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Current Challenges and New Methods for Maize and Sorghum Breeding

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CAN MACHINE LEARNING MODELS AND METHODS ENHANCE GENOME AND PHENOME PREDICTION ACCURACY IN PLANT BREEDING?

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In the last years genome and phenome models have been developed for the prediction of unobserved individuals using dense molecular markers, environmental covariables and images of different type. Models include single and multi-traits, and single and multi-environments as well as several high throughput phenotype (HTP) and near infrared spectroscopy (NIR) information with the objective of increase the prediction accuracy of grain yield, and other traits on unobserved individuals. Increase in prediction accuracy over the genomic best linear unbiased estimator (GBLUP) was achieved by means of the Gaussian kernel model with genomic × environments interaction (G×E). Models that incorporate other non-linear kernels can increase the prediction accuracy of G×E by 5-10% over the GBLUP. A Bayesian multi-trait multi-environment model can efficiently exploit correlated traits and environments and thus increasing the prediction accuracy over the single trait, single environment model. Bayesian models requires intense computational resources. Deep Machine Learner (DL) models and methods have been developed with densely connected network architecture with the objective of using fewer computing resources and accommodate extensive data sets. Results from DL were compared with those from GBLUP; results show that DL is a competitive method because it gave better accuracy than GBLUP in several data sets under a scenario that ignored covariates capturing G×E. However, when G×E was included, DL was inferior in terms of prediction accuracy to GBLUP. Although implementing the multi-trait DL models is feasible and practical in the genomic prediction context it is challenging due to the large number of hyper-parameters involved. To improve the computing speed of kernel method, Deep Kernel method with G×E have been studied and accuracy compared with other kernel methods employing genomic, and pedigree information.

THE GENETICS AND CONSEQUENCES OF MAIZE DOMESTICATION AND BREEDING: CAN WE MAKE A BETTER TASTING CORN?

Sherry Flint-Garcia*

US Department of Agriculture - Agricultural Research Service

The processes of domestication and breeding have had serious consequences on modern maize, particularly for the genes that are directly responsible for the transitions from the weedy teosinte ancestor to landrace populations to modern corn. These genes include genes involved in kernel size and composition traits, as these traits were targets of selection during domestication and breeding. In addition, the focus on yield as the primary trait of interest in the private sector for the last century has resulted in a loss of variation for food quality traits. My research program focuses on seed traits and seeks to integrate research on inbreeding and adaptation to develop better tasting and more nutritious food corn. We have initiated multiple projects to characterize the landraces (heirlooms) from the US and other regions around the world, improve several US heirlooms for agronomic characteristics, and are beginning to explore food properties of heirlooms to increase corn use in the human diet.

Keywords: *Landraces, Heirlooms, Diversity, Food Traits*

TACKLING MAIZE'S CONTRIBUTIONS TO CLIMATE CHANGE BY LEARNING FROM *ALL* PLANT GENOMIC DIVERSITY

Edward Buckler

USDA-ARS at Cornell University

Maize agriculture needs to increase yield, adapt to climate change, AND become a key element in carbon sequestration. We can accomplish this tall order if we start re-designing maize based on how related species function. Many wild ecosystems have excellent nutrient management and high soil carbon. Our research aims to understand how the dominant clade over 17% of the planet, the Andropogoneae grasses (includes maize, sorghum, sugarcane, and Miscanthus), have adapted to their environments and apply that understanding to maize and related forage crops. The current approaches for allele mining, mapping, genomic selection models are in efficient for scaling to numerous species. We use evolution and machine learning to develop robust models across the central dogma of molecular biology that work *across* species. We have applied this combination of evolution and machine learning to identify: distal regulatory elements, functional genes, chromatin structure, transcription, translation, and protein structure contribution to yield. The next stage is to apply these tools to understand Andropogoneae adaptations and leverage that knowledge into maize.

EXPERIMENTAL EVOLUTION: TWO DIVERGENT SELECTION STUDIES, 16 CYCLES FOR VEGETATIVE PHASE CHANGE AND 7 CYCLES FOR ENDOSPERM STARCH SYNTHESIS

William F. Tracy

University of Madison-Madison

Long term divergent selection studies can reveal correlated traits, loci that underlie the traits, and limits and power of selection. We have used this tool to study vegetative phase change and starch synthesis in the endosperm. Vegetative phase change is the transition from juvenile to adult vegetative growth stages in higher plants. We studied the morphological changes resulting from 16 cycles of divergent selection. Thirteen traits were evaluated, including the last leaf with juvenile wax (LLJW), plant height, leaf area infected by common rust, days to mid silk, ear characters, and number of tillers, leaves, and nodes with aerial roots. The direct effect of selection, LLJW, diverged from leaf 8 in cycle 0 to leaf 18 in cycle 16 late, and to leaf 5 in cycle 16 early. In a second study we used 7 cycles of divergent selection in a homozygous recessive *sugary1* background to alter endosperm appearance selecting for full, opaque kernels in one direction and wrinkled, glassy kernels in the other direction. Cycle 0 endosperm was 250 mg g^{-1} phytoglycogen and 290 mg g^{-1} starch. The C7 starchy population contains 35 mg g^{-1} phytoglycogen and 535 mg g^{-1} starch. We will discuss biochemical changes in key enzymes in the endosperm starch synthesis pathway.

GENETIC DIVERSITY MANAGEMENT AND BROADENING IN MAIZE BREEDING PROGRAMS USING GENOMIC SELECTION

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There is an increasing awareness that breeding programs should balance short- and long-term objectives by maintaining genetic diversity to cope with future challenges. The advent of high-density genotyping opened new avenues to further enhance the efficiency and sustainability of breeding. This involves the evaluation of genetic diversity in elite breeding pools, its efficient conversion into short- and long-term genetic gain and the efficient identification, improvement, and introduction of extrinsic variability.

Selection of parental crosses that generate superior progeny while maintaining sufficient diversity in next generation is a key success factor at short- and long-term. We derived analytical solutions to predict the joint distribution of a quantitative trait and genetic diversity in the progeny (UCPC). We used UCPC to extend the Optimal Cross Selection (OCS) that aims at maximizing the performance in progeny while maintaining diversity. In a simulated maize breeding program, UCPC proved to be more efficient than OCS to convert the genetic diversity into short- and long-term genetic gains.

The narrow genetic base of elite germplasm compromises long-term improvement. An efficient strategy to broaden elite genetic base is therefore required. Many genetic resources with different performance levels are accessible to breeders. We evaluated the interest of UCPC to improve genetic resources, to bridge them with elites, and to manage recurrent introductions into the elite population. In a simulated maize breeding program, we demonstrated that recurrent introductions of genetic resources through a bridging population maximize long term genetic gain while maintaining genetic diversity constant, with only limited short-term penalty.

Keywords: *Genetic Resources, Genomic Selection, Bridging, UCPC (Usefulness Criterion Parental Contribution), OCS (Optimal Cross Selection)*

MAIZE AND SORGHUM PHENOTYPING IN A CONTEXT OF GLOBAL CHANGE: WHAT TO MEASURE AND HOW TO DO IT

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High-throughput phenotyping, particularly under field conditions, is nowadays perceived as a key factor limiting crop genetic advance in maize and sorghum. Phenotyping not only facilitates conventional breeding, but it is necessary to fully exploit the capabilities of molecular breeding, and it can be exploited to predict breeding targets for the years ahead at the regional level through more advanced simulation models and decision support systems. In terms of phenotyping, it is necessary to determined which selection traits are relevant in each situation, and which phenotyping tools/methods are available to assess such traits. Remote sensing methodologies are currently the most popular approaches, even when lab-based analyses may be still relevant in some circumstances. On top of that, data processing and automation, together with machine learning/deep learning are contributing to the wide range of applications for phenotyping. On the other hand, the awareness of phenotyping as a breeding limitation is not only sustained by the lack of adequate approaches, but also by the perception that phenotyping is an expensive activity. This presentation will place emphasis on high throughput phenotyping aims to achieve reasonably priced solutions for all the components comprising the phenotyping pipeline. In that sense digital phenotyping with specific focus on onfarm measurements in a context of participatory breeding approach, will be also addressed.

NOVEL MAIZE BREEDING STRATEGIES ARE NEEDED TO FACE CLIMATE CHANGE

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Climate change will bring increasingly frequent events of severe drought or high temperatures. Selection for yield during the last 60 years led to substantial genetic gain in contrasting environmental scenarios, but it did not reduce the yield gap between optimal environmental conditions and expected adverse conditions. A phenomic and genomic-based retrospective analysis indicate that current strategies of plant breeding indirectly selected a limited number of traits, namely phenology and plant architecture, which in turn improved light interception and reproductive development with similar trends in all tested environmental conditions. Conversely, traits known for optimizing response to water or temperature stress were not improved: e.g., stomatal conductance, response of growth to water deficit or water use efficiency. The genomic regions that control these traits showed no signatures of selection, whereas those controlling phenological and architectural traits did. By selecting for yield, breeding has indirectly selected traits with stable effects on yield, but not adaptive physiological traits whose effects on yield can be positive or negative depending on environmental conditions. Because resilient and productive cultivars for sustainable intensification of agriculture under climate change are needed, it is crucial to develop novel strategies that enable effective use of the reservoir of alleles governing adaptive traits.

BREEDING MAIZE FOR STRESS TOLERANCE

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Maize is one of the most widespread crops; however, two main stresses affect maize production in Europe: 1) cold conditions at early sowing in northern latitudes and 2) drought in the south.
1) Maize is sensitive to cold conditions at early stages; the critical temperature threshold is about 10 ºC. A research program on maize cold tolerance has been developed at the Maize Genetics and Breeding Group of the *Misión Biológica de Galicia* for 25 years. We have found that maximum quantum yield of photosystem II (Fv/Fm) is useful for effective and reliable evaluations of large number of genotypes. Furthermore, evaluations have to combine controlled and field conditions. Breeding for cold tolerance in maize is not straightforward because there are several technical challenges for evaluation such as limited genetic diversity and complex regulation and interactions. Currently, we are conducting genomic and phenotypic selection programs for increasing quantum efficiency of photosystem II.
2) Drought is the main agricultural stress and will worsen with climatic change. Drought affects crops at all stages, causes yield losses up to 100% and increases pest and disease damages. We are coordinating the project *Capitalization of Mediterranean maize germplasm for improving stress tolerance* in which 12 research groups from nine European countries are involved. The aim of this project is to promote progressive transformations of the current agricultural systems into new environmental-friendly Innovative Farming Systems based on cultivation of maize varieties resilient to drought.

Keywords: *stress, cold, drought, maize, breeding*

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MODULATION OF WATER USE EFFICIENCY AND CARBON ISOTOPE DISCRIMINATION IN MAIZE

Sonja Blankenagel[1], Stella Eggels[1], Monika Frey[1], Erwin Grill[2], Eva Bauer[1], Corinna Dawid[3], Alisdair R. Fernie[4], Georg Haberer[5], Richard Hammerl[3], David Barbosa Medeiros[4], Milena Ouzunova[6], Thomas Presterl[6], Victoria Ruß[2], Rudi Schäufele[7], Urte Schlüter[8], Francois Tardieu[9], Claude Urbany[6], Sebastian Urzinger[1], Andreas P. M. Weber[8], Chris-Carolin Schön[1], Viktoriya Avramova[1,*]

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Improved water use efficiency (WUE) of crops is a key sustainability factor to mitigate the effects of climate change. WUE is defined as the amount of biomass production of a plant per unit transpired water and can be increased by limiting transpiration through stomata. However, the lower transpiration is very often accompanied by a reduction of photosynthesis. Here, we show that in maize, moderate changes in stomatal conductance was induced by natural allelic variation at single gene without substantial effects on photosynthetic parameters, thereby increasing WUE without growth reductions.

Using near-isogenic lines derived from elite maize breeding material, we genetically dissected a genomic region affecting WUE. This led to the identification of a causative gene encoding an enzyme that hydroxylates the phytohormone abscisic acid (ABA) and initiates its catabolism. Using CRISPR/Cas9, we knocked out the gene, which lead to increased leaf ABA levels and a consequent decrease in stomatal conductance resulting in WUE improvement without observable negative effects on plant growth in well-watered conditions. Moreover, physiological analyses revealed that the stomatal conductance, affected by ABA catabolism, caused variation not only in WUE, but also in the discrimination against the heavy carbon isotope ($\Delta^{13}C$) during photosynthesis. This led to changes in the isotopic signature in the maize kernels, which reveals a potential for $\Delta^{13}C$ to be used as an easy to screen proxy for WUE in maize. Therefore, our results establish a direct physiological and genetic link between WUE and $\Delta^{13}C$ through a single gene with the potential for application in maize breeding.

Keywords: *maize, water use efficiency, abscisic acid, carbon isotope discrimination*

ENDURING STRESS: TRANSLATIONAL APPROACHES TO SORGHUM ADAPTATION TO TROPICAL SOILS

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Acidic soils with low-pH result from intense weathering, and are thus prevalent in the tropical world, where food insecurity still exists. Major abiotic constraints for crop production on acidic soils have a chemical origin and are aluminum (Al) toxicity, due to root damage caused by ionic Al^{3+}, and phosphorus (P) deficiency, which results from P fixation into soil clays, reducing availability. Of ubiquitous occurrence and bound to get more and more prevalent due to climate change, drought stress interacts with Al toxicity and low-P availability and, in conjunction, these factors strongly influence yield stability on acidic soils. Our program seeds to identify the mechanisms leading to sorghum abiotic stress tolerance on tropical soils, identifying the underlying molecular determinants and finding out how they function in concert to provide adaptation. Finally, we seek to develop strategies to deploy those mechanisms to foster molecular breeding targeting sorghum adaptation to acidic soil regions. Two examples will be explored, both from the mechanistic and application perspectives. First, a single allele of the Al-activated citrate transporter, *SbMATE*, increases grain yield under strong Al toxicity in the soil. SbMATE detoxifies Al by promoting the exclusion of its ionic forms (Al^{3+}) from sensitive sites in the root apex, via formation of stable Al-citrate complexes. On the P acquisition side, we have shown that sorghum homologs of a serine/threonine receptor kinase, which was previously identified by other groups in rice, enhance phosphorus uptake and grain yield for sorghum cultivated on a low-P soil, via changes in root system architecture. We will also describe our efforts devoted to building a molecular genetic framework aimed at identifying novel determinants of abiotic stress tolerance as a prelude to in-depth validation approaches, and identifying the most appropriate breeding steps where these factors can be deployed to facilitate the development of cultivars with overall sorghum adaptation to acidic soils.

***Keywords**: abiotic stress, acidic soils, sorghum, molecular mechanisms*

FROM MYCO-KEY TO MYCO-TWIN: MYCOTOXIN MANAGEMENT ALONG FOOD/FEED CHAIN

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Fungal disease is one of the most important contributors to the occurrence and severity of mycotoxin contamination of crop plants. Phenotypic and metabolic plasticity has enabled mycotoxigenic fungi (MF) to colonize a broad range of agriculturally important crops and to adapt to a range of environmental conditions. New mycotoxin-commodity combinations provide evidence for the ability of fungi to adapt to changing conditions and the emergence of genotypes that confer enhanced aggressiveness toward plants and/or altered mycotoxin production profiles. Among diseases caused by MF, the most important are the result of attacks carried out by species complexes. Examples of these diseases are the *Fusarium* ear rot of maize, *Fusarium* head blight (e.g. wheat, barley, and oat), black point of wheat kernels by *Alternaria alternata* species complex and related species; and various rot caused by *Aspergilli*. Mycotoxins in plant products and in processed food and feed have a significant economic impact and pose a serious problem for animal and human health. The management of good agricultural practices in the pre- harvest is a key issue for minimizing the risk of mycotoxin accumulation in the crops before the harvest. Such practices can involve crop rotation, tillage, proper fertilization and fungicide or biological control distribution, variety selection, timely planting and harvests and the control of the insects which often facilitate the toxigenic fungal species infection. Moreover, it is extremely important to prevent post-harvest contamination and develop practical and effective post-harvest procedures for mycotoxin reduction in the food supply chains and to provide alternative and safe use options for contaminated batches. An update review will be given on integrated management of pre-and post-harvest practices aiming at the minimizing the risk of mycotoxin contamination along chain and main effective solutions developed by EU MycoKey (http://www.mycokey.eu/) and MycoTwin (https://www.mycotwin.eu/project) projects.

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THE PROGRAMME VASO: A LONG-TERM PARTICIPATORY MAIZE BREEDING FOR MAIZE BREAD

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Three Participatory Maize Breeding Projects have been discovered in the Global North, with VASO being the oldest.

In 1984, the VASO project began as a participatory plant breeding effort focusing on traditional germplasm used for human consumption, particularly maize bread.

