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Utilization of landraces
of European flint maize for breeding
and genetic research

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Content

General Introduction

Importance of maize and future challenges

Maize (Zea mays *L.*) is, together with rice and wheat, one of the leading crops cultivated for human nutrition, has the broadest cultivated geographical range of all crops, and not to be rejected an unprecedented level of diversity (Tenaillon et al., 2001; Gore et al., 2009). Maize takes an outstanding importance, since the production of 1,148 million tons of grain worldwide in 2019, is significantly ahead of wheat (765 million tons) and rice (503 million tons, milled equivalent), even if the production area covers a total of 197 million hectares worldwide and lies therewith behind wheat (215 million hectares) and just before rice (162 million hectares). The steadily increasing production and harvest area in Germany and Europe shows the continual importance of maize nowadays (DMK, 2021; FAO stat, 2021). Expanded cultivation practices and breeding efforts during the last decades led to the adaptation of a crop into new growing areas, which is able to cope with different climatic conditions (Frei, 2000), as well as the extensive use as silage crop for biogas production, feed and as a source of diverse industrially important products (Ranum et al., 2014). Maize yields are severely limited in many countries by abiotic and biotic stresses, besides other factors. Production may not be able to meet the demands of a growing world population without technological and policy interventions (Shiferaw et al., 2011) or uncontrolled area expansions. Another challenge for the long-term production will be the growth of maize in a changing global climate (Cairns et al., 2012). Furthermore, a steadily improvement of varieties and maintenance of a high production level with reduced use of fertilizers and pesticides, due to increasing prices and decreasing general public acceptance (Paul et al., 2019) will be a further objective. To increase the productivity and yield stability of maize, continuous attempts in plant breeding, variety development and research have to be undertaken. Since maize was the first fully sequenced allogamous crop (Schnable et al., 2009), it is at the front of genomic research and has also a long tradition as model species for basic research (Strable and Scanlon, 2009).

The role and potential of landraces

Maize has been domesticated in Central America about 9000 years ago from the grass teosinte (Beadle, 1939; Matsuoka et al., 2002; Doebley, 2004). Since that time, it has been

subject to selection and cultivation and like in other crop species, landraces formed the traditional type of variety. They were cultivated by farmers, who planted seeds saved from desirable cross-fertilized plants of the previous generation. Based on farmer's preferences and their needs, open-pollinated populations evolved, adapted to the local climatic and agroecological conditions (Gouesnard et al., 2005; Tenaillon and Charcosset, 2011). By this kind of selection unconscious use of different genetic factors has been made including mutations, genetic drift, or recombination events. The result was a broad diversity of open-pollinated populations adapted to different regions (Warburton et al., 2008).

Molecular evidence showed distinct paths of maize introduction to Europe: Caribbean Flint maize was first introduced to southern Spain by Columbus in 1493 (Rebourg et al., 2003; Brandolini and Brandolini, 2009). A further introduction of Northern Flint from North America to North-Western Europe took place during the 16th century (Rebourg et al., 2003). Comparing the material from Southern and Northern Europe reveals that populations from the Pyrenees and Galice show a large genetic distance to the North American material. Admixture between Northern and Southern germplasm resulted in novel germplasm that was the cradle of the European elite flint material, and a new germplasm pool resulted.

Until the mid of the twentieth century, maize played only a minor role in the agriculture of Central Europe (Schnell, 1992). This changed drastically with the introduction of hybrid breeding in the 1950s, when almost all existing landraces disappeared from their use as source material for breeding. Key factors for the success of hybrid varieties are beside a higher stability and uniformity a superior exploitation of heterosis (Barrière et al., 2006), as observed between the dent and flint heterotic groups (Messmer et al., 1993).

By recurrent selfing, prominent first-cycle lines such as F2 and F7 originating from the French landrace Lacaune, DK105 from the German landrace Gelber Badischer, or EP1 from the Spanish landrace Lizagarote were developed (Messmer et al., 1992). Even though the genetic resources in maize maintained in seed banks are one of the richest sources of all major crops (Ortiz et al., 2010), only a small fraction of the plethora of landraces became the source material of the flint heterotic pool used in maize breeding of Central and Northwestern Europe. Further, many genes for important traits such as drought tolerance or adaptation to low nutrient supply may have been lost by selecting cultivars for high-input environments (Schnable et al., 2011). According to molecular analyses a narrower genetic base is present in

the European flint pool, compared to the dent pool, since only a small proportion of the rich variation present in landraces is embedded there (Messmer et al., 1992; Dubreuil and Charcosset, 1999; Stich et al., 2005; Van Inghelandt et al., 2010). In comparison, the dent heterotic pool displays a very broad genetic diversity, since early maturing inbreds from breeding programs in the US as well as from Canada were used as source material (Messmer et al., 1992). Therefore, the genetic base of the dent heterotic pool is much broader compared to the flint heterotic pool, which is inevitably further reduced by selection. Efforts to broaden the narrow base of the flint breeding pool are needed urgently to avoid compromising the future selection gain, but they succeeded only moderately. Exploitation of the largely untapped reservoir of genetic variation in landraces could be an option to reverse the ongoing narrowing of the genetic basis (Strigens et al., 2013b). Therefore, it seems rewarding to revisit landraces and to assess their breeding potential in a profound manner.

More than 300,000 accessions of maize genetic resources are currently stored in gene banks and core collections worldwide (Table 1; FAO, 2010). Approximately one third of these accessions are landraces. The necessity to preserve genetic resources appeared when the importance of the conservation of national genetic patrimony was recognized (Edwards and Leng, 1965), and fortunately a huge number of maize landraces has been saved before their extinction (Dallard et al., 2000). Publicly managed gene banks are organizing the *ex situ* conservation, maintenance, and multiplication of landraces. Since landraces were collected in their growing locations, they are a treasure trove harboring alleles for important traits like tolerances and resistances, which are important for plant breeding and coping with the global challenges of agriculture (Moreno-Gonzalez et al., 1997; Peter et al., 2009; Mercer and Perales, 2010; Strigens et al., 2013b; Meseka et al., 2014; Melchinger et al., 2017; Romero Navarro et al., 2017; Mayer et al., 2020). Nevertheless, the characterization and description for important agronomic traits of the accessions is often times lacking, which impedes their use for practical breeding.

Table 1 Maize genetic resources stored in gene banks and collections worldwide (FAO, 2010)

Genebank	Total accessions		Landrace accessions	
	No.	%	No.	%
CIMMYT *International Maize and Wheat Improvement Center*	26.596	8	23.670	89
BPGV-DRAEDM *Portuguese Bank of Plant Germplasm*	24.529	7	1.962	8
NC7 *North Central Regional Plant Introduction Station, United States Department of Agriculture*	19.988	6	15.791	79
ICGR-CAAS *Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences*	19.088	6	0	
INIFAP *Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (Mexico)*	14.067	4	0	
VIR *N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry (Russian Federation)*	10.483	3	3.250	31
NBPGR *National Bureau of Plant Genetic Resources (India)*	6.909	2	1.105	16
NIAS *National Institute of Agrobiological Sciences (Japan)*	5.935	2	415	7
MRIZP *Maize Research Institute «Zemun Polje» (Serbia)*	5.475	2	3.011	55
CORPOICA *Centro de Investigación La Selva, Corporación Colombiana de Investigación Agropecuaria (Colombia)*	5.234	2	0	
BRGV Suceava *Suceava Genebank (Romania)*	4.815	1	3.322	69
IPGR *Institute for Plant Genetic Resources «K.Malkov» (Bulgaria)*	4.700	1	1.081	23
INRA-Montpel *Institut national de la recherche agronomique/Genetics and Plant Breeding Station (France)*	4.139	1	1.159	28
CENARGEN *Embrapa Recursos Genéticos e Biotecnologia (Brazil)*	4.112	1	0	
IR *Institute of Plant Production n.a. V.Y. Yurjev of UAAS (Ukraine)*	3.974	1	517	13
UNALM *Universidad Nacional Agraria La Molina (Peru)*	3.023	1	3.023	100
SSJC *Soutnern Seed Joint-Stock Company (Viet Nam)*	2.914	1	0	
RCA *Institute for Agrobotany (Hungary)*	2.765	1	1.051	38
BAP *Banco Activo de Germoplasma de Pergamino (Argentina)*	2.584	1	2.584	100
INIACRF *Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria,Centro de Recursos Fitogenéticos (Spain)*	2.344	1	2.227	95
UzRIPI *Uzbek Research Institute of Plant Industry*	2.200	1	0	
CERI *Cereal Institute, National Agricultural Research Foundation (Greece)*	2.048	1	0	
IPB-UPLB *Institute of Plant Breeding, College of Agriculture, University of the Philippines, Los Baños College (Philippines)*	2.013	1	2.013	100
EETP *Estación Experimental Pichilingue (Ecuador)*	2.000	1	0	
Others	145.997	45	42.339	29
Total	327.932	100	108.218	33

Limitations of landraces for breeding

In most crops, landraces remain widely unexploited since they are genetically and phenotypically unexplored. Only a limited amount of the genetic diversity of maize landraces worldwide has been used in European hybrid breeding programs so far (Dubreuil and Charcosset, 1999), even though they appear to be valuable genetic resources for broadening the genetic base of the elite flint pool. Therefore, it can be conjectured that those landraces, which did not serve as germplasm source for the current elite flint breeding pool, may contain useful untapped allelic variation. An important goal in regenerating gene bank accessions is, to avoid the loss of genetic diversity and favorable alleles resulting from genetic drift, inbreeding or the loss of viability (Dallard et al., 2000). Furthermore, their value for breeding will steadily decrease due to the increasing yield gap between the genetic resources and the elite breeding materials as well as their poor agronomic characters. Among others, this is a reason, why landraces cannot easily be used directly in a breeding program, making it urgent to continue and initiate new programs in the pre-breeding domain evaluating the breeding value of the genetic resources.

Since maize landraces are open pollinating populations, they are highly heterogeneous collections of individuals varying in the degree of heterozygosity harboring beside favorable alleles also detrimental alleles, called the genetic load (Hoisington et al., 1999; Strigens et al., 2013b). Hence, two problems arise: First, a main problem for gene banks is the genetic heterogeneity, which impairs the characterization of landraces. For monogenic traits, the exploitation of landraces is simple by using recurrent backcrossing, supplemented by marker-assisted selection to reduce the linkage drag (Frisch and Melchinger, 2001). However, for quantitative traits, in characterizing the potential of landraces the variation within landraces is generally neglected and only the population mean for agronomic (Mieg et al., 2001; Ferro et al., 2007; Malvar et al., 2007; Peter et al., 2009; Romay et al., 2010) and morphological traits (Gouesnard et al., 1997; Lucchin et al., 2003) is evaluated with high precision but not the performance of "immortalized" genotypes such as lines or clones. Second, since landraces are the foundation of hybrid breeding, the exploitation of the total variation in landraces requires the development of inbred lines, which was until now accomplished by recurrent selfing of the source material. However, in landraces the success of recurrent selfing was low since the genetic load caused poor vigor and the loss of lines due to fixation of detrimental and/or lethal

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alleles. Further, unwanted properties, which are tightly linked with the desired ones, might reduce the breeding value of the developed inbred lines, as negative properties will unintentionally be introduced into the breeding germplasm.

