



**3rd Conference of Cereal  
Biotechnology and Breeding**

November 2–4, 2015 • Berlin, Germany

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Cereal Biotechnology  
and Breeding / CBB3**

**November 2–4, 2015  
Berlin, Germany**

**Embassy of Hungary  
Unter den Linden 76, 10117 Berlin, Germany**

ISBN 978 963 05 9668 8

# Keynote Abstracts

## HEALTH RELATED QUALITY ATTRIBUTES OF WHEAT

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During the last one and half century the attributes for evaluating wheat quality significantly differentiated and expanded.

In recent years the new challenges originated from the demands of the industry and the consumer, altered the traditional priorities of quality related research and development in two directions. In the industry it become essential to implement processes capable to reduce the production cost of the end-products and therefore to use source materials, new genotypes which are suitable for these technological challenges. New requirements such as the need for wheats with less mixing energy requirement but stable, strong dough, or the increased demand for higher water absorption are good examples for this industry-driven trends. The other major revolutionary change in the term 'quality' is caused by the consumer's strong demand to get healthier, more nutritious but low-calorie containing baked goods in a wide product range satisfying even those individuals who are sensitive to gluten containing products.

This latter requirement forces the quality-improving professionals to characterize wheat proteins on a completely different level with the contribution of plant biology, including genomics, proteomic and bioinformatics studies, food science and technology moreover with the assistance of medical research. The types of the wheat sensitivity disorders with different mechanism but the same or similar symptoms are established recently, furthermore the amounts of the wheat proteins with the triggering toxic or allergen epitopes are much more clarified as the results of the intensive proteomic research in this area. In this context the extent of the toxicity/allergic effect of the wheat samples, the genetic background of the diseases and their prevalence also subjects of intensive research activity. It seems that cereal sensitivity is beyond cereal proteins but it is often directly related to carbohydrate composition. Completely new manufacturing and product development areas have been evolved for gluten-free bakery product manufacturing. Intensive research investigations are carried out regarding to seeking or to develop new wheat genotypes, wheat analogues at both levels of basic and applied research. According to the clinical results, for patients suffering from the most severe wheat caused disease (celiac disease) the only treatment is the gluten-free diet, while most of those who are just sensitive to wheat-made products are able to tolerate products manufactured from certain *Triticum spelta* genotypes.

An overview over these research achievements and the directions for further development is given.

## **VIRULENCE DYNAMICS AND GENETIC VARIATION IN PUCCINIA TRITICINA AND APPROACHES FOR DURABLE LEAF RUST RESISTANCE IN WHEAT**

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*Puccinia triticina*, the wheat leaf rust fungus, is widely distributed throughout the world on common wheat and durum wheat. Losses due to leaf rust can be significant if moisture and temperature conditions are suitable and if the wheat cultivars lack effective resistance. In the USA, over 50 races of *P. triticina* are identified on an annual basis. Wheat cultivars with seedling effective, race specific resistance genes have not provided long lasting resistance. In recent years wheat cultivars with genes *Lr17*, *Lr18*, *Lr39/41*, and *Lr21* have selected races with virulence to these genes, eroding the resistance of many cultivars. *P. triticina* populations in North America and worldwide were genotyped with 23 SSR markers to determine the genetic diversity of *P. triticina* within continental regions and the genetic relationship between continents. In North America, there were six groups of SSR genotypes that were genetically differentiated. The number of distinct SSR groups in the continental regions varied from two and three in China and Russia, respectively, to eight in Europe. All populations had a significant correlation of SSR genotypes with virulence, due to the clonal reproduction of urediniospores across different continents. Overall the populations of *P. triticina* from different continents were highly differentiated for SSR genotypes, with only a few SSR groups having similar genotypes. However, isolates with identical virulence and SSR genotypes were characterized from different continents, indicating the long distance transport of *P. triticina* urediniospores. In the USA, spring wheat cultivars with combinations of genes *Lr16*, *Lr23*, and *Lr34* have had long lasting resistance that has remained effective despite rapid changes in virulence in the leaf rust race population. Genes *Lr34*, *Lr46*, *Lr67*, and *Lr68* are race nonspecific partial resistance genes that are effective in the adult plant stage. Wheat cultivars with combinations of these genes have had effective durable resistance in North America and in CIMMYT germplasm. Genes *Lr34*, *Lr46*, and *Lr67* also condition resistance to stripe rust, stem rust and powdery mildew. Wheat cultivars with combinations of adult plant leaf rust resistance genes should also have effective durable resistance to these other diseases.

## **CEREALS GENOMICS AND GENOMIC SELECTION – NEW TIME OF MODERN CEREALS BREEDING**

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At the beginning of the 21st Century, mankind faces the dual challenges of providing enough food for a growing population with a background of reduced resources and a more variable climate. In this context, genomics and associated molecular marker technology must play an important role in developing new varieties better adapted to address these challenges. During the last decade, molecular marker technology has provided a wide range of novel approaches to improve selection strategies and together with the rapid accumulation of genomics tools and the emergence of high throughput technologies has facilitated practical implementation into cereal breeding programmes.

Genetic engineering allows the creation of gene combinations and other modifications not possible through classical breeding methods and when considered together with conventional and marker-assisted precision breeding technologies, provides a powerful set of tools for delivering the advances in efficiency we need. The complete restoration of pollen fertility and reduced ergot infection in hybrid rye and the enhancement of foliar disease resistances in wheat and barley are all prime examples where selection intensity and accuracy can be accelerated by the application of molecular marker technology.

The availability of new molecular tools and technologies is beginning to filter through the breeding programmes to have a significant impact on plant variety development and is proving to be the essential element required to accelerate this process. The results of specific applications of molecular markers, potential of genomic selection and the application of genomics and genetic engineering in cereals will be presented and discussed.

## **PROGRESS OF WHEAT NITROGEN USE EFFICIENCY AND GRAIN PROTEIN CONTENT**

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Australia is the fifth largest wheat exporter in the world. Due to sandy soil condition and undesirable rainfall distribution across wheat growth period, the Australian wheat is often having low protein problem. A research program was established in 2013 in Australia Murdoch University focusing on raising the wheat grain protein content through increasing the nitrogen use efficiency. A number research projects are currently underway in genetic and non-genetic aspects. The genetic projects including integration of the expressed Glu-Ay subunit into Australian wheat cultivars and survey the NAM gene architecture of both high and low protein germplasms. Currently, The Glu-Ay subunit has been successfully integrated into 8 Australian cultivars. Results revealed that the grain protein content increased by up to 8% and the %UPP by 14% through this integration, indicating that the Glu-Ay subunit significantly increases the grain protein content as well as optimises its components for better bread making. The NAM gene project revealed a highly complex architecture in both group 6 and 2 chromosomes. It can be speculated that simply utilizing the previously cloned GPC-B1 gene may not be adequate in raising the Australian wheat protein content. Our non-genetic approach focuses on utilising sulphur fertiliser to increase the nitrogen use efficiency. Results clearly showed that Under Western Australia conditions certain amount of sulphur fertiliser application can increase the wheat nitrogen use efficiency.

## THE UTILIZATION OF TRITICUM SPECIES IN WHEAT BREEDING IN NORTHEAST CHINA

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**Keywords:** synthetic hexaploid wheat, wheat breeding, *Triticum* species, stress, resistance

Spring wheat is an important crop in meeting the demand of local food habit in northeast China, where is rain-fed agriculture for most crops. The wheat varieties adapted here have to be resistant or tolerant to biotic and abiotic stress, including drought, pre-harvest sprouting, *Fusarium* head blight, spot blotch, powdery mildew, and so on.

In wheat improvement for resistance or tolerance to the stresses, narrow genetic background of crossing parents have been becoming bottleneck as many scientists expected, while number of wheat breeders enjoyed and have been achieved successful in crossing between varieties from similar genetic background or ecological conditions. Synthetic hexaploid wheat derived from tetraploid wheat and *Triticum tauschii*, as well as other *Triticum* species, are carrying numbers of desirable genes. The utilization of those species will definitely broaden the genetic background in wheat improvement, and achieving satisfied resistance or tolerance to the stress, quality as well as yield.

We evaluated the accessions of synthetic hexaploid wheat and *Triticum* species: drought tolerance evaluation were carried out in natural drought condition of Urumuqi (annual precipitation is about 200 mm or less); preharvest sprout tolerance, spot blotch resistance in lab and resistance to FHB and powdery mildew in greenhouse and field nursery. As results, a number of accessions are classified.

Based on the evaluation and classification of the accessions, different crossing formats have been employed to integrate the desirable genes from common wheat and other species of *Triticum*. It seems to us that 75% of common wheat genetic background is reasonable in the utilization of species of *Triticum* in common wheat improvement. In the field selection on progeny of the cross between common wheat and wild species, some disadvantage characteristics like hard glume, less tillers, shrivelled kernel and lodging, can be effectively discarded.

As results, a number of breeding lines performed satisfied tolerance to drought combining high yield potential and ideal plant height. A line derived from synthetics yielded 100% over local check variety in drought condition in Keshan, Heilongjiang, in 2010; a line derived from *T. dicoccoides*, with satisfied FHB resistance and drought tolerance, yielded 15% over local check variety by average of 3-year multi-location tests organized by provincial government. Several lines with short culm but satisfied tolerance to drought have been developed based on the evaluation in Urumuqi, China and Szeged, Hungary. Those secondary synthetics and lines derived from other species of *Triticum* were well evaluated and classified and play important role in wheat improvement in Northeast China as well as other wheat production area.

# Oral Presentations – Abstracts

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## **Biotic and Abiotic Stresses**

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### **RESPONSE OF BARLEY (*HORDEUM VULGARE* L.) TO SALINITY IRRIGATION IN KUWAIT**

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**Keywords:** barley, salinity, abiotic

The climate of Kuwait is characterized by very low annual rainfall and high degree of aridity. Drought stress has an adverse effect on the crops during the critical stages of crop development, and its combined effect with salinity makes the growth more detrimental. The Kuwait Institute for Scientific Research (KISR) aims at developing modern agricultural technologies to improve the production performance of promising crops to meet national food and forage demands. Barley is one of the most adapted cereals, which is grown under saline and drought conditions. Hence, KISR initiated this study in May 2012 to evaluate the performance of barley under the harsh environmental conditions of Kuwait. The physical and chemical properties of the soil were analyzed, and the soil was found sandy with low fertility and exposed to soil erosion. The response of barley lines to brackish water irrigation was evaluated, and the data generated was analyzed to draw satisfactory conclusions regarding the effect of brackish water irrigation on crop growth. Various growth determining parameters such as plant height, number of spikes, number of tillers, kernel weight, leaf area index, chlorophyll index, and grain yield were studied and the data showed that the brackish water decreased all the studied growth parameters significantly. The biochemical analysis of the carbohydrate and protein showed that brackish irrigation caused an increase in total soluble carbohydrates and decrease in protein content at the heading and maturity stages.

### **EFFECT OF ABIOTIC STRESS ON THE PRODUCTION OF DURUM AND BREAD WHEAT UNDER KUWAIT GROWING CONDITIONS**

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**Keywords:** bread and durum wheat, brackish water irrigation, abiotic stress

The scarcity of water resources challenges the agricultural sector in maximizing the forage productivity without degrading the environment and natural resources in Kuwait. The main irrigation water resources available in Kuwait are natural (groundwater) and artificial (desalinated seawater and treated wastewater). In an effort to overcome water shortages and satisfy the increasing water demand for agricultural development, the Kuwait Institute for Scientific Research (KISR) intended to evaluate the performance of bread and durum wheat promising lines under brackish water irrigation in Kuwait. The growth response of twenty promising lines under both fresh and brackish water irrigation was compared in a field experiment. Various growth determining parameters such as plant height, number of spikes, number of tillers, kernel weight, and grain yield were studied. Results showed that brackish water decreased all the studied crop growth and yield parameters significantly. It was observed that durum wheat showed lower grain yield than bread wheat. In addition, it was found that four bread wheat promising lines (12, 17, 11, and 8 lines) were the most tolerant under brackish irrigation, while only one durum promising line (line 19) was most tolerant under saline stress. Further studies are needed to investigate the stability and adaptability of the cultivars to harsh environmental conditions such as brackish water irrigation

## RESPONSE OF HULLED TETRAPLOID WHEAT TO SALINITY

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**Keywords:** ancient wheats, marginal environments, physiology, NaCl

A significant part of the world's land area is salt-affected, to an extent that it exerts negative impacts on the growth of most crops. Salinity may either be naturally present in the soil or it may have been caused by agricultural practices such as irrigation. Since irrigation is a necessity in arid and semi-arid regions, the salinity issue will remain one of the key threats to the global food production. A great degree of diversity exists between plant species in their capacity to tolerate salinity. The presence of diversity between and within cultivated species can be harnessed to explore new germplasm therein to potentiate increases in yield in saline conditions. Durum wheat (*Triticum turgidum* ssp. durum) is less salt tolerant than bread wheat. Hulled wheats are known as the ancestors of the present day modern tetraploid and hexaploid wheat cultivars. These wild and domesticated ancient relatives of modern cultivars of crop plants are thought to possess certain traits that may, potentially, help resolving the critical question of crop production under stressful environments. A series of field, hydroponics, pot and greenhouse studies were conducted on several hulled tetraploid wheat accessions of Central Iran, in which their responses to NaCl salt were compared to the durum wheat. Even though the hulled wheats were out-yielded by the durum wheat, irrespective of salt level, some indications of greater tissue tolerance to NaCl in these wheats, compared to durum wheat, were observed. Salt-induced decreases in grain yield appeared to be greater in the hulled wheats, relative to the durum wheat. In contrast to the grain yield, the salt-induced decreases in plant dry mass of the hulled wheat appeared to be smaller, compared to the decreases in

the free-threshing tetraploid wheat. Some probable physiological mechanisms of differential responses of the hulled wheats to NaCl and the prospects for the future of durum wheat production under marginal conditions are discussed.

