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**Abstracts of Oral Presentations and Posters**

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## KEYNOTE ABSTRACTS

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### GENOMIC STRATEGIES TO IMPROVE GRAIN QUALITY FOR DIVERSIFICATION OF CEREAL CROPS UTILIZATION

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The developing countries have over the last few decades seen the establishment of new food habits and sedentary lifestyles, when combined has led to an overconsumption of calories (>11.3 MJ/day) and an increase in diet-related diseases, such as diabetes, coronary heart disease and colorectal cancer. To stem the rising cost of healthcare in developing countries, a drastic reduction of the daily calorie intake will be required. On the other hand, people in developing countries need foods that will be completely digested to provide the maximum calories needed to meet the energy requirements for normal body functions. Therefore, grain quality is a dynamic concept which changes with the consumer.

More than 50% of the calories in human diet are provided by the cereal grains wheat, maize, rice, barley, sorghum and millets. Carbohydrates are the predominant food component in these grains and the major source of energy (9.2 MJ/day) in human diet. Most of the carbohydrates are found in the large endosperm tissue, where they occupy up to three-fourths of the space followed by proteins and lipids. The major carbohydrate starch is stored as an energy-dense and water-insoluble granule composed of one-quarter amylose and three-quarters amylopectin along with traces of lipids and proteins. Like most carbohydrates, starches of various botanical sources show structural diversity and small changes in their makeup can alter functional properties. For example, amylose deficient (waxy) and increased amylose wheat and barley grain starches show differences in the timing of energy release in form of calories when passed through the human digestive tract. Thus, starch is an important target for cereal improvement to satisfy the demand for lower and higher energy foods for different market needs. Some of the future targets for starch modification include alterations to starch granule size and amylopectin fine architecture which both influence starch digestibility.

Other grain genetic improvement targets for the developing world include structural polysaccharides, such as dietary fibre and beta-glucan, which have an effect on lowering the calorie uptake. Gene-based markers associated with high beta-glucan concentration in barley are being used to select suitable barley germplasm for breeding of barley with desirable beta-glucan content.

Anthropogenic activity caused climate change has resulted in increased global temperature and erratic precipitation. Precipitation during grain maturation and at harvest time results in-spike germination of physiologically mature grain, called Pre-harvest sprouting (PHS). Sprouted grain flour produce poor quality products, such as bread loaves with

large holes, sticky crumb and dark coloured crust and noodles with coloured spots. A genetic mapping strategy was used to identify genomic regions associated with PHS resistance to develop DNA markers for use in marker assisted selection to accelerate the development of PHS-resistant wheat varieties.

**Keywords:** wheat, barley, carbohydrates, starch digestibility, molecular markers

## HOW MODEL ORGANISMS HELP TO IMPROVE CEREAL STRESS RESISTANCE?

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According to the definition model organisms in general are all that are extensively studied to understand particular biological phenomena, with the expectation that discoveries in a particular organism will provide insight into the workings of others. In the focus of the lecture the above mentioned biological phenomena is the abiotic stress tolerance and the wide spectrum of organisms is narrowed to model plants. Many of the mono- and dicotyledonous plants could be listed here, but the lecture will concentrate only on those that provided the basis of major advances in the field and established a platform for the improvement of cereal stress tolerance.

As first in the line, tobacco (*Nicotiana tabacum* L.) has become the model system for tissue culture and genetic engineering more than 60 years ago. Besides tissue culture and protoplast fusion techniques, tobacco meant the basis of the establishment of optimized protocols for the genetic transformation with *Agrobacterium*. With the first sequenced chloroplast genome in 1986 this plant has started the “plant genome era” and since then this is the most often used model plant of the promising field of chloroplast transformation. Undoubtedly, *Arabidopsis thaliana* is currently the most popular model plant. Its small stature and short generation time facilitates rapid genetic studies, and many phenotypic and biochemical mutants have been mapped and available from stock centers. *Arabidopsis* was the first plant to have its nuclear genome sequenced. This, along with a wide range of information concerning *Arabidopsis*, is maintained by the several databases. Rice (*Oryza sativa*) is definitely the most important model for cereal biology. It has one of the smallest genomes of any cereal species, and sequencing of its genome is finished. *Brachypodium distachion* is an emerging experimental model grass that has many attributes that make it an excellent model for temperate cereals. *Physcomitrella patens* is a moss increasingly used for studies on development and molecular evolution of plants. It is so far the only non-vascular plant with its genome completely sequenced. Moreover, it is currently the only land plant with efficient gene targeting that enables gene knockout.

For the crop improvement through genetic manipulations hundreds of loci involved in the trait of environmental adaptation need to be identified in plants used as models. The

appropriate alleles for the major (able to have qualitative effects) loci will need to be identified and modified (e.g. increased expression, altered time/space expression, etc.). This will need a huge amount of gene expression regulatory sequence data that provide enough information even for artificial promoter design. Then the research should leave the lab and field experiments will be required. In field tests gene combinations will play a major role, probably modifying more than one of the physiological processes that contribute to abiotic stress tolerance. In the genotype evaluation the instrumental phenotyping will gain increasing importance; as moving out from the walls of the greenhouse to the field, this will be able to collect, analyze and synthesize the data of natural crop variants, breeding material and transgenic plants. This can serve as a platform to build up “virtual plants” where phenotypic effects of different abiotic stress genes and gene combinations can be not only evaluated but predicted also.

**Keywords:** model plants, abiotic stress tolerance, candidate gene, stress response profiling, genetic transformation

## PLANT PHENOTYPING AS A TOOL IN CEREAL BREEDING

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The ability to quantitatively analyze plant phenotypic traits (from single cells to plant level) and their dynamic responses to the environment is an essential requirement for genetic and physiological research, and the cornerstone for enabling applications of scientific findings to bioeconomy. Since molecular profiling technologies allow today the generation of a large amount of data with decreasing costs largely due to automation and robotics, there is an emerging need for understanding of the link between genotype and phenotype. This task requires the development of robust and standardized phenotyping applications based on specialised infrastructure, technologies and protocols. In the recent years there has been a rapid development in this area both at the level of academic research and biotech industry in Europe and overseas.

We have developed complex semi-robotic green house based plant monitoring systems, which are applicable for monitoring the growth and physiological status of middle sized plants both at the level of shoots and leaves, as well as roots. These include computer controlled precision watering of plants whose growth is monitored by digital photography imaging, which allows estimation of green biomass. The transpiration activity is followed by measuring leaf temperature with thermal-infrared imaging, while photosynthetic activity is followed by variable chlorophyll fluorescence imaging. Development of the root

structure is also quantified by digital photography imaging. These plant phenotyping systems have been successfully applied to study the drought tolerance of wheat in combination with other stress factors, such as N availability. The results have been validated by an outdoor rain shelter system. The combination of digital photography, chlorophyll fluorescence and thermal imaging has also been proven useful in studying the development and progress of leaf rust infection in wheat. The data obtained by the application of our phenotyping platforms can be used for pre-selection of promising candidates to enter into breeding programs.

**Keywords:** plant phenotyping, drought stress, wheat, RGB imaging, thermal imaging, chlorophyll fluorescence imaging

*Reference:*

Cseri, A., Sass, L., Törjék, O., Pauk, J., Vass, I., Dudits, D. (2013) Monitoring drought responses of barley genotypes with semi-robotic phenotyping platform and association analysis between recorded traits and allelic variants of some stress genes. *Australian Journal of Crop Science*, 7: 1560–1570.

## GENE DISCOVERY TOWARDS DROUGHT RESISTANCE – A QUEST FOR REALISM

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Plant breeding is being very successful in developing drought resistant varieties and hybrids of our cereal crops. This can be ascribed to conventional breeding programs interfaced with specific developments in plant and crop physiology as well as Mendelian genetics, agronomy, soil science and advanced phenotyping technology (see [www.plantstress.com](http://www.plantstress.com)). In the last two decades expectations from genomics contributions in this area of agriculture development were high in view of the high rate of publications concerning the real or potential contributions of genomics to drought resistance in plants. However, the fact remains that today the GM market is dominated mainly by herbicide, disease and pest resistant GM varieties and hybrids with only one probable drought resistant GM maize hybrid. This despite some 300 different drought resistant experimental transgenic plants reported to successfully express about 90 genes for drought resistance.

On the background of the available rich knowledge about the physiology, breeding and phenomics of drought resistance this presentation examines why gene expression work as a key activity in the genomics of drought resistance is very slow in delivering the promised results with regard to drought resistance improvement in the field [1]. The key problem considered here is the disparity between the protocols and methods used in gene expression studies towards drought resistance and the real expected utility of the resultant phenotype in the agricultural domain. Both the protocols for inducing gene expression and the phenotyping of the derived transgenic plants are all too often irrelevant towards drought stress and drought adaptation in the reality of the plant in the field.

This presentation is concluded by underlining two options for effectively using gene expression work interfaced with plant breeding in order to enhance probabilities for the improvement of drought resistant crops.

**Keywords:** drought resistance, drought stress, plant breeding, genomics, methods, protocols, gene expression

*Reference:*

1. Blum, Abraham (2011) Drought resistance – is it really a complex trait? *Funct. Plant Biol.*, 38, 753–757.

## WATER STRESS AND ITS REGULATION ON THE GRAIN FILLING OF RICE

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Modern rice (*Oryza sativa* L.) cultivars, especially the newly bred “super” rice, have numerous spikelets on a panicle with a large yield capacity. However, these cultivars often fail to achieve their high yield potential due to poor grain filling of later-flowering inferior spikelets (in contrast to the earlier-flowering superior spikelets). Conventional thinking to explain the poor grain filling is the consequence of carbon limitation. Our earlier results, however, have shown that carbohydrate supply should not be the major problem because they have adequate sucrose at their initial grain filling stage. The low activities of key enzymes in carbon metabolism may contribute to the poor grain filling. Proper field practices, such as moderate soil drying during mid and late grain filling stages, could solve some problems in poor grain filling. Further studies are needed by molecular approaches to investigate the signal transport, the hormonal action, the gene expressions and the biochemical processes in inferior spikelets.

## BREEDING STRATEGIES AND BREEDING RESULTS AGAINST FUSARIUM HEAD BLIGHT IN BREAD WHEAT

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In recent decades the toxigenic fungi have played an increasing role in food and feed safety. In breeding even now the yield is in the centre, other traits played only a secondary

role, especially toxigenic fungi that influence yield in most years. Most of the varieties and hybrids are much more susceptible than land races.

Resistance research has found in both wheat and maize close or very close relationships between disease severity and toxin contamination. In wheat from well planned experiments the relations are very close, so a breeding program that reduces susceptibility automatically decreases also toxin contamination. In the Szeged wheat breeding program we have bred numerous highly resistant lines and identified superior lines and cultivars among regular breeding material. The increased resistance might not be enough in highly epidemic years, but these genotypes can be protected effectively with fungicides securing toxin contamination below EU toxin limits.

Genetic research also helps this work, we detected that the 5A QTL from Sumai 3 (CM 82036) is as strong as the 3BS (*fhb1*). These QTLs protect against kernel infection and toxin contamination. They influence the whole disease process and have general significance, additionally they are not *Fusarium* species specific.

In Mini Mano/Frontana QTLs were detected that controlled visual symptoms, kernel infections a DON contamination at the same time; several other QTLs influenced only one or two of them. So the genetic regulation is not simple.

In maize the situation is more complex, in some hybrids resistance was found to all toxigenic species, in others resistance to one pathogen could be accompanied with susceptibility to others. Therefore the breeding system is more complicated; more *Fusarium spp.* should be controlled at the same time. As this trait is also polygenic, no major QTLs were described until now as it is the case for wheat, an effective breeding system will need strong efforts.

The resistance screening of cultivars and hybrids shows clearly a very large variability among them. For this reason the cultivar registration can help to exclude highly susceptible cultivars from commercial production to lessen the food and feed safety risks.

We see also that resistance alone will not solve the problems. Updated agronomy, keeping the regulation a Good Agronomy Praxis (GAP), strongly modernized storage systems and processing can secure that the good quality at harvest could be secured until the product is coming to our table.

**Keywords:** *Fusarium* head blight, wheat, maize, *Fusarium* ear rot, *Aspergillus flavus*, DON, fumonisin, aflatoxin

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## BREEDING VIA TRADITIONAL AND MODERN METHODS, NEW GENOTYPES

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Traditional breeding during the period from the discovery of Mendel's Laws to the present form one of mankind's success stories. Continuous efforts have led to the accumulation and preservation of favourable alleles for a better adaptation and agronomic performance of cultivated crops. The spectacular yield increases achieved from the sixties of the last century began to slow down during the last decade of the 20th century. This can be attributed to a number of factors, including the lack of coordination between enhanced yields and improvements in characters influencing yield stability [1]. Differences between alleles at multiple loci have become relatively small in the elite gene pool. It has become evident that the new wheat genotypes will need to be developed and that further crop improvement will require new breeding tools. The complex nature of new challenges means that traditional breeding methods need to be combined with new breeding technologies. This will include a new assessment of the existing wheat genetic resources in order to improve the sustainability, safety and quality of cereal-based food production.

The new wheat genotypes should satisfy several new challenges, like increased yield and quality stability, better nutritional quality traits, higher level of bioactive compounds. Better N, P and water use efficiency, thus improving ecological and economic sustainability. It should contribute to food safety and security through a higher level of durable stress resistance and/or tolerance, etc. To fulfil these challenges prebreeding programmes were established to use genebank collections, determine natural variation and generate new genetic variability. Marker assisted breeding is one of the tools which became widespread for breeding programmes. The efficient use of introgression lines by systematic backcrossing and pyramiding of marker-defined segments in a wheat genetic background became a routine technology. Further improvement of wheat genotypes will be necessary taking into consideration new challenges. Integration of novel technologies like transgenesis, cisgenesis, genomic selection, etc. into traditional breeding is necessary to identify and use of new genetic variation for the future progress.

**Keywords:** traditional breeding, genetic resources, prebreeding, marker assisted selection

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## RECENT DEVELOPMENTS IN HUMAN UTILIZATION OF TRITICALE

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Triticale (*Triticosecale* Wittm.) acreage shows a continuous growing trend all over the world in the last twenty-five years. In spite of the fact that both parental species (sp. *Triticum*, sp. *Secale*) are used for human food, the utilization of triticale in human consumption is still uncertain. The main focus of this paper was to study and compare technological and nutritional values of triticale and its parental species. Ten triticale (cultivars and experimental lines) along with one wheat and one rye varieties were used in the tests. Generally, grain and flour technological traits (Test Weight, hardness, flour yield, protein content, Zeleny volume) of the triticale entries positioned in between the parental wheat and rye attributes. However, thousand kernel weight of triticale cultivar GK Szemes and grain hardness of cv. GK Idus surpassed all other entries. The study revealed that – having considerable variation – triticale grains are very rich in beneficial elements such as P, K, Mg, Cu and Zn. Dietary fiber (DF) content of triticales were between the rye and wheat controls (10–15%). Total arabinoxylan content (TOTAX) of triticales were much closer to rye than and in some genotypes considerably expanded it (6.5–7%). AX consisted in 40–45% of total DF in rye and wheat while it reached as high as 50–55% triticale entries. Arabinose:xylose ratios (A/X) were 0.6, 0.65 and 0.67 for wheat, triticale and rye, respectively. A/X was found to be particularly high (0.75) in triticale cv. GK Szemes. Favorable water uptake and loaf volume were featured also by this variety. We concluded that this variety may be a valuable breadmaking component in triticale-wheat blends. More and more consumers trend to use increasingly valuable grain sources and fiber-rich products in their daily based diets. Because triticale flours per se perform poor attributes for baking industry, blends exploiting advantages of triticale and wheat features may be a suitable way to use this valuable crop in larger food relation.

