# GENETIC ANALYSIS AND MOLECULAR MAPPING OF QUANTITATIVE TRAIT LOCI (QTL) FOR DROUGHT TOLERANCE IN MAIZE (*Zea mays* L.)

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# GENETIC ANALYSIS AND MOLECULAR MAPPING OF QUANTITATIVE TRAIT LOCI (QTL) FOR DROUGHT TOLERANCE IN MAIZE (*Zea mays* L.)

by

**Amir Hossein Beiki**

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# CERTIFICATE

This is to certify that the thesis entitled "***Genetic Analysis and Molecular Mapping of Quantitative Trait Loci (QTL) for Drought Tolerance in Maize (Zea mays* L.)**", submitted to the Faculty of Post Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfillment of the requirements for the award of the degree of **Doctor of Philosophy in Genetics** by **Mr. Amir Hossein Beiki** embodies the results of *bona fide* work carried out by him under my supervision and guidance. No part of the thesis has been submitted by him for any other degree or diploma.

I further certify that any help or information received during the work on this thesis has been duly acknowledged.

BMPrasanna

Place: New Delhi
Date: January 4, 2005

**(B. M. Prasanna)**
Chairman
Advisory Committee

*Dedicated to*
*My Parents,*
*My Wife Shayesteh,*
*&*
*My Children Neda & Zoha*

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**Amir Hossein Beiki**

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# Introduction

Drought and low soil fertility are among the major abiotic stresses affecting the production and productivity of crop plants in several countries worldwide. The destructive impact of drought may grow as the specter of climate change becomes a reality. Climate change may increase flooding in some regions while further intensifying the frequency and magnitude of droughts in central Asia, northern and southern Africa, the Middle East, the Mediterranean region, and Australia (Ribaut *et al.*, 2003). Furthermore, the impact of climate change on precipitation will pose a serious problem for water supplies in many areas of the world, a potential crisis that will be exacerbated by population growth and the continuing migration of rural populations to urban areas.

Annual loss due to drought across the tropical maize-growing environments was estimated to be about 19 million tons, representing a 17% loss in production (Edmeades *et al.*, 1992). As per a study by CIMMYT (1994), India registered the highest absolute drought-prone area (2.5 million hectares), followed by Indonesia (2.2 m ha) and south China (1.15 m ha) respectively. In India, nearly 80 per cent of maize is grown as a *kharif* (rainy season) crop (Singh *et al.*, 2004). During the *Kharif* season in tropical/sub-tropical environments, inadequate soil moisture during the growth period, particularly during the flowering and grain filling stages, is one of the major limiting factors for production and productivity. In the tropics, where over 90% of all maize is grown, irrigation is often unavailable or not within the financial capacity of the farmers. Drought tolerance of crop plants, therefore, assumes considerable importance in the Indian context, where nearly 80 per cent of the maize is grown under rainfed conditions. Therefore, genetic enhancement of drought stress tolerance of maize in India is considered as one of the most important priorities of the National Agricultural Research System (NARS).

During the past three decades, CIMMYT (International Maize and Wheat Improvement Center) scientists devoted considerable efforts in improving drought tolerance in maize for the period during and after flowering. Extensive research has been conducted in the areas of breeding, physiology, agronomy, and most recently, biotechnology. (Beck *et al.*, 1996; Edmeades *et al.*, 2000; Ribaut *et al.*, 2002; Banziger *et al.*, 2004). In maize, when drought stress occurs just before and during the flowering period, a delay in silking is observed resulting in an increase in the length of the anthesis-silking interval (ASI) (Hall *et al.*, 1982; Westgate and Bassetti, 1990; Edmeades *et al.*, 2000) This asynchrony between male and female flowering was found to be associated with reduced grain yield under drought stress (Hall *et al.*, 1981; Westgate and Boyer, 1986; Bolanos and Edmeades,1993; Edmeades *et al.*, 2000). Selection for grain yield under drought was often found inefficient because of the increase in environmental variance relative to genetic variance, which decreases the heritability of complex traits like grain yield. Under these conditions, selection for secondary traits, such as ASI, which are highly correlated to grain yield and have relatively high heritability, was demonstrated to increase selection efficiency (Bolanos *et al.*, 1993; Edmeades *et al.*, 2000).

The complex expression of drought tolerance makes this trait difficult to analyze using traditional genetic and physiological methods. Although major progress, to date, has been achieved through conventional breeding (Edmeades, 1999; Banziger *et al.*, 2004), this approach remains slow and time-consuming. Combining conventional and molecular approaches might increase significantly the potential for genetic gain under water-limited conditions.

During the past two decades, molecular tools have advanced the identification, mapping, and isolation of genes in a wide range of crop species. The first DNA markers, restriction fragment length polymorphisms (RFLPs), have been widely used to construct linkage maps for several crop species, including maize (Helentjaris *et al.*, 1986), tomato (Paterson *et al.*, 1988). During the 1990s, a new class of markers based on the polymerase chain reaction (PCR) revolutionized molecular marker assays; they are easy to use and suitable for automation, thereby allowing large-scale and efficient screening of progeny. With the development of new PCR-based DNA markers such as simple sequence repeats (SSRs) (Powell et al., 1996), amplified fragment length polymorphisms (AFLPs) (Vos et al., 1995), allele specific marker-like molecular beacons (Bohnert et al., 1999), and most recently single nucleotide polymorphism (SNPs) (Gilles et al., 1999), molecular markers have become powerful tools to analyze the plant and animal genomes.

Molecular markers offer considerable advantages in genetic dissection of quantitative traits, such as plant yield, disease or insect pest resistance, and abiotic stress tolerance (Liu, 2002; Ribaut *et al.*, 2003). This specific application is popularly referred to as 'QTL mapping'. QTLs indicate the genomic regions controlling the expression of a specific quantitative trait, and are inferred by statistical analysis of genotypic data (generated through molecular marker assays on segregating experimental mapping populations) in conjunction with the phenotypic data (derived by evaluation of mapping populations for the target trait(s) of interest).

The utilization of molecular markers in quantitative trait loci (QTL) analysis and as tools to explore the genetic and phenotypic bases of complex traits is an important landmark in genetics and plant breeding. If individual genetic components associated with a complex trait can be identified, then research can focus on the function of each locus independently without the confounding effects of other segregating loci. Identification of molecular markers linked to .loci involved in drought stress tolerance could lead to a better understanding of various components of the trait and shall possibly lay the foundation for efficient molecular marker-assisted selection (MAS) strategies. The extensive knowledge generated through the application of molecular markers has given scientists the ability to identify genes and pathways that control important biochemical and physiological parameters, and to better understand how they are regulated.

QTL mapping and MAS for specific target traits is being practiced in several institutions across the world, enabling the breeders to exercise selections based on genotypic or DNA-based differences rather than phenotypic evaluations of segregating progenies (e.g., Ragot, 1995; Tanksley *et al.*, 1996; Ribaut *et al.*, 2004).

Under the All-India Coordinated Maize Improvement Project (AICMIP), a few cultivars like Harsha and Megha suitable for rainfed conditions were released (Dhillon and Prasanna, 2001). Increasing emphasis on breeding for drought tolerance in maize is now being placed in the NARS. However, published information on genetic variability in maize for drought stress tolerance in the Indian context is scanty. This study, therefore, aims at fulfilling this important research need. Besides, this investigation shall be the first attempt to utilize molecular markers for mapping loci influencing various component traits influencing the performance of maize under drought stress conditions in India. The Maize Genetics Unit at IARI, New Delhi, is at the forefront in applications of molecular markers for diverse purposes, including genetic diversity analysis, QTL mapping and MAS for genetic enhancement of maize in India for biotic and abiotic stress tolerance (Prasanna and Hoisington, 2003; Prasanna *et al.*, 2004). These research programmes on molecular breeding in maize are being carried out primarily under the Asian Maize Biotechnology Network (AMBIONET), facilitated by CIMMYT, Mexico.

With this background, the **objectives** of the present investigation were:

(i) To analyze the genetic variability for drought stress tolerance in maize germplasm under the Indian conditions;

(ii) To assay molecular polymorphisms among selected maize inbred lines with distinct responses to drought stress; and

(iii) To map molecular markers linked to loci conferring drought stress tolerance of maize in India.

# Review of Literature

Among the abiotic stresses, drought is considered as the most important limitation affecting production and productivity of crop plants worldwide. Improvement of drought tolerance in crops is a major objective for agriculture, particularly in the developing countries (Edmeades *et al.*, 1998). Because of its genetic complexity, drought tolerance is probably the most difficult trait to improve through conventional plant breeding (Ribaut *et al.*, 2002). Although impressive progress has been achieved through conventional breeding, the potential for genetic improvement for drought stress tolerance in crop plants like maize is still large. Such improvements are expected to be accelerated in the coming years, given the rapid increase in supply of new molecular tools, and the explosion in genetic information generated through genomic approaches.

Quantitative estimates of yield losses due to drought in the tropics are scarce and imprecise. Edmeades *et al.* (1992) estimated 17 per cent annual loss of maize grain yield due to drought in the developing countries. Drought tolerance is particularly important in the Indian ecosystems where farming is mostly at the mercy of weather conditions. Nearly 65-70 per cent of the maize grown in India is under rainfed conditions. Therefore, genetic enhancement of drought stress tolerance of maize in India is considered as one of the most important priorities of the National Agricultural Research System (NARS). In this chapter, salient aspects related to drought tolerance, including conventional and molecular breeding and genomics, with particular focus on maize, shall be reviewed.

## Definitions of Drought

Drought, in the meteorological context, is commonly defined as a period without significant rainfall (Turner, 1979). There are several definitions of drought based on precipitation, potential evapo-transpiration, and temperature and wind velocity for the whole year or a season. Despite more than 50 such definitions (WMO, 1975), it is difficult to define drought in the context of a crop at a given location. A workable or practical definition, in the biological context, could be "*The inadequacy of water availability, including precipitation and soil moisture storage capacity, in quantity and distribution during the life cycle of the crop to restrict expression of its full genetic yield potential*" (Chetti, 2002).

The two terms, water stress and drought stress are often used interchangeably. The term drought stress has the advantage over water stress, since it can only refer to a deficit, never to an excess of water (Levitt, 1980). Quisenbury (1982) defined drought as any period during which plant and/or soil water deficiencies affect the growth and developments of crop plants. These deficiencies may result either from a small moisture supply or a large moisture demand. The duration of this drought period will determine the amount of damage done to the plant's growth and development. To the plant breeder, the term drought tolerance/resistance is related to a moisture stress environment and means the ability of one genotype to be more productive with a given amount of soil moisture than another genotype.

Virtually all plant metabolic processes are affected by water stress, if the stress is severe or of long duration. Based on seasonal soil water availability patterns, Jordan and Miller (1980) have defined three types of drought conditions; (1) terminal, (2) unpredictable and (3) unpredictable and terminal (as in many Mediterranean regions).

### Plant's Ability to Cope with Drought Stress

Depending on the timing and the length of the drought stress, plants may exhibit different set of mechanisms to cope with the stress (Turner, 1981). For crops with adequate water at sowing but with a declining water supply particularly after flowering (terminal drought), mechanisms to conserve moisture loss are required during the vegetative phase. Mechanisms to adjust gradually to lowering soil and leaf water potential (for example, osmotic adjustment and dehydration) are required if there is an unpredictable water deficit at any growth stage, particularly in the late vegetative and flowering stages.

Drought tolerance is the ability of a genotype to grow and yield more satisfactorily in water-deficit prone areas than another. Characteristics that impose a limitation on crop productivity should be reversible if stress is likely to be relieved. This suggests that, if rainfall probability increases during a crop's life cycle, radiation shedding and stomatal closure would be of greater benefit than a reduction in leaf area from leaf senescence or shedding (Turner, 1982). Three different means of the plant's ability to cope with drought stress, in terms of drought escape, drought avoidance, drought tolerance and drought recovery, merit particular attention.

***Drought Escape***: Drought escape is a particularly important strategy of matching the phenological development with the period of soil moisture availability to minimize the impact of drought stress on crop production in environments where the growing season is short and terminal drought stress predominates (Turner, 1986). It is the ability of a plant to complete its development, including the reproductive cycle, before the onset of severe water deficit. Early maturity is one of the most important factors of drought escape (Chang *et al.*, 1986).

***Drought Avoidance***: It is the ability of a plant to maintain high water status during the drought period. Although the plant cannot alter an environmental stress by itself, it can prevent or decrease the penetration of the stress into its tissues. This kind of resistance is called 'stress avoidance' (Levitt, 1972). This can be broadly divided into two groups: maximizing water uptake by improving the capacity of the root system to acquire water, and optimization of the use of absorbed water for the production of dry matter.

The stress may be excluded or avoided, either partially or completely, either by means of a physical barrier which insulates its living cells from the stress or by a steady state or exclusion of the stress (a chemical or metabolic barrier). The water permeability of the leaf cuticle is determined completely by the amount of cuticular waxes. Plants with thick layers of cuticle and stomata are likely to avoid the effects of drought (Blum 1988).

***Drought Tolerance:*** Drought tolerance is defined as *"the mechanism(s) causing minimum loss of yield in a water-deficit environment relative to the maximum yield in a water constraint free management of the crop"* (Singh, 2002). This definition includes crop yield as an important component. Thus, it is the ability of a plant to withstand water stress as measured by the degree and duration of low plant water potential.

Drought tolerance, as an adaptive mechanism, is frequently mentioned in the literature, but it is difficult to identify and understand the mechanisms contributing to such ability. It is often possible to assume that the plant is actually suffering internal water stress and is exhibiting the capacity to withstand it. Chang and co-workers (1986) noted that the tolerance is attributable to less understood physiological adjustments in the plant tissues. Levitt (1980) suggested that stress tolerance occurs, not only via dehydration avoidance but also by dehydration tolerance, due perhaps to protein adaptations. Many mechanisms have been suggested as important in stress tolerance. These are mostly mechanisms that protect the structure of the cell and organelle membranes and the cell proteins (Lewin and Sparrow, 1975).

***Drought Recovery***: It is the ability of a plant to resume growth and yield satisfactorily after the drought stress. After drought stress, when the rainfall or supply of soil moisture does commence, the trait of rapid recovery and return to active growth and development of the plant is important. Good recovery ability in rice appears to be correlated with vegetative growth vigour, high tillering ability, a shallow root system, and rather long growth duration (Loresto *et al.*, 1976). According to Chang and coworkers (1986), drought recovery is partly related to vegetative growth vigour. In case of rice, considerable differences in the varietal recovery ability after drought stress were observed (IRRI, 1976).

## Breeding for Drought Tolerance in Maize

The progress in developing crop cultivars with drought stress tolerance in general, maize being no exception, has been slow, because of lack of knowledge of mechanisms of tolerance, poor understanding of inheritance of tolerance, low heritability and lack of efficient techniques for screening germplasm and breeding materials (Ribaut *et al.*, 2002; Prasanna and Ribaut, 2004). Nevertheless, encouraging progress has been made in the recent decades in maize with respect to genetic dissection of drought stress tolerance as well as development of germplasm and cultivars with varying levels of tolerance to water stress (e.g., Edmeades *et al.*, 1998; Banziger *et al.*, 1999).

The conventional approach of the plant breeder has been to select and breed varieties for high yield, disease resistance, quality, and other desirable traits. In general, breeders have conducted this selection and breeding under environmentally favorable conditions. There have been two important reasons for this. First, the breeders did not want environmentally stressful conditions to confound the results of breeding. Second, most modern plant breeding programmes have been conducted in areas and for areas that have relatively benign environments, or could be rendered so by fertilization, reclamation, and irrigation (Epstein and Rains, 1987).

The need for embarking on focused research programmes for improving abiotic stress tolerance of crop plants is now being emphasized worldwide. For developing superior crop cultivars for abiotic stresses, it is imperative that the nature of the stress condition is defined and the diverse range of germplasm be tested for response to the specific stress.

The choice of breeding approaches and methods may depend on the following considerations (Devine, 1982):

1. The method of reproduction (self-pollinated vs. cross-pollinated, vegetative or sexual, tolerance to inbreeding, seed increase methods etc.).
2. The mode of gene action (multigenic vs. monogenic, dominant vs.

recessive, heterosis, epistasis, etc.).

3. Sources of tolerance available (crop cultivars, uncultivated forms of the crop species, related species etc.).
4. Priority assigned to the goal in relation to the other agronomic traits (biotic stresses, quality factors, etc.).

Salient components of breeding for drought stress tolerance in a crop like maize are briefly discussed below.

***Screening for drought tolerance***: Accurate phenotyping of breeding materials for traits like drought tolerance is certainly one of the most important limiting factors in enhancing the efficiency of breeding for drought tolerance. It is not possible to define or measure drought tolerance with the same clarity or precision as it is for disease resistance or for morphological or physiological traits, nor is it easy to manage experimental drought environments with a high level of control and repeatability. Therefore, extra effort is needed in the conceptualization, design, and management of phenotyping programs for drought tolerance.

It is essential that the test environment represent the stress environment in which it is ultimately intended to grow the crop. CIMMYT researchers have successfully employed modern tools such as Geographical Information System (GIS) for characterization of TPEs and identification of test sites for drought screening in Sub-Saharan Africa. CIMMYT maize breeders in Sub- Saharan Africa, working towards improved drought tolerance, now have basic climatic (e.g., total precipitation during the growing season), topographic and soil surfaces available to assist them in their work. Using the Spatial Characterization Tool, it has been possible to map growing season length for the region, providing an initial level of data to assist in the process of determining appropriate areas for germplasm of differing maturities. This information, combined with ecological zoning of the region into mega-environments, enabled better definition of breeding priorities for the Sub-Saharan region and generation of appropriate germplasm and cultivars with drought tolerance (Banziger *et al.*, 2004).

The screening techniques vary with species and with particular stress(es) being imposed. It is essential that the test environment represent the stress environment in which it is ultimately intended to grow the crop. The test procedure(s) should be rapid and less expensive so that large number of genotypes is tested. In the tropical/sub-tropical regions of the world, scientists have used seasonally rain-free dry periods for screening under drought conditions. Efficient screening for drought tolerance calls for a rain-free environment where the timing and intensity of stress is modulated by irrigation, but where the effects of other factors such as disease, day length, temperature and soil characteristics have as small an effect as possible on the crop performance.

Two major test systems are used for this purpose.

1. The 'line source irrigation system' that allows to test materials over a gradient of drought (e.g. Mahalakshmi *et al.*, 1990) conditions. Here the analysis of the data allows comparing the response curves of the tested genotypes to a full range of stress conditions.
2. An orthogonal comparison of the tested genotypes between non-stress and appropriate stress condition or over a range of stress conditions. Such a test can be developed in one site by using irrigation to control stress (e.g. Mahalakshmi *et al.*, 1990), or it can be performed by testing materials over different locations which differ mainly in their water regime. Here data can be analyzed by the appropriate statistical procedure (ANOVA or linear regression), provided that a sufficient level of stress has been achieved in at least one test. This analysis can be supplemented by deriving a 'stress susceptibility index' (Fischer and Maurer, 1978) or by normalizing cultivar performance under drought stress for genetic variation in yield potential and phenology (Bidinger *et al.*, 1982). The value of these yield tests for deriving an estimate of drought resistance in terms of yield depends on the extent to which the main variable causing yield variations among tests is indeed the water regime.

Minimizing experimental error variance in the field trials for drought screening is a major challenge. This could be possibly achieved by: (i) selection and management of fields that are as uniform as possible; (ii) increasing the number of replicates in the experiment; (iii) ensuring that the experiment is bordered uniformly and adequately towards alleys in the field and field borders; (iv) using improved statistical designs such as alpha-lattice (rather than randomized block designs); (v) choosing an optimal field layout that reduces the variation within replicates; and (vi) using statistical analysis tools that consider spatial variation. The inclusion of points of reference, controls or standards is vital for the success of the field experiments (Banziger *et al*., 2000; Fischer *et al*., 2003). Two recent Manuals published in maize (Banziger *et al*., 2000) and rice (Fischer *et al*., 2003) provide highly useful tips in optimizing drought phenotypic assays.

***Target traits***: The genotype x environment (G x E) effects under stress conditions can easily bias selection if based on yield components only, especially if there is a range of flowering dates in the germplasm under evaluation (Banziger and Cooper, 2002). Most breeders, therefore, opt for a 'selection index' that incorporates various yield components and secondary morphological traits in their breeding programme to maximize the gain in grain yield and stress tolerance while minimizing the possibility of selecting the escapes. Proportional weights for each trait used in selection are calculated based on the phenotypic and genotypic covariance between that trait and grain yield (Banziger and Lafitte, 1997). In maize, considerable information is available on the secondary traits and their relevance in breeding for drought tolerance.

A suitable secondary trait is one that is (i) genetically associated with grain yield under drought; (ii) highly heritable; (iii) inexpensive and quickly measured; (iv) stable over the measurement period; (v) observed at or before flowering; and (vi) not associated with yield loss under unstressed conditions (Edmeades *et al*., 1998). A commonly used secondary trait for drought tolerance in maize is the asynchrony between silk emergence and pollen shedding under water-limited conditions which is referred to as 'anthesis-silking interval' (ASI). This secondary trait is highly correlated with grain yield; a short ASI is usually associated with good kernel set and high grain yield (Edmeades *et al.* 2000). ASI has a heritability that on average is slightly greater than that for grain yield under stress (Bolanos and Edmeades. 1996). An advantage to using ASI in breeding is that it is easily measured in the field, on either a plant or row basis, and data are available at flowering. Thus, it could be used to direct crossing in an accompanying well-watered nursery that was planted a few weeks later.

Stay-green is another trait of interest, because an active green canopy is essential to continued production under transient stress. In cereals, foliar senescence releases N required by the developing grain from the photosynthetic enzymes. Consequently, simultaneous selection for delayed senescence and enhanced sink size under drought may still result in accelerated senescence because the demands of the grain for N take precedence (Chapman and Edmeades, 1999). Greenness *per se* does not guarantee continued C assimilation (Thomas and Howarth, 2000) and may be detrimental if it indicates a weak grain sink and/or an inability to remobilize stem reserves (Blum, 1997). However, stay-green is generally associated with improved performance under drought in cereals since photosynthesis is maintained longer in stay-green types (Thomas and Smart, 1993). In sorghum, stay-green is considered the most important post-flowering secondary trait associated with grain production under water-limited conditions (Borrell, 2000).

Leaf temperature drops when water evaporates from leaf surfaces, a trait affected directly by stomatal conductance, which is affected by feedback mechanisms of other processes such as carbon fixation and vascular transport of water. Canopy temperature depression (CTD), the difference between the temperature of the air and the leaves of a field plot measured with an infrared thermometer, is another frequently used indicator of a genotype's ability to capture water. CIMMYT wheat breeders successfully used CTD measurements on small plots in their heat tolerance nurseries to identify the highest yielding entries (Reynolds et al., 2000a). When measured during grain filling under stress conditions, CTD correlated well (genetic correlation coefficients of 0.6-0.8) with final grain production in wheat (Reynolds et al., 2000b). Strong relationships between grain yield and canopy temperature have also been reported by Fischer *et al* (1989) among full-sib progenies of a maize population grown under drought conditions, though these have been weaker in inbred progeny trials (Bolanos and Edmeades, 1996).

The nature and adaptive value of these secondary traits are quite diverse. Some traits, such as stay-green, are of value to a wide range of crops, while others are specific to certain crops, such as ASI in maize. Another avenue of study has been the quantification of changes in key physiological parameters related to plant responses to water-limited conditions. Although most of the secondary morphological traits like ASI, stay-green, and CTD can be routinely used in breeding programs, this is not the case for most physiological parameters, such as osmotic adjustment (OA), ABA concentration, or the quantification of the late embryogenesis abundant (LEA) proteins, because their measurement is too time consuming and/or too expensive and those parameters generally show low correlation with yield components. Nevertheless, plant improvement for drought environments requires more complete knowledge of the genetic basis of both the morphological traits and the physiological parameters involved in the plant responses under water-limited conditions.

The mass and recurrent selections are important in accumulating genes for tolerance in the populations/genotypes against abiotic stresses such as drought. Full-sib family recurrent selection has been used extensively at CIMMYT to improve maize populations for drought tolerance. Replicated trial sets of full-sib progenies are evaluated under drought and well-watered conditions. Selection is made based on performance in all environments and considering other factors such as disease resistance, grain texture etc.

Eight cycles of recurrent full-sib selection for improved drought tolerance in a tropical maize population resulted in a yield gain of 500-800 kg/ha at all yield levels with only minor changes in maturity. Gains in drought tolerance and yield potential thus occurred at the same time. Selection did not result in changes in plant water status (Edmeades *et al.,* 1992). Yield gains were associated with increased ear growth rate prior to anthesis, rapid silk growth, reduced growth of the tassel and of surface roots, and higher harvest index, all manifestations of increased partitioning of photosynthates to the developing ear.

Estimates of progress from this selection approach have been established in maize using a wide range of germplasm (Bolaños and Edmeades, 1993a,b; Edmeades *et al.,* 2000a,b). Depending on selection scheme and selection intensity used, yield increases of 59 to 233 kg/ha$^{-1}$ cycle$^{-1}$ of recurrent full-sib or $S_1$ selection were obtained. Gains proved to be fairly similar across drought-stressed and well-watered conditions i.e., under conditions that produced average trial yields of less than 1 t/ha$^{-1}$ to more than 10 t/ha$^{-1}$. Although a considerable part of CIMMYT's drought tolerance research has focused on open-pollinated varieties (OPVs), evidence suggests that improvements carry over to lines and hybrids. Hybrids derived from drought-tolerant populations outyielded those derived from equivalent non-drought tolerant populations by 20%, at a mean drought-induced yield level of 1.6 t/ha$^{-1}$. The probability of obtaining a hybrid with a yield of 30-50% greater than this mean value was 3–5 times greater when lines were derived from drought-tolerant sources, compared with conventionally-selected source populations (Edmeades *et al.,* 1997a,b).

In CIMMYT's drought breeding programme, selection gains were largely the result of reduced barrenness under drought, and an associated increase in harvest index (Bolaños and Edmeades, 1993a). The ASI became shorter under drought, and selection seemed to have led to significantly faster spikelet and ear growth at flowering, but also to a reduction in final spikelet number. Fewer spikelets were formed, grew more rapidly, and were ultimately more successful in forming grain, especially under conditions of drought at flowering (Edmeades *et al.,* 2000).

In 1997, the maize inbred lines selected for drought tolerance at CIMMYT-Mexico were introduced to southern Africa and a product-oriented breeding programme was launched, in collaboration with the NARS in Angola, Botswana, Malawi, South Africa, Tanzania, Zambia, Zimbabwe. Between 2000 and 2002, 41 promising hybrids were identified and compared with 42 released and pre-released private seed company hybrids in 36-65 trials across eastern and southern Africa. Average trial yields ranged from less than 1 t/ha to above 10 t/ha. Hybrids from CIMMYT's stress breeding program showed a consistent advantage over the private company check hybrids at all yield levels. Several drought tolerant maize hybrids and varieties are now under cultivation in these countries. With over 30 institutions and 1000 farmers participating, collaborative trials have become an important mechanism for characterizing and comparing maize cultivars used in the SADC region and for promoting knowledge and the use of best cultivars by private companies, NGO's, extension personnel and farmers (Banziger *et al.*, 2004).

### Molecular Markers and Utility in Genetic Dissection and Breeding for Drought Stress Tolerance in Maize

Most of the traits considered in animal and plant genetic improvement programmes are quantitative traits, that is, they are controlled by many genes, influenced significantly by environmental factors, and the underlying genes usually have small effects on the observable phenotype. In classical genetic improvement programmes, selection is carried out based on observable phenotypes (of the candidates for selection and/or their relatives) but without knowing which genes are actually being selected.

With the advent of DNA-based markers in the late 1970s, the situation changed and researchers could, for the first time, begin to identify large numbers of markers dispersed throughout the genetic material of any species of interest and use the markers to detect associations with traits of interest. A milestone paper by Paterson and coworkers (1988) demonstrated that, given the availability of large numbers of genetic markers for the species of interest (tomato), how the effects and location of marker-linked genes impacting a number of quantitative traits (fruit traits in this case) could be estimated. This was based on an approach that could be applied to dissect the genetic make-up of any physiological, morphological and behavioral trait in plants and animals.

