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OPTIMIZING SELECTION EFFICIENCY IN MAIZE FOR THE DROUGHT PRONE EASTERN AND SOUTHERN AFRICAN ENVIRONMENTS

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1. General Introduction

Drought is one of the major constraints in maize production in Eastern and Southern Africa (ESA), where about 34 % of the maize is produced in areas that are characterized by recurrent occurrence of erratic rainfall and high temperatures (M'mboyi et al., 2010). Since rainfall is the only source of water supply in maize production, its fluctuation can highly affect crop growth and result in yield reduction. Annual yield loss is estimated to be 24 million tons, which is about 17 % of annual production in the tropics (Bänziger et al., 2000). Climate change and global warming are increasing the concern through raising the frequency of elevated temperature and unpredictable rainfall (Lobell et al., 2011). Thus, the need for development of drought tolerant maize genotypes becomes the highest priority in tropical maize breeding programs of the ESA and optimizing the breeding method for drought tolerance can increase efficiency in achieving that goal.

Maize in East and South Africa

Maize is an important crop in the ESA, where it is the main food source for more than 300 million Africans though its importance varies in different regions (M'mboyi et al., 2010). Maize is cultivated primarily for grain production and used as food and to some extent for making local beverages. The vegetative parts have lower values than the grain but can be used for animal feed, or burned as fuel and in some cases for mulching and manure. Area covered by maize production is over 25 million hectares of land, where the majority of the production comes from small scale farmers (Shiferaw et al., 2011). These farms are less mechanized and economically limited to use advanced agronomic practices, chemical fertilizers or pesticides than most large scale farms of the industrialized world. Further, expansion of agricultural land to marginal areas due to population growth and increase for maize demand made maize production vulnerable to water and heat stress, and poor soil conditions. Hence, average annual production of the region is 2.2 tons per hectare, which is very low when compared with 5.2 tons per hectare of the world's annual average production (FAOSTAT, 2010). This shows the need for development of stress tolerant genotypes and the improvement of selection methods in maize breeding.

Double haploids in tropical maize

CIMMYT (Spanish acronym for International Maize and Wheat Improvement Center) recently implemented the in vivo double haploid breeding technology from the University of Hohenheim for rapid and large scale production of homozygous lines in the tropics. Inbreds are homozygous lines developed through the continuous selfing of maize genotypes, usually for six generations. A new method of homozygous line production known as in vivo double haploid breeding technique has been developed to reduce the time required to obtain a new maize line (Röber et al., 2005). In addition to reduction of time for line development, i. e., two generations, this new method of breeding enables the development of totally homozygous lines, provide maximum genetic variance among lines, which is preferable for low heritable traits, and decrease the overall cost of line development.

The in vivo double haploid breeding technique involves the use of a special type of maize genotypes called inducers to pollinate source germplasm of interest and produce maternal haploid seeds. The haploid seeds from the harvested ears of the source germplasm are identified using a purple color embryo marker. Subsequently, the haploid seeds are treated with chromosome doubling agent to get double haploid (DH) plants and then selfed for the production of homozygous lines, commonly known as DH lines (see Geiger, 2009 for a review).

DH line production and their utilization in hybrid maize breeding has been commercialized in the maize breeding programs of the temperate zone while in the tropics it is in its initial stages. Studies on the enhancement of haploid induction rate and agronomic performance of inducers are extensive though both the inducer and the source germplasm are considered to be responsible for affecting haploid induction rate (Lashermes and Beckert, 1988; Sarkar et al., 1994; Shatskaya et al., 1994; Chalyk, 1999; Röber et al., 2005). Therefore, assessing the effect of source germplasm on haploid induction rate (HIR) is important to implement the technology in the tropical environments.

Environmental conditions are also considered as one factor that affects HIR in maize. Röber et al. (2005) found high variation in HIR when making induction in harsh (HIR = 2 %) and favorable (HIR = 16 %) environments in temperate maize germplasm. Geiger (2009) mentioned also in his review, the criticalness of favorable environmental conditions, such as keeping the growing conditions free of biotic and abiotic stresses to increase the success of induction.

CIMMYT introduced the DH breeding technology to the tropical environment through the Drought Tolerance Maize for Africa (DTMA) project to rapidly develop drought tolerant maize lines and hybrids. Studies concerning the HIR of tropical germplasm are limited. Further, the facilities of the DH breeding were established in a breeding station with two planting seasons, winter and summer. Identifying the optimum season for induction could maximize HIR. To my knowledge, no published reports are available on comparing source germplasm for their HIR and the seasonal variation in HIR in tropical germplasm.

Drought tolerance breeding for ESA

Genetic improvement of maize can play a crucial role in boosting productivity in those areas that are highly affected by recurrent drought occurrence. To alleviate yield reduction caused by drought stress, CIMMYT conducted trials under managed drought stress conditions and contributed greatly in the development of drought tolerant materials for the drought prone ESA region (Bänziger et al., 2006; Bolaños and Edmeades, 1993a, b). Altogether, the use of managed drought experiments succeeded in the improvement of maize for drought prone environments with a selection gain of 144 kg ha^{-1} $year^{-1}$ (Edmeades et al., 1999).