## IMPROVING FUSARIUM RESISTANCE IN BREAD AND DURUM WHEAT

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In the gene pool of bread wheat (*Triticum aestivum*) large variation for resistance to Fusarium head blight has been discovered, but strikingly less in current durum wheat (*T. durum*). Resistance to Fusarium head blight is a quantitative trait controlled by polygenes (QTL) and modulated by the environment. Numerous studies have been conducted to decipher the quantitative inheritance of Fusarium resistance in wheat mainly based on QTL mapping using segregating populations [1]. With the advent of high-density genotyping tools association genomics and genome wide prediction approaches became practicable recently [2].

Even in the era of genomics progress by selection depends heavily on accurate and powerful phenotyping. In a typical breeding situation large numbers of experimental lines need to be tested for resistance response. Genotype-by-environment interaction plays an important role in such trials and may result in low heritability. Therefore, measures need to be taken to obtain reliable resistance measurements, such as artificial inoculation, control of environmental conditions if feasible, and most importantly replication of trials within and across environments. In addition, it is not easy to separate active resistance responses from passive resistance due to plant morphological and/or developmental plant traits.

We report here about: 1) successful application of large effect QTL in marker assisted wheat improvement [3, 4]; 2) identification and genetic analysis of promising genetic resources for bread wheat and durum wheat breeding; 3) the association of morphological traits, especially plant height and the extent of anther extrusion with FHB resistance. The implications for resistance breeding will be discussed.

**Keywords:** Fusarium head blight, resistance, breeding, resistance genetics, phenotyping, trait associations, genomic assisted breeding

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**ORAL ABSTRACTS**

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**Section 1**

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**TRANSCRIPTOMICS: SIMULATENEUS GENE EXPRESSION AND SNP ANALYSIS OF BYDV RESISTANCE GENE *RYD4<sup>Hb</sup>* INTROGRESSED FROM *HORDEUM BULBOSUM* INTO BARLEY**

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Barley yellow dwarf virus (BYDV), a viral disease spread via aphids, is responsible for high yield losses world-wide. Complete resistance to the virus has been successfully introgressed from the secondary gene pool *via* interspecific crosses with the tetraploid wild species *Hordeum bulbosum* (*Hb*). The locus of the resistance was identified on barley chromosome 3HL and is governed by a dominant gene, *Ryd4<sup>Hb</sup>*. In the present study, Massive Analysis of cDNA Ends (MACE) and RNASeq were performed with bulks of the resistant introgression lines and susceptible wild-type genotypes after eliciting the resistance reaction by virus-aphid treatment, to 1) identify genes that were exclusively expressed in the resistant plants and 2) to identify SNPs characterizing the resistant plants.

More than 200 transcripts were exclusively expressed in the resistant plants and about 400 transcripts with unique sequence (SNPs, Alleles) were identified. From these, 3' UTR specific SNP-markers (MACE-markers) were developed. So far, all tested 35 MACE-markers were mapped on 3HL, three MACE markers are located with a distance of 0.1 to 0.4 cM to *Ryd4<sup>Hb</sup>* and are of potential use for marker-assisted breeding programs. Among the exclusively expressed transcripts, several had significant similarity to known genes involved in virus-resistance processes.

**Keywords:** genetic mapping, *Hordeum vulgare*, molecular markers, virus resistance

## ROLE OF THE *Hd1* FLOWERING TIME GENE ON DIVERSIFICATION OF CULTIVATED RICE

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After domestications, many crops have made dramatic evolutions to supply enough foods for human demands all over the world. Cultivated rice, *Oryza sativa* L., originating from a tropical region is an example of adaptation to extremely wide range of climatic conditions, from 53°N to 40°S latitudes. However, we do not know how to shape the adaptability to such regions where rice is never grown.

Flowering-time (heading date) is a major determinant of the adaptability in rice. In the last decade, many genes and quantitative trait locus (QTLs) for rice production have been identified that are involved in flowering-time control. Recent progress in genomics on quantitative traits has enhanced our understanding of the genetic and molecular bases on flowering-time control. Previously, we showed that allelic variation of *Hd1* has an important role in the shaping and diversification of adaptation during the domestication process [1]. *Hd1* is a key flowering time gene in rice and is orthologous to the Arabidopsis *CONSTANS* gene. In addition, a loss-of-function allele had an evolutionary force generating a genetically distinguished subpopulation in cultivated rice, which shows early heading and a distinct geographic distribution. In this study, we elucidated the role of *Hd1* on the wide adaptability of cultivated rice using varieties from Japan, 32°N to 44°N latitude. The results demonstrated that naturally occurred mutations during the diversification of cultivated rice were selected for the adaptation to local regions. I will discuss what is the gene targeted in rice breeding programs, *Hd1*, *Hd5* [2], and *qLTG3-1* [3].

**Keywords:** flowering time, adaptability, rice, breeding program

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## Section 2

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### REDUCED GRAIN STARCH PHOSPHATE INCREASES COLEOPTILE GROWTH

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Phosphate content of starch is related to properties such as viscosity and gelatinisation temperature, and is known to alter the starch degradation rates [1]. High phosphate starches are typically easier to digest, while plants with very low starch phosphate exhibit a starch excess phenotype due to the resistance to degradation these polysaccharides exhibit [2]. Most work in this field has focussed on transient (leaf) starch or on potato tubers. Our group has produced wheat lines with reduced endosperm starch phosphate to investigate its effect in cereals.

This grain specific reduction has produced lines with greatly altered growth characteristics from the early seedling stage to maturity, and here we examine alterations in the early and vital growth of the plants. We examine and describe the grain weight changes in these lines, demonstrating that the transgenic lines have a larger grain weight. We find that coleoptile length increases with grain weight, but that the transgene is also linked to a statistically significant increase in coleoptile length on top of these changes.

**Keywords:** starch, coleoptile, germination, phosphate

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### GENOTYPING BY SEQUENCING IN POOLED SAMPLES OF BARLEY DOUBLED HAPLOID LINES: A CASE STUDY

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Genotyping by sequencing (GBS) provides an economical and rapid approach to simultaneously identify and genotype large numbers of single nucleotide polymorphisms

(SNPs) in many individuals. In maize, barley and wheat, GBS has been shown to yield thousands of SNP markers on sets of 48 to 96 lines analysed in a single library [1, 2]. In a typical analysis, each line is given a separate barcode when constructing the GBS library, allowing for SNP genotyping to be performed on each line separately. In some applications, it could prove useful to analyse a set of lines as a composite sample by pooling equal amounts of each DNA. For example, if one is interested in determining the allelic frequencies at numerous SNP loci on a population level, it could prove more efficient to estimate the proportion of each allele simply by measuring the proportion of reads corresponding to one or the other allele rather than obtaining a genotype call in each line composing the population. In our case, we wanted to identify regions of segregation distortion in a number of populations of doubled haploid (DH) lines of barley. To explore the most efficient approach, we prepared a GBS library using the *PstI/MspI* protocol [2] for 75 DH lines of barley. From a single lane, we obtained 125 M 100-bp reads and these were used to genotype each of the lines at the SNP loci segregating in this population. Based on this conventional analysis, three regions of pronounced segregation distortion were identified. Using the same 125 M reads as if they had been obtained from a pooled sample of the same 75 DH lines, we explored how to best exploit these data to estimate allele frequencies. We determined the number of SNPs that could be called as a function of the number of reads used (5 M to 25 M reads) and the variability surrounding the estimated allele frequencies using 1–5 subsets of 5 M to 25 M reads. The resulting estimates of allelic frequencies obtained using an optimized approach on pooled reads were then used to explore segregation distortion. We found the same three regions of high segregation bias using the composite approach as was obtained by analysing lines individually. This result was obtained, however, using much fewer reads. Thus, it would be possible to examine multiple DH populations in this fashion rather than a single population for a given sequencing effort. The analyses conducted in this work likely apply to a number of other situations where the pooling of samples could prove more cost-effective than the analysis of individual samples.

**Keywords:** SNP markers, genotyping by sequencing, doubled haploids, barley, segregation distortion

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## ASSOCIATION MAPPING OF DROUGHT RESISTANCE AND MESOCOTYL ELONGATION IN RICE

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As rice is the most important staple food in China, prominent achievements in rice genetic improvement has been made by hundreds of breeders. A large number of rice cultivars have been released based on their excellent performances in grain yield, quality and disease/insect resistances. But drought resistance has been neither seriously considered as the target of selection in most rice breeding programs, nor included in character evaluation in national or provincial multiple environment tests (METs) for most cases. Only a few upland rice cultivars have been released by Ministry of Agriculture (MOA) of China or provincial governments, mostly in South-Western or North-Eastern part of China. Facing the double challenges of food security and water resource scarcity, theoretical studies and breeding practices in water-saving and drought-resistance rice have been taken by our team since the end of last century. A series of inbreds or hybrids have been developed that have high level of drought resistance together with advantages in yield potential, grain quality and disease/insect resistance.

In this study, a set of more than 200 rice genotypes, including core collection of Chinese rice germplasm and rice cultivars with drought resistance, were re-sequenced by ~5X coverage using new generation genome sequencing technology. More than 600K SNPs were identified and used as the genotyping data. Those materials were evaluated in trials with water regimes in drought screening facility for two seasons. Phenotypic data were obtained for a series of characters related to drought resistance, e.g. delayed heading date, leaf rolling, canopy temperature, spikelet fertility, grain yield, etc. Genome-wide association mapping were conducted using mixed linear model for those traits.

Direct sowing was widely used in rice production when water is not adequate for transplanting, or chosen by farmers for saving water and labour cost. High percentage of rapid, homogeneous seedling emergence became a key issue, especially when lower seeding rate was used to reduce the seed cost of hybrid rice. Mesocotyl elongation is an important character that accelerates seedling emergence through the cover layer of soil. The mesocotyl lengths of those genotypes were measured using both dark culture and sand culture methods. Rice germplasm with mesocotyl elongation were screened out to serve as parental lines in breeding program. Association mapping was used to identify genetic loci influencing mesocotyl elongation.

**Keywords:** *Oryza sativa* L., drought resistance, mesocotyl elongation, QTL, association mapping

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### Section 3

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#### IDENTIFICATION OF INFORMATIVE SNP MARKERS FOR ASSAYING FUNCTIONAL DIVERSITY IN RYE

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Rye (*Secale cereale* L.) is a major cereal in Northern Europe and can be grown under extreme climatic and poor soil conditions. This small grain cereal, which is traditionally used for food and feed, is well established as a bioenergy crop, and exhibits an outstanding resistance to biotic and abiotic stresses. Advanced breeding requires a more systemic view, linking phenotypes to genotypes as e.g. gene expression viz. transcript profiles, molecular markers and metabolites. Here, an integrated approach to breeding of energy rye is presented, and the applicability of novel selection tools is demonstrated.

RNA from two elite inbred lines of a hybrid breeding program, that were exposed to drought under controlled environmental conditions, was used for deepSuperSAGE transcript profiling. Differential expression data were correlated to single nucleotide polymorphisms (SNPs) identified in cDNA libraries from bulks of flag leaves, culms and ears of both genotypes in an independent experiment. We present results of an association study aiming at identifying genes responsive to drought stress in rye, and their use to develop SNP markers for practical rye breeding. The drought stress-regulated transcripts were integrated into the genetic map of rye. The resulting gene-based SNP markers complement the molecular toolbox available for rye, and will be backed up by QTL mapping and data from comprehensive field trials to approach complex inherited traits like biomass yield and yield components.

**Keywords:** deepSuperSAGE, Next Generation Sequencing, cDNA libraries, SNP marker, QTL mapping, abiotic stress, drought stress, bioenergy crop, hybrids, current elite breeding material

*Reference:*

Hackauf, B., Rudd, S., van der Voort, J.R., Miedaner, T., Wehling, P. (2009) Comparative mapping of DNA sequences in rye (*Secale cereale* L.) in relation to the rice genome. *TAG*, 118(2): 371–384.

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### DETECTION OF GENOME REGIONS AFFECTED BY SELECTION IN A BARLEY BREEDING PROGRAM

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The evolution of allele frequencies of molecular markers along breeding programs can be used to identify specific regions of the genome related to the trait(s) under selection. The regions with genes affected by to selection can be monitored by the detecting shifts of allelic frequencies from their expected values under a no-selection scenario. This approach has been named “selection mapping”, and its outcome are “selection QTLs” [1, 2]. We have attempted this approach for an elite cross of the Spanish barley breeding program. Allelic frequencies at the F2, before the onset of selection, were estimated with a set of microsatellites, to establish a baseline. Then, the genotypes of lines that reached F8 in the breeding program were assessed using a genotype-by-sequencing system (GBS). This last approach produced a number of markers large enough to allow a full genome scan of allelic frequencies, once they were positioned in the barley reference genome. Fourteen regions with selection QTL were declared, using a false discovery ratio of 5%. The nature of the genes that seem to underlie some of these regions, and the similarities with QTL found in other studies, offer new insights on the nature of barley adaptation for Mediterranean environments.

**Keywords:** barley, breeding, GBS, selection QTL

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## Section 4

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### THE DISCOVERY OF THE *CBF* GENES AS THE MAIN GENETIC DETERMINANTS OF THE FROST TOLERANCE AT THE *FR2* LOCUS AND THEIR POSSIBLE APPLICATION IN PLANT BREEDING

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Cold acclimation, which is ensured by the gradually decreasing temperature during autumn under natural conditions, is necessary for winter cereals to survive freezing temperatures in winter. Two main loci have been identified on chromosome 5 in wheat and barley that influence the capacity to over winter in temperate climates. The first major locus, designated to *FROST RESISTANCE-1 (FR-1)* co-segregates with *VRN-1* gene, which affects the vernalization requirement. The second locus, *FROST RESISTANCE-2 (FR-2)*, is approximately 30 cM proximal to *VRN-1* and includes a cluster of 11 (or more) *C-repeat Binding Factor (CBF)* genes. Transcription profiling and high-density mapping strategies have been used to identify possible candidate genes for frost tolerance within the *FR2-CBF* cluster. Three large deletions which eliminate six, nine, and all eleven *CBF* genes from the *Fr-B2* locus in tetraploid and hexaploid wheat have been discovered so far. In wild emmer wheat, *Fr-B2* deletions were found only among the accessions from the southern sub-populations. Indoor freezing tests showed that both the deletion of nine *CBF* genes in tetraploid wheat and the complete *Fr-B2* deletion in hexaploid wheat are associated with significant reductions in survival. Our results suggest that selection for the wild type *Fr-B2* allele may be helpful for breeders selecting for improved frost tolerance.