To assess the value of a landrace by evaluating its performance per se or its combining ability depends on the purpose of usage. While the breeding value of a genetic resource in line breeding relates mainly to its per se performance, the per se performance is only of secondary importance in hybrid breeding, because the value of parent lines is mainly determined by their combining ability with the opposite heterotic pool (Melchinger and Gumber, 1998). Identification of promising heterotic patterns among landraces was therefore focus in many studies (Malvar et al., 2004, 2005; Revilla et al., 2006). Nevertheless, a comprehensive study for the evaluation of the combining ability of flint landraces with testers from the dent heterotic pool was largely lacking.

DH technology for producing lines from landraces – DHL

To exploit most efficiently this largely untapped reservoir of genetic variation, Reif et al. (2005) proposed the use of the doubled haploid (DH) technology also for landraces. Historically, the DH technology replaced recurrent selfing for line development in elite materials (Prigge and Melchinger, 2012). The production of DH lines involves (i) haploid induction by pollinating the source germplasm with pollen of an inducer line to produce seeds with haploid embryos; (ii) the identification of haploid seeds by using a suitable marker; (iii) chromosome doubling of the haploid seeds with a mitotic inhibitor such as the alkaloid Colchicine, which is commonly used; and (iv) the selfing of the DH plants to increase the amount of seeds of the newly generated lines. This technology has been recommended for landraces since it perfectly captures all alleles present in the landrace, creates homozygous samples of the maternal gametes, and enables a more efficient and rapid access to the diversity harbored in landraces (Reif et al., 2005; Melchinger et al., 2017). However, information about the production of DH lines from landraces and assessment of their value in comparison with elite materials is scarce hitherto. First studies gave rise to hope, since only a moderate yield gap was observed between DH lines from landraces and commercial checks in European and tropical germplasm (Wilde et al., 2010; Prigge et al., 2012; Strigens et al., 2013b). Melchinger et al., (2017) showed that large-scale production of DH lines from

landraces is possible, albeit higher expenditures compared to elite materials have to be accepted. The advantages of the production of DH lines of landraces are obvious: (i) In the haploid or homozygous diploid stage the genetic load is uncovered and reduced, and the inbreeding process can be used to purge landraces from lethal or detrimental alleles (Crnokrak and Barrett, 2002; Eder and Chalyk, 2002). (ii) Immortalized gametes are produced and therefore, the alleles of the heterozygous individuals in landraces are fixed (Gallais, 1990). This also facilitates the maintenance for gene banks. (iii) A faster line development is achieved, and since lines are pure-breeding, multiplication and characterization is simplified and can be conducted ad libitum (Melchinger et al., 2005). (iv) The whole genetic variance within landraces is revealed within one step. Doubled haploid line libraries (DHL) from landraces therefore offer great promises to fix and preserve the genetic diversity, and to unlock the potential of landraces. Further, the level of linkage disequilibrium (LD) is expected to be low, which makes them an excellent research tool for association mapping. However, a thorough study, providing information about the production of DH lines in landraces and comparing the breeding potential of DHL from landraces with that of elite germplasm to broaden the genetic base of the flint heterotic pool, is urgently needed.

Association mapping and omics in DHL

High-throughput genotyping platforms and sequencing of individuals is routine since long time (Cao et al., 2011; Elshire et al., 2011; Ganal et al., 2011). Linking complex phenotypes to their genotypes in association mapping studies (for genes) becomes a routinely applied tool as well (Yu et al., 2006; McMullen et al., 2009b; Huang et al., 2015; Romero Navarro et al., 2017). Genetic mapping is a powerful tool to exploit the genomic information and to identify genetic determinants proving useful for crop improvement. Since marker density is not any more a major limitation (Stange et al., 2013), the ability to discover genetic determinants in a mapping study depends on the genetic diversity of the population and a high rate of genetic recombination (Mackay et al., 2009). In linkage mapping, where two inbred founders are crossed to produce genetically segregating progenies, populations provide a high statistical power for detection of quantitative trait loci (QTL) but suffer from a shortage of diversity and recombination events. In view of the tremendous progress in gene editing, intentions are nowadays to go beyond QTL down to the level of causal variants and underlying genes. With

increasing marker densities and reduced genotyping costs (Wallace et al., 2014) genome-wide association studies (GWAS) are promising approaches to detect genes and alleles of interest in germplasm collections. Compared to linkage analysis, GWAS offer three advantages: (i) higher mapping resolution; (ii) greater number of alleles and a broader reference population; and (iii) less research time in establishing an association between marker information and the causal genes (Buckler and Thornsberry, 2002; Flint-Garcia et al., 2003). Association mapping further benefits from high genetic diversity and the historical accumulation of recombination events (Dell'Acqua et al., 2015). Several techniques became available in GWAS for decoupling genetic associations with confounding factors (Yu et al., 2006; Sillanpää, 2011), such as population structure or cryptic relatedness leading to spurious associations (Astle and Balding, 2009). Different multi-parent cross designs, as the nested association mapping (NAM) design (Yu et al., 2008; McMullen et al., 2009b) or the multi-parent advanced generation intercrosses (MAGIC) population (Dell'Acqua et al., 2015; Huang et al., 2015), already employed and combined the advantages of both approaches. In comparison to traditional linkage mapping, in GWAS existing populations can be used and, given a high marker density and a fast decay in LD, a high mapping resolution can be achieved (Flint-Garcia et al., 2003).

A mine of genetic diversity can be found in a broad set of DH lines from landraces. Large phenotypic and genotypic variances can be expected since only a marginal degree of artificial selection took place. Conducting replicated trials makes it possible to estimate variance components as well as heritabilities to quantify the selection gain. Further, new advantageous characteristics might be identified in those genetic resources. Genotyping of DHL from landraces with high density single nucleotide polymorphism (SNP) marker platforms (Ganal et al., 2011) has already been done and gave insights into the diversity of landraces. A low degree of LD was observed in European landraces (Reif et al., 2005; Tenaillon and Charcosset, 2011; Mayer et al., 2017) as well as in DH lines derived from landraces (Brauner et al., 2018; Mayer et al., 2020). A combination of a high diversity and a dense marker coverage makes DHL perfect tools for GWAS. Most importantly, the decay of LD is expected to be much faster in DHL from landraces than in DH lines derived from elite breeding populations due to the ample opportunities for recombination attributable to panmixia for hundreds of generations (Strigens et al., 2013b; Mayer et al., 2017). Further, because DH lines represent immortalized genotypes, which can be multiplied and phenotyped with a high precision, a high QTL detection power should be possible due to a high heritability. The precise identification

of QTL and underlying candidate genes allows the introgression of favorable traits from landraces into elite material without the introduction of undesirable properties by linkage drag. Further, it allows a deeper understanding of the genetic architecture and understanding of physiological and metabolic pathways (Riedelsheimer et al., 2012).

A strategy to read out the plant physiological status is the plant metabolome, which is an intermediate between the genome and the phenome in plants (Luo, 2015). Since many metabolites are under the control of only few genes (Riedelsheimer et al., 2012), their architecture is probably less complex than for agronomic traits. Therefore, they could provide a link for dissecting the genetic architecture of complex traits. Metabolite profiling was carried out first in medicine (Suhre and Gieger, 2012), but can meanwhile be applied on a large scale with the invention of new techniques. Progresses in gas chromatography separation coupled with mass spectrometry (GC-MS) analyses with high-throughput platforms can identify and quantify a meaningful fraction of the entire metabolome in higher plants resulting in a wide coverage of central pathways (Lisec et al., 2006). An all-encompassing view on the potential of libraries of immortalized lines from landraces including phenotypic traits and metabolites is until now lacking and would complement the integrated view on the traditional variety types of maize.

Objectives

The goal of this thesis research was to evaluate the breeding potential of European flint maize landraces and use the advantages of the DH technique to unlock the diversity of these genetic resources by mining for new alleles by association mapping in DH lines derived from landraces. In particular, the objectives were to

(1) determine the genetic variation for testcross performance of European landraces of the flint germplasm pool in combination with elite dent testers

(2) evaluate the phenotypic and genotypic variation of immortalized lines within and among landraces

(3) compare the per se performance of those line libraries with elite lines as well as founder lines from the European flint germplasm pool

(4) analyze the breeding potential of immortalized lines from landraces in comparison with elite material to improve the narrow genetic base of the flint heterotic pool

(5) demonstrate the high mapping resolution of DHL from landraces in association mapping down to causal variants and underlying genes

(6) provide conclusions and guidelines for breeding and research using libraries of immortalized lines from landraces

Breeding Potential of European Flint Maize Landraces Evaluated by their Testcross Performance

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Abstract

A plethora of maize (Zea mays L.) landraces is stored in gene banks worldwide. However, information about their value in breeding is scarce and strategies for identifying the most promising landraces in pre-breeding are largely lacking. This study was conducted to (i) evaluate the testcross performance of 70 European flint landraces in combination with two elite dent testers and compare these results with the performance of modern hybrids for important agronomic traits, (ii) estimate the genetic variances among landraces and trait correlations for these two testcross series as well as the correlation between them, and (iii) devise a testing scheme for assessing the breeding potential of a large number of landraces for hybrid breeding. Grain yield of the landrace testcrosses was on average about 26% lower than modern hybrids. Genotypic variances among landrace testcrosses were significant for all traits, and genetic correlations were moderate to high for most trait combinations in both testcross series. Thus, it seems promising to tap this huge genetic reserve for enlarging the genetic base of the elite flint germplasm pool in Central Europe. Since the genetic correlation between the two testcross series exceeded 0.74 for all traits, we recommend assessing the breeding potential of landraces for broadening existing heterotic groups by evaluating their testcross performance in combination with one or two elite single-cross tester(s) from the opposite heterotic pool. Subsequently, doubled haploid lines from a few of the most promising landraces could be produced to exploit the large genetic variation within landraces to the full extent.

Tapping the genetic diversity of landraces in allogamous crops with doubled haploid lines: a case study from European flint maize

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Abstract

Landraces of maize (Zea mays L.) represent a huge reservoir of genetic diversity largely untapped by breeders. Genetic heterogeneity and a high genetic load hamper their use in hybrid breeding. Production of doubled haploid line libraries (DHL) by the in vivo haploid induction method promises to overcome these problems. To test this hypothesis, we compared the line per se performance of 389 doubled haploid (DH) lines across six DHL produced from European flint landraces with that of four flint founder lines (FFL) and 53 elite flint lines (EFL) for 16 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 σ_G^2 differed considerably among the DHL, resulting in different expected selection gains. Mean grain yield of the EFL was 25 and 62% higher than for 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 and grain yield (GY) in the top lines from both groups was similar. Our results corroborate the tremendous potential of landraces for broadening the narrow genetic base of elite germplasm. To make best use of these "gold reserves", we propose a multi-stage selection approach with optimal allocation of resources to (1) choose the most promising landraces for DHL production and (2) identify the top DH lines for further breeding.

High-resolution association mapping with libraries of immortalized lines from ancestral landraces

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Abstract

Landraces are traditional varieties of crops that present a valuable yet largely untapped reservoir of genetic variation to meet future challenges of agriculture. Here, we performed association mapping in a panel comprising 358 immortalized maize lines from six European Flint landraces. Linkage disequilibrium decayed much faster in the landraces than in the elite lines included for comparison, permitting a high mapping resolution. We demonstrate this by fine-mapping a quantitative trait locus (QTL) for oil content down to the phenylalanine insertion F469 in DGAT1-2 as the causal variant. For the metabolite allantoin, related to abiotic stress response, we identified promoter polymorphisms and differential expression of an allantoinase as putative cause of variation. Our results demonstrate the power of this approach to dissect QTL potentially down to the causal variants, toward the utilization of natural or engineered alleles in breeding. Moreover, we provide guidelines for studies using ancestral landraces for crop genetic research and breeding.

