Josef G. Knoll-Europäischer Wissenschaftspreisträger 2012

Josef G. Knoll-European-Science Award Winner 2012

Vanessa Prigge "Implementation and Optimization of the Doubled Haploid Technology for Tropical Maize (Zea mays L.) Breeding Programs", University of Hohenheim, 2012

Summary

Problem statement and research questions

Every day about a billion people go to sleep feeling hungry because they cannot afford to buy food. For many poor people maize is the food staple and in certain regions, for example in large parts of sub-Sahara Africa, more than 80% of a person's calory intake comes from maize-based meals. Maize production will have to be dramatically increased to achieve food security in the future and this will be particularly challenging in developing countries, because agricultural inputs are generally low in these countries and they are expected to severely suffer from the effects of global climate change and population growth. Hence, improved maize varieties that yield more with less or equal inputs than today and that show particular tolerance to prevalent biotic and abiotic stresses are indispensible to reduce hunger in poverty-stricken areas.

Interestingly, the keys to feeding the world in the 21st century may be two phenomena that are poorly understood on a molecular basis albeit extensively being exploited in maize breeding. These are hybrid vigor (or heterosis) and doubled haploids. Heterosis is best exploited by growing single cross hybrids, which maize breeders form by crossing two unrelated inbred lines. Though the exact mechanisms of heterosis remain obscure, it is well known that the F1 hybrid, i.e., the first generation of progeny, yields more than its two inbred parents. Hybrid and synthetic varieties have boosted global maize productivity since the 1950s and were a landmark achievement in plant breeding. Synthetics are developed by intermating not only two but several (10-20) carefully selected inbred lines followed by seed increase through open pollination. This variety type allows farmers to recycle the harvested seed for replanting in the following cycle without suffering severe yield losses. However, yield potential of synthetics is about 10-20% lower compared to single cross hybrids.

For hybrid breeding, the parental inbreds must be genetically stable (or homozygous) to be able to generate the same hybrid variety year after year and to ensure that the variety exhibits the same agronomic and quality properties every time it is grown. Traditionally, such homozygous inbreds were formed by continuous self-pollination for six to eight generations. Efficiency of breeding improved maize varieties can be considerably enhanced by using doubled haploid (DH) lines as components of hybrid and synthetic varieties. With this technology inbred lines can be generated in less than half the time required traditionally. It involves the induction of haploidy, such that haploid maize is generated that contains the gametic chromosome number in its somatic cells, and subsequent duplication of the haploids' chromosome set to re-establish diploidy in DH plants. Since the progenies obtained from selfing a DH plant derive from a single gamete, they are completely homozygous. Through the reduced period of inbred production, the development of improved hybrid varieties is

considerably accelerated. Additionally, maize breeding with DH lines has several quantitative genetic, economic, and logistic advantages and, therefore, has been adopted for inbred development in many public and commercial maize breeding programs in Europe and North America. However, before the initiation of this thesis research there were no published accounts of the adoption of DH technology in non-temperate areas.

In vivo production of DH lines involves four basic steps: (i) inducing haploidy by pollinating source germplasm with pollen of a haploid inducer; (ii) identifying putative haploid seeds (seeds with a haploid embryo) using a seed coloration marker; (iii) duplicating chromosomes of haploids by treating seedlings with a mitotic inhibitor; and (iv) self-pollinating DH plants to multiply their seed. Compared to in vitro approaches for DH line production such as anther or microspore cultivation, the in vivo method does not require tissue culture and is therefore also suitable for less sophisticated maize breeding programs in developing countries. However, it was not clear whether the protocols available for in vivo DH line production in temperate maize breeding programs were directly applicable to tropical conditions. All of the publicly available haploid inducers had been developed and mainly been evaluated for agronomic performance under temperate conditions. Hence, there was no information on whether the environment al conditions or the genomic composition and geographic origin of the source germplasm would influence the inducers' haploid induction rate (HIR), Le., the proportion of haploids produced among the total induction cross progeny. The biological mechanism underlying in vivo haploidy induction was still unknown and none of the modern haploid inducers with high HIR had been subjected to genome-wide analyses of the quantitative trait loci (QTL) controlling haploid induction ability.