**Keywords:** CBF, cold acclimation, frost tolerance, durum, bread wheat

*Reference:*

Stephen et al. (2013) Large deletions in the CBF gene cluster at the *Fr-B2* locus are associated with reduced frost tolerance in wheat. *Theor. Appl. Genet.* Online: DOI 10.1007/s00122-013-2165-y

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PROVING THE ROLE OF TWO WHEAT CBF TRANSCRIPTION  
FACTORS IN THE COLD ACCLIMATION PROCESS AND IN FROST  
TOLERANCE BY TRANSFORMATION OF WHEAT AND BARLEY

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Since winter cereals have to survive freezing temperatures in winter, the examination of cold-acclimation and the development of frost tolerance is a prominent research area. *CBF* (*C-repeat Binding Factor*) genes are among the most studied regulators in the plant kingdom, coding for transcription factors involved in cold and drought stress response. Our previous work and several further studies have highlighted that two CBFs (CBF14 and CBF15) have an outstanding role in the development of frost tolerance in wheat [1, 2]. The aim of our work was to confirm their function by transformation of economically important cereals. *TaCBF14* and *TaCBF15* genes were isolated from winter wheat (*Triticum aestivum* L. Cheyenne) and then transformed into spring barley (*Hordeum vulgare* L. Golden Promise) and spring wheat ('Cadenza'). Transgenic lines exhibited moderate retarded development, slower growth and minor late flowering compared to the wild type, with enhanced transcript level of a gibberellin catabolic gene. The frost tolerance of all the CBF over-expressing lines has been tested. Two different types of frost tests were applied; plants were hardened at low temperature before freezing, or plants were subjected to frost without a hardening period. Our analysis showed, that *TaCBF14* and *TaCBF15* transgenes improve the frost tolerance to such an extent that the best transgenic barley lines were able to survive freezing temperatures several degrees lower than that which proved lethal for the wild type [3]. The transgene expression and several cold-regulated downstream genes were studied by Real-Time RT-PCR method.

**Keywords:** CBF, cold acclimation, frost tolerance, wheat, barley, transformation

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## SURVIVING FLOODS: SUBMERGENCE TOLERANCE IN WHEAT

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According to the global climate projections, torrential rain in the growth season will increase both in severity and frequency leading not only to waterlogging but also complete submergence of cereals. In the U.S.A., crop loss due to flooding has increased 4-fold in the last decade and is in some years causing more crop loss than drought and diseases combined. European farmers are also feeling the changing rain patterns – latest with the devastating Central European spring floods in 2013 which led to tremendous crop losses. Consequently, we suggest that the scientific community and the commercial breeders form alliances with the overall goal of improving the flooding tolerance of major cereal crops. Under water, the 10,000-fold slower diffusion of gasses is the main physical constraint leading to anoxia in waterlogged soils and during complete submergence plant tissues can both experience severe O<sub>2</sub> and CO<sub>2</sub> deficiency. However, like rice and many other grasses, wheat has superhydrophobic leaves that retain a thin gas film when submerged into water. The gas films function as gills collecting gasses dissolved in the floodwater and once captured in the gas layer, CO<sub>2</sub> (in light) and O<sub>2</sub> (in darkness) may quickly diffuse to the nearest stomata and into the leaf tissues. Moreover, to aid internal aeration when facing anoxic waterlogged soils, many plants, including wheat, form highly porous adventitious roots as a response to waterlogging providing low resistance to O<sub>2</sub> diffusion.

As a first step in screening the submergence tolerance of wheat, we set out to evaluate the potential for underwater photosynthesis in 3 cultivars. We demonstrated the beneficial effect of a functioning leaf gas film as the underwater net photosynthesis (P<sub>N</sub>) at environmentally relevant light and CO<sub>2</sub> conditions was 6-fold higher in leaf segments with gas films present compared to segments where the gas film had been experimentally removed. At saturating light and CO<sub>2</sub> levels, the underwater P<sub>N</sub> was 22–28% of the rate in air showing that underwater photosynthesis can contribute significantly to carbohydrate production during a submergence event. Also in the dark, leaf gas films enhance gas exchange as the apparent resistance to O<sub>2</sub> uptake was 11-fold lower with the gas films present. Over time, however, the submerged leaves lose hydrophobicity and eventually the gas films vanish; after 5–7 days of complete submergence the gas films on the youngest fully developed leaves had completely disappeared leading to a decline in underwater photosynthesis of 85–87% compared to the rate immediately after submergence. Our study shows that

wheat possesses a capacity for underwater gas exchange and that the capacity is tightly linked to a functional leaf gas film. Consequently, we propose to include leaf gas film longevity when screening for traits conferring submergence tolerance in wheat and learn from the successful breeding of flooding tolerant rice varieties.

**Keywords:** *Triticum aestivum*, leaf gas film, underwater photosynthesis, submergence tolerance, flooding

*Reference:*

Winkel et al. (2013) *New Phytologist*, 197: 1193–1203.

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## Section 5

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### CLIMATIC VARIABILITY WIDENS POTENTIAL YIELD GAP IN MAIZE AND WHEAT-BREEDING IMPLICATIONS

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Agriculture in the days ahead will be faced with a double contrasting challenge. On the one hand the climate change that creates tremendous environmental variability and affects productivity adversely. On the other hand the demand for more food to sustain an ever increasing global population. As maize and wheat are major crops the impact of inter-annual variation on their stability is of particular concern. In both crops the harvested grain yield usually lags behind the potential yield leading to a considerable yield gap, thus depriving growers of potential income, but more importantly threatening crops' sustainability and food adequacy. A particular cause is population-dependence implying that the optimum for the potential yield population is either very high for some seasons or substantially variable across seasons. Population-dependence is an almost universality in maize, and becomes evident in wheat particularly at the Mediterranean type environments. Seasonal climatic variation is connected with variability in both yield potential and optimum population. For example, for dry seasons preference of low populations is a prudent decision, but for propitious seasons only high populations could accomplish the potential yield. However, at sowing time decision on a specific target population according to the expected yield level is a difficult issue. Because water regime is the predominant factor, inability of long-term weather forecast is a primary reason. Failure to obtain the most established population, nevertheless, ends up to a considerable yield loss. Indicatively, due to last summer's drought in the southern USA maize cultivation was a disaster. The so far consistent insistence on crowding to raise potential yield and neglect of the individual plant performance is a root cause. Henceforth breeding should prioritize single-plant yield potential targeting on population-independent cultivars (i.e. wide and of low threshold range of optimum population), able to effectively use resources at low populations under either marginal or prosperous environments, bridging thus the potential yield gap. Experimental data are available supporting that the pursuit is of sound task, and constitutes a viable option to serve the needs of a flexible agriculture and combat the implications of climate change on environmental variability.

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## INCREASING YIELD POTENTIAL IN WHEAT THROUGH ENHANCED ALLOCATION OF DRY MATTER TO THE GRAIN AND OPTIMIZED POST-ANTHESIS SOURCE-SINK BALANCE

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Increased demand for food, climate change and greater dependence on food imports in less developed countries represent a challenge to achieve the food required for the coming years. Wheat (*Triticum aestivum* L.) is the most widely grown crop and an essential component in ensuring global food security. It is foremost among all food crops as a direct source of food and energy for humans (Habib and Khan Salam, 2003). Therefore, breeders need to develop new varieties with higher grain yield potential. One physiological avenue to raise yield potential is by improving the allocation of assimilate to the spike during stem elongation to enhance grains  $\text{m}^{-2}$  and harvest index. Partitioning to spikes could be increased to enhance floret survival by reducing competition from alternative sinks, including roots, leaves, stems (structural and water soluble carbohydrates), and infertile tillers (Foulkes et al., 2011). However, some attention must also be paid to maintaining post-anthesis source (photosynthetic capacity) to ensure grain growth of new varieties with increased grain number is not source-limited. In this study, twenty nine CIMMYT spring wheat cultivars were evaluated in a field experiment with regard to competition of organs at anthesis, source and sink limitation and grain growth. The field experiment was carried out with optimal agronomy, in a lattice design with 2 replicates in 2010–11 and 3 replicates in 2011–2012 in NW Mexico. A subset of ten contrasting genotypes with a restricted range in anthesis date and representing the full range of dry matter partitioning was selected to be evaluated across the 2 seasons for detailed physiological assessments. A degreasing treatment was carried out at GS61+7–10 days. In this treatment 20 spikes per plot were trimmed in 2010–11 and 2011–12 by removing half of the spikelets from the top to the bottom down one side of the spike to assess grain weight variations against 20 control spikes. Grain weight responses to the degreasing treatment showed that grain growth was mainly co-limited by sink and source. The main plant organs competing with the spike during stem elongation appeared to be the leaf sheath and true stem. Responses to degreasing were associated with date of introduction; modern varieties were more source-limited. Grains per  $\text{m}^2$  showed a positive association amongst cultivars with the spike fertility index ( $R^2 = 0.56$ ,  $P < 0.001$ ) (grains per gram of spike DM at GS61+7d). The positive association between Grains  $\text{m}^{-2}$  and Spike Partitioning Index (SPI) was not significant. Source-type traits were positively associated with grain yield, senescence rates and post-anthesis radiation-use efficiency RUE had a positive association with grain yield as well.

*IN VITRO* REGENERATION, GENE TRANSFER AND SELECTION  
REGIMES FOR EFFICIENT GENETIC TRANSFORMATION  
OF SOUTHERN AFRICAN BREAD WHEAT CULTIVARS

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Genetic transformation of crops is a powerful biotechnology tool. Wheat (*Triticum aestivum* L.), the second largest staple food crop in the world, has in the past been a target of genetic engineers trying to improve its qualitative and quantitative traits. However, this has been a challenging task as transformation of wheat is not routine practise, especially in Southern Africa. Various problems exist with the transformation of wheat, such as explant production and recalcitrance towards tissue culture procedures, which is essentially genotype dependent. Additionally, the highly complex nature of the wheat genome contributes to the difficulties when conducting transgenic studies. An established transformation system for wheat will allow studying the function of useful genes to ultimately provide either gene markers to include in breeding programs or to create improved crops via gene transfer that are better adapted to the African environment. We report here the successful establishment of in vitro regeneration protocols for six SA wheat cultivars (*Tugela dn5*, *Tugela dn2*, *Palmiet*, *Palmiet dn5 x dn1*, *Gamtoos R*, *Gamtoos S*) and the establishment of optimal biolistic gene transfer parameters for the production of stably transformed wheat plants. Factors such as explant discovery, sterilization, growth media and growth conditions optimization were investigated and are reported.

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## Section 6

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### DEOXYNIVALENOL BIOSYNTHESIS RELATED GENE EXPRESSION DURING WHEAT-*FUSARIUM GRAMINEARUM* COLONIZATION

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*Fusarium* head blight (FHB) is a devastating disease of wheat and barley, mainly caused by *Fusarium graminearum*. During infection, the pathogen produces the trichothecene mycotoxin deoxynivalenol (DON), which increases fungal virulence. To date 15 DON biosynthetic genes have been characterized. The objective of this study was to examine the level of expression of *TRI4*, *TRI5*, *TRI9* and *TRI14* in resistant and susceptible wheat cultivars after inoculating with *F. graminearum* 3ADON and 15ADON isolates. Two wheat cultivars Glenn (MR) and Roblin (S) were grown in the greenhouse and inoculated with 3ADON and 15ADON isolates. The level of expression of DON biosynthesis related genes were evaluated at 0, 6, 12, 24, 48, 72 hrs and 7 days after inoculation. The relative expression of the above genes were analysed in comparison with the *GAPDH* control gene using qPCR. The expression of these genes was initiated at 72 hrs after inoculation and expression was greater at 7 dai when compared to 72 hai. The relative expression of *TRI5* was higher in the cv. Roblin as compared to cv. Glenn in most of the treatments. In several treatments, *TRI5* gene expression differed among the two isolates within the same chemotype group confirming the isolate variation during plant–pathogen interaction. Our results indicate that DON biosynthesis genes continue to express 7 dai. Consequently, wheat samples collected at 10 dai, 14 dai and 21 dai are now being analysed to examine the role of DON biosynthesis genes during wheat senescence.

### IDENTIFICATION AND CHARACTERIZATION OF WHEAT GENES CONTRIBUTING TO DEOXYNIVALENOL RESISTANCE

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We focus on identifying biochemical pathways involved in the wheat response to the *Fusarium* virulence factor deoxynivalenol (DON). Using functional genomics tech-

niques, DON-responsive transcripts were identified: these included transcripts encoding a basic leucine zipper transcription factor, a multidrug resistance protein ABC transporter, cytochrome P450s and novel proteins. Based on the results, candidate genes were silenced in wheat heads using virus-induced gene silencing (VIGS) [1]. We found that heads with reduced transcript levels developed more DON-induced bleaching as compared to control treatment.

These studies have also highlighted a novel, evolutionary divergent protein involved in the wheat response to DON. Transient expression and microscopy studies showed this protein fused to a fluorescent tag localised within punctate areas of the nucleus of wheat cells. Yeast two hybrid experiments suggest that this protein interacts with SnRK1 (SNF1-Related Kinase 1) and NAC transcription factors. Thus, it is likely that this novel protein is involved in genes expression regulation. We are currently characterizing these interactions and the role of this protein in plant stress responses.

**Keywords:** Fusarium, deoxynivalenol, wheat

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## Section 7

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### GWAS OF SEED QUALITY IN WHEAT AND BARLEY

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Taking advantage of the recent development in SNP genotyping of cereal crops and establishment of germplasm like the European Barley Core collection [1] we want to carry out gene-discovery by genome-wide association scan in order to identify novel genes and QTL controlling the concentration of important compounds in the cereal grain. In a collection of 205 European barley varieties we found a large variation in the resistant starch content but no statistically significant correlation between RS content and  $\beta$ -glucan content was found in a subset of 61 varieties. Ten SNP markers out of 40 associated with RS were located in genes with a known role in starch biosynthesis [2]. Analysis of 254 barley varieties showed negative correlation between amylose and beta-glucan and between amylopectin and beta-glucan. Many SNP were identified and some of these corroborated earlier mapped QTL for these compounds and were located in loci and chromosomal regions not previously identified to control the content of these three compounds in the grain [3]. These examples show the potential of obtaining SNP markers for dissecting know QTL as well for identification of new genes directly involved in the biosynthesis or accumulation of these compounds in the starchy endosperm. The study also revealed the interplay between the different pathways.

**Keywords:** amylose, amylopectin, beta-glucan, genome-wide association scan, phytic acid, resistant starch, waxy, high-amylose

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## TESTING TO ABIOTIC STRESS IN WHEAT BREEDING PROGRAMME IN CZECH REPUBLIC

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Testing to abiotic stress is one of important trait for successful breeding. Abiotic stresses can cause changes in quality, can affect the level of yield, and can affect development of plant diseases. Testing is based only on non-linear evaluation; we are only able to test the response to abiotic stress. We test response of varieties and lines to frost, drought and wet weather during harvest time. The winter hardiness is complex of stress (frost, winter drought, flooding, soil heaving and ice-encasement). We test frost resistance at pot in freezer and we use  $-12^{\circ}\text{C}$ ,  $-14^{\circ}\text{C}$ ,  $-16^{\circ}\text{C}$ . The degree of winter resistance is characterized on a 9 to 1 scale and with percent of survival plants. For testing response of varieties to drought we use measuring of electrical capacity of roots. Roots of plants are able to change their morphology during dry period, the part of plant aboveground is reducing and mass of roots are increased in the same proportion. The premise is that a variety with large root system would have higher electrical capacity of roots.

Germination of spike within the grain head before harvest is called pre-harvest sprouting (PHS). Visible indications of PHS include grain swelling, germ discoloration, seed coat splitting and the root and shoot emerging. Sprouting is influenced by the yearly and weather during ripening and pre-harvest time. Among the varieties are significant differences of resistance to sprouting. We evaluate visible marks of PHS.

**Keywords:** abiotic stress, wheat, winter killing, pre-harvest sprouting, drought, breeding

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DOUBLED HAPLOID PLANT PRODUCTION FOR TRITICALE  
(× *TRITICOSECALE* WITTM.) BREEDING

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The role of doubled haploid plant production methods have increased in applied research and breeding of crop plants to accelerate the production process of new marketable varieties.