La posta Sequia (LP) and Tuxpeno Sequia (TS) were the first two populations developed for drought prone tropical environments. These two populations served as source populations for the extraction of many of the currently used drought tolerant inbreds. Betrán et al., (2003) showed the type of gene action involved in drought tolerance with La posta sequia and Tuxpeno sequia population derived lines using a diallel analysis. Evidently, additive gene action was more important than dominance under severe stress conditions. Therefore, parental inbreds should have some level of drought tolerance for making drought tolerant hybrids.

Screening inbreds for their testcross performance (TP) under managed drought trials has been the method of developing drought tolerant inbreds at CIMMYT (Bolaños and Edmeades, 1993a). Line *per se* performance (LP) trials can give economic advantage for drought tolerance screening through decreasing cost of testcross production if LP can predict TP under drought. Previously, LP trials were conducted for highly heritable traits like flowering date, disease resistance or plant height (Mihaljevic et al., 2005). Yield potential, on the other hand, needs TP trials (Hallauer and Miranda, 1981). In optimum environmental conditions LP and TP have weak genotypic correlations whereas under stress conditions, higher genotypic correlations had been observed in low soil nitrogen stress (Presterl et al., 2002) and high plant density stress (El-Lakany and Russell, 1971) trials. Obviously, ranking of genotypes for performance under stress may differ from that based on performance or yield potential under optimum conditions. Thus, high genotypic correlation between LP and TP under drought stress could be a good indication to predict TP from LP trials.

The indirect selection efficiency (ISE) of LP trials, in comparison with direct selection in TP trials, is determined by the genotypic correlation between LP and TP trials and the heritabilities of the trait in the two environments used for comparison (Hallauer et al., 2010). ISE determines the predictive power of LP trials for TP trials and can be obtained from the ratio of the correlated response (CR) and direct response (R) to selection, *i. e.*, CR/R. Under the same selection intensity, ISE can be calculated as the genotypic correlation (r_g) multiplied with the square root of the ratio of heritabilities of the selection trait under LP (H_{LP}) trials and TP (H_{TP}) trials, [ISE = $r_g \times (H_{\mathrm{LP}}/H_{\mathrm{TP}})$]. Indirect selection is considered effective over direct selection when the value of ISE is near one; smaller values indicate that direct selection is more effective than indirect selection. In general, estimating ISE can be useful when (i) the trait under consideration has lower heritability in the direct selection environment than the indirect selection environment, or (ii) the indirect selection environment is more cost effective than the direct selection environment (Hallauer et al., 2010; Presterl et al., 2002).

Aspects of breeding for different regions: Mexico versus ESA

CIMMYT has been improving maize for the ESA and Latin American regions for the past four decades using its experimental stations in Mexico (Bolaños and Edmeades, 1993a, b). Though the drought tolerant germplasm developed in Mexico were proven to be useful in ESA (Bänziger et al., 2006), CIMMYT transferred its breeding program to Kenya and Zimbabwe in the early 1990’s to best serve the ESA region and improve its breeding efficiency (Hassan et al., 2001). The breeding programs in ESA are now well established and develop germplasm that might be useful for the Latin American region. Consequently, the exchange of germplasm between ESA and Mexico can allow breeders to take advantage of breeding materials developed in one region for use in the other.

To exchange germplasm efficiently, one has to assess whether the response to selection in one region is similar to the response in the other region. The selection response is highly dependent on the genotypic correlation between the two regions and their corresponding heritabilities, provided the same selection intensity is applied in both regions (Falconer and Mackay, 1996). Germplasm exchange is favored when there is high genotypic correlation between the two regions and heritability is higher for the selection region as compared to the target region, where the test results are going to be used. Another aspect to be considered before exchanging germplasm is regional adaptation. The presence or absence of regional adaptation can be assessed by partitioning the genotype × environment interaction variance into genotype × region interaction and genotype × environment within region interaction variance (Windhausen et al., 2012). Low estimate of genotype × region interaction facilitates germplasm exchange because performance in ESA will be similar with performance in Mexico and vice versa. To my knowledge, no published studies are available comparing the performance of germplasm in ESA and Mexico. Thus investigating these issues could benefit the breeding programs in the different regions and altogether improve the breeding efficiency of CIMMYT maize breeding program.