During the past two decades, molecular tools have advanced the identification, mapping, and isolation of genes in a wide range of crop species. The first DNA markers, restriction fragment length polymorphisms (RFLPs), have been widely used to construct linkage maps for several crop species, including maize (Helentjaris *et al.*, 1986), tomato (Paterson *et al.*, 1988), and rice (McCouch *et al.*, 1988). During the 1990s, a new class of markers based on the polymerase chain reaction (PCR) revolutionized the assays; they are easy to use and suitable for automation, thereby allowing the large-scale screening of progeny. With the development of new PCR based DNA markers such as simple sequence repeats (SSRs) (Powell et(Prasanna and Hoisington, 2003). For al., 1996), amplified fragment length polymorphisms (AFLPs) (Vos *et al.*, 1995), allele specific markers-like molecular beacons and most recently single nucleotide polymorphism (SNPs) (Gilles *et al.*, 1999), molecular markers revolutionized genetics and plant breeding, offering a palette of powerful tools to analyze the plant genome.

DNA markers have been enabling the identification of genes and genomic regions associated with the expression of numerous qualitative and quantitative traits, and have made the manipulation of such genomic regions feasible through marker-assisted selection (MAS) in various crop plants; maize is at the forefront of these developments simply inherited traits with high heritability and regulated by only a few genes, the use of molecular markers to accelerate germplasm improvement has been well documented (e.g., Ragot *et al.* 1995; Tanksley *et al.* 1996). Despite the dearth of MAS success stories related to germplasm improvement for polygenic traits, MAS has immense potential for improving even polygenetic traits (Ribaut *et al.*, 2004; Prasanna and Ribaut, 2004). As Young (1999) wrote in a recent review, "*Before the advent of DNA marker technology, the idea of rapidly uncovering the loci controlling complex, multigenic traits seemed like a dream. Suddenly, it was difficult to open a plant genetics journal without finding dozens of papers seeking to pinpoint many, if not most, agriculturally relevant genes*". MAS could aid in overcoming the difficulties associated with low heritability, recessiveness and difficult screening assays during the introgression of genes/genomic regions associated with drought stress tolerance in a breeding programme.

***Microsatellites or Simple Sequence Repeat (SSR) Markers and their Utility in Maize:*** Microsatellites are tandemly repeated DNA sequence elements found in abundance in the genomes of just about every known organism. They have come into prominence over the last decade because they have been found to be remarkably versatile molecular tools (Chambers *et al.*, 2000). The tandem repeats may include mono-, di-, tri-, tetra-, penta-. hexa- or compound nucleotide units that are arrayed throughout the genome (Powell *et al.*, 1996). The existence of dinucleotide repeats, Poly (C-A) - Poly (G-T) was first documented in 1982 by Hamada and coworkers. Later, Weber and May (1989) developed a general approach for detecting polymorphic microsatellites, by designing specific primers for PCR amplification and detection. These primers are sequence tagged sites and primers complementary to the conserved sequence flanking the repeat can be used to amplify the intervening microsatellite region in genotypes of target species. It has been estimated that, on an average, a repeat larger than 20kb in length occurs every 33 kb in plant nuclear genomes compared to every 6 kb in mammals. In plant genomes, the A-T repeat motif predominates whereas in humans, an A-C or T -G repeat is most common, and this appears to be a general feature distinguishing plant and animal genomes (Powell *et al.*, 1996).

Standard methods for isolation of SSRs involve (i) the creation of a small insert genomic library; (ii) library screening by hybridization; (iii) DNA sequencing of positive clones; (iv) primer design and locus-specific PCR analysis; and (v) identification of polymorphism (Powell *et al.*, 1996). The uniqueness and value of microsatellites as primers arise from their multiallelic nature, codominant transmission, ease of detection by PCR, relative abundance. extensive genome coverage and requirement of only a small amount of template DNA. Also these mapped markers can be distributed between labs as primer sequence. The major disadvantages of SSRs are (i) these are at present limited to crop plants where cloning and sequencing efforts have resulted in SSR primer database (in public domain); (ii) these markers are usually species-specific; and (iii) they require high resolution gels for separation of amplified products.

Chin *et al.* (1996) conducted a database survey of 576 maize sequences from Gen Bank and EMBL databases to determine the abundance of maize microsatellites, and identified 200 potential microsatellites. They reported that the vast majority of microsatellites were found in cDNAs, expressed sequence sites and known genes and hence reflect the occurrence of these repeats in or adjacent to maize genes. They saw an abundance of trimeric microsetellites within coding sequences. Of the 89 trinucleotides identified, 66% were found in coding sequence in which about 34% occurred within coding sequences. [n their search, they found CT/ AG as the most common dinucleotide motif, comprising 47% of all the dinucleotide repeats, 50% of which showed polymorphism.

Application of SSR markers by plant research community is relatively recent in comparison with animal genetics. Identification of SSR markers in plants is accelerating, and these loci are being incorporated into established genetic maps of an increasing number of crops. Mogg *et al.* (1999) showed that by sequencing the flanking regions of maize microsattelites, a SNP could be found every 40 bp. Given that the maize genome is estimated to be 2.5 x 109 bp in size, there is a potential for up to 62 million SNPs in maize. With the recent initiation of a (http://ww\\.zmdb.iastate.edll/zmdb/EST proiect.html), a new and potentially rich source of SNPs has been uncovered (Edwards & Mogg, 2001). While both SSRs and SNPs can be reliably applied on a large scale with only small quantities of DNA required for PCR amplification, SNPs are highly amenable for automation. And therefore, offer significant advantages for plant breeding purposes. SSRs however are the preferred choice when codominant, multiallelic information is required or when the infrastructure and resources are limited. At present, nearly 2000 SSR loci are identified in maize (MaizeDB; http://www.agron.missouri.edulssr.html).

## Mapping Quantitative Trait Loci (QTL) conferring Drought Tolerance in Maize

A 'Quantitative Trait Locus' is a *region* of the genome that is associated with an effect on a quantitative trait (Liu, 2002). The term 'QTL' was first suggested by Gelderman (1975) for genes that underlie quantitative traits. Conceptually, a QTL can be a single gene, or it may be a cluster of linked genes that affect the trait. Using the methods for QTL detection to be discussed here, we cannot resolve the difference, and use the term "locus" to refer to a chromosomal region. Our interest in QTL mapping is primarily to describe the effects of each genomic region on quantitative traits of interest. Excellent reviews dealing with various aspects of QTL mapping in crop plants are available (e.g., Liu, 2002). The first genome wide scan for QTL using RFLP was performed on tomatoes by Paterson *et al.* (1988), wherein mapping of QTLs for fruit mass concentration of soluble solids and fruit pH was carried out using backcross generation of inter specific cross between *Lycopersicon esculenlum* and a wild tomato *L. chmielewskii.* Since then, many such mapping studies based on DNA markers have been carried out successfully in different plant species.

'QTL analysis' is the phrase used currently to study the quantitative genetic variation to locate the genes responsible for them and to explain their effects and interactions. The enthusiasm for QTL mapping and analyses for applied research is strongly associated with the hope of using genetically linked DNA markers as the means of indirect selection for genomic regions affecting quantitative traits in plant breeding programs. It may also provide insights into facets of quantitative inheritance patterns and other complex processes and phenomena that have been exploited, but not yet understood (Lee, 1995).

The first comprehensive analysis of QTLs determining the responses of the maize plant to drought stress was carried out by Lebreton *et al.* (1995). This study led to mapping of QTLs for physiological traits associated with drought tolerance, namely stomatal conductance, ABA of different tissues, leaf water relations parameters, fluorescence, root pulling force, and nodal root number. Intensive research on drought tolerance in maize at CIMMYT led to the appreciation of the significance of reduced ASI as one of the key traits associated with drought tolerance. This was further supported by a series of QTL mapping experiments (Agrama and Moussa, 1996; Ribaut *et al.*, 1996; Ribaut *et al.*, 1997a,b), which also provided an opportunity to analyze the correspondence of QTL positions for ASI in different mapping populations. Four of the 5 QTLs for ASI detected by Agrama and Moussa (1996) appeared to map in the same chromosomal regions (chromosomes 1, 5, 6, 8) as those in Ribaut *et al.* (1996) who identified 6 QTL for this trait, which together accounted for 47 per cent of the phenotypic variance (Ribaut *et al.*, 1996). The highly significant phenotypic correlation observed between ASI and grain yield was confirmed at the genetic level by the co-location of four QTLs significant for both traits (Ribaut *et al.*, 1997a).

Several mapping populations for analysis of drought tolerance were developed at CIMMYT. To date, genetic dissection has been conducted in four different crosses, at different inbreeding levels (hybrids, $F_2$:$F_3$ families, and recombinant inbred lines (RILs), under different water regimes [well-watered (WW), intermediate stress (IS), and severe stress (SS) conditions) and in several different environments (Kenya, Mexico, and Zimbabwe) (Ribaut *et al.*, 2002). Initial experiments focused on flowering parameters, plant and ear height, leaf size and number, and yield components (Ribaut *et al.*, 1996; 1997a). As all the morphological traits, including yield, are complex and regulated by several genes, no major QTL (explaining more than 25% of the phenotypic variance) has been identified. The majority of the QTLs expressed 3–10% of the phenotypic variance and the total phenotypic variance expressed by combining all of the significant QTLs was generally 30 to 40%, and never surpassed 60%. Recent experiments in India related to mapping of QTLs conferring drought tolerance of maize confirmed this trend (Prasanna *et al.*, 2004).

Besides the morphological traits, physiological parameters like relative water content, osmotic adjustment, chlorophyll content, ABA content in the ear at the flowering stage, have also been systematically analyzed by CIMMYT researchers through inter-institutional collaborations, leading to identification and localization of several QTLs (Ribaut *et al.*, 2002, 2004). QTLs for maize-leaf ABA content under drought stress were also mapped by Tuberosa *et al.* (2002a,b) and Sanguineti *et al.* (1999). Sixteen QTL for ABA content corresponded with QTL for at least one of the following traits: stomatal conductance, drought sensitivity index, leaf temperature, leaf relative water content, ASI and grain yield. An increase in ABA content was generally associated with decreased stomatal conductance and grain yield but increased leaf temperature. However, the opposite effect was observed for a QTL on chromosome 7 that aligned with a QTL from a previous study for root pulling resistance suggesting that elevated ABA stimulated the development of a more extensive root system (Lebreton *et al.*, 1995).

An important outcome of the above studies was detection of correspondence of genomic locations of QTLs related to physiological and morphological traits in many cases. For example, a QTL for chlorophyll content was identified on chromosome 2 close to a QTL for ASI (under IS and SS) and grain yield (under IS only). Similarly, a QTL for relative water content on chromosome 6 corresponds exactly to a QTL for ASI (under IS and SS) and grain yield (under IS and SS) (Ribaut *et al.*, 2002). At the same chromosomal region, a *dehydrin* gene (*dhn1*) has also been located (Campbell and Close, 1997). The QTLs for physiological parameters were generally less significant compared to those obtained for morphological traits, and the total phenotypic variance was rarely greater than 25% for any given parameter. However, the QTL information generated for the physiological parameters is potentially valuable for: (i) developing a 'drought tolerance index' for selection of genotypes in MAS experiments; and (ii) serving as link between the data generated from functional genomics and morphological responses of the genotypes in response to drought stress.

An interesting approach for studying drought tolerance was proposed by the INRA (France) researchers using proteomics (de Vienne *et al.*, 1999; Prioul *et al.*, 1999). Using large-scale two-dimensional gel electrophoresis, they quantified the protein spot intensities and mapped these as 'protein quantity loci' (PQL). This approach was used to evaluate a maize RIL population under mild drought stress. Differentially expressed proteins from the leaf tissue were sequenced for identification of putative candidate genes. One of the proteins was an ABA/water stress/ripening induced protein located on chromosome 10 that was previously found to be induced by water stress in other species (de Vienne *et al.*, 1999). The location of this candidate gene corresponded with a QTL for xylem sap ABA content, leaf senescence, and ASI. Other PQL corresponding to the QTLs for drought responsive traits included those on chr.1 (Sh2–ADP-gulcose pyrophosphorylase), chr.2 (invertase), chr.5 (invertase), chromosomes 6, 8 (sucrose phospahte synthase), chromosomes 9 and 10 (Prioul *et al.*, 1999). Similarly, QTLs for invertase activity were found close to carbohydrate QTLs, and some of them formed 'stress clusters' (Pelleschi *et al.*, 1999).

## Molecular Marker-Assisted Selection (MAS) for Drought Tolerance in Maize

Although the information generated with respect to QTL mapping for drought tolerance in maize has been impressive and encouraging, very few successful experiments on marker-assisted selection (MAS) for improvement of drought tolerance in tropical maize have been published. This may be attributed to the limited number of major QTLs identified per trait, the reduced amount of phenotypic variance that they generally express individually, their interaction with the environment, plus the difficulty of epistasis evaluation (Ribaut and Hoisington, 1998). Besides the important limitation of having to select for several QTLs with less than 10% contribution to phenotypic variance, the technical handicap of handling restricted population sizes is a major factor, thereby limiting the flexibility and the power of selection. The latter constraint can be now overcome with the development of improved MAS protocols and availability of reliable PCR-based markers, which make screening of a substantial segregating population possible in a reasonable time period (Ribaut *et al.*, 1997b, 1999).

Taking advantage of the development of PCR based-markers, a drought-tolerant donor (Ac7643) was crossed to CML247 as a recurrent parent. After screening with molecular markers of about 2000 genotypes at two backcrosses and two self-pollinations, the best genotype was fixed from the donor line for five target regions. The 70 best $BC_2F_3$ plants were crossed to two CIMMYT tester inbreds (CML254 and CML274). These hybrids, as well as the $BC_2F_4$ families derived from the selected $BC_2F_3$ plants were evaluated in 1997-1998 under several water regimes. Results showed that the mean of the 70 selected genotypes performed better than the control crossed with the testers, and the best genotype among the 70 selected genotypes performed much better than the control under drought conditions. No yield reduction was observed under well-watered conditions for the hybrids derived from the MAS genotypes (reviewed by Ribaut *et al.*, 2002). Although some of the morpho-physiological traits associated with drought tolerance (such as ASI) can be effectively selected based on visual observations, molecular markers linked to QTLs for such key traits would undoubtedly aid in enhancing the efficiency of selection of drought tolerant genotypes as well as continuing a selection programme even in the absence of drought stress at flowering.

## Genomics of Drought Tolerance

Genomics represents an entirely new conceptual approach to the study and application of biology. Structural genomics uses the rapid generation of huge quantities of precise DNA sequence data to identify genes, and the structures of genes and other elements in a genome. The functions of these genes can be assessed by a number of high-throughput approaches, so-called "functional genomics." These techniques include the mapping of complex traits in very large populations, the characterization of correlated expression patterns of every gene within a species under all possible circumstances, and the use of reverse genetics and high throughput mutant screening to identify the phenotypes of mutations in all of the genes within a species. The ultimate goal of genomics is to find every gene and to determine the roles of each of these genes. Comparative genomics takes this goal several steps further: to identify and find the role of every gene in every species, to see what changes are significant in making one species different (in phenotype, growth habit, adapted environment) from another, and to determine how these changes came about.

Drought tolerance is a highly appropriate target for functional and comparative genomics because only such an information-rich approach is likely to unveil the key genetic contributors to the complex physiological processes involved. One of the important applications of genomics is to find and appropriately utilize the best drought tolerance alleles in nature, regardless of source, for crop improvement. The technology and biological materials needed to accomplish this ambitious goal now exist. All that is lacking are the appropriate teams and resources to undertake this important task.

Although the number of genes involved in plant regulation under abiotic stress is increasing (Bohnert *et al.* 2000) knowledge of their function, interaction and perhaps more importantly, the time frame they operate, remains poor. Recent developments in functional genomics should help overcome this problem and provide important information for evaluating the role of potential pathways, since they allow the simultaneous study of the expression of several thousand genes. To date, and partly because there are many constraints and uncertainties inherent in evaluating germplasm under controlled abiotic stress environments, few profiling experiments have reported changes in gene expression in maize grown under stress conditions in the field (Zinselmeier *et al.*, 2002) or in the glasshouse (Setter and Flannigan, 2001, Yu and Setter, 2002, Zinselmeier *et al.*, 2000).

***Expression Profiling for Identification of Drought-associated Genes***: By "traditional" molecular approaches (i.e., those that are in use from the late 1970's particularly till 2000), researchers identified a few genes that were induced or repressed by a particular environmental cue, such as drought. However, these technologies could never yield more than a subset of the genes involved, and were biased toward recovering genes with very major changes in expression that were observed over (usually) two chosen points in time. The genomic approach to this same question is to test all of the genes in a species for their RNA levels at all times before and after drought stress induction (Schena *et al.*, 1996; Ruan *et al.*, 1998). Because these characterizations are fairly quantitative, quite sensitive, and quick to perform, they allow the identification of genes that are induced or repressed by only a few fold, are expressed at low levels, and are altered in expression only during part of the exposure program. These genes with affected expression can also be located on the genetic map and, when they appear to co-localize with a QTL for drought tolerance, make attractive candidate loci (Byrne and McMullen, 1996).

Microarrays or DNA chip experiments, thus, generate a large amount of data on each sample and the expression levels of large numbers of genes presenting under contrasting experimental conditions can be observed (e.g. Seki *et al.* 2001, 2002). The greatest challenge of functional genomics approach is the organization and interpretation of these data to identify the most *informative* changes in gene expression, and evaluate their associated phenotypes (tolerant versus susceptible). In this regard, QTL data can help identify candidate genes responsible for changes in *plant* phenotype. Indeed, the correlation on a linkage map between a differentially expressed sequence tag (EST) cDNA and a QTL related to the same physiological parameter is a key step in identification of candidate genes. Combining information at three different levels: phenotypic characterization of the plant, QTL data, and changes in gene expression, for selected physiological pathways and morphological traits, provides an integrated approach for better understanding response to a water-limited environment. These three sources of information are complementary because the QTL characterization, which defines the genetic basis of the key physiological parameters, represents the link between differential gene expression mechanisms and the morphological plant response.

**Comparative genomics of drought stress tolerance:** A few studies carried out in the last few years suggested significant common gene content and colinear gene order in closely related dicotyledenous plants (Bonierbale *et al.*, 1988; Fatokun *et al.*, 1992; Kowalski *et al.*, 1994). Hence, different plant species appear to differ less in the genes they carry than they do in the alleles of genes that they contain. Moreover, comparative mapping studies suggest that the genetic differences responsible for very different morphologies and physiologies might be caused by differences in just a handful of genes, probably regulatory loci (Doebley *et al.*, 1995; Paterson *et al.* 1995). This suggests that critical and common biochemical pathways will exist in most plant species, and that the differences observed are due to possibly subtle changes in the timing, tissue, or magnitude of expression of such pathways. This conclusion makes it both possible and imperative to use the information from one species to enhance our understanding of every other species.

For drought tolerance, it is likely that maize and sorghum share the same basic tolerance pathways, but sorghum could have acquired superior allelic versions of the genes in that pathway as it evolved in drought-prone environments. Comparative genomics of these and other species will allow the identification of traits that are shared by all plants (and, hence, are required for general survival) and those that are only shared by some or all drought-tolerant individuals (and, hence, presumably are required only to survive in drought-prone regions). If one can identify the sorghum genes that are responsible for superior drought tolerance, then it is likely that these genes could function in maize to provide superior drought tolerance as well. This approach may be viewed as merely expanding the allelic variation available to maize, in a transgenic sense. Of course, the number of improved genes that would need to be added to have a significant effect remains an open question. Still, it is clear that using information (and eventually genes) from all pertinent species will provide a synergistic route for the improvement of any and all individual crops (Bennetzen and Freeling, 1997).

# Materials and Methods

The genetic materials and methodology employed for addressing each of the objectives of the present study are presented below:

## 1. Evaluation of Inbred Lines for Drought Tolerance

***Genetic Materials:*** A series of experiments were carried out over two years to evaluate a selected set of indigenous and exotic inbred lines at two different locations in India: (i) Acharya N.G. Ranga Agricultural University (ANGRAU) Maize Research Station at Amberpet, Hyderabad during Rabi (winter season) 2001-02; and (ii) ANGRAU Maize Research Station, Hyderabad during 2003-04. These experiments shall be hereafter referred to as Experiments I and II, respectively.

Experiment I comprised 18 inbred lines, including 9 lines developed by the maize research centers in India, 4 lines derived from the CIMMYT maize populations, and 5 lines obtained from the CIMMYT Applied Biotechnology Centre, Mexico. In Experiment II, a selected set of genotypes developed by the CIMMYT Maize Programme, specifically for drought stress tolerance, were evaluated. These include 39 DTPY lines (yellow kernel type) and 19 DTPW lines (white kernel type). The details of pedigree and sources of inbred lines analyzed in the present investigation are provided in Table 1.

### *Methodology*

***Field Screening:*** The selected maize inbred lines were evaluated against drought stress conditions imposed at Hyderabad under two irrigation regimes (normal and stress), whereas in experiment II inbred lines were evaluated only in stress condition, by following the drought screening procedures suggested by Banziger *et al.* (2000). The experiments were carried out in the winter (dry) season.

**Table 1. Pedigree and sources of inbred lines used in the present study.**

| S. No. | Inbred | Pedigree | Source of Seed Material* |
|---|---|---|---|
| 1 | Bio-5 | Derivative of LM5 | ANGRAU, Hyderabad |
| 2 | CM119 | Derivative of R-109 Ht | DMR, New Delhi |
| 3 | CM123 | Derivative of H3191-3 (LM2) | PAU, Ludhiana |
| 4 | CM138 | Derivative of IPA 21-10 (from AD6091) | IARI, New Delhi |
| 5 | CM139 | Derivative of (Tarun x MS1)-Y63 | PAU, Ludhiana |
| 6 | CM140 | Derivative of J617-61 | PAU, Ludhiana |
| 7 | CM207 | Derivative of Fla3H94 | DMR, New Delhi |
| 8 | FSA17 | FSA17-2-1-#-12-1-2-1-1 | IARI, New Delhi |
| 9 | IPA3 | IPA-3-20-f-6-1-1-# | IARI, New Delhi |
| 10 | IPA21A | IPA21-10-f-#-⊗ | IARI, New Delhi |
| 11 | IPA29A | IPA-29-f-2-#-f-1-# | IARI, New Delhi |
| 12 | IPA29B | IPA-29-3-18-1-#-f-# | IARI, New Delhi |
| 13 | IPA34 | IPA-34-f-106-2-#-# | IARI, New Delhi |
| 14 | CIM493 | Derivative of (CML159 x CML144) | DMR, New Delhi |
| 15 | CIM495 | 5406-119P28TSR.(SR)-3-1-2-2-B-# # #-BBBB-b-B | DMR, New Delhi |
| 16 | CIM500 | P27C5HC2/&-4-1-B-1-#-B-B-1BBBB-2-BB | DMR, New Delhi |
| 17 | CIM506 | P28TSR(S2)-3-1-3-1-# # #-B-B-B-B-2-BBB | DMR, New Delhi |
| 18 | CIM519 | Pool 26 Sequia (best synchronous)F2C2-## | DMR, New Delhi |
| 19 | Ac7643 | Derived from Population 43 (La Posta) | CIMMYT, Mexico |
| 20 | Ac7729 | Derived from Population 29 (Tuxpeno Caribb,) | CIMMYT, Mexico |
| 21 | K64R | Derived from 'Pride of Saline, South Africa | CIMMYT, Mexico |
| 22 | CML247 | Pool 24 (G24F119*G24F54)-6-4-1-1-BB-f | CIMMYT, Mexico |
| 23 | CML444 | – | CIMMYT, Mexico |
| 24 | DTPW1 | DTPWC9F2-3-1 | CIMMYT, Mexico |
| 25 | DTPW6 | DTPWC9F5-1-1 | CIMMYT, Mexico |
| 26 | DTPW43 | DTPWC9F18-1-1 | CIMMYT, Mexico |
| 27 | DTPW46 | DTPWC9F24-2-1 | CIMMYT, Mexico |
| 28 | DTPW51 | DTPWC9F24-4-1 | CIMMYT, Mexico |
| 29 | DTPW59 | DTPWC9F32-1-1 | CIMMYT, Mexico |
| 30 | DTPW79 | DTPWC9F55-2-1 | CIMMYT, Mexico |
| 31 | DTPW86 | DTPWC9F67-1-1 | CIMMYT, Mexico |
| 32 | DTPW88 | DTPWC9F67-2-1 | CIMMYT, Mexico |
| 33 | DTPW105 | DTPWC9F75-3-1 | CIMMYT, Mexico |
| 34 | DTPW125 | DTPWC9F92-2-1 | CIMMYT, Mexico |
| 35 | DTPW138 | DTPWC9F102-3-1 | CIMMYT, Mexico |
| 36 | DTPW165 | DTPWC9F119-1-1 | CIMMYT, Mexico |
| 37 | DTPW167 | DTPWC9F120-3-1 | CIMMYT, Mexico |
| 38 | DTPW176 | DTPWC9F126-1-1 | CIMMYT, Mexico |
| 39 | DTPW183 | DTPWC9F128-1-1 | CIMMYT, Mexico |
| 40 | DTPW195 | DTPWC9F137-3-1 | CIMMYT, Mexico |
| 41 | DTPW208 | DTPWC9F141-2-1 | CIMMYT, Mexico |
| 42 | DTPW211 | DTPWC9F141-4-1 | CIMMYT, Mexico |

(Contd.)

