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## BREEDING PROGRAM FOR MAIZE IMPROVEMENT

- Use of landraces as a valuable source for allelic diversity for maize breeding:

**Early vigor, flowering time, lodging tolerance, grain yield and opportunities to use haploid induction -**

*Grain yield, early vigor, flowering time and lodging tolerance are major traits under consideration by corn breeders. In this case study, strategies for using landraces for maize improvement will be debated by scrutinizing data from on-going selection programs.*

*Breeding methods based on new biotechnology methodologies will also be discussed.*

### **I. European maize landraces as a rich source of allelic diversity.**

#### **Context**

At the basis of selection, evolution and breeding, genetic variation is of crucial importance for crop improvement. Harnessing the allelic diversity of genetic resources is considered essential for overcoming the challenges of climate change and for meeting future demands on crop production. For most traits of agronomic importance, modern breeding material captures only a fraction of the available diversity within crop species. In the case of maize (*Zea mays* L.), today's elite germplasm went through several bottlenecks, first by geographical dispersion from its center of origin, second through the selection of only a few key ancestors sampled from a small number of landraces to establish heterotic groups, and third through decades of advanced cycle breeding with high selection intensities. For traits that were not targets of selection in the past, but are important today, like abiotic stress tolerance and resource-use efficiency, this might have resulted in the loss of favorable alleles during the breeding process. In addition, unfavorable alleles might have become fixed during the selection process due to drift and/or hitchhiking effects.

Maize landraces represent a rich source of allelic diversity, but their efficient utilization in breeding and research has been hampered by their heterogeneous and heterozygous nature and insufficient information about most accessions.

A library of ~1,000 doubled-haploid (DH) lines from landraces was produced to capture native genetic diversity for a multitude of quantitative traits and make it accessible for breeding. The DH lines were evaluated for nine agronomically important, quantitative traits in multi-environment field trials comprising seven locations and two years. The DH lines were genotyped with 600k SNP markers. After stringent quality filtering 500k markers remained for further analyses.

#### **Material and Methods**

##### *Population development*

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~1,000 doubled-haploid (DH) lines were derived from three pre-selected European maize landraces. The three landraces Kemater Landmais Gelb (KE, Austria), Petkuser Ferdinand Rot (PE, Germany), and Lalin (LL, Spain) were chosen for the production of DH lines because they showed phenotypic variation for early development as well as low levels of linkage disequilibrium (LD) and population structure within populations. They were selected from a set of 35 European maize landraces covering a broad geographical region of Europe. Together, they represented 95.0% of the molecular variance of the full set of 35 landraces. From the selected landraces, 1015 DH lines (516 KE, 432 PE, 67 LL) were produced and multiplied using the *in vivo* haploid induction method (Röber et al. 2005).

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### ***Guidelines for analysis***

1. *You will discuss the advantages of DH plant genetic resources for plant breeding.*

*“In vivo haploid induction (HI) triggered by pollination with special intraspecific genotypes, called inducers, is unique to *Zea mays* L. within the plant kingdom and has revolutionized maize breeding during the last decade.” From Hu et al., 2016*

2. *Based on the review article from Chaikam et al. (2019) and other research articles that you will find by yourself to support your statement, you will show how “In vivo haploid induction (HI) [...] has revolutionized maize breeding during the last decade.” You will also discuss the limits of this technology. As such, you will present the principles of In vivo haploid induction (HI) and discuss its advantages and drawbacks compared to the in vitro system.*