Objectives

The aim of my thesis research was to investigate different approaches for improving the breeding for drought tolerance in CIMMYT by (i) assessing the applicability of in vivo double haploid breeding technique with tropical germplasm, (ii) estimating the correlation between line *per se* and testcross performance for potential use of line *per se* trials in drought tolerance screening; and (iii) comparing the estimated selection responses of the principal breeding stations of CIMMYT in ESA and Mexico, for guiding breeding material exchange. The specific objectives of the study were to:

1. monitor the variation for HIR among diverse source germplasm in tropical maize,
2. determine the relative importance of general (GCA) and specific (SCA) combining abilities of the source germplasm for HIR,
3. investigate the influence of tropical summer and winter seasons and genotype × season interactions on this trait,
4. determine if LP is predictive of TP for yield under drought in sets of lines under development by the CIMMYT maize breeding program,

5. determine the genetic correlation between performance of lines per se under drought and testcrosses under optimal conditions for assessing its effect on yield potential,

6. examine the correlation between TP under well-watered and drought stress conditions to determine the efficiency of indirect selection under well-watered in comparison with drought stress trials,

7. determine the relative importance of regional adaptation of maize hybrids to Mexico and ESA by subdividing the genotype × environment interactions and determining genotypic correlations between both regions,

8. calculate the indirect selection efficiency for selecting materials based on test results from one region on the selection gain in the other region, and

9. identify the most suitable stage for exchanging breeding materials between ESA and Mexico.

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2. Effect of source germplasm and season on the in vivo haploid induction rate in tropical maize

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Abstract

For in vivo production of doubled haploid (DH) lines in maize, the rate of haploid induction is of crucial importance. Maternal haploid induction depends primarily on the inducer used as a pollinator. However, the source germplasm used as a maternal parent and the environmental conditions for induction may also influence haploid induction and these aspects have not been examined in tropical maize so far. The objectives of our study were to (i) monitor the variation for haploid induction rate (HIR) among diverse source germplasm in tropical maize, (ii) determine the relative importance of general (GCA) and specific (SCA) combining abilities for HIR, and (iii) investigate the influence of summer and winter seasons and genotype × season interactions on this trait. Ten inbreds were mated in a half diallel design. The resulting 45 F_1 single crosses were pollinated with the haploid inducer hybrid RWS × UH400 during the summer 2008 and winter 2009 seasons in a lowland tropical environment in Mexico. HIR of the single crosses averaged over seasons ranged from 2.90 to 9.66% with an overall mean of 6.74%. Mean HIR was significantly ($P < 0.01$) higher during the winter (7.37%) than summer season (6.11%). Significant ($P < 0.01$) variation was observed due to GCA effects of parental inbreds of single crosses but not for SCA, GCA × season and SCA × season interactions. Our study underpins that a higher HIR in tropical maize can be obtained by selecting appropriate source germplasm and undertaking pollination under favorable environmental conditions.

3. Relationship of Line per se and Testcross Performance for Grain Yield of Tropical Maize in Drought and Well-watered Trials

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Abstract

To optimize the efficiency of maize drought breeding, the ability to predict testcross performance (TP) under drought stress using line *per se* performance (LP) of the parental inbreds would be useful. We evaluated LP and TP of tropical inbreds in well-watered and drought environments in Kenya and Mexico. Our main objective was to determine if LP under drought stress was predictive of TP for grain yield under drought stress, and if selection for LP under drought stress would result in reduced yield potential for TP under well-watered conditions. Average yield reduction under drought stress was 77% for lines and 68% for testcrosses. Average genotypic correlations between lines and testcrosses under drought stress were positive and low (r_g = 0.48), but correlations increased with increasing levels of drought stress in both LP and TP trials. Averaged over all sets, indirect selection for LP was predicted to be only 57% as effective as direct selection for TP under drought stress, but was on average substantially higher in testcross sets where yield reduction due to drought was 70% or more. Thus, LP under drought stress could be used to develop hybrids for severely drought prone environments. Moreover, LP under drought stress was uncorrelated with TP for grain yield under well-watered conditions, showing that selection of lines *per se* for drought tolerance would likely not reduce yield potential of testcrosses.

4. Effectiveness of selection at CIMMYT's main maize breeding sites in Mexico for performance at sites in Africa and vice versa

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Abstract

The exchange of elite breeding materials across regions is an important way in which multinational maize breeding programs access new genetic variation, improve efficiency, and reduce costs. Our objectives were to examine whether CIMMYT's breeding programs for tropical and subtropical environments in Mexico and Eastern and Southern Africa (ESA) can effectively share materials. Sets of selected and unselected lines were evaluated for *per se* and testcross performance in multiple environments in Mexico and ESA for grain yield, days to anthesis, and plant height. Genotypic correlations between performance in Mexico and ESA as testcross and line *per se* were high ($\geq$ 0.72) for all experiments and indirect selection efficiency ranged from 67 to over 100% for all traits. Lines selected in ESA or Latin America performed equally well in each region, indicating selection was for broad rather than regional adaptation. Thus, breeding programs of CIMMYT in both Mexico and ESA can benefit tremendously by exchanging breeding materials and test results, and elite selections from each region should be fast-tracked for evaluation in the other.

