1st French-German Maize Breeders School

Genetic diversity in maize breeding programs

May 23 and 24, 2017

Stuttgart-Hohenheim,
Germany

DMK
Deutsches Maiskomitee e.V. (DMK)

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Program

Tuesday, May 23, 2017

11:00	Registration
12:15	Welcome addresses *Helmut Meßner, Deutsches Maiskomitee e. V. (DMK), Germany* *Albrecht E. Melchinger, University of Hohenheim, Germany*

Session 1 | Session chair: Alain Charcosset
Management and broadening of genetic diversity in breeding programs

12:30	Developing and deploying climate-resilient maize in the tropics: progress and prospects *Bodupalli Prasanna, CIMMYT, Kenya*
13:00	Diversity in a global breeding program. Status and possibilities for broadening *Rainer Leipert, KWS, Germany*
13:30	Lessons learned and expectations for broadening the diversity in elite breeding programs *Tobias Eschholz, Maisadour, Germany*
13:50	Breeding technologies help to describe and utilize genetic diversity in maize breeding *Carsten Knaak, KWS, Germany*
14:10	Managing genetic diversity in an elite breeding program *Alain Murigneux, Limagrain, France*
14:40	Practical aspects of introgression of landraces into elite pools: The Piemont-Pyreneen case study *Cyril Bauland, INRA, France*
15:00	Coffee break

Session 2 | Session chair: Chris-Carolin Schön
Tools and approaches for broadening the genetic diversity with genetic resources

15:40	Use of Doubled Haploids in the Context of Genetic Resources Exploitation *Thomas Lübberstedt, Iowa State University, USA*
16:10	Libraries of doubled-haploid lines: a new tool for harnessing landraces *Albrecht E. Melchinger, University of Hohenheim, Germany*
16:30	Association mapping of agronomic and metabolic traits in DH libraries of landraces *Juliane Böhm, University of Hohenheim, Germany*
16:50	Finding the optimum level of diversity for germplasm improvement *Manfred Mayer, Technical University of Munich, Germany*
17:10	GBS based comparison of USDA and INRA inbred lines collections *Brigitte Gouesnard, INRA, France*
17:30	DNA pooling for evaluating the originality of landraces *Stéphane Nicolas, INRA, France*
18:20	Departure evening program *Point of departure: Christkönigshaus, Paracelsusstr. 89, 70599 Stuttgart*
19:00–22.00	Reception and supper with wine tasting *"Collegium Wirtemberg", Uhlbacher Kelter Uhlbacher Str. 221, 70329 Stuttgart*

Wednesday, May 24, 2017

Session 3 | Session chair: Michaël Fourneau
Exploiting the genetic diversity in mapping populations and genomic prediction

08:30	Multiparental QTL mapping of biomass related traits *Adama Seye, INRA, France*
09:00	Choice of individuals for the training set in genomic prediction *Laurence Moreau, INRA, France*
09:30	Genomic selection in synthetics – results from a simulation study *Pascal Schopp, University of Hohenheim, Germany*
09:55	A comparison of criteria for optimizing gain from recurrent genomic selection *Dominik Müller, University of Hohenheim, Germany*
10:20	Coffee break

Session 4 | Session chair: Milena Ouzunova
Miscellaneous topics I

11:00	Omics-based Hybrid Prediction in Maize *Matthias Westhues, University of Hohenheim, Germany*
11:25	Identification of genes under differential selective pressure in temperate maize *Sandra Unterseer, Technical University of Munich, Germany*
11:45	Assessment of genetic heterogeneity using whole-genome regression models *Christina Lehermeier, Technical University of Munich, Germany*
12:10	Lunch

Session 5 | Session chair: Susanne Groh
Miscellaneous topics II

13.10	Alternatives to colchicine for chromosome doubling in maize *Willem Molenaar, University of Hohenheim, Germany*
13:30	A new system for identification of haploids in maize *Wolfgang Schipprack, University of Hohenheim, Germany*
13:50	Transcriptomic dissection of heterosis manifestation in young maize roots *Frank Hochholdinger, University of Bonn, Germany*
14:20	Variation for and inheritance of BYDV resistance in maize *Benjamin Stich, University of Düsseldorf, Germany*
14:50	Concluding remarks *Alain Charcosset, INRA, France*
15:00	End of meeting

Contents

Developing and Deploying Climate-resilient Maize Varieties in the Tropics: Progress and Prospects

Boddupalli Maruthi Prasanna

Maize contributes over 20% of total calories in human diets in 21 low-income countries, and over 30% in 12 countries that are home to a total of more than 310 million people. The crop is largely (about 80%) grown as a rainfed crop in sub-Saharan Africa (SSA), Asia and Latin America (LA), and is particularly vulnerable to an array of abiotic and biotic stresses; consequently, yields are usually less than half of those under irrigated systems. Maize yield losses and year-to-year variability in several agro-ecologies in sub-Saharan Africa and South Asia are related to climate-induced stresses.

