

Research Application Summary

**Genetic inheritance studies and early generation marker selection in maize breeding for drought tolerance**

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**Abstract**

It has been estimated that approximately US \$10 billion of primary food production is lost annually because of drought. Interventions like irrigation are options the majority of the smallholder farmers in sub Saharan Africa cannot afford. However, cultivar development through breeding of drought tolerant cultivars can be exploited to provide improved varieties for the resource constrained farmers. This study started with a diagnostic survey on the availability and use of drought tolerant cultivars, and assessed farmers' perceptions for input to development of drought tolerant varieties for drought prone areas in Zimbabwe and other parts of Southern Africa. Currently genetic studies on the drought tolerant inbred lines (variances components and heritability) are on-going while the last component of the study is looking into the possibility of improving efficiency and effectiveness in breeding for drought tolerance through the use of marker assisted recurrent selection (MARS) of secondary traits such as anthesis-silking interval (ASI), ears per plant (EPP), biomass, leaf rolling and leaf senescence using single nucleotide polymorphism (SNP) markers. Preliminary results show significant differences ( $P < 0.001$ ) for grain yield, anthesis-silking interval and ears per plant which are critical secondary traits used for selection under drought stress.

Key words: Drought tolerance, inbred lines, marker-assisted, recurrent selection, *Zea mays*

**Résumé**

Il a été estimé qu'approximativement 10 milliards de dollars américains alloués à la production des denrées alimentaires de première nécessité sont perdus annuellement suite à la sécheresse. Les interventions comme l'irrigation sont des options que la majorité des fermiers de petites exploitations en Afrique sub-saharienne ne peuvent pas se permettre de pratiquer. Cependant, le développement de cultivars par la sélection de cultivars résistants à la sécheresse pourrait être exploité pour fournir des variétés améliorées pour les agriculteurs aux ressources limitées. Cette étude a commencé par une analyse

diagnostique sur la disponibilité et l'utilisation des cultivars résistants à la sécheresse et a évalué les perceptions des agriculteurs pour contribuer à l'élaboration des variétés supportant la sécheresse pour des zones sujettes à la sécheresse au Zimbabwe et dans d'autres régions de l'Afrique australe. Actuellement les études génétiques sur les lignées tolérant la sécheresse (composants variances et héritabilité) sont en cours tandis que le dernier volet de l'étude se penche sur la possibilité d'améliorer l'efficacité et l'efficience dans la sélection liée à la tolérance face à la sécheresse grâce à la sélection récurrente assistée par l'utilisation du marqueur (MARS) des traits secondaires tels que l'intervalle anthèse-floraison (ASI), les épis par plante (EPP), la biomasse, les feuilles de laminage et la sénescence de feuille utilisant les marqueurs simples du polymorphisme de nucléotide (SNP). Les résultats préliminaires montrent des différences significatives ( $P < 0.001$ ) pour le rendement en grains, l'intervalle anthèse-floraison et les épis par plante qui sont des traits secondaires essentiels employés pour la sélection en vertu de la sécheresse.

Mots clés: Tolérance de sécheresse, souches pures, assisté par marqueur, sélection récurrente, *Zea mays*

## Background

In Eastern and Southern Africa drought stress incidences have been on the increase, due to global climate changes, the displacement of maize to more difficult production environments by high value crops and partly due to declines in soil organic matter and water holding capacity (Banziger *et al.*, 2002). As a result a single variety must be able to withstand a wide range of drought stress and limited nitrogen availability. A total area of 2 690 000ha in this region is drought prone and is planted to maize with the bulk of the area being in the mid altitude zones of Kenya and Zimbabwe, (CIMMYT-Zimbabwe 2000 Research Highlights). In an effort to get the most ideal drought tolerant genotypes for the farmers, research has now shifted focus from conventional selection methods to genetic analysis of drought components and use of biotechnology tools such as marker assisted selection. This particular study is examining the genetic basis of components that influence grain yield under drought stress and use of marker assisted recurrent selection (MARS) and genome wide selection using single nucleotide polymorphism (SNPs) as an option to improving selection and breeding for drought tolerance in maize.