5. General discussion

Breeding for drought tolerance can reduce yield losses from fluctuation of rainfall in ESA. CIMMYT conducts breeding for drought tolerance for the ESA regions since the 1970's with the main objective of developing maize genotypes that can perform well under drought stress and optimum conditions. To maximize gains from drought tolerance breeding, optimization of the breeding method can play a big role, both through fast delivery of drought tolerant materials to the farmers and reduction of breeding costs. We, therefore, aimed at improving drought tolerance breeding of CIMMYT in terms of (1) inducing haploids with tropical germplasm using the in vivo double haploid breeding technology, which is a pre-requisite for rapidly producing homozygous maize lines, (2) screening of genotypes for drought tolerance using line per se performance trials, and (3) determining indirect selection efficiencies of the principal breeding regions of CIMMYT in the tropics and subtropics for cost efficient exchange of breeding materials.

Haploid induction for DH line production of tropical germplasm

Hybrid maize breeding for drought tolerance in the tropics can be accelerated using large scale production of double haploid (DH) lines. DH lines can be produced using the in vivo double haploid breeding technique. The efficiency of producing DH lines at a large scale depends highly on the haploid induction rate (HIR) of the inducer as has been reported in previous studies on HIR (Lashermes and Beckert, 1988; Sarkar et al., 1994; Shatskaya et al., 1994; Chalyk, 1999; Röber et al., 2005). Inducers with HIR of 8-9 % made the DH breeding technique to be utilized for large scale DH line production (Röber et al., 2005). There are other important factors that were not assessed extensively but determine HIR.

Apart from the inducer, source germplasm and induction environment are the two important factors that can determine the success of implementing the DH technology in the tropical environments. Thus, we assessed a diverse source of tropical germplasm and their seasonal variation for HIR using a single inducer. We did not examine the interaction between source germplasm × inducer, since all inducers came from one origin Stock 6 (Coe, 1959); we assumed these interactions to be negligible.

The tropical source germplasms showed larger mean and range for HIR than previously reported by Lashermes and Beckert (1988) and Eder and Chalyk (2002), indicating better response of the tropical source germplasm for HIR and/or relatively higher HIR of the inducer used in our study (Röber et al., 2005). It is important to note that the comparison of the previous reports with our results may not give the exact picture due to the fact that source germplasm and inducers used were different in the different experiments. Hence, it should be treated with caution.

Evidently, source germplasm is important in the expression of HIR. This was underlined by the high genotypic variation and GCA effects, in our experiment (Kebede et al., 2011). Two inbreds, with comparatively higher HIR and high GCA, show potential for use in initial establishment of the DH breeding program in the tropics. These two inbreds were clustered very close to each other by the SNP based Roger's genetic distance (GD) though there was no significant association between GCA and GD. Nevertheless, source germplasm for establishing the DH technology should not be limited to these inbreds but more materials should be screened for high HIR and be used in the initial stages.

HIR can be enhanced in source germplasm because GCA was more important than SCA effects in our study and there was no significant interaction between combining ability and environment (Kebede et al., 2011). The implication of identifying source germplasm with high HIR would be to facilitate the initial production of DH lines in the tropics.

To achieve acceptable HIR, seasonal variation of HIR and MCR (Mis-Classification Rate) emphasized the importance of favorable induction environments. In our findings, the winter season in Mexico was more favorable than summer for induction of haploids because HIR was higher and MCR was lower for winter than summer (Kebede et al., 2011). Winter season was cooler than summer and had average temperature of 21.5 °C, which is similar with previously reported temperature for higher HIR (Röber et al., 2005). Chromosome elimination, which is the mechanism of haploid formation in maize, is affected by temperature in other crops as well, such as barley (Pickering, 1984; Pickering and Morgan, 1985). Optimum temperature during induction can result in high HIR though other environmental factors like keeping the maize field free of biotic and abiotic stresses are also considered to maximize HIR (Geiger, 2009).

The difficulty of identifying haploid seeds using the purple coloration of the seed was significantly variable for the different source germplasm tested in our study, suggesting the need for identifying a better haploid seed screening method. The R1-nj gene, which is controlling the purple seed color, resulted in high MCR in previous studies conducted on androgenetic haploid induction using female parent as inducer (Belicuas et al., 2007) as well as maternal haploid induction (Röber et al., 2005). The MCR was higher for certain source germplasm with high GCA for the trait, indicating the involvement of color inhibitory genes. Coe (1962) reported the C1-I gene to inhibit the expression of the purple seed color in maize. Further studies on the mechanism of inheritance of the inhibitory genes found in tropical germplasm and alternative haploid seed identification systems (purple root color) are currently being conducted at CIMMYT and the University of Hohenheim, respectively.

The overall result shows that the in vivo DH breeding technology can be applied in the tropics as effectively as in the temperate maize owing to the fact that tropical source germplasm gave acceptable HIR. Source germplasm with high HIR are expected to contribute more for the next generation and enhance the use of the DH breeding technology in the tropics. Desirable materials with lower HIR can be sustained by increasing the number of plants to be induced. Favorable induction environment, which is winter season in our findings, is important to maximize HIR. Haploid induction is the prerequisite for the development of DH lines in maize. The DH lines generated through this method can accelerate the breeding time required in developing drought tolerant materials. Hence, breeders can select drought tolerant lines with half the time required when compared with the conventional recurrent selfing method for generating inbred lines.