General Discussion

Breeding potential of European landraces

Evolution and domestication resulted in higher productivity of plants, but domestication also represented a bottleneck for their genetic diversity. The challenge of modern plant breeding is to create higher yielding and environmentally friendly varieties without harnessing further natural habitats for their production. This dissertation aims to utilize traditional variety types of maize as a source of genetic variation, which has been lost in modern elite germplasm, mostly by breeding activities. The hidden potential of maize germplasm resources including landraces is lying dormant in gene banks but considered essential to overcome future challenges of agriculture, especially those imposed by climate change, and to meet future demands on crop production (McCouch et al., 2013; Sood et al., 2014). Genetic studies can identify valuable traits of landraces, and introgression breeding can transfer the underlying genes to commercial breeding germplasm. If genetic resources remain idle in gene banks, the yield gap between them and the elite breeding material is continuously increasing (Duvick, 2005), which will decrease their future value in breeding.

Owing to the 'founder effect' in crop evolution, only a small fraction of the genetic variation present in their wild relatives is available in modern plant species (Langridge and Waugh, 2019). While landraces preserved this useful genetic diversity, they remained untapped, hitherto, due to genetic linkage between loci with useful and undesired alleles (Warburton et al., 2008). Nevertheless, they are natural candidates for introgression into the flint heterotic pool since the latter traces back to a small number of first-cycle lines extracted from a few European flint landraces at the beginning of hybrid breeding (Rebourg et al., 2001; Gauthier et al., 2002). Landraces can be incredible rich resources of genetic variation, especially for traits that are target of selection nowadays, like abiotic stress tolerance or those focusing on resource efficiency (Lobell and Tebaldi, 2014). Therewith, they can help to adapt crops to cultivation in the coming decades and cope with challenges such as new pests and the consequences of climate change.

Regarding the exploitation of landraces in allogamous crops, breeders are confronted with two major problems. First, they have to identify the most promising landrace accessions from the plethora of landraces for introgression into the known heterotic pools within their

breeding programs. Currently, this is mainly based on passport data provided by seed banks or data on their per se performance and/or testcross performance (Salhuana and Pollak, 2006; Böhm et al., 2014) but in the future, genotyping data can be used as complementary sources of information to monitor their molecular variance and uncover hidden relationships (Mayer et al., 2017). Further, since the majority of diversity is not lying between, but within landrace populations (Monteiro et al., 2016; Böhm et al., 2017; Mayer et al., 2017), the breeder has to mine the diversity within landraces. For later use in hybrid breeding, this should preferably be in the form of inbred lines, whereas landraces stored in gene banks are populations of heterozygous individuals. Consequently, there is a high need to characterize landrace populations and evaluate their breeding potential at the level of inbred lines extracted from them, so that the entries can be multiplied ad libitum and phenotyped with any desired degree of precision. In the present study, we used the advantages offered by the doubled haploid (DH) technology to get access to the genetic richness of landraces and to evaluate their breeding potential. The main objectives were to (i) propose a sampling strategy for breeders to improve the genetic diversity and performance of the current European germplasm pool, and (ii) evaluate the genetic potential of DH lines from landraces for direct use in breeding. With regard to genetic research, the objective was to demonstrate the use of doubled haploid line libraries (DHL) from landraces as a novel tool for association mapping to dissect candidate genes valuable for further breeding and research.

Sampling strategies

To initiate new or continue existing breeding programs in the pre-breeding domain, evaluating the breeding value of landraces for complex traits is crucial. Available information relates mostly to studies with comparatively small numbers of flint landraces and to their per se performance or classification into heterotic groups (Moreno-Gonzalez et al., 1997; Soengas et al., 2003, 2006). Since breeders have to choose from a large number of landraces the most promising ones for introgression into their heterotic pool, the combining ability of those genetic resources with the opposite heterotic pool is of primary interest.

Whereas maize landraces are highly heterogeneous populations, their potential in hybrid breeding depends not only on the population mean, but also on the variance within the population. Schnell (1983) proposed the usefulness criterion for comparing different

source populations, which combines information on the mean, the genetic variance and heritability with respect to the predicted response to selection. Thus, it is a suitable criterion to compare the breeding potential of different landraces, which usually differ in both the means and variances. Determining the population mean is generally less a problem but predicting the selection response requires also reliable estimates of heritability and the genetic variance, which requires sampling and evaluating a sufficiently large number of genotypes from a segregating population in replicated trials. As discussed by Böhm et al. (2014), there is ample room for making rapid breeding progress by selecting among lines within landraces, since testcross genetic variance with individual testers and the general combining ability variance of elite flint lines was of the same magnitude.

Different sampling strategies are devised to evaluate the breeding value of landraces, depending on the aim of the breeding program. One strategy will be to sample one or a few individuals from many landraces covering a wide range of genetic diversity, whereas contrary, one can sample many individuals from a few pre-selected landraces. The former will be applied when aiming to enlighten mechanisms of plant adaptation or identifying new alleles for disease resistances or quality traits, as conducted by Romero Navarro et al. (2017), where it is central to maximize the allelic diversity of the discovery panel. The latter might be promising for enlarging the genetic diversity of elite material for quantitative traits. This will only slightly reduce the level of molecular diversity (Mayer et al., 2017) compared to sampling from a more extensive set of landraces. The majority (> 70%) of the molecular (Sood et al., 2014; Mayer et al., 2017) and phenotypic (Strigens et al., 2013a; Böhm et al., 2017) variation is observed within those pre-selected landraces, and therefore, a high genetic variation for quantitative traits of interest can be assumed within a pre-selected set of landraces. Even if diversity parameters are varying between landraces, the majority of landraces are showing a high diversity while being collected and maintained in gene banks and by farmers. Therefore, for broadening the narrow genetic base of quantitative traits in pre-breeding programs, it seems more appropriate to sample a few highly selected landraces intensively rather than sampling from many landraces only a few genotypes each.

For doing this, I recommend a multi-step procedure described in Fig. 1. (i) In a first pre-selection step, a core set of landraces is identified by analyzing genotyping data for the presence of variation for the target trait(s) from accessions collected in their target region. (ii)

The selected collection will be screened in observation plots for adaptation and acceptable agronomic characteristics. During this step, the value of the tested set can be further increased by assuring that selected landraces are segregating for the trait of interest (Mayer et al., 2017). (iii) Landraces meeting these criteria best are evaluated in trials in multiple environments representative for the target region to evaluate their testcross performance with a single- or double-cross tester from the opposite heterotic pool as described by Böhm et al. (2014). In parallel, we recommend a comparison of the usefulness (U(α)) of the selected landraces to get information on the genetic variance in the key trait. Since this would require multi-location trials with at least around 30 entries per landrace, we recommend to take the molecular diversity within the landraces as a proxy for the genetic variance, following the proposal of Strigens et al. (2013). (iv) The most promising landraces out of those evaluated in the previous step are chosen, preferably based on U(α), to produce immortalized lines. This can preferably be accomplished by making use of the advantages of the DH technology to get access to the phenotypic and genetic richness of landraces and make it further available for research and breeding purposes. Hereby, the promising landraces could be evaluated for their success rate in producing immortalized lines and relate this information to molecular data for predicting the success rates in other materials. (v) Finally, a small number of the most promising lines from the DHL are used to tap novel genetic variation in the plethora of genetic resources.
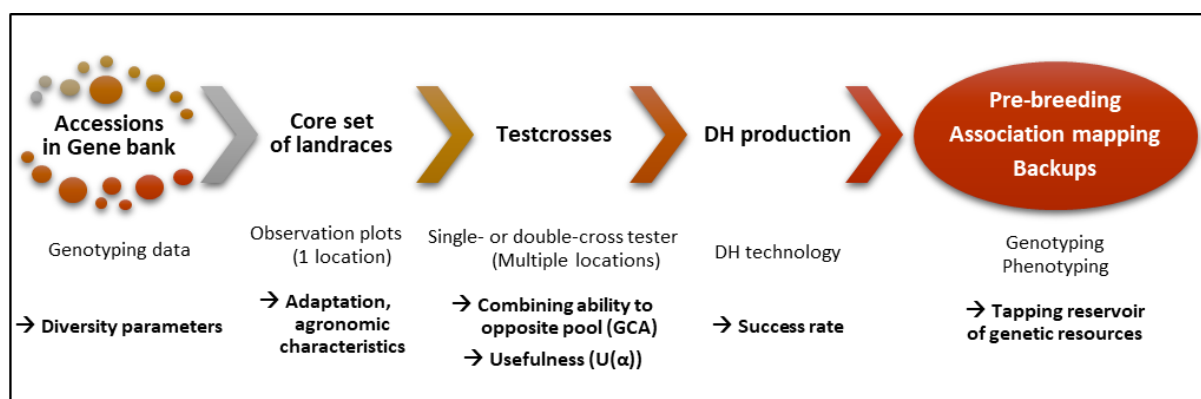

Figure 1   Multi- step procedure for identification of promising landraces

Using the DH technology to exploit the diversity of landraces

The difficulties for introgression of favorable alleles from genetic resources into elite material or their use in practical breeding programs are manifold as already stated. One method to alleviate some of these difficulties would be the development of maize inbred lines

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from the original population to generate reproducible units carrying the diversity of the source material. Nevertheless, the production of inbred lines in maize by recurrent selfing is time-consuming or not possible at all due to self-incompatibility or the high genetic load (Hallauer et al., 2010). Alternatively, the diversity of genetic resources can be tapped by gamete capture (Stadler, 1944). Here, a random sample of gametes of the source germplasm is used to pollinate elite genetic material, usually an inbred line, followed by recurrent selfing steps of the resulting offspring. A subsequent evaluation of the offspring can limit the usefulness of the gamete capture approach with respect to introducing new diversity, since a strong selection in favor of alleles contributed by the elite parent is probable (Sood et al., 2014). Producing DH lines from landraces circumvents the recurrent selfing process, and produces fully homozygous lines within one-step, helping to make genetic resources amenable to crop improvement by linking inventories of gene banks with meaningful phenotypes.

The diversity of landraces is perfectly maintained in DHL, since production of DH lines leads to a faithful representation of the allelic diversity in the original population (Melchinger et al., 2017). Thus, DH lines from landraces generally represent random samples of fairly unselected gametes and genes, apart from detrimental alleles purged in the haploid stage due to the moderate artificial selection in landraces over decades (Prigge et al., 2012). The huge phenotypic diversity as we observed in the DHL (Böhm et al., 2017) shows, that a substantial part of the genetic diversity in landraces is recovered in libraries of DH lines. This was also observed by Strigens et al. (2013b) and is contrasting to lines produced at the beginning of hybrid breeding, where only a few founder lines were developed from a few landraces and used for line production. Because of the allocation of variation within and between landraces, it seems reasonable to sample only a few landraces thoroughly rather than sampling a huge number of landraces with a few DH lines. The best lines from intensively sampled libraries of DH lines could be used directly or for producing synthetics either alone or in combination with elite lines and subject to several cycles of recurrent selection. Further, they provide kind of back up units to extend the genetic diversity of the narrow elite germplasm pool. Therefore, contrasting to elite germplasm, DHL of landraces harbor traits and characteristics eliminated during the last decades of modern breeding (Lauer et al., 2012; Li et al., 2021), which makes them additionally interesting for pre-breeding programs and association mapping approaches.