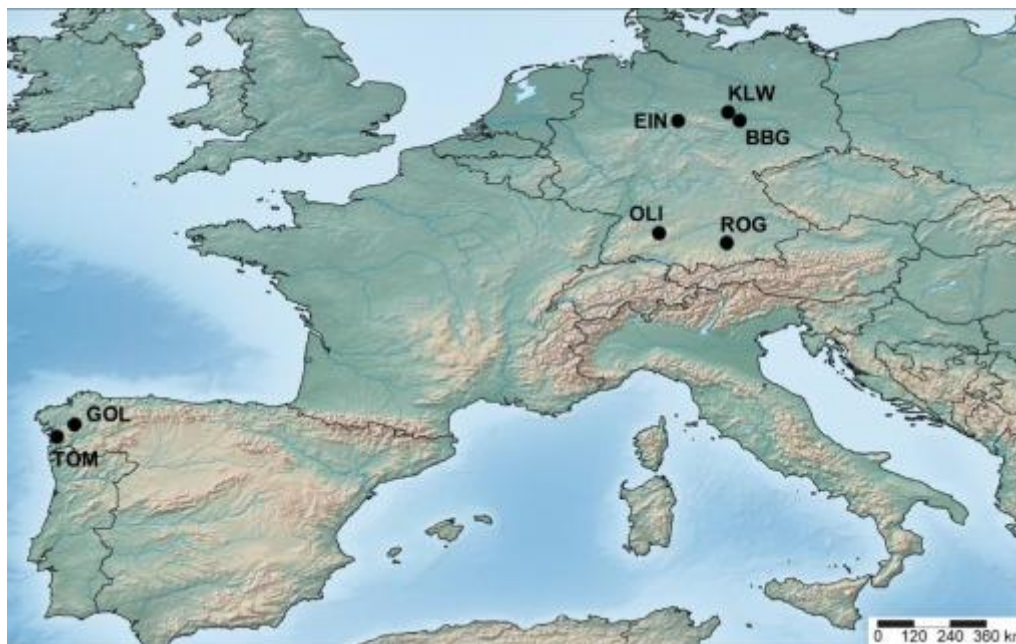
Chaikam, V., Molenaar, W., Melchinger, A.E. et al. Doubled haploid technology for line development in maize: technical advances and prospects. *Theor Appl Genet* 132, 3227–3243 (2019). <https://doi.org/10.1007/s00122-019-03433-x>

3. *You will present a flowchart of the methodology to create the library of doubled haploid (DH) lines (including the choice of the three founder landraces).*
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### ***Experimental design and phenotypic data collection***

The field experiments comprised 958 landrace-derived DH lines as well as 15 breeding lines and samples of the original landraces. Thereof, 899 DH lines (KE = 471, LL = 26, and PE = 402) passed the quality filtering based on genotypic data. The lines were evaluated for various traits in six and five locations in 2017 and 2018, respectively, resulting in up to eleven location-year combinations per DH line and trait (**Fig. 1, Table 1**).

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**Fig. 1. Locations of the field trials conducted in 2017 and 2018.** Einbeck (EIN; 2017/18), Rognstein (ROG; 2017/18), Bernburg (BBG; 2017), Klein Wanzleben (KLW; 2018), Oberer Lindenhof (OLI; 2017), Golada (GOL; 2017/18) and Tomeza (TOM; 2017/18).

**Table 1. Number of lines evaluated in each environment.** For each location-year combination, the number of entries evaluated, the number of DH lines after quality filtering and the number of checks (BL = breeding lines, LR = original landraces) are shown.

Location	Year	N entries	N lines after QC	N checks
Bernburg (BBG)	2017	1,000	461 KE 14 LL 393 PE	15 BL 3 LR
Einbeck (EIN)	2017	1,000	462 KE 14 LL 393 PE	15 BL 3 LR
	2018	800	365 KE 26 LL 365 PE	4 BL 3 LR
Golada (GOL)	2017	500	210 KE 7 LL 204 PE	15 BL 3 LR
	2018	500	222 KE 6 LL 240 PE	4 BL 3 LR
Klein Wanzleben (KLW)	2018	800	365 KE 26 LL 365 PE	4 BL 3 LR

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Location	Year	N entries	N lines after QC	N checks
Oberer Lindenhof (OLI)	2017	1,000	441 KE 13 LL 390 PE	15 BL 3 LR
Roggenstein (ROG)	2017	1,000	461 KE 14 LL 390 PE	15 BL 3 LR
	2018	800	365 KE 26 LL 365 PE	4 BL 3 LR
Tomeza (TOM)	2017	500	210 KE 7 LL 204 PE	15 BL 3 LR
	2018	500	222 KE 6 LL 240 PE	4 BL 3 LR

Total precipitation as well as average, minimum and maximum daily temperature during the vegetation period for each trial are described in Hölker et al. 2019.

The nine traits for which data are provided are:

- early vigour (EV; at growth stages V4 and V6, whole plot, 1–9 score),
- early plant height (PH; at V4 and V6, average over 3 plants, cm),
- final plant height (PH\_final; at R4, average over 3 plants, cm),
- days to male (MF) and female (FF) flowering (days until 50% of plants showed anthers/silks, d),
- lodging (LO; at R6, whole plot, 1–9 score)
- and tillering (TILL; at V9, whole plot, 1–9 score).