Use of line per se performance trials for drought tolerance breeding

Selection for drought tolerant materials has been conducted using testcross performance (TP) trials under managed drought stress and well-watered conditions. Selection was based on grain yield under drought, lower anthesis silking interval, higher number of ears per plant, and higher grain yield both under drought and well-watered conditions (Bolaños and Edmeades, 1993a, b; Bolaños et al., 1993). Lines per se performance (LP) evaluations are more promising for highly heritable traits like plant height, disease resistance, lodging resistance and moisture content. The applicability of LP trials for grain yield has been limited because there is low correlation between line performance per se and combining ability which in turn determines yield potential in hybrids (Hallauer et al., 2010). Previous studies, however, indicate that the correlation between LP and TP under drought stress is higher than under well watered conditions (Betrán et al., 1997). We therefore attempted to determine the correlations between LP and TP under drought stress and well-watered conditions, which enabled us to estimate to what extent LP can predict TP under drought and well-watered conditions.

Average genotypic correlation between LP and TP trials under well-watered conditions were very weak in our study (Kebede et al., 2013a), which is in agreement with previous findings (Mihaljevic et al., 2005; Eberhart et al., 1995; Hallauer et al., 2010). The reason for the low correlation could be the expression of heterosis in hybrids. Heterosis, which is caused by dominance and/or epistasis, is important in determining yield potential of an inbred line in hybrid combinations and can only be estimated using combining ability tests through evaluation in TP trials.

In contrast to well-watered trials, we found a moderate average genotypic correlation between LP and TP under drought stress that increases with an increase in drought severity (Kebede et al., 2013a). Betrán et al. (1997) also found higher correlation under severe drought stress conditions than under mild drought stress conditions. However, later studies by Betrán et al. (2003) indicated weaker relationship between LP and TP under drought stress though in this study, yield reduction was over 95%. Extreme severity can result in very low heritability estimates and, hence, low precision of the estimated genotypic means used for the correlation. Altogether, the result indicated that drought severity, which is expressed as yield reduction in drought stress trials as compared to well-watered trials, is an important factor in determining the strength of relationship between LP and TP.

Grain yield under other stresses like high density (El-Lakany and Russell, 1971) and low soil N (Presterl et al., 2002) have also been reported to result in stronger correlation between LP and TP trials. In contrast to these studies, Lafitte and Edmeades (1995) found for S2 lines only weak correlations between LP and TP in low soil N fertility trials, however, different levels of stress severity were not compared in their experiment. We reckon that, evaluation under different severity levels might have changed their conclusions because severity of stress appears to be an important factor affecting the strength of inbred-testcross correlations. The latter has been clearly demonstrated by Betrán et al. (1997) for drought tolerance. They found a very weak correlation between LP and TP under intermediate stress but high correlations under severe stress, and underpinned the importance of higher stress severity levels in generating higher correlations between LP and TP.

Fixing a certain threshold level would helpt to differentiate whether we are measuring yield potential or yield under drought. Blum (2006) suggested a severity level above 70% yield reduction for cereals like maize while Bänziger et al. (2000) suggested yield reduction ranging between 80-85%. In our experiment, the genotypic correlation between LP and TP reached its maximum when the severity level was above 70% suggesting that grain yield under drought has higher predictability in LP trials than yield potential (Kebede et al., 2013a). Further, the average indirect selection efficiency of LP in comparison with TP reached 100% when severity level was > 75%, which again shows the predictive power of LP for TP under drought conditions (Kebede et al., 2013a).

The potential utility of selection for LP of grain yield under stress in a hybrid development pipeline is supported by (i) somewhat higher heritability estimates for yield in drought stress for LP than for TP, indicating reliable detection of differences in drought tolerance among the inbreds in LP trials, (ii) high genotypic correlations between LP and TP under severe stress, and (iii) the fact that selection for drought tolerance in LP would not reduce yield potential. In most African countries, commercial companies and national research programs are interested in developing maize hybrids that perform well under both stress and non-stress conditions. Hence, the use of LP trials for identifying drought tolerant materials at an early testing stage can improve efficiency of drought tolerance breeding. However, because pre-selection for stress tolerance at the LP level would reduce the selection intensity that can be applied for yield potential, it appears that evaluation of LP under stress can be most useful when developing hybrids for the most stress-prone environments, i.e., when grain yield under stress is more important than yield potential in non-stress environments.

The current trend of inbred line development in maize is changing from recurrent selfing to double haploid line production through the in vivo double haploid (DH) breeding technology. The breeding program in CIMMYT can benefit from utilization of the DH breeding technology along with LP trials to evaluate and select drought tolerant genotypes. Drought is an important yield constraint in the tropics and subtropics of the Eastern and Southern African countries (ESA) and the use of LP trials with DH lines for drought tolerance breeding would boost CIMMYT's effort to rapidly and cost efficiently develop drought tolerant materials that can eventually be disseminated to the farmers.

