



**Josef G. Knoll-Wissenschaftspreisträger 2006**

**Josef G. Knoll-Science Award Winner 2006**

**Susanne Dreisigacker "Genetic Diversity in Elite Lines and Landraces of CIMMYT Spring Bread Wheat and Hybrid Performance of Crosses among Elite Germplasm", University of Hohenheim, 2004**

**Jochen C. Reif "Assessing the Genetic Diversity in Crops with Molecular Markers: Theory and Experimental Results with CIMMYT Wheat and Maize Elite Germplasm and Genetic Resources", University of Hohenheim, 2004**

Summary

Research problem

As the human population is steadily growing and the arable land is decreasing, the world faces a greater demand on agricultural output than ever before in history. In the past, this demand for increased agricultural productivity was met by a combination of genetic improvements, cultivation of more land, increased water supply, enhanced fertilization, use of pesticides, advanced mechanization, and favorable socioeconomic conditions. But as (i) freshwater reserves and petroleum resources, on which fertilizers and pesticides are based, are dwindling and (ii) problems caused by agricultural pollution are increasing, the current levels of agricultural inputs can hardly be enhanced or even maintained. Similarly, the existing farmland is decreasing due to urban and industrial development or natural phenomena such as expanding deserts. This leaves the genetic improvement of crops as one of the most viable and sustainable approach by which food production can attempt to keep pace with the anticipated growth of the human population. For the genetic approach to succeed, the genetic variation provided by nature and currently conserved in gene banks must be harnessed.

Research questions

Maize and wheat together amount for more than half of the cereals consumed in the developing world. Among the food crops, after rice, wheat and maize are the most important commodities both in terms of calorie consumption and value of production. They are significant staples in the diets of billions of poor in the developing world and variations in productivity can profoundly influence their well-being. The International Wheat and Maize Improvement Center (CIMMYT) plays a key role in the genetic improvement of wheat and maize and also in the conservation and exploitation of genetic resources of both crops. More than 70% of the wheat area in the developing world is now planted with CIMMYT-related wheat varieties covering more than 50 million ha. About 55% of the area planted with improved maize varieties, or more than 14 million ha of non-temperate maize, is now sown to CIMMYT-related cultivars.

The basic research question of our two dissertations was to optimize the conservation and use of CIMMYT's elite germplasm and genetic resources with the aid of molecular markers. The investigations comprised field trials and molecular analyses of maize and wheat. The wheat

project "Efficient management of genetic diversity in wheat: DNA markers for use in wheat breeding programs and gene banks" was funded by the Eiselen-Stiftung Ulm and the Bundesministerium für Wirtschaftliche Zusammenarbeit und Entwicklung.

### Objectives of the research

The objectives of our research were to

- develop a biometrical approach to analyze the molecular diversity (Dissertation Reif),
- examine the loss of genetic diversity during (i) domestication of bread wheat, (ii) transition from traditional landraces to modern wheat breeding varieties, and (iii) intensive selection over a sustained period of international wheat breeding (Dissertation Reif),
- investigate methods for a sophisticated exploitation of the genetic diversity untapped in the wild ancestors of bread wheat (Dissertation Dreisigacker),
- analyze the genetic diversity within and among traditional wheat landraces (Dissertation Dreisigacker),
- evaluate the CIMMYT concept of breeding for different mega-environments .., based on molecular markers (Dissertation Dreisigacker),
- assess the production of hybrid wheat for the increase of yield potential (Dissertation Dreisigacker),
- analyze the molecular genetic diversity of CIMMYT maize populations (Dissertation Reif),
- evaluate the usefulness of DNA markers for defining heterotic groups and patterns in tropical and subtropical maize germplasm (Dissertation Reif), and
- investigate strategies to optimize the exploitation of CIMMYT's maize genetic resources for breeding (Dissertation Reif).

### Methodological approach

#### Molecular analyses and field trials

Simple sequence repeat (SSR) markers have shown to be the most suitable marker system to measure the genetic diversity of wheat and maize populations. SSRs are abundant, genome specific, co-dominant in nature, and show a fairly uniform distribution over the genome. Therefore, we fingerprinted in total 633 *Triticum tauschii* accessions, landrace cultivars, synthetic wheats, and improved wheat varieties with up to 113 SSR markers. In addition, 22 wheat lines and their 112 factorial crosses were analyzed in replicated field trials.

In total 672 individuals were sampled from 23 CIMMYT maize populations and analyzed with 85 SSR markers. Furthermore, the maize populations and their diallel or factorial crosses were grouped according to their adaptation and evaluated in replicated field trials in different mega-environments. Per se and hybrid performance were analyzed and mid-parent heterosis was

calculated.