In our experiments, the efficiency of anther and isolated microspore culture were tested for androgenesis of triticale. The induction of androgenesis was more effective in isolated microspore culture than in anther culture. The number of embryo-like structures was nine times higher in microspore culture compared to anther culture and the number of regenerated plantlets was also three times higher. However, the regenerated plantlets from isolated microspore culture were mainly albinos. The production of green plantlets from anther culture was approximately three times higher than isolated microspore culture. In our experiments, the phenomenon of albinism did not hinder the green plant production in anther culture. The observed spontaneous rediploidization rate was low (~13%), so the in vivo or in vitro colchicine treatment is required for the efficient production of doubled haploid triticale plants [1, 2].

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**Keywords:** androgenesis, anther culture, haploid, microspore culture, Triticale, × *Triticosecale* Wittmack

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## THE EFFECTS OF LOCATION ON NUTRITIONAL FEATURES OF TRITICALE GENOTYPES

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Triticale (× Triticosecale Wittmack) is mainly used in animal feed and as a renewable crop for energy while its utilization for human food is still uncertain. In this study, eight triticale (cultivars and advanced lines) and reference wheat and rye were grown at two locations in Hungary (Kiszombor, Szeged). The samples were compared for important nutritional values (i.e. crude protein, crude fat, ash, dietary fibre /DF/, arabinoxylans /AX/, starch, and mineral composition). In most of our studies triticale characteristics were positioned between wheat and rye. In several components (ash, fibre, minerals), we found advantage of triticale entries if compared to wheat and these values were close to the value of rye control. However, on the average, protein and fat content was lower than those of wheat and rye. We found that beside the genotype, DF content (10.5–12.8%) was strongly affected by location. In triticale grains, AX was the main component of DF and some values (3.3–7.4%) were closer to rye than wheat. It was revealed that triticale grain is very rich in beneficial elements (Ca, Mg, P, K, Cu, Zn Fe). The effect of location was significant in formation of various element concentrations of the grains.

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## Section 8

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### INDUCED MUTATIONS FOR INCREASED DISEASE RESISTANCE: AN EXAMPLE OF RESISTANCE TO UG99 IN SPRING WHEAT

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Mutation induction is a successful method in providing genetic variation for disease resistance. The fungal race Ug99 of *Puccinia graminis* f sp *tritici* has overcome resistance genes to stem rust in wheat. Ug99 causes a devastating disease and threatens to affect wheat production in many regions. An IAEA inter-regional project (INT/5/150) was developed to generate genetic variation among contemporary and commercial wheat cultivars for Ug99 resistances *via* mutagenesis. Representatives of 21 countries (Algeria, Australia, China, Egypt, India, Iran, Iraq, Jordan, Kenya, Lebanon, Oman, Pakistan, Saudi Arabia, South Africa, Sudan, Syria, Tunisia, Turkey, Uganda, USA, Yemen) and 4 international institutions (CIMMYT, FAO, IAEA, ICARDA) participate in this project. Different cultivars were mutated and M<sub>2</sub> plants were first screened for resistance in 2009 at the University of Eldoret, Kenya, a hot spot for the disease. A range of disease resistance was recovered in mutated families, with 14 resistant families. In September of 2013, 2 new disease resistant advanced lines were exhibited to farmers and released to seed producers for subsequent cultivar release in Kenya. Genetic tests are being conducted to determine the total number of novel alleles generated within the project. At the PBGL, new tools are being developed for rapid characterization of mutant lines. This includes next generation sequencing approaches. Pilot tests are underway to develop exome capture methods designed for the efficient recovery of rare induced mutations. We have begun these tests in *Sorghum bicolor* due to its smaller genome size and annotation status. We envision this as a rapid technique for the validation of induced mutations causative for traits such as disease resistance, and also as an efficient reverse-genetics approach.

**Keywords:** black stem rust, Ug99, spring wheat, exome capture

MASSIVE ANALYSIS OF CDNA ENDS (MACE) OF STEM RUST  
RESPONSIVE GENES IN PERENNIAL RYEGRASS:  
RAPID IDENTIFICATION OF A PUTATIVE RUST RESISTANCE LOCUS

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The fungus *Puccinia graminis* causes the devastating stem rust disease of many cereals including the forage crop perennial ryegrass (*Lolium perenne*). Breeding for resistance against this and other pathogens is therefore of immense economical concern. Marker Assisted Selection substantially accelerates the tedious pyramidization of resistance genes against different pathogens in a commercial cultivar. Here we apply TransSNiPtomics, a versatile next-generation sequencing based strategy, for the rapid and cost efficient identification of molecular markers for rust-resistance breeding in the highly heterozygous perennial ryegrass. TransSNiPtomics employs Massive Analysis of cDNA Ends (MACE) for the simultaneous detection of expression and allelic diversity within transcripts. To this end, we combined disease-resistance rating and SSR markers from Italian Ryegrass [1] linked to the rust resistance locus LpPg1 [2] to select 20 susceptible and resistant progenies each, from an inbred full-sibling population segregating for rust resistance. Total RNA was isolated from rust-infected and non-infected leaves, respectively, from 20 susceptible and resistant individuals and pooled for bulked segregant analysis. Bulks were formed from the RNAs from the non-infected susceptible, the infected susceptible and from the non-infected and infected resistant individuals, respectively. TransSNiPtomics revealed whole-genome quantitative expression profiles of 57 million transcripts along with their allelic diversity. Of these, 401 transcripts and 56 SNPs occurred exclusively in the resistant bulks. BLASTing of these sequences against the genome sequence of the cereal model *Brachypodium distachyon* located the vast majority of them on a small region on *Brachypodium* chromosome 1 which is homoeologous to *L. perenne* chromosome 4 carrying the LpPg1 gene. The marker distribution suggested the presence of two closely linked resistance components at this locus. Fine mapping of the markers on the whole population of 395 individuals is underway. From these results we conclude that TransSNiPtomics is excellently suited to rapidly identify markers and candidate resistance genes for MAS in *L. perenne* and other crops.

**Keywords:** Stem rust resistance, LpPg1, perennial ryegrass, NGS, Massive Analysis of cDNA Ends (MACE), molecular markers

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IDENTIFICATION OF STEM RUST RESISTANCE GENES  
IN HUNGARIAN, AUSTRIAN, BULGARIAN, GERMAN, ROMANIAN,  
RUSSIAN AND SERBIAN WHEAT CULTIVARS

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Stem rust (*Puccinia graminis* f. sp. *tritici*) diseases of wheat cause serious yield losses worldwide. In the Middle European region, the occurrence of stem rust is less frequent, but due to the severity of infection, it can pose a great hazard for wheat production. The breeding and use of resistant cultivars offers an economical, safe and effective approach to protect wheat from these diseases. To use the advantages of breeding for disease resistance it is important for breeders to know the effects of diseases and the genetic background of the resistance in their cultivars. Resistance genes against stem rust like *Sr31* and *Sr36* played an important role in providing new resistance sources for wheat. In this study, 249 wheat cultivars developed in Hungary (Szeged and Martonvásár) (69 and 59, respectively), Austria (24), Bulgaria (30), Romania (23), Russia (12) and Serbia – (Novi Sad) (21) were investigated using PCR based molecular markers to determine the presence or absence and frequency of above stem rust resistance genes. We also assessed the severity and effect of stem rust on these wheat cultivars. The results indicated that *Sr31* which is located on 1BL.1RS wheat rye translocation was absent in Austrian and Serbian cultivars and widespread in Romanian, and German (22% and 27%, respectively) and especially in Russian and Hungarian (33% and 37%, respectively) wheats. Within Hungarian cultivars the occurrence of *Sr31* was extremely high (66%) in those ones which were developed in Martonvásár. *Sr36* which was also derived from an alien species, *Triticum timopheevii*, was detected in only in the Hungarian cultivars (23%) especially in those ones which were bred in Szeged (39% occurrence). Field and greenhouse artificial inoculation tests showed that both genes were still very effective. Data may help breeders to incorporate effective *Sr* genes into new cultivars.

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## POSTER ABSTRACTS

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### TRANSGENIC BARLEY WITH MODIFIED CYTOKININ LEVEL

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Barley, *Hordeum vulgare*, is an agriculturally important crop. New techniques of molecular cloning and plant transformation accelerate classical breeding techniques and produce barley plants with enhanced traits.

Cytokinins are plant hormones controlling many physiological processes including stress tolerance and root formation [1]. Cytokinins are irreversibly degraded by cytokinin dehydrogenase (EC 1.5.99.12; CKX), which is a principal factor to control cytokinin levels in plants [2].

For modulation of the cytokinin level in barley, constructs with cytokinin dehydrogenase with altered targeting under the control of potentially root specific promoter were prepared. All intended transgenic barley lines were prepared, confirmed and then analyzed in T2 generation of homozygous plants. Three unique transgenic barley lines with differently localized CKX showed distinct phenotypes.

**Keywords:** *Hordeum vulgare*, cytokinins, cytokinin dehydrogenase

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## OVEREXPRESSION AND SILENCING OF CYTOKININ DEGRADATION GENES IN BARLEY

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Improvement of yield and resistance against stresses remains the most important goal in the breeding of crops. The progress of molecular cloning accelerates classical breeding techniques and produce crop plants with multiply enhanced traits.

Genetic manipulation of plant hormones cytokinins may influence many physiological processes, besides others the stress tolerance, root formation and crop yield. Decreased cytokinin level in roots leads to enhanced root system, and increased cytokinin content in barley grains has positive affect on yield. These properties of cytokinins may be beneficial for agriculture. A good tool for regulation of cytokinin level is the enzyme cytokinin dehydrogenase (EC 1.5.99.12; CKX), that irreversibly degrades the side chain of adenine-derived isoprenoid cytokinins.

We obtained transgenic barley plants which overexpressed ZmCKX1 gene from maize under the control of two root specific promoters. As we suspected, transgenic plants had increased root system but surprisingly aerial part was negatively influenced and only one plant was fertile and gave T1 progeny. The expression of ZmCKX1 gene stayed increased in T1 generation of transgenic plants as well as CKX enzyme activity. For further research of root specific expression of CKX genes, the precise selection of promoter is required.

To follow the increasing of grain yield, transgenic barley with integrated silencing cassettes for HvCKX1, HvCKX2.2 and HvCKX9 were prepared. The transgenic plants have regenerated in vitro and will be analyzed in future.

**Keywords:** cytokinins, cytokinin dehydrogenase, barley, agriculture

## EFFECT OF SEAWEED AND SALT STRESS ON ANTIOXIDANT PARAMETER OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.)

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Salt stress is one of the most important environmental factors inducing biochemical changes in plants, the aim of this study is the improvement of tomato yield under saline conditions using seaweed fertilizer (SW) treatments. Significant effect were observed in activities of Catalase (CAT), Superoxide dismutase (SOD), Protease, Malondialdehyde (MDA) content, Proline and Protein amount in leaves of salt stressed plants. Seaweed treatment in absence and presence of NaCl had various changes on most of the assayed pa-

rameters. The results indicated that SW partially offset the negative impacts and increased resistance of tomato to NaCl stress by enhancing the activities of antioxidant enzymes, especially at 0.2%, markedly increased the activity of both CAT, SOD and amount of proline, however, content of both MDA concentration and proline level was significantly decreased. Generally, it could be concluded that SW have (to more extent) a beneficial regulatory role in plants grown under salt stress conditions by enhanced activities of SOD and CAT by SW addition may protect the plant tissues from salt induced oxidative damage, thus mitigating salt toxicity and improving the tomato growth. These results suggest that the scavenging system forms the primary defense line in protecting oxidative damage under salt stress in crop plants.

ALLELE VARIATIONS IN *PPD-B1* PHOTOPERIOD SENSITIVITY  
LOCUS, AND THEIR EFFECTS ON HEADING  
IN WHEAT (*TRITICUM AESTIVUM* L.)

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Flowering time is one of the most important adaptive characteristics of plants. The timing and duration of this plant developmental phase depends on the genetic background, on the environmental conditions (mainly temperature and photoperiod) and on their interactions [1]. In wheat, the most important genes regulating photoperiod sensitivity are *Ppd-A1* (2A), *Ppd-B1* (2B) and *Ppd-D1* (2D). In the case of *Ppd-D1* a large deletion in the promoter region of the PRR gene results in insensitivity, while in the case of *Ppd-B1* copy number variations stand behind the level of insensitivity [2]. In this study our major aims were [1] to characterize a collection of 683 wheat germplasm – collected from all over the world – for allelic variation at the *Ppd-B1* photoperiod genes using gene specific molecular markers and [2] to evaluate the possible interactive effects between the alleles of this gene and two developmental phases (DEV49 and DEV59) in field experiment (2011, 2012). Our results revealed that the *Ppd-B1* locus proved to be quite variable. In addition to the two genotypes found to have null copy of the *Ppd-B1* gene, nine versions of the insensitive *PPD-B1a* allele were identified based on the copy number, on the presence of a truncated copy and on the junction structure between two gene copies. 50.3% of the genotypes with the insensitive allele were copy number variation of the Chinese Spring basic type, 25.8% were of Recital, while 23.2% of the Sonora/Timstein basic allele type. The Recital type was always significantly the latest, while the Sonora type the earliest at each developmental stage in both years, while the Chinese Spring type was close to the Sonora

type. In the case of Chinese Spring type junction however the 4 copies resulted in already significant delay (at  $P = 0.05$  level) compared to the 2 copies.

**Keywords:** photoperiod response (*Ppd*), DEV49–DEV59 plant development phases, wheat (*T. aestivum* L.)

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## REGULATORY COMPONENTS INVOLVED IN COLD TOLERANCE OF BARLEY CELLS

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Micro- and macroclimatic changes fundamentally determine growth rate, development, crop production and geographical distribution of plant species. The existence of successful defensive mechanisms against the damaging effects of low temperature is essential for survival and sufficient seed production of plants.

In winter-type cereals cold acclimation process is activated by low temperature, and it leads to elevated level of resistance against harmful physiological effects of suboptimal temperature. One of the most important gene expression regulator units in this mechanism is the CBF-COR system. However, cold acclimation mechanism is a very complex phenomenon, the process is influenced by many factors, e.g. falling temperature, day length, spectral composition of irradiated light, as well as local and systemic internal signals. Because of this, realignment of the gene expression pattern connected to the cold acclimation mechanism and its phenotypical effects is very difficult to investigate excluding the influence of other factors with interfering action. Basic cellular and biochemical changes caused by only the low temperature, independently of other factors mentioned above are mainly undiscovered. Therefore, elemental cold response of the CBF-COR system was compared in seedlings and dark-grown, dedifferentiated, meristemoid callus cultures of winter barley. Detailed characteristics of CBF-COR induction and effects of cold-harden-

ing were also studied in barley callus cultures at the gene expression, hormone composition and freezing tolerance levels in the presence or absence of Dicamba, the exogenous auxin analogue used in tissue cultivation.

Our results suggest the presence of a basal, cold-responsive activation mechanism of CBF and COR genes with the highest influence on the evolvement of frost resistance, which is independent of the differentiated state of cells or chloroplast-related, light-induced and systemic signals. However, these factors seem to be required for reaching the maximum level of activation. The exogenous auxin analogue, Dicamba, seems to be rather a coinducer in this process, since it does not affect the initiation or the characteristic of the activation, only influences the magnitude of the response.