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## PHYSIOLOGICAL AND METABOLIC CHANGES IN DIFFERENT RICE (*ORYZA SATIVA* L.) CULTIVARS DUE TO WARMING NIGHTS

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**Keywords:** high night temperature, rice, metabolites, physiology, climate change

The productivity of crop plants is strongly dependent on environmental factors, which are expected to change during this century due to the global climate change. In the past century this was accompanied by an asymmetric warming with a broad decline in the diurnal temperature range, caused by a stronger increase of night compared to day temperatures. High night temperatures (HNT) are known to negatively impact crop yield. However, little is known about crop physiology and metabolic changes occurring during the early response of rice to HNT conditions.

We have analyzed the effects of HNT on 12 rice cultivars from both the *japonica* and *indica* subspecies and found a clear distinction between tolerant and sensitive cultivars, based on leaf chlorosis estimates. Physiological differences among tolerance classes in response to HNT were observed, as e.g. growth was increased in tolerant in comparison to sensitive cultivars, indicated by increased leaf length, fresh and dry weight.

Moreover, GC-MS metabolite profiling indicated distinct metabolic patterns under HNT compared to control conditions. In addition, the content of several metabolites of the central metabolism was increased under HNT specifically in sensitive cultivars. The TCA cycle, amino acid and polyamine biosynthesis were the main pathways that were influenced in this way. Targeted analysis revealed increased contents of all three stress related polyamines, putrescine, spermine and spermidine, under HNT restricted to sensitive cultivars. This was accompanied by enhanced expression of genes encoding enzymes catalyzing the first committed steps of polyamine biosynthesis.

Furthermore, tolerant cultivars showed a different metabolic constitution already under control conditions. Correlation analyses have uncovered candidate metabolites that may be suitable as metabolic markers to support breeding for HNT tolerance in rice.

## **EMMER WHEAT DIVERSITY IMPROVES THE WATER-USE-EFFICIENCY OF HEXAPLOID BREAD WHEAT**

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Drought significantly limits wheat yield globally and plant breeders require access to new genetic diversity to develop the next generation of water-use-efficient wheat cultivars. While genetic diversity on the D-genome has recently been exploited through the development and use of synthetic wheat, there has been less emphasis given to exploiting AB-genome diversity. A large number of *Triticum dicoccum* derived hexaploid wheat lines were developed and double haploids (DHs) were produced from these crosses. These DHs were screened for response to limited moisture in irrigated and drought stressed experiments in northern NSW in 2013 and 2014. Soil moisture was assessed to 1.34 m using neutron probes and environmental variables were recorded. Grain yield and a range of physiological, agronomic and phenological traits were assessed and the water-use-efficiency of individual genotypes, including parental materials, determined. Significant genotype x moisture regime interactions were observed for water-use-efficiency, grain yield, protein content, canopy temperature depression at heading, early vigour and normalized different vegetation index at heading. Several genotypes with significantly enhanced water-use-efficiency compared to parents and industry leading cultivars were identified. Genetic association analysis is underway to identify and validate those genomic regions linked to enhanced water-use-efficiency. Sixteen cultivars has been identified shows contrast performance in irrigated and drought stressed. Determining contrasting hydraulic behavior of root for these cultivars is also underway.

## **BREAKDOWN OF OXALATE BY OXALATE OXIDASE IN TRANSGENIC RICE PLANT IS AN EFFICIENT WAY TO ENHANCE RESISTANCE AGAINST THE SHEATH BLIGHT PATHOGEN *R. SOLANI***

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**Keywords:** rice, sheath blight resistance, *Rhizoctonia solani*, oxalate oxidase

Rice sheath blight, caused by *Rhizoctonia solani* Kuhn, is a worldwide important disease causing heavy yield losses specially in intensive production system which relies on high yielding varieties and high use of nitrogen fertilizer. Till date traditional breeding is not successful because no potential resistant donor is identified from both cultivated and wild relatives. The pathogen mainly attacks the green parts (leaf and stem) of the plant. The pathogen secretes

oxalate (OA) as its one of the virulence factors. In this study, a rice *oxalate oxidase* (*Osoxo4*) gene was overexpressed in rice plant in green tissue specific manner for sheath blight resistance. Integration and transcript level of *Osoxo4* was examined by southern hybridization and quantitative real time PCR, respectively. The protein in transgenic plants was able to breakdown oxalate and subsequent production of H<sub>2</sub>O<sub>2</sub> was evidenced by histochemical staining, in gel assay and in solution assay. Leaves from transgenic plants showed less yellowing area than did control leaves when exogenous OA was applied. Upon pathogen infection, transgenic plants exhibited enhanced expression of defense genes like phenylalanine ammonia lyase and rice chitinase 24. *Osoxo4* overexpressing transgenic rice lines showed significantly enhanced tolerance to the sheath blight pathogen. Moreover, transgenic plants were more tolerant to RS toxin (toxin secreted by *R. solani*) than control non-transgenic plants. The results indicate that green tissue specific expression of *Osoxo4* is an efficient strategy to enhance sheath blight tolerance by breaking down fungal virulence factor in one hand and H<sub>2</sub>O<sub>2</sub> (breakdown product) mediated activation of plant's own defense system on the other hand.

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## AUGMENTING THE RANGE OF METABOLITES ADVANTAGEOUS TO CEREAL CROP PRODUCTION USING EPICHLÖE FUNGAL ENDOPHYTES

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**Keywords:** *Epichloë*, endophyte, cereal, grasses, Hordeae, metabolites, alkaloids, symbiosis

*Epichloë* fungal endophytes colonise a number of grasses within the subfamily Pooideae. They form seed transmitted symbioses with their hosts and produce a range of alkaloidal secondary metabolites (Schardl, 2010). The production of these alkaloids by the fungus augments the array of metabolites available to the plant that might play a role in responses to both biotic and abiotic challenges. This advantage to the plant, conferred by the endophytic fungus, has been exploited in pasture-based agricultural systems (Johnson et al., 2013). Although *Epichloë* have not been identified in Pooideae cereal grasses such as wheat, barley and rye, they do occur naturally in genera within the Hordeae (Triticeae) tribe such as *Elymus* and tertiary gene-pool *Hordeum* species (Card et al., 2014). With a view to determining what metabolites might be added to cereal grasses to augment their response to biotic and abiotic stresses, we have examined a collection of *Epichloë*-infected *Elymus* and *Hordeum* species using GC and LCMS techniques. Among the range of endophyte-derived metabolites detected were examples of those previously shown to be of benefit in pasture-based agricultural systems. Efforts are being pursued to form synthetic symbioses, using these Hordeae-

sourced *Epichloë*, via laboratory-based artificial inoculation (Simpson et al., 2014). Progeny from successful inoculations are being tested to determine transfer of beneficial metabolites to wheat, barley and rye.

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## METABOLICS AND TRANSCRIPTOMICS ON RICE FLORAL ORGANS UNDER HEAT, DROUGHT AND COMBINED STRESS

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**Keywords:** heat and drought stress, rice, floral organs, combined stress, profiling

Heat and drought stress have an increasing impact on rice (*Oryza sativa* L.) yields and become major challenges in agriculture with increasing global climate change. Severe yield losses were observed when a single stress or a combination of stresses coincide with flowering, but the physiological responses of rice floral organs leading to spikelet sterility are not known. Metabolic and transcriptomic profiling of anthers, pistils before pollination and pollinated pistils was performed to investigate physiological responses determining fertility under heat and combined drought and heat stress using the heat- and drought-tolerant N22 and the heat-sensitive and drought-tolerant cultivar Moroberekan. Different metabolite patterns were observed between floral organs as well as between cultivars under non-stressed and stressed conditions. Several candidate metabolites were differentially accumulated in stressed anthers of N22 compared to Moroberekan under non-stress conditions. More genes were regulated under heat-drought stress than heat stress in anthers of both cultivars with N22 showing much less gene regulation under combined stress in comparison to Moroberekan. The functional class of genes related to stress was over-represented under all stress conditions in both cultivars while fewer bins were over-represented under heat stress compared to combined stress.

Combining metabolite and transcriptomic analyses identified sugar metabolism in anthers as crucial for tolerance or susceptibility to stress. In the future, identification of candidate metabolites and genes will be useful for marker assisted selection procedures to breed more stress tolerant crop plants.

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## Breeding Methods

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### SITE-DIRECTED GENETIC MODIFICATION OF BARLEY USING CUSTOMIZABLE ENDONUCLEASES

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**Keywords:** Cas9, CRISPR, genome editing, genome engineering, guide RNA, repair template, RGEN, TALEN

Genome engineering is a breakthrough technology that facilitates the functional validation of genes and offers versatile novel possibilities of crop improvement. Aiming to establish site-directed genetic modification in cereals, *GFP*-specific transcription activator-like effector nucleases (TALENs) were generated and expressed in barley lines that harbour a single copy of *GFP* used as experimental target sequence. Mutant plants were produced via *Agrobacterium*-mediated transfer of TALEN-coding expression units to embryogenic pollen. When mutagenesis events occurred prior to whole genome duplication in the initially haploid pollen-derived tissue, non-chimeric and homozygous mutants were immediately obtained, as was indicated by non-segregating progeny (Gurushidze et al. 2014). To allow for the optimization of endonuclease construct design and the routine prevalidation of target-specific constructs before embarking on time-consuming stable plant transformation, a test system was established based on transient expression, by which cleavage activity can be assessed via formation of indels in any endonuclease target motif translationally fused upstream of a reporter gene that features a shifted reading frame and whose functional capability is retrieved through loss or gain of basepairs. This assay method has proved to be useful for TALENs and RNA-guided Cas9 endonuclease. In addition, we exemplified homology-dependent genome editing in barley cells using a customized DNA-repair template (Budhagatapalli et al. 2015). This approach allows to precisely predetermine not only the concerned genomic locus but also the resultant DNA sequence.

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## Classical and Molecular Genetics

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### IDENTIFICATION OF YELLOW RUST RESISTANCE GENES IN EUROPEAN WINTER WHEAT CULTIVARS

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**Keywords:** molecular markers, *Puccinia striiformis*, *Yr9*, *Yr17*, *Yr18*

Yellow rust (*Puccinia striiformis* f. sp. *tritici*) is one of the most important diseases of wheat. Although this disease was rare in Hungary, heavy epidemics occurred here in the last three successive years. In the year of 2014, we observed 70% yield loss in the most susceptible cultivars. To effectively use yellow resistance genes (*Yr*), it is important for breeders to know the resistant genotypes in current cultivars. In this study, 34 winter wheat entries produced mainly in Central Europe were investigated using molecular markers to determine the presence and absence of three important *Yr* genes (*Yr9*, *Yr17*, and *Yr18*). In our study the natural infection (measured as leaf coverage by the fungus) of cultivars investigated ranged from 0% to 90% during 2014. Among the 34 entries three carried yellow rust resistance gene *Yr9*, four had *Yr17*, and *Yr18* was found in five cultivars. Field scoring of disease demonstrated the *Yr9* to be ineffective, while *Yr18* provided some protection. Among entries 11 proved to be resistant (infection values ranged from 0% to 10%), and all four cultivars carrying *Yr17* belonged to this group. Therefore, the rest of resistant cultivars might have other effective *Yr* genes. As more than fifty percent of cultivars investigated proved to be susceptible to yellow rust there is a need to introduce new, effective *Yr* genes into new wheat cultivars. The use of molecular markers is a convenient approach to identify effective yellow rust resistance genes in cultivars, and marker assisted selection can be efficiently applied to develop resistant wheat cultivars.

#### Acknowledgements

This study was supported by the Bilateral Hungarian-Chinese Science and Technology (S&T) Program (TÉT\_12\_CN) of the National Research Development and Innovation Office.