Delivering low-cost climate-resilient improved maize hybrids to smallholder farmers with limited purchasing capacity and market access requires innovative partnerships with national/regional partners. Under the Drought Tolerant Maize for Africa (DTMA) project (http://dtma.cimmyt.org/), CIMMYT and IITA, in close collaboration with national partners and private sector institutions in 13 countries in Africa, developed and deployed over 200 drought-tolerant (DT) improved maize varieties during 2007-2015, with close to 60% of these being hybrids. In 2016, over 67,000 tons of certified DT maize seed was produced and commercialized by nearly 100 seed company partners in Africa, covering approximately 2.5 million hectares, and benefiting an estimated 6.5 million African households (approx. 57 million people). The Water Efficient Maize for Africa (WEMA) Project (http://wema.aatf-africa.org/) is an important public-private partnership (PPP), that is intensively engaged in developing and deploying drought-tolerant and insect-resistant white maize varieties in five target countries in sub-Saharan Africa (Kenya, Tanzania, Uganda, Mozambique, and South Africa), through a combination of conventional breeding, marker-assisted breeding, and transgenes.

The USAID-funded Heat Tolerant Maize for Asia (HTMA) Project (http://www.cimmyt.org/project-profile/heat-tolerant-maize-for-asia/), led by CIMMYT and initiated in 2012, brings together public and private institutions based in South Asia (Bangladesh, India, Nepal and Pakistan). The project has been successful in developing some highly competitive heat tolerant maize hybrids adapted to Asian agro-ecologies. Thirty-seven heat stress tolerant maize hybrids, developed by CIMMYT, have been already licensed to public and private sector partners in the four target countries during 2015-2016.

The ability to develop, in a cost- and time-efficient manner, elite maize hybrids with high yield potential and necessary adaptive traits will be critical for improved productivity and diversification of cropping systems in the developing world. The breeding pipeline must be flexible enough to rapidly incorporate new tools/technologies/strategies, such as high-throughput and precision phenotyping, doubled haploid (DH) technology, genomics-assisted breeding, and decision support tools. CIMMYT Global Maize Program is intensively engaged in developing/validating/deploying impactful tools/technologies/strategies for increasing genetic gains in the tropics. As a part of this, the CIMMYT team demonstrated the utility of marker-assisted recurrent selection (MARS) and genomic selection for improving genetic gains in stress-prone environments of sub-Saharan Africa.

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Diversity in a global breeding program. Status and possibilities for broadening

Rainer Leipert

In the last 40 years the KWS maize breeding program developed from a European program into a global program, with activities in all major temperate markets in Europe, North America, and recently also China. In parallel to the activity range, the genetic diversity of the breeding material also increased significantly, with more than 15 different breeding programs, each focusing on a specific maturity in the different environments.

The genetic diversity is assessed by molecular markers (fingerprints), which are routinely used in all programs. Therefore, the available germplasm base can be structured according to the diversity of specific breeding programs as well as the diversity between programs. To actively broaden the diversity in a specific breeding program, material influx from other (mainly later) breeding programs is common. Additionally, available external germplasm sources can be exploited to increase the overall diversity or to overcome specific limitations in a breeding program. These external germplasm sources are mainly commercial, non-patented hybrids, “ex-PVPs” (old inbred lines with an expired patent in the US) and germplasm from the GEM program. Furthermore, germplasm from CIMMYT and gene banks has been available since the declaration of a “sMTA” agreement. The limitations and consequences of the use of these external sources are briefly discussed.

Four years ago, KWS broadened the scope of their breeding activities by the acquisition of local Brazilian breeding programs. These provide access to locally adapted (sub)tropical germplasm. Generally, this germplasm shows high genetic diversity with very good disease tolerance. The material is freely available for KWS, and therefore probably the most important new source for broadening the temperate germplasm base in the future.

The main problem for all described material sources is the low performance and adaptability compared to the elite material in the established breeding programs. This makes a direct use impossible. Therefore, the main question for a global breeding program in maize is not the genetic diversity itself, but its integration into a temperate breeding program. KWS opts for the efficient and targeted use of molecular markers to overcome this performance gap and simultaneously increasing the disease resistance in elite material following the introduction of exotic germplasm.

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Personal Notes

Lessons learned and expectations for broadening the diversity in elite breeding programs

Tobias Eschholz

Maize (*Zea mays* L.) is a crop species mostly cultivated in the form of hybrids. These hybrids are created by crosses of fixed inbred lines (or doubled haploid lines) of one heterotic group (or pool) with the inbred lines of a complementary heterotic group. For the adaptation of this originally tropical plant species to hybrid selection in European climate after 1945, the use of already present (after Columbus) open pollinated cultivars of the Flint type has proven very useful and shown good combining ability. Today the crosses between inbred lines of the European Flint type and inbred lines of the Iodent type are widely used for the creation of maize hybrids in temperate European climate for both grain and silage use.

When hybrid maize breeding was introduced to Europe, the successful combinations from the US Corn Belt showed little adaptation to the less continental and therefore harsher conditions for the maize plant in large parts of Europe. The observation of the presence of better adapted germplasm in the form of open pollinated varieties (OPVs) of the Flint type in several geographic regions of Europe was vital for the installation of successful hybrid maize breeding in Europe. The Flint material often serves as a source for superior early vigor and tolerance to cold spells. However it is often less productive in terms of seeds per ear and the grain does not dry down as well as most dent types of maize.

It has been shown that the transition phase from OPVs to elite inbred lines has caused a bottleneck effect in terms of variation and allelic diversity. This is probably enhanced within breeding companies by a further reduction of diversity through the selection based on combining ability to a specific complementary heterotic group. In the context of a maize silage breeding program Flints are an important source for good early vigor in spite of cold weather in suboptimal growing regions and for whole plant digestibility when used as feed stuff.

