nitrogen use efficiency compounds and GPC using 0, 50, 100, 150, 200 and 250 kgN/ha. Results indicated a significant interaction effect ($p \leq 0.001$) Year \times genotype \times N treatment for grain yield (GY), grain protein content (GPC) and nitrogen use efficiency (NUE). GPC was the most affected trait by nitrogen applied showing an increase of 25% under high N treatments (250 kgN/ha) compared to control plots without N treatments. A significant linear regression exists between GPC and NUE for the different N levels ($R^2 = 0.80$; $p < 0.001$). This effect was more pronounced for improved cultivars than landraces for all parameters. We conclude that NUE can be used to evaluate GY and GPC relations and to assess genotypic variation under different application of Nitrogen.

Keywords: Grain yield, grain protein content, nitrogen use efficiency

DISTRIBUTION, SURVIVAL AND GROWTHS RATE OF *PYRENOPHORA TRITICI REPENTIS* ON DURUM WHEAT UNDER CONSERVATION AGRICULTURE AND CONVENTIONAL TILLAGE IN TUNISIA

Manel Othmeni¹, Noura Omri¹, Med. Salah Gharbi¹, Mouhamed Jadlawi² and Amor Yahyaoui³

1 Institut National de Recherche Agronomique de Tunisie (INRAT) Tunisia

2 Institut National des Grandes Cultures (INGC)

3 International Maize and Wheat Improvement Center (CIMMYT), Mexico.

In Tunisia, the complex of foliar diseases has increased over the last decades due to continuous wheat cultivation and to shifts toward Conservation tillage practices. The prevalent durum wheat disease complex includes Septoria tritici blotch, spot blotch, and tan spot of wheat also known as yellow leaf spot, caused by the fungus *Pyrenophora tritici-repentis* (*Ptr*). This study addresses the survival and reproduction of *Ptr* on infected wheat straw and its subsequent spread by different types of spores. The fungus pseudothecia survives on host residue, which is considered the main source of primary inoculum. This report highlights the survival and growth rates of *Ptr* on wheat stubble under different agronomic practices. The information on the saprophytic survival of the pathogen could set up the level of parasitic damage of *Ptr* on the following wheat crop. Various levels of correlations were detected between survival rate of the pathogen under the two cultivation systems and the onset of the parasitic stage following exogenous and indigenous primary inoculum. The Survival rate of the fungus varied from 25 to 97% in fields where conservation agriculture has been practiced for 2 to 10 years in North and North western Tunisia. Survival rate at fields under conventional tillage was lower but not significantly different; it varied from 7 to 90%. Survival of *Ptr* on two- year old stubble and on clean field (straw plowed under) was significantly reduced to 25 and 7% respectively. The average growth rate of *Ptr* isolates collected from the same area was faster from conservation agriculture samples than that of conventional tillage. Infection rate is assessed and will be discussed. These findings could play an important role in the control of the disease; particularly in areas where continuous wheat cultivation is practiced, susceptible cultivars are grown, and minimum or no tillage is practiced.

REDUCED NITROGEN FERTILIZATION EFFECTS ON GRAIN CHARACTERISTICS AND END-USE QUALITY IN EIGHT DURUM WHEAT CULTIVARS

F. Lacoudre, C. André, D. Audigeos, C. Besombes, P. Braun, D. Cassan, A. Chiron, M. Cochard, J.C. Dusautoir, J.P. Kessler, M. Killmayer, T. Lefevre, J.P. Legoff, J.P. Leygue, P. Lonnet, G. Maraval, B. Ristuccio, M.F. Samson

Environmental rules and fertilizer price increase have pushed farmers to reduce nitrogen fertilization applied to the durum wheat crops. In order to optimize nitrogen application, cultivation practices such as splitting were promoted. These practices are not always sufficient to maintain elevated yield and grain quality required by milling and pasta making processes. The need to create cultivars with better nitrogen use efficiency is essential. Currently, for cultivar registration in the French national list, varieties are not tested under reduced fertilization conditions and it appears difficult to detect those which have the capacity to better use nitrogen. The objective of breeders is now to create new durum wheat cultivars able to grow up with limited nitrogen intake and to remobilize efficiently nitrogen from vegetative organs of the crop to grains during filling with the need to fulfil quality criteria required by pasta making industries. To achieve this goal a study was carried by French breeders associated with public research, development organizations and industrials in order to define minimal requirements in terms of protein quantity and quality to produce pasta able to satisfy consumers. Eight cultivars were grown during two years in the south of France with nitrogen intakes varying from 0 to 360 units. At harvest, grain characteristics (thousand kernel weight, specific weight, yellow berry...) were recorded and milling value was assessed with a micromill. Protein composition measured by chromatography and content were also evaluated along with gluten index, SDS sedimentation, tenacity. Semolina were produced and used to make pasta on a pilot plant. Pasta quality was appraised by different tests before and after cooking. A statistical analysis was then performed and shown that it is possible to reduce nitrogen fertilization to some extent without affecting dramatically pasta quality. Data also provide information useful to reconsider registration tests.