VASO was founded as an alternative approach to address the dilemma of how to enhance maize production using local germplasm tailored to farmers' needs in marginal areas where land is scarce, and multinationals have no market. This presentation gives an overview of VASO's history, concepts, and pioneers (VASO 1.0), followed by a second stage (VASO 2.0), in which agronomy, pre-breeding work, quantitative genetics, quality, food technology, and socioeconomics were all included in a transdisciplinary and multiactor approach to the valorisation and use of maize genetic resources.

Keywords: *maize, participatory plant breeding, genetic resources, maize bread*

MAIZE ENDOSPERM VITREOUSNESS: GENETIC ANALYSIS AND THE IMPACT IN STARCH BIOAVAILABILITY DURING ENSILING

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The kernel is a major contributor of energy for maize forage. Starch digestibility is affected by the amount and types of proteins that surround the starch granules forming the starch-protein matrix which also conditions physical properties of the endosperm such as vitreousness and density. The first goal of this research is to identify genomic regions controlling endosperm vitreousness, total kernel density and total protein. A set of 809 inbred lines from a maize diversity panel and 536 inbreds from the MAGIC population were evaluated using replicated field experiments across two seasons in Wisconsin. A set of 423,117 RNA-sequencing derived markers and 91,798-exome capture based genetic markers were used for the diversity panel and MAGIC population, respectively. Several significant associations were identified for the three traits with overlapping regions between vitreousness and density. The second goal of this research was to study the effect of altering endosperm vitreousness on the composition, nutritive value and starch digestibility of maize forage mixes during ensiling. Near-isogenic lines (NILs) with three different endosperm mutations (*opaque-2*, *floury-2* and *soft endosperm*) were treated with different pollinations regimes to form different allelic combinations of the mutations and trigger contrasting endosperm phenotypes through allelic dosage effect. The experiment was configured as a split-plot and silage samples were harvested as silage maturity and ensiled for five different times. Significant differences were found in fresh sample kernels for endosperm vitreousness and prolamin (zein) profiles among NILs with different pollination treatments. Zein content was negatively correlated with *in-vitro* starch digestibility (ivSD) and progressive ensiling times reduced both total zein and α-zeins concentrations. ivSD increased with ensiling time for all genotypes. The ability to regulate kernel composition characteristics has the potential to improve animal nutritional efficiencies and have environmental impact related to N_2O emissions.

BIODIVERSITY OF AND DIAGNOSTIC TOOLS FOR TOXIGENIC *ASPERGILLUS* AND *FUSARIUM* SPECIES ON MAIZE

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Maize is one of the most cultivated crops worldwide, and a staple food for human and livestock worldwide. Maize can be colonized by several fungal pathogens, including species belonging to *Aspergillus* and *Fusarium* genera, of particular concern since they cause devastating maize fungal diseases, and significant losses of products. Species of both genera produce a wide range of mycotoxins, harmful secondary metabolites that accumulate in plants throughout their growth, especially in the mature kernels in the field. Aflatoxins and fumonisin B_2 are the most important mycotoxins accumulated by *Aspergillus* species, while fumonisins B_1, B_2, B_3, deoxynivalenol, zearalenone and, a less toxic mycotoxin, beauvericin, are the most important accumulated by *Fusarium* species. Accurate identification of all these mycotoxigenic species is key aspect for health risk assessment, since each toxigenic species can have its own mycotoxin profile. We carried out several studies devoted to develop diagnostic tools for both an early, correct toxigenic species identification, and achieving new techniques of early detection and identification of toxigenic fungi that would allow also an intra-specific distinction between toxigenic and non toxigenic strains. Therefore, we report here the investigations on:

(i) the appropriate molecular marker for species identification, identifying calmodulin gene as single taxonomically informative entity;

(ii) the genetic differences in the housekeeping gene TEF1a, exploited to design species-specific PCR primers for correct species identification of the two phylogenetically sister species *F. subglutinans* and *F. temperatum*, that differ in their ability to produce beauvericin;

(iii) 3 single nucleotide polymorphisms (SNP) in bea1 gene occurring in both *F. subglutinans* and *F. temperatum* leading to the understanding the base of the difference in beauvericin production between the two species;

(iv) the microbial-derived volatile organic compounds (mVOCs) emitted by toxigenic and non-toxigenic strains of *A. flavus*, allowing to establish a relationship between some VOCs and aflatoxin B1 production.

Keywords: *Calmodulin, F. subglutinans, F. temperatum, SNP, VOCs*

FROM SEED SCIENCE TO ISTA RULES FOR SEED TESTING

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ISTA’s mission is “Uniformity in seed quality evaluation worldwide“.
The ISTA test methods are based on scientific knowledge and the accumulated experience of those working in seed testing and quality control. This expertise is provided by the members of ISTA’s Technical Committees. All test methods proposed for inclusion in the ISTA Rules have gone through the ISTA Method Validation Programme. To stay update, ISTA is revising its Rules annually.
A positive impact on food and feed supply can be achieved by improved seed breeding processes and cultivation techniques. They always require high seed quality, which can only be achieved by uniform seed testing.
Seed quality is influenced by several factors and ISTA plays an important role in maintaining and improving the testing methods.
Seed is a living biological organism, and its behavior cannot be predicted with the certainty that characterizes the testing of inert, non-biological material.
Characteristics such as trueness to variety, germination, physical purity, viability, vigour, are important aspects for breeders, seed producers and farmers as final users of the seeds.
The above-mentioned characteristics and not only, will lead to a uniform crop and successful establishment and support to avoid weedy, diseased crops and to avoid uneven maturity. Among the most relevant aspects, the mechanical damages may affect seed germination, health and vigour.
One of the strategic goals of ISTA is to develop tests and methods based on science and new technologies and facilitate the application of new or emerging technologies into current methods. Some achievements are the introduction of the DNA-based methods for variety testing, X-radiography, vigour tests (e.g., radicle emergence) and GMO testing (detection, quantification, or confirmation of the presence of genetically modified organism seeds).
Corn and grain sorghum are among the top cereal crops worldwide, and are key for global food security. Both species are considered in the ISTA Rules.

Keywords: *seed, seed quality, testing methods, ISTA*

SHOULD MAIZE BREEDERS ROUTINELY DO RECURRENT SELECTION?

Rex Bernardo

University of Minnesota

Yes.

Keywords: *maize breeding, recurrent selection, doubled haploids, genomewide prediction*

TRANSITIONING FROM SELECTION OF MAIZE GRAIN FOR HIGH PROVITAMINA CAROTENOIDS FOR SUB-SAHARAN AFRICA TO SELECTION FOR HIGH MACULAR AND TOTAL CAROTENOIDS FOR USA

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Vitamin A deficiencies result in blindness or death for 250,000 to 500,000 children annually. Considerable progress has been made on the release of new higher provitamin A (proVA) maize hybrids and open-pollinated varieties in eleven Sub-Saharan countries, India, and China. These high proVA materials are designed to help address the serious Vitamin A deficiency problem. This effort benefits from the selection of favorable alleles of genes in the carotenoid biosynthetic pathway, particularly a weak allele of *beta-carotene hydroxylase 1* (*crtrb1*), which results in higher levels of proVA carotenoid beta-carotene. The USA essentially does not have Vitamin A deficiencies, yet there are deficiencies in the macular carotenoids lutein and zeaxanthin, which are associated with visual health. Age-related macular degeneration is increasing in the USA and is a leading cause of blindness. Thus efforts are ongoing to breed for higher levels of lutein and zeaxanthin, which are naturally predominant in maize grain and accumulate in the macula of the eye thereby providing protection. We have identified SNPs in favorable alleles of carotenoid biosynthetic genes associated with higher levels of lutein and zeaxanthin. Yet presently, we are very effectively using visual, colorimetric, and HPLC-guided introgression and selection for higher levels of macular carotenoids in former plant variety protected inbreds associated with higher grain yields in F1 hybrids. In parallel, feeding high carotenoid orange maize grain to poultry increased levels of lutein and zeaxanthin in egg yolks, which should provide a highly bioavailable source of macular carotenoids to consumers. Furthermore, health benefits to poultry, such as reduction of the unsightly sores associated with footpad dermatitis, have been associated with feeding orange maize grain. The work for USA is in collaboration with NutraMaize, LLC startup company which has a mission of making maize grain more nutritious and bringing more nutritious products to the consumer.

Keywords: *Maize, macular, carotenoids, orange, poultry*

RECENT ADVANCES IN MAIZE DOUBLED HAPLOID TECHNOLOGY

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Doubled Haploid (DH) technology is used for rapid inbred line development, marker-assisted backcrossing, gene stacking, gene editing, population improvement schemes, exploitation of genetic resources, and facilitates variety protection, genetic analyses and gene identification. DH lines can be produced by in vitro and in vivo techniques. To date, in vivo maternal haploid induction is most successful in maize. Consequent screening and development in maize converted haploid induction from an obscure phenomenon to a key technology with reasonably high rate of haploids over several decades. From the first haploid inducer Stock 6 to modern inducer lines, induction rates increased with an apparent upward trend relative to the year of release. Most recently, the first maize gene involved in maternal haploid induction has been isolated. Haploid identification is still mostly done using the R1-nj kernel marker. Additional markers such as red root mediated by Pl1, as well as automated kernel sorting procedures based on high oil xenia effects have been implemented, and respective inducers developed. Use of colchicine is the established procedure for artificial genome doubling. However, genotypes with high levels of spontaneous haploid genome doubling (SHGD) ability have been identified and may overcome the need of using artificial genome doubling and transplanting, and thus increase the efficiency of DH line development substantially. Importantly, a major quantitative trait locus (QTL) affecting SHGD has been identified on maize chromosome 5. Utilization of this major QTL and more generally application of SHGD in plant breeding and in the context of genetic resource introgression will be discussed.

***Keywords**: Maize, Doubled Haploid, Haploid Induction, Haploid Genome Doubling*

IMPROVING GENOMIC PREDICTIONS WITH INBREEDING AND NON-ADDITIVE EFFECTS IN TWO ADMIXED MAIZE HYBRID POPULATIONS IN SINGLE AND MULTI-ENVIRONMENT CONTEXTS

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In maize (*Zea mays*), hybrid selection has led to the structuration of genetic diversity into complementary heterotic groups. This structuration is convenient for creating good performing hybrids but the reduced gene flow between groups increases the risk of intra-group genetic erosion associated with drift and selection, which may hinder long term breeding efficiency. It also complexifies the introduction of diversity from material such as landraces or admixed individuals derived from intergroup hybrids, that cannot be assigned to a specific heterotic group. Crossing admixed lines can generate contrasted levels of inbreeding in the hybrid population. This can reveal substantial non-additive effects, which impact performance and variation for traits showing heterosis. These non-additive effects raise specific issues for the implementation of genomic selection. Recent methodological developments modelling inbreeding and non-additive effects with the natural and orthogonal interaction approach (NOIA) have been proposed in order to increase genomic prediction accuracy in hybrid populations. Using two maize populations of hybrids admixed between dent and flint heterotic groups, we compared genomic prediction models incorporating (or not) inbreeding and non-additive effects in single and multi-environment contexts. Both populations showed a strong impact of inbreeding on plant yield, height and flowering time, which was supported by the superiority of prediction models incorporating this effect. The model including additivity, dominance, epistasis and inbreeding effects appeared to be the most robust for prediction across traits and populations and was advantageous when predicting hybrids not observed yet in any environment. Overall, comparing variance decompositions was helpful to guide model selection for genomic prediction. Finally, we recommend the use of models including inbreeding and non-additive parameters following the NOIA approach to increase prediction accuracy in admixed populations. Based on these results we will discuss the opportunities offered by genomic selection to revisit maize hybrid breeding.

Keywords: *Admixture, inbreeding, non-additive effects, genomic selection, maize*

CURRENT LIMITATIONS IN QUANTITATIVE GENETICS, AND POTENTIAL SOLUTIONS FOR ROBUST GENOMIC PREDICTION AND BIOLOGICAL INFERENCE

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In plant breeding, genomic technologies have been useful to rapidly screen populations, and predict agronomic traits from genome-wide marker data. However, the state-of-the-art methods employed in these applications typically learn patterns in genomic variability that are specific to certain populations of individuals, with little ability to extrapolate to new populations and little guarantee that learned associations have any biological meaning. In this talk, I will present a strategy to address these caveats, for more accurate predictions across plant populations and more interpretable biological inferences. My collaborators and I have pursued these goals through: (i) the detection of causal variants by evolutionary measures of fitness effects; (ii) the description of genomic variability by gene function rather than marker data; and (iii) the estimation of genetic interactions due to different populations or environments. In maize, we used comparative genomics to detect and understand the effect of DNA mutations on fitness-related traits, at higher resolution compared to traditional quantitative genetics methods. In maize and sorghum, we used haplotype information and gene expression to represent genomic variability, with more interpretable features compared to the DNA markers typically used in quantitative genetics. In maize and switchgrass, we used machine learning techniques (kernel machines or neural networks) to make predictions of agronomic traits across biological contexts (environments or populations), which are more accurate compared to the usual linear regression models. Our results motivate research to further improve quantitative genetics approaches, for precise detection of causal genetic effects, interpretable inferences about their biological basis, and robust prediction across biological contexts.

I Characterize, maintain and broaden genetic diversity

THE SEQUENCE AND GEOGRAPHICAL DISTRIBUTION OF THE MYSTERIOUS B CHROMOSOME IN MAIZE

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B chromosomes are dispensable, supernumerary and “selfish” genetic elements found in representatives of plants, animals and fungi. In a population, they are present only in some individuals. Interestingly, they do not follow rules of Mendelian inheritance and can accumulate through a process called nondisjunction. The maize B chromosome is one of the first discovered (a hundred years ago) and most studied. We combined available genomics tools to assemble its reference sequence. While it had been believed for decades that B chromosomes lack genic sequences, annotation of the maize B chromosome identified more than seven hundred genes. The analysis of the closest orthologs/paralogs of B-localized genes in the genomes of maize and *Sorghum bicolor* reveal a lack of purifying selection, rapid gene degradation, and that any potential progenitor chromosome cannot be recognized. Gene Ontology analysis indicated a prevalence of GO-terms linked to B-chromosome behavior among those over-represented on the B chromosome compared to the A-chromosome complement. Further, we developed molecular markers to detect the presence of the B chromosome in a collection of 770 landraces. This analysis revealed that the B chromosome is widespread in Americas, being found in 307 (40%) screened accessions. The abundance of the B chromosome in the analyzed set makes it an attractive target for follow-up studies to describe additional maize diversity.

Keywords: *B chromosome; reference sequence; evolution; nondisjunction; landraces*

EVA - EUROPEAN EVALUATION NETWORK HARNESSING MAIZE GENETIC DIVERSITY PRESENT IN EUROPEAN GENEBANKS

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The European Cooperative Programme for Plant Genetic Resources aims at ensuring conservation and sustainable use of plant genetic resources in Europe through collaborative activities. The ECPGR European Evaluation Network (EVA, https://www.ecpgr.cgiar.org/european-evaluation-network-eva) was established in 2019, bringing together stakeholders in public-private partnerships to characterize and evaluate crop accessions present in European genebanks, which are often poorly characterized and consequently underutilized.

One of five crop-specific networks operating at present, the EVA Maize network joins 18 partners from nine countries, including eight breeding companies, working together at a pre-competitive stage to increase knowledge about valuable traits of publicly available maize germplasm, with a view to introducing these into public and private breeding programs.

In evaluation cycles lasting three years, maize accessions are first multiplied by holding genebanks, then undergo an initial characterization using standardized protocols and data templates and are genotyped. In a second phase of the evaluation cycle, a subset of accessions is selected for in-depth evaluation and test-cross populations are created to assess the landraces in hybrid crosses.

The current project provides evaluation of up to 750 maize accessions, mostly contributed in-kind by network partners who bring their relevant expertise to the project. They generate a wealth of standardized evaluation data with a focus on biotic and abiotic stress traits as well as agronomic characteristics tested in multiple environments across the continent. EVA thus assists breeders in broadening the genetic basis and developing maize suitable for diverse agro-ecologies and able to cope with the challenges of a changing climate.

As a framework for successful public-private partnerships, EVA promotes sustainable use and provides perspectives for an improved management and valorization of maize genetic resources.

Keywords: *characterization, evaluation, EVA, maize genetic resources, public-private partnerships*

Acknowledgements: EVA Maize is supported by the Government of Germany through grant agreement GenRes2019-2. The EVA Maize network consists of partners Agroscope, Bayer, CREA-CI, CSIC, Delley DSP, INIAV, INRAE, ESA Coimbra, IPK, KWS, Lidea, Limagrain, MAS Seeds, MRIZP, RAGT, Suceava Genebank and University of Zagreb.