Through a thorough GBS-based diversity study comparing Maisadour elite breeding material with publicly available material of the Amaizing and Panzea projects, we found evidence for allelic and haplotype diversity existing in the Flint group that is not yet part of our breeding programs. In order to introduce part of this diversity and enable the Flint material to adapt to changing environments, to changing end use of the hybrids and to improve resistance to diseases we apply several breeding approaches at Maisadour Semences. One of them is Haploid Recurrent Selection. This method allows for the increase of favorable additive or epistatic allele combinations in a given population through a haploid generation grown in field conditions which automatically removes the lethal or rather negative allele combinations and subsequent backcross with the precedent generation of diploid plants. As the field conditions of today differ from conditions during the 1940s and 1950s, we expect to mine different allele combinations than what was favored in the early days of European maize breeding. In addition to that it can be expected that the resulting Flint lines will be better adapted to haploid induction. The approach allows for the production of doubled haploids at different cycles of the process, giving us the possibility to sample and test the level of improvement achieved. All the resulting lines will be evaluated for combining ability with our relevant complimentary heterotic pool through production and trialing of test cross hybrids.

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Breeding technologies help to describe and utilize genetic diversity in maize breeding

Carsten Knaak

The recent and ongoing maize public genomic research has released a huge volume of genomic resources. For several diverse European maize genotypes the genomic sequences covering > 50 million single nucleotide polymorphisms became available (Synbreed, BMBF GER; Amaizing ANR, France) and facilitated the development of cost attractive genotyping technologies as Illumina Maize 50K Bead Chip (Ganal et al. 2011) and Affymetrix Maize 600K array (Unterseer et al. 2014). In maize breeding the availability of these technics in combination with the implementation of genomic selection was the start of an extended continuous genotyping of maize lines. To investigate the diversity of maize, extended comparisons of old founder lines and new germplasm are ongoing. In addition, also studies to describe the actual diversity within and across breeding cycles started as well. First results of using continuously applied genotyping and diversity studies in breeding will be presented.

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Managing diversity in an Elite breeding program

Alain Murigneux

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The development of commercial varieties involves a complex network of research activities. Among many other criteria, a lot of attention must be paid on the Genetic diversity that is present in the germplasm used. The talk will present a schematic structure of a breeding program and will illustrate the need of Germplasm diversity at various steps. We will then have a look at several parameters that influence the process of Genetic diversity maintenance: what kind of Genetic diversity is available and usable? – what are the different processes available to introduce the diversity in the given set of Germplasm and how efficient they are? – how this diversity is maintained or not throughout the process of cultivar development? Practical examples of introduction of diversity into elite Germplasm will be presented. Description of several alternative type of objectives will also be discussed whether the project is driven or not by the introduction of a specific trait. Finally we will discuss the whole picture of the organization of the research that covers the full scope of cultivar development, namely from "Diversity collect and maintenance" to "Genetic resources diversity characterization", to "pre-breeding" all the way to "elite cultivar development". How can we better balance the research effort in these different steps and how may we increase global efficiency by promoting public-private partnership at some steps along this chain.

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Practical aspects of introgression of landraces into elite pools: The Piemont-Pyreneen case study

Cyril Bauland

The genetic group of European flint corn has been allowed the increase of the maize hybrids in the north of Europe. The genetic base of this group is very narrow and lead genetic progress in this group more difficult to achieve. INRA Previous studies highlighted a sub-group of flint European, named „the Pyrenees-Galicia Flint“, for its different genetic origin based on the classification by molecular markers. INRA agronomic studies have showed the interest of the pyreneen population for the characters of silking (female flowering) precocity and forage feeding value, both major breeding targets for north European maize.

To our knowledge, pedigree selection in the Pyreneens Flint population did not led to elite inbred lines involved in commercial hybrids.

With the collaboration of the French private breeders ProMaïs association, we designed a scheme for using those populations to introduce original alleles controlling quantitative economical traits into elite flint inbred lines. The same S1 offsprings of these populations were introgressed in a flint elite line of each partners in order to release BC1S1. The whole 900 BC1S1 were crossed to one Iodent tester and evaluated for silage in a 2 years trials network. We sorted out the population S1 with the overall introgression ability and for each partners the population S1 with a specific introgression ability with their elite line. The population S1 were also topcrossed with the tester and were evaluated in the same trials. We were able to test the correlation between the combining ability of the population S1 and their introgression ability.

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Use of Doubled Haploids in the Context of Genetic Resource Exploitation in Maize

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Haploids are an effective tool to eliminate recessive genes leading to lethality or sub-vitality. The main application of (doubled) haploids (DHs) is production of completely homozygous and homogeneous lines in short time. Potentially masking genetic variation within lines or families is completely eliminated within DH lines. The usefulness of DH lines is significantly increased over F2:3 or F3:4 families, due to a significantly enhanced genetic variation among DH lines. Finally, substantial progress has been made in development in novel inducers and for understanding the genetics of haploid induction and response of donor plants to the induction process. The objective of this presentation is to study and discuss possibilities for using DH technology to support the germplasm enhancement in maize (GEM) allelic diversity project (http://www.public.iastate.edu/~usda-gem/). The GEM diversity project using DH lines to evaluate exotic introgressions in elite background taps into a wide diversity of maize races including Highland germplasm. No selection is made for agronomic performance in the ongoing GEM diversity project, which may be an advantage to capture novel allelic diversity. Currently, 54 races (including 12 Highland races above 2,500 meters elevation) are represented in the DH materials. As a case study, results from a genome-wide association study for agronomic traits related to nitrogen use efficiency using a panel of GEM-DH lines, will be reported in detail.