Since open pollination is the propagation system of maize landraces ever since and random mating with a large sample size reduces linkage disequilibrium (LD) (Falconer and Mackay, 1996), a fast decay of LD even between tightly linked loci can be expected, making DHL ideal populations for genome-wide association studies (GWAS). Using DHL as immortalized genotypes allows precise phenotyping, resulting in a high heritability, which benefits detection of quantitative trait loci (QTL) with those approaches. The genetic variation between genotypes is even larger than in bi-parental populations created with extreme parents and the LD decay is much faster (Zhu et al., 2008; Strigens et al., 2013b; Brauner et al., 2018). Nevertheless, a high population size is required to achieve a sufficient power of QTL detection in genome-wide association mapping approaches, which in combination with the high mapping resolution enables to narrow down the number of candidate genes in a QTL region. Moreover, QTL detection power is increased when conducting association mapping with DHL of maize instead of the landraces themselves and the advantage that detrimental QTL alleles contributing to the genetic load are largely purged from the landraces during the DH production step might be exploited. Even if there are only little evidence that this occurs commonly (Melchinger et al., 2017; Zeitler et al., 2020), detected QTL alleles with negative effects could help to find variants of useful alleles in elite germplasm and eliminate negative alleles.

Ancestral landrace association mapping in DHL to identify new alleles

Association mapping has opened new avenues for a better understanding of trait expressions and direct interactions between QTLs and the environment. Different approaches were evaluated showing different potentials and limitations depending on the diversity of the panel used, each affecting the mapping resolution and the power to detect a QTL. To increase the mapping resolution, it is essential to have a fast decay of the LD. In landrace populations of maize, this rapid LD decay was found, compared to elite lines (van Inghelandt et al., 2011; Mayer et al., 2017; Brauner et al., 2018). An even faster LD decay than in landraces could be observed in diverse world-wide collections of maize, but issues of strong population structure and insufficient adaptation to test environments could be an issue (Yan et al., 2009). To maximize the diversity within a single landrace, which supports getting a higher mapping resolution, I recommend using less related lines for association mapping. This can be achieved

by excluding immortalized lines, recovered from the same S_0 plant, showing high relation to each other based on marker data. To increase the QTL detection power in association mapping, a certain sample size of the mapping population is required. This is highly limited by the resources needed to produce DHL and phenotype them for the target trait(s). Since most of the variation lies within landraces and less between them (Goodman and Holland, 2014; Böhm et al., 2017), it depends on the aim that is pursued. If a specific target trait is in focus which is rare to find or may be present in only one landrace, I recommend using a single landrace only. Whereas it could be more promising to establish libraries for several landraces if all of them show variation for the target trait(s). Generally, evaluating several landraces increases the chance to detect QTL, especially if the genetic architecture of the trait is varying. To warrant sufficient power to detect a QTL, the sample size of each landrace should be balanced with the number of landraces used for association mapping. We recommend a minimum of e.g. N = 250 lines per landrace. Choosing multiple landraces will increase the number of segregating QTL, as well as the mapping resolution and gives information about stable expression of a detected QTL across diverse genetic backgrounds. Further, a maximum marker density should be aimed to increase the QTL detection power. Therefore, approaches of whole-genome sequencing should be applied.

The higher resolution of association mapping, compared to linkage mapping, increases the plausibility of candidate genes identified (Strigens et al., 2013a). Using ancestral landraces for an association mapping approach (Würschum et al., 2021) allowed us to reach an unprecedented power of QTL mapping compared with other types of mapping populations. As mentioned, QTL detection power depends on the number of families segregating for the QTL and the association between the marker and QTL which is limited e.g in the NAM population (McMullen et al., 2009a). There, the mapping panel is less or unequally adapted to the test environments and artifacts arising from a poor adaptation can occur. Further, a limited number of founder parents, as well as the use of a central parent restrict the QTL detection power, since the LD decay within families occurs at a lower level, compared to association mapping with ancestral landraces. The MAGIC design (Dell'Acqua et al., 2015; Huang et al., 2015) has more parallels to our approach. Nevertheless, LD in founder lines can persist over long distances, resulting in lower mapping resolution. What makes the MAGIC population interesting is the fact that the chances of segregating QTLs could be increased by choosing parents showing diverse phenotypes for the target trait. As a third example one can

name the recently proposed FOAM design (Romero Navarro et al., 2017), where from a very large number of landraces, one plant per landrace is collected for genotyping, and testcross progenies with a common tester are used for phenotyping to perform association mapping. Like in the NAM approach, the problem of having confounding effects of adaptation to the target region is coming up with this highly diverse panel. Nevertheless, when using population sizes as large as used in the NAM or FOAM populations, an even higher power of QTL detection in association mapping with ancestral landraces can be expected.

For quantitative traits, efficient strategies for their targeted utilization are needed to make the native diversity for developmental traits accessible for elite germplasm. Mayer et al. (2020) reported a genome-based strategy to make the diversity of maize landraces accessible for elite germplasm improvement by discovering beneficial haplotype-trait associations for quantitative traits. Therewith, they showed a first step in the direction of creating plants with new combinations of alleles. The ancestral landrace association mapping approach (Würschum et al., 2021) showed, that it was possible to dissect the genetic architecture of oligo- or polygenic traits down to the causal variant. Detected QTLs often explain only a minor proportion of the genetic variance, even if they have a large effect. This was also the case for the candidate genes found in Würschum et al. (2021), where e.g. the DGAT1-2 was confirmed as a target for uncoupling oil content and quality from other traits by using natural or engineered alleles in maize breeding (Liu et al., 2020). Therefore, the genes detected must be validated and characterized before further use in breeding. Once confirmed, the information could be used for genome editing or search for allele mining in gene banks (Voytas, 2013; Boglioli and Richard, 2015; Huang et al., 2016). Identification of QTLs and underlying genes in an association mapping analysis allow the introgression of these genomic regions by marker-assisted selection using methods described by Frisch and Melchinger (2005). They might also be included as fixed factors in models for genomic prediction (Bernardo, 2014). Usage of such methods would drastically reduce the developing time and circumvent the problems of linkage drag under conventional backcrossing.

To complement the expensive phenotyping of test candidates, genomic prediction emerged as a powerful tool in breeding programs (Crossa et al., 2017). Brauner et al. (2018) showed for the same materials as used by Würschum et al. (2021) that genomic prediction could be promising within DHL from landraces, but larger training sets than commonly used

in segregating families from elite materials are required to achieve a decent prediction accuracy. The reason is that unlike in elite germplasm, there is only weak relatedness among DH lines extracted from a landrace so that LD between marker and QTL is the main source of information which contributes to the accuracy of genomic prediction.

Metabolites represent together with transcripts an important and intermediate biological layer in the cascade from genotype to phenotype (Mackay et al., 2009). Even if only a small subset of all suspected metabolites of plants occurring in nature (Fernie, 2007) are captured by recent metabolite-profiling technologies (Xu et al., 2016), they play an important role in trait expression (Sackton and Hartl, 2016). Based on the assumption that they have simple genetic architecture and their expression is associated with agronomic traits, we included metabolites as traits in our association mapping study (Würschum et al., 2021). While the correlation between agronomic traits and metabolites was generally weak, including them in the association mapping approach proved promising. For example, we were able to identify for allantoin the enzyme allantoinase as candidate gene (Würschum et al., 2021) and by this the basis for an exploitation of the allelic diversity controlling the allantoin content. Further, including metabolites in association mapping approaches could be a way to uncover the role of the currently large number of metabolites with unknown function. Their role could be elucidated in a bottom-up approach by cloning the genes involved in the biochemical pathways to clarify their function.

To summarize, libraries of DH lines derived from maize landraces showed a huge phenotypic and genetic variation. The diversity of landraces is perfectly maintained in DHL, allowing a precise mapping of new genes and alleles. With recent progress in the DH technology, it seems possible to achieve the necessary population sizes required for association mapping as well as for compiling training sets for genomic prediction. Therefore, libraries of DH lines represent a powerful tool to broaden the narrow genetic base of the flint heterotic pool and open the avenue to cope with the future challenges of agriculture.

Summary

Maize is one of the most important crops species for agriculture worldwide. Since its domestication, landraces formed the traditional type of variety. Selection and genetic factors formed a broad diversity of open-pollinated populations well adapted to local conditions. This changed with the introduction of hybrid breeding when nearly all existing landraces disappeared from their use in agriculture and as source material for breeding. Molecular analyses showed a narrow genetic base of the flint heterotic pool compared to the dent pool. Since genetic resources in maize are one of the richest of all major crops, the exploitation of this untapped reservoir of genetic variation in landraces could be an option to reverse the ongoing narrowing of the genetic basis to meet the demands of a growing world population as well as new challenges under a changing global climate and reduced inputs.

The main goal of this study was the evaluation of European flint maize landraces to unlock their genetic diversity. In detail our objectives were to (i) determine the variation for testcross performance of European maize landraces; (ii) evaluate the phenotypic and genotypic variation of immortalized lines within and among landraces; (iii) compare the per se performance of those line libraries with elite lines as well as founder lines from the European flint germplasm pool; (iv) analyze the breeding potential of immortalized lines from landraces in comparison with elite material to improve the narrow genetic base of the flint heterotic pool; (v) demonstrate the high mapping resolution of DH libraries from landraces in association mapping down to causal variants and underlying genes; and (vi) provide conclusions and guidelines for breeding and research using libraries of immortalized lines from landraces.

In a first experiment, we evaluated in multi-environment trials a broad collection of 70 European flint landraces for their testcross performance in combination with two elite dent testers. In comparison with the yield of modern hybrids, grain yield of the testcrosses of landraces was on average 26% lower, but a high genotypic variance among the landrace was observed for all traits and correlations were moderate to high for most trait combinations similar to those found in elite materials. Genetic correlations between the two testcross series exceeded 0.74 for all traits, suggesting that evaluation of testcross performance in combination with one or two single-cross tester(s) from the opposite pool is sufficient to assess the breeding potential of landraces.

In a second experiment, we produced libraries of DH lines from the most promising landraces identified in the first experiment. In total 389 DH lines from six European flint landraces were evaluated together with four flint founder lines and 53 elite flint lines for 16 agronomic traits in four locations. In general, the genotypic variance (σ_G^2) was larger within than among the DH libraries and exceeded also σ_G^2 of the elite flint lines. Furthermore, the means and σ_G^2 varied among the DH libraries resulting in large differences of the usefulness criterion. Mean grain yield of the elite flint lines exceeded that of the flint founder lines by 25% and DH libraries by 62%, indicating the impressive breeding progress achieved in the elite material and the substantial genetic load still present in the DH libraries. Nevertheless, the usefulness of the best DH lines was comparable to that of the elite flint lines for many traits including grain yield, underpinning the tremendous potential of landraces for broadening the genetic base of the elite germplasm.

