5th European Maize Meeting

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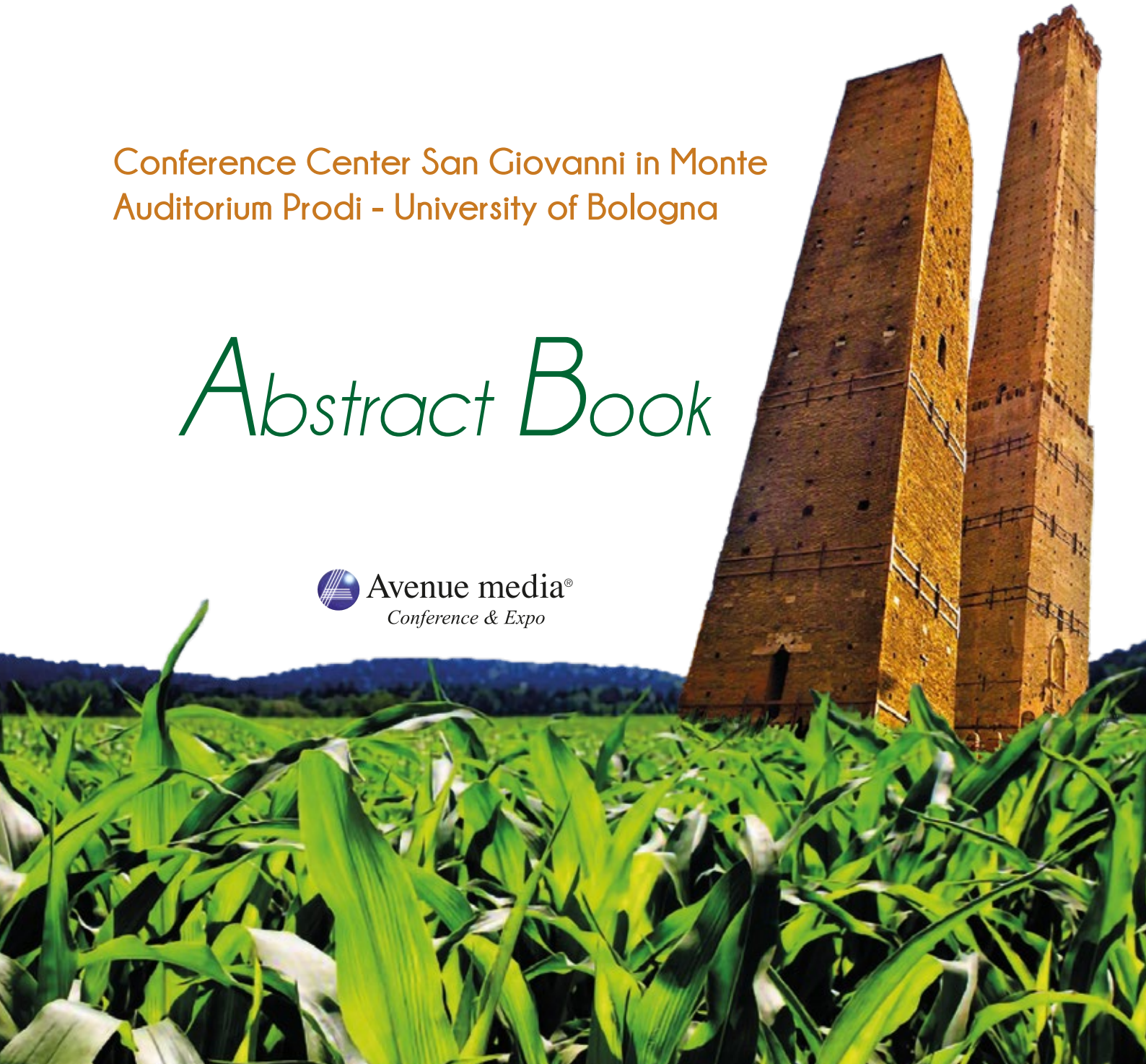
Abstract Book



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5th European Maize Meeting

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5th European Maize Meeting

INVITED SPEAKERS

Genetic control of maize meristem function
Andrea Gallavotti
Waksman Institute of Microbiology Department of Plant Biology Rutgers, The State University of New Jersey

Transposable elements contribution to maize tissue differentiation
Clémentine Vitte
CNRS, GQE-Le Moulon, Paris Saclay

The outlandishly dynamic LTR-retrotransposons and Helitrons in maize and other flowering plants
Jeff Bennetzen
University of Georgia, Athens, GA, USA

Genetic control and functional utility of root architectural and anatomical traits for soil resource capture
Hannah Schneider
Department of Plant Sciences – Wageningen University

Maize domestication contributes to root architecture and rhizosphere microbiome assemblage
Peng Yu
Emmy Noether Group Root Functional Biology, INRES, University of Bonn, Germany

High-throughput cistrome analysis links diversity in transcription factor binding to trait variation
Julia Engelhorn
Heinrich-Heine-Universität Düsseldorf /Max Planck Institute for Plant Breeding Research

Convergent selection of a WD40 protein that enhances grain yield in maize and rice
Xiaohong Yang
China Agricultural University, Beijing, China

European sustainable agriculture through Genome Editing - the role of scientists in policy making
Oana Dima
VIB Center for Plant Systems Biology, Ghent, Belgium

The outlandishly dynamic LTR-retrotransposons and Helitrons in maize and other flowering plants

Jeffrey L. Bennetzen1, Daniel C. Frailey1, Rocco J. Giarratano1 and Hongye Zhou1

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Along with polyploidy, transposable element (TE) action is the primary determinant both of plant genome structure and of de novo change in plant gene function. After insertion, many plant TEs (especially retroelements and Helitrons) lack the ability to excise, so they can be used to detect rates, types and outcomes of genome sequence variation in regions that are often selectively neutral. Comparison of the instability of genes to these "dead-on-arrival" (DOA) TEs can indicate the influence of selection on the mutations that persist in and near genes. Using PCR across pools of pollen grain, we were able to determine the rate of conversion of LTR (long terminal repeat) retrotransposons in maize into solo LTRs by homologous unequal recombination. This study also indicated the various factors that determine which LTR retrotransposons generate solo LTRs and which do not. By investigating DOA Helitrons, we were able to demonstrate the nature, mechanisms and astounding rates of maize genome change over the last few million years across 26 maize lineages, while consistent annotation of TE content across dozens of sequenced plant genomes indicated the patterns, or lack thereof, in the activation/amplification/silencing of specific TE families in flowering plants.

European Sustainable Agriculture Through Genome Editing - the role of scientists in policy making

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The EU has set ambitious targets in the Farm to Fork strategy of the Green Deal to make agricultural production more sustainable. Today, following pandemics and the current geopolitical situation, it is even more clear that we need immediate solutions for food security. Genome editing of plants has the potential to make a critical contribution to this transition because it enables the development of the next generation of crops with high precision and efficiency.
However, the current EU legislation subjects all crop varieties obtained using genome editing techniques under strict GMO regulations, which de facto blocks Europe from using and cultivating these crops. The European Sustainable Agriculture through Genome Editing (EU-SAGE) network, representing researchers from 150 leading European plant science institutes and learned societies, is convinced that Europe needs to enable applications of genome editing through developing science-based policies. Today, I will present to you how researchers in Europe are contributing to the policy environment for instance with the launch of an interactive, publicly accessible online database.

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SHORT SPEECHES

Unlocking the genetic architecture of local adaptation of European maize landraces using individual plants populations.

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One future breeding challenge for staple crops such as maize is heat and drought stress which can render the current commercial cultivars unfit for sustainable food production. Therefore, breeders are compelled to increase the fitness of current cultivars through continuous gene introgression. This breeding process is time ineffective thereby provoking our concept of using individual plants from segregating populations directly for breeding and selection.
We constructed a plant panel individual plants from 40 European maize landraces, preselected to capture the continent's geographical spread. Genotyping-by-sequencing (GBS) for randomly selected 340 individuals (approximately 10 individual plants per population) gave 233,002 SNP markers. Exploring this SNP data, we unravel the effect of elevation and latitudinal gradient on population structure giving rise to three main groups. These features (elevation and latitude) also influenced the genetic diversity parameters such as heterozygosity, inbreeding, and molecular variance within and between groups and populations. Interestingly, we adopted these features as traits and identified 37 and 41 marker-trait associations for elevation and latitude respectively. Search for candidate genes revealed 235 and 114 genes for elevation and latitude respectively with associated biological and molecular functions to local adaptation.
Very few studies have focused on characterizing European maize landraces, especially with high-throughput sequencing methods such as GBS. Genetic diversity properties as discovered in this study will help breeders and researchers to make informed selections of landraces in the future. More importantly, identified QTLs and genes can be further investigated for marker-assisted selection. Finally, we suggest the possibility of using individual plants from segregating populations for genome-wide association and prediction studies to optimize genetic gain in time for selection and breeding programs.

Ethylene response factor (erf) genes connect lateral root development and mycorrhizal symbiosis in maize

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Mycorrhizas are among the most important mutually beneficial interkingdom interactions between soil fungi of the subphylum Glomeromycotina and most land plants. The three-dimensional architecture of cereal root systems is a prerequisite for the efficient capturing of water and nutrients and the establishment of mycorrhizal symbioses. Nevertheless, the molecular mechanisms that underpin these morphogenetic processes during the establishment of mycorrhizal symbiosis are still unclear, particularly for non-legume plants such as the cereal maize. In the present study, genetic and molecular experiments identified members of the ethylene response factor (erf) gene family as central players connecting lateral root development and mycorrhizal symbiosis in maize. Multi-omics integration demonstrated that AM fungi established functional association with the developmental status of host roots via reprogramming of flavonoid metabolism and its homeostasis. High flavone content in root extracts and its exudates in turn led to an enrichment of Oxalobacteraceae and thereby positively affecting lateral root formation. The modulation of root system architecture in response to environmental resilience can be considered as an important target for crop genetic improvement by exploiting their beneficial interaction with soil microbes.