Further, it was important to investigate the applicability of the purple seed color marker used to detect haploids in tropical source germplasm, because in temperate germplasm inhibition of the purple color caused problems, particularly in source germplasm belonging to the temperate flint heterotic group, so that detection of haploids was not possible with this marker. In general, maize haploids are not expected to show symptoms of fertility such as silk and anther emergence due to disrupted gamete formation. Hence, duplication of their chromosome set to the diploid (doubled haploid) level is necessary to enable successful self-pollination and seed development on the selfed ear, aprerequisite for maintenance of the resulting DH line. However, in European germplasm several researchers observed significant genetic variation for male and female fertility of haploids. It was important to study this phenomenon in haploids of tropical origin as well, because a satifactory level of fertility would supersede artificial treatments, which still represent a bottleneck in the DH production pipeline due to the associated cost and labor inputs. Finally, there had been no proof-of-concept that viable and high-yielding DH lines can be developed by in vivo DH technology from tropical source germplasm.

Objective of the research

The aim of this thesis research was to furt her improve the in vivo DH technology to facilitate its implementation for tropical maize breeding programs at the International Maize and Wheat Improvement Center (CIMMYT). In particular, the objectives were to

- present a step-by-step documentation of the production of haploids and DH lines that can be used for capacity building purposes;

- examine the agronomic performance and haploid induction ability of temperate haploid inducers under tropical conditions;

- design and conduct a breeding program for developing haploid inducers that are well adapted to tropical environments;

- study the genetic architecture of in vivo haploid induction ability and assess the stability of QTL across various genetic backgrounds;

- explore options for exploiting fertility of maize haploids to avoid artificial chromosome doubling treatments during DH line production; and

- evaluate tropical DH lines for testcross performance to estimate quantitative genetic parameters and identify parental components for tropical hybrid and synthetic varieties.

Methodological Approach

Setting up a production pipeline for DH lines at CIMMYT-Mexico was the major aim of this thesis research and it required the amendment of the involved processes to local conditions and CIMMYT facilities. Apart from acquiring materials and establishing work flows that allowed performing the individual production steps in an efficient and cost-effective manner, particularly training of staff was essential for the successful technology transfer. As part of our capacity building efforts, we produced a detailed recipe-style protocol and a 15-minute training video to provide guidance on the production of doubled haploids in maize. The video is publicly available at http://www.youtube.com/watch?v=V2jOEuZjjrg.

Since inducers with tropical adaptation were initially not available, we tested the temperate inducers RWS, CH400, and RWSx CH400 developed by the University of Hohenheim for their suitability to induce haploidy in tropical source germplasm and evaluated their agronomic performance under tropical conditions. The inducers were used as pollinators in crosses with tropical germplasm of diverse origins (South and East Africa, Central and South America) and population types (single crosses and open-pollinated populations including accessions from CIMMYT's maize genebank) to estimate their HIR. Putative environment al influences were accounted for by conducting these experiments in three seasons and two locations in Mexico.

Simultaneously, we initiated a breeding program aiming at developing inducers that combine high haploid induction ability with adaptation to subtropical and tropical conditions for use in a wide range of cropping systems and agroecologies in developing country maize breeding programs. We crossed temperate inducers with tropical CIMMYT maize lines (CML) from Mexico and Zimbabwe and made backcrosses to both, inducer and GML, to investigate the effect of higher genome contributions by either parent on inducer performance. Selection criteria included agronomic characteristics such as abundance of pollen, plant vigor, resistance to tropical leaf diseases, and seed production capacity after selfing. Further, special characteristics pertaining to inducers were evaluated such as HIR and expression of the seed color marker required for identification of seeds with haploid embryo harvested on the induced source germplasm.