**Table 1 (Contd.)**

| S. No. | Inbred Line | Pedigree | Source of Seed Material |
|---|---|---|---|
| 43 | DTPY1 | DTPYC9F11-2-1 | CIMMYT, Mexico |
| 44 | DTPY5 | DTPYC9F13-2-1 | CIMMYT, Mexico |
| 45 | DTPY13 | DTPYC9F38-3-1 | CIMMYT, Mexico |
| 46 | DTPY18 | DTPYC9F38-4-1 | CIMMYT, Mexico |
| 47 | DTPY31 | DTPYC9F40-3-1 | CIMMYT, Mexico |
| 48 | DTPY39 | DTPYC9F46-1-1 | CIMMYT, Mexico |
| 49 | DTPY47 | DTPYC9F46-3-1 | CIMMYT, Mexico |
| 50 | DTPY57 | DTPYC9F53-3-1 | CIMMYT, Mexico |
| 51 | DTPY60 | DTPYC9F55-2-1 | CIMMYT, Mexico |
| 52 | DTPY62 | DTPYC9F65-2-1 | CIMMYT, Mexico |
| 53 | DTPY65 | DTPYC9F69-3-1 | CIMMYT, Mexico |
| 54 | DTPY71 | DTPYC9F71-2-1 | CIMMYT, Mexico |
| 55 | DTPY75 | DTPYC9F72-1-1 | CIMMYT, Mexico |
| 56 | DTPY79 | DTPYC9F74-1-1 | CIMMYT, Mexico |
| 57 | DTPY85 | DTPYC9F74-3-1 | CIMMYT, Mexico |
| 58 | DTPY91 | DTPYC9F81-2-1 | CIMMYT, Mexico |
| 59 | DTPY96 | DTPYC9F85-1-1 | CIMMYT, Mexico |
| 60 | DTPY97 | DTPYC9F86-1-1 | CIMMYT, Mexico |
| 61 | DTPY100 | DTPYC9F86-2-1 | CIMMYT, Mexico |
| 62 | DTPY105 | DTPYC9F87-1-1 | CIMMYT, Mexico |
| 63 | DTPY108 | DTPYC9F87-3-1 | CIMMYT, Mexico |
| 64 | DTPY116 | DTPYC9F103-5-1 | CIMMYT, Mexico |
| 65 | DTPY122 | DTPYC9F114-2-1 | CIMMYT, Mexico |
| 66 | DTPY128 | DTPYC9F114-3-1 | CIMMYT, Mexico |
| 67 | DTPY132 | DTPYC9F114-4-1 | CIMMYT, Mexico |
| 68 | DTPY135 | DTPYC9F116-2-1 | CIMMYT, Mexico |
| 69 | DTPY138 | DTPYC9F125-2-1 | CIMMYT, Mexico |
| 70 | DTPY149 | DTPYC9F132-2-1 | CIMMYT, Mexico |
| 71 | DTPY156 | DTPYC9F134-2-1 | CIMMYT, Mexico |
| 72 | DTPY162 | DTPYC9F134-3-1 | CIMMYT, Mexico |
| 73 | DTPY168 | DTPYC9F135-1-1 | CIMMYT, Mexico |
| 74 | DTPY174 | DTPYC9F138-1-1 | CIMMYT, Mexico |
| 75 | DTPY178 | DTPYC9F142-1-1 | CIMMYT, Mexico |
| 76 | DTPY184 | DTPYC9F142-3-1 | CIMMYT, Mexico |
| 77 | DTPY187 | DTPYC9F143-1-1 | CIMMYT, Mexico |
| 78 | DTPY194 | DTPYC9F143-5-1 | CIMMYT, Mexico |
| 79 | DTPY199 | DTPYC9F144-2-1 | CIMMYT, Mexico |
| 80 | DTPY202 | DTPYC9F145-3-1 | CIMMYT, Mexico |
| 81 | DTPY210 | DTPYC9F145-4-1 | CIMMYT, Mexico |

*ANGRAU: Acharya N.G. Ranga Agricultural University;
PAU: Punjab Agricultural University;
DMR: Directorate of Maize Research;
IARI: Indian Agricultural Research Institute;
CIMMYT: International Maize and Wheat Improvement Center

Trials were planted in the last week of November 2001 at Hyderabad (Experiment I) and in the first week of December 2003 at Hyderabad (Experiment II). The genetic materials in each experiment were evaluated in a randomized complete block design with two replications per genotype in the control as well as stress block (Experiment I) and with two replications per genotype only in the stress block (Experiments II and III). Each plot consisted of two rows (Experiment I) and one row (Experiment II), with a row length of 3m (15 plants per row), and a spacing of 15-20 cm between hills and 75 cm between rows. In all the experiments, the first two plants in the beginning and end of each plot were considered as 'border plants' and were not used for data recording. Agronomic practices for the control and stress blocks were as per the standard procedures, except for the irrigation schedule.

The irrigation schedules for the control and the stress blocks were same for the control and stress blocks till three weeks before flowering. The irrigation was stopped three weeks before flowering in the stress block, and no irrigation was applied until flowering was completed in the entries, leaving the barren genotypes. After the flowering period (around 100 days), both the control and the stress blocks were properly irrigated to ensure proper grain setting in the genotypes.

***Traits Analyzed*:** Measurements were taken on the following plant and ear characters: (i) plant stand; (ii) plant height (PH); (iii) ear height (EH); (iv) days to 50% male flowering or anthesis date (MFLW); (v) days to 50% female flowering or silking date (FFLW); (vi) anthesis-silking interval (ASI); (vii) tassel size (TS); (viii) number of ears per plant (ENO); (ix) leaf senescence (LS); (x) leaf rolling (LR); (xi) total grain yield per plot (TGY); (xii) ear length (EL); (xiii) ear diameter (ED); (xiv) number of kernels per ear (KNO); (xv) 100-kernel weight (HKW).

Plant height (in cm) was measured from the base of plant to base of the tassel. For all the trails male flowering and female flowering were measured on an individual plant basis. Male flowering was recorded as the number of days from sowing to the first anther extrusion from of the tassel glumes. Female flowering was the number of days from sowing to the first visible silk appearance. For Experiment I, ASI was calculated as the difference between the FFLW and MFLW on a plant-by-plant basis. In Experiment II, ASI was calculated as difference between FFLW and MFLW on a plot-by-plot basis. Ear height was recorded from the base of plant to base of the first ear.

Leaf rolling was scored on a scale from 1 to 5 (1 = unrolled, 2 = leaf rim starts to roll turgid, 3 = leaf has a V shape, 4 = rolled leaf rim covers part of leaf blade, 5 = leaf is rolled like an onion) as suggested by Banziger *et al.* (2000). Leaf rolling was measured before flowering when leaves were still more upright (Figure 1). Leaf senescence was scored on a scale from 1 to 10, as follows, dividing the percentage of estimated total leaf area that is dead by 10 (Figure 2).

| Scale | Dead leaf area | Scale | Dead leaf area |
|---|---|---|---|
| 1 | 10% | 6 | 60% |
| 2 | 20% | 7 | 70% |
| 3 | 30% | 8 | 80% |
| 4 | 40% | 9 | 90% |
| 5 | 50% | 10 | 100% |

Tassel size was scored on a scale from 1 (few branches, small tassel) to 5 (many branches, large tassel) (Figure 3).

The root capacitance of each genotype was measured in Experiment III on the DTPY and DTPW inbred lines using a BK Precision (Model 810c) Electrical Capacitance Meter (Figure 4), operating at a frequency of 1 kHz in range between 200pF and 2µF, as per the procedure suggested by van Beem *et al.* (1998). Electrical contact with the plant was established by connecting the negative electrode to the maize stem via a battery clamp at 6 cm above ground level, usually corresponding to the second internode. The positive electrode was connected to a battery clamp to a copper ground rod 55 cm in length inserted into the potting substrate to a depth of 15 cm and positioned 5 cm away from the stem base (Figure 4). The instrument was adjusted at the 20-nF level by setting the readout to zero with the zero adjust

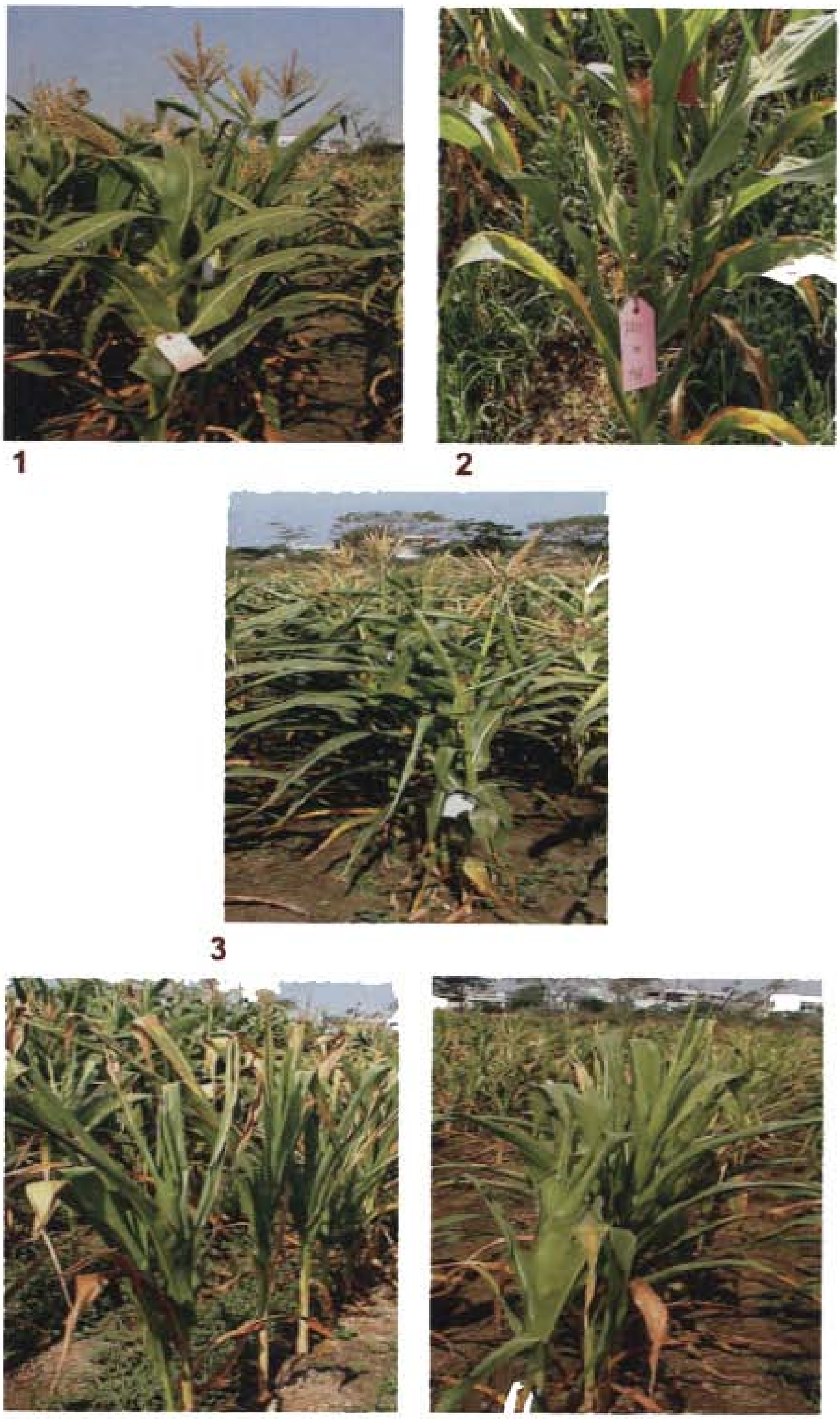


**Figure 1:** Scale of leaf rolling in genotypes under drought stress conditions
1. Unrolled, turgid; 2. Leaf rim starts to roll; 3. Leaf has V shape;
4. Rolled leaf covers part of the blade; 5. Leaf rolls like an onion.

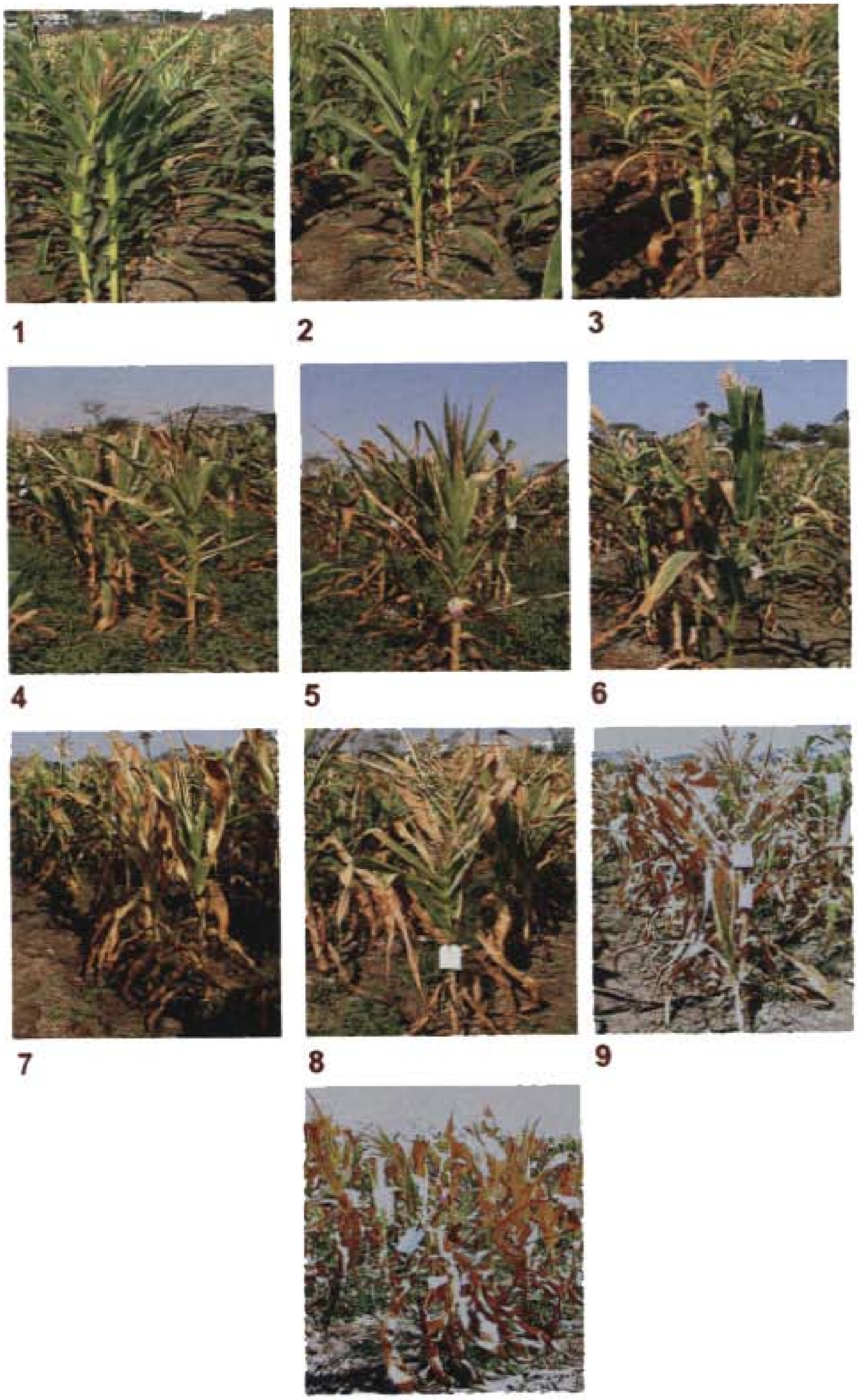


**Figure 2: Scale of leaf senescence in genotypes under drought stress conditions**
**1. 10% dead leaf area; 2. 20% dead leaf area; 3. 30% dead leaf area;**
**4. 40% dead leaf area; 5. 50% dead leaf area; 6. 60% dead leaf area;**
**7. 70% dead leaf area; 8. 80% dead leaf area; 9. 90% dead leaf area;**
**10. 100% dead leaf area**

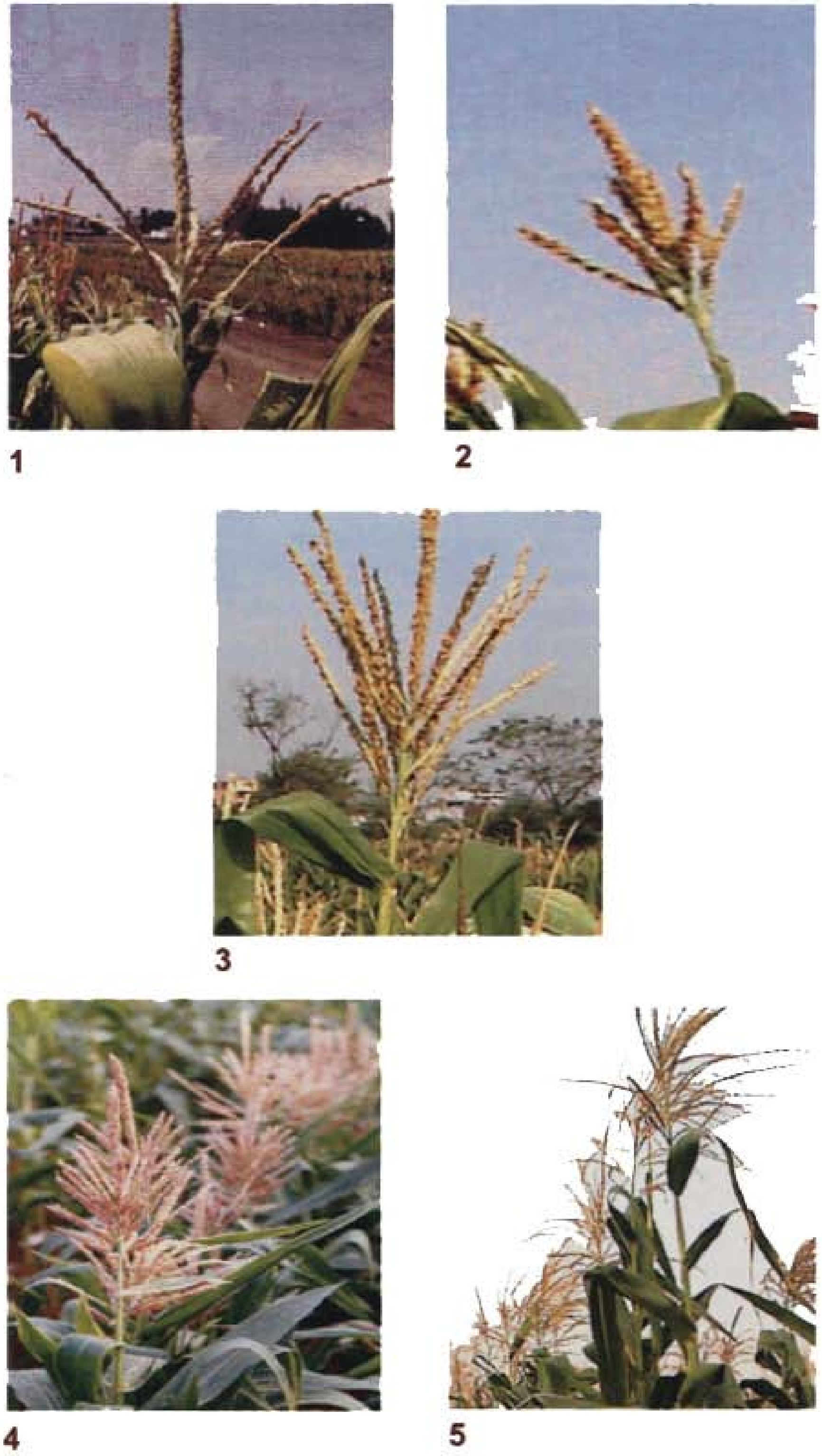


Figure 3: Rating of genotypes for tassel size under drought stress conditions based on a 1-5 scale (1 showing very few tassel branches to 5 showing many branches and large tassel)

Figure 4: Analysis of root capacitance of genotypes using a Root Capacitance Meter

knob. One capacitance measurement per plant was taken at 200nF after allowing 5 seconds for the system to stabilize (i.e., allowing the meter to reach a constant capacitance reading).

For grain yield per plant, all the plants in the plot were harvested and fresh dehusked ears were weighed. This was converted to ear weight at zero percent moisture by adjusting moisture content recorded at harvest. Grain yield per plot was then converted to grain yield per plant (in grams) at 15 per cent moisture content after making adjustments for shelling percent taken as 80 per cent using the following conversions:

Grain yield per plot (g) at 80% shelling (A) = Fresh ear weight (g)/plot x 80/100
Grain yield per plot at zero per cent moisture (B) = A x (100-M)/100
where M = moisture percent at harvest.
Grain yield per plot at 15% moisture = B x 100/85
Grain yield per plant = Grain yield per plot / Plant stand

The number of ears per plant was measured on the basis of the number of ears per plot divided by the plant stand, excluding the border plants.

***Statistical Analysis**:* The datasets from the three experiments were individually subjected to ANOVA using SAS version 6.12 statistical package. The general idea of ANOVA is to partition the total variability or total sum of square (SS) into components as specified by the underlying linear model:

$Y_{ijk} = \mu + R_i + G_j + \varepsilon_{ij}$

where $Y_{ij}$ denotes an observation for $j^{th}$ genotype in $i^{th}$ replication, $\mu$ denotes overall mean, $R_i$ refers to the effect of $i^{th}$ replication, $G_j$ denotes effect of jth genotype, $\varepsilon_{ij}$ refers to the random error associated with observation in $i^{th}$ replication and $j^{th}$ genotype.

Duncan's Multiple Range Test (DMRT) was carried out using SAS to compare the means of the 15 characters (as mentioned earlier) for the genotypes under study, separately for the two irrigation regimes (control and stress). DMRT uses several critical values for conducting the test, where the critical values for each subset of mean pairs are associated with the same number of steps, $R = 2,3, ..., k$. Duncan 's procedure also applies a different error rate, $R = (1 - \alpha)R$ for each subset associated with $R$ steps, $R = 2,3, ..., k$, in calculating all critical values. This procedure, thus, facilitated differentiation of the genotypes for a specific character based on statistically significant differences among the mean values.

***Derivation of 'Drought Tolerance Index' of the Genotypes:*** 'Drought tolerance index' (DTI) for each genotype was estimated on the procedure suggested by Banziger *et al.* (2000) was followed, with minor modifications. This index summarizes the worth of a genotype in terms of drought tolerance by making use of information from different traits such as grain yield, ASI, senescence etc. To consider together traits measured in different units, the phenotypic values, Pi, are usually standardized, as:

$$Pi = (x_{ij} - m_i)/s_i$$

where mi and si are the mean and standard deviation of trait i in a population and xij is the value of the trait i measured on genotype j. A selection index I in its simplest form can then be written as: $I = b_1P_1 + b_2P_2 + .... + b_nP_n$

where Pi is the observed standardized value of the trait i and bi is the weight given to that trait in the selection index.

Weights are chosen based on the relative economic value of each trait or based on the relative value of each trait as an indicator of drought tolerance (Banziger *et al.*, 2000).

| **Character** | **Weight** | **Sign** | **Desirable** |
|---|---|---|---|
| Grain yield | 5 | + | Increased grain yield |
| Ears per plot | 3 | + | Increased no. of ears per plot |
| ASI | 2 | - | Decreased ASI |
| Leaf senescence | 2 | - | Decreased leaf senescence |
| Tassel size | 2 | - | Decreased tassel size |
| Leaf rolling | 1 | - | Decreased leaf rolling |

While defining weights, one has to be careful about the right sign: positive where larger values are desired (e.g. grain yield), negative where lower values are desired (e.g. lodging and ASI).

## 2. Evaluation of Experimental Crosses for Drought Tolerance

***Genetic Materials:*** A selected of set of inbred lines were used to generate experimental crosses to generate 17 experimental hybrids (CM140 x CM207, Bio5 x CM139, CM140 x CM123, CM139 x Bio5, CM139 x 9424, 529 x IPA34, 529 x CM138, Bio-5 x Ac7729, Bio-5 x K64R, CM207 x IPA21A, CML247 x Bio-5, 500 x 519, Ac7729 x CML247, K64R x Bio5, and 530 x IPA21A). Due to significant variation in the flowering times between the Indian and exotic maize inbred lines, crosses could not be generated in a specific mating design.

***Methodology:*** The set of 17 experimental hybrids along with some of the parental lines were evaluated at ANGRAU Maize Research Station, Amberpet, Hyderabad during *Rabi* (winter season) 2002-03. The trial was planted in the last week of November 2002. The experimental materials were evaluated under two irrigation regimes (normal and stress) in an RBD experiment with two replications per genotype for each of the water regimes. Each plot consisted of two rows with a row length of 3m (15 plants per row), and a spacing of 15-20 cm between hills and 75 cm between rows. In all the experiments, the first two plants in the beginning and end of each plot were considered as 'border plants' and were not used for data recording. All other aspects of field and water management were as described earlier for the evaluation of inbred lines for drought tolerance (Experiments I and II). Details of trait measurement and statistical analyses were as explained previously, except that measurement on root capacitance was not recorded in this experiment.

## 3. Analysis of SSR Polymorphisms in Drought Tolerant and Susceptible Inbred Lines

***Genetic Materials***: A selected set of 12 inbred lines with distinct tolerance and susceptibility responses to drought stress at the flowering stage in maize were analyzed in this study. These include four DTPY lines (DTPY65, DTPY108, DTPY116 and DTPY194), three DTPW lines (DTPW51, DTPW105, DTPW183 and DTPW195), parental lines of a drought mapping population developed at CIMMYT, Mexico (Ac7643 and Ac7729), besides CM139 and CM140, which are the parental lines of a popular single cross hybrid in India 'Parkash'. These lines were selected based on: (i) distinct and consistent susceptibility/resistance reaction' responses against drought (based on the studies carried out during 2001 and 2003); and (ii) agronomic performance and current usage in maize breeding programmes in India and CIMMYT, Mexico.

***SSR Markers:*** Initially, 105 microsatellite or SSR markers were analyzed, of which 71 markers were found to be polymorphic (Table 2) *among the selected inbred lines.* Primers for these SSR loci were custom synthesized through Research Genetics Inc. USA or Sigma Aldrich, UK, based on forward and reverse primer sequence information available in public domain (MaizeDB; http://www.agron.missouri.edu) by the AMBIONET-India Lab, IARI, New Delhi.

The SSR markers were chosen based on their bin (genomic) locations to ensure, to the extent possible, uniform coverage of the genome. In addition, SSR markers located at specific bin locations with potential utility for QTL mapping of drought tolerance in maize (J.-M. Ribaut, personal communication) were utilized in the study. The maize SSR loci were previously designated as *'bnlg', 'phi', 'umc', 'nc'* or *'dupssr'* indicating the organization credited with their identification and characterization *(bnlg* Brookhaven National Laboratory, USA; *phi* - Pioneer Hi-Bred International, USA: *umc* - University of Missouri, Columbia, USA; *nc* - North Carolina State University, USA: *dupssr* - Dupont, USA etc.).

**Table 2. List of polymorphic SSR markers (along with bin locations) analyzed in the present study.**

| S. No. | Bin location | SSR locus | S. No. | Bin location | SSR locus |
|---|---|---|---|---|---|
| 1 | 1.01 | *phi097* | 37 | 6.01 | *phi077* |
| 2 | 1.02 | *bnlg147* | 38 | 6.01 | *umc1018* |
| 3 | 1.04 | *bnlg1016* | 39 | 6.02 | *umc1006* |
| 4 | 1.06 | *bnlg1556* | 40 | 6.03 | *phi070* |
| 5 | 1.08 | *phi037* | 41 | 6.03 | *umc1572* |
| 6 | 1.09 | *bnlg400* | 42 | 6.04 | *umc1014* |
| 7 | 1.10 | *bnlg1347* | 43 | 6.05 | *bnlg1154* |
| 8 | 1.11 | *bnlg504* | 44 | 6.05 | *bnlg1702* |
| 9 | 1.11 | *umc1331* | 45 | 6.05 | *mmc0241* |
| 10 | 2.01 | *bnlg1092* | 46 | 6.05 | *phi078* |
| 11 | 2.02 | *bnlg2042* | 47 | 6.05 | *phi102* |
| 12 | 2.02 | *bnlg1297* | 48 | 6.05 | *nc013* |
| 13 | 2.03 | *bnlg1064* | 49 | 6.08 | *phi089* |
| 14 | 2.03 | *umc1555* | 50 | 7.03 | *bnlg339* |
| 15 | 2.03 | *bnlg381* | 51 | 7.04 | *bnlg155* |
| 16 | 2.04 | *bnlg1613* | 52 | 7.06 | *phi116* |
| 17 | 2.04 | *bnlg1175* | 53 | 8.02 | *phi119* |
| 18 | 2.05 | *dup21* | 54 | 8.04 | *phi014* |
| 19 | 2.05 | *mage05* | 55 | 8.05 | *bnlg162* |
| 20 | 2.06 | *bnlg121* | 56 | 8.06 | *bnlg240* |
| 21 | 2.06 | *bnlg1138* | 57 | 8.06 | *bnlg1065* |
| 22 | 2.07 | *bnlg1045* | 58 | 8.08-8.09 | *phi015* |
| 23 | 2.08 | *bnlg198* | 59 | 8.08-8.09 | *phi080* |
| 24 | 2.09 | *bnlg1520* | 60 | 9.00 | *bnlg1272* |
| 25 | 3.05 | *bnlg420* | 61 | 9.01 | *phi033* |
| 26 | 3.08 | *phi088* | 62 | 9.03 | *bnlg1730* |
| 27 | 3.09 | *bnlg1182* | 63 | 9.05 | *bnlg1209* |
| 28 | 4.03 | *bnlg2244* | 64 | 10.00 | *phi041* |
| 29 | 4.05 | *bnlg1729* | 65 | 10.02 | *phi059* |
| 30 | 4.06 | *bnlg252* | 66 | 10.03 | *bnlg210* |
| 31 | 4.11 | *bnlg589* | 67 | 10.03 | *bnlg1655* |
| 32 | 5.02 | *phi113* | 68 | 10.04 | *bnlg1518* |
| 33 | 5.04 | *bnlg2323* | 69 | 10.04 | *bnlg1526* |
| 34 | 5.06 | *phi101* | 70 | 10.06 | *bnlg594* |
| 35 | 5.09 | *bnlg389* | 71 | 10.06 | *phi035* |
| 36 | 6.00 | *bnlg1043* | | | |

### *Methodology*

The following common procedures (unless otherwise specified) were followed for analysis of SSR polymorphisms in the selected genotypes.