DIVERSITY OF NUTRITIONAL QUALITY IN IFVCNS GRAIN SORGHUM COLLECTION

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The general concern for the development of functional foods has created the need to study and use new food ingredients that would be involved in improving health condition. Sorghum (*Sorghum bicolor* L. *Moench*) gains more interest since it has a gluten-free grain and represents a rich source of nutrients and biologically active compounds. The aim of this work was to test the nutritional quality of 178 genotypes from the sorghum grain collection of the Institute of Field and Vegetable Crops (Novi Sad, Serbia) produced in the agro-ecological conditions of Serbia. The main nutritional quality parameters that characterize the biological value of sorghum grain (according to CXS172-1989 are contents of ash-max. 1.5%, protein-min. 7.0%, fat-max. 4.0%, and total tannin-max. 0.5%) were analyzed in order to select genotypes suitable for human consumption. The grain of the tested sorghum genotypes had a total oil content (determined by the Soxhlet method) in the range of 1.55 to 6.62% of dry matter, while the total protein content (determined by the Kjeldahl method) ranged from 8.67 to 19.76% of dry matter, on average. The ash content ranged from 1.17 to 3.65% of dry matter. Sorghum grain has a wide array of phenolic compounds, out of which tannins are the most widely studied phytochemical constituents as an antinutritive factor. Total tannin content, obtained using the butanol/HCl assay, ranged from 0 to 1.43%. As a result of the research, sixteen high potential sorghum genotypes were selected, in terms of its for further processing in the food industry. This screening of valuable biochemical compounds shows that sorghum has a huge potential for its exploitation and development of healthy and functional food products.

Keywords: *breeding, nutrients, sorghum*

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CONTRIBUTIONS OF LINKAGE DISEQUILIBRIUM TO THE GENETIC VARIANCE IN PLANT BREEDING POPULATIONS

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Genetic variance determines the response to selection in plant breeding. Without dominance and epistasis, the genetic variance consists of the sum of the additive variances of individual loci and twice the sum of the additive covariances due to linkage disequilibrium (LD) between loci pairs. The expectation of the contribution of LD to the genetic variance due to these covariances is zero without selection, but its variance is not. This variance can be interpreted as the risk of an inflated or deflated genetic variance due to LD.

LD between loci without physical linkage, i.e. on different chromosomes is often not accounted for when interpreting genetic variances, but due to the large number of loci pairs on different chromosomes their sum can contribute significantly to the risk of LD contributing to the genetic variance.

In our simulation study based on genotypic data from published maize breeding experiments we generate synthetic populations with (1) source populations developed from elite breeding lines vs. from DH-lines from a landrace, (2) different numbers of parents sampled from the source populations, (3) different population sizes for subsequent intermating generations, and (4) different numbers of intermating generations. We show how these factors influence the LD between loci on the same and on different chromosomes contributing to the genetic variance. Our results suggest that for breeding populations with few large families one additional recombination step is beneficial for avoiding a potentially large contribution of LD between loci on different chromosomes to the genetic variance.

Keywords: *genetic variance, linkage disequilibrium, population structure, synthetic population*

THEORETICAL AND EXPERIMENTAL ASSESSMENT OF GENOME-BASED PREDICTION IN LANDRACES

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Genetic variation is the basis of selection and germplasm improvement. Most of the genetic variation in crop species rests in landraces that were formed by farmers prior to the beginning of systematic breeding. Given future challenges of agriculture, the targeted utilization of this rich reservoir of diversity is considered essential for securing sustainable crop production. However, knowledge-based strategies for introducing landrace material into elite populations are lacking.

Genome-based selection can accelerate the enrichment of favorable alleles in landraces, but its implementation still needs to be developed due to strongly reduced levels of relatedness compared to elite germplasm and the heterozygous and heterogeneous nature of landraces. We present two fundamentally different approaches for model training in genome-based prediction: i) the “pure” approach, deriving doubled-haploid (DH) lines directly from landraces; and ii) the “admixed” approach capturing gametes of the landraces in a cross with an inbred (capture) line. We developed a theoretical framework to translate molecular information on landraces and the capture line into expected genetic variances and to comparatively predict the success of genome-based selection in the two types of populations.

We validated theoretical considerations with extensive experimental data, comprising 873 DH and 544 gamete capture (GC) lines derived from two maize landraces. Our results demonstrate clear advantages of the DH over the GC approach, with generally higher prediction accuracies and without masking the effects of valuable landrace alleles. With prediction accuracies of 0.58 for dry matter yield, genome-based selection in landrace-derived DH populations is promising. For the GC approach, we show that the success of genome-based selection strongly depends on the choice of capture line.

Our findings allow an informed choice of landraces and their crossing partners and contribute towards the optimization of genome-enabled pre-breeding schemes in plant genetic resources.

Keywords: *landraces, genomic selection, doubled-haploids, gamete capture, genetic variation*

PROMAIS, FIFTY FIVE YEARS OF COLLABORATIVE RESEARCH WITH INRAE

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Promaïs is a French non-profit organization created in 1967 by the Seed industry with the objective to promote maize crop development and maize research through a tight partnership with INRAE. The talk will explain the context at the time of this creation and how this organization and this partnership was built and for which purposes. It will describe shortly how it is operating and will review the main scientific projects and domains it had been focusing on during these decades. Few examples of major achievement will be illustrated. Currently it is being discussed, among partners, on what are the domain that looks of the highest interest to be addressed through such private public partnership projects for the next decade. I will share the status of this process and will invite the room to react with comments and/or ideas - proposals.

PRESENCE/ABSENCE VARIATION CONTRIBUTES TO ADAPTATION, AGRONOMIC TRAITS VARIATION AND HYBRID PERFORMANCES IN MAIZE

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Structural variations (SV) are pervasive in plants. Maize displays a huge number of SVs, including Insertion/Deletion (InDel) or Presence/Absence variation (PAV) that lead to differences in gene content between individuals. These structural polymorphisms may contribute to trait variation, adaptation and heterosis by changing and complementing the gene/regulatory regions between individuals. To analyze the effect of InDels/PAVs on trait variation and adaptation, we genotyped 362 inbred lines representing a broad range of diversity with (i) a new Affymetrix® Axiom® array of 105,947 InDels ranging from 35bp to 129,7Kbp and (ii) the 600K SNPs Affymetrix Axiom array and by Genotyping by Sequencing to reach a number of 1 million SNPs. Half of InDels were not in strong linkage disequilibrium ($r2>0.8$) with any nearby SNPs. Accordingly, Genome wide association studies (GWAS) on 23 agronomic traits identified 13 new quantative trait loci (QTLs) with InDels as compared to 281 detected by SNPs. Genomic regions under selection between tropical, dent and flint lines were enriched in InDels polymorphisms with 56 out 188 genomic regions detected. These InDels/PAVs targeted genes involved in tolerance to biotic and abiotic stress and/or adaptive traits as flowering time. To study the effect of InDels on hybrid performance, we derived 287 hybrids by crossing 210 maize temperate lines from the previous panel and observed their female flowering, plant height and grain yield (GY) in four different environments. We performed GWAS with InDels/PAVs and SNPs and detected 78 and 133 QTLs with respectively an additive or dominance effect, respectively. Six additive and 11 dominant QTLs were exclusively detected by InDels, including one QTL for GY targeting a large cluster of InDels/PAVs on chromosome 6. However, Integrating InDels/PAVs information to estimate kinship to predict hybrid performance using an additive and dominant model did not increase predictive ability.

Keywords: *Structural Variation, GWAS, Genomic prediction, Heterosis, genotyping array*

MAINTAINING AND BROADENING THE GENETIC DIVERSITY OF SORGHUM THROUGH NOVEL POLLINATION CONTROL BAGS

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Controlling the gene-flow among accessions is a basic requirement of conserving, maintaining and broadening genetic diversity. Sorghum is predominantly self-pollinated crop but an average outcrossing rate of about 6%; ranging from 5% to 15%, means pollination control is needed to conserve and maintain diversity as intended. Two studies in sorghum demonstrate the higher efficiency of novel pollination control bags (PCBs) made of nonwoven fabrics compared to the commonly used kraft paper, parchment paper or plastic bags in controlled gene-flow systems, as measured by greater panicle weight, seed weight, seed yield and mould rates. First, a two-year EMBRAPA (Brazil) study on winter sorghum compared novel materials SG1 polyester and SG2 polypropylene PCBs to standard paper bags. In 2015, the paper bags suffered greatest bird damage while SG1 and SG2 suffered none. SG1 followed closely by SG2 showed increased panicle weight by 25%, seed weight by 27% and average seed weight per panicle by 34%. In 2016, the novel bags (SG1, SG2) exhibited 195-652% greater seed yields to Kraft paper bags. Second, ICRISAT (India) compared rainy season sorghum breeding for two years using three nonwoven bags (SG1, SG2 and SG3) and standard paper bags. No bird damage was observed in the nonwoven fabrics PCB unlike the high damage in paper bags. The mould infection was the highest in the paper bags and the lowest in SG1 bags. The SG1 bags showed significantly higher 100-seed weight than either the paper bags or no bagging. Both studies established that novel nonwoven PCBs can provide better results than the paper bags regarding pollen proofing, protection against birds, and beneficial micro-environment for output of high yields of healthy seeds. These novel bags offer new options for maintaining the genetic identity of landraces and also for broadening the genetic diversity through better-controlled outcrossing for sorghum breeders' manoeuvres.

Keywords: *Genetic diversity, maintenance, selfing, crossing, pollination control bags, nonwoven fabrics*

IDENTIFICATION OF NEW SOURCES FOR EARLINESS AND LOW GRAIN MOISTURE AT HARVEST THROUGH MAIZE LANDRACES *TEST-CROSS* PERFORMANCE

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Early maturity is very important factor in the yield, grain moisture and plant density game, contributing to cost-efficient maize production. Landraces conserved in gene banks present a promising basis to enrich diversity of early maize breeding material. To start and speed-up the mobilization of currently scattered, unknown and underused maize genetic resources maintained in *ex situ* Maize Research Institute Zemun Polje gene bank collection, 63 landraces, as new potential sources of earliness and low grain moisture at harvest, were selected for evaluation of their *test-cross* performance with two divergent early testers. The majority of landraces (29), with prevailed flint type, exhibited heterosis for yield in crossings with BSSS-ID tester (102NS), where 20 *top-crosses* expressed grain moisture below defined threshold value (21.1%). The best performing landraces can be used as a starting-point of new pre-breeding programme for flint maize breeding material broadening. In parallel, nine landraces expressed simultaneous heterosis in crossings with flint tester (14NS), exhibiting grain moisture above threshold value. Simultaneous heterotic effect with two divergent inbred testers implied to the existence of an independent heterotic pool. These findings will contribute to broadening of maize breeding material for earliness and low grain moisture at harvest as important goals in maize breeding.

Keywords: *genetic diversity, grain yield, heterotic pattern, pre-breeding, Zea mays L.*

CREA MAIZE GENEBANK: GERMPLASM ENRICHMENT AND INNOVATION THROUGH ITALY – BOLIVIA COOPERATION PROJECT

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Italian maize germplasm is one of the largest for original inputs and differentiation of local forms. Our country is in fact characterized by many different climatic and orographic environments, which produced a wide set of landraces, showing flint or semi-flint kernels, a higher flour yield and being particularly adapted to food production.

The Genebank of the CREA Research Centre for Cereal and Industrial Crops in Bergamo (Italy) contains the largest maize collection in Italy and one of the richest in Europe: more than 5,700 accessions are maintained *ex-situ* at 7°C and regenerated in the fields.

In the framework of the different national and international research programs, CREA takes care of the conservation, use and valorisation of maize genetic resources from Italy and other countries.

In the frame of the cooperative Project between Italy and Bolivia “P.S.G.O. Bolivia km 0”, funded by the Italian Agency for Cooperation and Development, innovative maize pigmented genotypes were developed. Some Italian maize local varieties stored at the CREA Genebank (Bergamo) were crossed with germplasm of Bolivian type “Morado” and Mexico type ‘Azul’ at CREA Experimental Farm, Bergamo (45°68’N; 9°64’E) and selected during cycles from 2015 to 2021. The materials derived from field trials were manually harvested at the end of each season, ears were dried at 40°C for 7 days up to 14 % relative humidity; then were shelled and kernels stored a 4°C until research analyses. Two pigmented landraces, violet and blue, rich in anthocyanins and functional molecules with antioxidant properties were already described (Suriano et al., 2021). A set of new Italian maize pigmented genotypes developed in this research will be presented and described; the materials enriched in bioactive compounds represent genetic resources suitable to be introduced into advanced breeding programs aimed to enhance and valorize maize biodiversity potential.

Keywords: *pigmented maize, germplasm, valorisation, international cooperation*

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POPULATION STRUCTURE AND DIVERSITY IN FORMER ZP MAIZE BREEDING PROGRAM ASSESSED WITH SNP MARKERS

Nikola Grčić*, Ana Nikolić, Nenad Delić, Marko Mladenović, Zoran Čamdžija, Sofija Božinović, Snežana Mladenović Drinić

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Maize breeding in Maize research institute “Zemun Polje” was conducted for more than 75 years resulting in the creations of numerous maize hybrids with superior traits and performance. The source breeding material used in the breeding programs was very diverse and varied from the domestic landraces to the introduced inbred lines. This resulted in a very diverse and complex structure of the breeding pools used in commercial maize breeding in “Zemun Polje” Institute. As genetic material in this study, 24 maize inbred lines of different origin, heterotic and maturity goups were selected were selected from the 3rd and 4th cycle of breeding in Maize research institute “Zemun Polje”. These are inbreds that were commonly used in breeding programs during the 1980s and were parental components of most important commercial maize hybrids. The genotyping was performed using a Maize 25k Infinium array. The neighbor-joining cladogram, PcoA and Structure were used to analyze the genetic structure and diversity of the panel. Population structure analysis of the panel initially showed a clear divide between Lancaster and Non-Lancaster inbred lines, revealing the dominant heterotic pattern in the breeding program. Further analysis with an increased number of population (k) showed a more complex structure of the non-Lancaster group of inbred lines with best number of ancestral population being k=5. The average genetic distance in the panel was 0,385, ranging from 0,039 to 0,544, with paternal inbreds in most of commercial hybrids being the most distant ones. The genotyping results of the Zemun Polje maize panel were further merged with DROPS European panel of 247 dent maize inbred lines in order to view the results of the diversity analysis of the Zemun Polje panel from a broader perspective.

Keywords: *maize breeding, inbred lines, genotyping, population structure, diversity*

RECIPROCAL EFFECT ON GRAIN YIELD AND YIELD COMPONENTS IN SINGLE-CROSS MAIZE HYBRIDS

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Reciprocal effect in maize refers to the phenotypic difference between reciprocal F1 hybrids. The aim of this study was to estimate influence of reciprocal crosses on grain yield and eight yield components. Five single-cross hybrids, their reciprocal crosses and six parental inbred lines were selected and all hybrids belong to late maturity group (FAO 500-600) with Lancaster ZPL-7 line as mutual parent. The experiment was set up in two replications using a completely-randomized block design in 2016 and 2017, at a total of seven environments. Reciprocal effect significantly influenced only grain yield and ear length, while factors hybrid and location were significant for all the examined traits. All hybrids individually displayed significant reciprocal effect for grain yield, ear length, number of kernels per row and 100 kernel weight, two hybrids for kernel length and thickness, while three hybrids showed reciprocal effect on kernel width. The effects were both positive and negative depending on the genotype itself. Furthermore, there were no significant differences for ear row number and ear width between normal and reciprocal variants. The highest difference for grain yield was between ZP 606 reciprocal (12.06 t/ha) and ZP 606 normal (11.28 t/ha). Pearson correlations were calculated between examined traits. All correlations between grain yield and other yield components were found significant and positive, except for the number of rows per ear (no correlation) and kernel width (negative correlation). Reciprocal effect has strong influence on the measured yield traits, but it is genotype specific. Therefore, in the future we should examine normal and reciprocal variants of all commercial maize hybrids.

Keywords: *grain yield, reciprocal effect, single cross, Zea mays L.*

GENETIC DIVERSITY WITHIN A COLLECTION OF ITALIAN MAIZE INBRED LINES: A RESOURCE FOR MAIZE GENOMICS AND BREEDING

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Knowledge of genetic diversity in maize lines is key information for breeders to decide which sources of diversity to integrate in their specific programs; favourable alleles for traits of interest can be identified through association mapping in such collections. A panel of 634 maize genotypes, preserved at CREA Bergamo Genebank, (603 inbred lines derived from traditional Italian varieties and 31 Elite Inbred Lines) was analysed. A subset of 384 lines was subject to extensive SNP search through the tGBS® Genotyping by Sequencing technology. A set of 797,368 SNP sites was initially produced; following an imputation and filtering process based on percentage of missing data, redundant markers and frequency of the rarest allele, a final dataset of 15,872 SNP markers physically mapped was defined for further analyses. Linkage disequilibrium (LD) decay evaluated at genome wide level was of 52.4 kb for r^2 0.1, indicating the collection as a valuable resource for genome wide association mapping. The pattern of LD was depending on chromosomal regions and showed some variations across the ten maize chromosomes. The population structure analysis, carried out with *ADMIXTURE*, was performed on a set of non-redundant markers following a pruning with r^2 thresholds of 0.8 and 0.5, and showed that grouping statistics stabilized at K > 4, with differentiation between inbred lines derived from the same cultivar. A prevalence of Nostrano dell'Isola and Isola Basso lines was observed in the groups 1 and 4, while the majority of elite and other lines derived from American breeding genepool were present in group 3. The genotypic information allowed us to characterise the panel for genetic diversity. Based on these data, the CREA Italian maize collection once genetically characterized represents an important tool for the identification and study of useful traits/alleles and for their use in maize breeding.