To support traditional inducer selection schemes, which are based on time-consuming and labor-intensive phenotyping protocols, we resumed studies on the genetic architecture of haploid induction ability which had been initiated more than a decade age at Prof. Geiger's group at the University of Hohenheim. Identification of the genomic regions that harbour QTL

affecting the trait involved: 1) developing a large segregating population from two inbred parents with contrasting HIR levels; 2) phenotyping the progeny for HIR under field conditions; 3) genotyping the progeny with DNA-based markers densely covering the genome; and 4) applying sophisticated statistical analysis tools to relate polymorphisms detected in the DNA sequence with the HIR levels of individuals. To assess the stability of QTL across different genetic backgrounds, which is a prerequisite for marker-based trait introgression programs, we conducted these QTL analyses in four populations comprising between 113 and 185 individuals each. The populations were formed from four cross combinations of elite inbreds involving two the temperate inducers UH400 and CAUHOI as well as one temperate and two tropical inbreds without induction capacity.

While the previous three studies focused on optimizing the first two steps of the DH production pipeline, namely haploidy induction and detection of haploids, the fourth study aimed at exploring alternatives to artificial chromosome doubling for the production of homozygous, fertile inbreds from these haploids. To assess genetic variation for male fertility of haploids, we screened 260 haploid maize populations derived by haploidy induction of diverse temperate and tropical source germplasm comprising various maturity groups, heterotic groups, and variety types under field and greenhouse conditions in two tropical environments in Mexico and three temperate environments in the U.S. corn belt. We monitored anther emergence and performed self-pollinations when possible. After harvest, we estimated the proportion of fertile haploids per population and determined the average number of intact seeds produced on selfed ears by fertile populations.

Finally, our last study made use of tropical DH lines that had been produced in the frame of this thesis research at CIMMYT during 2007 to 2009. We selected DH lines from five open pollinated populations (including one genebank accession from Brazil) and five elite single crosses from Zimbabwe and Colombia for field evaluations to estimate quantitative genetic parameters for teste ross grain yield and dry matter content. This allowed comparison of these population types with regard to their suitability for maximizing response to selection in tropical maize breeding programs employing DH lines. In addition, we genotyped the DH lines to assess the molecular diversity captured from the corresponding source germplasm.

Results and Discussion

Evaluation of temperate haploid inducers in Mexico revealed HIR of 8-12% on average, which is comparable to the rates commonly obtained under temperate conditions in Germany and Chile. Induction of haploidy worked well in diverse tropical source germplasm irrespective of population type or geographic origin, yet some sour ce germplasm were more responsive to haploidy induction than others. The level of expression of the purpie seed coloration, which is inherited from the inducers and used to discriminate seeds with haploid embryo from diploids, also varied among sour ce germplasm but the method worked well in tropical dent and flint germplasm alike. These results suggested that temperate inducers could be readily employed for haploidy induction during initiation of DH breeding programs in the tropics. However, the temperate inducers exhibited only decent pollen and seed production capacity and showed strong symptoms of susceptibility to prevalent tropical leaf diseases. Therefore, their permanent deployment in tropical DH breeding programs was not recommendable.

Fortunately, promising results were obtained in our tropical inducer breeding program. Through targeted recombination of temperate inducers and tropical CML and by applying phenotypic selection based on results from extensive field trials, we managed to develop

tropical inducer candidates combining HIR of up to 10% with notably improved agronomic performance under tropical conditions. Specifically, these putative tropical inducers showed improved plant vigor and abundant pollen production, reduced disease susceptibility, delayed anthesis dates which better synchronized with flowering dates of the tropical source germplasm, and improved seed production capacities. These results are currently being confirmed by assessing the performance of these tropical inducers in multiple environments at CIMMYT's regional maize breeding hubs in India, Kenya, Zimbabwe, and Colombia.

The comparative QTL analyses revealed several new insights into the genetics of haploid induction ability: (1) The trait is likely controlled by one major QTL on chromosome 1 which is stably expressed in populations of inducer x non-inducer crosses. This confirmed findings of previous studies and suggested that the identified chromosomal region is very suitable for positional cloning of the corresponding gene(s). (2) Additional QTL with smaller effects may act as modifiers suppressing or enhancing the expression of the major locus and may be responsible for variable HIR depending on the genetic background. Since these small-effect loci will be very difficult to select far phenotypically, marker-based approaches such as genomic selection may be necessary to capture them for inducer breeding. (3) At loci in the vicinity of the major QTL on chromosome 1, inducer alleles were underrepresented, the observed segregation ratios at these loci deviated significantly from the expected Mendelian segregation ratios. This means that the inducer's gametes are not transferred properly during meiosis and, therefore, inducer genotypes may gradually lose their haploid induction ability through sexual recombination. Consequently, monitoring of HIR levels is crucial during maintenance breeding of inducers to ensure that only genotypes with high HIR are kept as inducer stocks. Once the underlying genes have been elucidated and molecular markers closely associated with them have been designed, application of marker-based trait introgression has the potential to rapidly convert any germplasm into a haploid inducer, thereby enabling the development of custom-made inducers that can be used in a wide range of agro-ecologies.