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THE PHENOTYPIC ARCHITECTURE OF TETRAPLOID WHEAT (*TRITICUM TURGIDUM* L.): EFFECTS OF DOMESTICATION AND POST-DOMESTICATION UNDER CONTRASTING NITROGEN FERTILISATION

Tania Gioia, Kerstin A. Nagel, Romina Beleggia, Mariagiovanna Fragasso, Donatella Bianca Maria Ficco, Roland Pieruschka, Pasquale De Vita, Fabio Fiorani, Roberto Papa

The process of domestication has led to dramatic morphological and physiological changes in crop species due to adaptation to cultivation and to the needs of farmers. To investigate the phenotypic architecture of shoot- and root-related traits and quantify the impact of domestication and post-domestication, we examined a collection of 36 wheat genotypes under optimal and nitrogen-starved conditions. These represented three taxa that correspond to key steps in the recent evolution of tetraploid wheat (i.e., wild emmer, emmer, and durum wheat). Overall, nitrogen starvation reduced shoot growth of all genotypes, while it induced the opposite effect on root traits quantified using the automated phenotyping platform GROWSCREEN-Rhizo. We observed an overall increase in all shoot and root growth traits from wild emmer to durum wheat, while emmer was generally very similar to wild emmer, but intermediate between these two subspecies. While the differences in phenotypic diversity due to the effects of domestication were not significant, the post-domestication transition from emmer to durum wheat was marked by a large and significant decrease in the coefficient of variation. In particular, this reduction was very strong at optimal condition, and less intense under nitrogen starvation. Moreover, while at optimal condition both root and shoot traits showed significantly reduced diversity due to post-domestication, under nitrogen starvation the reduced diversity was significant only for shoot traits. Overall, a considerable amount of phenotypic variation was observed in wild emmer and emmer which could be exploited for the development of pre-breeding strategies.

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COMPARATIVE RESPONSE OF YIELD, YIELD COMPONENTS AND PHYSIOLOGICAL TRAITS IN DURUM WHEAT UNDER CONTRASTING WATER IRRIGATION

Zoubeir Chamekh, Sawsen Ayadi, Chahine Karmous, Youssef Trifa, Ali Sahli, Hajer Amara and José Luis Araus

Grain yield, plant height, biomass, ion accumulation, and the natural abundance of the stable isotope compositions of carbon ($\delta^{13}\text{C}$) and nitrogen concentration of mature kernels were measured in 25 durum wheat genotypes (five landraces and five modern cultivars) growing in three semi-arid sites differed by their irrigation water salinity: Echbika (S1, 6 dS m⁻¹), Barrouta (S2, 12 dS m⁻¹) and Sidi Bouzid (S3, 18 dS m⁻¹). Water salinity was the main environmental factor affecting yield and $\delta^{13}\text{C}$. Compared with control site (S1) all other treatments (S2 and S3) decreased grain yield. The genotypic effect was significant for all measured traits. A significant linear regression exists between grain yield at control site S1 and grain at saline site S2 ($R^2 = 0.79$; $P < 0.001$) and saline site S3 ($R^2 = 0.36$; $P < 0.001$). Plant height, biomass and nitrogen concentration were correlated with grain yield. However, the trait that correlated best with genotypic differences in grain yield was $\delta^{13}\text{C}$. This trait was the first variable chosen at each of the two salinity levels in a stepwise analysis.