Suitable stage for breeding material exchange in a multinational breeding program

CIMMYT conducts breeding trials for the tropical and subtropical regions in its experimental sites in Mexico for breeding efforts targeting the Latin American countries and its experimental sites in Kenya and Zimbabwe for the target environments in ESA. A multinational maize breeding program such as CIMMYT could highly benefit from breeding materials exchange between these two regions if selection in one region can result in gains in the other. We therefore examined testcross performance of unselected (Exp. 1) and preselected lines (Exp. 2) that originated from genetically different background and employed different testers and *line per se* performance of preselected lines (Exp. 3) for assessing regional adaptation and estimated the indirect selection efficiencies of one region for selection gains in the other, whereby the intention was to determine the ideal stage of breeding material exchange.

In order to use germplasm developed in one region in another without investing additional breeding efforts, there should be high correlation between the performances of genotypes in the two regions and there should also be a high correlation between the rankings of the genotypes. Our estimates of the genotypic correlation for the means and rankings between Mexico and ESA were high for grain yield, days to anthesis and plant height in both unselected (Exp. 1) and selected (Exp. 2 and 3) lines. This highly increases the indirect selection efficiency (ISE) of one region for selection gains in the other given the heritability of the indirect selection region is greater than the heritability of the direct selection region (Falconer and Mackay, 1996). In our experiment, heritability estimates for Mexico were consistently higher than ESA, which increased the ISE of Mexico over 100% so that it was always higher than for ESA with only one exception (Kebede et al., 2013b). Even if the ISE of ESA was lower than Mexico, it was still greater than 78%. This indicates that selection decisions for performance in ESA can be based on available test results from Mexico and vice versa. This was true for both line *per se* and testcross performance experiments.

The other important aspect to consider when thinking of breeding material exchange is the magnitude of regional adaptation of the genotypes. In order to quantify the regional adaptation we partitioned the genotype × environment interaction variance across all regions into genotype × region (σ^2_{gs}) and genotype × environment within region ($\sigma^2_{ge(s)}$) interaction variances. We observed a 5% σ^2_{gs} as compared to $\sigma^2_{ge(s)}$ for grain yield in both Exp.1 and Exp. 2 and 0% σ^2_{gs} for days to anthesis and plant height only in Exp. 2. However, the estimate of σ^2_{gs} for days to anthesis and plant height in Exp. 1, which was substantial, might be due to the development of the unselected lines only in Mexico. There is a latitude difference between Mexico and ESA, which could have been the reason for the difference. These situations were different for the preselected lines in Exp. 2, which were selected based on their performance both in Mexico and ESA and, hence, have broader adaptability with a similar response in both regions. Although σ^2_{gs} for lines *per se* was higher than for testcrosses, it was still 15% or less as a proportion of total genotype × environment interaction variance, indicative of limited specific adaptation to regions. The overall result indicated that regional adaptation for both unselected and preselected lines was negligible to affect breeding material exchange at an advanced stage of breeding.

High genotypic correlation and ISE between regions and negligible regional adaptation would be advantageous if we want to maximize the selection gain for broadly adapted genotypes (Atlin et al., 2000, Windhausen et al., 2012). Furthermore, it would be advantageous if we want to exchange advanced stage breeding materials because it would reduce costs that would otherwise be required to test materials in each one of the regions. It is important to note that adaptive diseases in Mexico and ESA differ. Marker assisted selection or genomic selection with high density SNP assays could play a significant role to facilitate the introgression of disease resistance in selected lines and facilitate the exchange of advanced breeding material between Mexico and ESA.

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6. Summary

Breeding for stress tolerance is the most cost effective way of avoiding drought-induced yield reduction in the tropics. Optimizing breeding for drought tolerance at CIMMYT could enhance the effectiveness of this multi-national breeding program and warrant fast delivery of drought tolerant materials to the farmers. Thus, the overall aim of my study was to improve the efficiency of drought tolerance breeding of maize at CIMMYT for the rapid and cost effective advancement of drought tolerant materials for the drought prone regions of the ESA (Eastern and Southern African countries).

We screened a diverse source of tropical germplasm for their haploid induction rate (HIR) and the seasonal variation of this trait. We then compared various managed drought and well watered experiments conducted as line *per se* performance trials (LP) and testcross performance trials (TP) in Kenya and Mexico. Further, we estimated the relative selection efficiency of the principal breeding regions of CIMMYT for the tropics in ESA and Mexico with unselected and selected breeding materials. The specific objectives of my study were to (1) monitor the variation for HIR among diverse source germplasm in tropical maize, (2) determine the relative importance of general (GCA) and specific (SCA) combining abilities of the source germplasm for HIR, (3) investigate the influence of tropical summer and winter seasons and genotype × season interactions on this trait, (4) determine if LP is predictive of TP for yield under drought in sets of lines under development by the CIMMYT maize breeding program in Kenya and Mexico, (5) determine the genetic correlation between performance of lines *per se* under drought and testcrosses under optimal conditions and assessing its effect on yield potential, (6) examine the correlation between TP under well-watered and drought stress conditions for potential indirect selection efficiency of well-watered conditions in comparison with drought stress, (7) determine the relative importance of regional adaptation of maize hybrids to Mexico and ESA by subdividing the genotype × environment interactions and determining genotypic correlations between both regions, (8) calculate the indirect selection efficiency for selecting materials based on test results from one region on the selection gain in the other region, and (9) identify the most suitable stage for exchanging breeding materials between Mexico and ESA.