**Keywords:** barley, callus, CBF, COR14b, cold stress, Dicamba, hormone

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## DESIGNING A NEW METHOD TO EFFICIENTLY ASSESS DAMAGE DUE TO AFRICAN RICE GALL MIDGE (AFRGM)

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The African Rice Gall Midge (AfRGM), *Orseolia oryzivora* Harris and Gagné (Diptera: Cecidomyiidae), is an endemic rice pest found throughout Africa. The failure of different control methods imposes the need to breed for crop resistance using marker assisted breeding (MAB) to achieve durable AfRGM resistance in rice. Efficient insect pest damage assessment is a prerequisite for gene mapping and further MAB. A study was initiated to develop an accurate method for assessing damage caused by AfRGM in order to efficiently determine AfRGM resistance genes' modes of action, the behavioural pattern of progenies with resistance to AfRGM attack, Simple Sequence Repeat (SSR) or SNP markers flanking genes or QTLs linked to resistance to AfRGM. The approach consisted in comparing four methods of assessment including the IRRI's Standard Evaluation System (ISES) for rice and three methods based on resistance index (RI) assessments differing in the computing of the percentage of tillers with galls on a resistant check variety.

The RI-based assessment (RI-BA) methods consistently provided a better evaluation of AfRGM damage than the ISES, regardless of the trial size. Within RI-BA methods,

RI-BA2 was always more accurate than RI-BA1 and RI-BA3 when the plot was large. RI-BA2 and RI-BA3 were equally accurate when the plot size was small, and they provided better estimates than RI-BA1. When the plot was of medium size, RI-BA2 was more accurate than RI-BA3 and RI-BA3 was itself better than RI-BA1. The best method of assessing AfRGM damage was RI-BA2, regardless of the plot size.

In addition, RI-BA2 was used to state that 1) the AfRGM resistance expression studied is governed by two genes, 2) the tolerance to AfRGM is under complex mechanisms of control, 3) narrow-sense heritability estimates of resistance to AfRGM were low in populations involving tolerant varieties and were high in populations involving resistant varieties, 4) progenies behaved in 4 manners being either more resistant than the resistant check entry at 45 DAT and 70 DAT or more resistant at 45 DAT and susceptible at 70 DAT or susceptible at both 45 DAT and 70 DAT or susceptible at 45 DAT and resistant at 70 DAT, 5) two SSR and SNP markers were found to be associated to AfRGM resistance. This paper describes in details the methodology used to efficiently assess damage caused by AfRGM and also pointed out some of the milestones achieved using this methodology.

**Keyword:** AfRGM, insect pest resistance, gene mapping, *Orseolia oryzivora*, resistance index-based assessment

## BREEDING STRATEGIES FOR RESISTANCE TO FUSARIUM HEAD BLIGHT ON SPRING BARLEY IN LATVIA

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Occurrence of *Fusarium* species in cereals in different parts of Latvia was investigated in 2006–2012 by the Latvian Plant Protection Research Centre. Traditional morphological identification and molecular diagnostic methods showed the presence of *F. culmorum*, *F. poae*, *F. graminearum*, *F. avenaceum*, *F. sporotrichioides* and *F. langsethiae* in the Latvian population of *Fusarium* species associated with fusarial head blight (FHB) in small grain cereals. The dominant species *F. culmorum*, *F. poae* and *F. langsethia* founded in 2012 prevailed in oats. In field experiments an effect of fungicides and the influence of infection with *F. culmorum* on contamination of grain of spring barley by mycotoxins were investigated. Trials designed with four replicates using randomized blocks, a plot size of 30 m<sup>2</sup>. Influence of *F. culmorum* on accumulation of mycotoxin DON in grain was evaluated by artificial infection. Plots were inoculated at anthesis stage by conidium of *F. culmorum* propagated on PDA. Infection of grain with *Fusarium* spp. was recorded after harvesting by sterile conditions according to the ISTA methods. Contamination of grain with mycotoxin deoxynivalenol (DON) was determined by using

Method NDC-T-012-057-2007 in the Institute of food safety, animal health and environment “BIOR”, Latvia. Trial data were analyzed by ARM and GenStat program. The infection of grain of spring barley with *Fusarium* species ranged from 0.3 to 12.0%. In field trials the significant differences were found among spring barley cultivars in their resistance to FHB by natural and artificial infection.

**Keywords:** spring barley, *Fusarium* species, cultivars, resistance

## TRANSFORMATION OF BREAD WHEAT TO ENHANCE APHID RESISTANCE

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Global wheat production loss is caused by multiple abiotic and biotic factors, including pests and pathogens that greatly affect crop yield and quality of the endproduct. Russian wheat aphid (*Diuraphis noxia*, Kurdjumov, RWA) is such an economically important pest, with a narrow host range restricted mostly to wheat and barley. Previously, it was found that ascorbate peroxidase (*APX*) and glutathione-S-transferase (*GST*), both known to be associated with the hypersensitive response, are under differential regulation in RWA-infested wheat plants. Therefore, the focus of this study is to confirm the function of these hypersensitive-related genes in RWA resistance using a reverse genetics approach. To achieve this, gene fragments for both *APX* and *GSTF6b* were isolated from wheat, and sequenced to confirm their identity. Gene fragments *APX* and *GSTF6b* were cloned into the pUBI-510 transformation vector in the antisense orientation, suitable for knockout studies. Silencing constructs containing these gene fragments were bombarded into four-to-six-day-old wheat embryogenic tissues, respectively. The embryogenic tissues were obtained from immature seeds of Gamtoos-S (*Dn7<sup>-</sup>*) and Gamtoos-R (*Dn7<sup>+</sup>*) cultivars collected 11 to 16 days after anthesis, followed by subculturing of embryogenic tissue on different mediums for different time periods. From a starting total of 371 bombarded embryogenic tissues, 58 died on the shoot elongation medium and only three plantlets were able to withstand the entire selection process. To validate if the surviving plants were transgenic, qPCRs were conducted to confirm the presence of the transgene in the respective plants, and also quantify the expression of the genes with/without RWA infestation. An almost 50% reduction in *APX* and *GSTF6b* expression when compared to the control was observed in the respective plants.

**Keywords:** Russian wheat aphid, hypersensitive genes, transgenics

DEFENSE RESPONSE TO FUNGAL PATHOGENS *ASPERGILLUS FLAVUS*, *FUSARIUM PROLIFERATUM* AND *FUSARIUM SUBGLUTINANS* IN MAIZE CO354 SUSCEPTIBLE GENOTYPE

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Developing caryopses of maize genotype CO354 (susceptible line) (1, 2), were inoculated with fungal species *Aspergillus flavus*, *Fusarium proliferatum* and *Fusarium subglutinans*, at 15 days after anthesis. During this early stage of caryopsis development, when the embryo is formed, investigation on some defense systems was carried out. At 72 hours after infection, on non-inoculated and inoculated caryopses, the ascorbate-glutathione cycle and the specific activity of catalase, soluble and wall peroxidases (PODs) enzymes were analyzed to verify their effectiveness and involvement level in these pathosystems. In addition, the redox state of protein thiols as well as the hydrogen peroxide and malondialdehyde contents were studied to evaluate the oxidation level in developing maize caryopses before and after infection.

The three pathogens induced an enhancement of all defense components analyzed (ascorbate, glutathione, dehydroascorbate reductase, glutathione reductase, soluble and wall PODs, catalase) except monodehydroascorbate reductase. Particularly, in caryopses inoculated with *Aspergillus flavus*, the contemporaneous increase of H<sub>2</sub>O<sub>2</sub> content and wall PODs activity was responsible of higher lignification and strengthening of cell wall, first target of fungal attack. In addition, in these caryopses, the largest increase in reduced glutathione content, due to elevated glutathione reductase activity, indicated high defensive capability to counteract the pathogen toxicity. Relatively to caryopses inoculated with *Fusarium subglutinans*, the highest increase in ascorbate, a defense metabolite like so the glutathione, and soluble PODs and catalase, underlined greater defense capability against invading pathogen than caryopses inoculated with *Fusarium proliferatum*. Finally, in inoculated caryopses, the pattern of total proteins changed, indicating an alteration and insufficient maturation of the proteins of kernels in presence of the three different pathogens. Summarized, our data provide an important basis for further investigation of defense gene functions in developing caryopses in order to aid an improved tolerance to fungal pathogens and reduced losses of yield in maize.

**Keywords:** maize, defense systems, *Fusarium*, *Aspergillus*

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## NATURAL VARIABILITY IN WHEAT RESPONSE TO TRANSIENT DROUGHT STRESS: A METABOLIC PERSPECTIVE

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Water deficit is a common environmental stress limiting crop productivity worldwide. As such, improving crop tolerance to water deficit is a major goal in current breeding programs. The implementation of this requires wide exploitation of potential genetic resources and a detailed understanding of adaptive mechanisms, including reorganization at the metabolic level. The current study investigated the physiological and metabolic response of six wheat cultivars (Barnir, Gedera, Galil, Zahir, Yuval, and Rutah) to drought and recovery at tilling and grain-filling stages.

Drought stress did not significantly affect transpiration in any of the cultivars during grain filling. Nevertheless during recovery Gedera, Galil and Rutah showed significant decrease in transpiration compared to their control. GC-MS based metabolite profiling was used to access the metabolism during tilling and grain filling stage. The data showed that generally conserved metabolic responses to drought included the increase in stress related metabolites. Specifically accumulation of proline, valine, myo-inositol, sucrose, galactose, galactinol, glucose and ascorbate and its degradation products and may function as osmoprotectants during water stress. TCA cycle intermediates were decreased in response to drought in all cultivars, except Barnir, which increased the abundance of TCA cycle intermediates compared to its control plants. These results are in support with physiological measurement. For example, Yuval and Zahir responded to drought stress and recovery by reduction of plant height and transpiration, while these parameters were not significantly affected in Barnir. This may suggest that, while Yuval and Zahir closed their stomata to reduce transpiration and thereby decreased carbon assimilation, Barnir maintained the production of photo-assimilates to sustain growth during drought. The analysis highlights cellular mechanisms of different cultivars response to drought stress and the strategy for recovery which will aide in defining original strategies for drought-tolerance improvement in wheat plant.

## GENETIC ARCHITECTURE FOR YIELD, ITS COMPONENTS AND SALT TOLERANCE IN RICE (*ORYZA SATIVA* L.)

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The experimental material, comprising of four females (lines), ten males (testers) and their 40 hybrids developed through line  $\times$  tester mating design along with two checks, was grown in a randomized block design with three replications at Coastal Soil Salinity Research Station, Danti-Umharat, Navsari Agricultural University, Navsari, Gujarat during *kharif* 2012–13. The main objective of the experiment was to study heterosis, combining ability and nature of gene action for yield and its component traits under coastal salt affected soil.

Analysis of variance indicated significant differences among the parents and hybrids for all the characters studied, which revealed existence of variability in the genotypes. Based on mean performance, Gurjari, GNR-3 and IET-15429 among females and GNR-2, IR-55179 and GAR-1 among males were found superior for grain yield and most of the yield attributing traits. Among hybrids, Gurjari  $\times$  GAR-1, Gurjari  $\times$  IET-19347 and IET-21734  $\times$  Jaya exhibited highest mean performance for grain yield and the major yield components.

In general, the parents showing superior performance gave superior hybrids and higher magnitude of heterosis in hybrid combination. Among 40 hybrids, the maximum value of heterobeltiosis for grain yield per plant was observed in cross IET-21734  $\times$  Jaya, Gurjari  $\times$  GAR-1 and IET-21734  $\times$  IET-19347. On the other hand 14 hybrids exhibited significant positive standard heterosis over Dandi, while only 6 hybrids depicted significant positive standard heterosis over CST-7-1. The highly heterotic response in these hybrids for grain yield per plant resulted due to significant positive heterosis for yield contributing characters like panicle length, productive tillers per plant, number of grains per panicle and 1000-grain weight.

Combining ability analysis indicated the importance of both additive and non-additive gene action for the inheritance of all the characters; however, the ratio of  $\sigma^2_{gca}/\sigma^2_{sca}$  revealed the preponderance of additive gene action for plant height, days to 50% flowering and kernel length while remaining characters showed predominance of non-additive gene action. An observation of the general combining ability effects suggested that female parent Gurjari was found to be good general combiner for majority of the traits, while male parents viz., IET-19347, GNR-2, GAR-1 and IR-71907 were best general combiner for grain yield per plant and also for number of yield components and biochemical characters. GNR-3 and IR-55179 were identified as the most promising parents for imparting dwarfness and earliness, respectively.

Estimates of sca effects did not reveal any specific trend. However, the comparison of sca effects with per se performance of crosses indicated that at least one good general combiner was necessary for better per se performance along with sca effects. The hybrids

which recorded maximum significant positive sca effects for grain yield were IET-21734 × Jaya, Gurjari × GAR-1 and GNR-3 × IET-22608.

In a view of the preponderance of non-additive gene effect and presence of high magnitude of heterosis for grain yield and its important traits, the exploitation of hybrid vigour on commercial scale is suggested. However, to exploit the additive genetic variance present in sizable proportion for yield and yield components, biparental approach in the segregating generations or population approach as suggested by Doggett (1972) may be adopted.

Higher  $K^+/Na^+$  ratio in shoot and root were found in salt tolerance genotypes, these biochemical traits can be utilized as marker characters in salt tolerance breeding programme in rice.

On the basis of per se performance, heterotic response, combining ability estimates and nature of gene action for grain yield per plant and its attributes, hybrids viz., Gurjari × GAR-1, Gurjari × IET-19347, IET-21734 × Jaya and IET-21734 × GNR-2 were found most promising and could be exploited commercially after critical evaluation for its superiority and stability across the locations over years.

## CELL VIABILITY AND MEMBRANE FLUIDITY IN RELATION TO DROUGHT TOLERANCE OF INDIAN WHEAT CULTIVARS

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Drought stress is an important production constraint of wheat during grain-fill period in India and in other parts of the world where the moisture stress become high during anthesis to maturity stage of plant growth. Drought induced damage of plasma membrane was assayed by membrane thermo-stability (MTS), which measure electrolyte leakage from leaf tissues after exposure to high moisture stress. Cell membrane stability (CMS) and tetrazolium triphenyl chloride (TTC) test, drought susceptibility index (DSI), drought response index (DRI) and grain yield were used to evaluate these genotypes under normal and drought stress conditions for two years. Mean value for grain yield per plant under drought stress ( $6.94 \pm 0.43$  gm) was significantly lower than that under normal conditions ( $11.56 \pm 0.67$  gm) indicating significant influence of drought stress. The varieties 'Seri' and 'Raj 3765' had significantly higher mean values for TTC ( $67.57^*$  and  $62.53^*$ , respectively) CMS ( $73.18^*$  and  $70.01^*$ , respectively) under drought stress and significantly higher grain yield under both environments indicating a desirable combination of high yield potential and drought tolerance, while, 'WH 730' and 'WH 533' were better only for TTC and CMS. The genotypes 'Kauz' ( $9.29^*$  gm) and 'PBW 373' ( $9.24^*$  gm) were significantly higher for grain yield under drought stress conditions as well as better in drought

resistance/tolerance parameters. This revealed that high grain yield under drought stress in these genotypes might have been contributed by the other traits other than the TTC and CMS. Correlation coefficients revealed that the genotypes having high cell viability (TTC) also had high CMS ( $r = 0.83^*$ ) high grain yield ( $r = 0.46^*$ ) under drought stress and high value of DRI ( $r = 0.54^*$ ) (Table 2). CMS was also significantly positively related with DRI ( $r = 0.59^{**}$ ). Significant positive association of TTC and CMS with DRI revealed that these characters appeared to contribute towards drought tolerance. High grain yield under normal environment was associated with early heading ( $r = -0.46^*$ ) and susceptibility to drought stress (DSI,  $r = 0.48^*$ ), while high grain yield under drought stress was contributed by drought escape ( $r = -0.60^{**}$ ) and combination of both, i.e., high grain yield potential and drought escape (DSI,  $r = -0.56^*$ ), and drought tolerance (DRI,  $r = 0.49^*$ ). The varieties ‘Seri’ and ‘Raj 3765’ had a desirable combination of cellular drought tolerance (TTC and CMS), drought tolerance (DRI) and high grain yield potential under drought stress, while ‘WH 730’ and ‘WH 533’ were better in cellular drought tolerance and drought tolerance. The varieties ‘PBW 373’ and ‘Kauz’ also performed better under drought stress in terms of grain yield and DSI/DRI. The varieties ‘Kanchan’, ‘PBW 373’, ‘NIAW 34’ and ‘GW 173’ were avoiders/escapers, ‘Seri’ and ‘HUW 234’ were tolerant to drought stress, while ‘WH 730’, ‘WH 533’, ‘Nesser’, ‘Raj 3765’ and ‘Kauz’ showed the combination of both. Correlation coefficients revealed that DRI was the most important trait followed by TTC because the genotypes having high DRI also had high grain yield and better mitochondrial viability and membrane stability under drought stress.