Keywords: *maize, germplasm collection, genetic diversity*

CHARACTERIZATION OF CHEMICAL AND BIOACTIVE PROPERTIES OF THE GRAIN OF NEW MAIZE INBRED LINES

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The objective of this study was to early identify genotypes with increased potential for the development of hybrids with high nutritional and functional value, suitable for food production, by assessing the grain quality parameters of eleven new maize inbred lines. The inbreds, including nine standard yellow and two red kernel lines, were grown in the experimental field of the Maize Research Institute at the location of Zemun Polje, Serbia in 2020. Wholegrain maize flour was obtained by a dry grind process on a laboratory mill. The assessment of the chemical composition and content of certain bioactive compounds, as well as total antioxidant capacity, was conducted using standard laboratory procedures. The highest starch content (73.73%), was determined in Line 8, while Line 10 had the highest protein content (12.82%). The lowest oil content was determined in red kernel Line 7, namely 4.87%. Among soluble proteins, the α-zein fraction was dominant in most of the lines, ranging from 0.92% to 3.57%. The glutelin and albumin fraction were present in a lower percentage, followed by the globulin fraction. The highest content of total fibers (NDF) was determined in red kernel Line 9 (15.77%). Line 8 was the richest in total carotenoids (21.08 µg βCE/g d.m.), while Line 7 had the highest total antioxidant capacity (34.30 mmol Trolox/kg d.m.), which can be explained by the presence of anthocyanins in the red grain. Line 1 had the highest content of total sugars (3.36%), and Line 4 had the lowest (1.44%). All samples of new inbred lines investigated in this study showed good quality parameters regarding chemical composition and bioactive properties. The obtained results may provide some valuable guidelines needed in the following stages of maize breeding as well as open up various possibilities for the utilization of wholegrain maize flour in the food industry.

Keywords: *maize, inbred lines, grain properties, wholegrain flour, food.*

SUITABILITY OF MAIZE HYBRIDS FOR THE ENRICHMENT OF MARINATED BABY CORN WITH ANTHOCYANINS EXTRACTED FROM BLACK SOYBEAN

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Maize (*Zea mays* L.) is the third most grown cereal crop in the world, after wheat and rice. Immature dehusked maize ears harvested before fertilization, within 2-4 days after white silk emergence are classified as baby corn. The objective of this study was to investigate the suitability of baby corn cobs obtained from different maize hybrids for their enrichment with anthocyanins extracted from the black soybean seed coats. Three different maize hybrids, namely, a popcorn hybrid ZP 6119k, a sweet hybrid ZP 553su, a semi-flint with a short vegetation period (105 days) ZP 161, as well as black soybean genotype “Black Tokyo”, were used in this study. Plant material was grown in the experimental field of the Maize Research Institute at the location of Zemun Polje, Serbia in 2021. Maize was harvested after the emergence of silk from the ears (silking stage), while soybean plants were harvested in the stage of full maturity (R8). The baby maize cobs differed in length (9.5-20.0 cm) and girth (12-21 mm), the number of kernel rows (12-20), color, and length of the silk outside the husk (3-7 cm). Soybean seeds were manually dehulled and seed coat was ground in a laboratory mill prior to the extraction of anthocyanins. After dehusking and measuring, the baby corn cobs were washed and blanched for two minutes in boiling water, and then cooled. Subsequently, the baby corn cobs were immersed in a marinating brine enriched with anthocyanins extracted from the seed coat of black soybean, pasteurized, and kept in closed glass jars in a cool dry place for 30 days. After chemical and sensory analyzes, the product obtained from popcorn hybrid ZP 6119k was chosen as the most suitable for the preparation of the marinated baby corn product enriched with anthocyanins from black soybean.

Keywords: *maize hybrids, baby corn, anthocyanins, black soybean.*

MOLECULAR VARIABILITY OF MAIZE LANDRACES ESTIMATED BY SSR MARKERS

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In order to provide continuous progress in maize breeding, it is necessary to expand the genetic base of breeding working collections by introgression of new germplasm into existing heterotic groups. Landraces can be valuable donors of desirable traits despite a large performance gap compared to current commercial varieties. Information on the heterotic pattern of landraces necessary for decision on their incorporation into pre-breeding and breeding programmes, have been made. Molecular characterisation of landraces can provide important information on their diversity before evaluation of their combining ability through crosses to divergent testers. In this study, molecular characterisation of 31 landraces and five divergent testers was performed using 29 polymorphic Simple Sequence Repeat (SSR) markers, in order to examine their genetic divergence. According to cluster analysis, landraces were grouped into four clearly defined groups and one separate branch. The MB1509 landrace differed significantly from all other landraces and testers used. The testers were grouped according to known information on their pedigree. The correspondence analysis pointed out the complexity of mutual relations of landraces and testers. Based on the results obtained by molecular markers, it can be, with high reliability, concluded with which testers the landraces will not give heterosis, making field research more effective (by reducing the number of crosses). The results indicated that the variability of landraces is significant, and that belonging to a certain heterotic group enables the correct selection of the initial material for pre-breeding and their use in breeding programmes.

Keywords: *genetic divergence, SSR markers, landraces,heterotic groups, Zea mays* L.

POTENTIAL AND ADVANTAGES OF COLORED MAIZE HYBRIDS APPLICATION IN BAKERY PRODUCTS

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Apart from its significant influence on the technological properties of bread, pigmented maize has gained newfound attention from a nutraceutical perspective due to its potential health benefits. The present study was carried out to demonstrate the effects of different maize flour on the physical, textural and sensory properties of composite breads. Chemical characterization of composite flours made with four different types of color maize was assessed. Furthermore, the content of total phenolics, flavonoids, anthocyanins, in composite flours was determined. Composite flour with blue maize had a higher total phenolic content (1380.07 mg GAE/kg d.m.) than those with yellow and red maize (1037.28 and 1129.45 mg GAE/kg d.m., respectively) as well as with light blue maize (976.45 mg GAE/kg d.m.). The total flavonoid content did not vary greatly among composite flour samples. Results demonstrated a maximum anthocyanin level in the blue maize composite flour (286.79 mg CGE/kg d.m.), which was higher by approximately 70% and 38% than the level measured in the light blue and red maize composite flours, respectively. In the yellow maize composite flour anthocyanins were not detected. The data for the physical and textural analysis of composite bread reveal that the loaf volume of the colored maize composite bread samples ranged from 182.50ml to 188.01ml. Red maize composite bread showed the lowest crumb hardness, the highest loaf volume and specific volume as well as the highest springiness value. Maize composite bread loaves had a typical and pleasant odor and taste, and the aroma was very intensive. Results obtained might be beneficial to further study how ingredient proportion, improvers or processing conditions affect the textural and sensory attributes of the final product from composite maize flour.

Keywords: *coloured maize, bread, textural properties, sensory properties.*

ENCAPSULATION OF ANTHOCYANINS FROM BLUE MAIZE IN ORDER TO CREATE FUNCTIONAL CARRIERS WITH APPLICATION IN THE FOOD AND PHARMACEUTICAL INDUSTRY

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Colored grains are rich sources of anthocyanins that could play an important role in the prevention of various diseases associated with oxidative stress. The food and pharmaceutical industry are aiming to exploit the novel sources of valuable bioactove compounds and their increased utilization is one of a researches goal. Anthocyanins-rich colored grains could be used as a functional food ingredient that provides health benefit to a large part of human world's population.

The aim of the present research was to develop and examine microencapsulation systems of blue maize flour using conventional wall material (biopolymers) as maltodextrin (MD), in combination with novel one, hydroxypropyl-β-cyclodextrin (HPCD), in order to obtain powder with appropriate functional, organoleptic and pharmacological characteristics, uniform in size, which could be applied in the food and pharmaceutical industry. Waste product of blue maize processing was used for anthocyanins extraction. Liquid blue maize extract was spray dried with and without adding carrier agents: MD (30%), HPCD (30 %) and combination of both carriers (15% MD and 15% HPCD). The obtained samples were analyzed for the powder property parameters (powder yield, moisture content, rehydration time, powder densities, flowability, cohesiveness, pH and color) and for the content of phenolic compounds.

Powder bulk and tapped densities are important parameters which determine the quality of final product in pharmaceutical process. Bulk density values were in the range from 0.23 to 0.33 g/ml and tapped densities from 0.37 to 0.55 g/ml, demonstrating good powder properties. Rehydration properties of blue maize microencapsulated powders was in accordance with results of their flowability and cohesiveness. Powder obtained with MD was characterized with significantly longer rehydration time athough in combination with HPCD rehydration time was half time shortened. The average content of anthocyanins and total phelolic compounds in microincapsulates was high, 10.677 µg CGE/g and 31.380 µg CE/g, respectively.

Keywords: *Blue maize, anthocyanins, encapsulation, spray drying, functional carrier*

II Environmental adaptation to face climate change and new agricultural constraints

circRNAs INVOLVED IN CHILLING STRESS RESPONSE DURING EARLY DEVELOPMENTAL STAGES IN MAIZE

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Circular RNAs (circRNAs) are endogenous, single-stranded, covalently closed RNA molecules, generated by back-splicing events. Their role as regulators of different processes in animal cells has been widely studied and proven, however functional studies on circRNAs in plants are still few and far between. Here we attempt to bring light to their role in abiotic stress response – particularly chilling stress, in young maize seedlings (*Zea mays* L.) using high-throughput sequencing methods. One of the strategies of avoiding yield loss is earlier maize sowing as to escape exposure to extremely high temperatures and drought in the summer. This means submitting maize plants to suboptimal temperatures during earlier developmental stages.

Two maize inbred lines of contrasting tolerance to chilling were selected and marked as LT (tolerant), and LS (sensitive). Sterilized seeds of the two lines were germinated for five days, in the dark, in optimal conditions and after the germination period was finished, the 5-d old seedlings were exposed to chilling conditions for 24h (temperature: 10/8° C; photoperiod: 12/12h). Sampling was done after 6h and 24h of treatment. After total RNA isolation and purification, quality check, and cDNA library preparations were finished, paired-end 150 sequencing was performed (Illumina® Novaseq 6000). Custom pipeline was used for circRNAseq data analysis and included their identification and quantification (CIRCexplorer2, v2.0.5), differential expression analysis (edgeR Bioconductor v4.1) and functional enrichment (GO, KEGG, psRobot).

Processing of this data is still underway, and by now several thousand circRNA molecules and over a thousand 5' and 3' alternative back-splicing sites have been identified. Significant number of those circRNA have shown expression dependent on chilling, as well as genotype, but further studies are necessary to confirm their role in this process, find their target molecules and unravel regulatory networks they create with other types of RNAs.

Keywords: *circRNAs, chilling, abiotic stress, RNAseq*

FORTIFYING AND ENHANCING RESILIENCE IN C4 CROPS FOR CURRENT AND FUTURE CLIMATE CHANGE ADVERSITIES

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Global climate change, towards higher temperature, greater aridity and more frequent erratic climate events, become a major threat to agricultural sustainability and food security, particularly in the Mediterranean regions but also in central and northern Europe. Crops with a C4 photosynthetic pathway will be favoured over C3 crops. These plants are seeing strong resurgence in Europe for human food and animal feed production purposes. Hence, enhancing crop resilience to climate change is a major challenge facing the global agricultural community. This is the purpose of C4FUTURE (Fortifying and Enhancing Resilience in C4 Crops for Current and Future Climate Change Adversities), a SusCrop ERA-Net Cofound Action in the field of Sustainable Crop Production. C4FUTURE exploits the natural variation in maize and sorghum to enhance adaptation to nitrogen and water stresses, considered as the most important limitations to yield stability. First, mineral nitrogen fertilization is used to increase crop yield, but excessive concentrations of nitrate are detrimental to the environment and the human health. Second, as already mentioned, the impact of climate change on crop yield is a reality in Europe and drought is the main driver of losses for cereal production. A special emphasis is on the optimization of the root system architecture to enhance the plant capacity for soil exploration and, thus, nutrient and water acquisition. The consortium will identify crop characteristics and breeding technologies to sustain cereal productivity and value chains in Europe.

Keyword: *Climate change, C4 cereals, Natural variation, Resource Use Efficiency, Root system architecture.*

DROUGHT RESPONSE QTLs DETECTED ON PHENOTYPIC RATIOS CONTRIBUTE TO THE GENOTYPE X ENVIRONMENT INTERACTION

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Drought stress can considerably affect maize yields, which is a source of concern for maintaining global food security in the coming years. Identification of quantitative traits loci (QTLs) involved in the genotype x environment interaction (GxE) is a way to increase maize resilience to drought through marker-assisted breeding or candidate gene identification. However, these QTLs generally have low effects, which make them difficult to detect. We hypothesized that computing ratios from phenotypic values measured in two contrasted watering conditions would help in identifying QTLs involved in GxE interaction that would not be detected when considering each condition separately. To test this hypothesis, we used previously published data containing phenotypic measurements for six drought-responsive ecophysiological traits acquired on 254 maize hybrids grown under two watering conditions, well-watered (WW) and water deficit (WD), in four greenhouse experiments (Prado et al., 2018). We conducted a genome wide association study on the trait values obtained in each watering condition as well as on the WD/WW ratios, taking into account an effect of the experiment. A total of 140 QTLs were detected, 102 (73%) were detected in one of the two watering conditions and 38 (27%) were detected only on the ratios. We quantified the contribution of the detected QTLs to the variations of the traits by fitting statistical mixed models including fixed effects of the QTLs and random effects of the genotype, genotype x condition interaction and genotype x experiment interaction. This showed that the QTLs detected in each watering condition mostly contributed the genotype variation (39%-65% of the variance captured depending on the trait) while the QTLs detected only for the ratios mainly contributed to the genotype x condition interaction (69%-100% of the variance captured). Altogether, these results show that ratio-specific QTLs are complementary to the QTLs detected in each condition separately and are worth being considered to explain the GxE interaction.

Keywords: *maize, drought response, QTLs detection, genotype x environment, ratio*

DECONSTRUCTING MALADAPTATION TO MINE DIVERSITY IN MAIZE

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Exotic crop varieties harbor unique climate adaptations, but phenotypic plasticity and genetic complexity impede their incorporation across geographical zones. When exotic material is grown outside its native environment, estimating genetic merit for most traits is confounded by extreme flowering times due to maladaptation, requiring long periods of breeding for preadaptation before alleles that are worth using can be identified. We used experimental evolution in maize across a 3100 km (28 degree) latitudinal transect to understand how extreme flowering time is shaped by the intersection of genetics, environment, and selection. Selected lineages from eight locations were evaluated in a common garden experiment across the original locations where selection took place. In addition, parental haplotype maps were constructed for 12,000 samples and used to dissect the genetic architecture underlying phenotypic change across latitude and generations. We detect different degrees of parallelism at phenotypic and genetic scales, with evidence for genetic heterogeneity and locally adaptive alleles that have negligible effects in other environments. On some chromosomes, unfavorable recombination-selection dynamics are found that constrain genetic progress. These findings are being used to develop genomics-assisted strategies to accelerate breeding for the preadaptation.

Keywords: *adaptation, experimental evolution, flowering time, extreme mapping, genomic selection*

THE RESPONSE OF MAIZE LINES TO FOLIAR HERBICIDES

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Herbicide application and weed control in seed maize production requires special attention due to maize lines larger sensitivity to herbicides compared with hybrid maize. The introduction of foliarly applied herbicides on the market resulted in new possibilities for weed control, while causing problems more than ever to sensitive maize lines. The aim of study was to examine and define the sensitivity/tolerance of the five maize inbred lines (belonging to different heterotic groups), based on visual estimation of injury, morphological response (height, weight, and biomass), as well as in alterations of secondary metabolites from maize leaves (phenolics, protein sulfhydryl groups, phytic and inorganic phosphorus). Two herbicide groups were applied in the study: triketons (mesotrione and topramezone) and sulfonylureas (rimsulfuron and foramsulfuron). Lines from independent heterotic group, which were sensitive to herbicides, expressed higher visible damages together with significant reduction of grain yield, biomass, weight and leaf area reduction, mainly induced by sulfonylurea herbicides. Parallel with that, significant increase in phenolics, phytic and inorganic phosphorus, and drop in protein sulfhydryl groups were observed in their leaves. Tolerant lines (belonging to Lancaster group) had mainly insignificant grain yield reduce, also no reduction of measured morphological parameters, together with lesser variations in sulfhydryl groups, content of phytic and inorganic phosphorus, as well as increase in phenolics content. Among examined secondary metabolites, phytate was the main factor, contributing to herbicide tolerance in maize lines. Owing to lesser yield decrease and variation in content of examined secondary metabolites, expressed in treatments with triketone herbicides, their usage could be safe in maize lines. The obtained data suggest that mesotrione can be applied in seed maize for weed control, while with sulfonylurea herbicides, application must follow guidelines and instructions obtained by field and greenhouse monitoring.