Live cell imaging of meiosis in maize

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Meiosis is a specialized cell division characteristic of eukaryotes and essential for sexual reproduction. During meiosis, one round of DNA replication is followed by two chromosome segregation events which eventually lead to the formation of four daughter cells with half the genetic material of the parental cells and with a new assortment of alleles. Hence, meiosis is key to genetic diversity and lies at the heart of plant breeding since new, possibly advantageous, allele combina%ons can be generated. So far, most studies of meiosis in plants have relied on genetic analyses and on cytological observations of fixed material, which, despite informative, can capture the underlying cellular dynamics only to a small degree. Here, we present our set-up of a live cell imaging system to follow meiosis in maize. The method relies on the observation of stable transgenic lines producing fluorescently labelled proteins that highlight specific aspects of meiosis. Spikelets from plants carrying the construct of interest are sampled, cultured on medium and imaged over time by confocal laser scanning microscopy. Our first live-cell imaging data rely on the concomitant observation of two reporter lines, DSY2 to visualize chromosomes and TUB2 to monitor microtubules. Their dynamic accumulation pa1ern sets the basis for the establishment of a landmark system to precisely analyze meiotic progression, temporally dissect meiosis and eventually define a cytological framework of meiosis in a crop model system, such as maize.

BREEDIT: a multiplex genome editing strategy to improve complex quantitative traits in maize

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BREEDIT aims at developing a flexible pipeline that combines multiplex gene editing of 60 genes related to growth and yield-traits and different crossing schemes to generate plants with modified traits (**Lorenzo et al., 2022**). By using a Cas9-expressing maize line, called EDITOR, 12 candidates genes were simultaneously targeted using one transformation construct, named SCRIPT. The single SCRIPT edited populations displayed (on average) 5% –10% increases in leaf length and up to 20% increases in leaf width compared with controls. Single SCRIPT top performers were chosen for selfing and/or crossing to stack up to 24 gene edits. After the cross, edited alleles in the interscript became heterozygous and new transgenerational gene editing occurred, expanding the edit profile. Selfing was used to increase gene dosage or plants were subjected to another round of crossing with other single SCRIPT line to expand the total gene space of edited alleles in a triple SCRIPT population (up to 36 edits). While screening the populations of double and triple interscript plants unexpected phenotypes were observed. All of these quantitative and even qualitative data were subjected to AI models to determinate the nature of the causative genes affecting the desired trait.

Double fertilization defects in maize haploid inducer lines

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Sexual reproduction in flowering plants involves a unique process named double fertilization. It is characterized by two separate fusion events between the male and female gametes. A maize line first reported in the 60s deviates from this classic pattern: Crosses using pollen from this so-called haploid inducer line, trigger the development of the egg cell into a haploid embryo with only the maternal genome. Although this in planta haploid induction is a powerful tool for maize breeding, the underlying molecular mechanism remains elusive.
To understand the double fertilization defects occurring during haploid induction, a specific protocol was developed to visualize the sperm cell nuclei within the maize embryo sac. Pollination by a fluorescent sperm cell marker line was combined with ovule fixation and clearing technics to characterize maize double fertilization by confocal imaging. This cell biology pipeline was applied for two mutants involved in haploid induction disabled either for the phospholipase NOT-LIKE-DAD (NLD, also known as MTL or ZmPLA1) or the DUF679 domain membrane protein ZmDMP. Our work demonstrates that both mutants have fertilization defects an increased number of unfused sperm cells. Interestingly, nld single and nld/dmp double mutants show central cell single fertilization, which could explain the correct endosperm formation in kernels having a haploid embryo. Altogether, by combing genetics and cell biology approaches, we provide new insights into maize double fertilization and in the mode of action of haploid induction.

Mapping photosynthetic efficiency 'from pot to plot' using a maize multiparent population

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Improving photosynthesis is recognized as an underutilized strategy to boost crop productivity. In maize, slight improvements of yield can result in significant impacts on global food security. Within the framework of the H2020 project CAPITALISE, we map photosynthetic efficiency in maize, using an eight-way maize Multi-parent Advanced Generation Inter-Cross (MAGIC) population.

We selected and genotyped 347 MAGIC RILs along with their founders with Single Primer Enrichment Technology (SPET). The same lines were phenotyped at high-throughput in the IPK non-invasive phenotyping system for large plants: kinetic Chlorophyll fluorescence on dark-adapted plants was measured once a week, for five weeks while daily imaging data were collected during the whole experiment (32 days).

The panel was also grown in two field experiments at NIAB (UK), in 2021 and 2022. Here, high resolution photosynthetic parameters, including light-saturated photosynthetic gas exchange, leaf reflectance and NPQ induction and relaxation were measured after silking.

BLUPs from both approaches were used for QTL mapping that allowed to identify common regions underling large variation on photosynthetic efficiency. Identified signals vary in intensity at different daily and weekly time points as well as different spectra measurement stages from data of field phenotyping. Parental coefficients estimated at QTL loci were used to test differential expression using founders' transcript levels at fourth leaf stage. We identified a set of candidate genes, including components of the antenna complex of photosystem II. Results showed that combining phenomics approaches can support mapping of complex traits.

Keywords: MAGIC population, QTL mapping, photosynthesis.

Analysis of genetic components to improve cuticle-dependent leaf permeability in maize.

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Plant resistance to drought conditions implies several mechanisms, among which the control of water loss. During drought events, stomatal opening is reduced to minimise transpiration and the rate of water loss is determined by the transpiration mediated by the cuticle, the outer hydrophobic barrier covering the epidermis. The cuticle is composed of two main layers, an inner layer composed by cutin embedded with waxes and an external layer formed by epicuticular waxes. The chemical composition of both layers varies, among organs, and across developmental stages. In maize, cuticle undergo profound changes in young and adult leaves and their different chemical composition might exert a different impact on leaf permeability. To address this question, mutants in two key transcription factors have been characterized in this work.
GLOSSY15 (ZmGL15) is an APETALA transcription factor that regulates the transition from the juvenile to the adult vegetative phase. FUSED LEAVES1 (ZmFDL1) is a MYB transcription factor controlling cuticle deposition in juvenile leaves. Our results show that in juvenile leaves lacking ZmGL15, the observed glossy phenotype, which is peculiar to adult leaf, is ascribable to changes in cuticle biosynthesis. Moreover, the transcript quantification, the chemical analysis and the analysis through chlorophyll leaching assay suggest that ZmGL15 and ZmFDL1 interact to regulate the deposition of the juvenile cuticle.
The chemical analysis of the different genotypes has also allowed to identify those cuticle components that cause a reduction in leaf permeability and thus improve cuticle properties, i.e., cuticle capability to response to drought stress.

Non-renewable phosphorus in agriculture: the potential of low-phytate maize mutant and two approaches to restore seed germination.

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Conventional agriculture relies on chemical inputs, and phosphorus (P) is obtained from rock phosphate, a limited and non-renewable resource. In seeds, the main reserve of P is phytic acid (PA), a strong anti-nutrient: monogastric animals assimilate only 10% of PA in the feed, while 90% is excreted, becoming a pollutant, and causing eutrophication. Hence, the reduction of PA in seeds has become an important challenge in breeding programs and many low phytic acid (lpa) mutants have been isolated in major crops. In maize, lpa1-1 is the most promising, showing a 66% reduction in PA, followed by a proportional increase in free P. Nevertheless, the reduction of PA leads to many adverse pleiotropic effects that can affect seed germination and, in general, plant performance.

Here I present the main results obtained with lpa1-1, highlighting the benefits and challenges of low-phytate mutants. A multi-year field experiment was set up and it was found that lpa1-1 had a comparable (or even better) seed weight/ear than the wild-type; the main problem was the reduced field emergence (~40%), which consequently led to lower yields. To restore germinability, two possible approaches have been proposed: i) a conventional breeding approach; ii) a seed priming approach. It was found that germinability improved by 20% in lpa1-1 seeds with a hydropriming treatment, suggesting a possible role of seed priming in restoring germination rates. Overall, low-phytate crops have the potential to improve the long-term P sustainability in agriculture, but more research and support is needed to optimize their performance.

Evaluation of genome and base editing tools in maize protoplasts

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Despite its rapid worldwide adoption as an efficient mutagenesis tool, plant genome editing remains
a labor-intensive process requiring often several months of in vitro culture to obtain maize mutant plantlets. To avoid a waste in time and money and to rapidly test the efficiency of molecular constructs or novel Cas9 variants (clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9) prior to stable transformation, rapid analysis tools are helpful. To this end, a streamlined maize protoplast system for transient expression of CRISPR/Cas9 tools coupled to next generation sequencing analysis and a novel bioinformatics pipeline was established. Mutation types found with high frequency in maize leaf protoplasts had a trend to be the ones observed after stable transformation of immature maize embryos. The protoplast system also allowed to assess the activity of two Cas9 variants, xCas9 and Cas9-NG, with relaxed PAM (protospacer adjacent motif) sequences, which increase the choice of target sites for genome editing, albeit with decreased frequency of mutation. Finally, efficient base editing in maize could be achieved for certain but not all target sites.
Phenotypic analysis of base edited mutant maize plants demonstrated that the introduction of a stop codon but not the mutation of a serine predicted to be phosphorylated in the basic helix loop helix transcription factor ZmICEa (INDUCER OF CBF EXPRESSIONa) caused abnormal stomata, pale leaves and eventual plant death two months after sowing. Altogether, an optimized maize protoplast system was developed to rapidly evaluate CRISPR/Cas9 tools before engaging resource-consuming stable transformation.

Selective enrichment for alleles conferring adaptation to Green Revolution practices in gene bank maize accessions of Southeastern Europe

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Maize cultivation in Southeast Europe (SEE) is comparable to that in the Corn Belt region of the United States, with a similar dent germplasm. Historically, this region has undergone several genetic material swaps, including the significant importation of accessions through US aid programs after WWII. These accessions were admixed with previously adapted germplasm to support the transition to single-cross breeding. A subset of this historical germplasm, stored in the Maize Gene Bank of the Maize Research Institute Zemun Polje (MRIZP) between 1960s and 1980s, was genotyped using the Affymetrix Axiom Maize Genotyping Array with 616,201 polymorphic variants. Admixture analysis revealed seven ancestral populations, with the absence of Iodent germplasm in the subset of inbreds with SEE origin, reflecting its historical context. Further analysis identified several signatures of selection at specific chromosome regions, including chromosomes 1, 3, 6, 7, 8, 9, and 10. These regions were mined for protein-coding genes and used for gene ontology (GO) analysis, which showed a highly significant overrepresentation of genes involved in response to stress. Our findings suggest the accumulation of favorable allelic diversity in the genetic resources of SEE, particularly in the context of changing climate and the adaptation to Green Revolution practices. Overall, our results provide insights into the genetic diversity of maize in Southeast Europe and its potential for contributing to the development of new maize varieties with improved adaptation to changing agricultural practices and environmental conditions.