## GENETIC ELUCIDATION OF *Mat-b* AND *Mat-c*, TWO HYPOTHETICALLY CO-LOCALIZED GENES DETERMINING FLOWERING TIME IN BARLEY

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**Keywords:** flowering, earliness, mutant, near isogenic line, genotyping-by-sequencing

Understanding the genetic basis of flowering time control is important for the improvement of cereal crop cultivation worldwide, as flowering time is a key factor for adaptation and directly influences yield. Flowering time in barley is a complex trait that exhibits almost continuous variation. A number of quantitative trait locus (QTL) studies have identified mainly three classes of genes that regulate flowering time in barley: those conferring photoperiod sensitivity, requirement for vernalization, and earliness per se genes. Several major QTLs have been mapped to the pericentromeric region of chromosome 2H. However, until now, these loci remain poorly defined, presumably due to their confounding effects. In the present study, we explored barley mutant resources to provide powerful Mendelian solution to the characterization of these loci. We analyzed two near-isogenic lines, BW507 and BW508, that were reported to carry two independent early-flowering mutant loci: *mat-b.7* and *mat-c.19*, respectively [1]. Both introgression segments of BW507 and BW508 are co-localized in the pericentromeric region of chromosome 2H [1]. High-resolution bi-parental mapping allowed us to assign BW507 mutation to a 0.27 cM interval and identify it as a null mutation in an ortholog of *Antirrhinum majus* *CENTRORADIALIS* and *Arabidopsis thaliana* *TERMINAL FLOWER1*, designated in barley as *HvCen* [2]. Our data shows that both BW507 and BW508 carry mutation in *HvCen*, which corresponds to *Mat-c* locus. We complement earlier studies on *Mat-c* by identifying *mat-c.19* mutation and seven additional *mat-c* alleles that previously failed to be characterized. Moreover, we explored the X-ray structures of *HvCen* orthologous proteins to predict the functional effects of the identified amino-acid substitutions. In order to determine the true chromosomal localization of the *Mat-b* gene, we performed genotyping-by-sequencing (GBS) on two doubled-haploid mapping populations derived from crosses of the original *mat-b.7* mutant. We established the definitive chromosomal allocation of *Mat-b* and proposed potential candidate genes.

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## Functional Genomic and Breeding

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### CYTOKININ DEHYDROGENASE ACTIVITY AND WHEAT YIELD

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CKX (cytokinin dehydrogenase) enzymes control local concentrations of cytokinins, thereby affecting the processes regulated by these phytohormones. They catalyse reaction of irreversible degradation of cytokinins by isopentenyl side chain cleavage. CKX are encoded by multigene families, in wheat and barley ten genes have been identified.

The main objective of this study was to verify correlation between *TaCKX* expression level, CKX enzyme activity and yield components in selected wheat genotypes.

The hypothesis of this work: “decreased *CKX* genes expression, lowered CKX enzyme activity correlates with increased plant productivity” was verified in our previous study on barley.

Ten breeding stocks and two modern wheat cultivars were tested. The genotypes were selected based on the differences in yielding. In this study we profiled the expression patterns of all known *TaCKX* genes in seedlings’ roots, young leaves, immature inflorescences and developing spikes collected 0, 7 and 14 days after pollination (DAP). CKX activity was measured in seedlings’ roots and 7 DAP spikes. Another set of plants of selected lines, grown to maturity in containers, were characterized for their aboveground height and weight, grain yield, thousand kernels weight, number of kernels per container and the number of fertile tillers.

The direct comparison of cumulative expression of nine *TaCKX* genes in developing spikes with yield components supported the assumed hypothesis (i.e. lower *TaCKX* expression = higher yield and thousand kernels weight) in the majority of the tested lines. The expression level of all *TaCKX* genes was also consistent with CKX activity in 7DAP spikes. Based on qPCR results of all tested *TaCKX* genes the expression of *TaCKX1* was the highest indicating that it is the key gene involved in cytokinin degradation in developing wheat spikes. Its expression was negatively correlated with yield, thousand kernels weight and number of kernels per container.

The cumulative expression of nine *TaCKX* genes in roots was inversely proportional to the aboveground weight as well as the yield and thousand kernels weight. In roots an equal expression of all genes was observed (except not expressed *TaCKX2*).

In case of leaves positive correlations were found between *TaCKX* genes expression and plant height, weight of aboveground part and the number of fertile tillers. The expression of *TaCKX10* was the highest among all tested genes in all examined tissues indicating that it is the major gene involved in cytokinin degradation in leaves.

Based on these results it can be presumed that the activity of CKX or expression of *TaCKX1* in 7DAP spike may be helpful in selecting high yielding materials.

#### Acknowledgements

The research financed by Ministry of Agriculture and Rural Development, PB No. 5

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## Quality and Utilization

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### QUALITY PARAMETERS OF TRITICALE VARIETIES IN LONG-TERM FERTILIZATION TRIALS

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Hexaploid triticales (*X Triticosecale* spp.) are man-made cereal species from crossing of wheats (*Triticum* spp.) and rye (*Secale cereale*). Acreage of this species has been spectacular as it is grown on more than 4 million ha across the world to date, with 120–140 thousand ha in Hungary. Generally, its quality traits are in-between wheat and rye, even exceeds the ancestors in some characteristics. Triticale pronounces a specific combination of protein- and pentosan-components sourcing from parental species. Pentosans (mainly arabinoxylans) improve the water absorption capacity and the nutritional quality, and extend the shelf-life of the bakery products. Less favorable protein characteristics compared to wheat (special composition of rye- and wheat-origin high molecular weight glutenins, weak gluten, high viscosity and high alpha amylase activity) in triticale may deteriorate the rheological traits limiting its use in the milling and baking industry. For that reason, the use of triticale for bakery industry is suggested in blends wheat flour above all. The quality of the flour blends is influenced not only by the applied ratio but also by the quality of the flours to be mixed. This work focused on the assessment of the qualitative and rheological potential of the triticale varieties as influenced by fertilizer rates in a long term trial.

Grain samples of two triticale varieties were analyzed in this study for milling and baking characteristics. Cultivars *GK Rege* and *GK Szemes* were grown on calciferous meadow soil in Fülöpszállás, Hungary, in a long-term fertilizer trial in 2013 and 2014. The microanalysis of whole and crushed grains were done by PERTEN SKCS 4100 and MININFRA – 5 NIR instruments: the grain hardness, 1000 kernel weight, protein content were measured. White flour was produced by Brabender Senior mill and whole-meal samples were ground by Fluormill A500MSM stone mill. Falling number, Zeleny sedimentation, farinographic (water absorption, quality number) and extensographic properties (stability =  $R_{\max}$  and extensibility = Ext.) were calculated by the standard methods. Sixteen nitrogen (N), phosphorus (P) and potassium (K) fertilizer level were applied in various, elevating combinations.

Elevating macronutrient dosage and year had different effects on most of the tested properties: grain hardness (GK Rege 4–49, GK Szemes 20–60), 1000-grain weight (GK Rege 35–46 g, GK Szemes 38–52 g), Zeleny sedimentation (GK Rege 9–12 ml, GK Szemes 11–13 ml), and the farinographic water absorption (GK Rege 52.6–55.3%, GK Szemes 54.1–54.4%), farinographic quality number, white flour (GK Rege 11–30, GK Szemes 10–28), and farinographic quality number, whole-meal (GK Rege 39–150, GK Szemes 37–150), extensographic  $R_{\max}$ , white flour (GK Rege 309–385, GK Szemes 294–305), extensographic  $R_{\max}$ , whole-meal (GK Rege 550–861, GK Szemes 447–748), Ext., white flour (GK Rege 84–102, GK Szemes 111–115), and Ext., whole-meal (GK Rege 50–61, GK Szemes 51–69).

Rheology-properties of the white flour were not influenced; however, whole-meal properties changed significantly by the applied macronutrient fertilizer treatments. Elevating macronutrient levels – particularly P and K – advanced the farinograph properties of whole meals. In both varieties, each PK ratio led to changes having the maximum curve character in the farinograph values; nevertheless, higher N-rates within the individual PK rates caused reduction in these values. Extensibility (Ext.) of whole-meals diminished to 50% and the stability ( $R_{\max}$ ) became 1.5–2.5-folds higher if compared to white flours. Magnitudes showed year-dependence, the changes were more pronounced in 2014 than in 2013.

#### **Acknowledgments**

Funding from the GOP 1.1.1.-11-2012-2014 project is gratefully acknowledged.

## **CHANGING THE OF FREE DEOXYNIVALENOL (DON) IN SOURDOUGH WITH AND WITHOUT YEAST**

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**Keywords:** masked DON, sourdough, yeast

The presence of free DON mycotoxin in cereals could cause several health problems and is strictly regulated by EU legislation. However, plants have versatile systems to transform DON to different and more or less toxic bounded or masked mycotoxins. These DON forms are not detected routinely together with DON nor regulated by legislation. However, still can cause problems as precursors. As the process is not irreversible, from these DON derivatives free DON could be released by several food processing technology such as fermentation or even some microorganisms in the gut are able to hydrolase them. The mostly found and examined DON derivatives are the DON-3-glucoside compounds, but glutathione conjugated-DON or DON-sulphate were also reported and other unknown DON derivatives still can exist. The 13- and 15-acetyl-DON forms are not masked, they are produced from different chemotypes of the fungus. However also they jeopardize food safety.

In the baking industry where the traditional technologies become again more popular making bread with sourdough could be preferred and thus long fermentation method is used.

In our study we have tested two kind of flours (milled from moderately resistant and moderately sensitive wheat samples which were naturally infected by *Fusarium*) making sourdough with yeast and determined their DON content. Though the flour samples didn't contain considerable DON-glucoside or acetyl-DON conjugates an increase in the amount of DON was detected during formation of sourdough. The origin of the DON increase, and the influence of the added yeast and/or the natural microorganisms of flour to the appearance of free DON was tested. As the problem has food safety aspects, it is significant also for the baking industry.

#### Acknowledgements

The authors thank the project GOP 1.1.1 for the financial support of the work.

## EFFECT OF HMW-GS ANALOUGE PROTEINS ON THE POLYMER MATRIX STRUCTURE OF THE RICE DOUGH

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**Keywords:** wheat analogue and storage proteins, rice, dough

Wheat prolamins are seed proteins expressing in the endosperm. HMW glutenin subunit (HMW-GS) is a unique subgroup of prolamins, due to their effect on dough mixing and bread making quality. HMW-GS proteins have a common structural characteristic by comprising a flexible central repetitive domain and cysteine-rich terminal domains. A set of artificial HMW-GS analogue protein (ANG) was established to study the molecular pattern of polymer protein structure of the flour. The central domain of these proteins derived from hordein, a prolamin homolog gene. The proteins are named after the number and the location of cysteine residues in the protein (1N, 1N2C, 2N2C). The ANG proteins were incorporated into rice flour to monitor their effect on the mixing parameters of rice dough. Dough mixing parameters – mixing time, maximum resistance, resistance breakdown – were analysed by a 4g micro-valorigraph. The effect on polymer distribution in the rice doughs was studied by SE-HPLC. The number of cysteine residues determines the ability of the ANG proteins to incorporate into the protein polymer matrix during dough mixing. The incorporation of 1N and 2N1C proteins resulted weaker dough, while 2N2C proteins provided stronger and more stable dough. 1N protein has a terminating effect on the polymer chain, while 1N2C and 2N2C may enlarge and branch the polymer. The amount of polymer protein fraction was decreased in the 1N containing dough, indicating that the polymer formations of this protein were terminated. Protein 2N2C had an opposite effect than 1N and the ratio of the polymer protein fractions were increased in the rice dough containing 2N2C proteins.

The HMW analogue proteins (1N and 2N2C) were labelled by FITC (Fluorescein Iso-ThioCyanate). The distribution of FITC labelled ANG proteins in rice dough was studied by CLSM microscopy. Microscopy studies demonstrated that 1N can bind to the proteins of rice on the surface of different compound. Hence 2N2C protein with an even number of cysteine residues may enlarge the polymer. The observed changes indicate that 2N2C was built into the polymer matrix of the rice dough. Due to the low allergenicity of rice proteins it is a promising field to study the bread making and dough mixing parameters of rice flour, and understanding the special characteristics before and after exogenous protein addition.

#### **Acknowledgements**

The work was supported by the Hungarian Scientific Research Fund (OTKA PD 101330).

## **HIGH AMYLOSE WHEAT: FROM GENETICS TO BENEFITS**

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Globally, diet-related diseases such as coronary heart disease, type II diabetes and bowel disorders are on the rise. Reducing starch assimilation in the upper gut is an important strategy for addressing these major public health problems. Resistant starch (RS) is starch which escapes digestion and absorption in the human small intestine. Accordingly, increasing the content of this particular type of fibre in foods, especially dietary staples, offers potential long term health advantages for consumers. Wheat with >80% amylose content in the endosperm starch was developed through an integrated conventional breeding strategy as a source of dietary fibre rich in RS. We demonstrate that the high amylose wheat flour is rich in RS whereas the wild type wheat flour contains a negligible amount of this important type of dietary fibre. Our analyses also revealed that breads made from refined and wholemeal high amylose wheat flour had almost 300% more total dietary fibre than corresponding products made from conventional wheat flour.