(a) Isolation of genomic DNA and DNA quantification;

(b) DNA amplification using specific SSR primers by polymerase chain reaction (PCR);

(c) Agarose gel electrophoresis, using super fine resolution (SFR) agarose, for resolving the amplified products; and

(d) Molecular marker data analysis.

**(a) Genomic DNA Isolation, Quantification and Quality Analysis:** DNA was extracted from maize seedlings using the CTAB procedure developed by Saghai-Maroof *et al.* (1984) with suitable modifications. Healthy leaves from 3-4 week old seedlings (15-20) were collected, and ground into a fine powder (2g) .The powder was transferred to labeled polypropylene centrifuge tube containing 8 ml of warm CT AB extraction buffer and incubated for 1h at 65°C with occasional stirring. The tubes were removed, the contents were allowed to cool, and an equal volume (8 ml) of chloroform: isoamylalcohol (24:1) was added and mixed thoroughly by gentle inversion. The contents were centrifuged at 3500 rpm for 15 min at room temperature. The supernatant was transferred into new tubes and 6 ml of chilled isopropanol was added. The contents were mixed gently by inversion till strands of precipitated DNA were visible, and were left at 4°C overnight for effective precipitation of DNA. The precipitated DNA was hooked out and washed with 1 ml of 70% ethanol and 1.3 ml of 7.5M ammonium acetate. The pellet was air dried at room temperature and the dry pellet was dissolved in adequate amount of Tris EDTA (TE) buffer. RNase A (2.5 µl from a stock of 10 mg/ml) was added to the dissolved DNA and incubated at 37°C for one hour. DNA was again precipitated with the addition of two volumes of absolute ethanol and 1/10*th* volume of l0 mM ammonium acetate, and later on pelleted by high-speed centrifugation for 10 min. The pellet was rinsed with 1 ml of 70% ethanol for 20 min, air dried and dissolved in adequate amount of TE.

Agarose gel electrophoresis (0.8% agarose) was carried out for DNA quantification and quality analysis, using standard protocol, by comparison with λ DNA samples of known quantity (20 ng / 40 ng / 80 ng per µl) run on the same gel. DNA working stocks were prepared for PCR from the original stocks by diluting them to a concentration of ~10 ng/ µl of DNA.

**(b) PCR and Resolution of SSR Amplification Products:** PCR was carried out using SSR primers (forward + reverse) in MJ Thermal Cycler, USA (Model PTC-I00). The components of the PCR reaction mixture and thermal cycling conditions were as follows:

| Stock | Final Conc. | 15 µl Reaction Mix |
|---|---|---|
| 10X PCR Buffer (MBI Fermentas) | 1X | 1.20 |
| 25mM $MgCl_2$ (MBI Fermentas) | 0.75mM | 0.36 |
| 10mM dNTPs (MBI Fermentas) | 0.1mM | 0.12 |
| SSR primers F+R (1 µl) | 0.25 µm | 3.00 |
| Taq DNA Polymerase (3 µ/ µl) (MBI Fermentas) | 0.025 µ/ µl | 0.10 |
| $ddH_2O$ | | 4.22 |
| DNA | | 3.00 |

PCR cycling consisted of initial denaturation at 94°C for 2 min, followed by 30-35 cycles of amplification, at 94°C for 1 min, 55-65°C (based on annealing temperatures standardized for different SSR primers) for 2 min, and 72°C for 2 min; the final extension step at 72°C for 7 min was followed by termination of the cycle at 4°C.

The amplified products were resolved in a 3.5% SFR (Amresco) agarose gel at 100-125V for 1.30 h using a submarine gel electrophoresis system (Biorad), as per the procedure suggested by Kassahun and Prasanna (2003). The SFR agarose has the capacity to resolve amplified fragments ranging from 75 to 750 bp. A I00-bp DNA ladder or φXI74/HaeIII ladder was run alongside the amplified products to determine the approximate size of each SSR allele.

**(c) SSR Allele Scoring:** The gels were photographed using a CCD camera (Sony XC-75 CE) attached to a gel documentation system with the Biocapture software (Vilber Lourrnat, France), and scoring was carried out manually. The SSR allele sizes were determined depending on the positions of the bands relative to the ladder. Band patterns for each of the SSR markers were recorded for each genotype by assigning a letter to each band, like a1, a2 etc. sequentially from the largest to the smallest sized band. Any band thought to be an artifact or bands which are either too diffuse or highly faint or those that are too difficult to score were considered as 'missing data'.

The SSR amplification patterns were scored, and the data was entered in MS Excel in a binary mode "0" for absence of an allele; "1" for the presence of an allele; and '9' representing the missing data, if any, for a 'SSR marker.

**(d) Statistical Analysis of SSR Data:** The data from the 12 inbred lines for various polymorphic SSR loci was subjected to statistical analysis as follows. For each polymorphic SSR marker, **'Polymorphism Information Content' (PIC)** was determined as described by Senior *et al.* (1998) and Kassahun and Prasanna (2003). PIC is a measure of allele diversity at a locus and is equal to $1 - \Sigma(P^2{ij})$, where $Pij$ is the frequency of the $j$th allele for $i$th locus summed across all alleles in the locus. PIC is synonymous with the term "gene diversity" as described by Weir (1996).

**Genetic similarity** (GS) based on SSR data can be calculated for all possible pairs of genotypes using various coefficients; this, in turn provides information about the genetic distance between genotypes as Genetic Distance (GD) = 1 - GS. In the present study, Jaccard's (1908) coefficient (J), based on the formula, $J = N_{11} / (N_{11} + N_{10} + N_{01})$ was used to estimate genetic similarities, where $N_{11}$ is the number of bands present in both individuals; $N_{10}$

is the number bands present only in one of the pair (individual *I*); $N_{01}$ is the number of bands present only in the individual *j*.

Values of GS may range from '1' (identical profiles for all markers in the two inbreds) to '0' (no bands in common). The similarity matrix was further analyzed using NTSYS-pc 2.11 (Rohlf, 1992), by employing UPGMA (Unweighted Paired Group Method using Arithmetic averages) with average linkage (Sneath and Sokal, 1973). To test the goodness of fit of a clustering to a set of data (in this case, the SSR data) **'cophenetic correlation coefficient'** or cophenetic value was estimated using the COPH and MXCOMP options in NTSYS-pc program. The degree of fit can be interpreted subjectively as follows:

| Level | Interpretation |
|---|---|
| $0.9 \leq r$ | Excellent fit |
| $0.8 \leq r < 0.9$ | Good fit |
| $0.7 \leq r < 0.8$ | Moderate fit |
| $r < 0.7$ | Poor fit |

**'Bootstrap' analysis** is a general resampling procedure for estimating the distribution of a statistic based on independent observations (Efron, 1965). The technique resamples the actual data to reveal some its subtler patterns. The basic notion is that the data themselves, viewed as a frequency distribution, represent the best available image of the frequency distribution from which they were drawn. Bootstrapping can be effectively utilized for estimating the statistical support to the internal branches in a tree (Felsenstein, 1985). In the present study, bootstrap analysis was carried out on the SSR dataset to determine the statistical support to the internal branches of the dendrogram. The WINBOOT software developed by IRRI, Philippines (http://www.irri.org) was utilized for bootstrap analysis.

**Principal component analysis (PCA)** is an ordination technique that produces a visual representation of the relative positions of genotypes in a space of reduced dimensions, thus indicating spatial relationships among genotypes (Mohammadi and Prasanna, 2003). In the present investigation, PCA was employed to summarize the variation of the original SSR dataset, and to make a graphical presentation of the genetic relationships among the 12 selected inbred lines. The analysis was carried out using NTSYS-pc 2.11.

## 4. Mapping QTLs conferring Drought Tolerance in Maize

***Mapping Populations:*** For the purpose of QTL mapping of drought tolerance, 236 RIL families and 240 $F_3$ families were developed at CIMMYT-Applied Biotechnology Center, Mexico, using Ac7643$S_5$ (drought tolerant) as female parent and Ac7729/TZSRWS5 (drought susceptible) as male parent, with the respective lines showing considerable contrasts especially for anthesis-silking-interval (ASI) under drought condition. The RILs were derived by single seed descent method from the $F_2$ plants (Ribaut *et al.*, 1996, 1997; Fracheboud *et al.*, 2002).

***Phenotyping of Mapping Populations:*** "Phenotyping", in the context *of* QTL mapping, refers to analysis or screening of the mapping population (in the present case, RILs) for the target trait(s) of interest. The RIL families, along with the parental lines, were evaluated under an alpha-lattice design with two replications per genotype, and one row per replication, in two different water regimes (control and stress conditions), during *rabi* (dry season) 2001-2002 at ANGRAU Research Station at Karimnagar.

Measurements were taken on the following plant and ear characters: (i) plant height (PH); (ii) ear height (EH); (iii) days to 50% male flowering or anthesis date (MFLW); (iv) days to 50% female flowering or silking date (FFLW); (v) anthesis-silking interval (ASI); (vi) tassel size (TS); (vii) number of ears per plant (ENO); (viii) leaf senescence (LS); (ix) leaf rolling (LR); (x) grain yield per ear (GY); (xi) kernel number per ear (KNO); and (xii) 100-kernel weight (HKW), following the procedures described earlier in this Chapter.

The RIL families were also evaluated under drought stress conditions during *rabi* 2003-2004 at the Maize Winter Nursery, Amberpet, Hyderabad, using an alpha-lattice design with two replications and one row per replication. Field management and data recording were as per the procedures mentioned earlier. In stress conditions, the irrigation regime was designed such that drought at flowering was severe enough to delay silking and cause ear abortion.

The phenotypic datasets from Karimnagar and Hyderabad were analyzed separately. The datasets were subjected to ANOVA using Alpha and SAS statistical packages. Pearson correlation coefficients were calculated, as per the standard procedure, between the pairs of characters traits separately for the datasets from the control/well-watered and drought stress blocks of the Karimnagar Experiment, and for the drought stress dataset for the Hyderabad Experiment.

**Genotyping of Mapping Populations and Linkage Mapping:** 'Genotyping' refers to analysis of the individuals constituting a mapping population using DNA markers that were found polymorphic between the parental lines. The mapping populations (RILs) were genotyped at CIMMYT-Applied Biotechnology Center, Mexico, earlier by Ribaut *et al.* (1996, 1997), using 138 RFLP markers, and the procedure was described by Fracheboud *et al.* (2002). A linkage map of a total length of 2250 cM with an average density of 17cM was constructed using Mapmaker Version 3.0 (Lander *et al.*, 1989). The longest distance between two consecutive markers (72.2 cM) was located on chr.4 between 12.3 and 84.5 cM. Apart from this large gap, five other gaps of 40-50 cM were identified on chr.3 (two of them), 5, 6 and 10.

**QTL Mapping:** The genotypic data and the phenotypic data for each environment (Karimnagar/Hyderabad) and the mapping population (RILs) were analyzed using composite interval mapping models (Zeng, 1994) map QTLs and to estimate their genetic effects. A series of four models was used in the analysis: Model I, which is simple interval mapping (Lander and Botstein, 1989 or Model III of Zeng, 1994), was used for the selection of cofactors; Model II, which is composite interval mapping with unlinked markers as cofactors (Model 11 of Zeng, 1994), was used to maximize QTL detection; and Models III and IV (Model I of Zeng, 1994) with selected markers as cofactors were used to confirm the QTLs detected in Model II. Model III uses two markers flanking the interval with a minimum map distance of 30 centimorgan (cM) (size window = 30cM) while Model IV uses linked factors within a minimum map distance of 20 cM (size window = 20cM). Details of the principle behind each of these models were provided by Zeng (1994).

The linkage disequilibrium between a marker and an adjacent chromosomal region, indicating the presence of a QTL, is inferred on the basis of likelihood ratio (LR) peaks. The general relationship between LOD ($\log_{10}$ of the odds) and the LR is as follows: LR = 2 ln(l0)LOD. LOD scores are the ratio of the probability that data show linkage at a specific recombination frequency, versus the chance that the data show no linkage; therefore the recombination frequency is 0.5. A LOD score threshold of 2.4 gives a probability of less than 5% that even a single false positive will occur anywhere in the genome.

For a single trait analysis using a RIL mapping population, and for the LOD scores of 2.0, 2.5 and 3.0, the corresponding critical LR values are 9.21, 11.51 and 13.82, respectively. This is applicable for the Type I error rates (α level) of 0.01, 0.0031 and 0.001 for LOD=2.0, LOD=2.5 and LOD=3.0, respectively. The Type I error rates (α level) at different LOD thresholds (2.0, 2.5 and 3.0) applicable for mapping populations were estimated using chi.sas package developed at CIMMYT-Applied Biotechnology Center, Mexico; the principle behind a level is as follows:

Assume that:

$\alpha$ = Prob {LR $\geq c$ at a fixed locus}

where $c$ is assumed to be a critical value used in the test. Then, assuming the total number of independent tests over the whole genome is k, the genome-wise error rate with the same critical value $c$ would be:

$\acute{\alpha}$ = Prob {LR $\geq c$ at $k$ independent loci}

= l-(1-a) k ≈ $ka$

We are required to control the genome-wise error rate to a certain level, such as $\alpha = 0.05$. We may use the approximation to obtain $\alpha \approx \acute{\alpha} / k$. The exact value of $k$, is however unknown in this case and we have to obtain, an approximate value for practical use. Assuming each of the chromosomes in a maize genome is 2M in size, one marker on each chromosome arm would be assumed to segregate independently. Therefore, we may use $k = 20$, $\alpha = 0.0025$ for $\acute{\alpha} = 0.05$, which is approximately equivalent to the $\alpha$ values corresponding to LOD=2.5 in case of a mapping population. In the present study, the threshold used for QTL detection was set at a LR value corresponding to LOD>2.0, 2.5 or 3.0.

Besides the critical threshold, the decision whether a QTL was present a given chromosomal position was based on the following: (i) A QTL was declared as present when a significant likelihood ratio (LR) peak was detected by Model II and Model III also yielded an LR peak at the same or in adjacent position. In this case, it was not required that the LR peak found by Model III exceed the critical threshold; (ii) if the LR peak identified by Model II at a specific position was not confirmed by Model III, the hypothesis of the presence of a putative QTL at this position was rejected. This result was taken as indicative for the presence of multiple linked QTLs in adjacent regions; (iii) when separated by at least two markers and a minimum distance of 20 cM, two peaks on one chromosome were considered as two different QTLs. Otherwise, the higher peak was chosen to represent the QTL; (iv) a QTL solely detected with Model **III** was regarded as significant without further confirmation; and (v) a QTL was declared whenever the LR is significant in individual analysis in a specific environment and/or in the joint analysis over all environments.

# Results

## 1. Analysis of Genetic Variability in the Maize Inbred Lines for Drought Tolerance

Experiments were carried out at the Acharya N.G. Ranga Agricultural University (ANGRAU) Maize Research Station at Amberpet, Hyderabad during *Rabi* (winter season) 2001-02 and 2003-04 to evaluate the responses of maize inbred lines from India and CIMMYT, Mexico, for their responses to drought stress at the flowering stage. These experiments shall be hereafter referred to as Experiment I and Experiment II, respectively.

The characters recorded in these experiments were: (i) plant height; (ii) ear height; (iii) anthesis-silking interval (ASI); (iv) tassel size; (v) number of ears per plant; (vi) leaf senescence; (vii) leaf rolling; (viii) grain yield per plot; (ix) grain yield per plant; (x) grain yield per ear; (xi) 100-kernel weight; (xii) number of kernels per ear; (xiii) ear length; and (xiv) ear diameter.

**Experiment I**: In this experiment, a set of 18 inbred lines, including 9 lines developed by different maize research centers in India, 4 lines derived from the CIMMYT maize populations, and 5 lines obtained from the CIMMYT Applied Biotechnology Centre, Mexico, were evaluated both under well-watered (control) and stress conditions.

Analysis of variance (ANOVA) revealed significant differences between the two treatments (control vs. stress) for all the characters except ear height (Table 3). The differences among the genotypes for several characters were found to be significant, except for ear height, grain yield and number of kernels rows per ear. ANOVA for the dataset from the stress block indicated significant differences among the genotypes for all characters, except plant height, ear height, tassel size, leaf rolling, leaf senescence and ear diameter (Table 4). No significant differences between the replications were noticed for the traits under study, except for leaf senescence.

Table 3. ANOVA for various characters in the inbred lines evaluated at Hyderabad (2001-02; Experiment I) under two different water regimes

| Characters | Mean Sum of Squares | | | |
|---|---|---|---|---|
| | Error | Treatment | Replication | Genotype |
| Plant height | 454.03 | 5794.26** | 10.81 | 1741.14** |
| Ear height | 183.97 | 23.72 | 28.03 | 320.56 |
| ASI | 4.18 | 169.9** | 8.40 | 10.17** |
| Tassel size | 0.55 | 4.50** | 0.89 | 1.32** |
| Leaf rolling | 0.44 | 8.68** | 0.01 | 0.59 |
| Leaf senescence | 1.61 | 82.34** | 5.01 | 5.73** |
| No. of ears per plant | 0.07 | 2.10** | 0.07 | 0.14* |
| Yield per plot | 410.64 | 21139.79** | 1.86 | 1369.50** |
| Grain yield per ear | 72.63 | 191.52 | 0.93 | 227.36** |
| Ear length | 20.11 | 557.93** | 5.48 | 63.43** |
| Ear diameter | 2.19 | 179.20** | 1.04 | 11.18** |
| No. of kernel rows | 8.03 | 108.83** | 18.93 | 13.41 |
| No. of kernels per ear row | 27.77 | 2213.72** | 1.67 | 135.13** |
| Kernel number per ear | 7456.00 | 467789.24** | 614.18 | 29485.02** |
| 100-kernel weight | 13.77 | 236.95** | 1.19 | 36.69** |

*Significant at P = 0.05 ; **Significant at P = 0.01

Table 4. ANOVA for various characters under drought stress conditions in the inbred lines evaluated at Hyderabad (2001-02; Experiment I)

| Characters | Mean Sum of Squares | | |
|---|---|---|---|
| | Error | Replication | Genotype |
| Plant height | 371.53 | 0.00 | 778.55 |
| Ear height | 141.55 | 62.22 | 150.41 |
| ASI | 3.74 | 13.81 | 17.25** |
| Tassel size | 0.89 | 1.36 | 0.97 |
| Leaf rolling | 0.91 | 0.03 | 0.83 |
| Leaf senescence | 2.15 | 13.44* | 3.38 |
| No. of ears per plant | 0.01 | 0.00 | 0.05** |
| Yield per plot | 46.21 | 56.7 | 265.34** |
| Grain yield per ear | 16.9 | 4.07 | 113.33** |
| Ear length | 3.02 | 0.00 | 16.59** |
| Ear diameter | 4.35 | 1.90 | 8.30 |
| No. of kernel rows | 4.11 | 2.69 | 19.47** |
| No. of kernels per ear row | 10.98 | 1.19 | 39.77** |
| Kernel number per ear | 2092.06 | 575.18 | 9772.89** |
| 100-kernel weight | 8.53 | 1.47 | 21.73* |

*Significant at P = 0.05; **Significant at P = 0.01

Duncan's Multiple Range Test (DMRT) was carried out to compare the mean values for each character for the genotypes under study. A comparison of the mean values under the control (Table 5) and stress conditions (Table 6) revealed significant effects of drought stress on plant and ear characters of the maize genotypes under study. For example, among the inbred lines Ac7729 recorded a mean plant height of 149.7cm in the control block, whereas under stress conditions the mean plant height was only 119cm. Significant variation for plant height (67.5cm in IPA21A to 129.9cm in CML444) could be noticed among the genotypes under stress conditions; however, the range with respect to ear height was not considerable.

ASI, which is considered as one of the key secondary traits influencing the performance of maize under drought stress conditions, ranged from 0 days in CM207 to 12 days in CIM500, whereas the range for ASI was 1.7 days (Ac7729) to 2.9 days (CM139) under control/well-watered conditions. The lowest ASI values under drought stress conditions (0, 1.6 and 3.4 days) were recorded in CM207, CIM506 and FSA17. Interestingly, low ASI values in CM207 and CIM506 had not resulted in superior yield performance, as compared to FSA17, although genotypes showing very high ASI values like CIM500, Ac7729 and IPA34 recorded very poor grain yields under drought stress conditions.

As indicated by ANOVA, the variation with respect to tassel size, leaf rolling and leaf senescence were not significant among the genotypes analyzed, indicating their highly limited utility in differentiating drought tolerant lines from the susceptible ones. For number of ears per plant which is an indicator of productivity of the genotype, Bio-5, IPA29B and CIM506 recorded the highest values (1.05, 1.00 and 0.95, respectively) under control conditions, whereas FSA17, IPA29B and CM140 were found to be relatively more promising than other lines under drought stress conditions with the highest values (0.60, 0.50 and 0.30, respectively).

Analysis in terms of grain yield under control conditions revealed good *per se* performance of IPA29B, CIM500 and CM139. Under the drought stress conditions, the performance of IPA29B, FSA17, Ac7643 and CM140

**Table 5. Performance of inbred lines for yield and yield-related characters under normal conditions (Experiment I; Hyderabad 2001-02)**

| S.No. | Inbreds | PHT | EHT | ASI | TS | LR | LS | ENO | YLD | EL | ED | NKR | KPR | KNO | 100WT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | CIM506 | 98.7 | 55.1 | 2.4 | 5.0 | 1.0 | 1.0$^{I}$ | 0.9$^{I}$ | 634.0 | 11.7 | 11.1 | 13.7 | 24.7 | 338.2 | 16.6 |
| 2 | CIM495 | 135.4 | 77.1$^{II}$ | 1.8 | 4.5 | 1.0 | 2.5 | 0.6 | 350.0 | 10.0 | 10.2 | 13.6 | 16.9 | 229.8 | 13.0 |
| 3 | Ac7643 | 134.4 | 66.2 | 2.2 | 5.0 | 1.0 | 2.0 | 0.2 | 407.0 | 33.2$^{I}$ | 12.6 | 15.6$^{I}$ | 27.5 | 423.0$^{II}$ | 16.5 |
| 4 | FSA17 | 121.3 | 61.6 | 2.5 | 3.0$^{II}$ | 1.0 | 2.0 | 0.5 | 470.0 | 10.4 | 10.7 | 14.2 | 20.7 | 298.1 | 11.8 |
| 5 | IPA29A | 98.9 | 53.1 | 2.7 | 4.5 | 1.5 | 5.5 | 0.2 | 200.0 | 9.0 | 10.4 | 8.9 | 19.4 | 158.4 | 10.2 |
| 6 | CM207 | 92.6 | 54.5 | 2.0 | 4.0 | 1.0 | 1.5$^{I}$ | 0.7 | 760.0 | 10.1 | 10.8 | 14.7 | 18.0 | 264.8 | 19.3 |
| 7 | CM140 | 93.3 | 48.9 | 1.8 | 3.5 | 1.0 | 4.0 | 0.5 | 447.0 | 13.8 | 10.4 | 10.9 | 29.2$^{III}$ | 319.5 | 15.1 |
| 8 | IPA34 | 100.2 | 55.7 | 2.4 | 3.5 | 1.0 | 3.5 | 0.1 | 90.0 | 7.1 | 8.1 | 11.2 | 13.3 | 149.3 | 10.7 |
| 9 | Bio-5 | 109.5 | 70.8 | 2.2 | 4.5 | 2.0 | 5.0 | 1.0 | 800.0 | 13.1 | 13.0$^{III}$ | 14.4 | 26.0 | 368.9 | 20.7 |
| 10 | CM139 | 117.5 | 60.2 | 2.9 | 5.0 | 1.5 | 2.0 | 0.8$^{II}$ | 1350.0$^{II}$ | 14.1 | 11.9 | 13.8 | 23.2 | 319.1 | 19.3 |
| 11 | IPA29B | 139.7$^{III}$ | 71.7$^{III}$ | 1.7$^{III}$ | 5.0 | 1.0 | 3.5 | 0.6 | 2025.0$^{I}$ | 16.5$^{III}$ | 13.9$^{I}$ | 13.6 | 35.1$^{I}$ | 477.4$^{I}$ | 23.0$^{I}$ |
| 12 | K64R | 137.6 | 69.6 | 2.2 | 3.5 | 1.0 | 1.5$^{I}$ | 0.6 | 980.0 | 12.8 | 11.2 | 13.3 | 28.8$^{III}$ | 382.2 | 18.1 |
| 13 | CML444 | 149.7$^{III}$ | 35.1 | 1.5$^{II}$ | 3.5 | 1.0 | 1.0$^{I}$ | 0.3 | 521.0 | 11.0 | 10.0 | 12.8 | 22.9 | 293.4 | 18.6 |
| 14 | CIM493 | 64.0 | 78.5$^{II}$ | 5.2 | 2.5$^{I}$ | 0.5$^{I}$ | 3.5 | 0.2 | 65.0 | 14.5 | 6.1 | 13.9 | 14.6 | 32.7 | 22.6$^{II}$ |
| 15 | Ac7729 | 177.2$^{I}$ | 98.6$^{I}$ | 1.1$^{I}$ | 4.0 | 1.0 | 5.5 | 0.8$^{II}$ | 977.5 | 13.0 | 12.0 | 13.2 | 28.8 | 380.2 | 17.9 |
| 16 | CIM500 | 151.4$^{II}$ | 79.0$^{II}$ | 2.5 | 4.0 | 1.0 | 1.5$^{I}$ | 0.8$^{II}$ | 1410.0$^{II}$ | 27.9$^{II}$ | 12.6 | 13.5 | 30.3$^{II}$ | 409.2$^{III}$ | 21.1$^{III}$ |
| 17 | CML247 | 126.6 | 59.7 | 2.6 | 3.5 | 1.0 | 2.5 | 0.5 | 475.0 | 13.5 | 13.4$^{II}$ | 14.6 | 25.8 | 383.5 | 18.3 |
| 18 | IPA21A | 85.1 | 45.5 | 2.8 | 4.0 | 1.0 | 3.5 | 0.5 | 440.0 | 9.4 | 10.7 | 16.2$^{I}$ | 19.8 | 317.9 | 13.6 |

PHT: Plant height (cm); EHT: Ear height (cm); ASI: Anthesis-silking interval; TS: Tassel size; LR: Leaf rolling; LS: Leaf senescence; ENO: ear number per plant; YLD: Grain yield per plot (g); EL: Ear length (cm); ED: Ear diameter (cm); NKR: No. of kernel rows per ear; KPR: Kernels per ear row; KNO: Kernel number per ear; 100WT: 100-kernel weight (g); Numbers in superscripts are DMRT ranks