Keywords: maize genomics, high-density, selective sweep, admixture

Unravelling the genetic architecture of lateral root length in maize

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In maize, the genetic control of root traits is largely unknown, despite their importance for adaptation and yield potential. In order to disentangle the genetic architecture of lateral root (LR) length in maize, we crossed two doubled-haploid (DH) lines contrasting for the trait, generating large mapping populations that we phenotyped in different developmental stages in controlled and field conditions, including a low phosphorous (low P) treatment.

We mapped QTL on several chromosomes for LR length overlapping in reproductive stages R2 and R6 that we could not find in the vegetative stages (V3, V5), indicating that different genes are affecting LR length in different stages of root development, while their effects remain stable from flowering (R1) until senescence. The low P treatment in the field had a significant, albeit moderate to small effect on the traits early vigour, chlorophyll content, root weight, and grain water content. LR length is positively correlated with root dry weight (r=0.6) and we observed a weak, positive correlation of these root traits with plant height in stage R2, flowering time, chlorophyll content in stage V6 and R2 and shoot biomass.

We will fine map the major QTL for LR length to describe the gene(s) involved in trait expression and the underlying molecular mechanism(s). Furthermore, we will test the nitrogen use efficiency of genotypes contrasting for LR architecture in the field, with the final goal of using novel alleles from maize landraces for elite germplasm improvement with a targeted approach.

Toward a sustainable maze cropping: X-omics support the understanding of the effects of some biotechnological practices

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The adoption of more sustainable practices in maize cropping may be hampered by the challenges posed by climate change if the same do not also improve the resilience of the system to more stressing environment. The exploitation of beneficial microorganisms as bioinoculants appears as an environmental-friendly biotechnological tool in maize cropping as they can reduce requirements of inorganic fertilizers by positively influencing soil fertility. Biochar addition to soil is a promising strategy for climate change mitigation and soil fertility improvement. The objectives of this study were to unravel the synergistic effect of co-applied biochar and synthetic microbial consortia (SMC) or arbuscular mycorrhizal fungi on maize growth, soil microbiome and grain metabolome. Field experiments were carried out in two growing seasons in Italy. At different vegetative growth stages, both physiological parameters and soil chemical composition were evaluated. At harvest, yield performance was assessed, and maize kernels were collected to perform global metabolomic profiling. Rhizosphere microbial ecosystem was investigated by 16S metabarcoding sequencing and bioinformatic tools. SMC application did not significantly affect the microbial communities in terms of diversity and richness of species, with a low risk of a long-term impact on the ecology of the indigenous microbial population. However, biochar exerted a great impact on rhizosphere soil microbiome, suggesting that functionalization of biochar with SMC seems a promising approach for microbiome modulation and for enhancing plant growth also in limiting environments. Larger effects were found on the grain at metabolomic level on the presence of different fatty acids, aminoacids, and lipids.

A Common Resequencing-Based Genetic Marker Dataset for Global Maize Diversity

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Maize (Zea mays ssp. mays) populations exhibit vast amounts of genetic and phenotypic diversity. As sequencing costs have declined, an increasing number of projects have sought to measure genetic differences between and within maize populations using whole genome resequencing strategies, identifying millions of segregating single-nucleotide polymorphisms (SNPs) and insertions/deletions (InDels).

Unlike older genotyping strategies like microarrays and genotyping by sequencing, resequencing should, in principle, frequently identify and score common genetic variants. However, in practice, different projects frequently employ different analytical pipelines, often employ different reference genome assemblies, and consistently filter for minor allele frequency within the study population. This constrains the potential to reuse and remix data on genetic diversity generated from different projects to address new biological questions in new ways. Here we employ resequencing data from 1,276 previously published maize samples and 239 newly resequenced maize samples to generate a single unified marker set of ~366 million segregating variants and ~46 million high confidence variants scored across crop wild relatives, landraces as well as tropical and temperate lines from different breeding eras. We demonstrate that the new variant set provides increased power to identify known causal flowering time genes using previously published trait datasets, as well as the potential to track changes in the frequency of functionally distinct alleles across the global distribution of modern maize.

From a proteomic dataset to characterization of novel plant peptides

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Despite their recognition as key players in defense, development, physiology, and cell-to-cell communication, thus far only a few peptides have been discovered and functionally characterized in crop plants. Recent proteomic and transcriptomic studies on different stages of maize male flower development implicate peptides as coordinators of growth and cellular behavior from inception through meiosis to dispersal of mature pollen. Evidently, a correct organ-developmental program is a prerequisite for the biotrophic corn smut fungus Ustilago maydis to successfully infect its host. These observations indicate that yet unexplored and functionally uncharacterized peptides play pivotal roles in male flower development and susceptibility to pathogens.
With the aim to identify novel peptides, we selected CANDIDATEs (CANs) from maize proteomic data. These CANs were subjected to a screening pipeline, which utilized the recently established U. maydis-based Trojan horse approach among other experiments. Up to now we identified six novel peptides. Further analysis of two CANs revealed unique features, such as conservation among Angiosperms, protein localization, and phenotypes in planta. Here, we will present latest results on the characterization of these two CANs.

Characterization of exosome-like vesicles from maize pollen and their potential role in sRNA trafficking

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In plants, small RNAs (sRNAs) generated in vegetative cells have been reported to have regulatory activity in sperm cells, but the mechanisms of sRNA trafficking remain unknown. To explore mechanisms of sRNA transport in pollen, we isolated sperm cells and vesicles from mature maize pollen and performed sRNA-seq and proteomics analysis. Intact membrane vesicles were purified and fractionated by sucrose density gradient ultracentrifugation.
Comparative analysis of sRNA content in fractionated vesicles, sperm cells, and whole pollen revealed that the sRNA present in sperm cells is significantly enriched in multiple fractions compared to that in the whole pollen. The size distribution of enriched sRNA varies among fractions, with the 1.10g/ml density fraction of vesicles being particularly distinctive, as it was found to be enriched with the 22-nt RNA detected in sperm cells. LC/MS analysis showed that the 1.10 g/ml fraction was enriched in sRNA-binding proteins such as Argonautes and DEADbox ATP-dependent RNA helicases, which were previously found in Arabidopsis exosomes. Components of COPII vesicles, which are known to bud from the endoplasmic reticulum (ER) were also enriched in this fraction. Negative staining TEM revealed that four types of vesicles were present in this fraction and two of them were budding from ER like structures. Further experiments are required to determine whether sRNA-containing vesicles are derived from ER and are destined to sperm cells. However, our study provides first evidence of vesicles containing sRNA in pollen, and suggests their involvement in sRNA transport.

Trans-acting small RNAs as a positional signal in specifying the embryonic shoot stem-cell niche in maize

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Unlike the highly stereotyped pattern of cell divisions and cell fate decisions typical for the Arabidopsis thaliana embryo, embryogenesis in monocots follows a relatively random, unpredictable cell division pattern to establish a multicellular early embryo with apical-basal and radial polarity. In addition, adaxial-abaxial polarity, characteristic for the monocot embryo, is evident at the subsequent transition stage and triggers initiation of the shoot stem cell niche and the elaboration of the embryonic shoot-root axis at the face opposite to the endosperm. This critical event in embryogenesis is instructed by lateral positional signals. The identity of these essential positional signals and the genetic regulatory networks that these signals connect into, however, remain poorly understood. Here, we show that the mobile small RNA tasiARF forms an expression gradient at the presumptive shoot apical meristem (SAM) of the transition stage maize embryo. tasiARF creates a local expression minimum of its ARF3 transcription factor targets that is critical for formation of the embryonic SAM at the adaxial face. Mutations in tasiARF biogenesis components lead to ARF3 misexpression in the presumptive SAM and fail to allow the embryonic SAM close to the epidermis via effects on expression of regulators involved in maintenance of meristem properties, organ patterning, auxin transport and signaling, and cell wall stiffness in particular. Interestingly, these defects are quantitatively tuned by natural variation at a locus coding for a microtubule-associated protein, whose expression is repressed in tasiARF biogenesis mutants and essentially contributes to embryonic SAM formation, implying its direct regulation by misexpressed ARF3. Our research thus provided a mechanistic framework of trans-acting small RNA-instructed initiation of the shoot stem-cell niche in the monocot embryo.

Genetic diversity within a collection of Italian maize inbred lines: a resource for maize genimics and breeding

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Core collections are an essential tool to dissect and analyse genetic diversity in crops. A panel of 384 lines, a subset of a larger one preserved at CREA Bergamo Genebank, which includes inbred lines derived from traditional Italian maize landraces and advanced breeding ones (Elite Inbred Lines), was subject to extensive SNP search through the tGBS® Genotyping by Sequencing technology. Following an imputation and filtering process based on the percentage of missing data, redundant markers and the frequency of the rarest allele, a final dataset of 15,872 SNP markers that were physically mapped was defined for further analyses. In particular, the inbred lines panel was characterized for linkage disequilibrium (LD), genetic diversity, population structure and genetic relationships. The analysis of the population structure, carried out with different clustering methods, showed stabile grouping statistics for four groups, which could be mainly referred to groups of 'Insubria', 'Microsperma', 'Scagliolino', and a fourth group with prevalence of elite lines derived from Italian and U.S. breeding programs. LD decay at genome wide level indicates the collection as a valuable resource for genome wide association mapping (GWAS). Indeed, a preliminary GWAS analysis was carried out on data derived from a field trial in which a subset of 109 lines has been evaluated for main agronomic traits in an exceptionally dry season. On the basis of these data, the CREA Italian maize collection once genetically characterized can represent an important tool for the identification and study of useful traits/alleles and for their use in maize breeding.

