Project number		M191/13
Project title		Development of maize inbred lines and cultivars
Project manager		AP Fourie
Co-worker(s)	Internal	K Mashingaidze, CMS Mienie, TJ Malala, IP Mokowe
	External	Commercial Seed Industry, SANSOR
Project status		Complete
Duration		01/04/2009 to 31/03/2012

FINAL ABSTRACT

Maize breeding is a never-ending challenge of developing new inbred lines and cultivars with improved yield, as well as with tolerance to a diverse spectrum of biotic and abiotic stress conditions, e.g. diseases, pests, low soil fertility and drought. New cultivars must also conform to changing industry requirements such as grain quality for milling and ethanol production. Climatic changes such as global warming pose new challenges to breeders to develop cultivars that will perform well under changed conditions. The objective of the project was to develop elite inbred lines with good combining ability and provide them to the private seed sector for commercialisation. At the end of the project, ARC-GCI made 618 inbred lines available to recognized maize seed companies through SANSOR. The inbred lines were characterised using DNA marker technology, and genetic distance data are available on request. Acquisition of the inbreds can be negotiated with the ARC-GCI for a research fee acceptable to both ARC and the seed company.

Keywords

Breeding, inbred line, inbreds, combining ability, drought, biotic, abiotic, genetic distance, pedigree, hybrid, gene, allele, microsatellites, marker

INTRODUCTION

Maize breeding constitutes a never-ending challenge to develop new inbred lines and cultivars with improved yield, as well as tolerance to a diverse spectrum of biotic and abiotic stress conditions, e.g. diseases, pests, low soil fertility and drought. New cultivars must also conform to changing industry requirements such as grain quality for milling and ethanol production. Climate changes such as global warming pose new challenges to breeders with regard to developing cultivars that will perform well under changed conditions. Although seed companies produce their own hybrids they remain dependant on foundation-seed suppliers for inbred lines and even sometimes locally adapted hybrids. ARC-GCI is seen as a foundation seed provider and its inbred lines are sought after due to their combining ability for yield and tolerance to biotic and abiotic stresses. New inbred lines have been supplied on a regular basis to local commercial seed companies for commercialisation. Inbred lines have also been supplied to international stress breeding projects like Water Efficient Maize for Africa (WEMA) and Improved Maize for African soils (IMAS).

This project (M191/13) was a continuation of two classic hybrid-maize breeding (M191/10) and hybrid-yield testing (M161/10) projects that were started during the 1940's by the then Department of Agriculture. The aim was to develop maize inbred lines for incorporation into maize hybrid cultivars. This was done to replace the low-yielding, open-pollinated maize cultivars that were used in the early days of maize production in South Africa. From these two pioneer projects were developed the first inbred lines incorporated into maize hybrids in South Africa, ca. 1950 by the Department of Agriculture. Since the inception of this national programme it has over the years contributed significantly to the continuous supply of newly improved maize inbred lines for use in commercial hybrid seed production. A commercial maize seed industry has developed in South Africa that is unparalleled by any other country on the continent.

The fundamental discoveries of Darwin and Mendel established the scientific basis for plant breeding and genetics at the turn of the 20th century. Similarly, the recent integration of advances in biotechnology with conventional plant breeding practices has created the foundation for *molecular plant breeding*, an inter-disciplinary science that is revolutionising 21st century crop improvement (Moose and Mumm, 2008).

The genomic revolution provides the scientific community with tremendous opportunities for improving the pace and scale of plant breeding progress and thereby helping to solve some of the world's most serious agricultural and food security issues. For example, molecular

markers can be used for (1) determining genetic distances between inbred lines and constructing heterotic groups; (2) screening germplasm for novel genes or superior alleles; and (3) constructing representative subsets of core collections for specific traits. Reif *et al.* (2003) concluded that “SSR markers provide a powerful tool for grouping germplasm and are a valuable complementation to field trials for identifying groups with satisfactory heterotic response”. Knowledge of the genetic diversity of breeding lines gives plant breeders an indication of possible crosses to make to obtain high levels of heterosis in creating new hybrids. Several techniques are used worldwide, with microsatellites (SSR) and single nucleotide polymorphisms (SNP) as the most prominent marker types used in maize. Although SNP marker technology is available, it is still too expensive to be used routinely in South Africa to genotype maize germplasm.

A study on the genetic diversity of maize inbred lines has several benefits, including the establishment of heterotic groups, because combining ability for grain yield still remains the most important selection trait in a maize breeding programme. Inbred lines with superior combining ability for grain yield provide the vehicles to carry novel genes into marketable maize hybrids. Therefore, DNA fingerprinting provides an excellent tool for sorting inbred lines into more manageable heterotic patterns.