**Keywords:** cell viability, membrane fluidity, drought tolerance, wheat

## DNA MARKERS ASSOCIATED WITH ERGOT RESISTANCE IN DURUM WHEAT

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Ergot caused by the fungus *Claviceps purpurea*, is a disease of durum wheat (*Triticum turgidum* var *durum*) characterized by the production of ergot bodies that contain toxins. The ergot bodies contaminate grain which causes down grading and a loss of value to farmers. The identification of resistance to ergot and the development of markers to the resistance would be valuable in breeding. The objective of this study was to discover markers associated with resistance to ergot in durum wheat. Recombinant inbred line (RIL) and doubled haploid (DH) populations from the cross of the resistant durum line Greenshank (RIL population) or a resistant progeny line of Greenshank (DH population) with susceptible AC Avonlea were inoculated with *C. purpurea* and genotyped using DArT. The

progeny segregated in a manner that indicated the presence of a major resistance gene, and a marker was found to be located within 11 map units of the resistance. The results will be useful for further fine mapping and marker assisted breeding of Greenshank ergot resistance.

**Keywords:** Ergot, durum, wheat, marker, *Triticum turgidum*, *Claviceps purpurea*, resistance, disease

## ULTRA-SPACED MAIZE INBREDS: OVER-LOCATION AND WATER REGIME G×E INTERACTION

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Exploitation of G×E interaction has essentially contributed to improve yield. However, multi-environmental experimentation to handle it is a high-cost process. Objective of this study was to assess the degree of environmental influence on maize genotype comparative evaluation in the absence of competition. Thirty one inbred lines were tested in 2012 at 0.74 plants/m<sup>2</sup> and across three locations spread from west to east, in the northern maize belt in Greece, at normal and water-stressed regimes. Traits analyzed were: the mean grain yield per plant, the respective coefficient of variation (CV), as well as their product value B used as prediction criterion of genotype performance at normal farming densities (see also Tzantarmas et al., this congress). In overall, the 31 genotypes yielded from 102 up to 697 g/plant, depicting huge phenotypic expression and differentiation. The CVs ranged from 0.38–0.81, implying that lines substantially differed in genetic background to counteract the environmental forces, attributable to difference in load of deleterious genes which are still present in maize inbreds [1]. To estimate genotype consistence in performance and thus, approach heritability, linear correlations for each of the three particular traits were computed. Noteworthy, all the 15 *r* values were very high ( $r > 0.83$ ,  $P < 0.001$ ) for mean yield per plant, implying absence of G×E interaction and high heritability. On the other side, it was observed strong G×E interaction for CV, since out of the 15 *r* values only four were significant but relatively low ( $r = 0.38–0.47$ ,  $P < 0.05$ ). Accounting for the specific genotype adaptability by the load of deleterious genes, inconsistency behaviour is a presumed implication. However, for integrated B values, consistence and high heritability were again present, since all the 15 *r* values were strongly positive and significant ( $P < 0.001$ ), varying from 0.65 up to 0.92. From the above results, it is hypothesized that the integrated B value is a statistic measure that damps down the G×E interaction. Given that it is assumed as a useful prediction criterion of genotype performance at the

normally farming densities, these findings open the possibility of evaluation and selection conducted at a single environment could succeed in fairly general adaptability.

**Keywords:** adaptability, deleterious genes, heritability

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## ULTRA-SPACED MAIZE INBREDS: CORRELATION VERSUS DENSE STAND FOR YIELD PERFORMANCE

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Intense selection among ultra-spaced individual plants has been suggested to tackle the environmental heterogeneity and the negative relation between yielding and competitive ability [1]. It was asserted that relative crop yield (CY) potential of a genotype can be predicted from ultra-spaced plants through a novel equation that incorporates two components into a single value (value B), the first based on plant yield (PY) potential and the second based on the coefficient of variation (CV) of individual plants. The first component describes the yield potential and the second one, reflecting load of deleterious genes and vulnerability to environmental forces, the stability of performance. Therefore, theoretically CY is expected to be connected positively with PY and inversely with CV. The goal of this study was to investigate in maize inbred lines for these two components and their product value B, the correlation of ultra-spaced plants (0.74 plants/m<sup>2</sup>) versus CY at 6,666 plants/m<sup>2</sup>. Experimentation involved 31 inbred-line genotypes and conducted at three locations of north Greece. PY exhibited a consistent positive correlation with CY (*r* values of 0.59, 0.76 and 0.91, all significant at  $P < 0.001$ ). In contrast, CV and CY weakly correlated just in one location ( $r = -0.38$ ,  $P < 0.04$ ). It seems that the first component meets perfectly its scope. The second one falls short of a tight connection with CY. Load of deleterious genes assumed still present at high levels in maize inbred lines, is regarded a reasonable cause of unstable behaviour (see also Ninou et al., this congress). However, B values were found to strongly correlate with CY (*r* values of 0.53, 0.74 and 0.89, all significant at  $P < 0.001$ ). Moreover, the over-location analysis showed that either the two components or the B value significantly correlated to CY, i.e. highly for YP ( $r = 0.91$ ,  $P < 0.001$ ), mod-

erately for CV ( $r = -0.45$ ,  $P < 0.02$ ), and highly for B value ( $r = 0.87$ ,  $P < 0.001$ ). The conclusion was that the B product combining yield and stability is a useful integrated statistic when breeding is carried out at ultra-low density, supporting previous research where found verified to match the agronomic sense of stability [2].

**Keywords:** Coefficient of variation, Stability, Yield

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## GENETIC AND AGRONOMIC CHARACTERIZATION OF RICE COLLECTION FROM KAZAKHSTAN

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Rice (*Oryza sativa* L.) is one of the strategic cereal crops worldwide, including in Kazakhstan. The rice is third important crop in Kazakhstan with sowing area 100 thousand hectares, and with average grain yield 3.5 t/ha.

New DNA genotyping technology was applied in order to improve local breeding program towards resistance to abiotic stresses. The collection of 96 rice accessions (predominantly *japonica* type) from Kazakh Rice Research Institute in Kyzylorda, Kazakhstan, was studied by using genome wide SNP (single nucleotide polymorphism) analysis. The collection was consisted from 65 local accessions, including 12 commercial cultivars and 4 landraces, and 31 foreign accessions from West Europe, Russia, Central Asia, Far East and South-East Asia. The oligonucleotide pooled assay was used to genotype rice samples by 384 SNPs distributing over all 12 chromosomes. PCR, hybridization, and scanning were performed according to GoldenGate genotyping assay protocol (Illumina Inc.) at the Institute of Plant Science and Resources, Okayama University, Japan. The Neighbor joining tree suggested separation of accessions to four large clusters. The accessions from Kazakhstan were well presented in all four clusters suggesting broad usage of world germplasm in the breeding activities.

In addition, the collection was grown in triplicate randomized blocks in experimental plots of the Kazakh Rice Research Institute. The collection was studied by ten agronomic traits and ten grain quality parameters. The Pearson's correlation analysis suggested high association of grain yield with number of kernels per plant, 1000 kernels weight, size of

kernels, and density of panicles. Generated genetic and agronomic data will be used for genotype–environment interaction studies and for association mapping for genes related to abiotic stress resistance.

**Keywords:** rice, SNP, genotyping, agronomic traits, grain quality

## PINORESINOL AND SECOISOLARICIRE SINOL INHIBIT TRICHO THECENE BIOSYNTHESIS IN *FUSARIUM GRAMINEARUM*

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Lignans are a group of diphenolic compounds with anticancer and antioxidant properties that are present in various grains. Pinoresinol and secoisolariciresinol are considered to be among the most prominent wheat lignans, however, their effect on toxigenic fungi has been poorly examined to date. The impact of different levels of plant lignans pinoresinol and secoisolariciresinol on trichothecene biosynthesis by six *Fusarium graminearum* strains of different chemotypes was examined *in vitro*. RT-qPCR analyses of tri4, tri5 and tri11 genes encoding the first steps of trichothecene biosynthesis pathway revealed decrease in tri mRNA levels in lignan treated fungal cultures. Correspondingly, decreased accumulation of toxins in lignan treated cultures was confirmed by GC-MS analysis. Pinoresinol seemed to have a stronger inhibitory effect on trichothecene biosynthesis than secoisolariciresinol. The average decrease in total trichothecene content for all *F. graminearum* strains tested was 90% and 81% for 1.25 mg/L and 5.0 mg/L of pinoresinol, respectively. The same doses of secoisolariciresinol decreased total trichothecene content by 56% and 61%, respectively. This is the first study to demonstrate the inhibitory effect of both pinoresinol and secoisolariciresinol on trichothecene biosynthesis in *F. graminearum*.

**Keywords:** pinoresinol, secoisolariciresinol, *F. graminearum*, trichothecenes

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## GENOTYPING AND PHENOTYPING OF OAT COLLECTION

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Oat is important cereal worldwide for human food consumption and animal feeding. In this work the collection of oat accessions was studied by use of 22 DNA microsatellite markers, or SSRs (simple sequence repeats), and phenotypic traits. The collection consisted from 26 commercial oat cultivars and perspective lines from Kazakhstan and 199 foreign accessions from Northern and Southern America, Western Europe, Asia, Russia and Ukraine. It was found that 14 SSRs were polymorphic for this collection out of 22 SSRs selected from Becher et al (2007) and studied in this work. Neighbor joining phylogenetic tree suggested separation of all accessions to 4 large clusters. Most accessions from Kazakhstan formed a separate sub-cluster in Cluster III. Shannon information index for samples from Kazakhstan was 1.042 and comparable to samples from other regions of the world.

The collection of oat was also studied for agronomic traits in three different regions of Kazakhstan – Kostanai (Northern), Karaganda (Central), and Almaty (South-Eastern) in 2011–2013. The accessions were grown in randomized 1m blocks in three repetitions. Samples were characterized by following traits – vegetation time length, plant height, stem length, number of nodes per stem, number of fertile stems, spike length, number of kernels per spike, thousand kernels weight, and grain yield. Several statistic methods, such as AMMI and GGE Biplot, were applied for studies genotype × environment interaction patterns. The accessions with high grain yield components were identified for the improvement of local breeding programs for development of new cultivars with high productivity and resistance to abiotic and biotic stresses.

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Becher, R. (2007) EST-derived microsatellites as a rich source of molecular markers for oats. *Plant Breeding*, 126(3): 274–278.

**Keywords:** oat, DNA microsatellites, genotyping, genetic diversity, phenotypic variation

## THE EXPRESSION OF THE *CBF* GENES IN CEREALS DEPEND ON THE LIGHT SPECTRUM

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Winter hardiness of cereals depends on many factors, such as temperature, light intensity and day length. The *CBF* genes, encoding transcription factors are induced by short cold exposure. As transcription factors, they regulate other genes, thus initiating processes that lead to the formation of frost tolerance. Because of their high yield, winter cereals are economically important, so it is necessary to understand the whole frost acclimation process thus helping the work of molecular breeders. In *Arabidopsis* it was proven that light-quality influences *CBF* expression, leading to increased level of frost tolerance.

In our experiment the effect of monochromatic blue, red and far-red light was examined first on the winter habit *Triticum monococcum* 'G3116' line. The expression level of several *CBFs* and a cold-inducible effector gene, *Cor14b* was detected by quantitative real-time PCR method. Our results prove that there is interaction between the *CBFs* and the light-quality in cereals as well.

In the second experiment, frost tolerance was tested on the winter barley 'Nure', the winter wheat 'Cheyenne' and the winter einkorn 'G3116'. Beside white light, additional red or far-red or blue light was given to the plants and the membrane injury in the leaves was investigated by electrolyte leakage measurement at different freezing temperatures. Expression levels of several target genes were also detected. Whole plants were frozen at the end of the treatments and regeneration ability, survival rates were also calculated. It was obviously proven that the far-red light treated plants showed higher frost tolerance and expression level of several frost-related genes.

**Keywords:** *CBF* genes, frost tolerance, light quality, spectrum, red/far-red ratio

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## EVALUATION OF MAIZE INBRED LINES FOR TOLERANCE TO DROUGHT USING PHYSIOLOGICAL CHARACTERISTICS

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Improvement for maize drought tolerance has always been a significant objective for breeders and plant physiologists. Nowadays, this is becoming more important, where climate change sets new challenges to major crop adaptation under stressful environments. Therefore, the measurement of physiological traits related to the response of maize to drought stress could contribute to finding the mechanisms that are involved to drought tolerance improvement. The objective of the present study was to investigate the performance of maize inbred lines at ultra-low density conditions and under different water regimes (normal and drought conditions) using physiological characteristics such as the gas exchange parameters (net assimilation rate (A), transpiration rate (E), stomatal conductance ( $g_s$ ), intercellular CO<sub>2</sub> concentration leaf). For this purpose, 31 maize inbred lines were evaluated across two locations under normal and drought stress conditions at plant density of 0.74 plants/m<sup>2</sup>. The results showed that net assimilation rate (A) was reduced by the water stress to both locations affecting in a similar way the examined inbred lines. Significant differences were recorded among the examined maize inbred lines to both locations. This parameter was higher at the physiological stage of full bloom compared to the measurements taken at dough stage. At both locations water regimes did not affect the  $c_i$ , although differentiation among genotypes was observed. At both locations the reduced water availability has diminished the stomatal conductance depicting the plant reaction to drought stress. Additionally, the stomatal conductance was differentiated between the two measurements. There was a clear sign that the gas exchange parameters have the potential to be used for the selection of stress-adaptive genotypes and may permit modern maize to be grown at wider range of environments addressing the climate change scenarios.