# Poster Presentations – Abstracts

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## Biotic and Abiotic Stresses

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### IDENTIFICATION OF WHEAT GERMPLASM RESISTANT TO CADMIUM FOR GROWING ON CONTAMINATED SOILS

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**Keywords:** wheat, heavy metals, metal resistant germplasm, physiological parameters, promising forms

Use and development of varieties that are resistant to pollutants, especially to heavy metals, is a constituent part of environmentally-friendly technologies which allow receiving clean agricultural products on contaminated soil. Plants, grown in contaminated soils, exhibit significant interspecies differences in responses to pollution. The available literature data showed the significant positive correlation between the concentration of heavy metals in the grain and genotypes, indicating the possibility of breeding varieties with a low potential for accumulation of heavy metals [1]. We investigated different genotypes of winter wheat in the East Kazakhstan agrocenoses to evaluate the accumulation of such a priority for the region pollutant as cadmium. Field studies were carried out for the determination of physiological parameters. Heavy metals in soil and in plant samples were determined by atomic absorption spectrophotometry. Determination of the content of this metal in the soil of rhizosphere of studied genotypes showed that the amount of cadmium exceeded the MPC for soil. Investigation of the distribution of cadmium in the organs of winter wheat showed that it accumulates mainly in roots and leaves, it is also present in the stems and seeds. Cadmium is a toxic element, and therefore, the presence of this metal in food is a problem for food security. The reduction of cadmium in the grain is one of the priorities of breeding programs [2]. Estimation of cadmium accumulation in seeds of investigated genotypes showed that the amount may exceed the MPC for seeds and the number of investigated heavy metal depends on the genotypic features of the variety. Cadmium content in seeds of winter wheat varieties Ming-2 and Komsomolskaya 56 do not exceed the MPC for the grain. These varieties can be recommended for further use in breeding for resistance to cadmium. Winter wheat variety Ming-2 is a promising variety – it accumulates little amount of cadmium, has a good development, overwintering, yield, and it can be recommended for growing in contaminated soils. Screening of spring wheat varieties in the laboratory conditions allowed to reveal metal resistant forms. These varieties can be used for research in the field to identify the most promising forms that combine resistance to adverse environmental conditions and productivity.

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**MUTATION IDENTIFICATION IN FREEZING TOLERANCE-RELATED GENES IN WINTER WHEAT TILLING POPULATION**

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**Keywords:** freezing tolerance, HRM, *Triticum aestivum* L.

Freezing temperatures is one of the most severe abiotic stresses limiting winter wheat growth, productivity, and distribution. Freezing tolerance of winter cereals is associated with the occurrence of cold-hardening which is triggered by the induction of cold responsive genes after exposure of plants to low non-freezing temperature for certain periods of time (Winfield et al., 2010). TILLING population of two winter wheat varieties ('Kena DS' and 'Gaja DS') was developed in order to create mutant forms of the candidate genes to verify their role in freezing-tolerance formation. The *Sucrose synthase 1 (Ss1)* gene, transcription factor *TaWRKY71* gene and *Vernalization gene 2 (Vrn2)*, were chosen as candidates for mutation identification by High Resolution Melting (HRM) analysis in wheat TILLING population composed of 946 M2 plants. A total of 540.7 kb of DNA was screened resulting in an overall mutation density of one mutation per 36 Kb in the population. HRM analysis resulted in the identification of 2, 7 and 6 new allelic variants for *Ss1*, *TaWRKY71* and *Vrn2*, respectively. All mutations identified were shown to be transitions of the type C→T or G→A as expected for treatment with EMS, which acts via alkylation of G residues. Two novel alleles of *Ss1* gene exon 8 were identified, of which 1 was silent and 1 non-sense (premature stop codon) mutation. Of the 7 novel alleles of *TaWRKY71* gene exon 1 that were characterized, 2 were mis-sense and 5 silent. Six novel alleles were identified for the *Vrn2* gene exon 1 of which 3 were mis-sense and 3 silent. qPCR analyses were performed to estimate how these mutations affect the expression level of *Ss1* gene in crown and leaf tissue during cold acclimation. Putative knock-out mutant M631 had significantly lower relative expression of *Ss1* gene in non-acclimated leaves as well as in crowns and leaves collected at 2, 4 and 6 weeks of cold acclimation compared with the wild type winter wheat line 'Gaja DS'. Freezing tolerance test of the wild type and M631 winter wheat was further carried out to determine effect of the premature stop codon mutation in *Ss1* gene on the freezing tolerance development during cold acclimation. M631 plants hardened at 4 °C for four weeks had substantially lower freezing tolerance (LT50 difference of –3.2 °C) than the wild type plants at the same hardening stage. This suggests that the *Ss1* gene is involved in the process of cold acclimation of winter wheat. Our results show that this reverse genetic tool combined with genetic and phenotypic analy-

sis is very good approach to verify low temperature induced genes involvement in freezing tolerance formation in plants.

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## COMPARATIVE STUDY OF XANTHINE DEHYDROGENASE IN SOFT WHEAT AND *BRACHYPODIUM* FOR INTRODUCTION OF THE LATTER INTO THE BREEDING PRACTICE

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**Keywords:** *Brachypodium*, rust, wheat, xanthine dehydrogenase

Wheat main economically important cultivated crop in Kazakhstan, one of the world leaders for production of marketable grain, is strongly affected by the fungal diseases. Most dangerous and common pathogen of grain is brown leaf rust. Xanthine dehydrogenase (XDG) is considered as a water-soluble enzyme having physiological function in the metabolism of reactive oxygen species. It is known that the rate of purine degradation controlled by XDG increases during wheat infection with *Puccinia recondita*. From the literature search it was revealed that *Brachypodium* (Bd) halophyte now frequently used as a modern system for cereals research, which unlike *Arabidopsis* might also serve as a host for rust diseases caused by the pathogens from *Puccinia* genus [1, 2]. The aim of the current research is the comparative study of XDG enzyme in soft wheat and Bd.

Objects of research – two local varieties of spring wheat and Bd (line 21 seeds provided by RIKEN BioResource Center, Japan). Degree of resistance or susceptibility to leaf rust served as main reason for selection of wheat varieties. In two leaves growth stage, plants were subjected to experimental inoculation with rust urediniospores (provided by Kazakhstan SRI for Biological Safety); with untreated plants serving as control. Symptoms of plant infection were recorded on the 7th day. They appeared on the upper side of leaves, sometimes on leaf sheaths in the form of brown pustules with a diameter of 0.5–2.0 mm. Estimation of XDG activity was performed by the method of native gel electrophoresis. Leaves were extracted in an appropriate buffer (in ratio of 1:4 and 1:3 for leaves and roots, correspondingly), using a rotar, pestle and shaker vortexing. The buffer consisted of 250 mM Tris-HCl (pH = 8.48), 1 mM EDTA, 14 mM GSH, 4 mM DTT, 5 mM L-cysteine, 0.05 mM Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O solution, 0.1 mM PMSF, 0.001 mM pepstatin A and 250 mM sucrose. Extracts were centrifugated at 14,000 rpm under 40 °C for 20 minutes. XDG activity was evaluated by the relative intensity of formazan bands staining using the ImageJ processing of digital images of gels obtained on Epson Perfection V750 PRO scanner.

According to the results in Bd leaves a negative correlation by 36% between XDG activity and infection is observed, while for roots it is generally positive. This demonstrates that the main burden in halophyte adaption in terms of XDG activity is performed by roots. XDG inhibition may in turn lead to increased cell tolerance to bacterial pathogens.

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## ESTIMATION OF QUANTITATIVE GENETIC PARAMETERS FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WINTER WHEAT

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**Keywords:** winter wheat, Fusarium head blight, resistance, artificial infection, heritability

Each year Bc Institute d.d. performs screening of its most improved winter wheat breeding lines for Fusarium head blight (FHB). The 18 best breeding lines are planted in field trials (RCBD, four replications) together with five standard winter wheat cultivars (Lucija, Poncheau, Renan, Roazon and Žitarka) and three FHB infection methods (spray method, deployment of Fusarium infected corn stalks and natural infection) are applied. Disease severity ratings are scored as visual rating index (VRI) and % of Fusarium damaged kernels (FDK). In the present study, results obtained for five standard cultivars during four experimental years (from 2011 to 2014) are presented. Cultivars and years were modelled using the PROC GLM in SAS/STAT (SAS Institute Inc. 2003). Components of variance were estimated using the PROC VARCOMP. Genetic correlations ( $r_G$ ), calculated as proposed in Falconer and MacKey (1996), as well as Pearson's correlation coefficients ( $r_p$ ), computed using PROC CORR, were calculated over four years between the methods of infection and two disease ratings. Also, for both disease ratings and each method of infection broad sense heritability ( $h^2$ ) for disease resistance was calculated according to Hallauer et al. (2010). Among the cultivars significant differences for FHB resistance were found for all methods of infection and both disease ratings except for FDK under conditions of natural infection. The highest FHB severity scored both as VRI and FDK was observed using the spray method of infection. Deployment of FHB infected corn stalks, what simulated the narrow crop rotation production practice where winter wheat is planted after maize, generally resulted in lower FHB scores than spray method, but it does not require the production of inoculum and could be applied even in situations where breeding nursery is not near the laboratory. The Pearson's correlation coefficients among the methods of infection and disease ratings were all positive, and

the strongest was determined between VRI scored using the spray method of infection and VRI using the deployment of corn infected stalks ( $r = 0.84^{**}$ ). The highest  $h^2$  for both disease ratings (0.84 for VRI and 0.78 for FDK) was observed using the spray method of infection, while the smallest (0.24 for both VRI and FDK) was observed under conditions of natural infection, confirming the necessity for performing artificial infection in FHB selection trials.

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## THREE YEARS OF STRIPE RUST (2013–2015) IN HUNGARY

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**Keywords:** *Puccinia striiformis*, natural infection, yield loss

Stripe rust caused by *Puccinia striiformis* var. *striiformis* is major biotic threats in many wheat growing area of the world. Infection intensity varies depending on climate conditions, sensitivity of wheat varieties and agricultural practices. In Hungary the fungus caused severe epidemics in 1933, 1977, 1985, 1994, 1995, 2000, 2001, 2013, 2014 and 2015. In 1977 and 2014, the most notable epidemics of stripe rust occurred in Hungary.

Usually the initial symptoms of yellow rust can appear in May. In 2013, the strength of the nationwide epidemic was poor, moderately strong or strong, depends on the locations. The mild winter without hard and long frost given rise to the first symptoms could be identified in early March of 2014. The spread of disease was quick and the urediospores could be also observed on the spikes. In 2015, the nationwide stripe rust epidemic was not as bad as that in 2013.

In 2013, 2014 and 2015, the effect of stripe rust to yield was observed in six, eighteen and three untreated locations in Hungary. The maximum yield loss was determined to be 75% on susceptible wheat varieties compared to the main average in 2014.

Four treatments of 34 winter wheat entries, including untreated control were evaluated for control of stripe rust in the field in Szeged-Óthalom location in 2014. The fungicide tested was epoxiconazole and was applied i) none (control) ii) once (heading) iii) twice (tillering and heading) iv) three time (tillering, heading and flowering). Stripe rust severities were scored using 0–100 percent. Grain weight measurements were taken after harvesting. The trend of reduction on stripe rust severity was already maintained after the once fungicide application. Zero rust severities was observed after twice and three time spraying. The main

grain yield among the treatments ranged from 5.55 to 6.01 t/ha which were 18 to 29% higher than the untreated control.

#### Acknowledgements

We give thanks for the publication to the Variety Testing Innovation Council.

This study was supported by the Bilateral Hungarian–Chinese Science and Technology (S&T) Program (TÉT\_12\_CN) of the National Research Development and Innovation Office.

## OPTIMIZING PROTOCOL FOR SOMATIC EMBRYOGENESIS AND BIOLISTIC GENETIC TRANSFORMATION OF BREAD WHEAT (*TRITICUM AESTIVUM* L.)

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**Keywords:** bread wheat, somatic embryogenesis, picloram, 2, 4-D, plantlets regeneration, biolistic, *Hardy* gene, drought, salinity tolerance

In this study, the effects of media, varieties and their interaction on callus induction and plant regeneration in bread wheat using immature and mature embryos as explants were studied. Four Moroccan bread wheat varieties ‘Achtar’, ‘Amal’, ‘Mehdia’ and ‘Rajae’ were cultivated on four media (M1 to M4; M1 [2, 4-D], M2 [picloram], M3 combinations [2, 4-D+picloram] and M4 [MS without hormone]). A significant effect of variety, medium and variety x medium interaction were observed for callus induction from both immature and mature embryos. All tested media induced embryogenic callus for all the varieties. For plantlet regeneration, the induction media used for callus induction had a significant effect on plantlets regeneration ( $p < 0.001$ ). Medium with picloram (M2) induced the highest embryogenic callus and the callus subsequently regenerated the highest number of plantlets when used immature and mature embryos as explants. Regeneration of embryogenic callus from M2 medium resulted in 1.4168 plantlets per embryogenic callus and 8 plantlets per regenerating callus for immature embryos derived callus whereas for matured embryo derived callus, for the same medium, it was 0.8427 of plantlets per embryogenic callus and 7 plantlets per regenerating callus. M2 medium was subsequently used for biolistic transformation of Arabidopsis gene *Hardy* involved in drought tolerance under the control of *CaMV35S* or *RD29A* or Rice *APX* promoter using *bar* as selectable maker. The number of bombardment and nature of phytohormones used (auxin or cytokinin + gibberellic acid) had an effect on the regeneration of the transformed calli.