In a third experiment the materials from the 2nd experiment were genotyped with the MaizeSNP50 BeadChip from Illumina® and seeds of all genotypes were used for extracting and analyzing 288 metabolites with GC-MS. Data for agronomic traits and metabolites were used for a novel association mapping study. The much faster decay of linkage disequilibrium for adjacent markers in the DH libraries compared with the elite flint lines resulted in unprecedented map resolution. This was strikingly demonstrated by fine-mapping a QTL for oil content down to the phenylalanine insertion F469 in DGAT1-2 as the causal variant. Further, for the metabolite allantoin, which is related to abiotic stress response, promoter polymorphisms as well as differential expression of an allantoinase were identified as putative causes of variation despite a moderate size of the mapping population. These results are very encouraging to use DH libraries from landraces for association mapping and dissect QTL potentially down to the causal variants. However, larger population sizes of each DH library are recommended, similar to those commonly used with other approaches such as the NAM design, for detection of QTL explaining only a small portion of the genetic variance. This opens a new avenue for utilization of natural and/or engineered alleles in breeding.

In conclusion, the genetic variation present in European flint maize landraces represents a unique source to reverse the ongoing narrowing of the genetic basis of the elite germplasm of this heterotic pool. For identifying the most promising landraces, we propose a multi-stage approach, where based on an assessment of the molecular diversity about one

hundred landraces are evaluated in observation trials for agro-ecological adaptation and testcrosses with one single-cross tester are used for evaluating their general combining ability with the opposite heterotic pool. For a small number (< 6) of landraces a large number of DH lines are developed, which are phenotyped and genotyped for further use in association mapping and genomic selection with the ultimate goal to make these "gold reserves" accessible for maize breeding with modern approaches.

Zusammenfassung

Mais ist eine der wichtigsten Kulturarten für die Landwirtschaft weltweit. Seit seiner Domestikation bildeten Landrassen den traditionellen Sortentyp. Durch Selektion und genetische Faktoren entstand eine breite Diversität an panmiktisch vermehrten Populationen, die gut an lokale Bedingungen angepasst waren. Dies änderte sich mit der Einführung der Hybridzüchtung, als nahezu alle Landrassen in der landwirtschaftlichen Produktion und als Ausgangsmaterial für die Züchtung verschwanden.

Molekulare Analysen zeigen eine enge genetische Basis des Flint Pools im Vergleich zum Dent Pool. Genetische Ressourcen im Mais gehören zu den umfangreichsten aller Nutzpflanzen. Die Nutzung dieses bislang ungenutzten Reservoirs an genetischer Diversität in Landrassen bietet eine Möglichkeit, um der fortschreitenden Einengung der genetischen Basis entgegenzuwirken und somit den Aufgaben der Pflanzenzüchtung im Hinblick auf eine wachsende Weltbevölkerung sowie den Herausforderungen des Klimawandels und reduzierten Inputs im Anbau gerecht zu werden.

Übergeordnetes Ziel dieser Studie war die Evaluierung europäischer Flint-Mais Landrassen, um deren genetische Vielfalt nutzen zu können. Im Speziellen waren die Ziele (i) die Variation in Testkreuzungen europäischer Mais-Landrassen zu bestimmen; (ii) die phänotypische und genotypische Variation der Linien innerhalb und zwischen Landrassen zu beurteilen; (iii) die Eigenleistung dieser Linien mit Elite-Linien sowie Founder-Linien aus dem europäischen Flint-Pool zu vergleichen; (iv) das Potential von doppelhaploiden (DH) Linien aus Landrassen im Vergleich zum Elitematerial für die Züchtung zu analysieren, um die enge genetische Basis des Flint-Pools zu erweitern; (v) die Verwendung von DH-Bibliotheken aus Landrassen für die Assoziationskartierung bis hin zur Eingrenzung kausaler Gene zu demonstrieren; und (vi) Schlussfolgerungen und Leitlinien für die Züchtung und Forschung zu erörtern , um DH-Linien aus Landrassen nutzbar zu machen.

In einem ersten Versuch wurde eine umfangreiche Kollektion von 70 europäischen Flint-Landrassen mehrortig in Kombination mit zwei Elite Dent-Testern auf ihre Testkreuzungsleistung hin untersucht. Verglichen mit dem Ertrag moderner Hybriden war der Kornertrag der Testkreuzungen der Landrassen im Durchschnitt um 26 % geringer, jedoch wurde eine hohe genotypische Varianz zwischen den Landrassen für alle Merkmale

beobachtet. Die Korrelationen waren mittel bis hoch für die meisten Merkmalskombinationen und entsprachen denen im Elitezuchtmaterial. Die genetische Korrelation der beiden Testkreuzungsserien überstieg 0,74 für alle Merkmale. Dies zeigt, dass es ausreicht die Leistung von Testkreuzungen in Kombination mit einem oder zwei Testern - bestehend aus Einfachkreuzungen des anderen Gen-Pools – zu bewerten, um das Potenzial von Landrassen für die Züchtung zu beurteilen.

In einem zweiten Versuch produzierten wir Bibliotheken von DH-Linien der vielversprechendsten Landrassen des vorigen Versuches. Insgesamt wurden 389 DH-Linien aus sechs europäischer Flint Landrassen zusammen mit vier Flint Founder-Linien und 53 Elite Flintlinien auf 16 agronomische Merkmale an vier Standorten geprüft. Die genotypische Varianz (σ_G^2) innerhalb der DH-Bibliotheken war größer als die zwischen den Bibliotheken und übertraf auch σ_G^2 der Elite Flintlinien. Darüber hinaus variierten die Mittelwerte und σ_G^2 zwischen den DH-Bibliotheken, was zu großen Unterschieden im Brauchbarkeits-Kriterium („usefulness") führte. Der mittlere Kornertrag der Elite Flintlinien übertraf den der Flint Founder-Linien um 25 % und der DH-Bibliotheken um 62 %, was auf den beträchtlichen Zuchtfortschritt im Elitematerial hinweist sowie auf die erhebliche genetische Bürde, welche in den DH-Bibliotheken vorliegt. Die Brauchbarkeit der besten DH-Linien war trotzdem für viele Merkmale, einschließlich dem Kornertrag, mit der von Elite Flintlinien vergleichbar. Dies zeigt das enorme Potenzial, Landrassen zur Verbreiterung des genetisch engen Elite Flint-Pools zu verwenden.

In einem dritten Versuch wurden das genetische Material des vorherigen Versuches mit dem MaizeSNP50 BeadChip von Illumina® genotypisiert und Samen aller Genotypen zur Extraktion und Analyse von 288 Metaboliten mit GC-MS verwendet. Sowohl die agronomischen Merkmale als auch die Metabolit-Daten wurden für eine Assoziationskartierung verwendet. Der schnelle Abfall des Kopplungsungleichgewichts benachbarter Marker in den DH-Bibliotheken im Vergleich zu den Elite Flintlinien führte zu einer hervorragenden Auflösung in der QTL-Kartierung, was durch die Feinkartierung eines QTL (= quantitative trait locus) für Ölgehalt bis zur Phenylalanin Insertion F469 in DGAT1-2 als kausale Variante demonstriert werden konnte. Darüber hinaus wurden für den Metaboliten Allantoin, der im Zusammenhang mit abiotischem Stress steht, Promotorpolymorphismen sowie die Expression einer Allantoinase als vermutete Ursache der Variation identifiziert. Dies

gelang trotz der moderaten Größe der Kartierungspopulation. Diese Ergebnisse sind ermutigend, um DH-Bibliotheken von Landrassen für die Assoziationskartierung zu verwenden und QTL bis auf die kausalen Varianten zu entschlüsseln. Eine Erweiterung der Populationsgrößen der DH-Bibliotheken, ähnlich wie sie in anderen Versuchsdesigns in der Literatur verwendet wurden, ist hierbei zu empfehlen, um mit diesem Ansatz QTL zu detektieren, welche lediglich einen kleinen Teil der genetischen Varianz erklären. Dies eröffnet neue Wege zur Nutzung natürlicher und/oder neu geschaffener Allele in der Züchtung.

Zusammenfassend zeigen die Ergebnisse dieser Arbeit, dass die genetische Variation europäischer Landrassen bei Flint-Mais eine einzigartige Quelle darstellt, um die fortschreitende Verengung der genetischen Basis des Elitematerials in diesem Gen-Pool umzukehren. Um vielversprechende Landrassen zu identifizieren, schlagen wir folgenden zweistufigen Ansatz vor: (i) Basierend auf der Bewertung der molekularen Diversität werden etwa hundert Landrassen in Leistungsprüfungen auf ihre Anpassungsfähigkeit für die Zielregionen evaluiert und ihre Kombinationsfähigkeit mit dem entgegengesetzten heterotischen Gen-Pool in Testkreuzungen mit einer Einfachkreuzung als Tester bewertet. (ii) Für eine geringe Zahl (< 6) von Landrassen wird anschließend eine große Anzahl von DH-Linien erstellt, welche für die Nutzung in der Assoziationskartierung und/oder genomischen Selektion phänotypisiert und genotypisiert werden, um diese „Goldreserven" für die Maiszüchtung mit innovativen Methoden zugänglich zu machen.

References

Astle, W., and D. Balding. 2009. Population Structure and Cryptic Relatedness in Genetic Association Studies. Stat. Sci. 24(4): 451–471 Available at http://discovery.ucl.ac.uk/104582/.

Barrière, Y., D. Alber, O. Dolstra, C. Lapierre, M. Motto, A. Ordas, J. Van Waes, L. Vlasminkel, C. Welcker, and J. Monod. 2006. Past and prospects of forage maize breeding in Europe. II. History, germplasm evolution and correlative agronomic changes. Maydica 51: 435–449.

Beadle, G.W. 1939. Teosinte and the origin of maize. J. Hered. 30(6): 245–247.

Bernardo, R. 2014. Genomewide Selection when Major Genes Are Known. Crop Sci. 54(1): 68–75 Available at https://doi.org/10.2135/cropsci2013.05.0315.

Boglioli, E., and M. Richard. 2015. Rewriting the book of life: A new era in precision gene editing.

Böhm, J., W. Schipprack, V. Mirdita, H.F. Utz, and A.E. Melchinger. 2014. Breeding potential of European flint maize landraces evaluated by their testcross performance. Crop Sci. 54(4): 1665–1672.

Böhm, J., W. Schipprack, H.F. Utz, and A.E. Melchinger. 2017. Tapping the genetic diversity of landraces in allogamous crops with doubled haploid lines: a case study from European flint maize. Theor. Appl. Genet. 130(5): 861–873.

Brandolini, A., and A. Brandolini. 2009. Maize introduction, evolution and diffusion in Italy. p. 233–242. *In* Maydica.

Brauner, P.C., D. Müller, P. Schopp, J. Böhm, E. Bauer, C.C. Schön, and A.E. Melchinger. 2018. Genomic prediction within and among doubled-haploid libraries from maize landraces. Genetics 210(4): 1185–1196.

Buckler, E.S., and J.M. Thornsberry. 2002. Plant molecular diversity and applications to genomics. Curr. Opin. Plant Biol. 5(2): 107–111 Available at https://linkinghub.elsevier.com/retrieve/pii/S1369526602002388.

Cairns, J.E., K. Sonder, P.H. Zaidi, N. Verhulst, G. Mahuku, R. Babu, S.K. Nair, B. Das, B. Govaerts,

M.T. Vinayan, Z. Rashid, J.J. Noor, P. Devi, F. San Vicente, and B.M. Prasanna. 2012. Maize Production in a Changing Climate: Impacts, Adaptation, and Mitigation Strategies. Adv. Agron. 114: 1–58 Available at https://www.sciencedirect.com/science/article/pii/B9780123942753000067.