Insights into the regulatory mechanisms of a major flowering time QTL in maize

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Flowering time is an essential adaptative trait for crop breeding to local environments and environmental stresses avoidance. A major quantitative trait locus (QTL) for flowering time and number of nodes, qVgt3.05 (Vgt3) was previously identified on chr. 3 by using a mapping population deriving from the cross between B73 and the extremely early flowering Canadian – origin landrace Gaspé Flint. Vgt3 was finely mapped to a 380-kb genomic region including the already known flowering time gene Mads69 by positional cloning. The involvement of Mads69 in flowering time gene was confirmed by overexpression and downregulation experiments. Examination of sequence alignment for the Mads69 coding sequence of Gaspe Flint and B73 did not reveal polymorphisms. Comparative genomics analysis showed Mads69 expression variation, both in terms of quantity and alternative splicing, is associated with structural variation. The earliness-inducing allele carried by Gaspé Flint appears of ancient origin, is present in tropical maize and likely contributed to adaptation to high-land and high-latitude environments, whereas the late-inducing allele was likely subsequently derived by transposon insertions.

NDH mediated cyclic electron flow is required for cold tolerance in the Austrian maize landrace 'Kemater Landmais Gelb'

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Maize (Zea mays L.) has the second highest production quantity and harvested area of all crops worldwide (FAO 2021) but its productivity remains limited in temperate European climate due to frequent cold events during early growth. In a genome-wide association study (GWAS) we identified a QTL overlapping for the cold-tolerance related traits early plant height, early vigor and maximum potential quantum yield of Photosystem II (Fv/Fm) in the Austrian landrace 'Kemater Landmais Gelb' (KE). The photosynthetic machinery of maize is prone to cold stress thus we assume a common underlying factor and dissected the QTL. We validated the association with early development in field experiments in a bi-parental population derived from two doubled-haploid KE lines contrasting for the target QTL. Further, we confirmed an effect on Fv/Fm in a controlled cold-stress experiment and fine-mapped the QTL to a 0.64 Mb genomic segment, comprising eight gene models. The candidate region contains two copies of ZmNdhM, a structurally important component of NAD(P)H dehydrogenase-like complex (NDH). Analysis on RNA and protein level indicate that one copy of ZmNdhM is alternatively spliced between the parents of the population, likely causing the differences in early development and cold tolerance due to reduced cyclic electron flow. In the future, we will validate our findings by mutant analysis, evaluate allelic effects of the selected candidate gene and develop makers to use them in breeding. Our work is a successful example on how to extract genes associated with quantitative traits of interest from locally adapted landraces.

Immature leaves are the dominant volatile sensing organs of maize

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Plants perceive herbivory induced volatiles and respond to them by upregulating their defenses. So far, the organs responsible for volatile perception remain poorly described. Here, we show that responsiveness to the herbivory induced green leaf volatile (Z)-3-hexenyl acetate (HAC) in terms of volatile emission, transcriptional regulation and defense hormone activation is largely constrained to younger maize leaves. Older leaves are much less sensitive to HAC. In a given leaf, responsiveness to HAC is high at immature developmental stages and drops off rapidly during maturation. Responsiveness to the non-volatile elicitor ZmPep3 shows an opposite pattern, demonstrating that hyposmia is not driven by defective canonical defense signaling. Neither stomatal conductance nor leaf cuticle composition explain the unresponsiveness of older leaves to HAC, suggesting perception mechanisms upstream of canonical defense signaling as driving factors. Finally, we show that hyposmia in older leaves is not restricted to HAC, and extends to the full blend of herbivory induced volatiles. In conclusion, our work identifies immature maize leaves as dominant stress volatile sensing organs. The tight spatiotemporal control of volatile perception may facilitate within-plant defense signaling to protect young leaves, and may allow plants with complex architectures to explore the dynamic odor landscapes at the outer periphery of their shoots.

The European mutant resource BonnMu and its application in gene function analyses in maize

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BonnMu is a European transposon-tagged mutant collection for forward and reverse genetics analyses in maize. To establish the resource, we crossed active Mutator (Mu) lines into the inbred lines B73, Co125, F7, EP1, and DK105. By now, we generated 7,992 mutagenized F2-stocks and sequenced them by the Mu-seq approach. To identify Mu insertion sites in the F2-stocks we created an automated Mutant-seq workflow utility. Up to now, we identified 553,967 unique Mu-insertions affecting 36,612 of 39,756 (92%) high-confidence gene models of maize. Mu transposons showed obvious preferences regarding their insertion sites in the genome, because the majority of insertion sites (94%) was located in genic regions. Such insertions most likely disrupt gene function. Only 6% of the insertions tag non-coding intergenic sequences of the genome.

In a forward genetic screen of 10-day-old seedlings of the Mu-tagged F2-stocks we identified amongst others the magenta root dwarf1 (mrd1) mutant. In comparison to the wildtype, mrd1 mutants are characterized by a dwarfed shoot and an over-accumulation of anthocyanins in primary, seminal, and crown roots. Preliminary work identified a constitutive photomorphogenesis 9 (cop9) signalosome complex subunit 4 (csn4) as a causal candidate gene that underlie the mrd1 phenotype. Currently, we are validating that mrd1 encodes csn4 by novel mrd1 mutant alleles generated by genome editing via CRISPR/Cas9.

Small RNAs as potential regulators of heterosis in maize

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Heterosis is defined as the superior performance of an F1 hybrid compared to its parents. Although heterosis is widely used in agriculture, it is poorly understood at the molecular level. The main models of heterosis postulate that genetic and epigenetic differences between the parents drive changes in gene regulatory networks, leading to a non-additive phenotype in the F1 hybrid. To complete this picture, we investigated the relationship between small RNAs (sRNAs) and heterosis, for sRNAs can regulate gene expression through different pathways. To identify heterosis-associated sRNAs (ha-sRNAs), we used grain yield heterosis data from 598 maize F1 hybrids and sRNA expression data from their 95 parents. We classified the hybrids into low and high-grain yield heterosis and identified sRNAs differentially expressed in the parents and preferentially associated with one heterosis group. We could identify about ~4.5 million unique ha-sRNAs, including ~30% that map to ribosomal DNA (rDNA). About 70% of ha-sRNAs mapping to rDNA are non-canonical in size, i.e., outside of the 21-24 nucleotide length range. Genome-wide, canonical and non-canonical ha-sRNAs are positively and negatively associated with heterosis, respectively. However, ha-sRNAs mapping to rDNA are predominantly negatively associated with heterosis. These results indicate that a significant fraction of ha-sRNAs is mapping to rDNA, non-canonical in length, and negatively associated with heterosis. We currently investigate the potential biological function of these ha-sRNAs in heterosis.

POSTER

PRIMA-DROMAMED: identifying new sources of adaptation to heat and drought within Mediterranean maize germplasm

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Key words: germplasm; climatic change; drought; heat stress; genetic resources; sustainable agriculture; maize

Maize, one of the most important worldwide crops, is particularly vulnerable to drought in a scenario of climate change. DROMAMED, a three years project (2021-2024) financed by the PRIMA call, intends to 1) valorize maize Mediterranean germplasm pooling and evaluating varieties from the national collections for adaptation to a large diversity of stressful environments, 2) capitalize current and new knowledge about mechanisms of tolerance to stresses, and 3) develop selection tools to improve breeding approaches enhancing tolerance.
With the aim to capitalize the diversity of maize for cultivation in the Mediterranean area, germplasm including accessions adapted to dry areas were pooled to identify a subset of landraces tolerant to abiotic stresses representative of biodiversity of the national collections.

The subset, including 215 genotyped landraces from dryland of Algeria, France, Portugal, Spain and Italy, was tested in multisite field experiments (6 locations) during 2022 in Italy, Tunisie, Morocco. In addition, breeding populations selected for abiotic stress tolerant traits were evaluated in Turkey, Algeria and Spain. Finally, Spanish populations included in the European Maize Landraces Core Collection (EUMLCC) and Tunisian populations were tested in Spain. Preliminary results about field trials will be reported. Plants experienced heat waves and diverse range of water deficit scenarios. Appreciable variation for plant development and ear set was observed.
Combined analysis of collegial field trials for key adaptation traits, together with marker association analysis, will allow us to capitalize new genetic variability from which extrapolate the varieties with greater resilience to face challenges derived from climate change.

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Regulation of Single-Parent Expression complementation in an IBM-RIL backcross population

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Genes active in one parental inbred line but inactive in the second lead to an extreme instance of gene expression complementation in maize (Zea mays L.) hybrids. This specific type of expression, which is denoted as single-parent expression (SPE), is in line with the classical dominance model of heterosis. The dominance model of heterosis explains the superior performance of F1-hybrids by the complementation of deleterious alleles by beneficial alleles in many genes. In this study, we aim to understand the regulation of single-parent expression complementation and its association with the phenotypic manifestation of heterosis. We survey the transcriptomic and phenotypic plasticity in a backcross population consisting of the two female inbred line B73 and Mo17, 112 genetically diverse recombinant inbred lines of the IBM-RIL syn4 population as male parents and their resulting F1-hybrids during early primary root development. Across all parent-hybrid triplets, we consistently observed expression complementation (i.e. activity) of hundreds of such genes, resulting in higher number of totally active genes in the hybrids than their parental inbred lines. Furthermore, we established that the number of SPE genes is significantly associated with mid-parent heterosis (MPH) for phenotypic traits. In the following steps, we aim to identify the nature and functions of regulatory elements controlling SPE genes and by constructing gene expression networks based on regulatory genes and their regulatory eQTLs.

The ABA-Hydroxylase gene family influences water use efficiency by modulating stomatal properties in maize

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In order to enhance sustainability in agriculture, it is crucial to have high-yielding crop cultivars with improved water use efficiency (WUE) and drought resistance. Research has shown that the development of leaf stomata and the movement of stomatal guard cells affect WUE. The concentration of the phytohormone abscisic acid (ABA) is a key factor in determining these stomatal characteristics. ABA levels in turn are controlled by ABA-8'-hydroxylases (ABH), which are the key enzymes involved in ABA catabolism. In maize (Zea mays L.), there are five ZmAbh genes, that code for five ABH homologs. To investigate the effects of all ZmAbh genes on drought-related traits, we generated CRISPR/Cas9 mediated null mutants. We could show that ZmAbh4 is predominantly expressed in fully developed maize leaves and two abh4 mutants showed reduced stomatal conductance and improved intrinsic WUE (iWUE), without apparent penalties in plant growth or changes in stomata development. Our most recent results suggest, that ZmAbh1 and ZmAbh2 alter stomatal development, thus playing an important role in regulating plant water balance in the leaves. To understand the additive effects of ZmAbh4, ZmAbh1 and ZmAbh2, we are characterizing double and triple mutants of these genes. In further experiments, transcription factors known to play a role in stomatal development and to be influenced by altered ABA levels will be investigated in double and triple ZmAbh mutants.