As the above studies showed, induction of haploidy is not a limiting factor for production of DH lines in tropical maize breeding programs. To further simplify the methodology and thereby increase the chance that small breeding programs in developing countries will adopt DH technology, we explored one option of abolishing artificial chromosome duplication treatments by screening a diverse set of untreated haploids for male fertility and seed production capacities. On average, about 1% of the haploids were fertile, i.e., they produced pollen and silks and were responsive to self-pollination as shown by a certain number of seeds produced on selfed ears. This is much lower than the average proportion of 5-10% fertile haploids commonly obtained when applying chemical treatments for artificial chromosome duplication. However, we detected significant genetic variance for male fertility among the untreated haploids, with up to 70% of fertile, seed-producing haploids being recorded for some source germplasm. Presence of genetic variation is the main driver for gain from selection and, therefore, our results imply that significant improvements of haploid fertility can be achieved by recurrent selection. Thus, we anticipate that germplasm with notably improved haploid fertility will enable abandoning the costly and labor-intensive artificial chromosome doubling treatments in the long run.

Finally, the field evaluation of DH lines generated from tropical elite single crosses and open-pollinated populations provided the ultimate proof-of-concept that high-yielding varieties can rapidly be generated from tropical source germplasm by application of the D H technology. The development and subsequent testcross evaluation of inbreds in a single 3-year PhD project has hardly been achieved before. The induction crosses were made in summer 2008, followed

by artificial chromosome doubling and seed multiplication in winter 2008/2009, production of testcross seed in summer 2009, and field evaluation of testcross hybrids in winter 2009/2010. When analysed across three environments, no significant differences where observed between the mean testcross grain yields of DH lines derived from single crosses of elite inbreds and those derived from heterogeneous open-pollinated populations. Hence, both population types are suitable source germplasm for tropical inbred development. In fact, higher genetic variance was observed among DH lines from open-pollinated populations and this trend was also confirmed by molecular diversity indices based on DNA marker analyses. This demonstrates that genetic variation contained in maize genetic resources such as genebank accessions and improved open-pollinated populations can effectively and rapidly be made accessible to tropical maize breeders by means of DH lines, which promises to greatly accelerate breeding progress and, accordingly, the arrival of improved stress-resilient maize varieties at the resource-poor farmers' fields.

Relevance of the submitted thesis research with regard to the objective of the award

Considering the growing global population and the gradual decline of natural resources such as arable land, water, and phosphorus, plant breeders play a crucial role in mitigating poverty and food shortages by developing stress-resilient and high-yielding maize varieties for resource-poor farmers. Abiotic and biotic stresses that hamper maize production in developing countries include droughts and floods, heat, nutrient-depleted or saline soils, and a variety of insects and diseases. Reducing the time to variety development will be essential to be able to rapidly react to changing requirements and upcoming stresses, as recently exemplified by the aggressive spreading of the wheat stern rust race Ug99. In vivo production of maize DH lines therefore is a key component of future maize variety development, as it allows combining novel traits into high-yielding varieties at much higher speed than previously. This thesis research no less than provided the ultimate proof-of-concept for the applicability of DH technology to tropical maize breeding programs. CIMMYT's use of this technology is a prominent example of putting advanced technologies at the service of disadvantaged, small-scale farmers.