Source germplasm and induction season affected HIR and MCR (mis-classification rate) considerably in tropical maize. Source germplam with high HIR and low MCR could be used in the initial stage of implementing the DH technology in the tropics. GCA effect was more important than SCA or genotype × season interaction effects for HIR in tropical maize. Thus, enhancing HIR in source germplasm can be achieved through cyclical breeding or recurrent selection. Winter season was considered the best season for induction because it provides suitable environmental conditions for higher HIR and lower MCR. Overall HIR was high enough to apply the *in vivo* DH technology in the routine breeding activities in tropical maize.

There were moderate genotypic correlation and ISE (Indirect Selection Efficiency) values between LP and TP under drought that increased with an increase in stress level. Hence, LP trials were predictive of TP trials particularly under severe drought stress. Furthermore, screening of lines for LP under drought stress did not compromise yield potential. TP under well-watered conditions were not predictive of TP under drought stress emphasizing the need of managed drought trials to identify drought tolerant materials. With the current shift of inbred development to large scale DH line production, LP evaluations can reduce the cost of making large numbers of testcrosses and optimize breeding for drought tolerant hybrids in the tropics.

The exchange of breeding materials between ESA and Mexico can be done with early and late generation materials. This is because there was negligible genotype by region interactions as compared to genotype by location interactions within each region and high genotypic correlations between the two regions. Further, ISE estimates for trials conducted in Mexico and in ESA were high. Adaptive diseases for each location might hamper the exchange of materials, however, with current molecular marker tools like marker assisted selection and genomic selection, the problem of selecting for disease resistance in the region where the disease is not prevalent seems promising.

In conclusion, there are ample opportunities in the CIMMYT maize breeding program to optimize breeding for drought tolerance in the tropics through rapid and large scale production of DH lines and evaluation of these lines for LP in managed drought trials. Moreover, breeders from ESA and Mexico could benefit from each other’s materials and test results by regular exchange of breeding materials at both the early and late stages of testing.

7. Zusammenfassung

Die Züchtung auf Stresstoleranz ist der kosteneffektivste Weg, um Trockenheitsstress bedingte Ertragsverluste bei landwirtschaftlichen Kulturpflanzen zu minimieren. Eine Optimierung der Züchtungsstrategien am CIMMYT bezüglich Trockenstresstoleranz von Mais könnte die Effektivität dieses multi-nationalen Züchtungsprogramms enorm verbessern und eine schnellere Bereitstellung von trockenstress-tolerantem Material an die Farmer in den Zielregionen garantieren. Aus diesem Grund war das übergeordnete Ziel dieser Forschungsarbeiten die Verbesserung der Effizienz der Züchtung von Mais auf Trockentoleranz am CIMMYT, um schneller und kostengünstiger Fortschritte bei der Entwicklung von trockenstresstolerantem Material zu erzielen, insbesondere für die durch Trockenheit stark gefährdeten Regionen im östlichen und südlichen Afrika (ESA, Eastern and Southern Africa).

Mittels der Technologie zur Erzeugung von Doppelhaploiden(DH)-Linien wurde ein breites Spektrum von tropischem Material bezüglich seiner Haploiden-Induktionsrate (HIR) und der saisonalen Variation in diesem Merkmal untersucht. Weiterhin wurden vergleichende Experimente unter kontrollierter Trockenheit als auch unter optimaler Wasserversorgung durchgeführt, bei denen sowohl die Linieneigenleistung (LP = line *per se* performance) als auch Testkreuzungsleistung (TP = testcross performance) in Kenia und Mexiko erfasst wurde. Zusätzlich wurde die relative indirekte Selektionseffizienz (ISE) für die beiden tropischen Züchtungsregionen des CIMMYT, nämlich ESA und Mexiko, an unselektiertem und vorselektiertem Material untersucht.