### Statistical analyses

We investigated the genetical and mathematical properties of 10 dissimilarity coefficients. The relationships among different coefficients were determined with a procrustes analysis. The molecular and field data of wheat and maize were analyzed using appropriated statistical methods such as analysis of variance, multiple regression analysis, principal coordinate analysis, estimation of gene diversity, Rogers' and modified Rogers' distances and their standard errors using bootstrap procedures. In addition, the coefficient of parentage was calculated as an indirect measure of genetic diversity among genotypes.

### Empirical background and results

#### Diversity coefficients

Choice of suitable diversity measures is important to facilitate the interpretation of findings from DNA marker studies on a theoretically sound basis. In a theoretical study, 10 dissimilarity coefficients widely used in germplasm surveys were investigated, with special focus on application in plant breeding and gene banks. The mathematical properties of the dissimilarity coefficients were investigated as well as the underlying genetic models. Based on these findings, appropriated dissimilarity measures were chosen to study the molecular diversity of CIMMYT's wheat and maize accessions.

#### Flux of diversity in wheat

Bread wheat was domesticated 12 000 years ago in the Fertile Crescent. It is postulated that the size of the founder population of bread wheat was limited, causing a domestication bottleneck. We observed a significant decrease in the diversity from *T. tauschii* accessions - one of the wild ancestors of bread wheat - to landrace cultivars. This reduction in genetic diversity is probably the product of a relatively young history of the wheat crop, the presumably small founder population, and the intensive long-term selection for agronomic traits. Thus, the initial steps of crop domestication caused a severe population bottleneck.

During the past century, traditional landrace cultivars were continuously replaced by modern wheat cultivars, which culminated in only about 3% of the wheat growing area currently sown with landrace cultivars. Modern wheat cultivars were bred with a limited number of landrace cultivars in their pedigree and it is postulated that modern wheat cultivars contain less genetic diversity than landrace cultivars. In accordance with this expectation, a substantial genetic diversity loss was observed from landrace cultivars to modern wheat cultivars. This outcome can be explained by (i) the limited number of landrace cultivars used as the germplasm base for the development of modern wheat cultivars and (ii) selection and drift during the breeding of modern wheat cultivars. The loss of genetic diversity may indicate an elimination of undesired or even deleterious alleles, but may also reflect an erosion of alleles valuable for plant improvement and future demands of producers and consumers. The latter hypothesis was supported by various surveys reporting the potential of landrace cultivars as a source of novel useful allelic variation.

It has been claimed that plant breeding reduces genetic diversity in elite germplasm, thereby jeopardizing future selection gain in crop improvement. Genetic diversity measured as average Rogers' distances between individuals was narrowed from 1950 to 1989, but was slightly

enhanced from 1990 to 1997. The reduction in the diversity can be explained by the "Early Green Revolution", which was characterized by breeding high yielding new semi-dwarf varieties based on a limited number of key parents. The increase in diversity from 1990 to 1997 indicate that breeders averted the narrowing of the genetic base of the elite breeding pool through the introgression of various novel wheat materials.

Summarizing, a drastic diversity loss occurred from *T. tauschii* to landrace cultivars and from landrace cultivars to modern wheat cultivars. Consequently, the genetic base of the modern wheat cultivars can be increased by introgression of both landrace cultivars and *T. tauschii* accessions. This broadening would guarantee a high genetic variation and thus a sustainable genetic improvement of wheat.

#### Exploitation of the diversity present in the wild ancestors of bread wheat

The creation of synthetic wheats is a very promising approach for the introduction of genetic variation from wild relatives into the elite wheat germplasm pool. In this study, the synthetic wheats were produced from artificial crosses between tetraploid durum wheat and the wild species *T. tauschii*. The resulting hybrids were hap-loid and required embryo rescue and artificial chromosome doubling. The synthetic wheats are in general highly tolerant against many biotic and abiotic stresses but their agronomic performance is overall marginal. Therefore, they were backcrossed once or twice with improved varieties to combine the useful novel alleles with the high yield potential of the elite varieties. Selection within and among the produced backcross families resulted in lines showing superior performance than the improved recurrent parents. It is unknown which proportion of germplasm in these selected backcross lines is derived from the wild ancestors and where the introductions are located in the genome. This knowledge would allow breeders to introgress the observed favorable alleles directly in different genetic backgrounds.

The synthetic wheats, their parental lines, the improved recurrent parents, and the backcross families were genotyped with SSR markers. With this approach we were able to trace the flow of alleles from the wild ancestors to the improved backcross lines. We mapped several preferentially inherited *T. tauschii* alleles in the synthetic wheats by testing for the presence of selection. Consequently, the findings of our study clearly showed that the applied procedure allows to identify chromosomal regions harboring favorable alleles of the wild ancestors. This information can be used to introgress *T. tauschii* alleles in elite varieties through marker-assisted selection.