**Table 6. Performance of inbred lines for yield and yield-related characters under drought stress conditions (Experiment I; Hyderabad 2001-02)**

| S.No. | Inbreds | PHT | EHT | ASI | TS | LR | LS | ENO | YLD | EL | ED | NKR | KPR | KNO | 100WT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | CIM506 | 68.7 | 52.8 | $1.6^{II}$ | 3.5 | $1.0^{I}$ | $3.5^{I}$ | 0.2 | 79.0 | 7.2 | 6.8 | 10.2 | 9.8 | 100.0 | 9.0 |
| 2 | CIM495 | $120.9^{II}$ | 59.3 | 3.5 | 3.0 | $1.0^{I}$ | $3.0^{I}$ | $0.3^{II}$ | 156.5 | $9.8^{I}$ | 8.8 | 11.8 | 8.3 | 97.7 | 11.3 |
| 3 | Ac7643 | 100.6 | 66.1 | 4.1 | 4.0 | 1.5 | 4.5 | $0.2^{III}$ | $216.5^{II}$ | $10.6^{I}$ | $9.5^{I}$ | 11.9 | 10.2 | 132.9 | 15.6 |
| 4 | FSA17 | 108.5 | 51.5 | $3.4^{III}$ | $2.5^{I}$ | 2.0 | $4.0^{I}$ | $0.6^{I}$ | $219.5^{II}$ | 8.7 | $9.2^{I}$ | $14.4^{I}$ | 13.0 | $187.5^{III}$ | 9.3 |
| 5 | IPA29A | $113.5^{III}$ | 69.5 | 3.9 | 4.5 | 2.0 | 6.0 | $0.3^{II}$ | 119.5 | 7.2 | 8.9 | $13.1^{II}$ | $15.9^{I}$ | $208.6^{II}$ | 11.4 |
| 6 | CM207 | 86.0 | 48.6 | $0.0^{I}$ | 3.5 | 2.0 | 5.5 | 0.1 | 49.0 | 5.3 | 6.8 | 9.6 | 5.9 | 56.7 | 15.1 |
| 7 | CM140 | 75.7 | 52.5 | 4.5 | 4.0 | 2.0 | 6.0 | $0.3^{II}$ | 187.5 | 9.0 | 9.4 | 12.0 | $15.2^{I}$ | $182.4^{III}$ | 13.2 |
| 8 | IPA34 | 86.7 | 60.4 | 9.5 | 3.5 | 1.5 | 5.5 | 0.0 | 12.0 | 1.7 | 3.7 | 3.0 | 4.5 | 15.0 | 14.5 |
| 9 | Bio-5 | 80.0 | 54.5 | 4.7 | 3.5 | 2.5 | 4.5 | 0.1 | 80.0 | 8.3 | 8.7 | 12.6 | 9.8 | 126.3 | $16.1^{III}$ |
| 10 | CM139 | 87.0 | 60.0 | 5.9 | 4.0 | 2.0 | 5.0 | 0.1 | 73.0 | $9.9^{I}$ | 8.8 | 11.0 | 8.5 | 95.0 | $16.6^{II}$ |
| 11 | IPA29B | 110.5 | 72.0 | 3.8 | 5.0 | 1.5 | $4.0^{I}$ | $0.5^{I}$ | $597.5^{I}$ | $12.0^{I}$ | $10.9^{I}$ | $14.0^{I}$ | $21.0^{I}$ | $293.5^{I}$ | 14.0 |
| 12 | K64R | 101.2 | 63.1 | 6.2 | $2.5^{I}$ | $1.0^{I}$ | $2.5^{I}$ | 0.2 | 45.0 | 7.8 | 6.5 | 8.4 | 5.0 | 43.0 | 13.0 |
| 13 | CML444 | $129.9^{I}$ | 70.6 | $3.2^{III}$ | 3.5 | 1.5 | 4.5 | 0.1 | 125.5 | 9.2 | 8.9 | 11.2 | 12.2 | 138.2 | 13.7 |
| 14 | CIM493 | 95.7 | 74.0 | 6.1 | $2.5^{I}$ | 1.0 | 4.5 | 0.0 | 12.0 | 3.0 | 3.5 | 4.0 | 4.0 | 32.0 | 5.0 |
| 15 | Ac7729 | $118.8^{II}$ | 73.5 | 8.6 | 3.0 | 2.0 | 8.0 | 0.1 | 49.0 | 6.9 | $9.4^{I}$ | 10.1 | 1.0 | 14.0 | 10.5 |
| 16 | CIM500 | 105.9 | 59.3 | 12.0 | 4.0 | 3.5 | 5.0 | 0.1 | 82.5 | 7.6 | 7.2 | 10.7 | 8.8 | 97.1 | 12.6 |
| 17 | CML247 | $130.8^{I}$ | 71.9 | 8.6 | 3.0 | 2.5 | 6.0 | 0.1 | 137.5 | 9.0 | 8.9 | $13.0^{II}$ | $14.5^{I}$ | 141.0 | 24.0 |
| 18 | IPA21A | 67.5 | 51.3 | 7.2 | 4.0 | 2.0 | 6.0 | 0.1 | 55.0 | 5.1 | 6.8 | 11.3 | $13.5^{I}$ | 153.2 | 11.4 |

PHT: Plant height (cm); EHT: Ear height (cm); ASI: Anthesis-silking interval; TS: Tassel size; LR: Leaf rolling; LS: Leaf senescence; ENO: ear number per plant; YLD: Grain yield per plot (g); EL: Ear length (cm); ED: Ear diameter (cm); NKR: No. of kernel rows per ear; KPR: Kernels per ear row; KNO: Kernel number per ear; 100WT: 100-kernel weight (g); Numbers in superscripts are DMRT ranks.

was encouraging (Figure 5). These genotypes were also relatively superior with respect to kernel number per ear, although they were not among the top-ranked genotypes for 100-kernel weight. IPA29B and FSA17 were also promising in terms of ear length and ear diameter under drought stress conditions. Overall, based on DMRT ranking for different characters influencing the performance of the genotypes under control/well-watered as well as drought stress conditions, FSA17, IPA29B, Ac7643 and CM140 can be considered as promising among the inbred lines analyzed in Experiment I. Among the highly susceptible lines were IPA34 (Figure 5), CIM500 and Ac7729 (Table 7).

'Drought tolerance index' (DTI) for each genotype was calculated using the model suggested by Banziger *et al.* (2000), with some minor modifications, as explained in 'Materials and Methods'. The model gives different weightage for traits such as grain yield, ASI, leaf senescence etc., based on the relative value of each trait as an indicator for drought tolerance. Based on DTI values, FSA17 (9.90) and IPA29B (7.90) can be considered as drought tolerant, whereas Ac7643 (4.80), CM140 (2.80) and CIM506 (2.30) were moderately tolerant. A comparison of the yield performance of the genotypes under both control and stress conditions, in conjunction with % yield penalty over control (Table 7), revealed that almost all genotypes were severely affected by drought stress at flowering stage. Nevertheless, IPA29A, Ac7643 and FSA17 were relatively less affected compared to other genotypes. Taking into account both DTI and % yield penalty over control, the study revealed the potential of IPA29B, FSA17, Ac7643 and CM140. It is important to note that elite maize lines such as Bio-5 (derivative of LM5), CM207 and CM139, were highly vulnerable to drought stress at the flowering stage and so were inbred lines derived from CIMMYT maize populations, like CML247, Ac7729, K64R, CML444, CIM493, CIM495, CIM500 and CIM506.

**Experiment II**: In this experiment, a selected set of genotypes developed by the CIMMYT Maize Programme, specifically for drought stress tolerance under tropical/sub-tropical maize-growing regions, were evaluated under

**Table 7. Comparison of yield performance and drought tolerance indices of inbred lines under control and stress conditions [Experiment I; Hyderabad 2001-02)**

| S. No. | Inbreds | Grain Yield (in g) (Control) | Grain Yield (in g) (Stress) | % yield penalty over control | DTI* |
|---|---|---|---|---|---|
| 1. | CIM506 | 634.0 | 79.0 | 87.5 | 2.3 |
| 2. | CIM495 | 350.0 | 156.5 | 55.3 | 1.8 |
| 3 | Ac7643 | 407.0 | 216.5 | 46.8 | 4.8 |
| 4. | FSA17 | 470.0 | 219.5 | 53.3 | 9.9 |
| 5. | IPA29A | 200.0 | 119.5 | 40.3 | -1.4 |
| 6. | CM207 | 760.0 | 49.0 | 93.6 | 0.1 |
| 7. | CM140 | 448.0 | 187.5 | 58.1 | 2.8 |
| 8. | IPA34 | 90.0 | 12.0 | 86.7 | -13.0 |
| 9. | Bio-5 | 800.0 | 80.0 | 90.0 | -0.9 |
| 10. | CM139 | 1315.0 | 73.0 | 94.4 | -5.1 |
| 11. | IPA29B | 2025.0 | 597.5 | 70.5 | 7.9 |
| 12. | K64R | 980.0 | 45.0 | 95.4 | 1.1 |
| 13. | CML444 | 522.0 | 125.5 | 76.0 | 1.1 |
| 14. | CIM493 | 65.0 | 12.0 | 81.5 | -5.1 |
| 15. | Ac7729 | 977.5 | 49.0 | 94.9 | -8.1 |
| 16. | CIM500 | 1410.0 | 82.5 | 94.1 | -12.0 |
| 17. | CML247 | 475.0 | 137.5 | 71.1 | 1.2 |
| 18. | IPA21A | 440.0 | 55.0 | 87.5 | -7.5 |

*Drought Tolerance Index (DTI), based on the model suggested by Banziger *et al.* (2000)

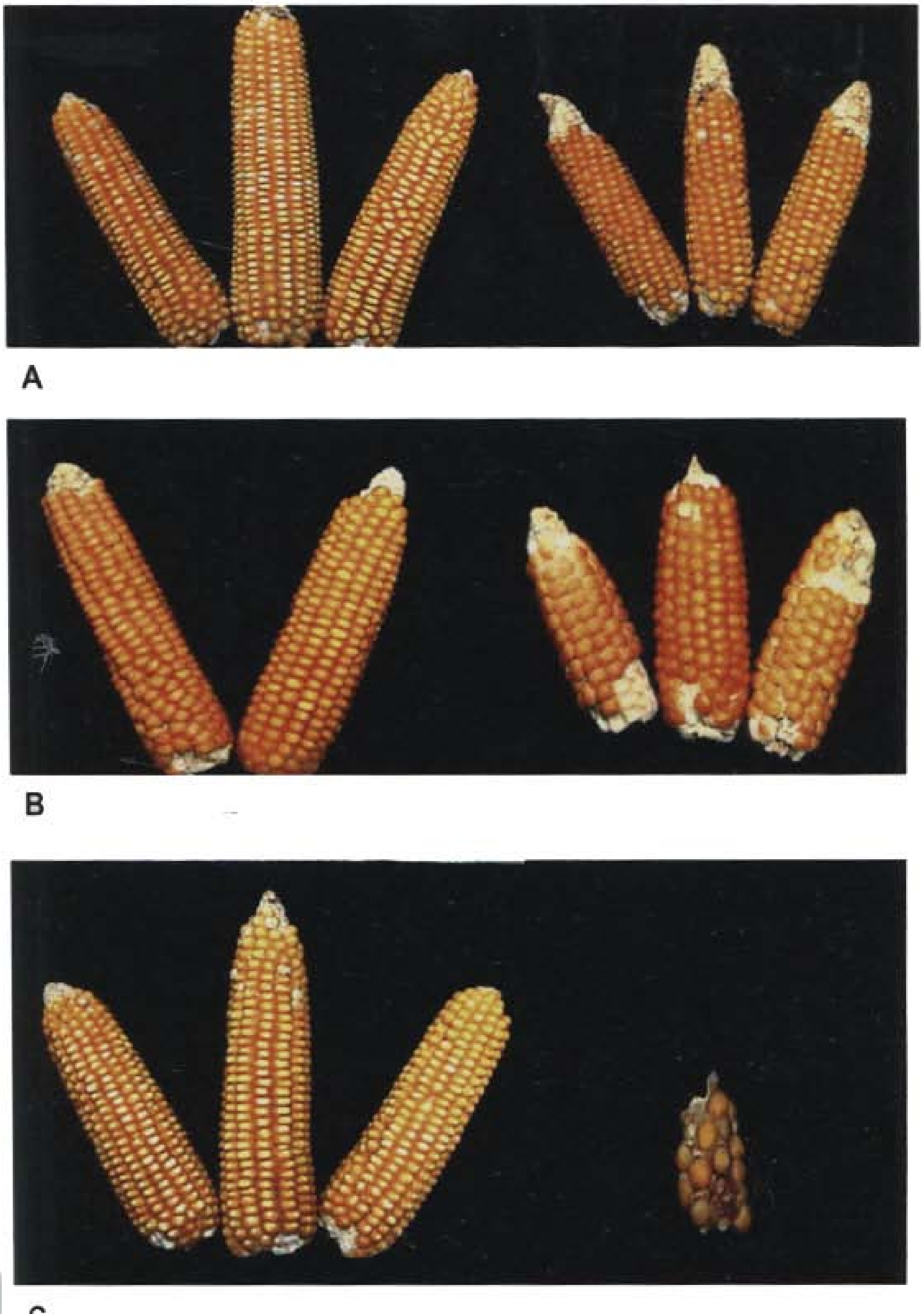


Figure 5: Performance of maize inbred lines under drought stress conditions (Left: Control/Well-watered; Right: Drought stressed)
A. IPA29B; and B. CM140, showing moderate drought tolerance;
C. IPA34 showing severe susceptibility

drought stress conditions at Hyderabad (2003-04). These included 39 DTPY lines (yellow flint kernel type) and 19 DTPW lines (white flint kernel type). Due to paucity of seed material, these lines could not be simultaneously evaluated under control/well-watered conditions.

**Performance of the DTPY Lines:** Thirty-nine DTPY inbred lines along with one check (CM140) were evaluated in Hyderabad (winter/dry season 2003-04). The responses of the inbred lines under drought stress were evaluated for the following characters: ASI, grain yield per plot, grain yield per ear, ear number per plant, number of kernel rows per ear, number of kernels per ear row, ear length, ear diameter, kernel number per ear and 100-kernel weight. ANOVA revealed significant differences among the genotypes for ASI, grain yield per plot, ear length and number of kernels per row. The effect of replication was found to be non-significant for all characters (Table 8).

The mean values with respect to various characters in the DTPY lines and the check (CM140) under drought stress conditions, along with ranking of genotypes on the basis of DMRT, were presented in Table 9. Most of the DTPY lines recorded male flowering 75-78 days after planting. ASI in majority of the DTPY lines was near 4.5 to 5 days, thereby affecting the grain yields. The lowest mean ASI values were recorded in DTPY122, DTPY199 and DTPY5 (-0.5, -0.5 and 1.0 day, respectively). While DTPY1, DTPY18 and DTPY132 did not produce any ear with seed set, DTPY100 and DTPY174 were highly promising in terms of ear number per plant.

With respect to grain yield per plot and grain yield per ear, the performance of DTPY116, DTPY65 and DTPY149 under drought stress was highly encouraging among the DTPY lines analyzed (Table 9). These genotypes were also significantly superior to CM140 (check). However, CM140 recorded significantly higher seed set (kernel number per ear) in comparison with the DTPY lines. Among the DTPY lines, DTPY199, DTPY5 and DTPY47 recorded high mean values for kernel number per ear.

IPA29B and FSA17 were also promising in terms of ear length and ear diameter under drought stress conditions. Overall, based on DMRT ranking

**Table 8. ANOVA for various characters under drought stress conditions in the DTPY inbred lines evaluated at Hyderabad (2003-04; Experiment II)**

| Characters | Mean Sum of Squares | | |
|---|---|---|---|
| | Error | Replication | Genotype |
| ASI | 2.30 | 0.05 | 4.90* |
| No. of ears per plant | 0.16 | 0.13 | 0.15 |
| Yield per plot | 2880.00 | 96.60 | 4709.89** |
| Grain yield per ear | 104.680 | 23.69 | 160.31 |
| Ear length | 33.30 | 6.05 | 964.40** |
| Ear diameter | 12.40 | 4.30 | 17.80 |
| No. of kernel rows | 19.30 | 0.51 | 31.03 |
| No. of kernels per ear row | 29.70 | 7.80 | 79.70** |
| Kernel number per ear | 4761.00 | 5080.00 | 11603.00** |
| 100-kernel weight | 46.60 | 28.20 | 42.90 |

*Significant at P = 0.05 ; **Significant at P = 0.01

**Table 9. Performance of DTPY lines for yield and yield-related characters under drought stress conditions (Experiment II; Hyderabad 2003-04)**

| Inbred | MFLW | FFLW | ASI | ENO | YLD | GYLDER | EL | ED | NKR | KPR | KNO | 100KW | DTI* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DTPY 1 | 75.4 | 80.6 | 5.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | -14.3 |
| DTPY 5 | 71.5 | 72.5 | 1.0$^{II}$ | 0.6 | 98.9 | 18.5 | 8.3 | 9.5$^{I}$ | 11.4$^{III}$ | 18.4 | 220.8$^{II}$ | 7.7 | 13.1 |
| DTPY 13 | 76.9 | 82.2 | 5.1 | 0.1 | 8.0 | 8.0 | 4.0 | 4.0 | 4.0 | 2.5 | 11.5 | 11.6 | -12.4 |
| DTPY 18 | 74.2 | 79.3 | 5.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | -14.3 |
| DTPY 31 | 76.9 | 72.0 | 5.1 | 0.6 | 57.9 | 15.2 | 7.1 | 8.4 | 10.6 | 11.3 | 126.0 | 12.1 | 1.3 |
| DTPY 39 | 74.2 | 79.3 | 5.1 | 0.4 | 28.1 | 10.8 | 3.3 | 3.1 | 3.7 | 4.6 | 21.2 | 11.0 | -6.2 |
| DTPY 47 | 76.2 | 80.9 | 4.5 | 0.5 | 49.0 | 16.8 | 8.8 | 8.6$^{II}$ | 11.2$^{III}$ | 20.7$^{I}$ | 220.7$^{II}$ | 8.1 | 2.7 |
| DTPY 57 | 76.3 | 81.4 | 5.1 | 0.2 | 27.5 | 9.2 | 5.0 | 4.5 | 6.0 | 9.0 | 150.0 | 6.2 | -5.5 |
| DTPY 60 | 76.9 | 82.0 | 5.1 | 0.3 | 18.3 | 6.1 | 5.5 | 4.2 | 4.0 | 6.0 | 51.5 | 6.3 | -7.9 |
| DTPY 62 | 74.8 | 79.4 | 4.5 | 0.3 | 44.8 | 17.1 | 8.0 | 8.0 | 10.3 | 15.6 | 152.7 | 10.3 | -2.3 |
| DTPY 65 | 77.0 | 79.0 | 2.0 | 0.7 | 156.1$^{II}$ | 30.2$^{II}$ | 9.3 | 10.6$^{I}$ | 11.1 | 19.6$^{III}$ | 201.0 | 15.5 | 18.0 |
| DTPY 71 | 77.1 | 81.5 | 5.1 | 0.2 | 36.6 | 9.1 | 4.3 | 3.7 | 5.5 | 6.4 | 75.0 | 6.0 | -6.0 |
| DTPY 75 | 78.0 | 81.0 | 3.0 | 0.4 | 28.3 | 10.1 | 6.7 | 7.5 | 10.0 | 11.1 | 94.5 | 10.5 | -2.2 |
| DTPY 79 | 78.0 | 81.0 | 3.0 | 0.7 | 86.3 | 17.3 | 8.9 | 8.5 | 9.8 | 13.7 | 117.0 | 13.4 | 7.3 |
| DTPY 85 | 78.5$^{III}$ | 83.0 | 4.5 | 0.6 | 92.8 | 25.7 | 10.6$^{II}$ | 9.2$^{I}$ | 10.4 | 13.7 | 159.9 | 16.2$^{II}$ | 6.3 |
| DTPY 91 | 75.1 | 80.4 | 5.1 | 0.4 | 25.7 | 9.6 | 9.8 | 8.4 | 8.6 | 6.3 | 54.1 | 16.1$^{II}$ | -6.5 |
| DTPY 96 | 75.6 | 80.7 | 5.1 | 0.2 | 5.1 | 5.1 | 6.0 | 3.0 | 1.0 | 1.0 | 5.0 | 5.8 | -10.9 |
| DTPY 97 | 77.3 | 80.6 | 5.1 | 0.1 | 23.6 | 7.9 | 5.0 | 3.4 | 4.0 | 5.5 | 40.8 | 8.5 | -9.3 |
| DTPY 100 | 75.8 | 79.9 | 4.0 | 1.2$^{I}$ | 137.5 | 24.0 | 8.5 | 9.3$^{I}$ | 10.8 | 17.9 | 176.4 | 12.6 | 18.9 |
| DTPY 105 | 75.8 | 80.9 | 5.1 | 0.3 | 37.0 | 13.9 | 6.6 | 6.5 | 8.0 | 12.3 | 122.0 | 11.1 | -4.4 |
| DTPY 108 | 76.9 | 82.0 | 5.1 | 0.4 | 53.6 | 8.9 | 5.0 | 4.3 | 3.2 | 4.1 | 28.9 | 11.2 | -3.4 |
| DTPY 116 | 77.8 | 70.4 | 5.1 | 0.7$^{II}$ | 172.6$^{I}$ | 30.3$^{II}$ | 10.7$^{II}$ | 8.4 | 9.6 | 19.0 | 190.5 | 14.9 | 15.9 |
| DTPY 122 | 76.0 | 75.5 | -0.5$^{I}$ | 0.1 | 8.0 | 2.7 | 1.1 | 2.7 | 4.0 | 2.5 | 19.8 | 5.6 | -4.2 |
| DTPY 128 | 77.8 | 79.4 | 1.5 | 0.7 | 29.1 | 14.5 | 6.9 | 8.1 | 10.5 | 15.0 | 151.2 | 8.0 | 4.4 |

| Inbred | MFLW | FFLW | ASI | ENO | YLD | GYLDER | EL | ED | NKR | KPR | KNO | 100KW | DTI* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DTPY 132 | 77.6 | 82.2 | 4.5 | 0.0 | 0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | -13.6 |
| DTPY 135 | 75.5 | 80.6 | 5.1 | 0.2 | 15.8 | 5.3 | 2.8 | 3.1 | 4.3 | 5.8 | 53.6 | 4.4 | -9.6 |
| DTPY 138 | 78.0 | 81.0 | 3.0 | 0.7$^{II}$ | 99.6 | 30.3$^{II}$ | 6.9 | 7.5 | 9.3 | 13.7 | 199.0 | 13.4 | 11.3 |
| DTPY 149 | 76.8 | 80.9 | 4.0 | 0.7 | 138.3$^{III}$ | 31.5$^{I}$ | 9.6 | 9.9$^{I}$ | 15.3$^{I}$ | 13.3 | 201.0 | 13.4 | 13.7 |
| DTPY 156 | 77.3 | 80.6 | 5.1 | 0.2 | 18.0 | 6.0 | 18.9$^{I}$ | 3.6 | 4.3 | 5.1 | 46.65 | 6.9 | -9.5 |
| DTPY 162 | 77.2 | 81.8 | 4.5 | 0.4 | 100.9 | 24.7 | 8.3 | 8.8$^{II}$ | 8.7 | 9.4 | 93.6 | 15.2$^{II}$ | 3.3 |
| DTPY 168 | 74.8 | 79.4 | 4.5 | 0.3 | 17.6 | 5.8 | 3.1 | 3.4 | 4.0 | 5.1 | 61.3 | 4.9 | -7.1 |
| DTPY 174 | 75.5 | 80.6 | 5.1 | 1.0$^{II}$ | 17.0 | 8.5 | 5.2 | 4.5 | 4.5 | 3.0 | 27.0 | 6.0 | -1.2 |
| DTPY 178 | 73.8 | 77.4 | 3.5 | 0.4 | 39.0 | 14.5 | 6.5 | 8.3 | 12.0$^{III}$ | 11.2 | 153.6 | 9.3 | -0.6 |
| DTPY 184 | 75.5 | 79.0 | 3.5 | 0.7 | 100.5 | 18.1 | 7.4 | 9.2$^{I}$ | 10.6 | 15.1 | 147.8 | 11.5 | 9.5 |
| DTPY 187 | 75.0 | 80.0 | 5.0 | 0.7 | 110.8 | 22.2 | 7.5 | 8.6$^{II}$ | 11.0 | 11.9 | 134.3 | 15.2 | 7.7 |
| DTPY 194 | 78.5$^{II}$ | 81.5 | 3.0 | 0.3 | 62.5 | 12.5 | 4.3 | 4.8 | 5.6 | 6.7 | 60.1 | 8.5 | 0.1 |
| DTPY 199 | 79.5 | 79.0 | -0.5$^{I}$ | 0.7 | 131.1 | 26.5$^{III}$ | 9.3 | 9.0$^{I}$ | 11.6$^{III}$ | 17.7 | 212.5$^{III}$ | 13 | 19.2 |
| DTPY 202 | 71.0 | 72.5 | 1.5$^{III}$ | 0.2 | 7.5 | 7.5 | 3.2 | 4.7 | 6.0 | 7.0 | 41.0 | 4.65 | -6.1 |
| DTPY 210 | 77.5 | 80.5 | 3.0 | 0.3 | 20.0 | 10.7 | 6.6 | 7.5 | 8.0 | 5.6 | 33.8 | 18.5$^{I}$ | -5.1 |
| CM140 (Check) | 82.0$^{I}$ | 80.6 | 5.1 | 0.5 | 102.2 | 20.7 | 8.4 | 8.5 | 12.7$^{II}$ | 22.4$^{II}$ | 364.0$^{I}$ | 9.15 | 10.0 |

MFLW: Days to male flowering; FFLW: days to female flowering; ASI: Anthesis-silking interval; ENO: ear number per plant; YLD: Yield per plot (g);
GYLDER: Grain yield per ear (g); EL: Ear length (cm); ED: Ear diameter (cm); NKR: No. of kernel rows per ear; KPR: Kernels per ear row;
KNO: Kernel number per ear; 100WT: 100-kernel weight (g);
Numbers in superscripts are DMRT ranks.
*Drought Tolerance Index (DTI), based on the model suggested by Banziger *et al.* (2000)

for different characters influencing the performance of the genotypes under drought stress conditions, FSA17, IPA29B, Ac7643 and CM140 can be considered as promising among the inbred lines analyzed in Experiment I.

A comparison of 'Drought tolerance index' (DTI) values for the DTPY lines (Table 9) indicates that most of the genotypes (23 out of 39 DTPY lines) were severely affected by drought stress at the flowering stage, thereby recording negative DTI values; the check (CM140) recorded a DTI value of 10.00. Nevertheless, the performance of DTPY199 (19.2), DTPY100 (18.9), DTPY165 (18.0), DTPY116 (15.9), DTPY149 (13.7), DTPY5 (13.1) and DTPY138 (11.3) was notable among the inbred lines evaluated in this experiment.

**Performance of the DTPW Lines:** In this experiment 19 DTPW inbred lines along with CM140 (check) were evaluated in Hyderabad during the winter/dry season 2003-04. The responses of inbred lines for drought resistance were recorded in the drought stress block with respect to the following characters: ASI, root capacitance, ear per plot, grain yield per plot, grain yield per ear, kernel rows, number of kernel per row, ear length, ear diameter, total seed per plot, and 100-kernel weight.

ANOVA revealed significant genotypic differences for various characters analyzed, except for root capacitance, grain yield per ear and number of kernel rows per ear. The effect of replication was found to be significant only for kernel number per ear (Table 10).

The mean values with respect to various characters in the DTPW lines (besides the check, CM140) under drought stress conditions, along with ranking of the genotypes on the basis of DMRT, were presented in Table 11. Unlike the DTPY lines, there was significant variation among the DTPW lines for male flowering, with the lowest mean value (69.50 days in DTPW208) and highest mean value (80.50 days in DTPW46).