Keywords: *Grain yield; durum wheat, salt stress, $\delta^{13}\text{C}$*

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NEW APPROACHES TO DEAL WITH COMPLEX GENOMES SEQUENCING: THE CASE OF DURUM WHEAT

Sergio Lucretti

Many important food crops such as wheat, barley, rapeseed, potato, strawberry, are polyploids and made of more and somewhat diverse homeologous chromosome copies. Genomics tools are more apt on haploid and diploid organisms than on complex genomes. Large genomes and/or high levels of heterozygosity require high sequencing data coverage, big computational resources and are tough to phase (individually deciding which single chromosome variants occurs). Plus, many plant genomes are highly repetitive and Next Generation Sequencing data could not just fit those long repetitive sequences into NGS small reads, demanding for long DNA libraries. An original genome complexity reduction strategy has been applied to durum wheat, using a “flow molecular cytogenetic” approach. This is built on Fluorescent *In Situ* Hybridization In Suspension (FISHIS) labeling of durum wheat chromosomes by microsatellites and repeated sequences combined with flow cytometry analysis and sorting. We have applied FISHIS to the glorious durum wheat Senatore Cappelli, obtaining the bulk-separation of the A- and B- sub-genomes resolving the polyploidy-problem, thus allowing preparation of Illumina libraries for each sub-genome. Attempts have been made to obtain long span mate-pairs and a whole genome optical map using HMW gDNA. An assembly of the A genome has been generated and is being annotated using RNA-Seq data. We point to obtain high quality (L50 \geq 300 kb), annotated assemblies of both A and B genomes from a valuable wheat variety. These data will be useful for re-sequencing modern varieties and wild relatives, and “to accelerate the development of improved varieties and to empower all aspects of basic and applied wheat science” (ref. IWGSC and Intn. Durum Wheat Sequencing Initiative). FISHIS proved already to be effective in a number of plants (e.g. *Triticale*, *A. sativa*, *T. monococcum* and *D. villosum*) virtually extending the WGS² (Whole Genome Sequencing by Sorting) approach to other large genome crops.

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INTEGRATED DURUM BREEDING FOR WATER STRESS TOLERANCE IN AUSTRALIA: THE POTENTIAL OF MIRNAOMICS

Liu H, Searle I, Watson-Haigh NS, Baumann U, Mather DE, Able AJ & Able JA

Significant yield losses, due to the lack of rainfall in early reproductive stages are a major concern for durum breeders in Mediterranean environments. MicroRNAs are endogenous small noncoding RNAs that are known to play critical roles in plant abiotic stress responses. Genotypic differences in the miRNAome between water deficit tolerant and sensitive durum varieties, particularly under water deficit stress will be described. From an initial panel of 20 durum wheat genotypes, water deficit stress tolerant (Tamaroi and Yawa) and sensitive (Bellaroi and Tjilkuri) varieties were identified by evaluating various yield, morphological and physiological responses to pre-anthesis water deficit stress. A total of 96 small RNA libraries were then constructed from flag leaf and developing head tissues of these four durum varieties. Next generation sequencing and subsequent statistical analysis has provided the first indication that many conserved and novel microRNAs show differential expression patterns in response to water deficit stress during the early reproductive stages in durum. MicroRNAs were also differentially expressed across durum genotypes with different levels of stress sensitivity or between different tissues. Expression profiling of a selected group of stress responsive miRNA were confirmed by qRT-PCR, while northern blotting validated the presence of novel miRNAs. The present study provides insight into potential miRNAs that can regulate determinants of tolerance to water deficit stress in durum. Further functional analysis of stress responsive miRNAs and their interaction with mRNA targets will assist in efforts to develop durum germplasm with water deficit stress tolerance.

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COMPARATIVE ANALYSIS OF DROUGHT-RESPONSIVE MICRORNAS OF WILD EMMER AND MODERN WHEATS

Hikmet Budak

Molecular Biology, Genetics & Bioengineering Program, Faculty of Engineering and Natural Sciences, Sabanci University, Istanbul, Turkey

Wheat has been thoroughly studied in terms of its miRNA repertoire and miRNA expression profiles under various conditions. Despite the extensive genetic diversity of wild germplasms and landraces, miRNA studies have been limited to modern durum or bread wheats so far. We compared the miRNA repertoires of modern durum wheat and its wild relatives under control and drought conditions through RNA-Sequencing. The modern durum variety, *Triticum turgidum* ssp. durum var. Kızıltan has a moderate drought tolerance, while its wild relatives, *Triticum turgidum* ssp. dicoccoides genotypes TR39477 and TTD-22 exhibit extreme tolerance and sensitivity against drought, respectively.

Transcriptomes of these three genotypes revealed a total of 66 miRNAs expressed in control and drought conditions; of which 46 and 38 had not been previously reported in modern and wild wheats, respectively. In particular, genotype- and/or stress-specific miRNAs expand our understanding of the miRNA regulated drought response and provide candidates to be exploited in wheat breeding programs.