#### Conservation and use of traditional wheat landraces

Landrace cultivars have proven to be an excellent source of favorable alleles absent in the elite breeding pool. Nevertheless, breeders rarely resort to landraces cultivars because little is known about their genetic variation. Evolutionary history, such as geographical sites and the nature of population establishments or the amount of seed used for regeneration in gene banks, highly influence this genetic variation. Molecular markers are an interesting tool to characterize the genetic diversity of gene bank accessions and, consequently, represent a first step for the exploitation of the genetic variation present in wheat landraces. A considerable high genetic variation was revealed within and between the selected landraces cultivars in our research. Infra-specific diversity was in particular characterized by a high number of unique alleles and major heterogeneity. The high number of unique alleles refers to diverse selective influences. High heterogeneity within landraces is important for genetic buffering and adaptation of landraces to different environments with various biotic and abiotic stresses. Our

results clearly confirmed the value of landrace cultivars for the improvement of bread wheat and gave important implications for their further use and conservation.

Evaluation of the concept of breeding for different mega-environments based on molecular markers

The impact of CIMMYT's wheat breeding program in developing countries is enormous. In 2002, the proportion of the total wheat area planted to varieties containing CIMMYT-related germplasm totaled 97% in East and South Asia, 83% in Latin America, 63% in Eastern and Southern Africa, and 57% in West Asia/North Africa. In these vastly varying areas wheat production experiences a range of biotic and abiotic stresses and crop improvement thus requires precise focusing on corresponding needs. To aid in this task, CIMMYT follows a concept of breeding for different mega-environments. A mega-environment is defined as a large, not necessarily contiguous area, occurring in more than one country and frequently transcontinental, with similar abiotic and biotic stress conditions, cropping systems, and consumer demands.

The SSR and coefficient of parentage analyses of present wheat breeding varieties indicated that CIMMYT's breeding concept has not yet resulted in genetically diverse germplasm groups specific to the targeted mega-environments. Several reasons might explain this observed absence of genetic differentiation: (i) selection based on mega-environment adaptation has not been practiced long enough to differentiate the germplasm, (ii) genes conferring fitness to one mega-environment are not unique and may confer fitness to several mega-environments, and (iii) adaptation to mega-environments is not based on an accretion of random genes but rather a limited set of specific genes. Our results might additionally be associated with observed discrepancies between mega-environment designations and their respective test locations in Mexico. To overcome this issue, the decision for a global expansion of test locations in the CIMMYT wheat breeding program took place in 2005. Despite lacking genetic differentiation, adequate genetic variation was found within the germplasm targeted for each mega-environment. This is fundamental as each mega-environment still encompasses millions of hectares with varying diseases and quality demands. Sufficient genetic variation assures that cooperating national research programs in each respective mega-environment are able to reselect and release cultivars for their own local needs.

Hybrid wheat for the increase of yield potential

Inspired by the enormous success of hybrids in maize, the production of hybrid wheat has been extracted as an additional option for wheat improvement at CIMMYT. The improved tolerance to abiotic stresses in marginal environments, the rapid response to new disease problems, or certain specific end use properties, which are required in many specialty markets in developing countries, have been secondary incentives for CIMMYT to consider hybrid wheat development. The observed levels of heterosis in our research did not lead to significant gains of hybrid wheat over leading standard cultivars. Highest levels of heterosis were observed by crossing parents with high per se performance. The genetic distance between parents did not play a major role in predicting heterosis. The successful dissemination of hybrids in wheat could therefore not be considered promising, especially if the costs of hybrid seed production remain high.

Conservation and use of CIMMYT's maize germplasm

CIMMYT's maize germplasm base consists of two main groups: (i) diverse but low yielding traditional populations of maize based on materials from a single racial complex (further referred to as races) and (ii) modern high yielding elite populations most of them established combining several racial complexes. The elite germplasm has been grouped according to its adaptation into four mega-environments and subsequently improved through recurrent intra-population selection. These improved populations have played an important role in maize breeding and production in developing countries.

The SSR analysis of CIMMYT's elite maize populations revealed that most of the genetic diversity is within the populations and just a minor part between the populations. This reflects CIMMYT's breeding policy and the establishment of this germplasm. Most elite maize populations were composed of germplasm from several racial complexes and have been improved with intra-population breeding methods. Our results indicate that the applied procedures to handle the broad range of germplasm was suboptimal with regard to (i) maintaining maximum genetic diversity within the populations and (ii) conserving genetic diversity between the populations. It is rather likely that desired alleles, which occurred with high frequency in just one racial complex, are lost by mixing different germplasm sources. Consequently, the conservation of the genetic diversity within and among CIMMYT's maize germplasm can be optimized by considering the racial complexes of the populations.