ASI in the DTPW lines ranged from -0.50 (DTPW208) to 6.50 (DTPW88 and DTPW176). DTPW208, DTPW51 and DTPW211 recorded the lowest ASI values among the genotypes analyzed. Root capacitance did not

**Table 10. ANOVA for various characters under drought stress conditions in the DTPW inbred lines evaluated at Hyderabad (2003-04; Experiment II)**

| Characters | Mean Sum of Squares | | |
|---|---|---|---|
| | Error | Replication | Genotype |
| ASI | 3.40 | 2.05 | 7.20* |
| Root capacitance | 48.90 | 54.30 | 58.30 |
| No. of ears per plant | 0.04 | 0.15 | 0.17** |
| Yield per plot | 86.00 | 183.00 | 484.00** |
| Grain yield per ear | 105.25 | 3.68 | 2.88 |
| Ear length | 5.00 | 8.60 | 13.40** |
| Ear diameter | 3.98 | 12.80 | 11.90* |
| No. of kernel rows | 26.70 | 0.19 | 46.00 |
| No. of kernels per ear row | 22.80 | 61.70 | 62.80* |
| Kernel number per ear | 3565.00 | 15764.00* | 15016.40** |
| 100-kernel weight | 26.90 | 0.96 | 62.20* |

*Significant at P = 0.05 ; **Significant at P = 0.01

**Table 11. Performance of DTPW lines for yield and yield-related characters under drought stress conditions (Experiment II; Hyderabad 2003-04)**

| Inbred | MFLW | FFLW | ASI | RC | ENO | YLD | GYLDER | EL | ED | NKR | KPR | KNO | 100KW | DTI* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DTPW1 | 77.50 | 80.50 | 3.00 | 14.77 | 1.08$^{I}$ | 248.50$^{III}$ | 42.22$^{II}$ | 11.70 | 9.60$^{I}$ | 11.40 | 14.65 | 182.90 | 20.40$^{II}$ | 11.90 |
| DTPW 6 | 74.00 | 76.00 | 3.00 | 35.27$^{I}$ | 0.50 | 63.05 | 19.14 | 8.00 | 8.75 | 9.65 | 9.80 | 97.80 | 13.45 | -2.78 |
| DTPW 43 | 78.50$^{II}$ | 81.50 | 3.00 | 25.90 | 0.41 | 82.80 | 26.85 | 9.20 | 9.00$^{II}$ | 9.50 | 16.25 | 167.50 | 15.30 | -2.78 |
| DTPW 46 | 80.50$^{I}$ | 82.50 | 2.00 | 36.00$^{I}$ | 0.83$^{II}$ | 86.70 | 21.68 | 10.05 | 8.85 | 9.50 | 10.00 | 69.90 | 17.45 | 2.85 |
| DTPW 51 | 71.00 | 71.50 | 0.50$^{II}$ | 24.00 | 1.05$^{I}$ | 320.10$^{II}$ | 33.40 | 10.25 | 9.70$^{I}$ | 11.80 | 20.30$^{I}$ | 261.00$^{II}$ | 14.90 | 17.65 |
| DTPW 59 | 76.50 | 79.00 | 2.50 | 33.32$^{I}$ | 0.17 | 29.40 | 14.70 | 9.80 | 7.15 | 10.50 | 15.00 | 221.50 | 7.65 | -7.15 |
| DTPW 79 | 77.50 | 82.75$^{II}$ | 5.30 | 26.15 | 0.23 | 22.25 | 15.58 | 7.85 | 7.15 | 8.35 | 8.25 | 91.50 | 12.90 | -9.91 |
| DTPW 86 | 71.50 | 74.00$^{I}$ | 2.50 | 32.82$^{I}$ | 0.78 | 152.55 | 29.27 | 10.30 | 9.15 | 10.40 | 19.00$^{II}$ | 231.70$^{III}$ | 14.25 | 4.81 |
| DTPW 88 | 78.25$^{II}$ | 84.75 | 6.50 | 24.80 | 0.75 | 19.65 | 19.65 | 7.50 | 8.75 | 7.00 | 7.00 | 44.50 | 23.90$^{I}$ | -6.00 |
| DTPW 105 | 78.50$^{II}$ | 83.25$^{II}$ | 4.80 | 19.55 | 0.60 | 129.60 | 33.48 | 12.40$^{I}$ | 9.15$^{II}$ | 25.00$^{I}$ | 15.75 | 174.15 | 17.45 | -0.54 |
| DTPW 125 | 74.00 | 76.00 | 2.00 | 29.10 | 0.71 | 82.70 | 19.25 | 8.30 | 8.35 | 11.20 | 14.55 | 131.70 | 13.70 | 1.37 |
| DTPW 138 | 78.00$^{II}$ | 81.00 | 3.00 | 28.85 | 0.44 | 84.70 | 12.10 | 4.00 | 4.45 | 5.40 | 7.00 | 78.10 | 7.20 | -2.42 |
| DTPW 165 | 77.50 | 82.75$^{II}$ | 5.30 | 32.57$^{I}$ | 0.21 | 24.15 | 8.05 | 3.60 | 4.10 | 4.35 | 5.85 | 40.15 | 10.15 | -9.98 |
| DTPW 167 | 71.00 | 74.75 | 3.80 | 33.75$^{I}$ | 0.76 | 127.90 | 27.04 | 8.30 | 9.10 | 13.80$^{II}$ | 15.90 | 186.90 | 14.45 | 2.14 |
| DTPW 176 | 75.60 | 82.10 | 6.50 | 30.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 21.10 | -14.67 |
| DTPW 183 | 78.50$^{II}$ | 82.25 | 3.80 | 28.60 | 0.52 | 32.90 | 9.37 | 6.30 | 7.10 | 6.70 | 7.30 | 36.60 | 20.85$^{II}$ | -4.75 |
| DTPW 195 | 73.75 | 78.50 | 4.80 | 24.60 | 0.89$^{II}$ | 405.00$^{I}$ | 47.71$^{II}$ | 11.75$^{II}$ | 10.10$^{I}$ | 13.00$^{II}$ | 21.20$^{I}$ | 311.86$^{I}$ | 20.15$^{II}$ | 15.34 |
| DTPW 208 | 69.50 | 69.00 | -0.10$^{I}$ | 32.52$^{I}$ | 0.60 | 202.10 | 37.18$^{III}$ | 10.65 | 9.40$^{I}$ | 11.40 | 17.10$^{III}$ | 221.10 | 14.00 | 8.06 |
| DTPW 211 | 73.00 | 64.00 | 1.00$^{III}$ | 26.62 | 0.47 | 46.15 | 11.54 | 8.35 | 8.10 | 9.50 | 11.10 | 113.00 | 14.15 | -1.69 |
| CM140 (check) | 74.00 | 79.00 | 5.00 | 32.12$^{I}$ | 0.47 | 145.70 | 28.87 | 10.40 | 8.95$^{II}$ | 11.55 | 17.45$^{III}$ | 230.90$^{III}$ | 10.03 | 9.05 |

MFLW: Days to male flowering; FFLW: days to female flowering; ASI: Anthesis-silking interval; ENO: ear number per plant; YLD: Yield per plot (g); GYLDER: Grain yield per ear (g); EL: Ear length (cm); ED: Ear diameter (cm); NKR: No. of kernel rows per ear; KPR: Kernels per ear row; KNO: Kernel number per ear; 100WT: 100-kernel weight (g); Numbers in superscripts are DMRT ranks

*Drought Tolerance Index (DTI), based on the model suggested by Banziger *et al.* (2000);.

show correspondence with the performance of the genotypes under drought stress conditions. The range was 14.77nF (DTPW1) to 36.00nF (DTPW46).

Many of the DTPW lines were comparable to the check, CM140 with respect to ear number per plant. Particular mention must be made of DTPW1, DTP51, DTPW195 and DTPW46, which showed higher mean values for ear number of plant than other genotypes under study. It is also significant to note that DTPW195, DTPW51 and DTPW1 were also highly impressive in terms of grain yield per plot and grain yield per ear, indicating their drought stress tolerance potential (Table 11). These genotypes were also significantly superior to CM140 (check) for these important traits. DTPW195 and DTPW51 also top-ranked for kernel number per ear, and were superior to CM140, which was third-ranked for this trait among the lines analyzed in this Experiment (Table 11).

The DTI values of the DTPW lines summarize the drought tolerance/susceptibility responses (Table 11). Eleven out of the 19 DTPW lines recorded negative DTI values, indicating their susceptibility to drought stress at the flowering stage, whereas the check (CM140) recorded a DTI value of 9.05. Nevertheless, the present study resulted in identification of three highly promising lines, DTPW51 (17.65), DTPW195 (15.34), DTPW1 (11.90), which recorded very high DTI values and were significantly superior to the CM140 (check).

## 2. Analysis of Genetic Variability in the Experimental Hybrids for Drought Tolerance

In this experiment, drought stress responses of 17 experimental hybrids were evaluated under two different regimes: well-watered (control) and severe water stress condition at the flowering stage during winter/dry season at Hyderabad in 2002-03. The characters recorded included both plant and ear characters, such as plant height, ear height, ASI, tassel size, leaf rolling, leaf senescence, grain yield per plot, number of ears per plant, grain yield per ear, ear length, ear diameter, number of kernel rows, number of kernels per ear row, kernel number per ear and 100-kernel weight.

As indicated in Table 12, ANOVA revealed highly significant differences between the two different water regimes (control vs. stress) for all the characters except ear height, tassel size, grain yield per ear and 100-kernel weight. ANOVA for the dataset from the stress block revealed that significant difference between the hybrids for grain yield per plot, ear length, ear diameter, number of kernel rows and 100-kernel weight (Table 13). The variation attributable to replication alone was non-significant for all the characters analyzed in this experiment.

The mean values for various characters in the experimental hybrids under control and drought stress conditions, along with ranking of genotypes on the basis of DMRT, were presented in Tables 14 and 15. Considerable variation could be noticed among the experimental hybrids under both well-watered and drought stress conditions for flowering characters, including male flowering, female flowering and ASI. The lowest mean ASI values under drought stress conditions were recorded in CM140 x CM123 (2.7 days), CIM500 x CIM519 (4.7 days), Bio5 x CM139 (4.7 days) and FSA17 x IPA34 (5.4 days). In the control block, Ac7729 x CML247, CIM500 x CIM519 and Bio5 x CM139 recorded the lowest ASI values.

There was no variation with respect to leaf rolling in the genotypes in the control block, while variation with respect to leaf senescence and tassel size was not high. The ranges of scores for tassel size and leaf rolling in the experimental hybrids in the stress block were relatively less in comparison to that observed for leaf senescence. Under drought stress conditions, FSA17 x IPA34 was least affected in terms of leaf senescence (score of 2.5) than other experimental hybrids under study. CM207 x IPA21A recorded the highest leaf senescence score of 7.0.

Bio5 x Ac7729, Bio5 x K64R, CM139 x IPA3 and FSA17 x IPA34 were the top-ranked genotypes for grain yield under control conditions (Table 14). However, as seen in Table 15, the performance of Bio5 x Ac7729 and Bio5 x K64R was only moderate under drought stress conditions. Particularly impressive was FSA17 x IPA34 (Figure 6) which was ranked second for grain yield under stress conditions, besides its good *per se* performance

**Table 12. ANOVA for various characters in the experimental crosses evaluated at Hyderabad (2002-03) under two different water regimes**

| Characters | Mean Sum of Squares | | | |
|---|---|---|---|---|
| | Error | Treatment | Replication | Genotype |
| Plant height | 191.54 | 9411.76** | 3.76 | 726.81** |
| Ear height | 88.45 | 133.28 | 57.99 | 375.41** |
| ASI | 2.72 | 292.12** | 1.49 | 4.25 |
| Tassel size | 0.54 | 0.01 | 0.72 | 0.71 |
| Leaf rolling | 0.31 | 2.48** | 0.13 | 0.29 |
| Leaf senescence | 1.14 | 72.06** | 0.00 | 2.70* |
| No. of ears per plant | 0.04 | 2.76** | 0.14 | 0.09** |
| Yield per plot | 328448.00 | 20549105.00** | 832730.00 | 1065613.00** |
| Grain yield per ear | 128.7195.32 | 95.32 | 70.15 | 166.96 |
| Ear length | 5.50 | 336.84** | 4.68 | 19.19** |
| Ear diameter | 3.23 | 196.88** | 2.90 | 10.28** |
| No. of kernel rows | 5.19 | 90.39** | 0.64 | 15.23** |
| No. of kernels per ear row | 31.21 | 31** | 7.89 | 124.57** |
| Kernel number per ear | 7159.6 | 773723.02** | 5648.08 | 29718.29** |
| 100-kernel weight | 15.34 | 836.71 | 0.05 | 56.36 |

*Significant at P = 0.05 ; **Significant at P = 0.01

**Table 13. ANOVA for various characters under drought stress conditions in the experimental crosses evaluated at Hyderabad (2002-03)**

| Characters | Mean Sum of Squares | | |
|---|---|---|---|
| | Error | Replication | Genotype |
| Plant height | 299.59 | 221.59 | 246.66 |
| Ear height | 139.77 | 69.46 | 128.30 |
| ASI | 3.75 | 6.67 | 7.60 |
| Tassel size | 0.97 | 0.47 | 0.38 |
| Leaf rolling | 0.64 | 0.26 | 0.59 |
| Leaf senescence | 1.28 | 0.47 | 2.87 |
| No. of ears per plant | 0.04 | 0.06 | 0.10 |
| Yield per plot | 144764.00 | 538273.10 | 150795.10* |
| Grain yield per ear | 215.75 | 94.25 | 306.63 |
| Ear length | 6.71 | 0.31 | 20.47* |
| Ear diameter | 2.15 | 1.09 | 15.19** |
| No. of kernel rows | 4.26 | 0.07 | 23.57** |
| No. of kernels per ear row | 61.99 | 2.04 | 89.81 |
| Kernel number per ear | 14724.57 | 0.93 | 17353.3 |
| 100-kernel weight | 11.56 | 2064 | 44.69** |

*Significant at P = 0.05 ; **Significant at P = 0.01

**Table 14. Performance of experimental crosses for yield and yield-related characters under control/well-watered conditions (Hyderabad 2002-03)**

| S. No. | Cross | PHT | EHT* | ASI | TS | LR | LS | ENO | YLD | EL | ED | NKR | KPR | KNO | 100WT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | CM140 x CM207 | 124.5 | 61.6 | 2.5 | 4.0 | 1.0 | 2.0 | 0.8 | 1186.0 | 13.1 | 12.0 | 12.8 | 24.0 | 307.0 | 19.3 |
| 2 | Bio5 x CM139 | 110.7 | 53.5 | 1.7$^{III}$ | 4.0 | 1.0 | 1.5 | 0.8 | 912.0 | 11.9 | 11.3 | 11.3 | 20.5 | 231.0 | 23.1 |
| 3 | CM140 x CM123 | 154.2 | 87.6$^{II}$ | 2.5 | 3.5$^{I}$ | 1.0 | 2.5 | 0.6 | 1675.5 | 16.3$^{I}$ | 12.2 | 12.4 | 33.8 | 419.2 | 23.4$^{II}$ |
| 4 | CM139 x Bio5 | 112.9 | 60.5 | 2.9 | 5.0 | 1.0 | 3.5 | 0.7 | 361.0 | 14.7 | 12.3 | 14.8 | 23.9 | 349.1 | 17.1 |
| 5 | CM139 x IPA3 | 165.9$^{I}$ | 91.6$^{I}$ | 3.2 | 5.0 | 1.0 | 1.5 | 0.3 | 2078.5$^{II}$ | 14.5 | 12.2 | 16.0$^{I}$ | 38.0$^{I}$ | 608.6$^{I}$ | 22.1 |
| 6 | CM119 x IPA34 | 130.5 | 69.4 | 2.1 | 4.5 | 1.0 | 2.0 | 0.8 | 1467.5 | 13.3 | 12.8 | 14.2 | 28.9 | 409.5 | 20.5 |
| 7 | FSA17 x IPA34 | 133.0 | 78$^{III}$ | 2.1 | 4.5 | 1.0 | 2.0 | 0.8 | 1708.5$^{III}$ | 13.4 | 13.8$^{III}$ | 15.4$^{II}$ | 28.2 | 433.4$^{II}$ | 23.4 |
| 8 | CM119 x CIM506 | 119.2 | 51.1 | 2.6 | 3.5$^{I}$ | 1.0 | 2.5 | 0.7 | 433.0 | 10.2 | 11.7 | 13.2 | 22.5 | 269.9 | 17.2 |
| 9 | CM119 x CM138 | 152.0 | 73.4 | 2.5 | 5.0 | 1.0 | 3.5 | 0.7 | 1475.9 | 14.7 | 12.9 | 14.9$^{III}$ | 32.7 | 478.8 | 20.3 |
| 10 | Bio5 x Ac7729 | 156.4$^{III}$ | 79.0$^{III}$ | 2.0 | 5.0 | 1.0 | 1.0$^{I}$ | 0.7 | 3406.5$^{I}$ | 16.9$^{I}$ | 14.6$^{I}$ | 14.2 | 36.8$^{III}$ | 522.7$^{III}$ | 30.1$^{I}$ |
| 11 | Bio5 x K64R | 143.9 | 73.5 | 2.6 | 4.5 | 1.0 | 1.0$^{I}$ | 1.0$^{II}$ | 3380.0$^{I}$ | 15.6 | 13.9$^{II}$ | 14.2 | 37.8$^{II}$ | 536.9 | 29.9$^{I}$ |
| 12 | CM207x IPA21A | 120.3 | 54.7 | 3.4 | 5.0 | 1.0 | 3.0 | 0.5 | 530.0 | 13.3 | 11.2 | 13.7 | 28.0 | 385.2 | 17.7 |
| 13 | CML247 x Bio 5 | 123.0 | 67.5 | 2.3 | 4.0 | 1.0 | 2.5 | 0.7 | 886.0 | 13.7 | 13.0 | 13.7 | 33.5 | 456.9 | 18.6 |
| 14 | CIM500 x CIM519 | 157.0$^{II}$ | 89.3$^{I}$ | 1.6$^{II}$ | 5.0 | 1.0 | 2.0 | 1.1$^{I}$ | 1130.0 | 13.9 | 13.7 | 14.5 | 30.2 | 437.9 | 26.0$^{II}$ |
| 15 | Ac7729 x CML247 | 157.7$^{II}$ | 85.9$^{II}$ | 1.3$^{I}$ | 4.0 | 1.0 | 2.5 | 0.8 | 701.0 | 14.7 | 12.7 | 13.4 | 34.4 | 460.6 | 17.5 |
| 16 | K64R x Bio5 | 133.7 | 77.9 | 2.6 | 4.5 | 1.0 | 3.0 | 0.7 | 1575.0 | 15.9 | 13.3 | 14.1 | 33.0 | 465.5 | 21.1 |
| 17 | CM123-4 x IPA21A | 128.9 | 62.0 | 2.9 | 4.5 | 1.0 | 2.0 | 0.8 | 1020.0 | 15.8 | 12.4 | 14.3 | 32.8 | 469.5 | 20.9 |

PHT: Plant height (cm); EHT: Ear height (cm); ASI: Anthesis-silking interval; TS: Tassel size; LR: Leaf rolling; LS: Leaf senescence; ENO: ear number per plant; YLD: Grain yield per plot (g); EL: Ear length (cm); ED: Ear diameter (cm); NKR: No. of kernel rows per ear; KPR: Kernels per ear row; KNO: Kernel number per ear; 100WT: 100-kernel weight (g);
Numbers in superscripts are DMRT ranks.

**Table 15. Performance of experimental crosses for yield and yield-related characters under drought stress conditions (Hyderabad 2002-03)**

| S. No. | Cross | PHT | EHT* | ASI | TS | LR | LS | ENO | YLD | EL | ED | NKR | KPR | KNO | 100WT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | CM140 x CM207 | 108.0 | 66.5 | 4.7$^{III}$ | 4.0 | 1.0$^{I}$ | 3.0$^{II}$ | 0.40 | 410.0 | 11.2$^{II}$ | 10.0$^{I}$ | 12.2 | 20.0 | 244.4 | 14.3 |
| 2 | Bio5 x CM139 | 104.7 | 54.7 | 6.7 | 4.5 | 1.5 | 4.0$^{III}$ | 0.05 | 61.5 | 9.5 | 10.5$^{I}$ | 14.0$^{II}$ | 8.5 | 106.0 | 19.4$^{I}$ |
| 3 | CM140 x CM123 | 110.8 | 66.5 | 2.7$^{I}$ | 4.0 | 1.5 | 4.5 | 0.70$^{II}$ | 1054.0$^{I}$ | 12.5$^{II}$ | 10.1$^{I}$ | 8.5 | 24.1$^{I}$ | 233.9 | 16.7 |
| 4 | CM139 x Bio5 | 97.6 | 53.0 | 5.7 | 4.5 | 2.0 | 5.5 | 0.20 | 37.5 | 6.8 | 6.5 | 9.3 | 7.7 | 70.9 | 6.9 |
| 5 | CM139 x IPA3 | 123.5 | 76.2 | 8.0 | 4.5 | 1.0 | 4.5 | 0.55$^{III}$ | 567.5$^{II}$ | 14.2$^{I}$ | 11.0$^{I}$ | 15.1$^{I}$ | 23.3$^{I}$ | 353.0$^{I}$ | 12.9 |
| 6 | CM119 x IPA34 | 96.3 | 64.1 | 8.2 | 4.0 | 1.5 | 6.5 | 0.15 | 159.5 | 8.8 | 8.6 | 11.8 | 13.0 | 173.2 | 15.7 |
| 7 | FSA17 x IPA34 | 122.2 | 74.6 | 5.4$^{III}$ | 5.0 | 1.0 | 2.5$^{I}$ | 0.50 | 582.0$^{II}$ | 10.0 | 11.1$^{I}$ | 13.6$^{II}$ | 19.7 | 267.9 | 17.2 |
| 8 | CM119 x CIM506 | 107.1 | 61.7 | 5.5 | 3.5 | 1.5 | 4.0 | 0.00 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 9 | CM119 x CM138 | 133.5 | 78.0 | 6.4 | 4.5 | 1.0$^{I}$ | 4.0 | 0.45 | 517.5$^{II}$ | 11.5$^{II}$ | 11.3$^{I}$ | 14.3$^{II}$ | 21.5$^{I}$ | 308.7$^{I}$ | 14.4 |
| 10 | Bio5 x Ac7729 | 119.5 | 72.0 | 7.9 | 5.0 | 1.0$^{I}$ | 4.0 | 0.35 | 272.5 | 9.2 | 9.1 | 11.1 | 17.4 | 196.8 | 18.7$^{I}$ |
| 11 | Bio5 x K64R | 114.0 | 72.7 | 6.9 | 5.0 | 1.0$^{I}$ | 3.0$^{II}$ | 0.30 | 289.5 | 10.3 | 9.8 | 12.4 | 16.3 | 203.5 | 16.8 |
| 12 | CM207x IPA21A | 96.3 | 64.6 | 5.9 | 4.5 | 1.5 | 7.0 | 0.25 | 89.0 | 13.5 | 8.3 | 11.6 | 11.0 | 129.9 | 15.4 |
| 13 | CML247 x Bio 5 | 110.5 | 70.8 | 7.4 | 4.0 | 3.0 | 5.0 | 0.15 | 132.0 | 9.8$^{I}$ | 11.4$^{I}$ | 13.5$^{III}$ | 21.2$^{I}$ | 290.3 | 18.4$^{I}$ |
| 14 | CIM500 x CIM519 | 120.7 | 69.5 | 4.7$^{II}$ | 4.5 | 1.0$^{I}$ | 3.0$^{II}$ | 0.85$^{I}$ | 221.5 | 10.5 | 10.3$^{I}$ | 13.2 | 21.0$^{I}$ | 277.2 | 13.9 |
| 15 | Ac7729 x CML247 | 128.9 | 78.0 | 5.6 | 5.0 | 1.0$^{I}$ | 4.0 | 0.25 | 129.0 | 11.4 | 11.5$^{I}$ | 12.5 | 23.7$^{I}$ | 296.5 | 16.2 |
| 16 | K64R x Bio5 | 120.5 | 81.7 | 8.5 | 4.0 | 1.0$^{I}$ | 4.0 | 0.35 | 430.5 | 9.9$^{II}$ | 10.0$^{I}$ | 12.8 | 19.6 | 256.8 | 17.0 |
| 17 | CM123-4 x IPA21A | 109.5 | 64.0 | 6.8 | 4.5 | 2.0 | 4.5 | 0.20 | 197.0 | 6.9 | 7.9 | 11.9 | 19.4 | 241.8 | 14.7 |

PHT: Plant height (cm); EHT: Ear height (cm); ASI: Anthesis-silking interval; TS: Tassel size; LR: Leaf rolling; LS: Leaf senescence; ENO: ear number per plant; YLD: Grain yield per plot (g); EL: Ear length (cm); ED: Ear diameter (cm); NKR: No. of kernel rows per ear; KPR: Kernels per ear row; KNO: Kernel number per ear; 100WT: 100-kernel weight (g);

Numbers in superscripts are DMRT ranks.

**Table 16. Comparison of yield performance and drought tolerance indices of experimental hybrids under control/well-watered and drought stress conditions [Hyderabad 2002-03)**

| S. No. | Experimental Hybrid | Grain yield (g) (Control) | Grain yield (g) (Stress) | % Yield penalty over control | DTI* |
|---|---|---|---|---|---|
| 1. | CM140 x CM207 | 1186.0 | 410.0 | 65.4 | 7.2 |
| 2. | Bio5 x CM139 | 912.0 | 61.5 | 93.3 | -8.3 |
| 3. | CM140 x CM123 | 1675.5 | 1054.0 | 37.1 | 14.4 |
| 4. | CM139 x Bio5 | 361.0 | 37.5 | 89.6 | -14.4 |
| 5. | CM139 x IPA3 | 2078.5 | 567.5 | 72.7 | 4.6 |
| 6. | CM119 x IPA34 | 1467.5 | 159.5 | 89.1 | -8.5 |
| 7. | FSA17 x IPA34 | 1708.5 | 582.0 | 65.9 | 7.6 |
| 8. | CM119 x CIM506 | 433.0 | 0.0 | 100.0 | -10.6 |
| 9. | CM119 x CM138 | 1475.9 | 517.5 | 64.9 | 5.3 |
| 10. | Bio5 x Ac7729 | 3406.5 | 272.5 | 92.0 | -2.3 |
| 11. | Bio5 x K64R | 3380.0 | 289.5 | 91.4 | -1.2 |
| 12. | CM207x IPA21A | 530.0 | 89.0 | 83.2 | -10.4 |
| 13. | CML247 x Bio5 | 886.0 | 132.0 | 85.1 | -3.0 |
| 14. | CIM500 x CIM519 | 1130.0 | 221.5 | 80.4 | 12.0 |
| 15. | Ac7729 x CML247 | 701.0 | 129.0 | 81.6 | 2.3 |
| 16. | K64R x Bio5 | 1575.0 | 430.5 | 72.7 | 5.3 |
| 17. | CM123-4 x IPA21A | 1020.0 | 197.0 | 80.7 | -3.7 |

*Drought Tolerance Index (DTI), based on the model suggested by Banziger *et al.* (2000)

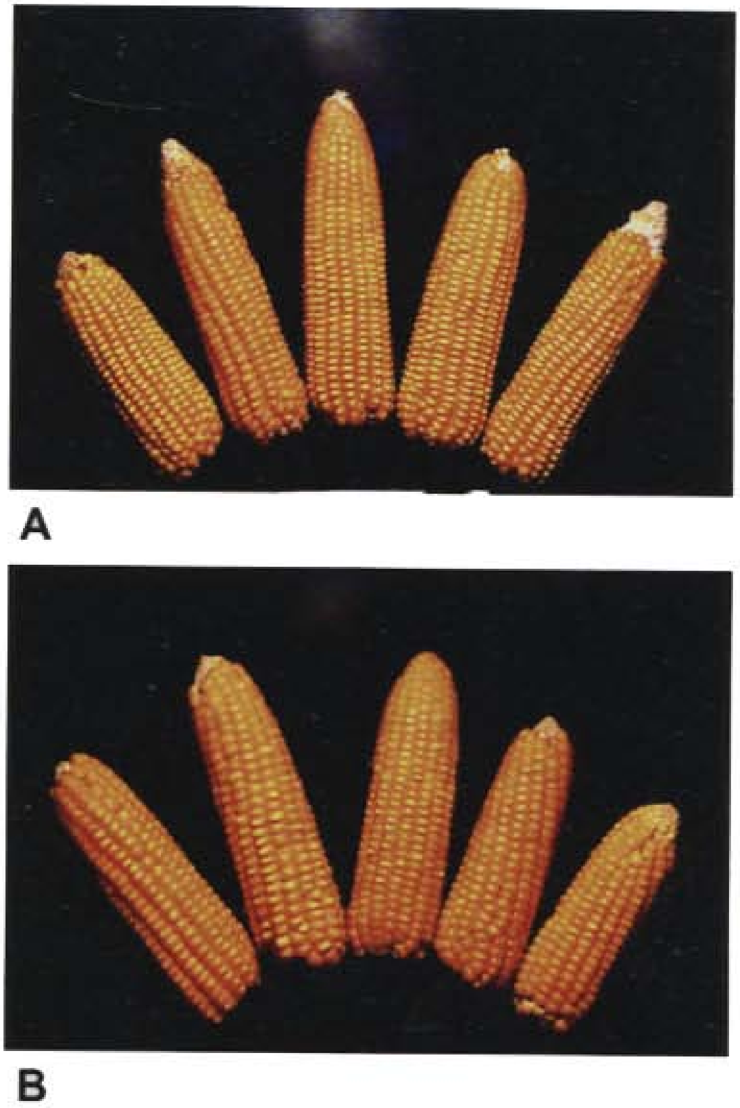


Figure 6: FSA17 x IPA34, an experimental hybrid, showing promising performance in both control/well-watered condition (A) and drought stress condition (B)

under the control/well-watered conditions. Among the various experimental hybrids analyzed in this experiment, CM140 x CM123 was top-ranked for grain yield, besides recording high ear number per plant, ear length and ear diameter. Equally impressive were CM139 x IPA3 and CM119 x CM138 which recorded good grain yield coupled with high kernel number per ear.