## ***Pch1* AND *Pch2* EYESPOT RESISTANCE GENES IN THE COLLECTION OF WINTER WHEAT (*TRITICUM AESTIVUM* L.) BREEDING LINES**

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Eyespot (strawbreaker foot rot), caused by necrotrophic fungi *Oculimacula yallundae* and *O. acufomis* is one of the most dangerous diseases of cereals in the temperate climate. However, only two eyespot resistance genes: *Pch1* and *Pch2* have been characterized and markers made available to plant breeders. The *Pch1* gene, transferred from *Aegilops ventricosa*, provides effective resistance to eyespot in wheat. *Pch2* is derived from the variety Cappelle Desprez and is located at the distal end of chromosome 7AL. *Pch2* has been shown to have limited effectiveness. An additional resistance has been reported on chromosome 5A of Cappelle Desprez, which confers resistance to eyespot in adult plants. In the present study, we investigated 168 genotypes of winter wheat obtained from various breeding programs. The *in vivo* inoculation tests enabled to evaluate the infection rate. Endopeptidase *EpD1*, *XustSSR2001-7DL* and *Xbarc97* markers were used to identify *Pch1* resistance gene. To track *Pch2* gene we have used *Xwmc525*, *Xwmc346* and *Xcfa2040* markers for 7A chromosome and *Xgwm639* for chromosome 5A. Statistical analysis showed that there is a correlation within infection rate and resistant/susceptible genotypes indicated in enzymatic and molecular analysis.

### **Acknowledgements**

This work was financed by the Ministry of Agronomy and Rural Development (HORhn 078/PB/29/15).

## AGRONOMIC AND METABOLIC CHARACTERIZATION OF RICE UNDER COMBINED HEAT AND DROUGHT STRESS AND POST-STRESS RECOVERY DURING FLOWERING

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**Keywords:** combined heat and drought stress, flowering, metabolite profiling, post-stress recovery, rice

Global climatic models predict the occurrence of heat waves and drought spells to increase in the future. Heat and drought stresses, which are identified as constraints to sustainable rice production, invariably occur simultaneously under field conditions; however, little is known about the effects of their interaction in rice. In addition, recovery from stress has not been well studied. To address these, three cultivars with contrasting tolerance to heat and drought stresses were assessed for their agronomic and metabolic responses to this stress combination under field conditions during the critical flowering period and subsequent recovery from drought. Yield was significantly reduced under stress, which could be traced to decreased spikelet fertility and grain weight. Increased grain chalkiness and lower head rice yield were also observed. The heat- and drought-tolerant cultivar N22 exhibited the lowest reduction compared to the drought-tolerant but heat-sensitive Dular and heat- and drought-susceptible Anjali. Metabolic analysis revealed spatial differences in the metabolite profiles of the three cultivars, wherein flag leaves were more responsive to combined stress compared to spikelets. Levels of most metabolites under stress were maintained during recovery, while some metabolites showed altered levels. Metabolites with increased pool sizes under stress and during recovery relative to control conditions possibly function in conferring tolerance to N22. These responses are currently being validated with data from additional field trials. Potential metabolite markers will aid in establishing high-throughput molecular phenotyping to identify more tolerant cultivars for breeding to increase combined stress tolerance in rice.

## GENETIC ENGINEERING OF ABIOTIC STRESS RESPONSE IN PLANTS

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Genome editing is utilized in genome modification becomes more widely in various plants including crops. We designed several gRNAs for the target genes related to signal transduction pathways and stress responses in tomato and Arabidopsis plants. We generated the CRISPR/Cas9 cassettes using the plant codon-optimized Cas9 driven by the constitutive promoter. The Cas9 and gRNA expression levels were monitored in the each transgenic line, and the selected plants or calli were used for the detection of newly generated mutations using Cel-1 assay and sequence analysis. The mutations were generated in the target genes with 12.5–60% efficiency. The reduction of the number of nuclear localization signals decreased the mutation efficiency. The mutations were successfully detected in the next generations and in the newly developed tomato shoots. Our data suggested that the CRISPR/Cas9-mediated mutation was successfully obtained. We will also focus on the further applications of molecular breeding to improve plant growth and stress responses.

## **IDENTIFICATION OF MARKERS LINKED TO *Pch1* AND *Pch2* EYESPOT RESISTANCE GENES IN THE HAPLOID BREEDING LINES OF WINTER WHEAT (*TRITICUM AESTIVUM* L.)**

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Eyespot, a fungal disease of wheat (*Triticum aestivum* L.) and other cereals is caused by fungi *Oculimacula yallundae* and *O. acufomis*. Severe infections can result in yield losses of up to 50%. Only two eyespot resistance genes: *Pch1* and *Pch2* have been characterized and markers made available to plant breeders. The most effective source of genetic resistance is *Pch1*, a gene derived from *Aegilops ventricosa*. *Pch2* is derived from the wheat variety Cappelle Desprez, which has been used as a source of the seedling-stage resistance. In breeding, the use of doubled haploids (DH) system together with molecular markers facilitates the identification of linkage between a marker and a trait of interests, shortens the selection of recombinants. In the present study, we investigated 57 genotypes of haploid winter wheat obtained by crossing selected resistant forms of wheat with maize (*Zea mays* L.). Endopeptidase *EpD1*, *XustSSR2001-7DL* and *Xbarc97* markers were used to identify *Pch1* resistance gene. SSR markers *Xwmc525*, *Xwmc346*, *Xgwm639* and *Xcfa2040* were used to track *Pch2* gene.

### **Acknowledgements**

This work was financed by the National Centre of Research and Development (PBS2/B8/0/2013).

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## Breeding Methods

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### PEARL MILLET WITH HIGHER STOVER YIELD AND BETTER FORAGE QUALITY: IDENTIFICATION OF NEW GERmplasm AND CULTIVARS

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**Keywords:** pearl millet, stover productivity, stover nutritional quality, germplasm, cultivars

Pearl millet is a source of both food grains for humans and dry fodder for their ruminant livestock in the semi-arid and arid tropics of Asia and Africa. Recently, pearl millet has occupied large areas during summer season in India and is also in great demand in central Asian countries for cultivation as forage crop, but very few cultivar options are available to farmers for this ecology. The objective of this study was to: 1) identify sources of higher stover yield and fodder quality traits, 2) initiate their utilization in breeding programs; and 3) identify OPVs and hybrids with higher stover yield and fodder quality. A set of 242 pearl millets from a minicore collection was evaluated for stover linked traits during summer and rainy seasons of 2013 at ICRISAT, Patancheru. Based on 2-season evaluation, about 10% best (21 accessions) having high fresh stover yield (23 to 38 tons ha<sup>-1</sup>) and dry stover yield (8 to 13 tons ha<sup>-1</sup>) at 85–90 days after planting were selected and further evaluated in the rainy season of 2014 along with checks at 6 locations in India. The dry stover yield of check OPVs varied from 15–21 tons ha<sup>-1</sup>, and in check hybrids from 16 tons (DFMH 30) to 19.7 tons ha<sup>-1</sup> (Nutrifeed). IP 22269 had highest dry stover yield (19 tons ha<sup>-1</sup>), followed by IP 20577 (18 tons ha<sup>-1</sup>) and IP 20409 (16.6 tons ha<sup>-1</sup>) and total of 11 accessions had dry stover yield of >14 tons ha<sup>-1</sup>. These accessions were also evaluated at ICRISAT during rainy season of 2014 along with earlier identified OPVs/germplasm accessions and top-cross hybrids. The identified 21 accessions had a wide range of stover nitrogen content (0.88 to 1.24%), *in vitro* digestibility (39.8 to 45.4%), and metabolizable energy (ME) (5.3 to 6.4 MJ·kg<sup>-1</sup>). Twenty of these identified accessions had *in vitro* digestibility of >40 %; and IP 14294 had highest *in vitro* digestibility (45.4%) along with highest ME (6.4 MJ·kg<sup>-1</sup>). These identified accessions were subjected to inbreeding yielding about 270 early generation inbreds (S<sub>1</sub>s-S<sub>3</sub>s), which will further be used for deriving breeding lines for the development of new hybrids and OPVs with high stover yield and fodder quality. Also, based on 2-year (2013 and 2014) multilocation evaluation at 5 locations in each year for stover yield and at one location (ICRISAT) for stover quality traits, five OPVs/germplasm accessions having high stover yield in the range of 16.3 to 17.8 tons ha<sup>-1</sup>, and *in vitro* digestibility from 42 to 45%; and five top cross hybrids having dry stover yield of 13.6 to 15.9 tons ha<sup>-1</sup>, with *in vitro* digestibility from 38.9 to 42.6% were identified; though top cross hybrids matured about 10–15 days earlier than the OPVs/

germplasm accessions. Dry stover yield had positive correlation ( $r = 0.420^{**}$ ) with *in vitro* digestibility for OPVs/germplasm accessions, while the low positive correlation ( $r = 0.339$ ) was observed for hybrids.

## EFFICIENCY OF QTL MAPPING BASED ON LEAST SQUARE METHOD

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**Keywords:** heritability, Quantitative Trait Loci, linkage map, chromosome

Popcorn is a food crop and its basic trait of quality is popping capacity. Pericarp thickness, endosperm starch type and moisture in the kernel have been attributed to the popping ability in popcorn. Markers on the genetic maps are the genes with variant alleles producing detectably different phenotypes. Objective of the study was to generate a simulated data of  $F_1$  and  $F_2$  generation for two contrasting inbred lines of 200 individuals followed by 50 simulations of 200 and 400 sample sizes and to analyze efficiency of least square method for QTL mapping. Genetic linkage map comprising of 10 linkage groups, 100 SSR markers and 12 estimated QTL were constructed using program Real breeding. The range of QTLs on single chromosome was from zero to three. Each estimated QTL explained approximately 2 and 4% of the phenotypic variance for the heritability's 30% and 70%. Using least square method we determined the power of QTL detection<sup>1</sup> (%) and average number of false QTLs in chromosomes with none, one and 2-3 true QTLs and Bias<sup>1</sup> (cM) between estimated and true position of identified QTLs using R package qtl for expansion volume, grain yield and days to maturity. Result showed high power of QTL detection<sup>1</sup> (%) and average number of false QTLs with 400 individuals and 0.7  $h^2$  regardless of the trait. Least square also declare that average number of false QTLs improved with high heritability, high population size in chromosome with high number of true QTLs (2-3 QTLs), while 95% CIw decreased. Bias<sup>1</sup> (cM) show close similarity with Power of QTL detection. We concluded that increasing the sample size and  $h^2$  will increase the power of QTL detection and Bias<sup>1</sup> (cM). Present study will provide basic knowledge in the area of mapping molecular markers and analysis of QTLs for quality trait in popcorn.

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## **USE OF GENES FOR BLUE ALEURONE, PURPLE PERICARP, AND THREE PISTILS IN A FLORET AS A VISUAL MARKERS IN WHEAT BREEDING**

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Abstract removed from the online version upon authors' request.

## HOW TO INCREASE THE EFFICIENCY OF CHROMOSOME DOUBLING IN ANDROGENIC REGENERANTS OF TRITICALE?

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**Keywords:** triticale, doubled haploid, chromosome doubling, androgenesis, antimitotic agents

For the doubled haploid (DH) approach to breeding to be successful, an economically effective production system is needed. It has to generate DHs in relatively large numbers to provide a fair chance of success in advancing breeding goals. Only sufficiently large populations of DH can offer proper representation of genetic variation between the parents and recombination events that generate new allelic combinations. Regardless of which method for the DH production is used, it has to be followed by a similarly effective and reliable method for doubling of the chromosomes number. Authors' experience indicates that the average rate of spontaneous chromosome doubling in triticale is ca. 20%, bringing certain urgency to the issue of chemical applications. There are certain costs associated with the standard use of colchicine, such as production of mixoploids, chimeras and individuals with higher chromosome numbers, as well as potentially mutagenic effects of colchicine itself, and low survival rate of the treated material. Alternative methods would be warmly welcome. Out of several options, antimitotic substances (herbicides) are worth a close look as they may successfully double the chromosome numbers without at least some side effects, and at lower concentrations and at low cost. This is due to their much greater affinity for tubulin than colchicine. An alternative to chemical chromosome doubling would be making use of the meiotic restitution mechanisms already available in triticale. Single division meiosis (SDM) reliably produces gametes with somatic number of chromosomes, and amphidiploid progeny.