Cao, J., K. Schneeberger, S. Ossowski, T. Günther, S. Bender, J. Fitz, D. Koenig, C. Lanz, O. Stegle, C. Lippert, X. Wang, F. Ott, J. Müller, C. Alonso-Blanco, K. Borgwardt, K.J. Schmid, and D. Weigel. 2011. Whole-genome sequencing of multiple Arabidopsis thaliana populations. Nat. Genet. 43(10): 956–963.

Crnokrak, P., and S. Barrett. 2002. Perspective: purging the genetic load: a review of the experimental evidence. Evolution (N. Y). 56(12): 2347–2358 Available at http://onlinelibrary.wiley.com/doi/10.1111/j.0014-3820.2002.tb00160.x/full.

Crossa, J., P. Pérez-Rodríguez, J. Cuevas, O. Montesinos-López, D. Jarquín, G. de los Campos, J. Burgueño, J.M. González-Camacho, S. Pérez-Elizalde, Y. Beyene, S. Dreisigacker, R. Singh, X. Zhang, M. Gowda, M. Roorkiwal, J. Rutkoski, and R.K. Varshney. 2017. Genomic Selection in Plant Breeding: Methods, Models, and Perspectives. Trends Plant Sci. 22(11): 961–975 Available at https://doi.org/10.1016/j.tplants.2017.08.011.

Dallard, J., P. Noël, B. Gouesnard, and A. Boyat. 2000. A network for the management of genetic resources of maize populations in France. p. 35–40. *In* IPGRI; FAO (ed.), Plant Genetic Resources Newsletter. No. 123.

Dell'Acqua, M., D.M. Gatti, G. Pea, F. Cattonaro, F. Coppens, G. Magris, A.L. Hlaing, H.H. Aung, H. Nelissen, J. Baute, E. Frascaroli, G.A. Churchill, D. Inzé, M. Morgante, and M.E. Pè. 2015. Genetic properties of the MAGIC maize population: A new platform for high definition QTL mapping in Zea mays. Genome Biol. 16(1): 1–23 Available at http://dx.doi.org/10.1186/s13059-015-0716-z.

DMK. 2021. https://www.maiskomitee.de.

Doebley, J. 2004. The genetics of maize evolution. Annu. Rev. Genet. 38: 37–59.

Dubreuil, P., and A. Charcosset. 1999. Relationships among maize inbred lines and populations from European and North-American origins as estimated using RFLP markers. Theor. Appl. Genet. 99(3–4): 473–480 Available at

http://www.springerlink.com/openurl.asp?genre=article&id=doi:10.1007/s0012200512
59.

Duvick, D. 2005. Genetic progress in yield of United States maize (Zea mays L.). Maydica 50: 193–202.

Eder, J., and S. Chalyk. 2002. In vivo haploid induction in maize. Theor. Appl. Genet. 104(4): 703–708 Available at http://www.ncbi.nlm.nih.gov/pubmed/12582677.

Edwards, R., and E. Leng. 1965. Classification of some indigenous maize collections from southern and southeastern Europe. Euphytica 14: 161–169 Available at http://link.springer.com/article/10.1007/BF00038982.

Elshire, R.J., J.C. Glaubitz, Q. Sun, J.A. Poland, K. Kawamoto, E.S. Buckler, and S.E. Mitchell. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One 6(5): 1–10.

Falconer, D., and T. Mackay. 1996. Introduction to quantitative genetics. 4th ed. Longmans Green, London.

FAO. 2010. Second report on the state of the world's plant genetic resources for food and agriculture.

FAO stat. 2021. Food and Agriculture Organization of the United Nations. Available at http://faostat.fao.org/site/339/default.aspx.

Fernie, A.R. 2007. The future of metabolic phytochemistry: Larger numbers of metabolites, higher resolution, greater understanding. Phytochemistry 68(22–24): 2861–2880 Available at https://linkinghub.elsevier.com/retrieve/pii/S0031942207004608.

Ferro, R.A., I. Brichette, G. Evgenidis, C. Karamaligkas, and J. Moreno-González. 2007. Variability in European maize (Zea mays L.) landraces under high and low nitrogen inputs. Genet. Resour. Crop Evol. 54: 295–308.

Flint-Garcia, S.A., J.M. Thornsberry, and E.S. Buckler. 2003. Structure of linkage disequilibrium in plants. Annu. Rev. Plant Biol. 54: 357–374 Available at http://www.ncbi.nlm.nih.gov/pubmed/14502995.

Frei, O. 2000. Changes in yield physiology of corn as a result of breeding in northern Europe.

Maydica 45: 173–183.

Frisch, M., and A.E. Melchinger. 2001. Marker-assisted backcrossing for simultaneous introgression of two genes. Crop Sci. 41(6): 1716–1725.

Frisch, M., and A.E. Melchinger. 2005. Selection theory for marker-assisted backcrossing. Genetics 170(2): 909–917.

Gallais, A. 1990. Quantitative genetics of doubled haploid populations and application to the theory of line development. Genetics 124: 199–206.

Ganal, M.W., G. Durstewitz, A. Polley, A. Bérard, E.S. Buckler, A. Charcosset, J.D. Clarke, E.M. Graner, M. Hansen, J. Joets, M.C. Le Paslier, M.D. McMullen, P. Montalent, M. Rose, C.C. Schön, Q. Sun, H. Walter, O.C. Martin, and M. Falque. 2011. A large maize (zea mays L.) SNP genotyping array: Development and germplasm genotyping, and genetic mapping to compare with the B73 reference genome. PLoS One 6(12).

Gauthier, P., B. Gouesnard, J. Dallard, R. Redaelli, C. Rebourg, A. Charcosset, and A. Boyat. 2002. RFLP diversity and relationships among traditional European maize populations. Theor. Appl. Genet. 105: 91–99.

Goodman MM, Holland JB, J.S.J. 2014. Breeding and diversity. p. 14–50. *In* Wusirika R et al (eds) (ed.), Genetics, genomes and breeding of maize. CRC Press, Boca Raton.

Gore, M. a, J.-M. Chia, R.J. Elshire, Q. Sun, E.S. Ersoz, B.L. Hurwitz, J. a Peiffer, M.D. McMullen, G.S. Grills, J. Ross-Ibarra, D.H. Ware, and E.S. Buckler. 2009. A first-generation haplotype map of maize. Science 326(5956): 1115–7 Available at http://www.ncbi.nlm.nih.gov/pubmed/19965431.

Gouesnard, B., J. Dallard, and P. Bertin. 2005. European maize landraces: Genetic diversity, core collection definition and methodology of use. Maydica 50: 225–234.

Gouesnard, B., J. Dallard, A. Panouille, and A. Boyat. 1997. Classification of French maize populations based on morphological traits. Agronomie 17(33): 491–498.

Hallauer, A., M. Carena, and J. Miranda Filho. 2010. Quantitative genetics in maize breeding. 3rd ed. Springer New York, Iowa State Univ. Press, Ames, IA.

Hoisington, D., M. Khairallah, T. Reeves, J.-M. Ribaut, B. Skovmand, S. Taba, and M.

Warburton. 1999. Plant genetic resources: What can they contribute toward increased crop productivity? Proc. Natl. Acad. Sci. 96(11): 5937–5943 Available at http://www.pnas.org/cgi/doi/10.1073/pnas.96.11.5937.

Huang, B.E., K.L. Verbyla, A.P. Verbyla, C. Raghavan, V.K. Singh, P. Gaur, H. Leung, R.K. Varshney, and C.R. Cavanagh. 2015. MAGIC populations in crops: current status and future prospects. Theor. Appl. Genet. 128(6): 999–1017 Available at http://dx.doi.org/10.1007/s00122-015-2506-0.

Huang, S., D. Weigel, R.N. Beachy, and J. Li. 2016. A proposed regulatory framework for genome-edited crops. Nat. Genet. 48(2): 109–111 Available at http://www.nature.com/doifinder/10.1038/ng.3484.

van Inghelandt, D., A.E. Melchinger, C. Lebreton, and B. Stich. 2010. Population structure and genetic diversity in a commercial maize breeding program assessed with SSR and SNP markers. Theor Appl Genet 120: 1289–1299.

van Inghelandt, D., J.C. Reif, B.S. Dhillon, P. Flament, and A.E. Melchinger. 2011. Extent and genome-wide distribution of linkage disequilibrium in commercial maize germplasm. Theor. Appl. Genet. 123(1): 11–20.

Langridge, P., and R. Waugh. 2019. Harnessing the potential of germplasm collections. Nat. Genet. 51(2): 200–201 Available at https://doi.org/10.1038/s41588-018-0340-4.

Lauer, S., B.D. Hall, E. Mulaosmanovic, S.R. Anderson, B. Nelson, and S. Smith. 2012. Morphological changes in parental lines of Pioneer brand maize hybrids in the U.S. central Corn Belt. Crop Sci. 52(3): 1033–1043.

Li, X., X. Quan, M. Mang, G. Neumann, A. Melchinger, and U. Ludewig. 2021. Flint maize root mycorrhization and organic acid exudates under phosphorus deficiency: Trends in breeding lines and doubled haploid lines from landraces. J. Plant Nutr. Soil Sci. 184(3): 346–359.

Lisec, J., N. Schauer, J. Kopka, L. Willmitzer, and A.R. Fernie. 2006. Gas chromatography mass spectrometry-based metabolite profiling in plants. Nat Protoc 1(1): 387–396.

Liu, J.-Y., Y.-W. Zhang, X. Han, J.-F. Zuo, Z. Zhang, H. Shang, Q. Song, and Y.-M. Zhang. 2020. An evolutionary population structure model reveals pleiotropic effects of GmPDAT for traits

related to seed size and oil content in soybean (F Costa, Ed.). J. Exp. Bot. 71(22): 6988–7002 Available at https://academic.oup.com/jxb/article/71/22/6988/5905248.

Lobell, D.B., and C. Tebaldi. 2014. Getting caught with our plants down: The risks of a global crop yield slowdown from climate trends in the next two decades. Environ. Res. Lett. 9(7).

Lucchin, M., G. Barcaccia, and P. Parrini. 2003. Characterization of a flint maize (Zea mays L. convar. mays) Italian landrace: I. Morpho-phenological and agronomic traits. Genet. Resour. Crop Evol. 50(3): 315–327.

Luo, J. 2015. Metabolite-based genome-wide association studies in plants. Curr. Opin. Plant Biol. 24: 31–38 Available at http://dx.doi.org/10.1016/j.pbi.2015.01.006.

Mackay, T.F.C., E.A. Stone, and J.F. Ayroles. 2009. The genetics of quantitative traits: challenges and prospects. Nat. Rev. Genet. 10(8): 565–577 Available at http://www.nature.com/articles/nrg2612.

Malvar, R.A., A. Butrón, A. Alvarez, B. Ordás, P. Soengas, L.P. Revilla, and A. Ordás. 2004. Evaluation of the European Union Maize Landrace Core Collection for resistance to Sesamia nonagrioides (Lepidoptera: Noctuidae) and Ostrinia nubilalis (Lepidoptera: Crambidae). J. Econ. Entomol. 97(2): 628–634 Available at http://www.bioone.org/doi/abs/10.1603/0022-0493-97.2.628.