Based on DTI values, which take into account simultaneously the performance of the genotypes for several key traits influencing drought tolerance, CM140 x CM123 (14.4) and CIM500 x CIM519 (12.0) are clearly superior over the rest of the experimental hybrids (Table 16). These hybrids were followed by FSA17 x IPA34 (7.6) and CM140 x CM207 (7.2). A comparison of the yield performance under control and stress conditions, and consequently the % yield penalty over control, indicate that most of the experimental hybrids were severely affected by drought stress. The lowest % yield penalty (37.1) was recorded by CM140 x CM123, while three other experimental hybrids, (CM119 x CM138; CM140 x CM207 and FSA17 x IPA34) showed the next best figures of 64.9%, 65.4% and 65.9%, respectively. All other hybrids suffered yield penalty of more than 70%, including CIM500 x CM519 which showed very high DTI value.

In conclusion, taking into account both the DTI and the % yield penalty over control, the present study led to the identification of three most promising experimental hybrids, namely CM140 x CM123, FSA17 x IPA34 and CM140 x CM207.

## 3. Molecular Characterization and Genetic Diversity Analysis of the Selected Maize Inbred Lines

A set of 12 inbred lines, namely DTPW195, DTPW51, DTPW105, DTPW183, DTPY65, DTPY116, DTPY108, DTPY194, CM139, CM140, Ac7729 and Ac7643, with distinct responses to drought stress at the flowering stage were analyzed for polymorphism at the molecular level using microsatellite (SSR) markers. Among the 12 inbred lines analyzed, DTPY116, DTPY65, DTPW51, DTPW195, CM140, Ac7643 showed promising performance under drought stress conditions, while DTPY108, DTPY194, DTPW105, DTPW183, Ac7729 and CM139 were drought-susceptible. Two of the inbred lines, CM139 and CM140 also served as 'reference lines' for SSR analysis, as they were extensively analyzed at the molecular level by Maize Genetics Unit, IARI under the Asian Maize Biotechnology Network (AMBIONET).

Molecular analysis of the selected set of inbred lines was carried out using 71 polymorphic SSR loci covering various bin (chromosome) locations. The objectives of this study were: (i) to effectively differentiate the selected inbred lines using SSR markers; (ii) to analyze the genetic relationships among these lines, which would in turn aid in their utilization in breeding programmes, including development of mapping populations and breeding materials.

The study revealed considerable polymorphisms for the SSR loci in the inbred lines analyzed (Figure 7). A total of 331 SSR alleles were detected across the 71 polymorphic SSR loci, at an average of 4.69 per locus (Table 17). The highest number of alleles was observed in *phi037* (1.08), *bnlg1064* (2.03), *dup21* (2.05), *bnlg121* (2.06), *bnlg1520* (2.09) and *bnlg1272* (9.00) with seven alleles each. Only two SSR alleles were found in case of *bnlg1347* (1.10). Nine SSR loci revealed six alleles each (Table 17). The SSR loci analyzed included those with di-repeats (54), tri-repeats (7), tetra-repeats (6), penta-repeats (2), and cmlex repeats (2).

Based on SSR allele frequencies, the PIC (polymorphism information content) values were estimated for different polymorphic SSR loci analyzed (Table 17). The PIC values ranged from 0.42 for *phi097* (1.01) to 0.93 for *bnlg594* (10.06). It is notable that out of the 71 polymorphic SSR loci, 10% recorded PIC values more than 0.80, 48% more than 0.70, 83% more than 0.60 and 93% more than 0.50. Only 7% of the SSR markers analyzed have recorded PIC values less than 0.50. The average PIC value across all the SSR loci analyzed was 0.68. Nine SSR markers (*bnlg1556, bnlg1064, dup21, bnlg121, bnlg1520, bnlg1182, phi080, bnlg1518* and *bnlg594*) showing PIC values equal to or more than 0.80 are particularly worth mentioning. Such markers are highly useful for genotype differentiation.

Table 17. Polymorphism information content (PIC) of SSR loci across the 12 inbred lines analyzed in this study

| S. No. | Bin location | SSR locus | Repeat type | No. of SSR alleles detected | PIC |
|---|---|---|---|---|---|
| 1 | 1.01 | *phi097* | TAG | 3 | 0.40 |
| 2 | 1.02 | *bnlg147* | CA | 6 | 0.70 |
| 3 | 1.04 | *bnlg1016* | AG | 4 | 0.67 |
| 4 | 1.06 | *bnlg1556* | AG | 6 | 0.81 |
| 5 | 1.08 | *phi037* | AG | 7 | 0.79 |
| 6 | 1.09 | *bnlg400* | AG | 4 | 0.74 |
| 7 | 1.10 | *bnlg1347* | AG | 2 | 0.44 |
| 8 | 1.11 | *bnlg504* | CA | 6 | 0.78 |
| 9 | 1.11 | *umc1331* | GGT | 6 | 0.75 |
| 10 | 2.01 | *bnlg1092* | AG | 5 | 0.66 |
| 11 | 2.02 | *bnlg2042* | AG | 5 | 0.72 |
| 12 | 2.02 | *bnlg1297* | AG | 4 | 0.72 |
| 13 | 2.03 | *bnlg1064* | AG | 7 | 0.80 |
| 14 | 2.03 | *umc1555* | TTCA | 5 | 0.69 |
| 15 | 2.03 | *bnlg381* | CA | 3 | 0.51 |
| 16 | 2.04 | *bnlg1613* | AG | 5 | 0.72 |
| 17 | 2.04 | *bnlg1175* | AG | 5 | 0.71 |
| 18 | 2.05 | *dup21* | AG | 7 | 0.84 |
| 19 | 2.05 | *mage05* | AG | 5 | 0.68 |
| 20 | 2.06 | *bnlg121* | CT | 7 | 0.84 |
| 21 | 2.06 | *bnlg1138* | AG | 4 | 0.71 |
| 22 | 2.07 | *bnlg1045* | AG | 4 | 0.48 |
| 23 | 2.08 | *bnlg198* | CT | 4 | 0.60 |
| 24 | 2.09 | *bnlg1520* | AG | 7 | 0.83 |
| 25 | 3.05 | *bnlg420* | CT | 5 | 0.69 |
| 26 | 3.08 | *phi088* | ACT | 4 | 0.65 |
| 27 | 3.09 | *bnlg1182* | AG | 6 | 0.80 |
| 28 | 4.03 | *bnlg2244* | AG | 5 | 0.73 |
| 29 | 4.05 | *bnlg1729* | AG | 4 | 0.65 |
| 30 | 4.06 | *bnlg252* | AG | 3 | 0.65 |
| 31 | 4.11 | *bnlg589* | AG | 5 | 0.70 |
| 32 | 5.02 | *phi113* | GTCT | 4 | 0.67 |
| 33 | 5.04 | *bnlg2323* | AG | 3 | 0.64 |
| 34 | 5.06 | *phi101* | ACT | 4 | 0.65 |
| 35 | 5.09 | *bnlg389* | CT | 5 | 0.70 |

**Contd.**

Table 17 (Contd.)

| S. No. | Bin location | SSR locus | Repeat type | No. of SSR alleles detected | PIC |
|---|---|---|---|---|---|
| 36 | 6.00 | *bnlg1043* | AG | 5 | 0.73 |
| 37 | 6.01 | *phi077* | AG | 4 | 0.74 |
| 38 | 6.01 | *umc1018* | CT | 5 | 0.74 |
| 39 | 6.02 | *umc1006* | GA | 5 | 0.74 |
| 40 | 6.03 | *phi070* | AGCTG | 5 | 0.73 |
| 41 | 6.03 | *umc1572* | GA | 5 | 0.74 |
| 42 | 6.04 | *umc1014* | GA | 4 | 0.68 |
| 43 | 6.05 | *bnlg1154* | AG | 5 | 0.57 |
| 44 | 6.05 | *bnlg1702* | AG | 3 | 0.54 |
| 45 | 6.05 | *mmc0241* | TA-N-TG | 3 | 0.69 |
| 46 | 6.05 | *phi078* | AAAG | 4 | 0.61 |
| 47 | 6.05 | *phi102* | AT | 3 | 0.58 |
| 48 | 6.05 | *nc013* | AG | 6 | 0.64 |
| 49 | 6.08 | *phi089* | ATGC | 4 | 0.71 |
| 50 | 7.03 | *bnlg339* | CT | 4 | 0.74 |
| 51 | 7.04 | *bnlg155* | CT | 4 | 0.52 |
| 52 | 7.06 | *phi116* | AGTG-ACG | 3 | 0.54 |
| 53 | 8.02 | *phi119* | AG | 3 | 0.72 |
| 54 | 8.04 | *phi014* | GGC | 5 | 0.45 |
| 55 | 8.05 | *bnlg162* | CA | 3 | 0.68 |
| 56 | 8.06 | *bnlg240* | AG | 6 | 0.42 |
| 57 | 8.06 | *bnlg1065* | AG | 4 | 0.65 |
| 58 | 8.08-8.09 | *phi015* | AAAC | 5 | 0.53 |
| 59 | 8.08-8.09 | *phi080* | AGGAG | 5 | 0.83 |
| 60 | 9.00 | *bnlg1272* | AG | 7 | 0.70 |
| 61 | 9.01 | *phi033* | AAG | 5 | 0.60 |
| 62 | 9.03 | *bnlg1730* | AG | 4 | 0.71 |
| 63 | 9.05 | *bnlg1209* | AG | 4 | 0.68 |
| 64 | 10.00 | *phi041* | AGCC | 4 | 0.70 |
| 65 | 10.02 | *phi059* | ACC | 5 | 0.69 |
| 66 | 10.03 | *bnlg210* | AG | 4 | 0.70 |
| 67 | 10.03 | *bnlg1655* | AG | 6 | 0.69 |
| 68 | 10.04 | *bnlg1518* | AG | 6 | 0.82 |
| 69 | 10.04 | *bnlg1526* | AG | 6 | 0.78 |
| 70 | 10.06 | *bnlg594* | AG | 5 | 0.93 |
| 71 | 10.06 | *phi035* | AC | 3 | 0.65 |

SSR profiles for some of the inbred lines displayed clear deviation from the expected pattern where inbred lines are assumed to be highly homozygous, and thereby should reveal only a single band (allele) per locus for a large majority of the loci, if not all. However, double bands could be clearly seen at a few of the SSR loci in all the inbred lines (Table 18) (Figure 7). However, it can be noticed from Table 18 that the frequencies of SSR heterozygosity were very low in all the inbred lines, indicating that the genetic materials analyzed were reasonably homozygous and 'genetically pure'. Among the lines, minimum SSR heterozygosity was observed in Ac7643 with a frequency of 0.028 and maximum in case of DTPY65, DTPY116 and DTPY194 with relative frequency of 0.10 (Table 18). The frequencies of SSR heterozygosity were very low in all the inbred lines indicating high homozygosity and genetic purity of all inbred lines in this study. There was no correspondence between a particular SSR locus and the heteroygosity, as the heteroygotes were found to be spread over various SSR loci analyzed.

Three of the 12 inbred lines DTPW195, DTPY194 and Ac7729 did not reveal any 'nulls' (no amplification product for that specific SSR primer pair), while the other inbred lines showed 1 or 2 nulls across the 71 polymorphic SSR loci. The exception in this case was Ac7643 with five nulls (Table 18). The occurrence of the null alleles was verified in each of these cases by reamplification using the same primer pair and resolution of amplification products to rule out the possibility of non-amplification because of experimental error.

Ninety-six out of 331 polymorphic SSR alleles (across 71 polymorphic SSR loci) were found to be 'unique' or 'rare'. In this case, the unique or rare allele is one that was found to occur in only one out of the 12 inbred lines. DTPW195 showed the maximum number of such rare SSR alleles (13), followed by CM139 (11), DTPW183 and DTPY116 (10 each), while DTPY194, DTPW105 and CM140 showed 9 rare alleles each. Analysis of the distribution of these 96 unique or rare alleles in various SSR loci (Table 18) revealed that the highest number was in *phi037* and *phi015* (4 alleles each)

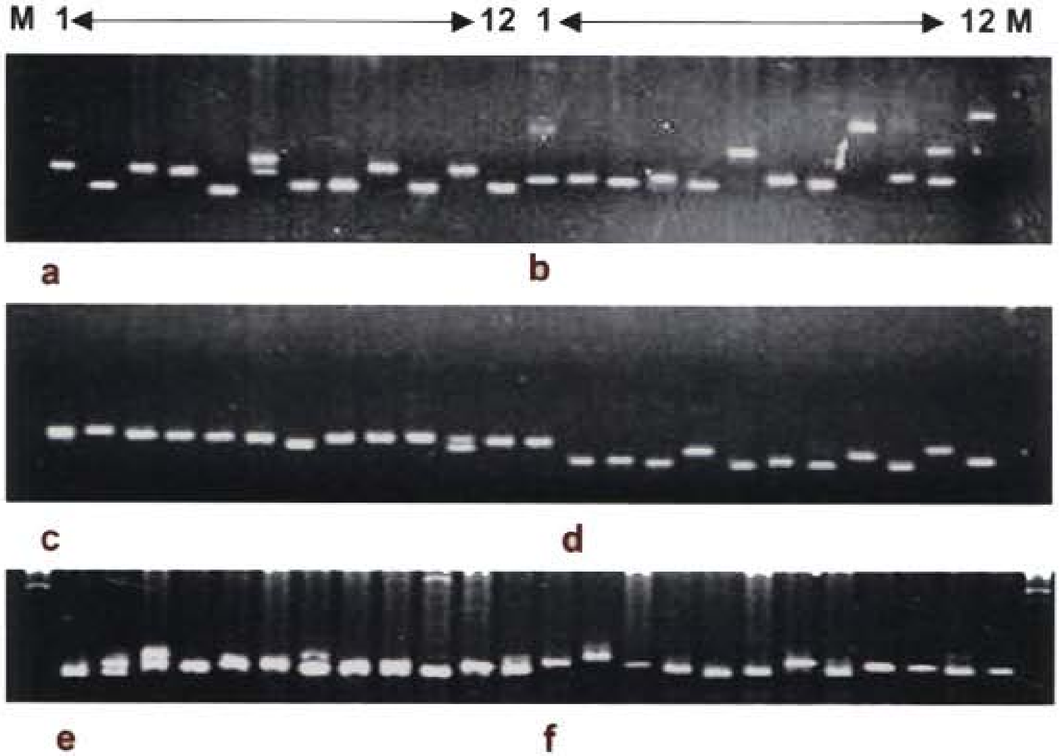


Figure 7: SSR polymorphism exhibited by the selected maize inbred lines.
a: *bnlg420*; b: *bnlg1182*; c: *phi101*; d: *bnlg389*; e: *bnlg1729*; f: *bnlg2244*
*Lane information*:
1: DTP195W; 2: DTP51W; 3: DTP105W; 4: DTP183W; 5: DTP65W; 6: DTP116Y; 7: DTP108Y; 8: DTP194Y; 9: CM139; 10: CM140; 11: Ac7729; 12: Ac7643. M represents the 100-bp molecular size standard.

## Table 18. Frequency of heteroygotes, nulls and rare SSR alleles in the inbreds

| Genotypes | No. of heteroygous SSR loci* | Frequency of heteroygous SSR loci | No. of 'nulls' for SSR markers* | Frequency of 'nulls' | No. of 'rare' SSR alleles* | Frequency of 'rare' SSR alleles |
|---|---|---|---|---|---|---|
| **DTPW195** | 4 | 0.06 | 0 | 0 | 13 | 0.18 |
| **DTPW51** | 4 | 0.06 | 1 | 0.01 | 6 | 0.08 |
| **DTPW105** | 6 | 0.08 | 1 | 0.01 | 9 | 0.13 |
| **DTPW183** | 2 | 0.03 | 1 | 0.01 | 10 | 0.14 |
| **DTPY65** | 7 | 0.10 | 1 | 0.01 | 5 | 0.07 |
| **DTPY116** | 7 | 0.10 | 2 | 0.03 | 10 | 0.14 |
| **DTPY108** | 5 | 0.07 | 1 | 0.01 | 3 | 0.04 |
| **DTPY194** | 7 | 0.10 | 0 | 0 | 9 | 0.13 |
| **CM139** | 4 | 0.06 | 2 | 0.03 | 11 | 0.15 |
| **CM140** | 4 | 0.06 | 1 | 0.01 | 9 | 0.13 |
| **Ac7729** | 6 | 0.08 | 0 | 0 | 4 | 0.06 |
| **Ac7643** | 2 | 0.03 | 5 | 0.07 | 7 | 0.10 |

*out of 71 polymorphic SSR loci analyzed among the SSR loci analyzed, whereas *bnlg147, bnlg1064, bnlg1526, bnlg420, nc013, phi119* and *phi080* showed 3 unique alleles each and 50 other loci showed 1 or 2 unique alleles each.

**Diversity Analysis using SSR Markers:** The SSR dataset was analyzed by computing pair-wise genetic similarities (GS) among the various entries using Jaccard's genetic similarity coefficient (Table 19). The GS matrix indicates the level of genetic divergence among the inbred lines under study. In general, the analysis revealed very high genetic divergence as reflected by the low GS values.

The highest GS value was between DTP194Y and DTP195W, whereas the lowest GS value was between DTP183W and CM139. On the base of average of similarity of inbred lines DTP194Y (0.20) and DTP108Y (0.145) showed maximum and minimum genetic similarity among the 12 inbred lines under study. GS values were very low for majority of the genotype pairs, in 98% of genotype pairs it was less than 0.20. Therefore, there was high genetic divergence among the genotypes analyzed.

The GS matrix was further analyzed by UPGMA clustering algorithm using NTSYS-pc version 2.11 (Rohlf, 1992). The dendrogram derived from this analysis was depicted as Figure 8. The 'cophenetic correlation coefficient' obtained using Mantel's test of correspondence between the GS matrix and the dendrogram was 0.78, indicating 'good fit' between GS matrix and the dendrogram.

The dendrogram obtained using the SSR data in the present study could be possibly 'cut' at different points to derive variable number of clusters. However, to arrive at an objective decision to determine the acceptable number of clusters, canonical discriminant analysis was carried out using SPSS9.0 statistical software. The analysis clearly revealed that there were four major clusters, with two sub-groups each in Group II and Group III (Figure 8). The analysis also revealed higher inter-group variance than intra-group variance.

### Table 19. Pair-wise genetic similarity values among the selected maize inbred lines based on analysis of SSR data using Jaccard's similarity coefficient

| Inbreds | DTP195W | DTP51W | DTP105W | DTP183W | DTP65Y | DTP116Y | DTP108Y | DTP194Y | CM139 | CM140 | Ac7729 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DTP51W | 0.12 | - | | | | | | | | | |
| DTP105W | 0.19 | 0.20 | - | | | | | | | | |
| DTP183W | 0.17 | 0.20 | 0.22 | - | | | | | | | |
| DTP65Y | 0.19 | 0.17 | 0.16 | 0.18 | - | | | | | | |
| DTP116Y | 0.15 | 0.13 | 0.17 | 0.17 | 0.18 | - | | | | | |
| DTP108Y | 0.12 | 0.13 | 0.23 | 0.14 | 0.14 | 0.12 | - | | | | |
| DTP194Y | 0.54 | 0.15 | 0.15 | 0.16 | 0.13 | 0.22 | 0.18 | - | | | |
| CM139 | 0.12 | 0.12 | 0.15 | 0.11 | 0.15 | 0.13 | 0.12 | 0.14 | - | | |
| CM140 | 0.16 | 0.17 | 0.14 | 0.14 | 0.16 | 0.19 | 0.12 | 0.19 | 0.20 | - | |
| Ac7729 | 0.12 | 0.20 | 0.13 | 0.12 | 0.19 | 0.14 | 0.14 | 0.19 | 0.21 | 0.18 | - |
| Ac7643 | 0.13 | 0.19 | 0.15 | 0.13 | 0.17 | 0.18 | 0.16 | 0.16 | 0.14 | 0.21 | 0.16 |

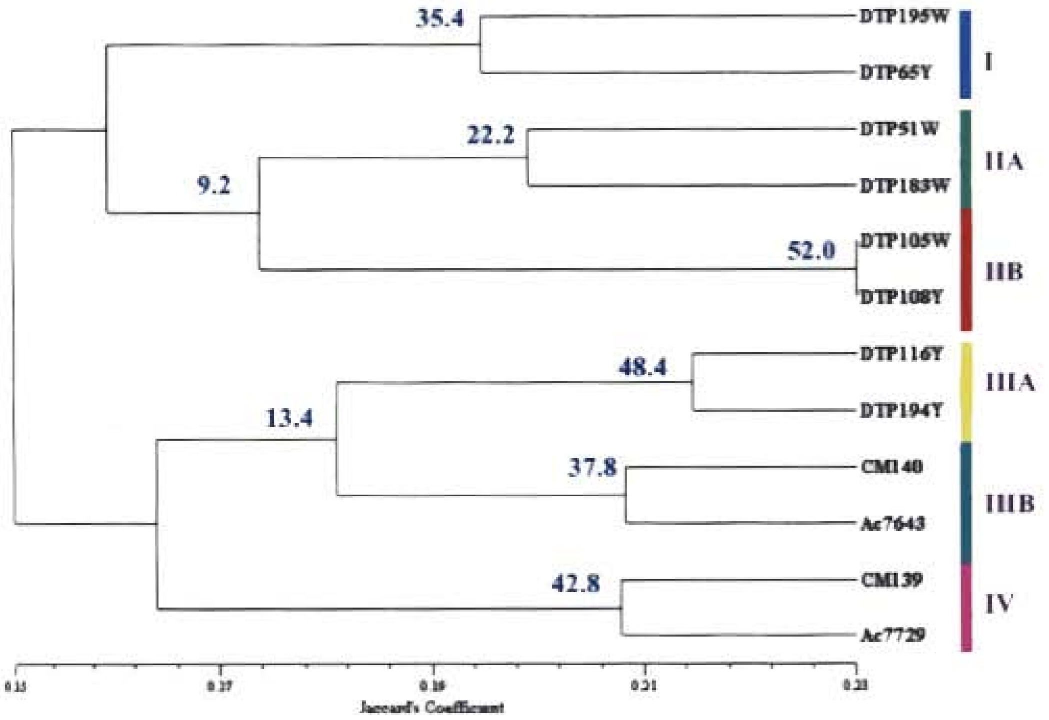


Figure 8: Dendrogram depicting the genetic relationships among the selected maize inbred lines. Bootstrap value is presented at every branch in the dendrogram.

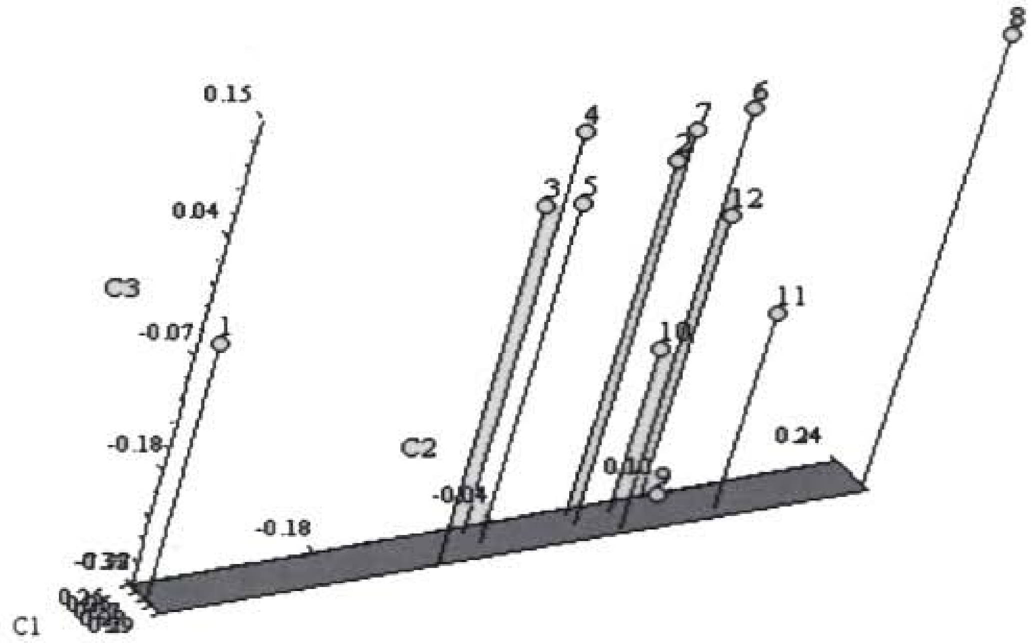


Figure 9: Three-dimensional illustration of the genetic relationships among the inbred lines based on Principal Component Analysis of the SSR dataset.

1: DTP195W; 2: DTP51W; 3: DTP105W; 4: DTP183W; 5: DTP65W;
6: DTP116Y; 7: DTP108Y; 8: DTP194Y; 9: CM139; 10: CM140;
11: Ac7729; 12: Ac7643

Group I included DTPW195 and DTPY65. Group II has two sub-groups: IIA including DTPY51 and DTPW183, and IIB comprising DTPW105 and DTPY108. Group III included two sub-groups: IIIA including DTPY116 and DTPY194 and IIIB comprising CM140 and Ac7643. Group IV which includes CM139 and Ac7729 was found to be genetically distinct from the other three groups.

Bootstrap analysis was carried out to ascertain the statistical strength of each of the nodes identified by cluster analysis. The analysis was carried out under high stringency (1000 permutations). Of the six nodes in the dendrogram, one was supported by large bootstrap values (more than 50%) and the others had moderate support (30-50%) (Figure 8). This clearly indicates that the SSR dataset generated in this study was robust and reliable enough to decipher the genetic relationships among the inbred lines analyzed.

To complement the cluster analysis in terms of understanding the relationships among the entries under study, Principal Component Analysis (PCA) was carried out. PCA further aided in depicting the relationships among the various entries in a three-dimensional mode (Figure 9). The analysis once again highlighted the distinctness of Ac7729 from rest of the inbred lines, and the close genetic similarity between the DTPW lines as compared to other lines. All the DTPY inbred lines are grouped with drought tolerant lines Ac7643 and CM140. Cluster analysis, bootstrap analysis and PCA revealed genetic relationships among the drought tolerant and susceptible lines. This study would aid in selection of proper parental lines for generation of mapping populations and segregating progenies for further utilization.

## 4. Mapping of QTLs for various traits influencing drought stress tolerance of maize in India

The two maize inbred lines used as parental lines to develop 210 the RIL families, Ac7643 (drought tolerant) and Ac7729 (drought susceptible) were tropical maize lines derived from La Posta and Tuxpeno Caribb, germplasm, respectively, at CIMMYT, Mexico. The RIL families along with the parental lines were genotyped at CIMMYT Applied Biotechnology Center, Mexico. A linkage map was constructed using the genotypic dataset based on analysis using 129 RFLP markers. The linkage map in this case spans a total length of 2250cM at an average molecular marker interval size of 17 cM.