Die spezifischen Ziele der Untersuchungen waren:

(1) die Erfassung der Variation für HIR von tropischem Mais verschiedenen Ursprungs;

(2) die Bestimmung der relativen Bedeutung von allgemeiner (GCA) und spezifischer (SCA) Kombinationseignung für HIR in einem di-allelen Kreuzungsschema mit verschiedenen tropischen Inzuchtlinien;

(3) die Untersuchung des saisonalen Einflusses und der Genotyp x Saison-Interaktionen bei diesem Merkmal;

(4) die Ermittlung der Vorhersageeignung von LP für den TP-Ertrag unter Trockenstress für Gruppen von Linien des CIMMYT-Maiszüchtungsprogramms in Kenia und Mexiko;

(5) die Schätzung der genotypischen Korrelation zwischen LP unter Trockenstress und TP

unter optimalen Bedingungen, bei Berücksichtigung der Auswirkungen auf das Ertragspotenzial;

(6) die Untersuchung der Korrelation zwischen TP unter optimaler Wasserversorgung vs. Trockenstress Bedingungen zur Ermittlung der indirekten Selektionseffizienz unter diesen Testbedingungen;

(7) die Ermittlung der relativen Vorzüglichkeit regionaler Adaptation von Maishybriden für Mexiko und ESA durch Aufteilung der Genotyp x Umwelt-Interaktionen und Schätzung der genotypischen Korrelation zwischen diesen beiden Regionen;

(8) die Schätzung der indirekten Selektionseffizienz in selektiertem Material anhand der Ergebnisse einer Region und dessen Auswirkung auf den Selektionserfolg in der anderen Region; und

(9) die Identifizierung geeigneter Stadien für den Austausch von Zuchtmaterial zwischen Mexiko und ESA.

Das Ausgangsmaterial und die Anbausaison beeinflussen HIR und MCR (Mis-Classification Rate) bei tropischem Mais. Ausgangsmaterial mit hoher HIR bei gleichzeitig niedriger MCR könnte als Ausgangsbasis für die Etablierung der DH Technologie in den Tropen dienen. Die GCA war wichtiger als die SCA oder die Genotyp x Saison Interaktionen für HIR. Aus diesem Grund kann ist die Verbesserung der HIR im Ausgangsmaterial durch rekurrente Selektion sehr aussichtsreich. Die Wintersaison war im Vergleich zur Sommersaison besser geeignet für die Haploiden-Induktion, da hier optimalere Umweltbedingungen für eine höhere HIR bei gleichzeitig geringer MCR vorherrschten. Zusammenfassend kann festgestellt werden, dass die HIR ausreichend hoch ist, um die *in-vivo* DH-Technologie in den üblichen Züchtungsablauf bei tropischem Mais zu integrieren.

Es wurden mittlere genotypische Korrelationen und ISE-Werte zwischen LP und TP unter Trockenstress gefunden, die sich bei einem Anstieg des Stressniveaus erhöhten. Dies bedeutet, dass LP-Ergebnisse einen hohen Vorhersagewert für TP haben, insbesondere bei extremem Trockenstress. Weiterhin konnte festgestellt werden, dass die Prüfung von Linien auf Eigenleistung unter Trockenstress deren Ertragspotenzial nicht maskierte. Demgegenüber war die TP unter optimalen Bedingungen nicht aussagekräftig für TP unter Stress. Dies unterstreicht die Notwendigkeit kontrollierter Trockenstress-Experimente, um trockenstress-tolerantes Material zu identifizieren. Mit dem Wechsel von der herkömmlichen Linienentwicklung zur serienmäßigen Produktion von DH-Linien ermöglicht die LP

Evaluierung eine Reduzierung der Kosten für die Herstellung einer großen Anzahl von Testkreuzungen; insgesamt ermöglicht dies eine deutliche Verbesserung der Züchtung trockenstress-toleranter Hybriden für die Tropen.

Der Austausch von Zuchtmaterial zwischen ESA und Mexiko kann sowohl in frühen als auch in späten Phasen der Materialentwicklung erfolgen. Dies ist möglich, weil die Genotyp x Region Interaktionen im Vergleich zur Genotyp x Orts-Interaktionen innerhalb der Regionen vernachlässigbar sind, bei gleichzeitig hoher genotypischen Korrelation zwischen den beiden Regionen. Zusätzlich waren auch die ISE-Werte für die Experimente sowohl in Mexiko als auch in ESA hinreichend hoch. Unterschiedliche Pflanzenkrankheiten in den beiden Zielregionen könnten allerdings einen Materialaustausch in Frage stellen. Mit den heute verfügbaren Techniken der modernen Pflanzenzüchtung, wie beispielsweise Marker-gestützte Selektion oder genomische Selektion, bestehen jedoch gute Aussichten, die in der jeweiligen Zielregion benötigten Resistenzgene in kurzer Zeit in das Zuchtmaterial einzukreuzen.

Als Schlussfolgerung ergibt sich, dass es vielfältige Möglichkeiten gibt, das Maiszüchtungsprogramm des CIMMYT zur Verbesserung der Trockenheitstoleranz in den Tropen zu optimieren. Insbesondere die Etablierung der DH-Technologie und die Prüfung der DH-Linien auf Eigenleistung unter kontrolliertem Trockenstress bieten vielversprechende Ansatzpunkte. Darüber hinaus könnten die Züchter in ESA und Mexiko von einem gegenseitigen Austausch von Zuchtmaterial und Ergebnissen sowohl in frühen aber auch späteren Stadien der Materialentwicklung enorm profitieren.

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9. Curriculum Vitae

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