Malvar, R.A., A. Butrón, A. Álvarez, G. Padilla, M.E. Cartea, P. Revilla, and A. Ordás. 2007. Yield performance of the European Union maize landrace core collection under multiple corn borer infestations. Crop Prot. 26: 775–781.

Malvar, R.A.R., P. Revilla, A. Butrón, B. Gouesnard, A. Boyat, P. Soengas, A. Álvarez, and A. Ordás. 2005. Performance of crosses among French and Spanish maize populations across environments. Crop Sci. 45(3): 1052–1057.

Matsuoka, Y., Y. Vigouroux, M.M. Goodman, J. Sanchez G, E. Buckler, and J. Doebley. 2002. A single domestication for maize shown by multilocus microsatellite genotyping. Proc. Natl. Acad. Sci. U. S. A. 99: 6080–6084.

Mayer, M., A.C. Hölker, E. González-Segovia, E. Bauer, T. Presterl, M. Ouzunova, A.E. Melchinger, and C.C. Schön. 2020. Discovery of beneficial haplotypes for complex traits in maize landraces. Nat. Commun. 11(1): 1–10 Available at

http://dx.doi.org/10.1038/s41467-020-18683-3.

Mayer, M., S. Unterseer, E. Bauer, N. de Leon, B. Ordas, and C.C. Schön. 2017. Is there an optimum level of diversity in utilization of genetic resources? Theor. Appl. Genet. 130(11): 2283–2295.

McCouch, S., G.J. Baute, J. Bradeen, P. Bramel, P.K. Bretting, E. Buckler, J.M. Burke, D. Charest, S. Cloutier, G. Cole, H. Dempewolf, M. Dingkuhn, C. Feuillet, P. Gepts, D. Grattapaglia, L. Guarino, S. Jackson, S. Knapp, P. Langridge, A. Lawton-Rauh, Q. Lijua, C. Lusty, T. Michael, S. Myles, K. Naito, R.L. Nelson, R. Pontarollo, C.M. Richards, L. Rieseberg, J. Ross-Ibarra, S. Rounsley, R.S. Hamilton, U. Schurr, N. Stein, N. Tomooka, E. van der Knaap, D. van Tassel, J. Toll, J. Valls, R.K. Varshney, J. Ward, R. Waugh, P. Wenzl, and D. Zamir. 2013. Feeding the future. Nature 499(7456): 23–24 Available at https://doi.org/10.1038/499023a.

McMullen, M.D., S. Kresovich, H.S. Villeda, P. Bradbury, H. Li, Q. Sun, S. Flint-Garcia, J. Thornsberry, C. Acharya, C. Bottoms, P. Brown, C. Browne, M. Eller, K. Guill, C. Harjes, D. Kroon, N. Lepak, S.E. Mitchell, B. Peterson, G. Pressoir, S. Romero, M. Oropeza Rosas, S. Salvo, H. Yates, M. Hanson, E. Jones, S. Smith, J.C. Glaubitz, M. Goodman, D. Ware, J.B. Holland, and E.S. Buckler. 2009a. Genetic properties of the maize nested association mapping population. Science 325(5941): 737–40 Available at http://www.ncbi.nlm.nih.gov/pubmed/19661427.

McMullen, M.D., S. Kresovich, H.S. Villeda, P. Bradbury, H. Li, Q. Sun, S. Flint-Garcia, J. Thornsberry, C. Acharya, C. Bottoms, P. Brown, C. Browne, M. Eller, K. Guill, C. Harjes, D. Kroon, N. Lepak, S.E. Mitchell, B. Peterson, G. Pressoir, S. Romero, M.O. Rosas, S. Salvo, H. Yates, M. Hanson, E. Jones, S. Smith, J.C. Glaubitz, M. Goodman, D. Ware, J.B. Holland, and E.S. Buckler. 2009b. Genetic properties of the maize nested association mapping population. Science (80-.). 325(5941): 737–740.

Melchinger, A.E., and R.K. Gumber. 1998. Overview of heterosis and heterotic groups in agronomic crops. p. 29–44. *In* Kendall, R.L., Staub, J.E. (eds.), Concepts and breeding of heterosis in crop plants. 25th ed. Crop Science Society of America.

Melchinger, A.E., C.F. Longin, H.F. Utz, and J.C. Reif. 2005. Hybrid maize breeding with doubled haploid lines: quantitative genetic and selection theory for optimum allocation of

resources. Proc. 41st Annu. Illinois Corn Breeders Sch. 2005 Urbana-Champaign, IL. 7-8 Mar. 2005. Univ. Illinois Urbana-Champaign: 8–21 Available at http://pba.ucdavis.edu/files/165479.pdf.

Melchinger, A.E., P. Schopp, D. Müller, T.A. Schrag, E. Bauer, S. Unterseer, L. Homann, W. Schipprack, and C.C. Schön. 2017. Safeguarding our genetic resources with libraries of doubled-haploid lines. Genetics 206(3): 1611–1619.

Mercer, K.L., and H.R. Perales. 2010. Evolutionary response of landraces to climate change in centers of crop diversity. Evol. Appl. 3(5–6): 480–493.

Meseka, S., A. Menkir, and K. Obeng-Antwi. 2014. Exploitation of beneficial alleles from maize (Zea mays L.) landraces to enhance performance of an elite variety in water stress environments. Euphytica 201(1): 149–160.

Messmer, M.M., A.E. Melchinger, J. Boppenmaier, E. Brunkhaus-Jung, and R.G. Herrmann. 1992. Relationships among early European maize inbreds: I. Genetic diversity among flint and dent lines revealed by RFLPs. Crop Sci. 32: 1301–1309.

Messmer, M.M., A.E. Melchinger, R.G. Herrmann, and J. Boppenmaier. 1993. Relationships among early European maize inbreds: II. Comparison of pedigree and RFLP data. Crop Sci. 33(5): 944 Available at https://www.crops.org/publications/cs/abstracts/33/5/CS0330050944.

Mieg, I.B., J. Moreno-Gonzalez, and A. Lopez. 2001. Variability of European maize landraces for forage digestibility using near infrared reflectance spectroscopy (NIRS). Maydica 46: 245–252.

Monteiro, F., P. Vidigal, A.B. Barros, A. Monteiro, H.R. Oliveira, and W. Viegas. 2016. Genetic distinctiveness of rye In situ accessions from portugal unveils a new hotspot of unexplored genetic resources. Front. Plant Sci. 7(AUG2016): 1–17.

Moreno-Gonzalez, J., F. Ramos-Gourcy, and E. Losada. 1997. Breeding potential of European flint and earliness-selected U.S. Corn Belt dent maize populations. Crop Sci. 37: 1475–1481.

Ortiz, R., S. Taba, V.H.C. Tovar, M. Mezzalama, Y. Xu, J. Yan, and J.H. Crouch. 2010. Conserving and enhancing maize genetic resources as global public goods–A perspective from

CIMMYT. Crop Sci. 50: 13–28.

Paul, C., A.K. Techen, J.S. Robinson, and K. Helming. 2019. Rebound effects in agricultural land and soil management: Review and analytical framework. J. Clean. Prod. 227: 1054–1067 Available at https://doi.org/10.1016/j.jclepro.2019.04.115.

Peter, R., T.W. Eschholz, P. Stamp, and M. Liedgens. 2009. Early growth of flint maize landraces under cool conditions. Crop Sci. 49: 169–178.

Prigge, V., R. Babu, B. Das, M. Hernández Rodriguez, G.N. Atlin, and A.E. Melchinger. 2012. Doubled haploids in tropical maize: II. Quantitative genetic parameters for testcross performance. Euphytica 185: 453–463.

Prigge, V., and A.E. Melchinger. 2012. Production of haploids and doubled haploids in maize. p. 161–172. *In* Loyola-Vargas, V.M., Ochoa-Alejo, N. (eds.), Plant Cell Culture Protocols. 3rd ed. Springer Protocols.

Ranum, P., J.P. Peña-Rosas, and M.N. Garcia-Casal. 2014. Global maize production, utilization, and consumption. Ann. N. Y. Acad. Sci. 1312(1): 105–112.

Rebourg, C., M. Chastanet, B. Gouesnard, C. Welcker, P. Dubreuil, and A. Charcosset. 2003. Maize introduction into Europe: the history reviewed in the light of molecular data. Theor. Appl. Genet. 106(5): 895–903.

Rebourg, C., B. Gouesnard, and A. Charcosset. 2001. Large scale molecular analysis of traditional european maize populations. Relationships with morphological variation. Heredity (Edinb). 86(5): 574–587.

Reif, J.C., S. Hamrit, M. Heckenberger, W. Schipprack, H. Peter Maurer, M. Bohn, and A.E. Melchinger. 2005. Genetic structure and diversity of European flint maize populations determined with SSR analyses of individuals and bulks. Theor. Appl. Genet. 111(5): 906–13 Available at http://www.ncbi.nlm.nih.gov/pubmed/16059732.

Revilla, P., A. Boyat, A. Álvarez, B. Gouesnard, P. Soengas, A. Ordás, and R. Malvar. 2006. Heterotic patterns among French and Spanish maize populations. Maydica 51: 525–535 Available at http://digital.csic.es/handle/10261/9620.

Riedelsheimer, C., J. Lisec, A. Czedik-Eysenberg, R. Sulpice, A. Flis, C. Grieder, T. Altmann, M.

Stitt, L. Willmitzer, and A.E. Melchinger. 2012. Genome-wide association mapping of leaf metabolic profiles for dissecting complex traits in maize. Proc. Natl. Acad. Sci. U. S. A. 109(23): 8872–7 Available at http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3384205&tool=pmcentrez &rendertype=abstract.

Romay, M.C., R.A. Malvar, L. Campo, A. Alvarez, J. Moreno-González, A. Ordás, and P. Revilla. 2010. Climatic and genotypic effects for grain yield in maize under stress conditions. Crop Sci. 50: 51–58.

Romero Navarro, J.A., M. Willcox, J. Burgueño, C. Romay, K. Swarts, S. Trachsel, E. Preciado, A. Terron, H.V. Delgado, V. Vidal, A. Ortega, A.E. Banda, N.O.G. Montiel, I. Ortiz-Monasterio, F.S. Vicente, A.G. Espinoza, G. Atlin, P. Wenzl, S. Hearne, and E.S. Buckler. 2017. A study of allelic diversity underlying flowering-time adaptation in maize landraces. Nat. Genet. 49(3): 476–480 Available at http://dx.doi.org/10.1038/ng.3784.

Sackton, T.B., and D.L. Hartl. 2016. Genotypic Context and Epistasis in Individuals and Populations. Cell 166(2): 279–287 Available at https://linkinghub.elsevier.com/retrieve/pii/S0092867416308558.

Salhuana, W., and L.M. Pollak. 2006. Latin American Maize Project (LAMP) and Germplasm Enhancement of Maize (GEM) project: Generating useful breeding germplasm. Maydica 51: 339–355.

Schnable, J.C., N.M. Springer, and M. Freeling. 2011. Differentiation of the maize subgenomes by genome dominance and both ancient and ongoing gene loss. Proc. Natl. Acad. Sci. U. S. A. 108(10): 4069–74 Available at http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3053962&tool=pmcentrez &rendertype=abstract.