Keywords: *corn, herbicides, tolerance, susceptibility*

NON-PARAMETRIC YIELD STABILITY ANALYSIS OF ZP MAIZE HYBRIDS IN SERBIA

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High and stabile yield in different production environments is priority in maize breeding. New statistical methods are constantly being sought to accompany analysis of variance, in order to achieve more reliable hybrid assessment. In this study non-parametric stability analysis is applied in order to assess GxE interaction for yield of 36 commercial maize hybrids. The experiment was set up at five locations in Serbia for three years according to the RCBD design in three replications. Yield stability of investigated genotypes was analysed by stability parameters $Si^{(1)}$, $Si^{(2)}$, $Si^{(3)}$, $Si^{(6)}$, TOP and RS. Analysis of variance identified highly significant F values for all experimental factors. Bredenkamp method confirmed the existence of non-crossover GxE interaction, for maize yield. Hybrid ZPH15 achieved the most stable yield based on parameters $Si^{(1)}$ and $Si^{(2)}$. According to parameter $Si^{(3)}$ it was ZPH5, while based on parameter $Si^{(6)}$ it was ZPH34. The highest overall yield achieved ZPH36 (11.18 t/ha), which was quite unstable (rank 24 in parameters $Si^{(1)}$ and $Si^{(2)}$), and very unstable (rank 34 in parameters $Si^{(3)}$ and $Si^{(6)}$). The most stable hybrids had average yields. In total, the hybrid ZPH23 had the best average rank (15.93). Based on TOP parameters, ZPH36 had the best rank (yield), followed by ZPH11, ZPH20, ZPH21 and ZPH9. However, RS parameter, revealed that ZPH21 was the most stable hybrid, so taking into account both TOP and RS parameters this is the most productive and the most stable hybrid. Based on this research, TOP and RS are the best parameters for selecting new maize hybrids for production in particular environment. In case of identical TOP value, the genotype with the lowest RS value should be selected. The parameters $Si^{(1)}$, $Si^{(2)}$, $Si^{(3)}$ and $Si^{(6)}$ can be used as alternative methods for the selection of genotypes with moderate yield and high stability.

Keywords: *maize, yield, GxE interaction, stability, non-parametric analysis*

MORPHOMETRIC AND COLOR ANALYSIS OF PLANTS IN RESPONSES TO DROUGHT USING HIGH-THROUGHPUT PHENOTYPING

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Water deficit is one of the most significant abiotic constraints decreasing crop productivity worldwide. Automated phenotyping has ambitions to contribute to the screening of crop genetic resources towards a higher water use efficiency and drought resistance of crops. The PlantScreen^{TM} phenotyping platform was applied to study the growth dynamics of different genotypes of sorghum in response to drought. Calculated morphometric parameters from RGB imaging in top and side view (with different angles) positions (such as plant area, growth height, relative growth rate, plant perimeter, compactness), as well color segmentation and greening index were used to discriminate between genotypes with different drought tolerance. The study was completed by measurements of leaf RWC and osmotic potential, the concentration of chlorophylls (spectrophotometrically and SPAD-index), and the calculation of water use efficiency. Detailed morphometric analysis showed that sensitive genotypes of both crops respond to drought by rapid growth decline, stress-induced senescence, and leaf chlorophyll breakdown. Colour segmentation on leaf area has been used to calculate the greening index via the user-defined algorithm clusters hues of green and chlorotic areas on leaves. This technique allowed us to show the dynamics of greening during plant ontogeny in well-hydrated conditions, as well as the effect of drought on the start of senescence of assimilation organs. Calibration of the selected scale of greens with the spectrophotometrically determined concentration of chlorophylls (and SPAD-index) allows the indirect evaluation of assimilatory pigments from RGB images. Therefore, the RGB imaging became an important tool in high-throughput plant phenotyping, and the final morphometric analysis can be reliably used to assess growth dynamics and plant fitness under stress situations.

Keywords: *High-throughput phenotyping, RGB imaging, greening index, sorghum, soybean, drought*

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INBRED SELECTION FOR INCREASED RESISTANCE TO KERNEL CONTAMINATION WITH FUMONISINS

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A pedigree selection program was performed with the aim of obtaining inbred lines with resistance to kernel contamination with fumonisins. Selection was done for reduced Fusarium ear rot (FER) because, in previous studies, correlation coefficients between damage severity and fumonisin content were moderate to high. F_2 populations were obtained from crosses EP31 x EP39 and F575 x EP65 (involving inbreds from the European heterotic group), B93 x Oh43 and A670 x H95 (from Lancaster group), and A630 x A635 and A654 x A666 (from Reid group). Plant selection was performed among F_2 plants and family and plant within family selection was carried out in three subsequent self-crossing generations.
Selection was done under kernel inoculation of the self-crossed ears. Ears were inoculated 7 days after self-pollination with a suspension of spores (10^6 spores/ml) of a toxigenic strain of *F. verticillioides*. At harvest, FER was estimated according to a scale of 1 (low) to 7 (high). The resulting 29 second-cycle inbred lines and their parental inbreds were evaluated in 2019 for FER and the two most promising second-cycle inbreds from each F_2 and the parental inbreds were analyzed for fumonisin content. In 2021, the evaluation of selected second-cycle inbreds and their parentals was repeated. Twenty-seven crosses among second-cycle inbred lines and four commercial hybrids were tested in an adjacent trial.
Preliminary results show that, in general, second-cycle inbred lines tended to improve resistance to FER and fumonisin contamination compared to their parental inbreds, although differences were not significant except for inbreds derived from F575 x EP65, that showed significantly ($p < 0.05$) less kernel fumonisin content than any of the parental lines. In addition, some crosses of these inbreds presented low values for FER, and grain yields comparable to those shown by commercial hybrids.

Keywords: *Fusarium ear rot, fumonisins, pedigree selection*

THE IMPACT OF CROP DENSITY ON GRAIN FILLING AND WATER RETENTION IN MAIZE GRAINS

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Sowing density affects not only crop growth, but also grain filling, including grain dry-down. Maize hybrids with upper-standing leaves allow to be grown in higher densities, what could affect some traits during ripening. An experiment with six maize hybrids (ZP388, ZP5550, ZP5601, ZP6263, ZP6364, ZP707), grown at 59,523 (D1) and 89,286 plants ha^{-1} (D2), during 2019 and 2020 was established. Maize cobs were sampled 15 days after pollination (DAP), according to 10 day time-schedule (five times), up to the harvesting. Fresh weight of grains, water percentage, as well as grain yield and shelling percentage at the end of vegetative cycle were determined.

Gradual and significant increase in grain fresh weight was noticed at D1, while at D2 greater values were obtained between 15th and 25th day, as well as between 45th and 55th DAP. At D1, continual increase in grain weight was observable for all hybrids, except for ZP6364, where drop 45th–55th day was observable. For ZP388, significantly higher values were noticeable at D1, at 55th DAP (31.83 g) and also at D2, 15th–45th DAP (from 24.06 to 32.02 g), including steeper drop to the 55th day (24.39 g), in regard to other hybrids. Significant and continual decrease in water content were noticed in grains of all of examined hybrids at D2, while at D1trend was slowed 45th–55th DAP, having the lowest values for ZP5550, ZP5601, and ZP6263. Significantly higher average grain yield achieved ZP6364 (10.05 and 11.35 t ha^{-1}, at D1 and D2, respectively), and D2, compared to D1 (>830 kg ha^{-1}). Similar trend was observable for shelling percentage with 0.71% greater value obtained at D2. ZP707 had the highest value, 82.80% and 90.11% for D1 and D2, respectively.

It could be concluded that, up to the 55th DAP, maize grain gained greater weight and retained higher water amounts at D1, while grain dry-down started from the 45th day and was greater at D2. This was followed with greater grain yield and shelling percentage. From this standpoint, ZP6263 expressed the best features, according to yield potential and grain dry-down.

Keywords: *grain yield, shelling percentage, grain weight, grain dry-down.*

BIORESPONSE OF MAIZE GENOTYPES TO European corn borer (*Ostrinia nubilalis* Hbn) ATTACK AND INSECTICIDE TREATMENTS

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During last years, the total losses from European corn borer (*Ostrinia nubilalis* Hbn.) can reach a high threshold of harmfulness although the different agro-technical measures that are being implemented. According to the sensitivity of hybrids, the damage may vary from only a few percent in tolerant hybrids, up to 30-40% on moderately intolerant hybrids. The aim of this study was to evaluate the variability and bioresponsiveness of four maize genotypes (ZP 427, ZP 434, ZP 600 and ZP 666) to applied insecticides and bioinsecticides and the interdependence of population dynamics. The research has been conducted in 2016 and 2018 in accordance with the EPPO method in field conditions in maize crops. Out of a total of 11.023 attacks in both years of research, in 2016. the largest number of plants (4.841) were with a broken stalk above the ear and at the height of the ear and belonged to categories C_3 to C_8. In 2018. 3.672 plants were dominated by leaf damage and perforation in the stalk type H_2. Analyzing the effectiveness of the tested insecticides and bioinsecticides by application times, an uniformity in the number of plants with recorded symptoms of damage was observed. Thus, foliar treatment with chlorantraniliprole insecticide proved to be the most effective with the least number of attacked plants with a high degree of damage. In contrast, bioinsecticide treatment of plants, in addition to a large number of infested plants, was dominated by high categories of damage that deviated from all other treatments as well as from the control. Within the definition of the degree of tolerance of maize hybrids, the influence of treatment on changes in secondary metabolites (phytins) in seeds was observed. It was noticed that there is a trend of positive correlation of the amount of phytin in the seed (mg/g) with the attack of moths of the first generation and the number of dissected larvae, as well as a trend of negative correlation with the percentage of attacks of moths of the second generation. Correlation coefficients are expressed: in the genotype ZP-600, while in the genotype ZP-666 the correlation is the lowest, ie. irrelevant. The obtained results may be of great importance in future work aimed at the selection of tolerant genotypes to attack corn flame, taking into account the role of phytin, as an antioxidant and phosphorus depot in maize grain.

Keywords: *maize genotypes, tolerance, Ostrinia nubilalis*

ECOLOGICAL STABILITY AND PLASTICITY ASSESSMENT OF EXPERIMENTAL MAIZE HYBRIDS

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The stability and plasticity of prospective maize hybrids for the grain yield trait from FAO groups of different maturity (500-600 and above) have been studied.

The research was conducted in 2020 under contrasting conditions at three different breeding locations: Maize Research Institute - Knezha, Institute of Agriculture and Seed Science Obraztsov Chiflik – Rousse, and Soybeans and Cereals Experimental Station – Pavlikeni. The ecological variety testing is performed by block method in three repetitions with a plot size of $10m^2$.

The statistical processing of the results of the study includes two-factor analysis of variance with repetitions under environmental conditions and parameters of stability and plasticity according to the method of Eberhart, S.A., Russel, W.A., (1966); Pakudin, VV, Lopatina, L.M., (1984).

The results of the analysis of variance demonstrate reliable differences between the conditions and the hybrids as well as more specific interaction between the genotype and the environment. The studied hybrids show different plasticity and stability dependent on their growing conditions. The hybrids E1, E3, E6, E13 and E16 from 500-600 FAO group have higher yields and high stability. Unstable and responsive to better growing conditions are the hybrids: E4, E8, E9, E12, E14, E17 and E18.

In the FAO group above 600, the studied hybrids having a higher and stable yield are: E2, E6, E7 and E13. The hybrids E1, E3, E4, E5, E8, E9, E12, E14 and E17 are with high and unstable yield.

Differentiation of the hybrids depending on their ecological evaluation allows more appropriate distribution across areas of growth.

Keywords: *hybrids, stability, plasticity, FAO groups*

STABILITY OF SOME ZP MAIZE HYBRIDS IN FAO MATURITY GROUP 700 BASED ON THE GENOTYPE × ENVIRONMENT INTERACTION

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Stability of yield or of any other trait depends on the ability of a given cultivar to response to changes in the environment, which is also referred to as phenotypic plasticity. The analysis of a genotype × location interaction is necessary to obtain information on the stable performance of genotypes.

This paper presents results of the genotype (G) main effect and the genotype by environment (GE) interaction, genotype plus genotype by environment interaction (G × GE) biplot analysis of a multi-environmental trial with eight maize hybrids of the FAO maturity group 700 and six different locations in Serbia conducted in 2018. The objective was to determine the effects of genotypes, six environments and their interactions on grain yield and to identify stable maize genotypes. The trial was set up in a randomised complete block design with three replications in each environment. The combined ANOVA indicated that the main effects of environments, genotypes and the genotype by environment interaction were highly significant. The G × GE effects were further partitioned using a GGE biplot model.

In the FAO maturity group 700 the first mega-environment contains environments E6 (Požarevac), E2 (Bečej) and E5 (Pančevo) with standard genotype G8 (ZP 1535) producing the highest yield; the second mega-environment contains environment E1 (Zemun Polje), E4 (Sremska Mitrovica) and E3 (Bajša) with genotype G6 (ZPSC 707) being the most yielding. GGE biplot view of this study identified the most productive and stable varieties – G8 (ZP 1535), G6 (ZP 707), G4 (ZP 8037), G2 (ZP 7704) and G3 (ZP 8702). Genotype G8 (ZP 1535) and G7 (ZP 707) is noticeable to the “ideal” genotype in terms of performance and stability.

Keywords: *AMMI stability value, GGE biplot, genotype, environment, maize.*

SALINITY-INDUCED RESPONSES OF PRIMARY PHOTOCHEMICAL REACTIONS OF SWEET SORGHUM GENOTYPES

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Since the sweet sorghum is one of the most popular crop used for bioenergetically purpose the study of its sensitivity to salinity stress is very important and actual. A little information exists about vulnerability of sorghum PSII photochemistry in relations to physiological mechanism of salt tolerance. In our study, we tested the effect of salinity on primary photochemical reactions of six sweet sorghum genotypes. The both, increase of Na+/K+ ratio as well reduction of plant leaf area after NaCl treatments were used for calculation of two salinity tolerance components: Na+ extrusion and osmotic tolerance, respectively. The increase in salinity significantly induced the accumulation of the proline and the decline of leaf osmotic potential. Expect for 100 mM NaCl concentration, salinity significantly decreased the leaf chlorophyll content and Fv/Fm. Increasing salinity led to higher accumulation of QB-non-reducing PSII reaction centers. Moreover, the Biolyzer software has been used for analysis the salinity effect on the parameters of energy fluxes within leaf. A remarkable finding was observation the K-step occurrence on JIP fluorescence transient in the most sensitive genotypes under high NaCl concentration. Finally, has been observed that the donor side of PSII is more affected by high salt concentration compared to the acceptor side of PSII in sensitive genotypes. Observed down-regulation the primary photochemistry in salt sensitive genotypes resulted from ineffective Na+ extrusion and raised the ionic imbalance. The studied sorghum genotypes responded differently to salinity stress. Thus, the study helps understand the plant tolerance mechanisms of different sweet sorghum genotypes to increasing salinity stress. The study also confirmed that the use of JIP-test is suitable for the identification of sorghum genotypes according to their growth under salinity stress.

Keyword: *fluorescence, JIP-test, photosynthesis, salinity, sorghum*

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SorgEnloS: SORGHUM FOR GRAIN USE IN HESSE: ESTABLISHMENT OF NEW, LOCALLY ADAPTED VARIETIES

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To adapt to climate change and increase biodiversity, it is necessary to establish new, robust crops in local agriculture. Grain sorghum is a drought-tolerant, multi-use crop with numerous agro-ecological advantages, which can also represent a risk protection for farmers during drought conditions and enable the establishment of new regional value chains. At present, however, no locally adapted varieties to Hesse and comparable agroecological regions in Germany are available. Further, there is a lack of information regarding the optimal agronomical practices (sowing densities, fertilization etc.) under local conditions.