The objective of the project was to develop elite inbred lines with good combining ability and provide them to the private seed sector for commercialisation.

MATERIALS AND METHODS

The two ARC research stations, Potchefstroom and Cedara, have been involved in maize breeding for approximately 60 years. In the breeding process two broad genetic germplasm pools, characterised by different traits have been developed. The Potchefstroom germplasm pool is characterised by medium to early maturing genotypes with adaptation to low and erratic rainfall conditions. The Cedara germplasm is characterised by late maturing material with good resistance to various maize leaf diseases. These two germplasm pools can be regarded as trait-based, *core collections*. Also, they complement each other with regard to biotic and abiotic stress traits. The germplasm base was also expanded with acquisition of elite publicly available maize genetic resources from organisations like CIMMYT, to further expand the accumulation of genetic traits.

Standard pedigree breeding and combining ability testing procedures were used to develop elite inbred lines. Major selection pressure was placed on earliness, prolificacy and tolerance to heat scorching because of the drought-tolerant advantage demonstrated by these traits. Emphasis was placed on using and improving the I, F and M heterotic groups, which were developed by ARC-GCI and played a prominent role in South African commercial maize hybrids. New inbred lines were test-crossed with elite tester parent lines in isolated crossing blocks. The resultant test-cross hybrids were evaluated in yield trials with specific emphasis on drought tolerance. Yield results were used to determine possible commercial application of inbred lines.

Elite inbred lines were fingerprinted to protect intellectual property as well as to determine genetic distances. DNA was extracted from lyophilized leaf tissue of each inbred line. DNA was analysed with SSR markers using publicly available primers. Methods used in the molecular analyses of breeding lines and hybrids were adapted from recommendations by the Applied Molecular Genetics Laboratory, CIMMYT, Mexico. Samples were used to optimize the fingerprinting technique using fluorescently labelled microsatellites for automatic analysis with an ABI3130xl genetic analyser. Electrophoresis results were analysed with Genemapper 4.0 software (Applied Biosystems). Statistical analyses were carried out with Powermarker v3.25 (Liu and Muse, 2005) using the Rogers (1972) parameter to determine genetic distances. The SSR markers represented a relatively even coverage of the maize genomic map. A total of 85 markers mapped at an average density of 8.5 markers per chromosome.

RESULTS AND DISCUSSION

The I group (I137TN heterotic group) of maize germplasm has over the past 30 years played a significant role in South Africa in the development of hybrid cultivars by commercial companies. The original I137TN material was released by the Department of Agriculture to the commercial sector during the 1970's, but initially it lacked prolificacy and drought tolerance. The I Group was also susceptible to Diplodia ear rot. Significant progress was made on the improvement of prolificacy and drought tolerance traits. Increased tolerance to Diplodia ear rot was also achieved. The combination of I Group and US Corn-Belt inbreds has proved to be a winning recipe in South Africa over the past three decades.

The ARC-GCI biotechnology laboratory quantified the genetic distances among 848 maize inbred lines, originating from the Potchefstroom and Cedara breeding programs. The resulting diallel table contains 359 128 data points, which represent the genetic distances among the 848 inbred lines in all possible combinations, reciprocals excluded. The genetic distances are an excellent breeding tool for the planning and implementation of maize breeding strategies in the development and commercialisation of improved maize hybrids. The genetic distances between inbred lines enable maize breeders to sort their breeding material into clearly defined heterotic groups. A major advantage of using genetic distances to sort inbred lines into heterotic pools before test-crossing is that it pre-empts the inclusion of genetically related material in single crosses for yield evaluation in yield trials. The exclusion of crosses among related parents will lead to considerable cost saving in yield evaluation programs of experimental hybrids.

Data from 2009/10 yield trials were used to determine to what extent the expression of genetic distances among inbred lines are reflected in the actual yields of their hybrids. The genetic distances between inbreds were correlated with the actual grain yields of their single-cross hybrids. The results indicated that 71.9% of the variability in grain yield was explained by the genetic distance between the two parental inbred lines in a single-cross hybrid. This showed that genetic distances could be used to predict heterotic crosses.

In 2012, the ARC-GCI made 618 inbred lines available to recognized maize seed companies through SANSOR. The inbred lines have been characterised using DNA marker technology, and genetic distance data are available on request. Acquisition of the inbreds can be negotiated with the ARC-GCI for a research fee acceptable to both ARC and the seed company.

PUBLICATIONS

MIENIE, C.M.S., A.P. FOURIE & L.A. MADUBANYA, 2011. Diversity studies using Marker technology in maize breeding at ARC-GCI in South Africa. Poster presented at XXII Eucarpia Maize and Sorghum Conference, 20 - 23 June 2011, Opatija, Croatia.

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