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Libraries of doubled-haploid lines: a new tool for harnessing landraces

Albrecht E. Melchinger, Juliane Böhm and Wolfgang Schipprack

Landraces of maize (*Zea mays* L.) evolved after the introduction of the crop to Europe over centuries of multiplication and selection by farmers in a diverse range of agro-climatic conditions. With the introduction of hybrid cultivars, many landraces disappeared from production, but most of them have been saved from extinction by seed banks. In the flint × dent heterotic pattern used for hybrid maize breeding in Central Europe, the genetic basis of the flint pool is rather narrow due to a small number of first-cycle lines originating from only a few flint landraces. Moreover, the already narrow genetic diversity of this germplasm pool is reduced by continuous selection. Therefore, countermeasures to increase its genetic diversity are urgently required. One option is to harness the huge reservoir of untapped genetic variation of flint landraces, because only a limited fraction of the diversity present in these materials was captured in the founder lines and modern elite flint lines.

Since landraces in maize are open-pollinated populations, they represent conglomerates of highly diverse, heterozygous individuals with a high genetic load. This entails problems for seed banks and prebreeding, because the genetic heterogeneity hampers characterization of landraces and the exploitation of the genetic variation available in landraces requires the development of inbred lines. However, the success rate of developing inbred lines from landraces by recurrent selfing was extremely low due to the high genetic load in these populations. During the past decade, the *in vivo* doubled haploid (DH) technology has largely replaced recurrent selfing for line development in maize for temperate elite germplasm. Thus, production of DH line libraries (DHL) from landraces by the *in vivo* haploid induction method offers great promise for unlocking their untapped genetic diversity. To test this hypothesis, we compared the line *per se* performance of 389 lines from six DHL derived from European flint landraces with that of four flint founder lines (FFL) and 53 elite flint lines (EFL) for 14 agronomic traits evaluated in four locations. The genotypic variance (σ_G^2) within DHL was generally much larger than that among DHL and exceeded also σ_G^2 of the EFL. For most traits, the means and differed considerably among the DHL, resulting in σ_G^2 different expected selection gains. Mean grain yield of the EFL was 25% and 62% higher than of the FFL and DHL, respectively, indicating considerable breeding progress in the EFL and a remnant genetic load in the DHL. Usefulness of the best 20% lines was for individual DHL comparable to the EFL. Altogether, our results corroborate the tremendous potential of landraces for broadening the narrow genetic base of elite germplasm.

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Association mapping of agronomic and metabolic traits in DH libraries of landraces

Juliane Böhm, Wolfgang Schipprack and Albrecht E. Melchinger

Maize (*Zea mays* L.) is the most important crop in global agriculture and has a long history as model species for basic research. Most of the research in maize was conducted with elite lines, whereas comparatively little attention has been given to genetic resources. However, many genes for important traits may have been lost by breeders in selecting high performing cultivars. Landraces, originally shaped by natural and artificial selection, served as source material for the development of first-cycle inbred lines during the introduction of hybrid breeding. Since then, their use as source material for breeding has drastically declined because of the high genetic load uncovered during recurrent selfing which encumbers the development of inbred lines. This problem could be overcome by the development of libraries of doubled-haploid (DH) lines from landraces. Since landraces of maize are genetically diverse, and they were random mated over hundreds of generations, a rapid decay of linkage disequilibrium (LD) can be expected. These preconditions propose doubled haploid line libraries (DHL) from landraces as a tool for high-resolution association mapping (AM). Nevertheless, a proof-of-concept for using DHLs as a biological resource for high-resolution AM is so far lacking in the literature. To test this hypotheses, we demonstrate the use of DHL in a joint AM study of metabolites and agronomic traits.

A total of 288 distinct metabolites were detected in the whole sample, including 118 metabolites with known chemical structure. Repeatabilities of metabolite measurements were on average 0.73. For all metabolites, the genotypic variance ($\overline{\sigma_G^2}$) within DHL was much larger than that among DHL. In addition, the σ_G^2 of the EFL was lower than that within DHL for the majority of metabolites. Correlations between agronomic traits and metabolites were medium to low. AM revealed significant associations for four of 16 agronomic traits (emergence, early vigour, fusarium ear rot resistance and seed set), as well as for 60 of 288 metabolites, of which 20 had known chemical structure. For certain metabolites we found clear peaks on single chromosomes, indicating SNPs that explained a relatively high proportion of the variance.