As hybrids perform better than open-pollinated varieties, CIMMYT decided to embark on a hybrid breeding program. The first fundamental step for the initiation of a hybrid breeding program consists in the establishment of heterotic groups. Therefore, CIMMYT conducted several diallel studies to identify heterotic groups and patterns among the populations. Some promising heterotic patterns were suggested, but a clear grouping of the maize germplasm on the basis of the field data was difficult due to the partially mixed origin of the populations.

If the mid-parent heterosis increases with increasing genetic distances of the parents, molecular marker-based genetic distances are a valuable tool to establish heterotic groups and patterns. The results of our study indicate that mid-parent increases with increasing genetic distance among the parent populations and that adaptation problems can cause deviations from this rule. Hence, if the populations are adapted to the target regions, genetic distance can be used as a criterion in the search for promising heterotic groups and patterns in addition to field trials.

Germplasm based on different racial complexes is useful for the improvement of open-pollinated varieties. Nevertheless, the significant positive correlation between genetic distances and heterosis supports the concept of clearly distinct heterotic groups. Populations with a mixed constitution are therefore not suitable for hybrid breeding. The reduced genetic diversity among the populations caused by admixture can only be recovered by long-term isolation or reciprocal recurrent selection programs. Therefore, only few CIMMYT elite populations based on one racial complex are suitable for hybrid breeding. For all mega-environments, a dent x flint heterotic pattern seems to be most promising. As the number of 'pure' flint and dent populations is limited, their genetic base can be broadened by introgression of (i) 'pure populations' conserved since the admixture, (ii) 'pure germplasm' from other mega-environments, and (iii) races of maize stored in the gene bank.

Strategy to optimize the exploitation of CIMMYT's maize genetic resources

A systematic exploitation of CIMMYT's maize genetic resources for breeding is lacking. A possible strategy could rest upon the natural relationships between the various races of maize. Goodman and Brown (1988) summarized the available information about the classification of races from the Western Hemisphere. This grouping of the races is only based on phenotypic and chromosome knob data. Further detailed investigations based on molecular markers are required to obtain precise information about the relationships among the different races and are currently undertaken in a joint project together with CIMMYT.

After having the races classified, they can be evaluated in field trials and first pre-breeding steps should be undertaken. One possible pre-breeding step consists in the decomposition of each race into a representative sample of haplotypes. The decomposition has two advantages: (i) the obtained genotypes are fixed and consequently phenotypic information can be collected for a specific genotype, which is for example important for association mapping, and (ii) deleterious alleles are eliminated during the inbreeding process. The haplotypes can either be generated by selfing a sample of selected genotypes or by using the newly emerging technique of producing doubled haploids in maize. This method has the advantage of a short time required to obtain fully inbred lines. As a second pre-breeding step, any infra-population selection method could be applied to enhance the agronomic performance of the haplotypes.

Based on the currently available data, it can be recommended to establish two broad-based breeding pools: (i) a dent and (ii) a flint composite. Each of these composites can be further divided into subpopulations for each mega-environment. For the short range, it might be best to start, with the more narrowly based 'pure' elite populations to establish the two breeding pools. Once the pools are established, any method of reciprocal recurrent selection could be applied to enhance their combining ability. The genetic base of the breeding pools can subsequently be increased by introgression of haplotypes of the various races.

Where open-pollinated varieties are desired, superior inbred lines from both breeding pools could be combined in a synthetic and further improved through infra-population improvement in the final target environment. The combination of the flint and dent germplasm pools into a single population should provide the best base to obtain a synthetic with high yield performance. However, hybrids present the most efficient way to exploit the strong natural heterotic pattern existing between the dent and flint complexes. Thus, if feasible and practical, hybrids should be generated by crosses between opposite heterotic breeding pools. The suggested strategy would guarantee (i) an optimal use of the promising dent x flint heterotic pattern for marginal environments via improved synthetics as well as for high input environments via hybrids and (ii) a systematic exploitation of the available genetic diversity in maize.

## Conclusions

Maize and wheat research is an important path to development, because maize and wheat are pivotal to nutrition, health, income, and environmental sustainability in low-income countries. In our dissertations, we led the foundation of the reorientation of CIMMYT's breeding strategies in maize and wheat. The suggested approaches included classical and novel molecular breeding strategies ensuring a sustainable improvement of both crops. Consequently, both dissertation significantly contribute to improve the food situation in developing countries.

Susanne Dreisigacker: Genetic Diversity in Elite Lines and Landraces of CIMMYT Spring Bread Wheat and Hybrid Performance in Crosses Among Elite Germplasm, Hohenheim, 2004

Jochen Reif: Assessing the Genetic Diversity in Crops with Molecular Markers: Theory and Experimental Results with CIMMYT Wheat and Maize Elite Germplasm and Genetic Resources, Hohenheim, 2004