Mutants in ZmFdl1 and ZmGl2 genes affect cuticle deposition and Fusarium verticillioides growth on maize silks

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Maize silks, the stigmatic portions of the female flowers, have an important role in reproductive development. Silks also provide entry points for ear rot pathogens, such as Fusarium verticillioides, into host tissues, since fungal conidia move along the silks to reach the developing kernel. The outer extracellular surface of the silk is covered by the hydrophobic cuticle, comprised of a complex array of long-chain hydrocarbons and small amounts of very long chain fatty acids and fatty alcohols. The silk cuticle protects against abiotic and biotic stresses, and might play a role in modulating plant-fungus interactions. ZmMYB94/fused leaves1 (fdl1) encodes a R2-R3 MYB transcription factor previously shown to regulate cuticle deposition in maize seedlings. The glossy2 (gl2) gene, a putative member of the BAHD superfamily of acyltransferases, was shown to be involved in the elongation of the fatty acid chains of the waxes in the juvenile leaves of maize seedlings. Both genes are highly expressed in silk. In this work the effect of fdl1-1 and gl2-ref mutations on silk cuticular wax composition and cuticle surface morphology has been analyzed. Furthermore, fungal growth rates on fdl1-1 and gl2-ref mutant and relative wildtype silks, following inoculation with a Fusarium conidia suspension, have been compared. Data obtained indicate that FDL1 retains a regulatory role in silk cuticle deposition, while the action of GL2 affects the abundance of specific cuticular wax metabolites. Their functional study also provides a first characterization of the molecular mechanisms underlying cuticle-mediated responses to F. verticillioides infection.

Functional analysis of RALF gene family members in maize and their evolution

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The evolution of land plants has resulted in a wide range of complexity, from the earliest algal ancestors, through the nonvascular bryophytes and vascular seedless plants, to the complex gymnosperms and angiosperms of today. Compared to ancient plants, gametophytic communication systems in reproductive organs of angiosperms are more complex due to the evolution of specialized sexual organs allowing reproduction in dry environments. Various reports indicate that RAPID ALKALINIZATION FACTORs (RALFs) play key roles in short range gametophyte cross-talk during pollen hydration, germination, tube growth and reception in Arabidopsis. Also in grasses like maize, we recently found that ZmRALF2/3 are also involved in regulating cell wall integrity during pollen tube growth in maize. Phylogenetic and expression analysis of the maize RALF protein family have shown that different members are highly and specifically expressed in pollen grains and during tube growth in maize. ZmRALF2/3 belong to Clade I RALFs, which include the typical RALFs found in ancient plants. However, ZmRALF1/5 belonging to Clade III generate about 50% RALF transcripts in maize pollen tubes. Unlike typical RALFs, ZmRALF1/5 lack the conserved S1P protease cleavage sites, YISY domain and YY domain, which are important for interaction with LRR-extensin cell wall proteins. An overview about the evolution of the RALF family and new function(s) of ZmRALF1/5 and their origin are important questions that will be addressed.

Boron deficiency leads to lateral root defects in maize

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Boron is an essential micronutrient for plants. Its deficiency can cause various growth and developmental defects such as thicker and shorter roots. In maize, while the importance of boron for shoot growth and productivity is well-established, there is a need of more research on boron deficiency-induced effects on roots. Boron deficiency-induced defects are often correlated with cell walls, where most cellular boron is located and crosslinked with pectin. Recent findings of boron deficiency induced cell division defects, and boron-phytohormone relations, open up exciting avenues to reveal the molecular mechanisms affected by boron deficiency. The experimental induction of boron deficiency is challenging, as boron is ubiquitous. Phenylboronic acid (PBA), with a structure similar to boric acid, has been proposed as a boron deficiency mimic. Our study aims to characterize boron deficiency-induced defects in the maize primary root, using PBA as a boron deficiency mimic.

Time course experiments showed, that PBA treatment and boron deficiency (boron-free Hoagland medium) both significantly reduced the number of lateral roots. The reduction in lateral root numbers was more severe in PBA-treated seedlings than in boron deficiency-treated ones. Histology results suggested that the reduction in lateral root numbers correlated with lateral root initiation defects rather than elongation defects. Overall, our study provides evidence supporting the use of PBA as an efficient boron deficiency mimic. Further characterization in cellular and molecular levels will shed light on the molecular mechanisms underlying the effects of boron deficiency on maize lateral root development.

Canopy architecture and nitrogen vertical distribution enhance yield performance in maize cultivars from old to new

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Reasonable canopy structure and leaf physiological characteristics are considered as important factors for improving canopy nitrogen (N) distribution by matching the available light resources and thus increasing the grain yield of maize. However, to which extent have light and nitrogen distribution in maize canopies changed during maize breeding remain poorly understood. To analyse the effect of genetic improvement on the light and nitrogen distributions within maize canopies, a 2-year field experiment was conducted with six cultivars selected between 1950 and 2010 in Jinlin province, Northeast China. Canopy architecture, the vertical distribution patterns of light and nitrogen among different cultivars from old to new were investigated. The leaf areas and angles of the upper leaves decreased much more than those of the other leaves, thus the changes in the vertical distribution of leaf area enhanced light interception efficiency in maize over generations of selection and significantly improved the radiation use efficiency. For the dynamic leaf area, the contribution of the different leaf rank to maize yield identified as middle leaves. These insights can inform future breeding strategies for continued NUE grains through improved conversion efficiency of accumulated plant N into grain yield. Coordinate the light and nitrogen distribution within the canopy to maximum canopy photosynthesis, and resulting in higher yield in modern maize.

Characterization of Nrf4-like, a homologue of Non-reduction in female4 (Nrf4), an apomixis candidate gene in maize

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Apomixis is an asexual mode of reproduction whereby seeds are formed without recombination and reduction of the maternal genome (apomeiosis) in the absence of fertilization (parthenogenesis). The resulting offspring is clonal, i.e., it possesses the exact same genetic material as the mother plant. The harnessing of apomixis in crop plants has tremendous potential for breeding and seed production. However, while apomixis occurs in about 500 wild species, it is absent from major crops, including maize. With the goal to engineer apomixis in maize, we undertook large-scale genetic screens to identify mutants that mimic the two elements of apomixis: apomeiosis and parthenogenesis.

We have shown that mutations affecting the Nrf4 gene of maize cause a phenotype similar to mitotic diplospory, a form of apomeiosis in which meiosis is replaced by mitosis that directly serves as the first gametophytic division. In nrf4 mutants, the embryo sac is almost always unreduced (>95%), but recombination is absent in only about one-third of them. The latter has successfully been used to generate clonal offspring by synthetic apomixis. However, for agricultural applications, the frequency of apomixis achieved by nrf4 (<35%) is not sufficient. As Nrf4 has a close homolog in the maize genome, we hypothesize that this Nrf4-like gene is partially redundant with Nrf4. Therefore, double mutants may suppress recombination at a higher frequency. We generated a set of CRISPR/Cas9-induced mutations in order to test this hypothesis and to characterize the function of the Nrf4-like gene.

Reduction in fertility after moderate heat stress application during silking of maize ears

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The reproductive phase in flowering plants is highly sensitive to ambient temperature stresses, with even a single hot day sometimes being fatal to reproductive success. Heat stress caused by climate change is predicted to reduce the productivity of crop plants. Many studies of heat stress on crop plants have shown that pollen development and fertilization often belong to the most sensitive reproductive stages. Less attention has been paid to heat sensitivity of female reproductive organs. We applied moderate heat stress to study the sensitivity and contribution of female organs to reproduction under heat stress in maize. We focused on the elongated stigma tissue (silk), which is in direct contact with ambient environment at the silking stage. We showed that moderate heat stress on female organs caused increased cell death and decreased cell vitality of silk hair cells. However, moderate heat stress on silk didn't affect pollen germination and early pollen tube growth inside the silk but caused late growth arrest of pollen tubes and likely cause also guidance leading to severe seed set reduction. In summary, our findings demonstrate that a short moderate heat stress affects silk cell vitality and pollen tube growth inside the silk, which consequently leads to severe yield losses in maize. We will further show the analysis and comparison of the transcriptome of heat stressed maize silk with and without pollination, respectively, in order to identify genes responsive to heat stress in silk tissue and growing pollen tubes. By applying sprayable dsRNAs that lead to the degradation of responsive regulator mRNAs we aim to prime maize silks to heat stress before stress application.

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Assessment of genetic diversity among maize (Zea mays L.) Italian landraces with the use of SNP markers

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Maize breeders have become increasingly aware of the need to maintain genetic diversity among varieties and to improve the management of genetic resources through the conservation of traditional populations. These represent both a valuable source of potentially useful traits and a starting point for the reintroduction of local maize varieties, as once grown in more rural areas. We are addressing the latter aspect in the framework of a PSR research project (VALOMAYS) financed by Regione Lombardia, through the genotyping of a series of local varieties using tGBS technology. Multiple single plant samples derived from 43 varieties (a total of 286 DNA samples) were analyzed and produced 2x1,075,208,363 reads with an average read count of 2x3,759,470 per sample. Among the 43 selected varieties, ten were either already part of the official conservation register of Regione Lombardia or prone to be registered, while the remaining varieties constituted a geographical representation of the landraces collected in Lombardy. After subjecting the obtained SNPs to quality controls, the filtered SNP set was used to perform a series of statistical analyses. Replicate samples, obtained from each variety, were either combined into a single sample representative of the maize landrace, or processed separately. Expected and observed heterozygosity indices and marker PIC values were calculated. Furthermore, PCA analyses were performed, and phylogenic trees were obtained with the filtered SNP set. AMOVA analyses were then performed to verify the distribution of the total variability present in the population across the groupings present in the phylogenetic trees.