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Finding the optimum level of diversity for germplasm improvement

Manfred Mayer[1], Sandra Unterseer[1], Eva Bauer[1], Natalia de Leon[2], Bernardo Ordas[3], Chris-Carolin Schön[1]

Genome-enabled strategies for harnessing untapped allelic variation of landraces are currently evolving. The success of such approaches depends on the choice of source material. Thus, the analysis of different strategies for sampling allelic variation from landraces and their impact on population diversity and linkage disequilibrium (LD) is required to ensure the efficient utilization of diversity. We investigated the impact of different sampling strategies on diversity parameters and LD based on high-density genotypic data of 35 European maize landraces each represented by more than 20 individuals. On average, five landraces already captured ~95% of the molecular diversity of the entire dataset. Within landraces, absence of pronounced population structure, consistency of linkage phases and moderate to low LD levels were found. When combining data of up to 10 landraces, LD decay distances decreased to a few kilobases. Genotyping 24 individuals per landrace with 5k SNPs was sufficient for obtaining representative estimates of diversity and LD levels to allow an informed pre-selection of landraces. Sampling strategies for harnessing allelic variation from landraces depend on the study objectives. If the focus lies on the improvement of elite germplasm for quantitative traits, we recommend sampling from few pre-selected landraces, as it yields a wide range of diversity, allows optimal marker imputation, control for population structure and avoids the confounding effects of strong adaptive alleles.

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GBS based comparison of USDA and INRA inbred lines collections

Brigitte Gouesnard

Genotyping by sequencing (GBS) is a highly cost-effective procedure that permits the analysis of large collections of inbred lines. We used it to characterize the diversity of 1191 maize flint inbred lines from the INRA collection, the European Cornfed association panel, and lines recently derived from landraces. We analyzed the properties of GBS data obtained with different imputation methods, by comparing them with a 50K SNP array. We identified 7 ancestral groups within the Flint collection (Dent, Northern Flint, Italy, Pyrenees-Galicia, Argentina, Lacaune, Pop corn) that are in agreement with breeding knowledge. It highlighted many crosses between origins and the improvement of flint germplasm with dent germplasm. We performed association studies on different agronomic traits, revealing SNPs associated with cob color, kernel color, and male flowering variation. We compared the diversity of our collection and USDA collection which was previously analyzed by GBS (Romay et al., 2013). Population structure of the 4001 inbred lines confirmed the influence of the historical inbred lines (B73, A632, Oh43, Mo17, W182E, PH207 and Wf9) within the dent group. It showed a distinctly different Tropical group and a Flint group separated from the sweet group (with Northern Flint in admixture) and sub-structured in Italian and European Flint (Pyrenees-Galicia and Lacaune) groups. Interestingly, we identified several selective sweeps between Dent, Flint and Tropical inbred lines that colocalize to SNPs associated with flowering time variation. The joint analysis of collections by GBS offers opportunities for a global diversity analysis of maize inbred lines.

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DNA pooling for evaluating the originality of landraces

Stéphane Nicolas[1], Mariangela Arca[1], Tristan Mary-Huard[1], Brigitte Gouesnard[2], Marie-Christine Le Paslier[3], Cyril Bauland[1], Valérie Combes[1], Delphine Madur[1], Alain Charcosset[1]

Maize landraces germplasm have a very large genetic diversity that is still poorly characterized and exploited in plant breeding programs. We studied the effect of both human selection and environmental adaptation on genome-wide diversity of landraces with a focus on landraces-hybrid transition in order to identify interesting source of genetic diversity to enlarge modern inbred lines pool. We developed a high-throughput, cheap and labor saving DNA pooling approach based on 50K SNP maize Illumina array and estimated thereby allelic frequencies of 23412 SNP in 156 landraces representing worldwide maize diversity. We compared diversity at both global and genome-wide scale level with a diversity panel of 336 inbred lines. Our new approach: (i) gives accurate allelic frequencies estimation that are reproducible across laboratories, (ii) protects both against detection of false allele presence within landraces population and against ascertainment bias. Modified Roger's genetic Distance estimated from 23412 SNP and 17 SSR on same DNA pool are highly correlated validating our approach. Accordingly, structuration analysis based on SNP give consistent results with SSR for highly level of structuration but give a slightly different pictures for more advanced structuration level suggesting that SNP and SSR could capture differently recent evolution. Gene diversity varies strongly globally and along the genome between the landraces and according to their geographic origins. We identified 376 SNP under diversifying selection unraveling a selective footprints in *Tga1/Su1* regions. While some maize landraces were closely related to several inbred lines and strongly contributed to modern breeding pool as Reid Yellow Dent or Lancaster Surecrop, some other have no related inbred lines and seems to have poorly contributed. Surprisingly, we identified few diversity loss or selective sweep between landraces and inbred lines excepted in centromeric regions. Theses landraces and genomic regions could be interesting to enlarge genetic diversity of modern breeding pool.

Keywords: Maize landraces, DNA pooling, Genetic diversity, Selection footprints, Prebreeding, germplasm

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Multiparental QTL mapping of biomass related trait