Phenotypic data are available in **Suppl. Table S1** (to be downloaded on Canvas). The provided data file described the plot-level data of the 899 DH lines and checks including the plot number, location, year, environment, the genotype names, the design factors (lattice, replication, block) and the corresponding phenotypic values for the nine traits.

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### ***Guidelines for analysis***

1. *What are the most discriminating traits which explain more variability between the different genotypes?*
2. *Are there any relationships between these traits? What are the possible biological explanations of these relationships? Which are the consequences for such relationships for plant breeding programs?*
3. *Are the studied agronomical trait subjected to GXE control? Which are the consequences for breeding and marketing strategies?*

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4. Which DH lines seem most interesting to initiate a breeding program aimed at creating a new improved maize variety with early vigor, early flowering and lodging tolerance?
  5. Are some of these DH lines interesting as genetic resources to initiate plant breeding program to create improved maize variety adapted for cultivation in the main maize production basin in Russia? if so, what would be the administrative procedures necessary to acquire these genetic resources?
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### Genotypic data

The DH lines were genotyped with the 600k Affymetrix® Axiom® Maize Array [1]. After stringent quality filtering 941 DH lines remained (501 KE, 409 PE, and 31 LL). The genotypic data provided consist of a filtered and imputed data file, comprising 941 DH lines and 501,124 markers ("SupplTableS2\_genotypes\_DHlines\_600k\_filteredImputed.txt.gz").

The data files contain 946 columns and 501,124 rows (header + individual markers), respectively. The first three columns contain the marker names and their physical position according to the B73 AGPv4 reference sequence. Unmapped markers are indicated by the value '0' for the chromosome. Information on SNP IDs, genome positions according to B73 AGPv2 (used for the array development), probe sets, and alleles are available at NCBI GEO as platform GPL18778 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GPL18778>). The landrace membership of individual lines is indicated by the respective abbreviation (KE, PE, LL) included in the column headers. The filtered data file contains only markers with the best quality class, "Poly High Resolution", and genotype scores coded as '0' and '2', without missing values. Columns 4 and 5 indicate the alleles corresponding to scores '0' and '2', respectively.

Genotypic data are available in **Suppl. Table S2** (to be downloaded on Canvas).

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### Guidelines for analysis

1. Based on available phenotypic and genotypic data, you will implement a genomic prediction analysis to assess the possibility to use such an approach in a breeding program aiming at creating a maize variety improved for early vigor, plant height, flowering time, lodging and tillering? Prediction accuracies will be computed.
2. You will propose a plant breeding schema aiming at introducing the desired traits in elite germplasm. You will discuss the opportunity to use different (possibly complementary) breeding approaches to make the best of the new genetic diversity brought by landraces. You will discuss the advantages and limits of
  - i. marker-assisted selection (MAS) to help in introgressing loci with major effect on the quantitative traits as identified by association genetics;
  - ii. haplotype-based breeding identifying superior haplotypes for improvement-related traits in landraces that can be introgressed into elite breeding lines;

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- iii. *crop breeding strategies based on genomic prediction to enhance crop productivity for the desired traits while avoiding the erosion of genetic diversity through optimal contribution selection (OCS)-based pre-breeding.*

Note: The three landraces can be assumed to be closest related to the European flint heterotic breeding pool, which therefore should be the target germplasm for introgression of novel beneficial alleles found in the DH populations.

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## **II. LinxTester analysis for grain yield**

The five most interesting new elite lines as obtained by the previous analysis using the DH lines as allelic sources for the traits of interest have been further studied through a LinxTester analysis for grain yield, a major trait for maize breeding.

### ***Experimental design and methodology***

Combining abilities to maximize heterosis for grain yield was investigated through a LineXTester analysis. A randomized complete block design (RCBD) comparing parents and one set of F1's, with 4 replicates, was set up to evaluate the grain of the five best maize DH as identified previously.

The three landraces are assumed to be closest related to the European flint heterotic breeding pool. Accordingly, the hybrid performance of the DH lines should be evaluated in testcrosses with complementary dent breeding lines. Here, three elite parental lines from the dent heterotic group usually used to derive F1 hybrid in combination with flint parental lines were used as tester.

Data for grain yield (arbitrary unit) are presented in **Suppl. Table S3** (to be downloaded on Canvas).

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### ***Guidelines for analysis***

1. *You will explain the concept of heterotic groups and present the classical approaches to defined them.*
  2. *Based on the analysis of the combining abilities of those five maize DH lines with dent tester, you will propose a strategy to create an improved variety with high productivity.*
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## **III. Certification procedure.**