**Keywords:** maize inbred lines, drought tolerance, physiological measurements

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## LOCATING QTLs FOR TURCICUM LEAF BLIGHT IN EARLY MAIZE

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Turcicum Leaf Blight (TLB) also known as Northern Corn Leaf blight is one of the important leaf diseases of maize and is caused by fungus *Exserohilum turcicum* previously called *Helminthosporium turcicum*. It is significant problem in the Eastern United States, Western Brazil, Latin America, China, India causing heavy yield losses which can exceed 50% if the disease appears before flowering. In current study an attempt has been made to Map QTLs responsible to TLB resistance, through approaches of Molecular Plant Breeding. Conventional Breeding is laborious, time consuming and dependent on environment conditions. Molecular breeding is an efficient alternative to the tedious work of phenotypic evaluation and allows for an efficient selection of complex traits. Quantitative Trait Locus (QTL) mapping using DNA marker is a highly effective approach for studying quantitative traits. So, a breeding program that includes QTL mapping procedures is better oriented, maximizing the chances in favor to overcome a disease via host-plant resistance. The Present study was conducted in two environments at VPKAS, Almora (Environment-I: E-I) and Zonal Agriculture Research Station, the hot spot for TLB at Nagenhelly, Mandya Karnataka. (Environment-II: E-II) About 10 maize inbred of early maturity group (85–100 days), which included resistance as well as susceptible inbreds, were subjected to screening in epiphytotic conditions for TLB diseases resistances and susceptibility in two hot spot environments viz. E-I and E-II. Based on evaluation and screening in two environments and past records these ten inbreds were classified into resistance (CM 104, CM105, CM118, CM145, and V336) and susceptible (CM212, CM126, V338, V 13 and V 178) Lines. These lines were subjected to Parental Polymorphism study with the help of about 500 SSR Primers obtained from Directorate of Maize Research, New Delhi, Applied Biotechnology Centre, CIMMYT and Asian Maize Biotechnology Network, AMBIONET. The Parental polymorphism studies revealed that about 101 primers exhibiting polymorphism between CM 212 (susceptible) and V336 (resistance), which was maximum among various groups of 10 inbred evaluated under present study. The mapping population ( $F_{2:3}$ ) of CM212  $\times$  V336 were raised and phenotypic data related to Lesion area, Disease Severity, Incubation Period, AUDPC Curve were generated in two environments (E-I and E-II). The genotypic data of all the genotypes of  $F_{2:3}$  populations in two environments were generated and the genotypic and phenotypic data were subjected to software to locate and map the QTLs for TLB in maize.

**Keywords:** Turcicum Leaf Blight, SSR markers, quantitative trait loci and maize

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## ANALYSIS OF GENETIC DIVERSITY WITHIN *SECALE* GENUS BASED ON *SCBX1* GENE SEQUENCE

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Benzoxazinoids (BX) are protective and allelopathic secondary metabolites that have been found in a large number of species belonging to *Poaceae* family, including the major agricultural cereals – maize, wheat and rye [1, 5]. Biosynthesis of these substances is a multi-stage process which is controlled by several genes *Bx1* – *Bx9* [1, 2]. *Bx1* gene can be considered as a “switch on/switch off gene”, because BX biosynthesis pathway is activated by the indole-3-glycerol phosphate lyase that catalyzes the transformation of indole-3-glycerol-phosphate into indole and is encoded by this gene [1]. In *Hordeum spontaneum* and most of *H. vulgare* varieties, the defensive system is based on indole alkaloids – gramines (3-(dimethylaminomethyl)-indole) and the *Bx1* gene is absent [3, 4].

The aim of this study was to study and define the genetic diversity within genus *Secale* based on SNP and INDEL polymorphisms within *ScBx1* gene sequence. Such an analysis has been performed in *Secale* for the first time. The plant material consisted of 192 rye accessions from different parts of the world, including wild *Secale* species, modern and historically important rye (*Secale cereal* L.) cultivars and landraces. Each accession was represented by 96 plants. DNA isolation was carried out using CTAB method by Murray and Thompson [6] with some modifications. Amplified *Bx1* sequences were sequenced and analyzed using NCBI bioinformatic tools, Sequencher, ClustalW and FGENESH programs. Based on the results of bioinformatic analyses, dendrograms showing genetic relations between investigated accessions were constructed.

**Keywords:** rye, genetic diversity, benzoxazinoids

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## THE EFFECT OF ELEVATED CO<sub>2</sub> ON FUSARIUM HEAD BLIGHT DISEASE OF WHEAT

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Carbon dioxide (CO<sub>2</sub>) is one of the most important greenhouse gases (GHG) and its concentration has been dramatically increasing since the industrial revolution due to the intensified anthropogenic GHG emissions [1, 3]. Wheat is one of the most important food crops in the world, but the productivity is highly dependent on environmental factors and the presence of pathogens. Evidence to date suggests that the higher level of CO<sub>2</sub> predicted in future climate estimations can positively affect disease development [2]. The aim of this study is to determine the effect of plant and pathogen adaptation to high CO<sub>2</sub> on the severity of *Fusarium* head blight (FHB) disease. FHB resistant and susceptible wheat cultivars (CM82036, Remus) were grown in Conviron BDW 40 walk-in controlled atmosphere and environment chambers adapted to normal (390 ppm) and high (780 ppm) CO<sub>2</sub> concentrations. The pathogen *Fusarium graminearum* GZ3639 strain was grown and subcultured over 20 generations under the same conditions. The adapted pathogen and plants were used for FHB experiments. Results show that elevated CO<sub>2</sub> and also pathogen adaptation to high CO<sub>2</sub> leads to higher disease levels, which suggests that future atmospheric CO<sub>2</sub> level will have a negative impact on disease resistance, food quality and production in the future.

**Keywords:** climate change, carbon dioxide, *Fusarium* head blight, wheat

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MOLECULAR CHARACTERIZATION OF BYDV RESISTANCE GENE  
*RYD4<sup>Hb</sup>* INTROGRESSED FROM *HORDEUM BULBOSUM*  
INTO BARLEY

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Barley yellow dwarf virus (BYDV) causes high yield losses worldwide. Thereby aphids play an important role as virus vectors. Yield losses caused by BYDV may be reduced using virus-tolerance genes from the primary genepool of barley. Additionally, complete resistance to the virus has been introgressed from the secondary genepool *via* interspecific crosses with the tetraploid wild species *Hordeum bulbosum* (*Hb*). Previous studies indicate that this resistance was introgressed to barley chromosome 3HL and is governed by a dominant gene, *Ryd4<sup>Hb</sup>*. In the present study, a BC<sub>2</sub>F<sub>6</sub> mapping population consisting of 450 individuals was used to establish a molecular-marker map for *Ryd4<sup>Hb</sup>*. To develop molecular markers specific for the introgressed *Hb* segment, three strategies are applied: (i) using anchor markers from the barley genome, (ii) exploiting the orthology of parts of the *Hordeum* genome and the model genome of *Oryza sativa*, and (iii) performing Massive Analysis of cDNA Ends (MACE) and RNASeq. The approaches provide a potential of approximately 250 markers for the 3HL introgression. So far, approximately 50 markers were mapped on 3HL including 35 MACE markers. Three MACE markers are located with a distance of 0.1 to 0.4 cM to *Ryd4<sup>Hb</sup>* and are of potential use for marker-assisted breeding programs.

**Keywords:** genetic mapping, *Hordeum vulgare*, molecular markers, virus resistance

## GRAIN EATING QUALITY AND YIELD OF RED-PERICARP NON-WAXY AROMATIC RICE MUTANTS IN TAIWAN

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The bran fraction of red-pericarp rice grain is endowed with many phytochemicals that exert beneficial effects on human health. The red-pericarp rice landrace Kuanfu waxy aroma is highly valued with its grain quality in Taiwan, but it has undesirable traits of awned rough grain and taller plant height. The present study compared the eating qualities of cooked rice and the grain yields of 11 awnless red-pericarp rice mutants with non-waxy endosperm mutated from Kuanfu waxy aroma and a non-waxy aromatic rice variety TNG71 with good eating quality. The results indicated that all the mutants exhibited reduced plant height and awnless grain traits compared with Kuanfu waxy aroma. Significant differences in palatability of cooked rice were also obtained from the tested mutants, with mutant AM-425 (70) and AM-430 (73.7) having higher palatability test scores than TNG71 (69). Mutant AM-425 also had higher aroma sensory test score (1.33) than aromatic TNG71 (1.17). Four crop seasons yield trials indicated that aromatic mutants AM-425 and AM-430 produced slightly lower grain yields than TNG71, but the produced grain yields were significantly higher than Kuanfu waxy aroma. It appears that the aromatic mutants AM-425 and AM-430 are suitable to produce the non-waxy polished rice grains for regular dietary consumption, and the separated bran fractions can be used to make high-value supplements for food and cosmetic uses.

## RICE EGG FUSION WITH *ECHINOCHLOA CRUSGALLI* (L.) BEAUV SPERM

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*In vitro* fertilization (IVF) techniques using flowering plant male and female gametes may also offer potential for creating new hybridized zygotes and ultimately plants by fusing isolated gametes from different species, which would be impossible to obtain using typical crossing techniques. Such hybridization through *in vitro* fertilization would avoid the effects or influence of somatic tissues of the stigma, style and ovule and may be also ploidy problems occurring during somatic fusion. The *Echinochloa crusgalli* (L.) Beauv is a familiar C4 weed in rice field with very strong viability and disease resistance. The hybridization of rice with *E. crusgalli* will make a chance create a distant hybrid that increases rice viability and disease resistance. Rice egg cells were isolated by enzyme digesting followed by mechanical dissection [1]. *E. crusgalli* sperm cells were isolated by pollen burst when it incubated in 10% mannitol solution. The electrical fusion method was

applied to induce the fusion of the isolated *E. crusgalli* sperm and rice egg cells. In a suitable solution containing 10% mannitol, 0.001% CaCl<sub>2</sub> and 0.04% BSA, 92.9% (224/241) sperm-egg could be induced fusion using alternating current (30–35 V, 10–25 s) to align sperm-egg, following direct current (400–500 V, 45–50 μs) pulse to perforate the plasma member of both sperm and egg cells. The artificial zygotes were also tried to culture but no success. The fusion conditions of inducing *E. crusgalli* sperm cells and rice egg cells are different from those of maize and rice.

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## GENOME-WIDE ASSOCIATION STUDY (GWAS) OF DROUGHT RESISTANCE IN RICE

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Genome-wide association mapping, a high-resolution method for mapping quantitative trait loci based on linkage disequilibrium, holds great promise for the dissection of complex genetic traits. It had been widely used in human genetic studies as well as in plants such as *Arabidopsis*, maize, soybean, rice, etc. Identification of the excellent allelic variation underpinning the phenotypic diversity in rice will have enormous practical implications in rice breeding.

More than 200 rice landraces and cultivars were collected from all over the world, including Asia, Africa and America. The materials were tested under two water regimes, namely normal water treatment and drought stress treatment, in the same drought resistance facility in 2011 and 2012. The drought stress were implemented at the early booting stage, and lasted for 35 days. Five categories of characters were investigated: i) agronomic traits including plant height, flowering time, tiller number, etc.; ii) water status related traits including leaf relative water content, leaf water potential; iii) antioxidant substance, including SOD, POD, CAT; iv) osmotic adjustment substances, including proline, soluble sugar, soluble protein; and v) yield related traits.

All materials were re-sequenced with the average coverage of ~5X. Total 1M SNPs were obtained and used as genotype data. The genome-wide association mapping was conducted by mixed linear model method for the traits mentioned above. Ten significant association sites were detected which are involved in grain yield under drought stress conditions, drought resistance coefficient, flowering time, plant height, relative rate of dead leaves, biomass and the content of soluble sugar. The location of other 3 sites, related with

grain length, grain width and apiculus color, were consistent with the location of GS3, qSW5 and OsC1, implying the reliability of the mapping results in this study.

**Keywords:** *Oryzasativa* L., drought resistance, GWAS

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## TRACKING THE FATE OF DIFFERENT STRAINS IN A MIXED INOCULUM OF *FUSARIUM GRAMINEARUM* USING 454 PYROSEQUENCING

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When performing artificial inoculations with *Fusarium graminearum* on barley and other cereals, it is common to use multiple strains that may differ genetically and in their chemotype. Although one can use equal amounts of each strain in initiating inoculum production (e.g. on maize kernels, as per [1]), it is not known if this equal abundance of the strains will persist throughout the production of the inoculum, its deployment in the plots of the nursery, the production of spores and the actual infection of various barley cultivars in the nursery that may differ in their heading date. To answer these questions, we have developed a pair of amplicons that allows the determination of the allele present at three different SNPs and the joint analysis of these three SNPs allows each strain to be identified. DNA was extracted from the pure strains, an equimolar mix of these strains used to inoculate sterilized maize kernels, batches of the resulting inoculum (infected maize kernels) at various stages of its use and infected barley seeds of four cultivars differing in heading date. PCR amplification was performed using specific primers to obtain the two amplicons from each sample and amplicons were mixed in equal amount for sequencing. Pyrosequencing was conducted on a Roche 454 sequencer and over 100,000 reads were used to calculate the relative abundance of each strain in each sample. The results of this analysis show that: 1) the three SNPs allowed a clear discrimination of the different strains; 2) the abundance of each strain in different preparations of inoculum varied substantially in both the freshly produced inoculum and in field-collected inoculum (weeks after application); and 3) the abundance of each strain also varied considerably in in-

ected barley kernels from the different cultivars. These results suggest that different strains can differ in their ability to colonize maize kernels, complete their life cycle under field conditions and succeed in infecting barley.

**Keywords:** *Fusarium graminearum*, barley, pyrosequencing, 454

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## BIOPHARMING AS A TOOL FOR PRODUCTION OF ANTIMICROBIAL PEPTIDES

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Antimicrobial peptides (AMPs) represent a diverse group of low molecular mass component of defence systems of probably all species of life. Different peptides have been demonstrated to kill various pathogenic bacteria, protozoa, parasites, enveloped viruses, fungi and even cancerous cells. AMPs are considered as alternative therapeutic agents due to their broad-spectrum activity, low cytotoxicity towards mammalian cells and rapid bactericidal activity. Moreover, use of AMPs can present a solution for the increasing incidents of pathogen resistance to conventional antibiotics, as unique mode of AMP action on microbial membranes hinders emergence of resistant strains.

One of the barriers to the success of AMPs in clinical use is the development of a cost-effective manufacturing platform. Direct isolation of AMPs from their natural sources, chemical synthesis, or microbial expression are expensive and limited in scope. Biopharming (using plants as bioreactors) provides tremendous benefits over other production systems. It is cost effective, offers large scale production capacity, and displays low to no risk of product contamination by human or animal pathogens. Additionally, biopharming allows heterologous production in a bioactive form, as eukaryotic post-translational modifications are performed in plant cells.

However, there are still technical limitations that must be overcome before biopharming can be exploited as a suitable and feasible alternative for AMP production. These limitations can be surmounted by optimization of parameters for enhancing the product yield (by boosting expression and increasing product stability) and development of methods for simple downstream processing. For this purpose, we have designed various plant transformation constructs that combine different purification tags, stabilization tags, and secretion signals, and assessed the effects of these fusion partners on AMP pro-

duction. We have used transient expression in *Nicotiana benthamiana* leaves and *Arabidopsis thaliana* cell suspension cultures. Additionally, we have aimed expression of AMPs in barley endosperm and investigated the effects of different seed-specific promoters on AMP production. The expression cassettes with different promoters have been used for transformation of barley immature embryos. The transgenic barley plants generated will be analysed in future. Overall, results of this study will improve biopharming in barley endosperm, which might present a prospective platform for large scale production of AMPs.

**Keywords:** *Hordeum vulgare*, antimicrobial peptides

## COMPARISON OF INOCULATION METHODS IN TESTING RESISTANCE TO FUSARIUM HEAD BLIGHT IN WHEAT

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*Fusarium* head blight (FHB) is a destructive disease of wheat (*Triticum aestivum* L.), causing not only yield losses, but also mycotoxin contamination. The main goal in FHB resistance breeding is the development of productive cultivars with low disease symptoms and low mycotoxin contamination despite high infection pressure. In the literature the comparison and stability of field inoculation methods used in wheat breeding programs and cultivar registration has not been shown in detail. This is especially true for the spawn method that becomes more popular as its costs are relatively low. The aim of this study was to compare the methods we thought to be most important in further use.