Therefore, the aim of the project is to develop stress-tolerant and locally adapted grain sorghum varieties in view of climate change. These varieties will help to increase biodiversity in agriculture and represent an important innovation, both as a new (in Germany) high quality animal feed and as a gluten-free 'superfood'. Since grain sorghum is a novel crop in Hesse, progress in breeding needs to be accompanied by knowledge extension of plant production measurements to farmers, and building of value chains. With this joint effort, the establishment of grain sorghum as a new robust crop seems feasible.

The project partners are institutions covering the aspects of breeding and agronomy on the one hand, and farmers conducting on-farm variety trials on the other hand.

- Breeding of grain sorghum for animal feed and as a gluten-free food (development and testing of new, locally adapted grain sorghum breeding material with enhanced cold tolerance)
- Optimization of agronomical practices in local grain sorghum production, including sowing density, row spacing and N-fertilization trials
- On-farm variety trials, including both commercial grain sorghum hybrids from other European countries and experimental hybrids of the University Giessen breeding program. Evaluation regarding their suitability under practical farming, and involvement of farmers in selection process.

This project is funded by the European Union in the framework of 'EIP-Agri' and by the federal state of Hesse.

Keywords: *Local adaptation, new varieties, cold tolerance, agronomic optimization*

DROUGHT-INDUCED ADJUSTMENT OF PRIMARY METABOLITES IN MAIZE HYBRIDS

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Primary metabolites are directly implicated in plant growth processes, photosynthesis, and respiration, allowing plants to acclimatize and recover from drought stress. Accordingly, this study aimed to determine alterations in content of sugars, total protein, seventeen amino acids and thirty two fatty acids in seeds of maize genotypes differing in drought tolerance in terms of improved plant productivity e.g. increased grain yield obtained under severe water deficit. The experiment was conducted under irrigation and rain-fed conditions. Long-term water deficit stress directly affected plant metabolism, resulting in profound changes in biosynthesis and transport of evaluated primary metabolites. Water deficit caused the inhibition of sugar metabolism (i.e. declined level of sucrose, glucose, fructose, maltose and lactose), bein more pronounced in seeds of drought-sensitive genotypes. Slight decrease in palmitic, linoleic and α-linoleic fatty acids were evidenced in drougth susceptible and medium drought-tolerant maize genotypes. However, significant increase was observed for oleic and especially stearic fatty acid content. In response to water deficit, medium drought-tolerant and tolerant genotypes exhibited amino acids content increase, especialy for methionine, lysine, isoleucine and leucine, with preserved incorporation of amino acids into protein chain. This was confirmed with highly significant positive correlations between total protein and amino acids content ($p \leq 0.01$). The increased protein content in drought tolerant genotypes is linked to altered C-partitioning, which changes the C/N ratio, to favor more N-assimilation, which was confirmed with highly significant negative correlations between sugars and protein content ($p \leq 0.01$), i.e. sugars and amino acids content ($p \leq 0.01$), respectively. Identification of sensitive sites (i.e. biochemical pathways for seed reserves) related to seed-filling processes in stressed plants under long-term water deficit, would provide useful cues in developing strategies to improve grain yield and its quality.

Keywords: *amino acids, fatty acids, sugars, water deficit, Zea mays L.*

SOBINEN: INSECT FRIENDLY ENERGY CROPPING SYSTEMS: COMBINATION OF SORGHUM WITH FLOWERING UNDERSOWN CROPS

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Many reasons are discussed for the current depletion of insects. The intensification of agricultural production, leading to a lack of flowering plants in agricultural landscapes, is one of them. Therefore, our interdisciplinary research project SoBinEn aims at developing suitable combinations of Sorghum bicolor dual-purpose type hybrids with insect-friendly seed mixtures and undersown crops. With this intercropping approach it is possible to extend the flowering period markedly, so that valuable nectar from the undersown species can be provided for bees and other pollinators, while the sorghum pollen itself serves as a valuable protein source sustaining bee brood raising (Siede et al. 2021). Further, these crop mixtures will improve the ecological significance of bioenergy crop rotations in terms of diversity, erosion protection, reduction of nitrate leakage during winter (in case of continuation of the undersown crops) and humus balance.

Five different institutions are involved in this joint research project, covering all aspects of agronomy, insect research and both academic and private (commercial) plant breeding. Principal work packages include:

- testing of a high number of different undersown species in mixed-cropping with sorghum for both insect attractivity and agronomical performance in multi-location field trials;
- agronomical optimization (e. g. sowing density and –techniques) of selected combinations;
- detailed evaluations of insect attractivity for the selected combinations, using both tent trials and free flying bee colonies;
- selection of undersown varieties with superior shade tolerance;
- development of novel erectophile (i. e. vertical roots and erect leaves) sorghum ideotypes, aiming at the reduction of light- and water use competition.

Altogether, this project will contribute to an enhanced sustainability of bioenergy cropping systems, and, in consequence, improve the acceptance of bioenergy crop cultivation for both farmers and the whole society.

Keywords: *Insect-friendly, undersown, sorghum, bioenergy, biodiversity*

Acknowledgment: This project is funded by the German Federal Ministry of Food and Agriculture with coordination by the Federal Agency for Renewable Resources (FNR).

TRANSCRIPTOME PROFILING OF MAIZE SEEDLINGS RESPONSE TO LOW TEMPERATURES

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The climate change associated abiotic stresses threaten to provoke even more pronounced reductions in grain productivity due to their increasing severity and frequency. In an attempt to overcome these challenging circumstances, different strategies, approaches and methodologies are applied. Maize, as one of the most important crop species is seriously affected by extremely high summer temperatures, causing yield reduction. Earlier sowing, as a good strategy for avoiding heat stress during critical phases for grain filling, exposes maize plants to unfavorable low temperatures in the early stages of development. Molecular mechanism of plant response to chilling stress in these stages are not fully defined and need further analyses. Broadening the knowledge and elucidation of these processes enables creating maize genotypes with improved tolerance to this stress. With the aim to achieve these goals, transcriptome profiling of a sensitive and a tolerant inbred line exposed to chilling stress was done. Five days old maize seedling were grown under control (25/20°C; 12/12h) and low temperature (10/8°C; 12/12h) conditions in growth chamber and sampled after 6h and 24h from the stress onset. Total RNA from all the samples was extracted and subjected to RNA-Seq on Illumina platform. Sequencing was followed by bioinformatic analyses. Different types of RNA were analyzed and preliminary results will be presented.

Keywords*: maize, total RNA sequencing, chilling stress*

TOXICOLOGICAL PROFILE OF PATHOGENIC SPECIES ON MAIZE IN SERBIA

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Fusarium and *Aspergillus* toxigenic species are considered to be the most common pathogens in maize. Agroecological conditions in Serbia favour the development of numerous pathogenic and toxigenic species of the genus *Fusarium* and *Aspergillus*. Global climate change may favour the contamination of maize with high levels of aflatoxins and fusariotoxins in temperate areas, such as Serbia. *Fusarium graminearum* synthesises mycotoxins from which the most important are type B trichothecene - deoxynivalenol (DON), its acetyl-ester derivatives (3-ADON and 15-ADON) and nivalenol (NIV). The two most important species of fungi responsible for aspergillosis kernel and ear rot are *Aspergillus flavus* and *Aspergillus parasiticus*, producers of aflatoxins (B1, B2, G1, and G2).

Isolates of *F. graminearum* and *A. parasiticus* identified from maize grain samples collected over 12 years from 40 localities in Serbia were analyzed. Production potential of isolates were determined by high-performance liquid chromatography method (HPLC).

Results of perennial research showed high variability in the concentrations of mycotoxins deoxynivalenol and aflatoxins in maize kernels. Detected concentrations of DON varied from 10,9 to 154.6 µg/g (average 47,2 µg/g), while 3ADON concentrations ranged from 3.42 to 71.6 µg/g (average 15,6 µg/g) and 15ADON from 5.4 to 121.3 µg/g (average 29,8 µg/g). Mycotoxin 15ADON were predominant in observed samples. The average synthesis of aflatoxin B1 was 4145.97 µg/kg (range 14.24 to 7361.03 µg/kg) by which the isolates of this group were classified into strong producers of AFB1. The average synthesis of aflatoxin B2 was 403.55 µg/kg, aflatoxin G1 3490.07 µg/kg and G2 198.29 µg/kg averaging, respectively.

Keywords: *Fusarium, Aspergillus, Maize, Mycotoxins*

EXPLOITATION OF STRESS TOLERANCE INDICES FOR THE IDENTIFICATION OF PROMISING MAIZE GENOTYPES

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Limiting resources, such as water and nutrients, is a major cause of yield loss for many important crops, including maize. Since increasing maize yield has been strongly associated with improving stress tolerance, seven stress indices were calculated to evaluate 13 genotypes under normal and low input conditions. The experiment was established in the farm of University of Western Macedonia, Florina, Northern Greece during the 2021 growing season. At the normal inputs' regime, the recommended irrigation and fertilization was applied while at the low inputs' regime only half of the recommended irrigation and fertilization was applied. Morphological and physiological parameters of the genetic material were measured, and the final seed yield was used for the calculation of the following indices, as selection criteria: Stress susceptibility index (SSI), stress tolerance (TOL), mean productivity (MP), stress tolerant index (STI), geometric mean of productivity (GMP), yield index (YI) and harmonic mean (HM). The genotypes exhibited a different yield performance across the two input treatments; the three top at the normal input regime differed from the three top under the low input regime. Thus, the genotype performance under normal conditions does not predict the genotype performance under stress conditions. Concerning the selection criteria, the Spearman's correlation coefficient indicated that MP, STI, GMP, and HM were associated with yield under the normal regime, and YI was connected with yield under the low input regime.

Keywords: *Corn, normal conditions, low inputs, stress indices*

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EVALUATING MAIZE GENOTYPES UNDER TWO INPUT REGIMES AFTER MYCORRHIZAL INOCULATION

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Maize is one of the most important crops globally due to its nutritional value, both for food and feed but also for its industrial uses. However, climate change and environmental variability affect maize growth and reduce global production. The aim of this research was to investigate the ability of maize genotypes to utilize available inputs and the development of suitable mycorrhizal inoculants to improve productivity. For this reason, an experiment was established in the farm of the University of Western Macedonia, Florina, Northern Greece, to evaluate 10 commercial hybrids and three open-pollinated lines under two regimes. The first regime (normal) included 8.889 plants per m^2 (15 cm plant spacing on each row), and the recommended irrigation and fertilization were applied. The second regime (reduced) included 5.333 plants per m^2 (25 cm plant spacing on each row), and only half of the recommended irrigation and fertilization was applied. Mycorrhizal inoculant for each genotype was seed coated with AMF spores of selected indigenous species in the low-input regime. Morphological and physiological parameters (stomatal conductivity, photosynthetic rate, chlorophyll content, and chlorophyll fluorescence) and their final seed yield were measured in selected plants from all genotypes in both experimental fields. According to statistical analysis, there were no differences between the two input treatments, emphasizing that the presence of mycorrhiza enables genotypes to perform satisfactorily even under low input conditions. Noteworthy, one open-pollinated line was included in the high yielding genotypes of this research. Finally, the correlation of all the recorded parameters with seed yield were estimated to identify the measurements that can predict the performance of maize genotypes.

Keywords: *Corn, mycorrhizae, low inputs, productivity*

Acknowledgment: This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code: T1EDK-00739).

IDENTIFICATION OF EARLY DECLINE OF SEED QUALITY BY VIGOR TESTS

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Seed vigor is the quality parameter which indicates germination under wide range of environmental conditions. This trait can be identified by standard germination test as so-called first count germination or germination energy. In addition, several tests for vigor evaluation were adopted by seed testing associations such as ISTA and AOSA. The main purpose for developing additional vigor tests is to obtain fast and reliable information on seed vigor which contributes to decision making in the inventory management. ISTA rules prescribes several tests for evaluating seed vigor, namely: conductivity test, accelerated ageing (AA) for soybean, controlled deterioration for Brassica seeds, Radicle emergence (RE) and Tetrazolium (TZ) vigor test for soybean. Seed testing laboratory of the Maize Research Institute is accredited by ISTA for RE vigor test, has internally developed cold-test and is capable for application of TZ vigor test for research purposes. These tests were applied on several maize seeds lots with declining germination energy and still highly rated final germination. Results of vigor tests were correlated with the standard germination quality parameters, germination energy and final count, in order to identify vigor test which provides the most approximate output to the standard germination test. Correlations were significant for all vigor tests, in average 0.882 and 0.713 for germination first and final count, respectively. The highest compatibility between vigor and germination tests was identified for cold test with correlation coefficients of 0.952 and 0.839 for germination first and final count, respectively. However, due to duration of cold test (2 weeks), it is not capable of providing fast information. Therefore, other two vigor tests, TZ and RE, which are shorter and have high correlation with germination energy (0.901 and 0.811, respectively) and final count (0.713 and 0.728, respectively) can be recommended for this purpose.

Keywords: *germination, seed vigor, radicle emergence test, tetrazolium vigor test, cold test*

FACTOR ANALYTIC APPROACH FOR THE ANALYSIS OF MAIZE EARLY TESTING MULTI-ENVIRONMENT TRIALS

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In early stages of maize breeding program, large number of new genotypes is grown in a set of field trial locations. The best ones are identified to move to the next stage so that in the final stages of breeding program small number of superior hybrids is grown in pre-registration and post-registration field trials across set of locations. Field trials were conducted in a partially replicated design, each genotype with two replications per location at six locations in 2020. The experiments included 150 hybrids obtained by crossing different S2 progenies with an elite inbred tester. These field trials were conducted to identify the best performing genotypes using appropriate statistical model. The factor analytic (FA) model accommodates the heterogeneity of genetic variance for locations and heterogeneity of genetic covariance between pairs of locations. Assuming that data from our field trials had high degree of the heterogeneity, we applied the FA model approach which resulted in accurate prediction of the hybrid by location effects. The FA (1) was identified as the best fitting model explaining about 80% of the hybrid × location interaction. The resulted genetic correlation matrix indicates moderate to high positive association among the locations. Using the FA (1) model, the most promising genotypes in terms of grain yield and yield stability were identified.

Keywords: *Grain yield, Yield stability, Maize breeding, Early testing, Hybrid × location interaction, Factor analytic model (FA)*

DYNAMICS OF GRAIN YIELD AND MOISTURE AT HARVEST AND PROGRESS OBSERVED IN AN EIGHTEEN-YEAR TESTING OF MAIZE HYBRIDS FROM 4 FAO GROUPS

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For a 18 year period (2001-2018) a total of 2459 hybrids from 4 maturity groups were tested. The dynamics of grain yield (*GY*), moisture at harvest (*GMH*) and performance index (P_i) were studied. The testing was conducted at 4 locations in Bulgaria - 3 without irrigation and 1 under irrigated conditions. The results from the analysis of variance indicates significant variances for the years of testing for all traits - GY, GMH, Pi. There are no significant variances for the FAO groups grain yield, which confirms the previous researches (Videnović et al., 2013).
Multiple correlation analysis shows significant values of the correlations between the three traits in all FAO groups, with equal chances for tandem selection. There is a steady tendency for the whole studied period that the grain yield increases at all maturity groups, the grain moisture at harvest to decrease and P_i to increase, respectively.
For all FAO groups, the average progress in grain yield is 76 kg / ha / year, confirming our previous research (Vulchinkov et al., 2016).
Grain moisture at harvest shows a decrease in all FAO groups with average value of 0.27% reduction per year. For the performance index (P_i) almost the same values are observed for all groups and an average increase for them of 0.12 per year.
The distribution of FAO groups in relation to the general average for grain yield (7330 kg / ha) for all environments - 4 locations and 18 years and to the average value of the regression coefficient *bi* regarding their stability puts in the high productivity zone (above average) the 400-500 and 500-600 FAO groups, and in the stable zone (bi <1.0) – the groups 300-400 and 500-600. The middle-late group (FAO 500-600) is relatively the most productive and stable under stress conditions.

Keywords: *maize hybrids, FAO groups, 18-year testing, breeding progress*

EVALUATION OF THE STABILITY OF EARLY MAIZE HYBRIDS BY PARAMETRIC AND NONPARAMETRIC METHODS

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Twenty-two maize hybrids from the early FAO group (300-400), tested at four locations, were evaluated for their phenotypic stability by seven different models, including parametric and non-parametric methods (Wricke, 1962; Eberhart and Russell, 1966; Shukla, 1972; Francis and Kannenberg, 1978; Kang, 1988; Vulchinkov, 1990; Purchase, 1997).

ANOVA results of grain yield show significant variances for all factors – hybrids, locations and their interaction, with locations having the largest share of the total variation. The differentiated variance of G x E points out that nonlinear interactions (balance) have a larger share. The correlation analysis between the yield and the different stability parameters indicates 64% reliable cases, most confirming previous studies.