Adama Seye, Cyril Bauland, Heloise Giraud, Alain Charcosset, Laurence Moreau

Maize (*Zea mays* L.) in Northern Europe is commonly used as silage for cattle feeding or for biogas production and improving whole plant biomass degradation is a major breeding objective. Hybrid varieties selected for silage or biogas generally result from crosses between Dent and Flint groups. It is therefore of key interest to identify in each group favorable alleles at QTL involved in silage quality traits to improve hybrid performances. Multiparental designs composed of several biparental populations proved to be efficient for broadening the diversity addressed in QTL detection studies compared to biparental population designs. However, they were mostly used so far to dissect variation within a given group using a single tester from the opposite group (Giraud et al. 2014). Tester-based designs necessitate to allocate phenotyping efforts between the different groups and do not permit to decompose hybrid value into its General (GCA) and Specific (SCA) Combining Ability components. To overcome this limitation, we implemented an original factorial design of 951 Flint x Dent hybrids obtained by crossing lines issued from a Dent and a Flint multiparental design. In each group, four founder lines were crossed to derive six connected biparental populations. Inbred lines were genotyped for 20K SNPs. Hybrids were phenotyped in eight environments for silage production (analysed by Giraud et al. submitted) and seven silage quality traits: the feeding value, the percentage of cell-wall, two cell wall *in-vitro* digestibility measures and three cell-wall components (cellulose, hemicellulose and lignin content). These traits were obtained from Near Infrared Reflectance Spectroscopy (NIRS) predictions on silage powder. For each trait, we estimated the GCA and SCA components of hybrid variance. We performed QTL detection using different allele coding considering either (i) alleles transmitted from the founder lines or (ii) alleles observed at SNP, assuming different or (iii) equal effects in each group. Predictive abilities of the different QTL models were compared using direct adjustment on the whole data-set and a cross-validation approach. We found a predominance of GCA compared to SCA that generally explained less than 10% of the hybrid variance for the traits considered. Depending on the QTL model, we detected between 74 and 80 QTLs across traits with only 2 QTL showing significant SCA effects at the whole genome level. The model assuming equal QTL effects in each group detected on average fewer QTL and explained a smaller percentage of variance, consistent with the known divergence between the Flint and Dent groups. Each QTL explained less than 10% of the within-population phenotypic variance and 96% of them co-localize with at least one QTL of another silage quality trait or silage production trait suggesting pleiotropic effects. For the best QTL model per trait, predictive abilities based on cross-validation ranged from 0.45 (NDF considering dominance effects) and 0.82 (for Lignin). This work opens new prospects for improving maize hybrid performances for both biomass productivity and degradability.

***Keywords:* Maize, hybrid value, silage, multiparental design, QTLs**

Reference:

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Choice of individuals for the training set in genomic prediction

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Genomic selection refers to the use of genotypic information for predicting the performance of selection candidates. It has been shown that prediction accuracy depends on various parameters including the composition of the calibration set (CS). Assessing the level of accuracy of a given prediction scenario is of highest importance because it can be used to optimize CS sampling before collecting phenotypes, and once the breeding values are predicted it informs the breeders about the reliability of these predictions. Different criteria were proposed to optimize CS sampling in highly diverse panels, which can be useful to screen collections of genotypes. But plant breeders often work on structured material such as biparental or multiparental populations, for which these criteria are less adapted. We derived from the generalized Coefficient of Determination (CD) theory different criteria to optimize CS sampling and to assess the level of risk associated to predictions in such structured material. These criteria were evaluated on two Nested Association Mapping (NAM) populations and two highly diverse panels of maize. They were efficient to sample optimized CS in most situations. They could also estimate at least partly the level of risk associated to predictions between NAM families, but they couldn't estimate the level of risk associated to the predictions of NAM families using the highly diverse panels as calibration sets. We illustrated that the CD criteria could be adapted to various prediction scenarios including inter and intra family predictions, resulting in higher prediction accuracies.

Keywords: Genomic selection, maize, calibration set, optimization, coefficient of determination, nested association mapping

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Genomic selection in synthetics — results from a simulation study

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Synthetics have played a pioneering role in quantitative genetic research and plant breeding and are currently rediscovered as a gateway for establishing genetically diverse source materials for selection and QTL mapping. Here, we employed extensive computer simulations to assess the usefulness of these populations for genomic prediction, which has not been systematically addressed hitherto. We generated synthetics by intermating *in silico* different numbers of parental inbred lines of maize (*Zea mays* L.) that originated either from source germplasm with short- or long-range linkage disequilibrium (LD). Using several customized simulation designs, we demonstrate that the information sources rendering genomic prediction superior to traditional pedigree-based BLUP converge from co-segregation to germplasm-wide LD as the number of parental components is successively increased. Co-segregation information dominates in synthetics generated from two to eight parents and provides high prediction accuracy even if marker density and training set size are only moderate. By comparison, capturing germplasm-wide LD information becomes mandatory when using larger numbers of parents, which requires a substantial increase of the resources devoted to phenotyping and genotyping. For prediction sets taken from unrelated synthetics, only germplasm-wide LD can be harnessed, which results in poor prediction accuracies unless strong long-range LD together with high marker density and large training sets are available. Our approach for analyzing prediction accuracy in synthetics provides new insights into the prospects of genomic selection for many types of source populations encountered in plant breeding.

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A comparison of criteria for optimizing gain from recurrent genomic selection

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Genomic selection offers the possibility to jointly estimate the effects of genome-wide molecular markers. These marker effects can be used to calculate genomic estimated breeding values (GEBVs) for individuals without phenotypes, which can serve as a selection criterion in recurrent genomic selection. However, the availability of marker effect estimates and other genomic information offers the possibility to develop alternative selection criteria to improve long-term genetic gain. We propose a novel criterion called expected maximum haploid breeding value (EMBV), which predicts the performance of the best among all gametes that an individual would contribute to the next generation. We used extensive simulations to examine the performance of EMBV in comparison with using GEBVs as well as two other recently proposed selection criteria. We considered different population sizes, numbers of selected candidates, chromosome numbers and levels of dominant gene action. Criterion EMBV outperformed GEBVs in terms of genetic gain after about 5 selection cycles, maintained higher genetic diversity in the population and eventually led to a higher long-term genetic gain. The two other selection criteria showed the potential to surpass GEBVs and EMBVs in advanced cycles of the breeding program, but yielded substantially lower genetic gain in early to intermediate selection cycles. Moreover, they were largely inferior in a scenario with completely dominant gene action. We conclude that EMBVs have the potential to be a promising alternative to GEBVs for recurrent selection.