Towards Understanding and Utilizing Environment-Sensitive PhasiRNA-Dependent Male Sterility (EPMS) in Maize

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An increase in temperature and extreme heat stress is responsible for the global reduction in maize yield. Thus, crops need adaptation to changing climates and new environment conditions. Small non-coding RNAs (sncRNAs) are a class of regulatory RNAs that play an important role in many biological processes. An increasing number of several classes of regulatory ncRNA (i.e., miRNA, siRNA, and lncRNA) related to heat stress responses have been reported. SncRNA also regulate DNA Methylation. DNA methylation is an important epigenetic modification, and plays a key role in the regulation of plant development and in response to abiotic stress in plants. Although many studies have revealed that abundant sncRNAs are expressed under heat stress, the molecular function in reproductive stage is still unclear. PhasiRNAs are a special subgroup of small RNAs that occur during male development in mainly monocot cereal crops. It is still unknown whether phasiRNA have specific targets and what their mode of action is. To study the function of phasiRNA in male devolopment, we propose to apply PARE-seq, Ribo-seq and TAIL-seq to isolated meiocytes at three stages (premeiosis, meiosis, tetrads). To test for conservation of phasiRNA action and its timing, we will perform the experiments in both the moderate reference-genome B73 line and the tropical CML228 line under both control and heat stress condition. At the same time, we also aim the other possibilities of sncRNAs in reponse to heat stress by performing sequencing in both B73 and CML228 line.

Dissecting Early Maize Embryogenesis through Single-Cell Transcriptomics

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Maize (Zea mays) is a globally significant crop, and understanding its embryogenesis is crucial for crop improvement and breeding programs. Compared to the model plant Arabidopsis embryogenesis in maize and other grasses is especially complex due to the formation of additional tissues like a coleoptile, mesocotyl, and scutellum as well as the formation of anlagen of first leaves and their primordia. Recent advances in single-cell omics technology have unlocked new opportunities for dissecting the cellular and molecular mechanisms underlying maize embryogenesis. We aim to provide a comprehensive overview of the transcriptomic dynamics during early maize embryogenesis, with a particular focus on symmetry breaking and meristem initiation using single-cell RNA sequencing (scRNA-seq). We currently establish methods to employ scRNA-seq to profile thousands of individual protoplasts at the transitional and coleoptilar stages. Our study will facilitate the identification of distinct cell populations, lineage-specific gene expression patterns, and regulatory elements driving the spatiotemporal organization of maize embryogenesis. Additionally, we intend to investigate the signal exchange mechanism between the early embryo and its surrounding endosperm. In maize, the embryo surrounding region (ESR), a specialized area of maize endosperm encircling the embryo, likely transfers nutrients toward the developing embryo and plays a crucial role in embryo defense and signaling. By analyzing esr1/2/3 mutants encoding CLE peptides and ESR cell capture sequencing, we hope to elucidate the role of ESR in embryogenesis. First results of this the ambitious and challenging study will be presented.

The impact of chilling and its recovery on cell division and expansion depends on the stage of maize leaf development.

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Maize (Zea mays) is highly sensitive to chilling which recurrently occurs during its seedling stage and negatively affects its end yield. Direct effect of chilling on maize is well-documented. However, one aspect that such studies have not addressed is the initial impact on and subsequent recovery of leaf growth following a shift from warm to low temperature. Our goal is to define the growth response to chilling and its recovery in leaves of different developmental stages by growth and kinematic analysis. We first studied the effect of a 3-day cold spell on leaf growth at the plant level. Then, a more detailed analysis at the cellular level was performed to analyse the contrasting impact of cold occurring just prior or after emergence of the same maize leaf. We determined the contribution of cell division and elongation to leaf growth during and after cold stress. Our results demonstrated that final leaf length of non-emerged leaves was more affected by cold than the emerged ones. This was not the result of better growth during cold but a slow and complete recovery of the leaf elongation rate. This difference was firstly due to a higher cell division rate and secondly to a higher cell elongation rate on the 1st and 2ndday recovery respectively.

Mountain areas as biodiversity hotspot: discovery and characterization of maize landraces in Valle d'Aosta region

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Despite the historical cultivation of maize (Zea mays L.) being well documented in Valle d'Aosta, no landrace from this mountain region was reported in the survey of maize carried out in 1949-1950. These materials are still cultivated and have high importance from a genetic and historical point of view.

Recently, 5 landraces from Valle d'Aosta have been collected and subjected to historic, morphologic and genetic characterization. These landraces were named after the sampling location as it follows: Arnad, Arnad-Crest, Chatillon, Entrebin and Perloz.

This study proved landraces' long presence on Valle d'Aosta territory through information and photographs found in local archives. Moreover, the genetic characterization, which involved the use of SSR markers, highlighted a significant genetic variability and differentiation among the investigated landraces. This may be explained by the long reproductive isolation experienced by these materials. Finally, morphological observations confirm the high diversity between landraces revealing that they generally have flint kernels, variable colour from yellow to dark red (Chatillon) while Perloz showed kernels with an apical beak, suggesting the belonging to the "Rostrata" group.

The present work confirms the importance of mountain areas in conserving agrobiodiversity and the richness of the Italian maize germplasm with materials well adapted to marginal areas. Such rich genetic variability may be characterized searching for useful traits for a more resilient agriculture.

Mapping QTL of drought related traits in a dent MAGIC population

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A multi-parent advanced generation intercross (MAGIC) DH population (N=495 DH) was established to access the genetic diversity of dent maize for drought tolerance in the project MAZE. Eight dent maize inbred lines from the "non-stiff stalk" heterotic group were selected as the MAGIC founders based on their diverging performance in drought and heat prone environments. Within MAZE, a large body of genotypic and phenotypic data was generated for the MAGIC population. The eight founders were genotyped with the 600k SNP array and high coverage (~50x) whole genome sequencing (WGS). The MAGIC DH lines were genotyped with a 15k SNP array and shallow-sequencing, on average 5x for each line. Variant calling, imputation, and genomic mosaic reconstruction were performed on the genotypic data. Nineteen field trials, seven as testcross and twelve as lines per se, were conducted in six European locations from 2020 to 2022, and important agronomic traits and drought related traits showed large genetic variation and high heritabilities. UAV-based phenotyping experiments were conducted in some locations to develop image-based traits for maize drought response. Haplotype-based mapping methods detected several QTL for drought related traits. Integration of these QTL from multiple environments and refining the QTL regions will be the next steps to decipher the genetic control of drought related traits in the MAZE diversity atlas.

Can functional-structural plants models contribute to improving maize nitrogen uptake?

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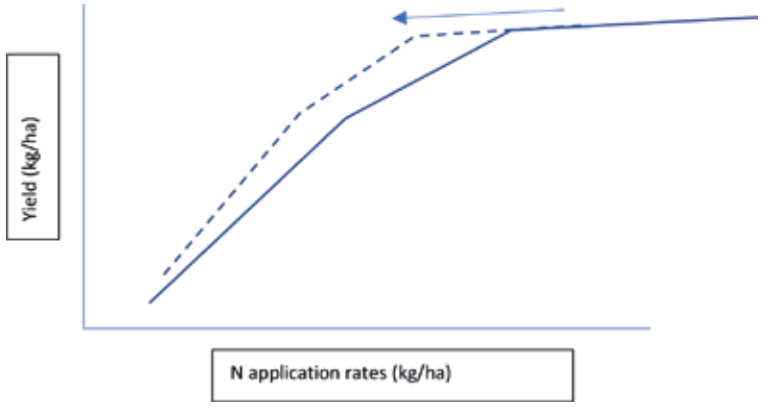
Maize (Zea mays) is the most widely grown and likely the most heavily fertilized crop in China. Reducing nitrogen input to agriculture is a prerequisite to reducing environmental externalities. Breeding for nitrogen-use efficient (NUE) maize could allow to maintain productivity at reduced input levels. Conceptually the figure below shows this higher NUE would be the broken line, we can reach maximum productivity at lower N input. Excessive input will not reduce losses to the environment. But we might reach the same production at lower inputs so it may help reducing the inputs. NUE is a complex trait, which depends on soil attributes, on plant architectural, physiological and developmental root and shoot traits, as well as plasticity in those traits to N availability. The relative contribution of crop traits and plasticity in those crops traits to NUE is not well known, but relevant to identifying breeding targets. Here, we developed a whole plant functional-structural plant (FSP) model of maize to mechanistically simulate the growth and development of root and shoot driven by temperature, light and sink-source relationships for carbon and nitrogen with plastic responses of root traits to plant N status. In addition, this FSP model has potential to upscale from plant to field level to further explore root behavior caused by plant-to-plant interactions. We parameterized and validated the model, and applied it to identify and quantify maize traits contributing to NUE.



Genetic variability for root system architecture (RSA) in Italian maize inbred lines

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Keywords: root system architecture RSA, selection criteria, maize germplasm, drought

Maize genotypes with efficient root systems are thought to better adapt to environments with frequent dry seasons. Preliminary investigations suggested that a fair amount of genetic variability for root system architecture (RSA) is still present among elite cultivars as well as in germplasm collections, which may result in drought stress tolerance. The evaluation of RSA led to the development of root ideotypes related to yield and stress tolerance. The objectives of the study are to explore variability for RSA in Italian maize germplasm and to develop phenotypic and genomic selection criteria for obtaining resilient varieties adapted to Northern Italy.

We studied 340 inbreds, 90 of which derived from local varieties grown in Italy before the 1950s and 250 elite inbred lines selected in the last 25 years within the CREA breeding programs and belonging to two breeding groups. In this study, all lines were evaluated for RSA in rhizotrones under controlled condition. Genome-wide association was performed with 20.397 SNPs. The results showed substantial variations for root traits, with medium-high heritabilities. Inbred lines were classified as shallow or deep rooted according to a multi-trait index, and crossed in partial North Carolina mating designs II. About 60 hybrids between shallow and about 60 between deep inbred groups were obtained and were evaluated in the field for agronomic traits under two water regimes. Preliminary results will be discussed. As applied output, this study may contribute to the setting of a selection criteria to identify hybrid combinations with improved root efficiency and stress tolerance.

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Regulation of the apical meristem in the maize shoot

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Plant architecture is dependent on the spatiotemporal regulation of the stem cell niche (SCN) in the shoot apex (shoot apical meristem, SAM). The SAM is organized into functionally distinct domains that reflect its primary functions: self- maintenance and organogenesis. The maintenance of SAM organization is crucial for its function and, as such, proper plant development. Although the general functions of the SAM are conserved between dicot and monocot species, the pathways controlling SAM homeostasis and maintenance appear to have diverged between these groups. Through a combination of genetics, imaging techniques and transcriptomic approaches, our work explores a potential novel role for the maize SAM epidermal layer as a signaling center concomitantly regulating SAM homeostasis and anchoring the SCN to the shoot apex in order to preserve its spatial organization and structural integrity. In addition, taking advantage of the rich SAM morphodiversity inherent in maize breeding stocks, we investigate relationships between meristem size and gene expression patterns, and potential scaling thereof. These works explore key, open questions of plant developmental biology.