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SEQUENCE-BASED GENETIC MAP USING DURUM WHEAT × WILD EMMER WHEAT POPULATION

Assaf Distelfeld, Raz Avni, Moran Nave, Inbal Hamerman, Omer Barad, Kobi Baruch, Gil Ronen and Abraham Korol

Genome sequencing using whole-genome shotgun strategy has difficulty to provide a linear order of contigs along individual chromosomes, especially in complex genomes. It was recently shown that sequencing progeny of segregating populations in barley and wheat allowed de-novo production of genetically anchored linear assembly of the gene space. As a step towards achieving a functional genome assembly of tetraploid wheat we hybridized wild emmer wheat (*T. turgidum* ssp *dicoccoides*, genomes BBAA) with durum wheat (*T. turgidum* ssp *durum*, cv. Svevo), and developed an F6 recombinant inbred line (RIL) population. Then, we combined data from the wheat 90K iSelect SNP genotyping assay along with a novel genotyping by sequencing (GBS) approach that requires relatively low sequence coverage (0.02×). Novel hierarchical ordering procedure enabled us to map 939,536 markers clustered to 3,732 recombination bins that represent the 14 chromosomes of tetraploid wheat. Next, we used few approaches to validate the order and integrity of our ultra-dense genetic map. For example, we compared all the short sequences produced by the GBS procedure to the recent draft sequence of the wheat A genome progenitor *T. urartu* and anchored 26,116 scaffolds to the seven A genome chromosomes of tetraploid wheat. The total length of the anchored *T. urartu* scaffolds was ~2Gb (~40% of *T. urartu* genome) corresponding to 104,191 A genome markers in our map (~24% of total A markers). These results demonstrate the efficiency and utility of our approach for improving the construction of functional draft linear assembly of complex genomes.

GENOTYPING BY TARGETED SEQUENCE CAPTURE IN DURUM WHEAT: APPLICATION TO DETECT QTL FOR WHEAT SPINDLE STREAK MOSAIC VIRUS RESISTANCE

Yan Holtz¹, V. Viader², S. Santoni², M. Bonnefoy³, D. Gouache³ and J. David¹

1 Montpellier SupAgro, UMR Amélioration Génétique et Adaptation des Plantes, 2 place Viala, 34060 Montpellier, France

2 INRA, UMR Amélioration Génétique et Adaptation des Plantes, 2 place Viala, 34060 Montpellier, France

3 Arvalis, institut du vegetal

Targeted sequence capture is a promising technology, which enables to lower the costs for sequencing numerous genomic regions on large sets of individuals. Bait sequences can be designed to capture alleles already discovered between parents or reference population in previous experiments. In our case, we studied a set of 184 RIL originating from the cross of a WSSMV resistant emmer cultivar 'Dic2' and a *durum* recent elite cultivar 'Silur'. Six thousands of sequence baits were designed on Dic2 vs. Silur polymorphisms discovered in a RNAseq study. These baits were exposed to the genomic DNA of the RIL population.

Here we report the results of this target enrichment for a tetraploid specie such as durum wheat. Data were integrated together with a set of 800 Darts and SSR markers to build a high quality genetic map of *Durum* Wheat. Finally, a QTL analysis was conducted for the resistance to Wheat Spindle Streak Mosaic bymovirus (WSSMV). The population was confronted with the WSSMV and observed for several years. Phenotyping was composed of several visual notations and one ELISA test each year. This study releases chromosomal zones and markers linked with the resistance to this virus.

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NOVEL AND CONSERVED MIRNAS INVOLVED IN PLANT DEVELOPMENT AND NITROGEN METABOLISM IN DURUM WHEAT

Diana L. Zuluaga, Domenico De Paola, Donatella Danzi, Michela Janni, Gabriella Sonnante

Institute of Biosciences and Bioresources, National Research Council, Bari, Italy

MicroRNAs (miRNAs) are small non-coding RNA molecules which down-regulate gene expression by mRNA degradation or translational repression. miRNAs play critical roles in diverse biological processes such as plant development and plant adaptive responses to nutrient deprivation. Nitrogen is the most important nutrient for the growth of plants since it is essential for protein and nucleic acids synthesis, and for other cellular constituents. A limitation of nitrogen in durum wheat plants generally results in a reduction of the yield and of protein quality, with negative effects on the attitude of pasta making.

In order to elucidate the role of miRNAs in durum wheat development and nitrogen metabolism, twelve small RNA libraries were generated from different tissues and developmental stages of Ciccio and Svevo cultivars growing under different nitrogen supply conditions. Following high throughput sequencing, conserved and novel miRNAs and their target genes were identified. Several miRNAs were expressed at different levels based on their abundance in Illumina libraries. Selected conserved and novel miRNAs and their corresponding target genes were subjected to quantitative PCR, and for some of them significant differences in expression profiles were confirmed.