In direct response to demand from Polish triticale breeders a series of experiments were conducted to determine how mitotic inhibitors, when added to the culture media at various stages of androgenesis, at different concentrations and for different periods of treatment, affect the explants survival rate and to determine the efficiency of chromosome doubling in typical breeding hybrids. The highest survival rate for explants was obtained after use of benfluralin, ethalfluralin, trifluralin and APM. So far, the highest chromosome doubling rate observed was 65 % which does not appear competitive with colchicine. It has been observed that making use of parental lines, that are lines generated via standard DH production methods, clearly increases the rate of spontaneous chromosome doubling among their androgenic progeny.

## **DETERMINANTS OF EFFECTIVE MICROSPORE EMBRYOGENESIS IN TRITICALE (*X TRITICOSECALE* WITTM.)**

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**Keywords:** androgenesis efficiency, microsporogenesis, oxidative stress tolerance, plant growth regulators

The discovery that stress can start an alternative, sporophytic program of microspore development created a very interesting model for biological studies. Moreover, haploid (n)/doubled haploid (2n) plants produced in the process are commonly utilised in multiple research domains and commercial breeding. However, in the case of many economically important plant species, including triticale, the efficiency of the process is not satisfactory and highly genotypic-dependent. Despite many investigations, the precise mechanism of microspore embryogenesis (ME) initiation and regulation has not yet been identified.

The fundamental requirement for effective ME initialization is a particular course of microsporogenesis leading to the formation of vital, properly developed microspores. In triticale, which possesses rather complex genetic organization because of its high ploidy level, large genome size and the distant relationship between the parental genomes, this condition is not always fulfilled. Various meiotic irregularities observed in triticale microspores could diminish the yield of isolation procedures and disturb further stages of embryogenic structure formation. Many abnormalities, e.g. disturbances in the symmetry of nucleus division, dispersion of chromatin in the vegetative nucleus, and the presence of micronuclei, have been revealed during mitosis, especially in ME-recalcitrant genotypes. Other important prerequisite for effective ME initialization is tolerance to stress applied as a trigger of sporophytic development, microspore isolation, and transfer to *in vitro* culture conditions. In triticale (Żur et al. 2014), this feature was highly dependent on antioxidative system efficiency, especially on the activity of H<sub>2</sub>O<sub>2</sub>-decomposing enzymes – catalase and peroxidase. Last but not least among the requirements is a specific endogenous homeostasis between various plant growth regulators (PGRs): auxins, cytokinins and ABA. In our studies (Żur et al. 2015), anthers of

ME-responsive genotypes after induction of ME were characterized by significantly lower concentration of IAA together with significantly higher IBA, *trans* zeatin and ABA level in comparison with recalcitrant genotypes.

#### Acknowledgements

The research was supported by the National Science Centre, Poland (project NN310452638) and institutional funding of the *F. Górski* Institute of Plant Physiology, Polish Academy of Sciences.

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## Classical and Molecular Genetics

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### VALIDATION OF SIMPLE SEQUENCE REPEATS ASSOCIATED WITH QUALITY TRAITS IN DURUM WHEAT

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**Keywords:** association analysis, simple sequence repeat (SSR), quality traits, core collection, *Triticum turgidum* ssp. *durum*

Over recent years, pasta-cooking quality has become an important commercial issue for durum wheat breeders. Modern breeding methods are most efficient for producing and supplying the best quality raw materials to the pasta industry. Marker-assisted selection (MAS) can improve plant breeding by saving time and labor in breeding programs. The objective of this study was to assess the effectiveness of molecular marker assisted selection for quality traits. For that purposes, DNA and biochemical markers were jointly used for the analysis of quality traits, affecting pasta-cooking quality in durum wheat genotypes. A total of 132 durum wheat (*Triticum turgidum* ssp. *durum*) Mediterranean landraces, international lines and Moroccan cultivars were analyzed for gluten strength, yellow pigment and protein content. Additionally, eighteen simple sequence repeat (SSR) markers covering a wide genome area were used, respectively: 10 GWM, 3 WMC, 2 CFA, 1 UHW, 1 GPW and 1 DupW. Of these, 14 (78%) were polymorphic and four monomorphic. Fifty-five bands were scored across accessions. Good concordance between quality traits and SSRs were detected. The present study showed the efficiency of SSR technology, which holds promise for a wide range of applications in marker-aided wheat breeding programs.

## IDENTIFICATION OF LEAF RUST RESISTANCE GENES IN MOROCCAN BREAD WHEAT GENOTYPES AND RILS POPULATION

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**Keywords:** disease resistance genes, *Puccinia triticina*, *Triticum aestivum*, molecular markers, Lr genes

Leaf rust caused by *Puccinia triticina* reduces the wheat yield and grains quality worldwide. Molecular markers help to determine leaf rust resistance genes (Lr genes). The aim of this study was to evaluate the presence and the effect of two slow-rusting genes Lr34 and Lr68 and two seedling resistance genes Lr19 and Lr21 in 21 Moroccan wheat genotypes and in F9 recombinant inbred population, from cross between resistant synthetic hexaploid wheat “syn6” and a susceptible Moroccan bread wheat cultivar Mehdiya, for resistance to leaf rust during three years in Moroccan field conditions at Sidi Allal Tazi and Merchouch stations. The application of markers linked to these genes indicates that the tolerance of 21 varieties was overcome, except for the Saada variety which showed a moderate field resistance. The 21 bread wheat genotypes studied lack the Lr19, Lr21 and Lr68 genes. The five wheat genotypes (Errihane, Massira, Amal, Mehdiya, and Baraka) have the Lr34 gene and only “Saada” carry the both slow-rusting genes Lr34 and Lr68. Seven RILs descendants, combining the three genes (Lr19, Lr21 and Lr34) showed a high level of resistance to leaf rust. Indeed the Lr34 gene is effective only if it combined with other genes. 35% of lines RILs with the genes (Lr19 and Lr21, Lr19 and Lr34, Lr34 and Lr21) have a moderate to high resistance. These resistance lines and Saada genotype are valuable sources to improve resistance to leaf rust of Moroccan Bread wheat genotypes.

## QTL-BY-QTL-BY-ENVIRONMENT INTERACTIONS FOR GRAIN LENGTH OF A RICE LARGE-GRAIN CULTIVAR DETECTED FROM NEAR-ISOGENIC LINES

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**Keywords:** additive effect, epistatic effect, genotype-by-environment interaction, grain length, rice

In quantitative genetics, interactions among different loci, or epistatic effects, have been considered as very complicated genetic processes and frequently neglected in standard genetic models, for example in diallel analysis [1]. On the other hand, the interactions between

genotype and environment have been evaluated extensively. Quantitative trait loci (QTL)-by-QTL interactions and QTL-by-environment interactions have already been estimated using appropriate data sets and software. However, QTL-by-QTL-by-environment (QQE) interactions have hardly been studied, probably because of their complexity. This study evaluated QQE interactions for five different QTLs controlling the grain length of a rice (*Oryza sativa* L., ssp. *japonica*) large-grain cultivar, BG 1, a model genetic system for agronomic traits. A set of near-isogenic lines (NILs) for grain length in the genetic background of a small-grain cultivar, Gimbozu, were used<sup>2</sup>. In these NILs, five individual QTLs, and 10 combinations of two and 10 of three of the five QTLs have large-grain alleles derived from BG 1, while all other loci have small-grain alleles from Gimbozu [2]. A total of 25 NILs and Gimbozu were cultivated in a paddy field with usual cultivation conditions for four growing years. The additive effects, and two-way and three-way interactions of these five QTLs were estimated as the following Eqs (1), (2) and (3), respectively, in each year:

$$X_{ir} = C + A_i + \varepsilon_{ir}, \quad (1)$$

$$X_{ijr} = C + A_i + A_j + I_{ij} + \varepsilon_{ijr}, \quad (2)$$

$$X_{ijk r} = C + A_i + A_j + A_k + I_{ijk} + \varepsilon_{ijk r}, \quad (3)$$

where  $X$ ,  $C$ ,  $A$ ,  $I$  and  $\varepsilon$  are observed values, genetic background (the mean of Gimbozu), additive effect, interaction and error term ( $\sum \varepsilon = 0$ ), respectively<sup>2</sup>. The additive effects and interactions of the QTLs were examined for their interactions with years. Results showed that the additive-effect-by-year interaction was not significant, whereas significant differences were detected in both factors. In contrast, the two-way and three-way QQE interactions were significant and large and showed environmental fluctuation of a cross-over type. These results strongly suggest that in rice grain length, and probably many agronomic traits, QTL-by-environment interactions are attributable mainly to the QQE interactions.

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## ASSESSMENT OF GENETIC DIVERSITY IN EMMER WHEAT [*TRITICUM TURGIDUM* L. SSP. *DICOCCON* (SCHRANK) THELL.] LANDRACES FROM TURKEY

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**Keywords:** cultivated emmer, *Triticum turgidum* L. ssp. *dicoccon*, genetic variation, landrace, SSR

Cultivated emmer wheat *Triticum turgidum* L. ssp. *dicoccon* (Schrank) Thell. (*BBA*;  $2n = 4x = 28$ ) is a primitive, non-free-threshing, allotetraploid species. Although it was the most

prominent cereal in the early farming villages of the Near East, today it is grown on a limited scale in Ethiopia, Iran, Italy, Transcaucasia, Turkey, and the Balkans. Being a source of useful genes, landraces of emmer wheat represent one of the most important genetic resources available to breeders for present and future genetic improvement of wheat. The genetic diversity in nine emmer wheat [*Triticum turgidum* L. ssp. *dicoccon* (Schrank) Thell.] landraces populations, originating from Turkey was investigated by using six microsatellite (SSR) markers, which are distributed overall six chromosomes. The number of alleles per locus ranged from 31 to 49, with an average 42.5. Null allele was observed for each of the SSR markers used in the analysis. The average value of polymorphic information content (PIC) was 0.87. The mean value of effective allele was  $n_e = 10.20$ . The mean value of expected heterozygosity was  $H_e = 0.88$ . the highest genetic diversity value  $H_e = 0.92$  was observed in population L, while the lowest genetic diversity value  $H_e = 0.73$  was observed in populations K and N. Considerably high amounts of variation were detected within and among the populations. The genetic differentiation between populations was  $F_{ST} = 0.14$ , while the mean value of gene flow was  $N_m = 1.59$ . Results obtained from this study can effectively be used in developing more efficient breeding programs to improve wheat genotypes, and to direct genetic resource conservation studies.

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## REGULATION OF *FLOWERING LOCUS C* (FLC) HOMOLOGS IN *BRACHYPODIUM DISTACHYON*

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Plants have evolved various mechanisms to sense environmental signals. These mechanisms help them to align growth and development to the environment so to maximize reproductive success. Winter cereals flower only after a prolonged exposure to cold (vernalization) and as such vernalization can have a major impact on crop yield. In *Arabidopsis*, *FLOWERING LOCUS C* (*FLC*) is the central repressor of flowering that is epigenetically silenced in response to cold (Bastow et al., 2004, Michaels and Amasino, 1999). However, in cereals, the vernalization response is thought to be regulated differently. We recently identified *FLC* homologs in monocots (Ruelens et al., 2013) and now we are investigating the functional role of *BdODDSOC2*, *BdMADS37* and *BdODDSOC1* (*FLC* homologs) in *Brachypodium distachyon*, a model grass species that is closely related to wheat and barley. We found that *BdODDSOC2* is responsive to cold and can be stably silenced in a summer and winter ac-

cession. We are now investigating the chromatin changes at *FLC* loci to understand whether they are epigenetically regulated through activation (H3K36me3) and repressive marks (H3K27me3) by using chromatin immunoprecipitation. Our study will help to understand epigenetic winter memory of cereals and will identify similarities and differences between vernalization pathways of cereals and *Arabidopsis*.

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Michaels and Amasino, 1999  
Ruelens et al., 2013

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## Functional Genomic and Breeding

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### ANALYSIS OF GENE COMBINATIONS DETERMINING GRAIN SIZE IN RICE

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Grain size in rice is one of the key factors determining yield and quality. A large number of genes are involved in the regulation of grain size parameters such as grain length and grain width. Six key genes known to influence grain size were investigated in this study: *GS3*, *GS5*, *GS6*, *GW2*, *qSW5/GW5*, and *GW8/OsSPL16*. Allele and grain measurement data were used to develop a regression equation model that can be used for molecular breeding of rice with desired grain characteristics. A total of 215 diverse rice germplasms, which originated from or were developed in 28 rice-consuming countries, were used in this study. Genotyping analysis demonstrated that a relatively small number of allele combinations were preserved in the diverse population and that these allele combinations were significantly associated with differences in grain size. Furthermore, in several cases, variation at a single gene was sufficient to influence grain size, even when the alleles of other genes remained constant. The data were used to develop a regression equation model for prediction of rice grain size, and this was tested using data from a further 34 germplasms. The model was significantly correlated with three of the four grain traits examined in this study. A regression equation model can be applicable to rice breeding programs for the development of new rice varieties with desired grain size and shape.