The hybrids were ranked by individual parameters, as well as ranking analysis by total ranking sum. There is a good coherence between the different methods determining the phenotypic stability of hybrids. The ranked three hybrids by minimum rank amount (G11, G18 and G3) occupy the most favorable quadrant in the distribution of hybrids regarding the average values of yield and AMMI stability value parameter. The first-ranked hybrid (G11) in our previous study with the same set of hybrids (Vulchinkov et al., 2021) is located at the center of the AMMI-2 biplot.

The rankings can be used successfully in selection of high-yielding new hybrids well adapted to different environments.

Keywords: *stability, maize hybrids, ranking by different methods*

HIGH-THROUGHPUT ANALYSIS OF BIOMASS ACCUMULATION AND GEOMETRY TO ASSESS STRESS RESPONSES AND WATER USE EFFICIENCY OF SORGHUM GENOTYPES

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High-throughput plant phenotyping enables collecting a large amount of raw data providing information about plant surface properties, plant geometry, and functional features that can be used to better understand plant growth and productivity in stressful environments. The Slovak PlantScreen Phenotyping Unit (SPPU) containing the PlantScreen^{TM} phenotyping platform was used to investigate growth dynamics and geometrical responses of four sweet sorghum genotypes cultivated in well-watered (WW) and gradual drought stress (DS) conditions. For calculating plant growth analysis parameters (AGR, RGR, NAR), the precision calibration of relations between automatically evaluated shoot area (from plant RGB imaging) and leaf area measured manually was used. The significant genotypic differences in above-ground biomass accumulation of plants growing in well-watered as well water stressed conditions have been observed. Further, the geometrical description of the plant has been done, such as height, circularity (roundness and isotropy), symmetry (eccentricity and RMS), and center distance (compactness and SOL) parameters. Based on data analysis, the genotypic differences in time-dependent dynamics of plant leaf area formation and growth rate (ARG and RGR) and NAR have been observed. We have identified that the oscillation of plant compactness calculated separately for side and top views provides essential information about leaf orientation. DS caused a significant decrease in the value of plant compactness in all genotypes, which can be attributed to more planophile-oriented leaves due to the decrease of the slope angle towards the horizontal plane by the turgor decrease. Thanks to detailed information on water use and environmental conditions, the high throughput data are useful to characterize drought responses and the water use efficiency of sorghum germplasm.

Keywords: *sorghum, genetic resources, high throughput phenotyping, drought, biomass*

Acknowledgment: The work was co-funded by projects VEGA 1-0683-20, VEGA 1-0664-22, APVV-18-0465, and bilateral project SK-SRB-21-0043.

III Diversifying the uses of maize and sorghum with specific varieties / breeding

GENOMIC REGIONS FOR MAIZE CELL WALL HYDROXYCINNAMATES USING A MAGIC APPROACH AND THEIR RELATIONSHIP WITH MAIZE USAGES

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Cell walls presenting great concentrations of hydroxycinnamates make maize tissues more recalcitrant to damage by insects, less digestible by ruminants, and less suitable for biofuel production. In a Genome Wide Association Analysis (GWAS) study carried out in a maize MAGIC (Multi-parent Advanced Generation Intercross) population, we identified 24 SNPs associated to esterified cell wall-bound hydroxycinnamates, that represented 15 Quantitative Trait Loci (QTL); thereafter we explore their relationship with SNPs associated to saccharification efficiency, digestibility of organic matter and corn borer damage in order to identify significant genetic variants useful to breed maize varieties for multiple uses. We identified new genomic regions associated to cell wall bound hydroxycinnamates in maize stover that could have an impact on their content across different genetic backgrounds. The high-resolution QTL described in this study could be valuable for addressing positional mapping of genes involved in hydroxycinnamate biosynthesis and could uncover genes implicated in the esterification of hydroxycinnamic acids to the arabinoxylan chains that are poorly understood. However, we found that genetic correlation coefficients between hydroxycinnamate content and economical important traits such as saccharification efficiency, animal digestibility and pest resistance were low to moderate, so modify specific hydroxycinnamates to indirectly improve the overall cultivar performance will be incompatible.

Keywords: *cell wall hydroxycinnamates, GWAS, MAGIC, stiffening*

CONNECTING TOTAL PHENOLIC COMPOUNDS AND AGRONOMIC TRAITS IN A DARK RED CORN POPULATION

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Dark red corn is rich in anthocyanins, which belong to phenolic compounds, one of the largest families of antioxidants. Due to numerous, beneficial biological activities of these compounds, this crop is rising in popularity. Unfortunately, the use of dark red corn in the temperate zone is limited, so the breeding activities in this sense are negligible. To maximize profitability and meet technological and growing demands, both biochemical and agronomic performances of such materials have to be considered. A preliminary study was conducted to test the variability of total phenolic compounds and some agronomic traits in NS01RB, a broad based population of dark red corn. 352 full sibs were tested in an incomplete block design with replicates in sets. Data were collected for grain yield, grain moisture, ASI, stay green, stalk lodging, ear rotting and grain color. Significant genetic variability existed among full sibs for all studied traits. A subset of 132 full sibs that showed the highest level of tolerance to ear rotting was selected for biochemical analysis. Mean values for the total phenolic compound ranged from 0.66 to 4.83 mg/g. Basic genetic parameters, relationships and mean values of the examined traits showed that the studied material represents a valuable source of phenolic compounds and could be used in a breeding program.

Keyword: *breeding, antioxidants, dark red corn, phenolics*

ESTIMATION OF PHENOLIC CONTENT IN YELLOW, RED, BLUE AND RED-BLUE MAIZE BY NEAR-INFRARED REFLECTANCE SPECTROSCOPY

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Red and blue colors in maize kernel are the consequence of anthocyanin pigments, responsible for the increased phenolic content and antioxidant activity in maize. Therefore, interest in pigmented maize as a novel functional food due to its possible health benefits and unique taste has grown. The red color in maize kernel originates from pericarp and is maternally inherited while the blue color originates from aleurone, which is triploid in nature and its mode of inheritance is more complicated. Red and blue maize are usually low-yielding populations, and at the Maize Research Institute Zemun Polje (MRIZP), commercial inbred lines have been converted to red and blue colors to produce high-yielding red and blue maize hybrids.

Maize extractable phenolic content (EPC) can be directly determined through wet chemistry and measures phenolic amides, glycerides, free phenolic acids, and some flavonoids. In this study, however, Fourier-transformed near-infrared reflectance spectroscopy (FT-NIRS), as a non-destructive, inexpensive, and rapid technique has been used to scan EPC profiles of 56 MRIZP maize samples (14 genotypes in two replications and two environments) differing in kernel color: yellow, red pericarp, blue aleurone and red pericarp with blue aleurone. Twenty samples with outlying EPC values have been directly analyzed for total phenolics and these values have been used to adjust and improve NIRS calibration.

EPC values ranged from 1.69 to 6.82 mg g^{-1} across genotypes and environments. Two red inbred lines had the highest EPC content in 2017, 6.82 and 5.11 mg g^{-1}, and, overall samples from 2017 had a higher content of EPC compared to the same genotypes grown in 2018. Red genotypes on average had the highest EPC content, followed by red-blue, while yellow and blue kernels had similar and the lowest content within the samples.

Key words: *colored maize, near-infrared spectroscopy, phenolics*

NUTRITIONAL AND COST EFFECTS OF ADAPTED QUALITY PROTEIN MAIZE ON BROILER FEEDING

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ZPQPM13 is a maize hybrid with improved protein quality, adapted to temperate climate growth conditions, developed by crossing a QPM version of ZPL5 commercial line (developed by marker assisted selection) and GS-6 (a QPM line with 50% tropical and 50% temperate germplasm). This hybrid has grain yield as commercial hybrids, over 90% of hard endosperm, contents of tryptophan and protein over 0.075% and 10%, respectively, and stability of tryptophan content over different locations and years. ZPQPM13 was tested for use in broiler feeds, with the aim to reduce utilization of costly synthetic lysine or soybean components. The feeding experiment on Ross 308 broilers was divided into two groups – control (fed with standard maize) and treatment (fed with QPM). In treatment group diets, ZPQPM13 was increased and soybean component decreased for 3%. Chemical analyses revealed higher contents of limiting essential amino acids in mercantile ZPQPM13 compared to standard maize – 0.44 vs. 0.25 % lysine, 0.077 vs. 0.070 % tryptophan and 0.39 vs. 0.27 % threonine. Duration of each feeding trial was 42 days, comprising three phases - starter (1-14 days), grower (15-35 days) and finisher (36-42 days). Feed intake (FI), body mass gain (BMG), average daily gain (ADG) and feed conversion ratio (FCR) were calculated at the end of each phase. In treatment group, FI was reduced, while BMG and ADG were higher in starter phase but lower in grower and finisher phases. FCR was lower in all three phases in treatment group – 1.13 vs. 1.30, 1.25 vs. 1.33 and 1.28 vs. 1.45. Although a small drawback is that final weight was 3.4 % higher in control broilers, comparison of feed and meat prices show that financial benefits of using QPM are still significant compared to using standard maize in feeds, as soybean is four times more expensive than maize.

Keywords: *broilers, diets, FCR, lysine, QPM*

LOCAL VARIETIES OF MAIZE FOR BREAD

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The commercial varieties of maize have not been selected for particular characteristics such as baking quality. Maize bread is a traditional product in the north of Spain and Portugal, and bakeries claim to recover the traditional varieties that have been selected by farmers for their flavor and quality. Thus, in cooperation with Da Cunha bakery, we have evaluated 10 local varieties in five environments in northwestern Spain during 2020. A randomized block design with three replications was used. We have taken agronomic traits: flowering date and grain moisture and yield and grain nutritional characteristics: moisture, protein, fat, ash, starch, and crude Fiber using the NIRs. We have selected six varieties that, together with four new varieties, were re-evaluated in 5 environments in 2021 following the same design as the previous year. Finally, three varieties were chosen that are suitable to be the base material for bakery products: 'Tuy' variety of flint and yellow kernels of cycle 400 FAO, 'Rebordanes' flint white maize of cycle 400 FAO and 'Oubiña negro' maize with flint and black grain, cycle 500 FAO. These three varieties can contribute to increasing the biodiversity of the maize grown in the area, as well as providing differential products of higher quality and greater added value.

Keywords: *Bread, maize, local varieties*

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SEARCHING FOR MAIZE PRE-BREEDING MATERIALS IN ORDER TO IMPROVE BOTH ANIMAL DIGESTIBILITY AND ETHANOL PRODUCTION

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In terms of agricultural land use and production, maize (*Zea mays* L.) is one of the most important crops worldwide. In addition to the general uses of maize grain as food, feed, or raw material for industrial derivatives, the whole plant can be used as silage for feeding cattle or the stover can be a profitable byproduct for ethanol production.

In the current study, we carried out both an inbred characterization for digestibility of the organic matter (DMO) (by Near-infrared spectroscopy) of the whole plant and for saccharification efficiency of the stover (SE) (by an automated saccharification robotic platform). Forage and stover yields were also estimated. The inbreds evaluated were selected attending to one or more of three specific reasons: (i) are parental inbreds of “Sugarcorn” hybrids used in ethanol production, (ii) show good performance in hybrid combination, including some inbreds with good general and specific combining ability for animal digestibility, or (iii) have been previously characterised in relation to pest resistance. A 20 genotypes set (A509, A632, A654, C103, CO348, CO384, CO442, CO444, EC212, EP17, EP42, EP47, EP53, EP86, EP105, EP125, F473, PB130, W64A, W182B) was evaluated for two years following a complete randomized block design.

One of the most promising inbreds was EP53 (from the Galician local variety Laro), with both great DMO and SE, however, forage and stover yields of this particular inbred were quite lower than the inbred average. In this sense, inbred A632 (Reid heterotic group) showed high DMO (71%) and SE (95 nmol mg^{-1} material^{-1} hour^{-1}) and yields up to the mean set. These inbred lines can be used as a promising base material for future breeding programs. In the short-term, they can be used directly to obtain the hybrid EP53 x A632 expected to have both good digestibility and ethanolic potential.

Keywords: *pre-breeding, digestibility, saccharification, MAGIC*

DONORS OF FAVOURABLE ALLELES FOR THE IMPROVEMENT OF THE EAR LENGTH IN MAIZE HYBRIDS

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Inbred lines derived through different cycles of recurrent selection of the synthetic populations BSSS and $BSCB_1$ were studied. The aim of the study was to evaluate which of observed inbreds had the highest relative values of favourable alleles for the improvement of the elite hybrid. The maximum μG^* allele values were detected in the inbreds B97(C_9) and B99(C_{10}) originating from the populations $BSCB_1$ and B14(C_0) and B37(C_0) originating from the population BSSS. The observed inbreds derived from the synthetic $BSCB_1$ were more related to the parental inbred ZPL1, while those originated from BSSS were more related to the parental inbred ZPL2. The significant differences between μG^* and μD^* were not established in the inbred lines B97(C_9) and B99(C_{10}), hence the initial population for selection was derived by direct self-pollination of F_1 generation developed from the crosses of B99 × ZPL1 and B97 × ZPL1. The inbred B37(C_0) was more related to the parental inbred ZPL_2 and in that case direct self-pollination could be performed from the cross B37 × ZPL2. Not only the μG^* values were high in inbred lines B14(C_0), B37(C_5), B84(C_7) and B91(C_8), but also differences between numbers of favourable dominant and unfavourable recessive alleles were significant. In this case, in order to develop the initial population, backcross of the F_1 generation (ZPL1 × Donor) × ZPL1 is recommended for inbreds B84(C_7) and B91(C_8), while backcross (ZPL2 × Donor) × ZPL2 is recommended for inbreds B14(C_0) and B73(C_5).

***Keywords**: inbred lines, recurrent selection, Zea mays* L.

COMBINING ABILITIES AND HETEROSIS FOR BIOMASS YIELD AND QUALITY RELATED TRAITS IN SINGLE-CUT FORAGE SORGHUM ADAPTED TO TEMPERATE CLIMATES

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Sorghum is among the world's most important cereals in terms of human and animal nutrition and it is currently considered as a promising crop to support several bio-based value chains (energy, bio-based materials). The development of varieties fitting the expectations of these different end-products requires a better understanding of the genetic determinism of the traits contributing to the targeted ideotypes. In this context, the objectives of this study were to estimate the combining abilities of the main target traits, explore their correlations and assess the heterosis levels achieved. To reach, these goals a factorial cross design has been developed based on 10 female and 16 male parents that were selected for their General Combining Abilities (GCA) for biomass production. One hundred and forty-seven hybrids were evaluated on 3 sites together with their parental lines. Heritability of 0.69, 0.79 and 0.90 were observed respectively for dry matter yield, lodging and biomass digestibility. Correlations analyses between hybrid genetic values and combing ability components showed that hybrid performance is mainly correlated with the male GCA and depends only weakly of the female GCA. Higher best parent relative heterosis (up to 49%) was observed for biomass yield compared to biomass digestibility (up to 9%) and a negative genetic correlation (-0.54 for the BLUP values and -0.6 for the GCA) was observed between biomass yield and digestibility. In addition to allow the identification of elite parental lines that will be used in future crosses, this study provided an access to a better understanding of the genetic determinism of key biomass related traits. The negative correlations between biomass production and quality related traits and the lack of obvious predictors of heterosis will need to be tackled to facilitate the development of varieties fitting the expectations of the different value chains.

Keywords: *Sorghum, Biomass yield, Biomass quality, Heterosis, Combining abilities*

VARIABILITY OF ANTIOXIDANTS IN WHITE KERNEL MAIZE VARIETIES AND HYBRIDS

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Undoubtedly yellow kernel maize is predominant all over the world, while the significance of the production and utilization of white kernel maize is highly dependent on cultural and specific nutritional preferences. Nevertheless white maize is commonly grown on large areas of South and Central America, East and South Africa. Likewise, in Serbia it is mainly produced for human consumption and traditionally, consumers prefer it over the yellow kernel maize. Thus, the utilization of white kernel maize has increasing trend. In this research we examined variability of some antioxidants in kernels of 16 open pollinated varieties and 4 white kernel maize hybrids (ZP552b, ZP74b, ZP775b, and ZP 887b). Antioxidant status was measured through the concentration of phytic P, phenolics, glutathione (GSH) and DPPH reduction capacity. Significant differences among genotypes were observed according to the content of GSH, phenolics and DPPH reduction capacity. There were no significant variations among genotypes regarding the concentration of phytic P. It must be underlined that kernels of open pollinated varieties were slightly higher in phytic P, and significantly higher in GSH and DPPH reduction capacity, in regard to hybrids, while kernels of hybrids had higher concentration of phenolics.