The step-by-step protocol and particularly the training video that were produced in the frame of this thesis research have already proven very useful for capacity building purposes during workshops in Kenya and Costa Rica as well as for training of staff at CIMMYT's own experimental stations. Positioning of the video at an open-access online platform is expected to help reach maize breeders and researchers lacking the funds for participation at specific workshops. Further, the six publications that resulted from this thesis research not only focused on reporting scientific novelties, but were designed to additionally foster sharing of practical experiences, i.e., successes and pitfalls alike, gained during implementation of the DH production pipeline at CIMMYT. This will assist maize breeders in developing countries in establishing the relevant processes at their breeding stations.

In combination with training material, the availability of tropical haploid inducers, which have been developed in the frame of this thesis research, promises to greatly increase adoption of the DH technology by smaller national maize breeding programs and seed companies in developing countries. Maintenance breeding of the inducers and handling of the induction cross es is simplified when deploying well-adapted inducers. For example, instead of growing one inducer plant for every two source germplasm plants to be pollinated, as was necessary with non-adapted temperate inducers in Mexico due to their very limited production of pollen, the number of inducer plants required may be greatly reduced when adapted inducers are used. This frees capacities for growing considerably more source germplasm plants on the same

space and, given that 2-5 DH lines can generally be derived from each source germplasm plant, this can lead to a significant increase in the number of DH lines rapidly produced in tropical breeding programs, which in turn accelerates the development of improved varieties.

There is no doubt that continuing crop yield improvements heavily depend on the availability of suitable genetic variation. In fact, one of the biggest benefits of DH technology to modern maize breeding may be the possibility to access the wealth of variation contained in maize genetic resources. CIMMYT is safe-guarding some 27 000 unique maize accessions in its genebank, the majority of which are likely to carry high-value loci that will prove very helpful in meeting the future demands of maize production and quality. Until recently, unlocking these loci from their genetic backgrounds, which often display undesirable agronomic characteristics, required many generations of inbreeding and often resulted in disappointment, because deleterious recessive alleles were only revealed after several years due to effects of masking heterozygosity. Effective purging of such deleterious alleles can be achieved with DH technology since there is no compensation for negative alleles in the haploid phase. As this thesis research has shown, viable DH lines can be generated from heterogeneous tropical open-pollinated populations including genebank accessions. Hence, uniform and reproducible units become available that immediately display the complete genetic variation and can systematically be characterized and more easily be reproduced and conserved. In light of the extensive maize germplasm collection held at CIMMYT, it will be crucial to apply cutting-edge technologies including DH technology to enable the identification of novel useful genetic variation and establish suitable pipelines for supplying maize breeding programs in developing count ries with new genetic stocks to face future challenges of food production under changing climatic conditions and increasing population pressure. In fact, recombining DH lines derived from farmer-preferred landraces into a synthetic variety, i.e., an improved version of the landrace that has been disburdened of all deleterious alleles, may be an elegant way of maintaining quality characteristics desired by farmers and consumers while nonetheless improving productivity.

In short, the many benefits of DH lines as parental components of improved hybrid and synthetic varieties are no longer withheld from tropical maize breeding programs. CIMMYT is now routinely producing DH lines from tropical and subtropical germplasm and is providing them to national agricultural research systems and seed companies in Africa, Asia and Central and South America. Several of the DH lines developed in the frame of this thesis research in Mexico are currently being extensively tested in breeding programs focusing on nitrogen-efficiency and drought-tolerance hybrids and synthetics in Kenya and Zimbabwe. Capitalizing on the success of this thesis research, a follow-up project is being jointly implemented by CIMMYT and the University of Hohenheim to support transfer of the DH technology to maize breeding programs at national agricultural research systems and seed companies in South and East Africa. In addition, a workshop is being jointly organised in June 2012 that will train maize breeders from Asian public and private breeding programs in the processes involved in DH line production. It is expected that these efforts for promoting the adoption of DH technology and providing maize breeders in the developing world with this powerful tool will have tremendous effects on breeding high-yielding, stress-tolerant maize varieties for small-scale farmers and thereby help reducing hunger among the world's poor -step by step.

Vanessa Prigge “Implementation and Optimization of the Doubled Haploid Technology for Tropical Maize (Zea mays L.) Breeding Programs”, University of Hohenheim, 2012

http://opus.uni-hohenheim.de/volltexte/2012/715/pdf/Diss_VPrigge_2012.pdf