Exploring new traits to evaluate maize organic varieties

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As partner of the European project "Organic seed and plant breeding to accelerate sustainable and diverse food systems in Europe" (LIVESEEDING) we are interested to introduce innovations in variety testing. In consideration of the European Directive 2022/1647 of 23 September 2022 the competent authorities should register also organic varieties to the national catalog. Our final aim is indeed to review, evaluate and update the protocols in force to register new varieties of wheat and maize identifying new traits of value in organic farming.
In this framework our research is focusing on the following activities: 1) identify and set up a methodology to assess the resistance to Aspergillus flavus in maize in laboratory; 2) test the phenomic methods and tools (PocketLAI smart app, RGB camera, SPAD) to be used to estimate the plant canopy architecture traits (canopy density, leaf area index, rate of leaf area development, and leaf distribution) as a measure of the ability to compete with weeds in wheat and maize varieties; 3) identify and set up a methodology to evaluate the ability of candidate varieties of wheat and maize to react to stress capitalizing on the close association between roots and specific groups of soil microbes with plant-beneficial properties: the level of enzymatic activity and microbial biomass of the soil (rhizosphere) affected by the root system will be measured.
Here we show some preliminary results on the characterization of resistance levels of maize varieties after inoculation of the kernels with a fungal strain belonging to *Aspergillus flavus*.

Characterizing heterosis in a set of recombinant intercrosses (RIXs) developed from a multiparental maize population.

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The exploitation of heterosis is one of the leading activities in maize breeding. Here, we developed an innovative recombinant intercross (RIX) population by crossing pairs of multi-parental maize recombinant inbred lines (RILs) to evaluate the heterotic response in a heterozygous multi-parental population. Field phenotyping was performed on 400 RIX genotypes considering 11 agronomic traits and the resistance to Fusarium Ear Rot (FER) in two consecutive years.

Thanks to the high level of allelic diversity available in parental genomes, a broad range of phenotypic diversity was observed. The broad-sense heritability (H2) was high for all traits (H2=0.48 to 0.89) illustrating a strong genetic basis. The heterotic response was computed based on RIL values as mid and best parent heterosis showing different magnitudes for different traits suggesting effects from partial dominance to over-dominance. GWAS detected 55 significant loci associated with agronomic traits across all chromosomes, with allelic effect ranging from 0.28 to 10.90 indicating the contribution of various sized QTLs. Several pleiotropic QTLs on chromosomes 8 and 3 were detected, indicating that these loci may contain valuable genes for multiple traits. RIX collection showed a moderate heritability of FER resistance and 7 putative QTL were associated to this trait on chromosomes 8 and 5. The allelic effect estimates indicated the presence of minor effect QTL with relatively small additive effects on disease resistance.

Our findings confirm the usefulness of the RIX population to decipher heterotic loci in maize and support utilizing this resource in future to accelerate crop improvement.

Characterization of the DCL2-homologous gene in Zea mays L.

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Small RNAs (sRNAs) control gene expression and epigenetic regulation. In maize, 22nt-long sRNAs are a class of relatively high abundance, compared to other plant species. Previously, we found an association of 22nt sRNAs with grain yield heterosis and a unique enrichment of the negatively associated ones in pericentromeric regions. Although most 22nt sRNAs of maize map to Gypsy transposable elements, they are not produced within the RNA-dependent DNA methylation pathway, which involves MEDIATOR OF PARAMUTATION 1 (MOP1). As mop1-1 knockout experiments showed, 22nt long sRNAs in maize must have an alternative origin. In other plants, like tomato or soybean, the abundance of 22nt sRNAs is linked to Dicer-like 2 (DCL2) homologs. The maize genome encodes a single DCL2 homolog (ZmDCL2), of which the functionality is unknown. Interestingly, RNA-seq data show strong evidence of a ZmDCL2-isoform specifically expressed in sperm cells. Together, these findings render the DCL2 homolog of maize an interesting candidate to study its function and potential role in heterosis and sexual reproduction.

The characterization of the ZmDCL2 is conducted as a gene-knockout experiment using the BonnMu resource. We could identify heterozygous plants with a Mu-insertion in exon 2 or the 5'-UTR of ZmDCL2. These plants need to be self-pollinated in order to achieve homozygosity for the Mu-inserted ZmDCL2. Additionally, we currently use RT-PCR for the characterization of isoforms and alternative splicing in different tissues and cell types.

Towards the identification of ubiquitinated targets important for meiotic chromosome dynamics in maize

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In plants, several protein-ubiquitination related genes have been reported to be essential for meiotic chromosome behavior and function, e.g., for meiotic recombination. However, little is known about the actually ubiquitinated target proteins. Taking an advantage of maize as a model system with large meiocytes, we report here our attempts to establish an analytical platform for the identification of ubiquitinated target proteins in meiotic cells. To this end, we have generated transgenic maize lines expressing FLAG-tagged UBIQUITIN (UBQ) gene under the control of the meiosis-specific DMC1 promoter from maize (pDMC1). In parallel, we have also generated maize mutants for three meiotic genes involved in protein-ubiquitination: 1) zygo1, a mutant for an Fbox protein gene important for telomere-bouquet formation and homologous chromosome pairing, 2) dsnp1, a mutant for an E3 ubiquitin ligase gene essential for accurate chromosome synapsis, and 3) hei10, a mutant for an E3 ubiquitin ligase gene supposed to function in crossover formation and distribution. In our strategy, the pDMC1-driven FLAG-UBQ transgene will be placed in the respective mutant background and the recovery of peptides from FLAGimmunoprecipitated fraction will be compared with wild-type situation to screen for potential targets of each protein-ubiquitination related protein. We hope our attempt will bring new and beneficial information for studies in meiotic chromosome dynamics and recombination, and with that also for plant breeding.

Maize resistance to Fusarium verticillioides by CRISPR/CAS9 gene editing approach of a WRKY gene and overexpression of a lipoxygenase gene.

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The maize WRKY transcription factors and the lipoxygenases (ZmLOXs) are well recognized as important players in plant defense against pathogens. In this regard, previous RNA-seq experiments reported the enhanced expression of ZmLOX genes in maize resistant genotypes and GWAS resulted in one SNP significantly associated with ZmWRKY125.
The Clustered Regularly Interspaced Short Palindromic Repeat/associated Cas9 (CRISPR/Cas9) editing of ZmWRKY125 and the transgenic overexpression of ZmLOX4 genes were carried out to investigate the possible implication of these two genes in the resistance mechanisms against F. verticillioides.
As regards ZmWRKY125, the CRISPR cloning was based on a double cloning using two sgRNA for one gene target. Agrobacterium tumefaciens mediated transformation was used for introducing in maize A188 line the construct under the maize promoter ZmpUBI in the binary vector p1609. Mutants from three different transformation events were obtained. For each event, T2 plants will be genotyped to find homozygous for the mutation that in turn will be phenotyped for F. verticillioides resistance and fumonisin content.
As regards ZmLOX4, the gene was cloned under a promoter involved in kernel development in the vector L1781, and the same transformation conditions adopted for the CRISPR/Cas9 editing of ZmWRKY125 were used. Mutants from two different transformation events were obtained. For each event, T2 plants were genotyped in order to find homozygous for the mutation. Homozygous plants will be further evaluated for F. verticillioides resistance, fumonisin accumulation, oxylipin content as well as for the expression analysis of the main genes involved in the jasmonic acid pathway.

Exploring maize biodiversity and microbiome in local varieties as strategic tools to face abiotic and biotic stresses

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Keywords: maize, stress resistance, biodiversity, microbiome

Local varieties are a valuable source of genetic diversity for addressing challenges such as climate change and soil degradation that threaten food security and agricultural sustainability. Italian maize varieties, maintained by farmers as open pollinated populations in diversified climatic zones, represent a strategic source because of their capability of adaptation to different local environments. In the GEMMA project (GEnotipi di Mais lombardo e MicrobiomA – Regione Lombardia), four Italian maize varieties, maintained at CREA Bergamo Genebank, have been characterized to highlight novel phenotypic and microbiological diversity and examine their involvement in agronomic traits as well as the plant response to abiotic and biotic stresses. Data related to plant development, productivity and seed nutritional quality (NIRS) of the four GEMMA genotypes cultivated in four different locations and in three seasons, will be presented. Their response to drought, measured in controlled conditions by growing plants in soils taken from the experimental sites, will also be compared considering soil chemical and biological differences.

To analyze the microbial diversity, both cultivation and NGS characterization of the embryo bacterial microbiota were carried out. 100 bacterial strains were isolated from the embryos, among which 12 showed high F. verticillioides growth inhibition in vitro, and 2 greatly reduced the infection also in vivo. The NGS characterization showed low diversity, with Enterobacteriaceae contributing more than 90% of the total bacterial reads in most genotype/field combinations.

The project dataset will constitute a comprehensive picture of selected maize accessions providing a model for future studies and valorization of crop biodiversity.

"The more, the better?": Investigating differential, non-additive and allelic expression in association with the phenotypic manifestation of heterosis

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Heterosis is the phenomenon, that cross-pollinated F1-hybrids are more vigorous than their parents. Hybrids produce more biomass, have a faster development and greater fertility. Maize, due to its cross-pollinating nature, shows this effect to an exceptional degree. Heterosis is quantified via the mid-parent value describing the deviation of a hybrid trait from the parental average of that trait. This phenotypic variation is often accompanied by transcriptomic variance. Previous transcriptomic studies showed that many genes show differences in their expression between the parents and the hybrid, including differential, non-additive and allele-specific patterns.

In the present study, we grew 112 different lines of the maize intermated B73×Mo17 recombinant inbred line (IBM-RIL) syn 4 population as parental lines and IBM-RIL lines outcrossed to the inbred lines B73 and Mo17 as F1-hybrids in parallel. The expression profiles of the inbred lines and their corresponding hybrids is analysed together with their phenotypic traits. Our analyses suggest, that almost all differentially expressed genes display high-parent expression. In further studies we will investigate if and how this is associated with hybrid vigour. The ultimate goal of this work is to obtain a better understanding of the molecular principles of heterosis and to identify candidate genes involved in the manifestation of heterosis.