## ASSOCIATION ANALYSIS BETWEEN AMYLOSE CONTENT AND WAXY GENE HAPLOTYPE IN USDA RICE MINI-CORE COLLECTION

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**Keywords:** amylose content, association analysis, USDA rice mini-core collection

USDA rice mini-core collection comprises 217 accessions that are considered to be representative of the entire USDA-ARS rice germplasm holdings. For efficient utilization of this valuable collection in rice breeding practice, many agronomic traits and their genetic bases need to be investigated. In this study, the extent of variation in apparent amylose content (AAC) among mini-core rice accessions was investigated using iodine colorimetric method. Our results revealed a huge variation in AAC in the mini-core rice, ranging from 0.6% to 30.7%, with a mean value of 22.5%. Most of the accessions belong to high or intermediate AAC group (48.7% and 27.4% accessions respectively). In addition, a total of eight *Wx* haplotypes were found in the mini-core rice based on G/T SNP in intron 1, 23 bp duplication in exon 2, A/C SNP in exon 6, and C/T SNP in exon 10 of *Waxy* gene. Three of these haplotypes had not been reported in previous studies. Our association analysis demonstrated that AAC variations could be explained by *Wx* haplotypes in over 70% cases. The results of this study can help rice breeders select suitable parental materials from the mini-core collection for quality improvement with a combination of these genetic markers.

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## Quality and Utilization

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### ASSESSMENT OF THE FUSARIUM OCCURENCE IN ORGANIC AND CONVENTIONALLY GROWN CEREALS

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**Keywords:** spring cereals, organic farming, *Fusarium*

Production of healthy food and feed and minimization of negative impacts on human and animal health as well as on the environment is the main goal of the current agriculture.

One of the most discussed issues is the content of mycotoxins in organic and conventional production. A lot of opponents argue that organic production should contain higher level of mycotoxins than conventional production due to the fact that regulations prohibit utilization of any fungicides. Contrary there are lot of articles published no differences in both productions. The main aim of our work was to assess the *Fusarium* occurrence in organic and conventional grown cereals and compare data with conventional data. The further aim was to evaluate the differences of mycotoxin contain in dehulled and hulled spelt grains.

#### Acknowledgements

This work was funded by the National Agency for Agricultural Research of the Ministry of Agriculture of the Czech Republic (Project No. NAZV QJ1310072).

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## GENETIC VARIABILITY ON GRAIN ZINC CONCENTRATION OF ADVANCED MUTANT LINES OF SPRING WHEAT OBTAINED THROUGH GAMMA IRRADIATION AND THEIR EVALUATION FOR YIELD-ASSOCIATED TRAITS

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**Keywords:** zinc enriched mutant  $M_5$  lines of spring wheat

Bread wheat (*Triticum aestivum* L.) is a staple crop with global economic importance and is a major cereal in both human and animal nutrition. It is a major crop that supplies the bulk of nutrients in the diet, therefore genetic enhancement with more of this micronutrient is one of the most cost-effective ways of solving the global micronutrient malnutrition problem. Zinc (Zn) deficiency associated with low dietary intake is a well-documented public health problem, resulting in serious health and socioeconomic problems. It is known that genotypic variation for grain Zn concentration among wheat cultivars relatively low. Induced mutation is a powerful tool for crop improvement. Seeds of the spring bread wheat variety Almaken (*Triticum aestivum* L.) were irradiated with 100 and 200 Gray doses from a  $^{60}\text{Co}$  source at the Kazakh Nuclear Centre. Promising advanced  $M_5$  mutant lines were obtained and evaluated for yield components traits such as grain number and weight per main spike, grain weight per plant and TGW and grain quality associated trait, zinc concentration.

Mutant lines showed wide variation in grain weight per plant (GWP) and thousand grain weight (TGW). Ten of the mutant lines (33.3%) showed significantly higher values for this trait than non-mutagenic cv. Almaken. Nineteen of the lines (63.3%) were characterized by significantly higher values for TGW than non-mutagenized variety. Three of the mutant lines and six of the mutant lines showed significantly higher values for three (grain number per main spike, GWP and TGW) and two of the lines (GWP and TGW) traits, respectively, than non-mutagenic cv. Almaken.

Considerable variation for grain zinc concentrations with a means of 3.4 to 45.0 mg/kg (mean =  $23.35 \pm 10.8$  mg/kg;  $n = 30$ ) was found in the  $M_5$  mutant lines. A significantly enhanced grain zinc concentrations relative to cv. Almaken were identified in fifteen  $M_5$  lines (50.0%). In these lines grain zinc concentrations exceeds 1.6 to 3.8 times as compared with cv. Almaken parental line. Eight of the single mutant lines showed positive correlation between grain zinc concentrations and yield component TGW ( $r = 0.45-0.99$ ). Hence, these mutant lines could be used as a donor parents in a breeding program and some of them can be recommended as candidates for new wheat varieties in Kazakhstan.

## BAKING QUALITY OF NEGLECTED WHEAT SPECIES DETECTED BY RHEOLOGICAL SYSTEM MIXOLAB

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**Keywords:** neglected wheat, rheological properties, baking quality, organic farming

Despite to organic farming development, farmers and processors looking forward to grow and process alternative crops. There is also large group of neglected species within genus *Triticum* L. Some of this wheat species could be perspective in organic farming growing. But there aren't information related to reaction of neglected wheat species to be grown in organic farming. The most important is also to analyses impact of organic farming management on technological quality of grain. Some examples from the past showed, that there is possible to enlarge portfolio of grown crops (eq. Khorassan wheat) (Amal et al. 2012). The present study investigated various underutilized spring wheat species with respect to baking quality detection. Twenty varieties of neglected wheat species (*Triticum cartlicum*, *T. polonicum*, *T. timopheevi*, *T. compactum*, *T. turgidum*, *T. sphaerococcum* and *T. macha*) were compared to common wheat in organic field trials in the Czech Republic and Austria. For the evaluation of baking quality was used Mixolab II. system (accepted as the ICC standard method No. 173 – ICC 2006), which makes possible to evaluate physical dough properties such as dough

stability or weakening, and starch characteristics in one measurement. For the comparison were used also standard ICC methods of baking quality analysis. Data were processed by the Statistica 9.0 (StatSoft. Inc., USA) program. *T. macha* had very similar rheological quality as bread wheat. *T. sphaerococcum*, *T. timopheevi*, *T. polonicum* and *T. compactum* had evidently worse rheological quality than *T. macha* and bread wheat. It was because of lower water absorption and also weak protein based on the mechanical work. On the other hand, in case of this wheat species was observed higher stability of hot-formed gel during starch evaluation. Weak protein and not stable starch had *T. turgidum*. High correlations were found between Mixolab characteristics and standard technological parameters during evaluation.

#### Acknowledgements

This work was funded by the National Agency for Agricultural Research of the Ministry of Agriculture of the Czech Republic (Project No. NAZV QJ1310072) and the University of South Bohemia in České Budějovice (Project No. GAJU 063/2013/Z).

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## THE EFFECT OF SELECTION UNDER HIGH AND LOW NITROGEN FERTILIZATION LEVELS ON GRAIN YIELD AND QUALITY OF WINTER WHEAT

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**Keywords:** winter wheat, bread-making quality, selection, fertilization

Nitrogen fertilization (N) increases grain yield and quality, but also represents a significant production cost and has a negative impact on the environment. Therefore, the plant breeders aim to create cultivars with higher N use efficiency. Breeding of N-efficient cultivars can be conducted either directly under N stress or indirectly under optimal conditions. Direct selection in the target environment is advantageous if a crossover interaction between genotype and production system can be detected (Ceccarelli, 1996). The aim of this study was to compare the effect of selection conducted at high (HN) and low (LN) levels of N fertilization on yield and indirect bread-making quality traits of winter wheat. Selection for increased protein content and grain yield was conducted in 2012/2013 selection trial among F<sub>6</sub> progenies of two crosses, Golubica × Emesse (G×E) and Verbunkos × Soissons (V×S). Out of 50 evaluated progenies 11 were selected from each cross at both LN and HN. The effect of selection was evaluated in F<sub>7</sub> generation grown in 2013/2014 evaluation trial at both N fertilization levels. The difference in the amount of N between the HN and LN was 100 kg/ha in both selection and evaluation trial. ANOVA revealed a significant effect of evaluation N level (N) on grain N yield (GNY), grain protein content (GPC), Zeleny sedimentation

value (ZSV) and wet gluten content (WGC) for both crosses and on grain yield (GY) for the cross V×S. Selection N level (Sel) significantly affected GY, GNY, GPC, ZSV and WGC of the cross V×S, while the N×Sel interaction was not detected. Averaged over selection N levels and crosses GY, GPC, GNY, ZSV and WGC were 6%, 7%, 12%, 14% and 9% lower under LN evaluation conditions than under HN evaluation conditions, respectively. On the other hand the gluten index (GI) and Pelshenke value (PV) had 6% and 3% higher mean values under LN evaluation conditions, but the differences were not significant. Averaged over evaluation N levels F<sub>7</sub> progenies of the cross V×S produced through HN selection had 12% higher GY, 4% higher GPC, 8% higher GNY and 10% higher WGC than F<sub>7</sub> progenies produced through LN selection with a higher response at LN evaluation level. For the cross G×E differences between two selection levels were not significant for all traits. The results indicate that selection conducted at high N level is more efficient than selection conducted at low N level in improving grain yield as well as quality traits when breeding for both high-N and low-N conditions.

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## FOOD COMPONENTS IN FRACTIONS OF QUINOA SEEDS

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**Keywords:** quinoa, seeds, proximate composition

Nutritional value of quinoa seeds (*Chenopodium quinoa* Willd) has been appreciated for its high protein content (12–18%) and wide range of minerals and vitamins. Special attention has been given to pseudo cereal quinoa, for people who are intolerant to gluten, as an alternative to cereals wheat, rye, and barley. Quinoa purified seeds of cultivar KV52 were compared with commercially available Bolivian quinoa seeds.

Quinoa variety used for investigation was KVL 52. It was selected by the University of Copenhagen and successfully trialed in typical agro-climatic conditions of Serbia. Whole seeds were first manually dehulled and then washed with water yielding quinoa purified seeds. Dehulling and subsequent washing was done in order to remove saponins that are giving a bitter aftertaste in food products. The main chemical and nutritive parameters of whole, dehulled and quinoa purified seeds that were estimated included: protein, crude fiber, oil, moisture and ash by standard AOAC methods. Total starch content was calculated.

Purified quinoa seeds of the investigated KVL 52 variety showed exceptionally high protein content of 16.69% that is much higher than that of Bolivian quinoa and superior to other pseudo cereals. Other nutrients were similar in both investigated seeds with the exception of crude fiber which was lower in Bolivian quinoa than in KVL 52 for more than 50%.

Chemical composition revealed the potential of quinoa seeds to be used as a healthy food or as a valuable ingredient in the preparation of cereal foods of improved nutritional characteristics.

#### Acknowledgements

This work has been supported by the Ministry of Education, Science and Technological Development of Serbia, projects: TR 31006, TR 31034 and by the EU FP7 project, Grant Agreement No.316004 (REGPOT-AREA)

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## NITROGEN- AND WATER-DEPENDENT ALTERATIONS IN QUALITATIVE GRAIN CHARACTERISTICS OF DURUM WHEAT-*TRITICUM DICOCOIDES* SUBSTITUTION LINES

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**Keywords:** *Triticum durum*, *T. dicoccoides*, nitrogen shortage, water use efficiency, protein content, rheological dough properties

Improved quality characteristics of the grain-derived end-products and better adaptation to adverse environmental conditions remain the major goals in breeding of the durum wheat. However, a relatively narrow genetic variation in the taxon and negative relations between yield and grain qualities may limit further progress in breeding programs. The introgression of novel genes such as wild taxa and exotic germplasms was frequently recommended as an alternate strategy to restore and enrich the gene pool.

The durum wheat cv. ‘Langdon’ (LDN), the donor species *T. dicoccoides* (DIC) and the set of thirteen Langdon-*dicoccoides* [LDN(DIC)] chromosome substitution lines were investigated in the factorial experiment arranged as split-plot design with two nutrition and water (optimal and reduced) levels and two replications. The effects of the substituted wild A and B (DIC)-chromosomes on qualitative and rheological grain parameters in the set of the substitution lines of *T. durum* under variable nutrition and soil moisture regimes measured on the whole-season long scale were studied.

Grain samples were analysed for protein and starch content, wet gluten content, Zeleny sedimentation value and alveograph parameter (*W*) using the near infrared transmittance technique. The differences between individual substitution lines appeared to be significant, for protein content, Zeleny test and alveograph parameter. The rheological parameters estimated using a reomixer, and Kieffer dough extensibility systems showed an appreciable

increase in dough-mixing properties, maximum resistance to extension ( $R_{max}$ ), and dough extensibility in the case of the occurrence of chromosomes DIC-2A, DIC-6B and DIC-7B. The DIC-chromosomes of the 1st, 2nd, 6th and 7th homologous groups were found to show the strong influence on the rheological parameters in the reduced N nutrition and low soil moisture. The obtained results suggest that the durum wheat with some (DIC)-chromosome substitutions has the potential to improve gluten polymer interactions and can be a valuable genetic resource for grain quality improvement.