## Literature Summary

In maize, flowering is the most crucial stage in terms of negative effects of drought on yield. During this stage, one single day of drought can potentially decrease yield by up to 8% (Shaw, 1977, Edmeades, 2008). Therefore, due to the high frequency of drought episodes at flowering, reproductive failure is the most important drought-related factor contributing to yield losses in maize. In this study random drought and managed drought stress evaluations will be done. Managed drought stress will be done off season and stress will be induced by withdrawing water for 2-4 weeks that bracket the flowering period. This will affect the flowering of the plants with the focus being the anthesis-silking interval (ASI) which is the derivative of the difference between the female and male florescence emergence. This will help in the study of the effects of synchronisation of flowering for effective pollination and consequently fertilisation in the process of grain yield formation of the maize and level of bareness to be calculated as ears per plant.

Marker-assisted recurrent selection (MARS) is the process of improving an  $F_2$  population by one cycle of marker-assisted selection (i.e., based on phenotypic data and marker scores) followed by three cycles of marker based selection (i.e., based on marker scores only) in an off-season nursery (Johnson, 2004). The marker scores are typically determined from about 20 to 35 SNP markers that have been identified, in a multiple-regression model, as significantly associated with one or more traits of interest. MARS for polygenic traits is mainly restricted to situations where phenotypic selection cannot be easily implemented such as for traits that are difficult to phenotype on individual plants or in off season nurseries such as drought tolerance. Once markers have been associated with target traits in a population, they can be used at any point in later generations, even when phenotypic selection is not effective. In addition the effectiveness of MARS relative to other approaches increases as the number of genes affecting the trait increases, and as heritability decreases which is typical of most traits measured under stress conditions. This is because MARS rapidly accumulates favourable molecular marker alleles linked to the desired QTLs in the breeding population.

## Study Description

The study is being conducted in two parts namely field evaluation of the testcrosses for combining ability or genetic studies and genotyping of the within heterotic group testcrosses that had been advanced to  $F_3$  families using the SNPs. In the SNP genotyping study nine maize populations derived from within

heterotic group crosses between CIMMYT Zimbabwe (African adapted) and CIMMYT Mexico 250 F<sub>3</sub> families per population are currently being genotyped with the 1536 SNP chips for the QTL mapping phase. It is planned to identify the best 20-50 markers associated with drought for use in markers-only selection cycles in 2 cycles. This is an on going study but the information from the latter will help in selecting the best families that should be recombined or selfed to produce the next generation.

### **Research Application**

In the genetic studies a total of 104 hybrids were developed from the parents used to constitute the nine populations in the genotyping study. The resultant testcrosses were evaluated under one low nitrogen (low N) stress (Harare: 1500masl; 31°E & 17.43°S), two optimum environments (ART: 1480masl; 31.5° E & 17.43°S and RARS) and two random drought stress environment (Kadoma 1155masl; 30.9°E & 18.32°S) and (Chiredzi: 429masl; 32. 14° E and 20.48° S). The testcross evaluation was done using the unbalanced block alpha 0.1 lattice design, one row plot, 4m long, with two replications and a spacing of 75cm x 25cm (53 000 plants/ha). The low N site was depleted of N over the last 7 years to below 25% of optimum N but all other nutrients were added relative to soil analysis results. Preliminary data analysis has been done using Fieldbook ASREML spatial analysis but a further (line x tester) analysis will be done using SAS 2004 program.

### **Recommendation**

Individual site analyses were done for two optimum, two random drought stress sites and one low N stress site. An across site analysis for traits with significant differences was done (data not shown). However preliminary results from individual site analysis show that there were significant differences ( $P < 0.001$ ) for grain yield, anthesis-silking interval and ears per plant. These are the most important secondary traits that are used in assessing drought stress tolerance in maize.

Testcrosses such a MAS[206/312]-23-2-1-3/CKL05005 appear promising under random drought and optimum (data not shown) environments. It also has very good nicking properties and can be a candidate for use as a single cross tester. However this is an on going study hence there is still need to evaluate the testcrosses under managed drought stress environments before an across site analysis and a further line x tester analysis is done to capture the best performing genotypes across

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environments as well as separate the various genetic components to enable calculation of heritability estimates.

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