Genetic transformation of tropical maize inbreds using leaf explants

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Maize (Zea mays) is one of the world's leading cereal crops and it is a plant model for gene_c and biotechnology research. However, gene_c transforma_on of maize by Agrobacterium tumefaciens faces barriers, such as the recalcitrance of many inbred lines and the difficult produc_on of the immature embryo explants used in current protocols. Our goal is to combine successful strategies to develop a gene_c transforma_on and gene edi_ng protocols suitable for recalcitrant inbred tropical lines, using: (a) young leaves as explants; (b) ternary vectors and (c) expression of morphogenic genes. For this purpose, we based our work on the protocol of Wang et al. (2023) to transform leaves of B104 and Fast-Flowering Mini-Maize. We have used Agrobacterium strain EHA105 recA- holding the ternary vector pVS1-VIR2, and a binary plasmid with the T-DNA containing morphogenic genes ZmBBM and ZmWUS2, and reporter gene GUS. Ini_al results showed a sa_sfactory transient GUS expression (6 days aher transforma_on) and rapid forma_on of Gus-expressing embryogenic callus. We are currently op_mizing the protocol for tropical inbred lines (CML444, CML577, CML532, CML607B, CML533 and CML578), and evalua_ng the regenera_on capacity of transformed calli. In the future, we would like to transform the most suscep_ble maize lines with vectors containing other morphogenic regulators (such as GRF-GIF) and assess the transforma_on efficiency. We an_cipate that this work will reduce the _me and cost of maize transforma_on, allow direct research in relevant varie_es, and pave the way for the transforma_on of other recalcitrant species.

Boosting drought tolerance in key cereals in the era of climate change

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Prolonged drought due to climate change has a severe impact on agriculture, requiring measures to secure yield stability under water shortage conditions. BOOSTER is a Horizon Europe project (101081770 - 1/05/2023 - 30/04/2027) with the aim to create climate resilient and drought tolerant cereals. Two synergistic strategies will be implemented. Firstly, a new approach will identify genomic variants in regulatory regions functionally associated with drought tolerance. Novel regulatory elements underlying resilience will inform efficient breeding efforts to create new drought tolerant cereal varieties. Secondly, novel seaweed extracts and microbial biostimulants will be developed as an eco-friendly approach for improving drought resilience.

The two strategies will be tested in two cereals with different responsiveness to drought: European maize and Ethiopian teff, a cereal with high genetic similarity to the desiccation tolerant Eragrostis nindensis. BOOSTER will improve drought tolerance in both maize and teff, while simultaneously exploring the potential for transferring species-specific drought responsive features. By exploiting natural genetic variation to achieve drought tolerant genotypes and by developing biostimulants derived from living organisms, BOOSTER will take advantage of the already available natural resources to steer our agriculture towards novel drought tolerant varieties. Importantly, BOOSTER approaches and results are transferable to other crops. The project will produce increased maize- and teff-derived biomass resources under harsh drought conditions, will lower irrigation requirement, will strengthen competitiveness of European and African agri-food industry, and will provide concrete examples for improving public awareness about a sustainable use of bio-based technologies.

Interdisciplinary approach to nitrogen use efficiency in maize combining combining genomics, multi/hyperspectral indices and machine learning

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Nitrogen (N) fertilization is a critical component of maize agrotechnical practice, but N depletion can limit yields, especially in low-input agricultural areas of Southeastern Europe. In maize there is a considerable variability in response to increasing N content, with some genotypes showing breakpoints in agronomic performance at lower N doses. Furthermore, limiting N fertilization could have beneficial effects on the agroecosystem, including preventing nitrate leakage and reducing greenhouse gas emissions, while retaining agronomic performance. In this study, we aim to construct the ideotypic genotype of maize by leveraging ecophysiological postulates such as Shelford's law of tolerance and utilizing novel tools such as multi/hyperspectral screening in a machine learning (ML) framework. We testcrossed 151 densely genotyped temperate dent maize inbred lines with two testers of Iodent and B37 background. Inbreds were genotyped by Illumina Infinium Maize SNP50K array, yielding ~49000 filtered (MAF<0.05, missing<0.05) and imputed positions for further analysis. The 238 resulting crosses will be phenotyped in 2023-2025 yield trials with three N fertilization levels, 0, 90 and 170 kg N/ha. We will use ML to analyze the genotypic and spectral data to identify the most nitrogen-use-efficient maize genotypes. We will also discuss the potential pitfalls of this interdisciplinary approach. Our findings will contribute to the development of sustainable maize production systems that balance high yields with environmental and economic considerations.

Keywords: maize genomics, artificial intelligence, quantitative genetics

Characterization of the Meiotic Cyclin SOLO DANCERS (SDS) in Maize

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Meiosis is a specialized cell division that produces haploid gametes after two subsequent rounds of chromosome segregation events. It ensures genetic diversity due to the random distribution of homologous chromosomes (homologs) and sister chromatids, as well as the exchange of DNA segments between homologs. Meiosis is also key in breeding as through meiotic recombination beneficial alleles can be combined. Thus, altering the frequency and distribution of meiotic recombination events is a very promising aim in plant breeding programs. However, our understanding of meiotic control is far from complete. Here, we have focused on the study of a cyclin, called SOLO DANCERS (SDS), which is well known from Arabidopsis thaliana to control together with its kinase partners, cyclin-dependent kinases (CDKs), progression and recombination in meiosis. Zea mays contains two putative SDS homologs, ZmSDS1 and ZmSDS2. To study their function, a double mutant was generated in the maize inbred line A188 using the CRISPR/Cas9 system. Consistent with a central function of SDS in maize meiosis, this double mutant was both male and female sterile. Chromosome spreads revealed partial chromosome synapsis and the presence of univalent in this double mutant. Ongoing work focuses on the expression and localization control of SDS in maize. Interestingly, the SDS function appears to vary between plant species possibly indicating a divergence between monocots and dicots and hinting at further differences in the control of meiosis in Arabidopsis versus major crops such as maize.

Live cell imaging reveals dynamics of the class I crossover protein HEI10 during maize meiosis

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Meiosis is a specialized cell division, which creates gametes with half of the chromosome number as in the parental organism by two consecutive chromosome separation events. Moreover, meiosis is important for the generation of genetic diversity through two mechanisms: First, homologous chromosomes (homologs) are assorted into new, yet complete sets. Second, homologs exchange DNA fragments (through crossovers), creating new allele combinations. These new allele combinations are the foundation of plant breeding which can be selected to create new plants with favorable traits, which are key to providing food security.

In this project, the dynamics of proteins important in meiotic recombination in maize are studied. The predominant class of crossovers are of the so-called type I while type II crossovers only account for XXX percent of meiotic recombination. A main actor in the class I crossover pathway is the protein HEI10. Generating a fluorescent marker for Hei10 has enabled us to study the dynamics of this protein by live cell imaging leading to new insights in the process of meiotic recombination.

In addition, a mutant of the maize HEI10 gene was made. Ongoing analysis of plants homozygous for the mutation, show a decrease in pollen viability and a reduction in bivalent chromosomes during meiosis. These defects, however, are not as severe as is known from other plant species, possibly hinting at a different regulation of meiotic recombination in maize.

The Proteomes that Feed the World

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Crop plants represent the nutritional basis of virtually all life on Earth and are essential for feeding an increasing human population, while facing challenges posed by climate change. While the genomes and transcriptomes of crops are increasingly elucidated, little is known about crop proteomes. In an effort to close this knowledge gap, we have assembled a large international group of partners within the international doctoral program "The Proteomes that Feed the World" which is funded by the Elite Network of Bavaria. One of the program's overarching aims is to map the proteomes of the major tissues and organs of the 100 crop plants most important for human nutrition. In preparation for the Crop Proteome Atlas project, a robust and reproducible protocol for the processing and analysis of a variety of plant tissues by liquid chromatography tandem mass-spectrometry (LC-MS/MS) was devised. This protocol constitutes a central component of the Crop Proteome Engine. All data will be made publicly available via the databases PRIDE and ProteomicsDB. In addition to creating the Crop Proteome Atlas, we will conduct several subprojects to demonstrate the usefulness of these data. For instance, we aim to explore intraspecies variation in the proteomes of maize lines, that are known to contrast in their cold stress responses, and connect them to variations previously found in their genomes and transcriptomes. Eventually, this will aid in the elucidation of mechanisms that contribute to differences in tolerance to cold stress.

Understanding the Molecular Mechanism of Parthenogenesis in Cereals

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Parthenogenesis, meaning "creation by virgin", is a key component of apomixis (asexual reproduction through seeds) and describes spontaneous embryogenesis from an unfertilized egg cell and thereby generates offspring genetically identical to the mother plant. Investigating parthenogenesis in crop plants not only has high potentials to immediately fix desired traits including heterosis and thus would create great economic values but help to understand how egg cell fate is determined for embrypgenesis initiation. The underlying mechanisms of parthenogenesis remain poorly understood. Here, we use the apomictic grass Tripsacum dactyloides to address these questions. As the closest wild relative of maize, Tripsacum is sexually reproducing as a diploid, but all polyploids display apomixis via parthenogenesis. We collected egg cells from diploid and tetraploid Tripsacum lines to compare their gene expression by maping RNA-seq reads to maize genome. We observed that parthenogenetic eggs possess relatively specific cell cycle gene expression pattern that confers division potentials. Transcriptional reprogramming considerably contributes via both ON/OFF and differential regulation modes primarily involved in cell differentiation and auxin signaling. Seemingly parthenogenesis and zygotic genome activation share similar gene expression alterations associated with RNA metabolisms at both transcriptional (via ZmBBM1) and post-transcriptional (via RNase exonuclease) levels. For those highly expressed in parthenogenetic eggs but completely silenced in sexual eggs, we sought to characterize their funtions via creation of egg cell-expressed lines and knock-outs in maize. Especially we used CRISPR-Cas9 to knockout ZmBBM1 and the homologous ZmBBM2 genes to resolve their functions in activating embryogenesis. Additionally, ZmBBM1 fused to mEGFP was highly detected in embryonic pro-vascular and root systems. The ultimate goal of this research is to gain mechanistic understandings of parthenogenesis and embryogenesis initiation in cereals and utilize the knowledge generated to contribute and improve the production of haploid maize lines or clonal seeds.

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