Schnable, P.S., D. Ware, R.S. Fulton, J.C. Stein, F. Wei, S. Pasternak, C. Liang, J. Zhang, L. Fulton, T.A. Graves, P. Minx, A.D. Reily, L. Courtney, S.S. Kruchowski, C. Tomlinson, C. Strong, K. Delehaunty, C. Fronick, B. Courtney, S.M. Rock, E. Belter, F. Du, K. Kim, R.M. Abbott, M. Cotton, A. Levy, P. Marchetto, K. Ochoa, S.M. Jackson, B. Gillam, W. Chen, L. Yan, J. Higginbotham, M. Cardenas, J. Waligorski, E. Applebaum, L. Phelps, J. Falcone, K. Kanchi, T. Thane, A. Scimone, N. Thane, J. Henke, T. Wang, J. Ruppert, N. Shah, K. Rotter, J.

Hodges, E. Ingenthron, M. Cordes, S. Kohlberg, J. Sgro, B. Delgado, K. Mead, A. Chinwalla, S. Leonard, K. Crouse, K. Collura, D. Kudrna, J. Currie, R. He, A. Angelova, S. Rajasekar, T. Mueller, R. Lomeli, G. Scara, A. Ko, K. Delaney, M. Wissotski, G. Lopez, D. Campos, M. Braidotti, E. Ashley, W. Golser, H. Kim, S. Lee, J. Lin, Z. Dujmic, W. Kim, J. Talag, A. Zuccolo, C. Fan, A. Sebastian, M. Kramer, L. Spiegel, L. Nascimento, T. Zutavern, B. Miller, C. Ambroise, S. Muller, W. Spooner, A. Narechania, L. Ren, S. Wei, S. Kumari, B. Faga, M.J. Levy, L. McMahan, P. Van Buren, M.W. Vaughn, K. Ying, C.T. Yeh, S.J. Emrich, Y. Jia, A. Kalyanaraman, A.P. Hsia, W.B. Barbazuk, R.S. Baucom, T.P. Brutnell, N.C. Carpita, C. Chaparro, J.M. Chia, J.M. Deragon, J.C. Estill, Y. Fu, J.A. Jeddeloh, Y. Han, H. Lee, P. Li, D.R. Lisch, S. Liu, Z. Liu, D.H. Nagel, M.C. McCann, P. Sanmiguel, A.M. Myers, D. Nettleton, J. Nguyen, B.W. Penning, L. Ponnala, K.L. Schneider, D.C. Schwartz, A. Sharma, C. Soderlund, N.M. Springer, Q. Sun, H. Wang, M. Waterman, R. Westerman, T.K. Wolfgruber, L. Yang, Y. Yu, L. Zhang, S. Zhou, Q. Zhu, J.L. Bennetzen, R.K. Dawe, J. Jiang, N. Jiang, G.G. Presting, S.R. Wessler, S. Aluru, R.A. Martienssen, S.W. Clifton, W.R. McCombie, R.A. Wing, and R.K. Wilson. 2009. The B73 maize genome: Complexity, diversity, and dynamics. Science (80-.). 326(5956): 1112–1115.

Schnell, F.W. 1983. Probleme der Elternwahl - Ein Überblick. p. 1–11. *In* Arbeitstagung der Arbeitsgemeinschaft der Saatzuchtleiter in Gumpenstein, Austria. 22.-24. Nov. Verlag und Druck der Bundesanstalt für alpenländische Landwirtschaft, Austria.

Schnell, F.W. 1992. Maiszüchtung und die Züchtungsforschung in der Bundesrepublik Deutschland. p. 27–44. *In* Vorträge Pflanzenzüchtung.

Shiferaw, B., B.M. Prasanna, J. Hellin, and M. Bänziger. 2011. Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. Food Secur. 3(3): 307–327.

Sillanpää, M.J. 2011. Overview of techniques to account for confounding due to population stratification and cryptic relatedness in genomic data association analyses. Heredity (Edinb). 106(4): 511–9 Available at http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3183892&tool=pmcentrez&rendertype=abstract.

Soengas, P., B. Ordás, R.A. Malvar, P. Revilla, and A. Ordás. 2003. Performance of flint maize

in crosses with testers from different heterotic groups. Maydica 48(2): 85–91.

Soengas, P., B. Ordás, R.A. Malvar, P. Revilla, and A. Ordás. 2006. Combining abilities and heterosis for adaptation in flint maize populations. Crop Sci. 46: 2666–2669.

Sood, S., S. Flint-Garcia, M. C.Willcox, and J.B. Holland. 2014. Genomics of plant genetic resources: Mining natural variation for maize improvement: Selection on phenotypes and genes. p. 467–487. *In* R. Tuberosa et al. (ed.), Genomics of Plant Genetic Resources. Springer Science+Business Media Dordrecht 2014.

Stadler, L.J. 1944. Gamete selection in corn breeding. J. Am. Soc. Agron. 36: 988–989.

Stange, M., H.F. Utz, T.A. Schrag, A.E. Melchinger, and T. Würschum. 2013. High-density genotyping: an overkill for QTL mapping? Lessons learned from a case study in maize and simulations. Theor. Appl. Genet. 126(10): 2563–2574 Available at http://link.springer.com/10.1007/s00122-013-2155-0.

Stich, B., A.E. Melchinger, M. Frisch, H.P. Maurer, M. Heckenberger, and J.C. Reif. 2005. Linkage disequilibrium in European elite maize germplasm investigated with SSRs. Theor. Appl. Genet. 111: 723–730.

Strable, J., and M.J. Scanlon. 2009. Maize (Zea mays): A Model Organism for Basic and Applied Research in Plant Biology. Cold Spring Harb. Protoc. 2009(10): pdb.emo132 Available at http://www.cshprotocols.org/lookup/doi/10.1101/pdb.emo132.

Strigens, A., N.M. Freitag, X. Gilbert, C. Grieder, C. Riedelsheimer, T.A. Schrag, R. Messmer, and A.E. Melchinger. 2013a. Association mapping for chilling tolerance in elite flint and dent maize inbred lines evaluated in growth chamber and field experiments. Plant, Cell Environ. 36(10): 1871–1887.

Strigens, A., W. Schipprack, J.C. Reif, and A.E. Melchinger. 2013b. Unlocking the genetic diversity of maize landraces with doubled haploids opens new avenues for breeding. PLoS One 8(2).

Suhre, K., and C. Gieger. 2012. Genetic variation in metabolic phenotypes: study designs and applications. Nat. Rev. Genet. 13(11): 759–769 Available at http://www.nature.com/articles/nrg3314.

Tenaillon, M.I., and A. Charcosset. 2011. A European perspective on maize history. Comptes Rendus - Biol. 334(3): 221–228.

Tenaillon, M.I., M.C. Sawkins, a D. Long, R.L. Gaut, J.F. Doebley, and B.S. Gaut. 2001. Patterns of DNA sequence polymorphism along chromosome 1 of maize (Zea mays ssp. mays L.). Proc. Natl. Acad. Sci. U. S. A. 98(16): 9161–6 Available at http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=55390&tool=pmcentrez&rendertype=abstract.

Voytas, D.F. 2013. Plant genome engineering with sequence-specific nucleases. Annu. Rev. Plant Biol. 64: 327–350Available at http://www.ncbi.nlm.nih.gov/pubmed/23451779.

Wallace, J.G., S.J. Larsson, and E.S. Buckler. 2014. Entering the second century of maize quantitative genetics. Heredity (Edinb). 112(1): 30–38 Available at http://dx.doi.org/10.1038/hdy.2013.6.

Warburton, M.L., J.C. Reif, M. Frisch, M. Bohn, C. Bedoya, X.C. Xia, J. Crossa, J. Franco, D. Hoisington, K. Pixley, S. Taba, and A.E. Melchinger. 2008. Genetic diversity in CIMMYT nontemperate maize germplasm: Landraces, open pollinated varieties, and inbred lines. Crop Sci. 48: 617–624.

Wilde, K., H. Burger, V. Prigge, T. Presterl, W. Schmidt, M. Ouzunova, and H.H. Geiger. 2010. Testcross performance of doubled-haploid lines developed from European flint maize landraces. Plant Breed. 129(2): 181–185.

Würschum, T., T.M. Weiß, J. Renner, H. Friedrich Utz, A. Gierl, R. Jonczyk, L. Römisch-Margl, W. Schipprack, C.C. Schön, T.A. Schrag, W.L. Leiser, and A.E. Melchinger. 2021. High-resolution association mapping with libraries of immortalized lines from ancestral landraces. Theor. Appl. Genet. Available at https://doi.org/10.1007/s00122-021-03963-3.

Xu, S., Y. Xu, L. Gong, and Q. Zhang. 2016. Metabolomic prediction of yield in hybrid rice. Plant J. 88(2): 219–227 Available at https://onlinelibrary.wiley.com/doi/10.1111/tpj.13242.

Yan, J., T. Shah, M.L. Warburton, E.S. Buckler, M.D. McMullen, and J. Crouch. 2009. Genetic characterization and linkage disequilibrium estimation of a global maize collection using SNP markers. PLoS One 4(12).

Yu, J., J.B. Holland, M.D. McMullen, and E.S. Buckler. 2008. Genetic design and statistical power of nested association mapping in maize. Genetics 178(1): 539–551.

Yu, J., G. Pressoir, W.H. Briggs, I. Vroh Bi, M. Yamasaki, J.F. Doebley, M.D. McMullen, B.S. Gaut, D.M. Nielsen, J.B. Holland, S. Kresovich, and E.S. Buckler. 2006. A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat. Genet. 38(2): 203–8Available at http://www.ncbi.nlm.nih.gov/pubmed/16380716.

Zeitler, L., J. Ross-Ibarra, and M.G. Stetter. 2020. Selective loss of diversity in doubled-haploid lines from european maize landraces. G3 Genes, Genomes, Genet. 10(7): 2497–2506.

Zhu, C., M. Gore, E.S. Buckler, and J. Yu. 2008. Status and Prospects of Association Mapping in Plants. Plant Genome J. 1(1): 5 Available at https://www.crops.org/publications/tpg/abstracts/1/1/5.

Acknowledgements

Curriculum Vitae

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Work experience

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Juliane Renner

Langquaid, den 6. Juni 2022

Erklärung

Eidesstattliche Versicherung über die eigenständig erbrachte Leistung gemäß § 18 Absatz 3 Satz 5 der Promotionsordnung der Universität Hohenheim für die Fakultäten Agrar-, Natur- sowie Wirtschafts- und Sozialwissenschaften

1. Bei der eingereichten Dissertation zum Thema *„Utilization of landraces of European flint maize for breeding and genetic research"* handelt es sich um meine eigenständig erbrachte Leistung.

2. Ich habe nur die angegebenen Quellen und Hilfsmittel benutzt und mich keiner unzulässigen Hilfe Dritter bedient. Insbesondere habe ich wörtlich oder sinngemäß aus anderen Werken übernommene Inhalte als solche kenntlich gemacht.

3. Ich habe nicht die Hilfe einer kommerziellen Promotionsvermittlung oder -beratung in Anspruch genommen.

4. Die Bedeutung der eidesstattlichen Versicherung und der strafrechtlichen Folgen einer unrichtigen oder unvollständigen eidesstattlichen Versicherung sind mir bekannt.

Die Richtigkeit der vorstehenden Erklärung bestätige ich. Ich versichere an Eides Statt, dass ich nach bestem Wissen die reine Wahrheit erklärt und nichts verschwiegen habe.

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Juliane Renner

Langquaid, den 6. Juni 2022