In four experimental years (2009–2012) 40 wheat genotypes were tested with three inoculation methods: the spray inoculation of 15–20 heads which is followed by a covering with polyethylene bags for 48 hours to secure humidity for inoculation (1); the spray inoculation combined with 48 hours of mist irrigation (2) and the spawn method with 48 hours of mist irrigation (3). In every year genotypes were inoculated with four *Fusarium graminearum* or *Fusarium culmorum* isolates. We examined the severity of *Fusarium* head blight (FHB), the rate of *Fusarium*-damaged kernels (FDK) and the deoxynivalenol (DON) content to measure *Fusarium* resistance level.

In most cases the data for all the examined traits were the highest achieved with the spray inoculation method combined with polyethylene bag covering, and showed large genotypic variation. The other methods gave occasionally higher indices, but often the FHB severity, FDK rate or DON content was so low that the useful differentiation of the genotypes was not possible. The data set showed the closest correlations for the spray and bag covering methods. Most of the FHB data of spray and bag covering method correlates significantly with the data of other methods. The lowest correlations were found between

spawn and other methods, but several significant correlations were also found. The ANOVAs considering the whole data set show that the genotype differences are highly significant and the genotype  $\times$  environment (years, isolates, methods) interactions are much smaller than the main genotype effects for all traits.

This means that the genotype ranking will be influenced to some extent, especially at lower infection severities, but any of the methods can be used when the ecological risks can be treated well. For this reason in drier climate conditions the spray inoculation method combined with polyethylene bag covering seems more reliable, in other areas the other two methods can also be applied successfully.

**Keywords:** Fusarium head blight, wheat, inoculation method

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## EXAMINATION OF RESISTANCE LEVEL OF MAIZE HYBRIDS AGAINST FUSARIUM SPECIES

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Several fungal genera are pathogens, able to infect maize in the field and cause various disease symptoms. Many of their members also produce mycotoxins, secondary metabolites, which are harmful to animals and humans. Contaminated grain also affects international economy, regulatory, and trade policies. The most problematic mycotoxins in maize are aflatoxin, which is mainly produced by the fungus *Aspergillus flavus*, fumonisins produced mainly by *Fusarium verticillioides*, trichothecenes and zearalenon produced by several *Fusarium* species like *F. graminearum*.

Field test included 37 hybrids, inoculated by one-one isolates of each species of *F. graminearum*, *F. culmorum* and *F. verticillioides*. Three replicated random block designs were used with four row plots of 5 m length. Both toothpick and silk channel resistance tests were applied. *Aspergillus flavus* infection was rated only in 2012 from natural infection. Non-inoculated rows served to evaluate natural infection. 15 ears per row were individually rated for coverage by the fungi as percentage values.

The inoculation results were at medium level, the hybrid differences were well expressed and the resistance to different species was also clearly differentiated. The kernel resistance test (toothpick method) gave 10.75 mean severity (range 5.79–16%, LSD 5% 2.14), the silk channel test only 2.76 (range 0.68–6.54%, LSD 5% 1.07). This latter test differed the genotypes much less effectively. The interannual correlations were only moderate. The silk channel and kernel resistance data showed a medium level correlation ( $r = 0.46$ ). However, there were genotypes that showed susceptibility in kernel resistance and

good resistance in silks, others behaved in the opposite way, and several produced similar data at different resistance or susceptibility levels. It seems that the two resistance mechanisms occasionally correlate well; therefore both methods have significance for breeding. In relation to *Aspergillus* only one year data are present, further data will be necessary to draw solid conclusions.

A selected set of genotypes was subject of toxin analysis, which is in progress.

**Keywords:** *Fusarium*, *Aspergillus*, maize, resistance, inoculation method

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## DESIGNING HIGH-YIELDING WHEAT IDEOTYPES FOR A CHANGING CLIMATE

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Global warming is characterised by shifts in weather patterns and increases in climatic variability and extreme events. New wheat cultivars will be required for a rapidly changing environment, putting severe pressure on breeders who must select for climate conditions which can only be predicted with a great degree of uncertainty. To assist breeders to identify key wheat traits for improvements under climate change, wheat ideotypes can be designed and tested in silico using a wheat simulation model for a wide range of future climate scenarios predicted by global climate models. A wheat ideotype is represented by a set of cultivar parameters in a model, which could be optimised for best wheat performance under projected climate change. As an example, high-yielding wheat ideotypes were designed at two contrasting European sites for the future 2050(A1B) climate scenario using the Sirius wheat simulation model. Simulations showed that wheat yield potential can be substantially increased for new ideotypes compared with current wheat varieties under climate change. The main factors contributing to yield increase were improvement in light conversion efficiency, extended duration of grain filling resulting in a higher harvest index, and optimal phenology.

**Keywords:** wheat, crop modelling, climate change impacts, Sirius, LARS-WG

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ADVANCES IN OAT BREEDING.  
EFFORTS FOR TISSUE CULTURING

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Oat breeding at our institute follows successive strategy. After the pre-screening process of the basic genetic material from different genotype collections then introduction of targeted crossing programmes start with selected oat genotypes. During the last two years, 420 oat cultivars, composite lines, landraces and advanced lines were examined in micro-parcels. Beside *A. sativa*, *A. sterylis*, *A. byzantina*, *A. murphy* and nuda-type oat genotypes were also screened. The spring or facultative oats from boreal climatic region were fall-sown in the nursery to predict their winter hardiness associated with extra grain, biomass and straw yield capacity in case of an average winter. Seed germination, frost resistance, homogeneity, plant height, resistance to lodging, virus and fungal disease symptoms, slab structure and tendency to shatter were visually characterized. After manual harvest, the yield and TKW, threshing properties and the cleaning loss were measured. Analytical examinations like lipid and protein content were measured by NIR method.

The parents for the targeted crossing have been selected based on the complex analysis of the yield, quality contents and resistance to biotic and abiotic factors. A few parts of these crossing programs have been realized, partly in the green-house and in the field. 35 crossing combinations (20 in F<sub>2</sub> and 15 in F<sub>1</sub>) were created with the use of pre-selected oat genotypes. The main targeted populations fit the actual market expectation: high yielding cultivars, hull-less types for human and bio use, cultivars for silage, specific fodder oats (etc. racehorses), high yielding winter oats, cultivars with complex expectation (black winter, black hull-less, etc.).

Homozygous doubled haploid (DH) plants and lines can be used in cereal breeding programmes. Methods based on androgenesis induction are a common way to produce homogenous basic material for variety development and genetic research purposes. Anther culture is a relatively simple and rapid method for DH production in case of major cereal crops like barley and wheat. Oat is known recalcitrant species for tissue culture response specially anther and microspore culture. Its low frequency DH production limits extensive application in breeding. Our aim is to develop an improved protocol to generate acceptable number of DH lines for breeding.

**Keywords:** oat breeding, DH production, anther culture

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MORPHOMETRY OF EINKORN (*TRITICUM M. MONOCOCCUM*)  
SEED POPULATIONS FOR PROVIDING DIGITAL TOOLS  
FOR CULTIVATION AND VARIETY REGISTRATION

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Einkorn (*Triticum monococcum* ssp. *monococcum*) was introduced into the Carpathian Basin by Neolithic tribes [1]. Now, its cultivation is reduced and grown mostly on small, isolated mountain regions. However, for human consumption, there is a new attempt to use it for brewing. The grain has high microelement and essential amino acid contents especially *lysine*, nevertheless its carbohydrate content is very low.

Here we applied Computer Assisted Seed Morphometry [2] analysis of digital images of seed populations of *eleven einkorn varieties and landraces* with a final aim to provide *new digital tools* for variety registration. We also conducted gene and protein sequence analysis *in silico* (nuclear gene *gapdh*, and cytoplasmic cpDNA) to estimate the evolutionary distance among the ‘ancient’ diploid (2n) einkorn compared to the tetraploid (4n) and hexaploid (6n) wheats (*Triticum* ssp.). Comparative field tests of the einkorn accessions were also conducted measuring the vigor of plant development, weed control characteristics, and the effects of soil quality on the yield.

During the diseases and pest control, leaf beetle (*Lema melanopa*) and grain beetles (*Anisoplia austriaca*) were detected. Significant differences in the yield production were found. However, all einkorn varieties and landraces can be suggested to grow in organic farming even in the areas with low production potentials, as their production technology can be easily integrated. The best varieties were provided to plant breeders for further studies, and suggestions were given towards a digital registration system based on seed morphometry.

**Keywords:** Einkorn (*Hung.* Alakor), seed morphometry, cultivation

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## MEASURING DIVERSITY: MORPHOMETRIC MONITORING OF VARIATION IN WHEAT (*TRITICUM* SSP.) SEED POPULATIONS

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Computer Assisted Morphometry (CAM) involves quantitative measurements of morphological parameters (e.g. size, shape and tomography measurements) for statistical analysis and classification routines [1]. Morphological measurements are based on pixel counts occurring within the margin of each binary object multiplied by calibrated pixel size with adjustments for specific measurements. Object morphometry and object recognition are achieved using five parameter categories: dimension of size, dimensionless ratio of shape, harmonic analysis, fractal dimension and topology. The shape of a distribution patterns derived from such measurements shows if the distribution is *continuous*, *normal* (bell-shaped), *bimodal*, *multimodal* or *non-parametric*.

In the previous studies, we have studied over a thousand species/varieties; measured hundreds of thousands of seeds and assembled a database of about 3 million measurements [2]. First, we attempted to find the minimum and optimum reference samples needed to identify *unknown seeds* by plotting incremental data points until an expected *bell-curve* distribution was obtained. We failed — because the *natural distribution* of variation is rarely, if ever, *bell-shaped*. Significantly, *domestic seed population* distributions were typically *normal* because they are subjected to artificial, human control of evolutionary selection. Results also showed that different patterns of variation, *bell-shaped*, *bimodal*, *multimodal*, *non-modal*, etc. may occur in any specific species for any parameters measured. Moreover, neither the probability of mean values nor the probability of the frequency of measured values was *predictable*. Such consistently inconsistent results constitute an “*Unpredictable Variation Principle*” in *Evolution*. Thus, probability-based statistics, especially *parametric statistics*, are not appropriate for accurate analysis. We present new methods of quantitative analysis, i.e. “*Variometric Analysis*”, that make no probability assumptions in assessing patterns of variation.

In the study presented we aimed to conduct Computer Assisted Seed Morphometry (CASM) of *Triticum* species to find correlations between evolutionary stages of ploidy levels (2n, 4n, and 6n) and seed parameters using digital images. In total 33 parameters were measured: *Adjusted\_Count.*, *Area* (cm<sup>2</sup>), *Aspect Ratio*, *Breadth* (cm), *Circum. Rad.* (cm), *Convex Area* (cm<sup>2</sup>), *Convex Perim.* (cm), *Convexity*, *Elongation*, *Equiv. Diam.* (cm), *External Perim.* (cm), *Filled Area* (cm<sup>2</sup>), *Form Factor*, *Fractal Dim.*, *Inscrib.Rad.* (cm), *Length* (cm), *Mean\_Ext. Bran. Len.* (cm), *Mean\_Fiber Width* (cm), *Moment\_Angle*, *Perimeter* (cm), *Radius Ratio*, *Roundness*, *Skel.Branch\_Points*, *Skel.End\_Points*, *Skeleton Length* (cm), *Solidity*, *Symmetry*, *Width\_Std.Dev.* (cm), *X-Centroid* (cm),

*X-Geom.Center* (cm), *X-Weighted\_CG* (cm), *Y-Centroid* (cm), *Y-Geom.Center* (cm), *Y-Weighted\_CG* (cm) by the CAM program used [2].

The PCA study (*Principal Component Analysis*) conducted revealed that seven parameters of the 33 parameters measured, the *Length* (cm), *Breadth* (cm), *Perimeter* (cm), *Roundness*, *Solidity*, *Convexity* and *Symmetry* of seeds are discriminative and appropriate for parametric analyses. When cumulative parameters were plotted, wheat seeds showed not a linear evolution with the *endosperm hypotrophy* (i.e. seed sizes) from the diploid wheat ( $2n = 2x = 14$ ) *T. monococcum*, through tetraploids ( $2n = 4x = 28$ ) of *T. carthlicum*, *T. dicoccoides*, *T. durum* and *T. kamut* to the hexaploid ( $2n = 6x = 42$ ) *T. aestivum*.

**Keywords:** wheat, seed morphometry

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## ASPECT OF UTILIZATION OF MOLECULAR MARKERS TO FHB RESISTANCE IN WHEAT BREEDING PROGRAMMES IN CZECH REPUBLIC

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Selection through to molecular markers (MAS) might be an efficient breeding tool special for programmes with strategic importance, where used testing procedures are not effective, costly or difficult. The advantage of molecular markers is also time, when breeder can receive the information about the location of wanted gene in plant or progeny. Nobeoka Bozu was widely studied and Sumai3 based markers are used but further study is required. Some varieties selected in the classical breeding programme are considered to be moderately resistant (MR).

**Keywords:** *Triticum aestivum*, FHB, selection

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## IMPROVING YIELD POTENTIAL IN BARLEY USING YIELD-RELATED GENES

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High yield has been a major breeding target in cereals, including barley (*Hordeum vulgare* L.), one of the most important food crops and model monocot of the small grain cereals. Grain size is the most important components of grain yield and is controlled by quantitative trait loci (QTLs) derived from natural variations in crops. However, the molecular roles of these QTLs in the regulation of grain characteristics have not been fully elucidated. Here, we report the cloning and characterization of barley homologues of the previously analyzed rice *GW2* gene that encodes a RING-type protein with E3 ubiquitin ligase activity, which is known to function in the degradation by the ubiquitin-proteasome pathway. The pleiotropic effects of the mutation have shown that *GW2* may be involved in the development of other tissues or organs, beyond its influence on grain size [1]. Our database searches have revealed that the barley genome encodes at least two *GW2* homologues. These differ from each other mainly in the substrate-binding region; this may show that these E3 ubiquitin ligases share the same E2 ubiquitin conjugase enzyme during their action. Based on these results, we have made an extensive database search for proteins encoded by *GW2* homologue genes. Our results have demonstrated that *GW2*-like proteins can be classified into three groups (dicot, *monocot I* and *monocot II*) in mono- and dicot species. Dicots contain two *GW2* proteins with high level of homology, while monocots contain at least two *GW2* proteins that shares considerable homology only in the RING-domain. We must note that until now no information is available on the function of the second group of monocot *GW2* proteins, all published results were based on the characterization of *GW2* genes coding for the proteins belong to the *monocot I* group. In this project transgenic barley plants with lowered expression of both barley *GW2* homologues were created using antisense approaches. Until now, we verified the lowered expression of these genes by RT-PCR and cultivate the T2 generation of the plants for seed collection for further characterization. Although the phenotypes of the primary transformants cannot allow firm conclusions, we have observed prolonged seed filling for antisense *HvGW2.1* transformants and increased leaf surface for antisense *HvGW2.2* transformants. Observations on phenological parameters – like days to heading, days to maturity – and morphological attributes of grain yield, plant height, number of tillers per plant, number of spikelets per spike, awn length, and dry weight per plant were recorded. The obtained information demonstrated that lowering the *GW2* gene expression is a useful tool for increasing grain yield, because it resulted in increased grain weight (15–20%)

in both of antisense HvGW2.1 and HvGW2.2 transformants, compared to the wild type plants.

**Keywords:** barley, grain weight, grain size, antisense, yield-related genes

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