Keywords: *kernel quality, phytic P, phenolics, glutathione, DPPH reduction capacity*

IV New avenues in selection and breeding through integration of molecular tools

IDENTIFYING QTLS INVOLVED IN HETEROTIC GROUP COMPLEMENTARITY: NEW GWAS MODELS APPLIED TO A FACTORIAL AND AN ADMIXED DIALLEL MAIZE HYBRID PANEL

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In traditional selection schemes, maize inbred lines are structured into heterotic groups to maximize the inter-group hybrid performances through the genetic complementarity between parental lines, which guarantees the expression of heterosis. This structuration of the genetic diversity in heterotic groups is associated with differences in terms of allelic frequencies at causal or neutral polymorphisms, linkage disequilibrium or local epistatic interactions. To understand how these variations are involved in the heterotic group complementarity, we proposed a general GWAS framework disentangling the allelic effect from the heterotic group origin of the alleles, and compared it with a conventional additive/dominance model. An application is presented on two hybrid panels with different genetic compositions: i) a factorial between lines from a dent and a flint panels and ii) a diallel panel obtained by crossing admixed dent-flint lines. The panels were evaluated for male and female flowering time, grain moisture and yield in several locations allowing two different layers of analysis: within each environment and in a multiple environment context. We identified several strong additive QTLs for all traits, including some well-known additive QTLs for flowering time (in the region of Vgt1/2 on chromosome 8). Moreover, we underlined the importance of dominance effects for grain yield. Most of the dominant QTLs identified in the diallel showed overdominance or, more likely, pseudo-overdominance effects. The comparison between environments revealed a higher stability of additive QTL effects compared to non-additive ones. We identified multiple QTLs with interaction with the local genetic background and revealed large chromosomic regions involved in the effect of the heterotic origin of the alleles. Altogether, our results illustrated the interest of the admixed panel and our GWAS model revealed new causal polymorphisms hidden in classical factorial hybrid panels and inaccessible with traditional modelling.

Keywords: *Maize, GWAS, heterotic groups, panel comparison, genetic background*

EFFICIENT FLOWERING CLASSIFICATION BASED ON DEEP LEARNING AND MARKER DATA IN MAIZE INBRED LINES

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The advent of deep learning methods such as convolutional neural networks (CNN) represents a new avenue in analysis of biological data in the “Breeding 4.0” era. The power of this approach lies in its ability of feature extraction, combined with architecture having layers of interconnected neurons sharing fragments of information. Such information flow coupled with powerful means of dimension reduction based on spatial coherence (linkage disequilibrium) and pooling might provide good method for analysis of dense genotypic data. Total of 1066 maize inbred lines developed at the Agricultural Institute Osijek were screened for distinctness, uniformity and stability (DUS), and their flowering window was classified compared to checks to groups 1 to 7, with earliest inbreds such as CM7 belonging to group 1, most European flints to group 2 PHJ40 to group 3, PHP02 to group 4, Oh43 to group 5, B73 and Mo17 to group 6, and the latest flowering inbreds F118 and HBA1 to group 7. All inbreds were genotyped with Illumina MaizeSNP50 array. Missing and heterozygous positions were filtered (5% and 2.5%) leaving 48734 markers that were imputed with LinkImpute and one-hot recoded. Convolutional neural network was setup with Tensorflow 2 in Python. Model validation with external dataset showed >93% classification accuracy, while all of the ~7% misses were classified to neighboring groups (±1). A priori classification of germplasm can facilitate improvement of germplasm-environment compatibility. Novel machine learning algorithms show promise in analysis of complex nonlinear data and their deployment in breeding programs needs to be further studied.

Keywords: *machine learning, neural network, maize, SNP 50k array, flowering time*

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GENOMIC *VS* PHENOTYPIC SELECTION FOR RESISTANCE AND TOLERANCE OF MAIZE TO STEM BORERS

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Genomic selection (GS), which uses all marker information to calculate genomic estimated breeding values, is presented as an emerging alternative to phenotypic and marker-assisted selections for improving complex traits controlled by many genes with small effects. Therefore, although phenotypic selection has been effective for increasing resistance and yield under high infestation with maize stem borers, higher genetic gains are expected to be obtained through GS based on the complex architecture of both traits. Our objective is to test whether GS is more effective than phenotypic selection for improving resistance and/or tolerance to maize stem borers. For this, we compare different breeding programmes based on phenotype and genotypic value for a single trait, resistance or yield, and for both traits together.

As results, we obtained that GS for yield achieved the highest genetic gain, but did not work to increase resistance to stem borers, meanwhile phenotypic selection for yield was the programme that achieved the highest reduction of tunnel length, but was ineffective for increasing yield. However, phenotypic or genomic selection for increased resistance may be more effective in improving both traits together; although the gains per cycle would be small for both traits.

Keywords: *Maize, stem borer, resistance, genomic selection, phenotypic selection*

GENOMIC PREDICTION OF HYBRID PERFORMANCE: COMPARISON OF THE EFFICIENCY OF FACTORIAL AND TESTER DESIGNS USED AS TRAINING SETS IN A MULTIPARENTAL CONNECTED RECIPROCAL DESIGN FOR MAIZE SILAGE

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In maize breeding, the selection of the candidate inbred lines is based on topcross evaluations using a limited number of testers. Then, a subset of single-crosses between these selected lines is evaluated to identify the best hybrid combinations. Genomic selection enables the prediction of all possible single-crosses between candidate lines but raises the question of defining the best training set design. Previous simulation results have shown the potential of using a sparse factorial design instead of tester designs as the training set. To validate this result, a 363 hybrid factorial design was obtained by crossing 90 dent and flint inbred lines from six segregating families. Two tester designs were also obtained by crossing the same inbred lines to two testers of the opposite group. These designs were evaluated for silage performances in eight environments and used to predict independent performances of a 951 hybrid factorial design. At a same number of hybrids and lines, the factorial design was as efficient as the tester designs, and, for some traits, outperformed them. All available designs were used as both training and validation set to evaluate their efficiency. When the objective was to predict single-crosses between untested lines, we showed an advantage of increasing the number of lines involved in the training set, by (i) allocating each of them to a different tester for the tester design, or (ii) reducing the number of hybrids per line for the factorial design. Our results confirm the potential of sparse factorial designs for genomic hybrid breeding.

Keywords: *Maize silage, Hybrid breeding, Genomic selection, Factorial design, Tester design*

IDENTIFICATION OF SNP MARKERS ASSOCIATED WITH PHENOTYPIC TRAITS IN ZP ELITE MAIZE INBRED LINES

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Maize is the most important crop in Serbia and among three most important crops in the world. Nowadays yield improvement of maize through plant breeding is essential in terms of global food security, especially due to predictions of growing human population and global warming. Considering that grain yield and other important quantitative agronomic traits are polygenic and mostly controlled by numerous minor loci with small individual effects, their improvement through conventional breeding is difficult. Today, availability of the reference maize genome, high-throughput genotyping techniques and appropriate bioinformatics tools all together provide better insights into complex genetic architecture underlying quantitative traits in maize. Such modern multidisciplinary approaches are being commonly used in maize breeding for improving important agronomic traits. In this research, genotype–phenotype association study was conducted with aim to identify SNP markers which show strongly statistically supported relationship with favorable values of important quantitative traits in maize. These results would provide knowledge for improving breeding efficiency and making breeding decisions more precise. A panel of 46 elite maize inbred lines of different heterotic origin and maturity, used in current maize breeding programs of Maize Research Institute Zemun Polje, were selected for genotype–phenotype association study. Chosen inbreds were subjected to total RNA-Seq using Illumina MiSeq platform. Total RNAs isolated from third leaf at V4 growth stage in controlled greenhouse conditions were sequenced. Bioinformatics pipeline for data manipulation and analysis was custom made. Processed whole-transcriptome sequencing data were used for single nucleotide polymorphisms (SNPs) identification. In total, 943,023 polymorphic SNP loci were detected. Thereof, 163,140 SNPs with minor allele frequencies >0.05 were used for further analysis. Phenotyping was done for 13 important agronomic quantitative traits in eight environments. With the aim to identify SNP marker–trait associations, genotype-phenotype association analysis was performed using TASSEL software. Association of particular SNP markers with traits is discussed.

Keywords: *maize, RNA-Seq, SNP markers, quantitative traits, marker-trait association.*

QTL MAPPING IDENTIFIES NOVEL MAJOR LOCI FOR EAR FASCIATION, EAR PROLIFICACY, AND TILLERING IN MAIZE

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Ear fasciation originates from abnormal proliferation of the ear meristem and usually manifests as a multiple-tipped ear, ear flatness, and/or disordered kernel arrangement. Ear prolificacy expresses as multiple ears per node and has developmental similarities with tillering, both resulting from shoot branching. In this study, the genetic control of ear fasciation and shoot branching was analyzed using two recombinant inbred line populations (Lo1016 x B73 and Lo1016 x Lo964) with Lo1016 and Lo964 as donors of ear fasciation and prolificacy, respectively. Kernel row number (KRN), ratio of ear diameters, ear fasciation, ear ovality and kernel row disorder, and prolificacy and number of tillers per plant were phenotyped in at least two-year field experiments. The ear-fasciation related traits showed relatively high heritability ($h^2 > 0.5$) and were significantly correlated. QTL mapping identified four QTLs for ear-fasciation related traits, on chr. 1, 5, and 7, and five QTL for KRN. The QTL with the strongest effect was *qKRN2* (LOD = 7.76; PVE = 23%) which did not associate with fasciation. However, *qFAS7* on chr. 7 included QTL peaks for fasciation index, ear ovality, kernel row disorder, and KRN. Overlaps between QTLs and known candidate genes for ear fasciation highlighted *ramosa1* (*ra1*) as a possible candidate gene for *qFAS7*, however, comparisons between B73, Lo964, and Lo1016 genome sequences did not show difference in *ra1*. Additional candidate genes are being evaluated for *qFAS1.2*. Three and four QTLs were mapped for ear prolificacy and tillering, respectively, with no overlaps between the two traits QTL. Our study uncovered new ear fasciation, ear prolificacy, and tillering loci, which are still segregating in elite materials. Further validation is in progress to confirm the association between candidate genes and QTL for these important agronomic traits.

Keywords: *ear development, kernel number, QTL, tillers, yield components*

GENOTYPING OF MAIZE USING OPTIMIZED SNP ARRAYS

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Genotyping for plant genetic studies, germplasm and variety characterization, and plant breeding requires accuracy, good genetic resolution and low costs per sample. In order to develop such genotyping arrays for maize, we have assembled public SNP (single nucleotide polymorphism) marker sets that (i) comprise of SNP markers that are of high technical quality selected from the 50K and 600K maize genotyping arrays, (ii) display a good level of polymorphism over a wide range of adapted and wild material based on many hundreds of genotyped public lines; (iii) are not in perfect linkage disequilibrium with each other; (iv) are evenly distributed along the chromosomes based on recombination. Especially, for plant breeding purpose such as Genomic Selection and other aspects of marker-assisted breeding in combination with Imputation using the genotyping data of parental lines with large arrays or genome sequencing, we have further reduced our optimized 25K maize genotyping array to a smaller 9K maize genotyping array that can be used in a very cost-efficient way. Such optimized genotyping arrays for maize are now widely used through our services within genetic research and accelerated plant breeding both from the academic and private sector.

DO GENETIC ARCHITECTURE OF TRAITS MEASURED IN PHENOTYPING PLATFORM FACILITATE YIELD PREDICTION?

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Indoor phenotyping platforms allow measurement of tens of traits at single plant level over hundreds of plants (e.g. plant architecture, light interception, leaf expansion and its responses to environmental conditions), and of physiological measurements in reproducible conditions (e.g. stomatal conductance, transcripts of key genes or hormone concentrations). To what extend there are predictive of yield in the field? We carried out a study with four experiments in a phenotyping platform with 246 maize hybrids that were also grown in 27 field experiments. The variance of yield in the field was largely accounted for by traits and QTLs measured in the platform. The contribution of traits QTL to yield was higher than that of random regions of the same size, and differed between environmental scenarios: QTLs of stomatal conductance had a positive effect on yield in favourable conditions and negative in water deficit; QTLs of water use efficiency had a positive effect under high evaporative demand only. Conversely QTLs of plant architecture positively contributed to yield and grain number in cool and well-watered scenarios. This opens the way for the use of crop models predicting yield in thousands of fields, based on traits and parameters measured in phenotyping platforms for hundreds of genotypes and, potentially, thousands of genotypes if parameters can be derived by genomic prediction.

GENETIC DIVERSITY OF MAIZE INBRED LINES ASSESSED BY SNP MARKERS

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Information on molecular genetic diversity of maize inbred lines provides more efficient usage of local germplasm for determining breeding strategies. In this study, the assessment of genetic diversity among fifteen perspective maize inbred lines, developed in Maize Research Institute, was done utilizing 25k SNP Illumina Infinium Array. The results of these analyses showed that the lowest and the highest genetic distances among chosen maize genotypes were 0.08 and 0.48 respectively, with an average of 0.40, thus indicating a high level of dissimilarity among inbreds. Polymorphism Information Content (PIC) was in a range from 0.12 to 0.38, with an average of 0.29. According to PIC values, most of the markers were moderatly infomative. The SNPs diversity ranged from 0.12 to 0.50. Gene diversity for most of the SNPs (88.3 %) fell between 0.30 and 0.50. Observed heterozygosity values ranged from 0.00 to 0.07 and only 2% of markers were heterozygous. Only one inbred expressed heterozygosity of 2%. Mean observed heterozygosity of 0.002 and high inbreeding coefficient confirmed a high level of inbreeding per genotype. The clustering, PCA and STRUCTURE analyses divided inbred lines in groups generally consistent with defined heterotic groups.

Keywords: *homozigosity*, *Single Nucleotide Polymorphism*, *Zea mays* L.

MOLECULAR CHARACTERIZATION OF POPCORN INBREDS USING 25K SNP ARRAY

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Popcorn is a type of specialty maize and plays important role in the history and the spread of this species. This type of maize historicaly received little atention in genetic diversity studies while the fact is that information on genetic variability and population structure is of crucial importance for improvement of breeding programs. The genetic variation of popcorn maize was mainly analysed using SSR markers, but there are not many studies applying high-density SNP markers with lower genotyping errors. The aim of this study was the assesment of genetic diversity and population structure of popcorn germplasm which represents the genetic resources currently used in breeding programs in Maize Research Institute „Zemun Polje“. Molecular characterization of 44 popcorn genotypes was done using 25k SNP Illumina Infinum Array for maize. After quality control filtering, 12335 SNPs were selected for downstream analyses. Genetic distance detected ranged from 0.001 to 0.51 with an average of 0.33. The majority of genetic distances (70.6%) fell between 0.30 and 0.51 revealing significant genetic variability in a set of maize genotypes under study. SNP markes were moderatly informative with an average PIC value of 0.26. Also, for more than 50% of SNPs, PIC value was greater than 0.30. The average observed heterozygosity was 0.04 ranging from 0 to 0.11. The clustering, principal component and model-based STRUCTURE analyses showed similar grouping of popcorn lines, generally in accordance with their genetic background. The results of this research gave valuable information for initiation of future breeding strategies.

Keywords: *popcorn maize, SNPs, genetic diversity, population structure*

GENETIC DISTANCE OF MAIZE INBREDS BASED ON SSR MARKERS FOR PREDICTION OF HETEROSIS

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Microsatellites are a powerful tool in detecting the genetic diversity of maize inbred lines and classifying them into different heterotic groups which is very important in maize breeding programs. It is well known that estimated genetic distances between parental lines might be a good indicator of the heterosis level of their hybrids. The objective of this study was to evaluate the possibility to predict grain-yield heterosis of maize hybrids using the estimated genetic distances between their parental inbred lines obtained by SSR molecular markers. Seven maize inbred lines of different heterotic origin and maturity groups and twenty-one maize hybrids developed by crossing these seven lines according to the half-diallel mating system were selected for this study. Estimation of genetic distances (GDs) between inbred lines was performed using 34 SSR primers which detected a total of 118 alleles with an average of 3.47 alleles per marker. The yield trials of both lines and hybrids were set up in a randomised complete block design with three replications at two locations during 2017. ANOVA showed a statistically significant influence of both location and genotype factors on grain yield. High-parent heterosis (HPH) for all hybrid combinations was calculated based on yield results. Pearson's correlation coefficient between GD and HPH was positive and statistically significant (r=0.6496). The highest estimated GD (0.55) was recorded between lines ZPL2 and ZPL6, and HPH value of their hybrid was 197.66%. On the other hand, both ZPL1xZPL4 and ZPL2xZPL4 hybrids showed the highest HPH values (251.36% and 264.83%, respectively) of all evaluated hybrids, while the estimated GDs between their parental lines were lower (0.47 and 0.44, respectively) compared to estimated GD between lines ZPL2 and ZPL6. The results indicated that estimated genetic distances among parental inbred lines obtained by SSR markers could only partially predict grain-yield heterosis of their hybrids.

Keywords: *inbred lines, heterosis, genetic distance, SSR markers*

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