Some miRNAs were predicted to regulate genes involved in plant development, in important biochemical processes such as photosynthesis, and in nitrogen metabolism.

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WILD EMMER WHEAT NATURAL POPULATIONS DISPLAY HIGH SEQUENCE CONSERVATION OF THE BROAD SPECTRUM STRIPE RUST RESISTANCE GENE *YR36 (WKS1)*

Lin Huang¹, Tamar Kis-Papo¹, Hanan Sela², Lihua Feng¹, Tamar Krugman¹, Jorge Dubcovsky³, Tzion Fahima¹

1 Institute of Evolution and Department of Evolutionary and Environmental Biology, University of Haifa, Haifa 31905, Israel

2 Institute for Cereal Crops Improvement, Tel-Aviv University, Tel Aviv 69978, Israel

3 Department of Plant Sciences, University of California, Davis, CA 95616, USA

Natural wild emmer wheat (*Triticum dicoccoides*) populations are dispersed over a wide range of habitats in Israel and vicinity, where they are challenged by the devastating stripe rust disease of wheat, caused by the pathogenic fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*). During the long history of plant-pathogen coevolution, wild emmer populations developed various strategies to cope with stripe rust attacks. One of them is exhibited by the wild emmer gene, *Yr36 (WKS1)*, previously cloned by our group, which confers partial resistance to a broad spectrum of stripe rust races. *WKS1* shows a unique gene architecture combining a kinase and a START lipid-binding domains which is found only in the Triticeae tribe. The analysis of the sequence variation and distribution of *WKS1* in accessions from a broad range of wild emmer natural populations revealed that *WKS1* is present only in the Southern distribution range of wild emmer natural populations in the Fertile Crescent (Israel, Jordan, Syria, and Lebanon). The distribution of *WKS1* in Israel was found to be clustered around the Sea of Gallilee in the northern part of Israel. Analysis of full length *WKS1* (>7 kb) from 47 wild emmer wheat accessions identified very low nucleotide diversity ($\pi = 0.0002$), with a higher nucleotide polymorphism in the intron regions of *WKS1* than in the coding regions. Analysis of the coding region of *WKS1* revealed only four haplotypes among all tested accessions, encoding three different putative *WKS1* proteins (designated P1, P2, and P3) in addition to the functional *WKS1* (P0). Stripe rust infection tests showed that partial resistance response was displayed by 43 accessions that carried the P1 haplotype of *WKS1* and the two with P3, while only one accession carried P2 haplotype and was susceptible to stripe rust. These results suggest that the *WKS1* proteins P0, P1 and P3 may be functional in conferring resistance to the stripe rust disease and can be utilized for wheat improvement.

FROM SEED TO PASTA & BEYOND

A Sustainable Durum Wheat Chain for Food Security and Healthy Lives

IDENTIFICATION OF A NOVEL DOMESTICATION LOCUS ON WHEAT CHROMOSOME 4B CONTROLLING UNIFORMITY OF GRAIN DIMENSIONS AND GERMINATION

Moran Nave¹, Raz Avni¹, Iago Hale², Assaf Distelfeld¹

In the wild, seed dormancy can increase the probability of progeny survival under unstable environmental conditions. In crop wild relatives, patterns of within-plant variation in seed dormancy are associated with both grain dimension differences as well as differences in position within the spikelet. In wild emmer, for example, smaller grains, located lower within the spikelet, exhibit deeper dormancy than the larger, upper grains which germinate readily. Here, we elucidate the genetic control of differential grain dimensions and dormancy within spikelets of tetraploid wheat (*Triticum turgidum* L.). The genetic dissection of within-spikelet variation in grain dimensions and dormancy was performed using a RIL population (wild emmer x durum wheat) and a high-density genetic map based on the Infinium 90K SNP chip array. Phenotypic data from two field experiments were used for quantitative trait locus (QTL) analysis. We identified a single major locus on the long arm of wheat chromosome 4B that explains more than 40% of the observed variation between the wild and domesticated parents in terms of differential grain dimensions and seed dormancy within spikelets. Our results show that in wild emmer, the lowest grain within the spikelet is about 30% smaller and takes significantly longer to germinate compared to the larger, upper grain that germinates usually within two days. These differences are unaffected by spikelet position along the spike or seed coat characteristics. The domesticated variant of this novel QTL on chromosome 4BL, probably fixed during the process of wheat domestication, favors spikelets with uniform grain size and germination.