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## DISTRIBUTION OF BETA-AMYLASE IN SOY PROTEIN PRODUCTS OBTAINED DURING TOFU PRODUCTION

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**Keywords:** HTC processing, soybean, soymilk, tofu, tofu whey,  $\beta$ -amylase

The most popular soy-foods are soymilk and tofu. Okara and tofu whey are the main by-products of soymilk and tofu production. The distribution of enzyme nutritionally valuable  $\beta$ -amylase from six soybean genotypes (Nena, Krajina, Novosadjanak, Balkan, ZPS-15 and Lana) in protein extracts of okara and tofu as well as in soymilk and tofu whey after hydrothermal cooking (HTC) of soymilk were assessed.

Soaked soybeans were ground and cooked by steam injection system (soybeans:water = 1:6) at 110 °C/1.8 bar/8 min. Content of  $\beta$ -amylase was determined by densitometry of SDS-PAGE electrophoresis gels. Data were assessed by analysis of variance (ANOVA – the Tuckey multiple range test).

The  $\beta$ -amylase isolated from soybean flour consists of a single polypeptide chain of molecular weight of 55,000–57,000. Content of  $\beta$ -amylase in investigated soy protein products (soymilk: 3.10–3.98%; tofu: 4.15–5.74%; tofu whey: 4.94–5.60) was high and similar to the content in soybean genotype (3.94–4.99%). Content of  $\beta$ -amylase in okara (0–7.17%) depended on respective soybean genotype. These results lead to the conclusion that the applied HTC treatment significantly affected protein fractions of higher molecular masses. In protein extracts of okara from genotypes Balkan, ZPS-15 and Lana the presence of  $\beta$ -amylase was not registered. This is consistent with our previous results which showed that the majority of extracted proteins from okara produced by hydrothermal processing of soymilk were low molecular mass peptides with molecular masses below 40,000. This data could be important for technology practice, also pointing out to new possibilities of protein by-products use.

### Acknowledgements

The study was financed by the Serbian ministry of Education, Science and Technological Development (TR31022) and the EU FP7 project Grant Agreement No.316004 (REGPOT-AREA).

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## WORKING VALUE OF OAT VARIETIES GROWN USED WITHIN LOW-INPUT FARMING

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**Keywords:** naked grain oat, glume grain oat, seed volume weight, 1000 seed weight, low-input farming

Oat is an ideal crop to be used within organic farming and other low-input farming methods. The most evident difference between both oat types lies in 1000 seed weight, which is 20–22% higher by the glume grain varieties. Also the gross grain yield of glume grain varieties is 25–28% higher, which after grain scalping changes. Naked grain oat features 22–27% higher yield of peeled oats which means commercial food production (Moudrý, 2003). In years 2012–2013 several small-area experiments were established on the experiment field of the Faculty of Agriculture in České Budějovice (380 m above sea level, pseudogley cambisol soil type, sandy-loam soil, pH 6.4, moderate temperature region, year average temperature 7.8 °C, annual rainfall 620 mm). Used 4 naked grain oat varieties were ABEL, IZAK, OTAKAR, SAUL and 4 covered (glume) grain oat varieties ATEGO, NEKLAN, VOK, ROZMAR. Particular genotypes were twice repeatedly sown on area of 10 m<sup>2</sup> using small-area exact. These experiments were based on low-input farming principles. As a working value – seed volume weight and 1000 seed weight, as an additional feature is mentioned grain yield. Achieved results were analysed by the two-way variance analysis using software SAS System. An average 1000 seed weight was very similar in both years, naked oat varieties reached values about 25 g and glume grain varieties about 32.7 g. Highest value of 1000 seed weight shown variety Abel (26.2 g), lowest values shown variety Izak (22.8 g). Among glume grain varieties highest values of the same parameter shown variety Neklan (33.8 g), followed by varieties Atego, Vok and Rozmar, which had 31.5 g. 1000 seed weight parameter showed very strong dependence on genotype and glume presence with low values variability. The dependence of year was not statistically proven. Seed volume weight significantly varied between both years. In year 2012 reached this factor higher values compared to year 2013 and was near to average values for oat. In year 2012 this value amounted 661 g·l<sup>-1</sup> for naked grain oats on average and

518 g·l<sup>-1</sup> for glume grain oats. In year 2013 reached seed volume weight 571 g·l<sup>-1</sup> for naked grain oat and 471 g·l<sup>-1</sup> for glume grain oat. Average seed volume weight was 616 g·l<sup>-1</sup> for naked grain oats and 495 g·l<sup>-1</sup> for glume grain oats.. Glume presence rate significantly affects seed volume weight while there was statistically proven slight dependence of year on seed volume weight only. 1000 seed weight- the difference was predominantly caused by glume absence by naked oat varieties. Differences between years were insignificant. When compared grain yields, there was found out significant difference between both oat types, whereas the naked grain varieties reach only 58% of glume grain varieties.

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## BAKING QUALITY OF HULLED WHEAT SPECIES DETECTED BY RHEOLOGICAL SYSTEM MIXOLAB

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**Keywords:** einkorn, emmer, spelt, wheat, rheological properties

Hulled wheat species (einkorn, emmer, spelt) are presumed to be an alternative to bread wheat in organic agriculture since the yield penalty is less pronounced under organic farming and generally higher prices can be achieved for specialty grains (Konvalina et al., 2014). Despite to organic farming development in EU, farmers start to grow hulled wheat species, but processors need more information related to the technological quality. In the past, landraces of various wheat species have been used to improve protein content and other specific quality traits (Vaccino et al. 2010). The present study investigated various underutilized spring wheat species with respect to baking quality detection. Einkorn, emmer and spelt wheat were compared to common wheat in organic field trials in the Czech Republic. For the evaluation of baking quality was used Mixolab II. system (accepted as the ICC standard method No. 173 – ICC 2006), which makes possible to evaluate physical dough properties such as dough stability or weakening, and starch characteristics in one measurement. For the comparison were used also standard ICC methods of baking quality analysis. Data were processed by the Statistica 9.0 (StatSoft. Inc., USA) program. Einkorn and emmer had evidently worse rheological quality than spelt and bread wheat. It was because of lower water absorption and also weak protein based on the mechanical work. On the other hand, in case of einkorn and emmer was observed higher stability of hot-formed gel during starch evaluation. Spelt was very similar in both, protein and starch measurements, to bread wheat. High correlations were found between Mixolab characteristics and standard technological parameters during evaluation.

**Acknowledgements**

This work was funded by the National Agency for Agricultural Research of the Ministry of Agriculture of the Czech Republic (Project No. NAZV QJ1310072) and the University of South Bohemia in České Budějovice (Project No. GAJU 063/2013/Z).

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# Author Index

- Aadel, H. 25  
Abdelwahd, R. 25, 35  
Ács, E. 16, 17  
Ács, K. 16, 17  
Al-Menaie, H. S. 6  
Al-Ragam, O. 6  
Al-Shatti, A. 6  
Alybayeva, R. 20, 40  
Amallah, L. 34  
Arhbal, A. 35  
Armonienë, R. 21  
Askanbayeva, B. 22  
Asrandina, S. 20, 40  
Atabayeva, S. 20, 40  
Atkari, D. G. 29
- Bao, J. 39  
Barac, M. 45  
Békés, F. 1, 18  
Belter, J. 26, 28  
Berbezy, P. 19  
Bird, A. 19  
Birtáné Vass, Zs. 24  
Blümmel, M. 29  
Boczkowska, M. 15  
Bona, L. 16  
Bramley, H. 9  
Brazauskas, G. 21  
Budhagatapalli, N. 12  
Bukan, M. 23, 42
- Capouchová, I. 39, 41, 47  
Chapron, S. 19  
Chi, Y. 5  
Csósz, M. 13, 24
- Danielewicz, J. 26  
Datta, K. 9  
Demin, M. 43  
Demir, S. 36
- Diria, G. 34, 35  
Dochy, N. 37  
Doktyrbay, G. 40  
Douira, A. 25  
Dubas, E. 33  
Dziurka, M. 33
- Ehsanzadeh, P. 7  
El Mouhtadi, A. 25  
Erban, A. 8, 11, 27
- Falusi, J. 24  
Fonseca e Silva, F. 30  
Fu, D. 13
- Gaboun, F. 25, 34, 35  
Ganoudi, M. 35  
Gawłowska, M. 26, 28  
Geuten, K. 37  
Ghouse, S. K. C. 29  
Glaubitz, U. 8, 11  
Górny, A. 44  
Grausgruber, H. 41  
Gunjača, J. 42  
Gupta, S. K. 29  
Gurushidze, M. 12
- Hansson, M. 14  
Hassikou, R. 34  
Hensel, G. 12  
Heuer, S. 11  
Hiekel, S. 12  
Hincha, D. K. 8, 11, 27  
Horibata, A. 35
- Ibriz, M. 25  
Ikić, I. 23, 42  
Iraqi, D. 25, 35
- Jagadish, K. S. V. 11, 27
- Jakab, T. 24  
Jan, H. U. 30  
Jang, S. 38  
Janovská, D. 39, 47  
Janowiak, F. 33  
Johnson, R. 10  
Jovanovic, Z. 43  
Jukić, K. 23, 42
- Kaczmarek, Z. 26  
Kapás, M. 13, 24  
Kato, T. 35  
Kenzhebayeva, S. 20, 40  
Kim, B. 38  
Koh, H.-J. 38  
Kolmer, J. 2  
Konvalina, P. 39, 41, 46, 47  
Kopka, J. 8, 11, 27  
Korbas, M. 26  
Korzun, V. 3  
Kovács, Zs. 16  
Krzewska, M. 33  
Kumlehn, J. 12  
Kwiatiek, M. 26, 28
- Lajtár, T. 13  
Langner, M. 44  
Langó, B. 16  
Lantos, Cs. 13  
Lawas, L. M. F. 11, 27  
Lee, C.-M. 38  
Lee, G. 38  
Li, D. 5  
Li, K. 39  
Li, T. 5  
Li, X. 11, 27  
Lovrić, A. 23, 42
- Ma, W. 4  
Ma'arup, R. 9

- Mace, W. 10  
 Majka, M. 26, 28  
 Makowska, K. 32  
 Malo, R. 11  
 Maričević, M. 23, 42  
 Martinek, P. 31  
 Matuz, J. 24  
 Matyszczyk, I. 14  
 Mauleon, R. 11  
 McManus, M. 10  
 Mesterházy, Á. 17  
 Milovanovic, M. 43  
 Molla, K. A. 9  
 Moudry, J. 46  
  
 Nadolska Orczyk, A. 15  
 Nowicka, A. 33  
  
 Oleszczuk, S. 32  
 Omirbekova, N. 22  
 Onyšk, A. 15  
 Orczyk, W. 15  
 Osakabe, Y. 27  
 Oszvald, M. 18  
 Óvári, J. 24  
 Özbek, Ö. 36  
  
 Park, J. 38  
 Pauk, J. 13  
 Pesic, M. 45  
 Petróczi, I. M. 16, 24  
 Pfrieme, A.-K. 12  
 Polya, S. 18  
 Poós, B. 24  
  
 Purnhauser, L. 13, 24  
  
 Rajchel, I. 15  
 Regina, A. 19  
 Rhríb, K. 34  
 Ruelens, P. 37  
  
 Safonov, D. 22  
 Salmanowicz, B. P. 44  
 Šarčević, H. 23, 42  
 Schedel, S. 12  
 Seo, J. 38  
 Sharma, N. 37  
 Sharp, P. 9  
 Shi, W. 27  
 Shinozaki, K. 27  
 Simpson, W. 10  
 Soriano Viana, J. M. 30  
 Stanojevic, S. 45  
 Stehno, Z. 41  
 Štěrba, Z. 46, 47  
 Stikic, R. 43  
 Stoličková, M. 47  
 Suchý, K. 41  
 Sugano, S. S. 27  
 Sun, L. 5  
 Sun, Mei 39  
 Sun, Minglong 5  
 Svobodová, L. 39  
  
 Tabatabaei, S. 7  
 Taghouti, M. 34  
 Tamas, L. 18  
 Tar, M. 13, 24  
  
 Tashenev, D. 40  
 Tomoskozi, S. 18  
 Tóth, B. 24  
 Trethowan, R. 9  
  
 Udupa, S. M. 25  
 Ueta, R. 27  
  
 Vagi, P. 18  
 Varga, M. 17  
 Véha, A. 16  
 Vida, Gy. 24  
 Vucelic-Radovic, B. 43, 45  
 Vyhnanek, T. 31  
  
 Waligórski, P. 33  
 Wang, X. 5  
 Watanabe, T. 27  
 Williams, W. 10  
 Wiśniewska, H. 26, 28  
 Wu, Jiajie 13  
 Wu, Jingzheng 13  
  
 Zelkowski, K. 12  
 Zhang, H. 13  
 Zharasova, D. 40  
 Zhunusbayeva, Z. 22  
 Zhussupova, A. 22  
 Zimny, J. 32  
 Zmijewska, B. 15  
 Žur, I. 33  
 Zuther, E. 8, 11, 27