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Omics-based Hybrid Prediction in Maize

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The development of high-density SNP chips has enabled whole-genome prediction (WGP), but further progress is limited due to its inability to incorporate intricate epistatic interactions. Interactions within and between preceding biological strata are expected to be, at least partially, integrated by downstream 'omics' predictors. Tapping their information is therefore anticipated to improve upon currently realized predictive abilities. Here, we evaluate the prospects of forecasting agronomic performance in maize hybrids using several 'omics' predictors – genomic, transcriptomic and metabolic data – collected on their parent lines at early development stages. The combination of genomic and transcriptomic data yielded superior predictive abilities across multiple traits, thereby leveraging the success rate in the correct prediction of untested hybrid progeny. An integration of SNP information from both, adjacent and distant locations relative to the expressed genes, was revealed. These results suggest a manifestation of physiological epistasis in downstream predictors, which is transmitted from parent lines to their hybrid progeny. In conclusion, exploiting downstream 'omics' data promises to integrate novel information that can lead to higher and more accurate predictions crucial for efficient hybrid breeding.

Personal Notes

Identification of genes under differential selective pressure in temperate maize

Sandra Unterseer

Understanding genomic differences between maize germplasm pools may contribute to a better understanding of the complementarity in heterotic patterns and of mechanisms involved in adaptation to different environments. To elucidate how selection shaped the pool-specific genomic diversity of maize, divergence of two major germplasm pools exploited in maize breeding, Dent and Flint, was investigated on a genome-wide scale. By screening a panel of 136 temperate maize lines for extreme allele frequencies over extended linked sites, candidate genes under differential selective pressure in Dent and Flint were identified. The significant enrichment in derived allele frequencies for these genes provided strong indication that the candidate regions represented selective sweeps. The identified candidates included genes associated with traits that are known to differentiate Dent and Flint like cold tolerance and flowering time. By investigating the effect of the flowering time candidates in a Dent-Flint introgression library, it was shown that the Flint haplotypes of these candidates promoted earlier flowering. Within the flowering network of maize, a Flint-specific enrichment of genes associated with endogenous pathways was discovered in contrast to Dent, where selection seemed to act predominantly on genes involved in the response to environmental factors. Low levels of differentiation of Flint flowering time candidate genes between European Flint elite lines and European landraces indicated a major contribution of landraces from France, Germany, and Spain to the candidate gene diversity of the Flint elite lines. The findings of this study highlight the role of genomic regions that have undergone intense selection and contributed to the differentiation of temperate Dent and Flint. The identification of pool-specific selection signatures enabled insights into the patterns of diversity of temperate Dent and Flint and provides new targets for future functional analyses and crop improvement.

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Assessment of genetic heterogeneity using whole-genome regression models

Christina Lehermeier, Chris-Carolin Schön, Gustavo de los Campos

Plant breeding populations exhibit varying levels of structure and admixture. These features are likely to induce heterogeneity of marker effects across subpopulations. Traditionally, structure has been dealt with as a potential confounder, and various methods exist to “correct” for population stratification. However, these methods induce a mean correction that does not account for heterogeneity of marker effects. Multivariate models can be used to infer the extent to which marker effects vary among clusters in a heterogeneous population and to predict population-specific breeding values. This information allows characterizing genetic heterogeneity between subpopulations. Our results suggest that the extent to which effects change from subpopulation to subpopulation depends not only on the genetic distance between subpopulations but also on the trait of interest. Finally, we will discuss the effect of linkage disequilibrium on genomic correlations between subpopulations and investigate alternative estimation approaches.

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Alternatives to colchicine for chromosome doubling in maize

Willem Molenaar and Albrecht E. Melchinger

Hybrid maize breeding can benefit tremendously from efficient methods for production of doubled-haploid (DH) lines. Especially the effectiveness of chromosome doubling agents has a major impact on the cost per DH line. Although colchicine, the standard chemical used to double chromosomes, is effective, it is highly toxic. Therefore, new developments in chromosome doubling of haploid seedlings are needed to increase efficiency and cost effectiveness while reducing risks, both to personnel handling chemicals and to the environment. Especially in developing countries, safety aspects of chemicals used for chromosome doubling are important, because chemical disposal is difficult.

As alternatives to colchicine treatment, we evaluated new procedures for chromosome doubling based on herbicide cocktails or treatment with N_2O gas. Experimental results showed that out of all evaluated mitosis-inhibiting herbicides, amiprophos-methyl (APM) and pronamid were the most effective, with chromosome doubling success rates a few percent points lower than colchicine. Treatment of seedlings with N_2O gas yielded success rates at the same level as colchicine. In herbicide and N_2O treatments, the concentration, chemical or gas mixture, and treatment duration had significant effects on the effectiveness of treatments. Acute toxicity of the herbicides used was much lower than colchicine. N_2O gas is also relatively safe, and its disposal simply involves venting into the atmosphere. In conclusion, the developed alternative herbicide seedling treatments are easy to implement in existing colchicine treatment infrastructure, however, their effectiveness has not surpassed colchicine yet. In contrast, the developed N_2O seedling treatment requires a high-pressure treatment chamber; however, the effectiveness of N_2O seedling treatment seems promising in comparison with colchicine treatment.

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A New System for Identification of Haploids in Maize

Wolfgang Schipprack and Albrecht E. Melchinger

In vivo haploid induction has become a routine tool for rapid line development in maize. However, distinguishing haploid *(H)* from diploid crossing *(C)* seeds is problematic for many germplasm due to poor expression or suppression of the currently used *R1-nj* embryo marker.

Xenia effects on oil content (OC) of seeds have been proposed as an alternative for distinction of *H* from *C* seeds (Rotarenco et al., 2007). Melchinger et al. (2014) investigated this proposal and showed, that, using a normal oil inducer with OC=3%, OC distributions of H and C fractions overlap too much for reliable sorting.

Therefore, Melchinger et al. (2013) proposed a new method for sorting *H* and *C* seeds, based on pollination with inducers having high oil (HO) content in the seeds, and presented its theoretical foundation. In a proof-of-concept ten source germplasm of different genetic structure were pollinated by a high-oil (HO) inducer with OC=10.8%. The average difference between the mean OC of *C* and *H* seeds was more than twice the standard deviation within each fraction and sorting of *H* and *C* seeds based on their OC was generally more reliable than based on the *R1-nj* embryo marker.

For clarification, whether reliable sorting of *H* and *C* seeds could possibly be achieved by measuring oil mass (OM) alone, Melchinger et al. (2015) compared the distribution properties of *H* and *C* seeds from 11 induction crosses with high-oil inducers for seed weight (SW), OM, and OC. Since misclassification rates for sorting of *H* and *C* seeds were generally smaller for OC than for OM and the optimal bivariate discrimination function on the basis of OM and SW yielded similar error rates as a univariate discrimination function on the basis of OC alone, it is recommended to use OC as a simple and robust criterion for sorting of *H* and *C* seeds.

Altogether, induction crosses with HO inducers hold great promise for promoting the DH technology in maize, especially for many flint and tropical germplasm, which suppress anthocyanin pigmentation of the embryo. To take full advantage of this novel approach an automated high-throughput platform for sorting seeds is recommended.

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Transcriptomic dissection of heterosis manifestation in young maize roots

Frank Hochholdinger, Jutta Baldauf, Caroline Marcon

Distantly related maize inbred lines exhibit an exceptional degree of structural genomic diversity, which is probably unique among plants. Heterozygous F1-hybrid progeny of such inbred lines are often more vigorous than their homozygous parents, a phenomenon known as heterosis. Single-parent expression (SPE) is an extreme form of complementation on the gene expression level in hybrids. SPE describes the observation that a gene is expressed in the hybrid but in only one of its two parental inbred lines. In previous work, we demonstrated that hybrids express more genes than their parental inbred lines as a consequence of SPE (Paschold et al., 2012). Moreover, we studied the plasticity of SPE in different root tissues of maize (Paschold et al., 2014) and in roots grown under drought stress (Marcon et al., 2017). These initial studies were performed in the inbred lines B73 and Mo17 and their reciprocal hybrids.

We now aim to extend and generalize our previous observations of transcriptomic patterns to a more diverse panel of maize inbred lines and their hybrid progeny and to different stages of maize root development. For this purpose we investigated how the genetic divergence of seven selected parental inbred lines (B73, Mo17, A554, H84, H99, Oh43, W64A) is reflected in the transcriptomic landscape of primary roots of their hybrid progeny during development. A RNA-seq experiment was designed to maximize the number of direct comparisons among the parent-hybrid pairs and simultaneously to ensure a high degree of precision for indirect comparisons. For each developmental stage and each parent-hybrid combination we observed hundreds of SPE genes. Evolutionary aspects of these results and implications for hybrid vigor will be discussed.

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Variation for and inheritance of BYDV resistance in maize

Benjamin Stich

The impact of viruses transmitted by insects to crop plants, such as barley yellow dwarf virus (BYDV), is expected to increase with increasing winter temperatures. Breeding for resistance is the best strategy to control the disease and break the transmission cycle of the virus. The objectives of our studies were to determine genetic variation for BYDV resistance in different genetic materials of maize and to identify molecular markers genetically linked this trait.

The expression of disease symptoms and BYDV resistance were evaluated in field and greenhouse experiments which were artificially inoculated with BYDV. The inheritance of these traits has been studied by linkage and association mapping approaches.

A considerable genotypic variance as well as a high heritability on a plot basis were observed for BYDV resistance and disease symptoms in different genetic materials. A major QTL was identified on chromosome 10 by linkage mapping in connected segregating populations. This QTL was fine mapped to the gene GRMZM2G018027 using association genetics.

Our results suggested that a high gain of selection for BYDV tolerance and resistance of maize can be reached. Furthermore, our findings indicate that BYDV resistance is an ideal candidate for marker assisted selection as trait evaluation is costly and a molecular marker is available that explains a high proportion of the phenotypic variance.

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