The 210 RIL families, along with the parental lines, were evaluated first at the ANGRAU Research Station at Karimnagar, during winter/dry season 2002-03. The trial was conducted under two different water regimes (control/well-watered and drought stress) in an alpha lattice design with two replications per genotype under each treatment/water regime. The responses of entries were recorded for several characters influencing the performance of the genotypes under drought stress conditions. The same set of RIL families, along with the parental lines, was phenotyped at DMR Winter Nursery, Amberpet Farm, Hyderabad during winter/dry season 2003-04. The procedures followed for undertaking the trials were similar at Karimnagar and Hyderabad, except that the Hyderabad trial was undertaken only under drought stress conditions and not simultaneously under control/well-watered conditions due to paucity of the seed material.

**Analysis of the RIL Dataset from Karimnagar (2002-03):** The Karimnagar location, where the experiment was undertaken during 2002-03, was consistently dry during the entire winter season (November 2002 to May 2003), thus allowing control of stress severity by irrigation. In the stress block, irrigation was withdrawn three weeks before flowering. After the severe stress during flowering, irrigation was resumed at the end of the flowering stage, until harvest to allow grain filling of the pollinated embryos.

ANOVA revealed significant differences among the genotypes for all the characters analyzed under both control/well-watered and drought stress conditions (Table 20).

***Correlations among various characters:*** Pearson correlation coefficients were computed between different plant and ear characters recorded in the

**Table 20. ANOVA for various characters in the RILs evaluated at Karimnagar (2002-03) and Hyderabad (2003-04) under drought stress conditions**

| Characters | Mean Sum of Squares | | | |
|---|---|---|---|---|
| | Karimnagar | | Hyderabad | |
| | Error | Genotype | Error | Genotype |
| Plant height | 278.3 | 604.0** | 203.8 | 486.0** |
| Ear height | 108.1 | 250.7** | 73.1 | 157.8** |
| MFLW | 3.2 | 10.3** | 17.2 | 29.3** |
| FFLW | 12.1 | 15.9* | 31.6 | 49.8** |
| ASI | 7.1 | 8.4* | 12.2 | 19.6* |
| Leaf rolling | 0.6 | 1.1** | 0.3 | 0.7** |
| Root capacitance | - | - | 212.1 | 273.4* |
| Tassel size | 0.6 | 0.9** | 0.3 | 0.5** |
| Leaf senescence | 2.1 | 3.8** | 1.3 | 2.4** |
| No. of ears per plant | 14.6 | 17.9* | 0.1 | 0.1** |
| Kernel number per ear | 1052.7 | 3406.3** | 1051.3 | 4072.9** |
| 100-kernel weight | 11.3 | 26.2** | 92.3 | 119.1* |
| Grain yield per ear | 2416.8 | 5322.1** | 3940.4 | 13725.7** |

*Significant at P = 0.05; **Significant at P = 0.01.

Note: 1. The variation due to replications was not significant for any character analyzed at locations. 2. Root capacitance of the genotypes was not analyzed at Karimnagar.

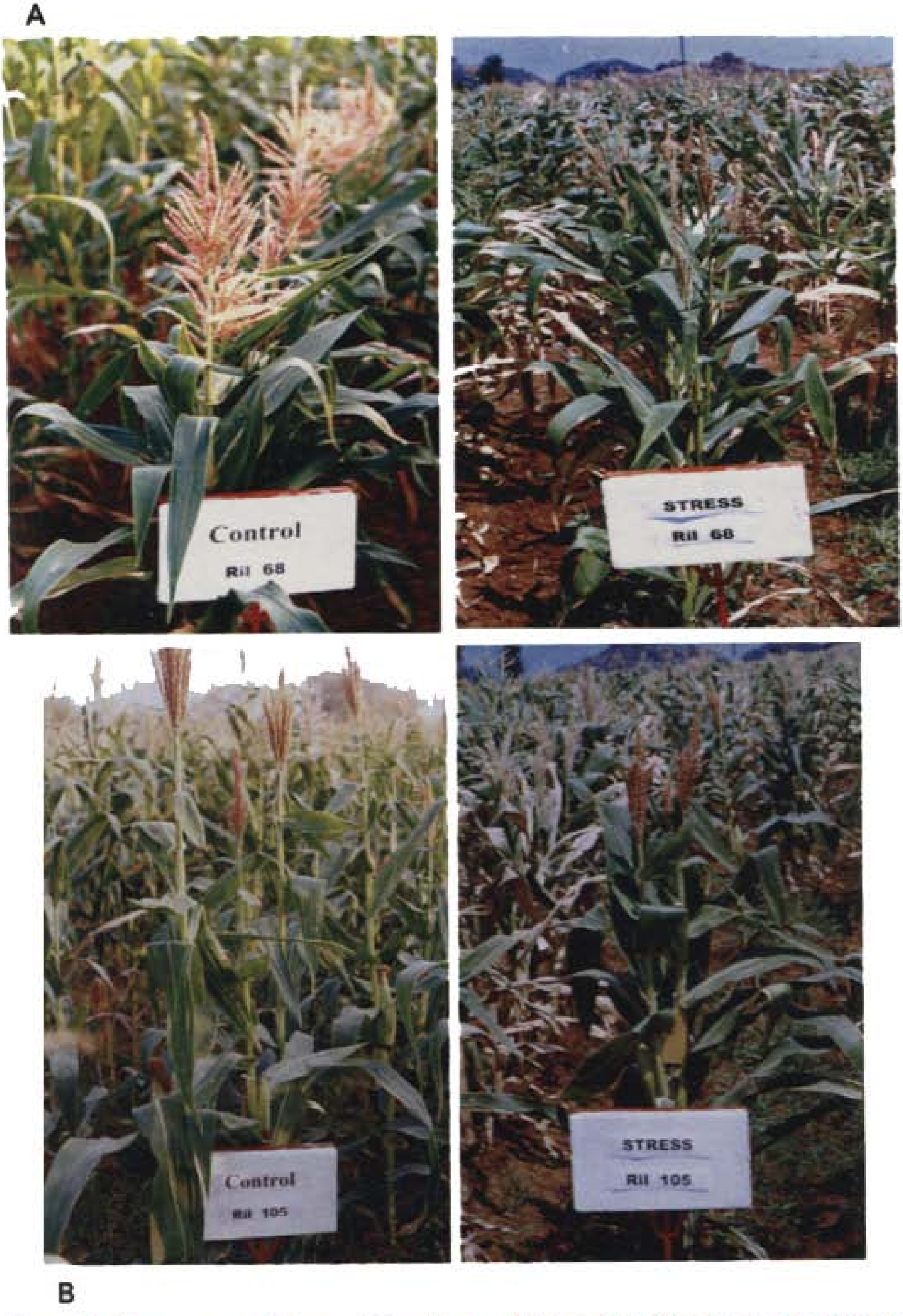


Figure 10: Responses of Recombinant Inbred Lines (RILs) to drought stress at flowering stage (Karimnagar 2002-03)
A. RIL68 under control/well-watered and drought stress conditions;
B. RIL105 under control/well-watered and drought stress conditions

RIL mapping population at Karimnagar; the analysis was done separately for the datasets from the control/well-watered (WW) and stress blocks (Table 21). MFLW and FFLW were found to be highly correlated under WW as well as drought stress conditions (r = 0.77 and 0.61, respectively). Correlation between ASI and MFLW was non-significant under stress and also under the WW condition. On the other hand, the correlations between ASI and FFLW in both the water regimes were positive and significant (r = 0.33 and r = 0.34, respectively).

A comparison of the average ASI value in stress condition (1.08 days) versus the same under WW condition (3.4 days) indicates the effect of drought stress on ASI. The average MFLW values in the WW and stress blocks across the RIL families were comparable (68 and 67 days, respectively). However, drought stress led to a distinct delay in silk emergence as revealed by the mean FFLW values under WW and stress conditions (69 and 71 days, respectively). Therefore, it was the delay in FFLW that led to a higher mean ASI value in the stress block as compared to the ASI value in the control.

Grain yield is the ultimate criterion to judge the *per se* performance of a genotype under drought stress conditions. Under the WW conditions, grain yield recorded significant correlations with all characters, except leaf rolling. With MFLW, FFLW, ASI and leaf senescence, the correlations of grain yield were found to be negative and significant. In contrast, grain yield recorded significant correlations with all characters, except ear height under drought stress condition. The correlations were negative and significant with MFLW, FFLW, ASI, leaf rolling and leaf senescence. The most important characters showing strong correlation with grain yield under drought stress conditions were ear number per plant, kernel number per ear and ear diameter.

Ear number per plant, which indicates the productivity of the genotype, recorded highly significant and positive correlation with grain yield under both normal and stress conditions. The correlation of ear number per plant with all other characters, except leaf rolling, was found to be significant under WW conditions. Under the drought stress conditions, the same character

### Table 21. Correlation coefficients among various characters in the RILs under control/well-watered and drought stress conditions (Karimnagar 2002-03)

| Characters | PHT | EHT | MFLW | FFLW | ASI | LR | TS | LS | ENO | KNO | HKW | GYLD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PHT** | - | 0.83** | 0.11* | -0.02 | -0.17** | -0.23** | 0.22** | -0.01 | 0.28** | 0.20** | 0.22** | 0.32** |
| **EHT** | 0.74** | - | 0.18** | 0.03 | -0.23** | -0.28** | 0.13** | -0.01 | 0.27** | 0.18** | 0.13* | 0.27** |
| **MFLW** | -0.16** | -0.07 | - | 0.77** | -0.02 | -0.18** | -0.55** | 0.09 | -0.19** | -0.30** | -0.25** | -0.32** |
| **FFLW** | -0.25** | -0.15** | 0.61** | - | 0.33** | -0.13* | -0.52** | 0.18** | -0.35** | -0.43** | -0.29** | -0.47** |
| **ASI** | -0.16* | -0.12* | -0.02 | 0.34** | - | 0.16** | 0.19** | 0.11* | -0.40** | -0.28** | -0.14* | -0.37** |
| **LR** | -0.16** | -0.09 | 0.16** | 0.14* | -0.06 | - | 0.13* | 0.02 | -0.03 | 0.05 | 0.11* | 0.07 |
| **TS** | 0.27** | 0.21** | -0.34** | -0.19** | 0.26** | -0.12* | - | -0.04 | 0.18** | 0.48** | 0.38** | 0.57** |
| **LS** | 0.11* | 0.15** | -0.23** | -0.14** | 0.13* | 0.08 | 0.09 | - | -0.25** | -0.12* | -0.18** | -0.17** |
| **ENO** | 0.06 | -0.002 | -0.02 | -0.18** | -0.38** | -0.31** | 0.04 | -0.47** | - | 0.49** | 0.23** | 0.80** |
| **KNO** | 0.22** | 0.12* | -0.20** | -0.26** | -0.13* | -0.04 | 0.34** | -0.09 | 0.32** | - | 0.15** | 0.78** |
| **HKW** | 0.24** | 0.06 | -0.12* | -0.08 | 0.02 | -0.09 | 0.02 | -0.14** | 0.19** | 0.10 | - | 0.35** |
| **GYLD** | 0.20** | 0.10 | -0.11* | -0.22** | -0.25** | -0.11* | 0.28** | -0.27** | 0.56** | 0.66** | 0.30** | - |

*Significant at P=0.05; **Significant at P = 0.01

Values above the diagonal are correlation coefficients under control/well-watered conditions, and values below the diagonal were for the drought stress conditions

Abbreviations: PHT: Plant height; EHT: Ear height; MFLW: Male flowering; FFLW: Female flowering; ASI: Anthesis-silking interval; TS: Tassel size; LR: Leaf rolling; LS: Leaf senescence; ENO: ear number per plant; GYLD: Grain yield per plot; EL; KNO: Kernel number per ear; HKW: 100-kernel weight displayed significant and negative correlations with FFLW, ASI, leaf rolling and leaf senescence, and positive and significant correlations with kernel number per ear, 100-kernel weight and grain yield.

***QTL Analyses:*** QTL mapping was performed using adjusted means of the RIL dataset for various characters. The localization of QTLs for each trait is primarily based on linkage disequilibrium between the markers and a chromosomal region (QTL) contributing to the target trait of interest. The map distances in the linkage map developed by analysis of genotypic dataset of the 210 RIL families along with phenotypic trait means and molecular marker data for each individual in the mapping population, was utilized for QTL analysis by a QTL mapping software developed by Zeng (1994). This powerful statistical package utilizes a 'family' of four interval mapping models: Model 1 uses a Simple interval mapping (SIM) approach that does not include any marker as cofactor. The next three models are based on Composite Interval Mapping (CIM), which uses a mixed model and likelihood methods. In Model 2, pre-selected unlinked markers, closely linked to significant QTL peaks identified through SIM (Model I), are used as cofactors to reduce the residual variation throughout the genome. In Models 3 and 4, markers flanking the tested interval are used as cofactors to block the effects of possible QTL that are close to the interval of interest. These two models tests two different "window" sizes: 30cM and 20cM, respectively.

QTL detection was inferred on the basis of likelihood ratio (LR) peaks. In the context of interval mapping, the log likelihood ratio is called the LOD score (Lander and Botstein, 1989).The general relationship between LOD ($\log_{10}$ of the odds) and the LR is as follows: LOD=0.217 LR. For a single trait analysis using a RIL mapping population, and for the LOD scores of 2.0, 2.5 and 3.0, the corresponding critical LR values are 9.21, 11.51 and 13.82, respectively. This is applicable for the Type I error rates (α level) of 0.01, 0.003 and 0.001 for LOD=2.0, LOD=2.5 and LOD=3.0, respectively. At each significant QTL, allelic effects and additivity (applicable to RILs) were obtained directly from the output of the software. QTL identification was thus achieved by successively running the four models and comparing the different outputs.

A total of 52 QTLs, including 22 QTLs under the control/well-watered condition and 30 QTLs under drought stress conditions (as listed in Table 22), were detected through the above-mentioned analysis of the RIL dataset from Karimnagar. These QTLs were distributed on almost all chromosomes, except chr. 7 (Table 22) (Figure 11). No significant QTLs at LOD>2.0 could be identified for leaf rolling, leaf senescence, tassel size, root capacitance and 100-kernel weight.

***Plant Characters:*** Five QTLs were detected for plant height, with one on chr. 1 (188 cM), three on chr. 3 (three QTLs on 29 cM, 104 cM and 197cM) and one on chr. 8 (88 cM), together explaining 25 per cent of total phenotypic variance for this trait under drought stress conditions. In contrast, only one QTL could be detected under the WW regime, located on chr. 9 (81 cM). Nevertheless, this QTL accounted for about 11% of the phenotypic variance. The QTLs that contributed to an increased plant height in the RIL mapping population under WW and stress conditions were from the drought-susceptible parent (Ac7729), as demonstrated by the negative value of the mean additivity of those QTLs. In contrast, one QTL on chr. 3 (197 cM) that led to an increase in plant height was from the drought-tolerant parent (Ac7643), indicated by the positive value.

Several QTLs could be identified for ear height both under W and drought stress conditions. Of the 4 QTLs detected for ear height under WW conditions, three were located on chr. 4, of which the one at 15 cM was at repulsion phase with the rest. Under the drought stress conditions, six QTLs could be detected of which three were on chr. 3 (two in repulsion phase). Significantly, one of the QTLs in chr. 4 (167 cM) was exactly the same as that in the WW conditions. Also, the six QTLs identified in the stress data accounted for nearly 31% of the phenotypic variance.

Three QTLs were detected for MFLW, one each on chr. 4 (142 cM), 6 (110 cM) and 9 (132 cM) under WW conditions, whereas under drought

**Table 22. QTLs detected for salient yield components under control/well-watered and drought stress conditions at Karimnagar (2002-03; RIL dataset)**

| Chr. | cM | LR | Markers | $R^2$(%) | Additivity |
|---|---|---|---|---|---|
| ***Plant height - Control*** | | | | | |
| 9 | 81 | 19.90 | *umc114-umc95* | 11.05 | -3.73 |
| | | | **Total $R^2$** | **11.05** | |
| ***Plant height - Stress*** | | | | | |
| 1 | 188 | 13.30 | *umc33-umc83* | 2.08 | -1.74 |
| 3 | 29 | 9.63 | *umc313-umc50* | 5.54 | -1.61 |
| | 104 | 11.60 | *umc372-bnl8.01* | 6.93 | -1.84 |
| | 197 | 19.11 | *umc39-umc17* | 2.26 | 2.43 |
| 8 | 88 | 19.24 | *umc152-umc120* | 5.24 | -2.23 |
| | | | **Total $R^2$** | **25.00** | |
| ***Ear height - Control*** | | | | | |
| 4 | 15 | 10.90 | *php20725-bnl5.46* | 3.20 | -1.67 |
| | 152 | 14.98 | *umc318-umc66* | 5.34 | 2.09 |
| | 167 | 10.15 | *umc104-umc133* | 5.65 | 1.75 |
| 9 | 106 | 11.96 | *umc95-umc366* | 5.25 | -2.27 |
| | | | **Total $R^2$** | **16.62** | |
| ***Ear height - Stress*** | | | | | |
| 3 | 30 | 17.04 | *umc313-umc50* | 3.21 | -1.73 |
| | 111 | 17.27 | *umc372-bnl8.01* | 3.34 | -1.64 |
| | 199 | 29.00 | *umc17-umc96* | 5.20 | 2.43 |
| 4 | 167 | 10.43 | *umc104-umc133* | 4.37 | 1.26 |
| 8 | 96 | 20.15 | *umc120-umc346* | 6.82 | -2.00 |
| 9 | 127 | 10.67 | *umc358-bnl14.28* | 2.51 | -1.31 |
| | | | **Total $R^2$** | **31.33** | |
| ***Male flowering - Control*** | | | | | |
| 4 | 142 | 9.97 | *umc156-umc318* | 4.57 | 0.32 |
| 6 | 110 | 12.08 | *umc341-umc369* | 5.60 | 0.35 |
| 9 | 132 | 13.88 | *umc358-bnl4.28* | 3.86 | -0.38 |
| | | | **Total $R^2$** | **14.01** | |
| ***Male flowering - Stress*** | | | | | |
| 1 | 259 | 9.33 | *bnl6.29-umc147* | 4.37 | 0.28 |
| 2 | 108 | 17.56 | *umc8-umc55* | 5.62 | 0.40 |
| 4 | 153 | 10.56 | *umc318-umc66* | 5.10 | 0.28 |
| 9 | 113 | 10.63 | *umc366-umc358* | 2.49 | -0.30 |
| | | | **Total $R^2$** | **17.93** | |
| ***Female flowering - Control*** | | | | | |
| 4 | 141 | 12.61 | *umc156-umc318* | 4.53 | 0.23 |
| | | | **Total $R^2$** | **4.53** | |
| ***Female flowering - Stress*** | | | | | |
| 8 | 138 | 11.21 | *umc89-umc30* | 5.74 | -0.22 |
| | | | **Total $R^2$** | **5.74** | |
| ***Anthesis-silking interval - Control*** | | | | | |
| 2 | 84 | 21.49 | *umc34-umc371* | 8.22 | -0.15 |
| 10 | 56 | 10.09 | *bnl7.49-umc354* | 5.12 | -0.11 |
| | | | **Total $R^2$** | **13.12** | |

**Table 22 (Contd.)**

| Chr. | cM | LR | Markers | $R^2$ (%) | Additivity |
|---|---|---|---|---|---|
| ***Anthesis-silking interval - Stress*** | | | | | |
| 10 | 68 | 9.34 | *umc354-umc182* | 3.89 | -0.23 |
| | | | **Total $R^2$** | **3.89** | |
| ***Grain yield – Control*** | | | | | |
| 1 | 153 | 9.42 | *umc119-umc33* | 4.00 | 16.48 |
| 4 | 146 | 9.88 | *umc156-umc318* | 3.51 | -16.44 |
| | | | **Total $R^2$** | **6.99** | |
| ***Grain yield - Stress*** | | | | | |
| 2 | 169 | 11.54 | *bnl6.29-umc137* | 3.11 | 5.44 |
| | 192 | 11.97 | *umc150-umc344* | 4.73 | 5.18 |
| 3 | 220 | 13.17 | *umc17-umc96* | 5.66 | 5.76 |
| 5 | 103 | 14.76 | *bnl6.22-bnl5.71* | 6.06 | -5.68 |
| | | | **Total $R^2$** | **18.90** | |
| ***Ear number per plant - Control*** | | | | | |
| 1 | 160 | 6.75 | *umc119-umc33* | 2.50 | 0.15 |
| 2 | 89 | 6.51 | *umc34-umc371* | 2.37 | 0.17 |
| | | | **Total $R^2$** | **4.76** | |
| ***Ear number per plant - Stress*** | | | | | |
| 3 | 236 | 13.13 | *umc17-umc96* | 4.92 | 0.17 |
| 5 | 103 | 25.03 | *bnl6.22-bnl5.71* | 11.33 | -0.20 |
| 10 | 68 | 9.54 | *bnl7.49-umc354* | 3.69 | 0.12 |
| | | | **Total $R^2$** | **18.13** | |
| ***100-kernel weight - Control*** | | | | | |
| 4 | 101 | 18.08 | *bnl5.46-bnl5.71* | 8.04 | -0.38 |
| | | | | **8.04** | |
| ***100-kernel weight - Stress*** | | | | | |
| 2 | 166 | 9.23 | *bnl6.29-umc137* | 3.17 | 0.40 |
| | | | **Total $R^2$** | **9.59** | |
| ***Kernel number per ear - Control*** | | | | | |
| 1 | 135 | 9.89 | *umc167-bnl5.59* | 5.40 | 9.49 |
| 2 | 75 | 9.47 | *umc34-umc371* | 0.94 | 8.17 |
| 3 | 192 | 11.77 | *umc39-umc17* | 3.87 | 8.80 |
| 4 | 149 | 9.66 | *umc318-umc66* | 4.27 | -7.14 |
| 10 | 96 | 13.05 | *umc354-umc182* | 5.50 | 9.40 |
| | 167 | 12.57 | *umc334* | 4.85 | -7.82 |
| | | | **Total $R^2$** | **24.62** | |
| ***Kernel number per ear - Stress*** | | | | | |
| 2 | 122 | 13.50 | *umc8-umc55* | 2.52 | -7.72 |
| | 170 | 9.42 | *bnl6.29-umc137* | 2.96 | 6.15 |
| | 205 | 10.51 | *umc150-umc344* | 4.81 | 6.10 |
| 10 | 56 | 13.29 | *bnl7.49-umc354* | 6.83 | 8.15 |
| | 78 | 9.47 | *umc354-umc182* | 4.90 | 7.12 |
| | | | **Total $R^2$** | **19.21** | |

*LR: Likelihood ratio

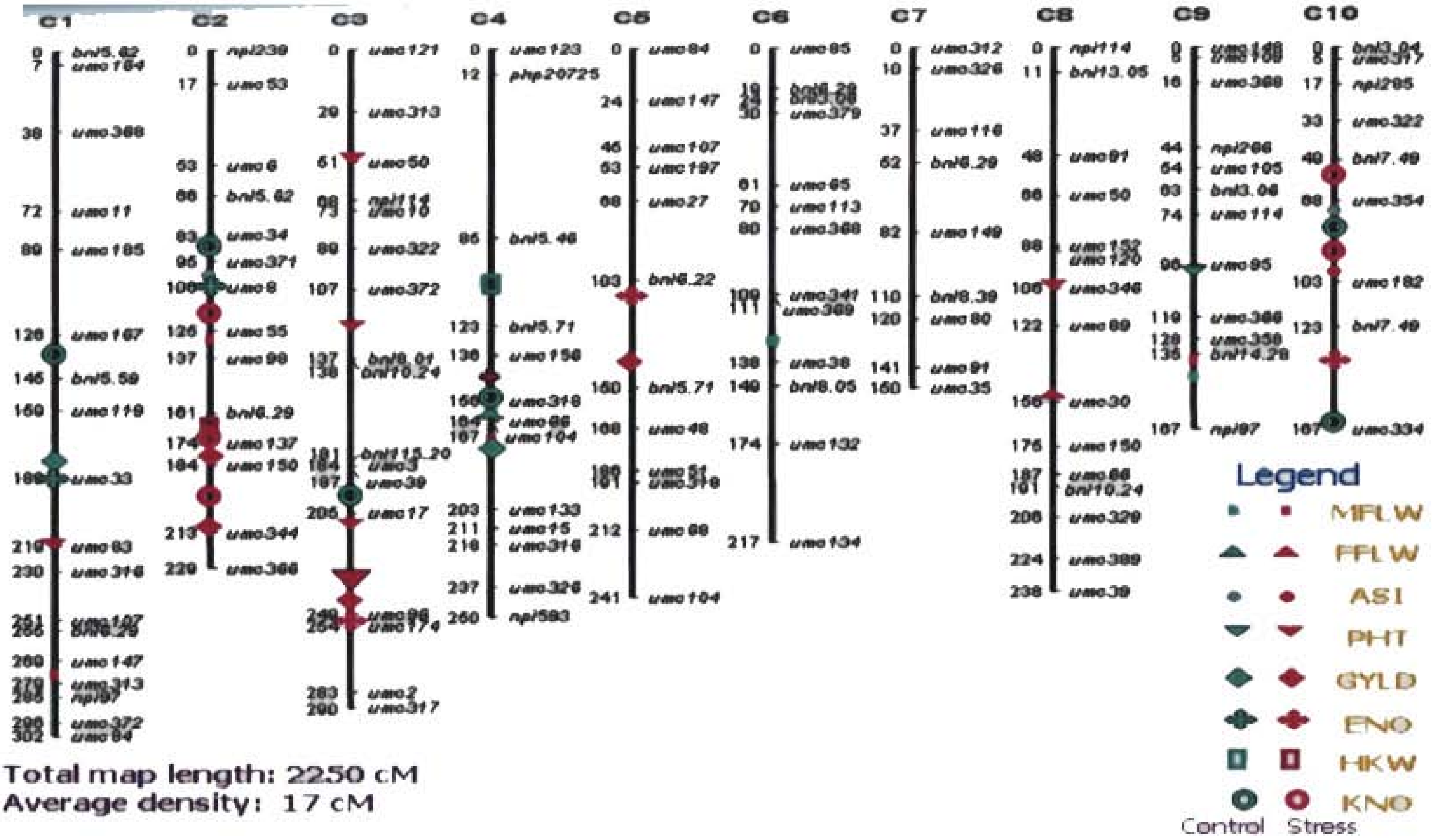


Figure 11: QTLs detected for different traits in the RIL mapping population evaluated in the drought experiment at Karimnagar (2002-03); A. Green colored symbols depict QTLs under normal/well-watered condition; B. Red colored symbols depict QTLs under drought stress condition
MFLW: Male flowering; FFLW: Female flowering; ASI: Anthesis-silking interval; GYLD: Grain yield;
ENO: Ear number per plant; HKW: 100-kernel weight; KNO: Kernel number per ear

stress, four QTLs were identified, with one each on chr. 1 (259 cM), 2 (108 cM), 4 (153 cM) and 9 (113cM). These QTLs collectively accounted for 14.01% and 17.93% of phenotypic variance ($R^2$), respectively, under WW and stress regimes. Except for the QTLs detected on chr.9 that were contributed by the drought susceptible parent (Ac7729), the rest were from the drought tolerant parent, Ac7643, as can be inferred from the + and – signs of additive variances (Table 22).

In contrast to MFLW, only one QTL each were detected for FFLW under WW and stress conditions. These were located on chr. 4 (141 cM) in WW dataset and chr. 8 (138 cM) in the stress dataset, with very low $R^2$ values. However, the QTL on chr. 4 in this case corresponded exactly with that detected for MFLW.

In case of ASI, two QTLs were identified, one each on chr. 2 (84 cM) and chr. 10 (56 cM), for the WW data, and one on chr. 10 (68 cM) for the stress data. While the two QTLs detected for WW data explained 13% of the phenotypic variance, the one on chr. 10 in the stress dataset accounted for only about 4% of the phenotypic variance. The QTLs that contributed to an increase in the ASI were all from Ac7729.

***Ear Characters:*** The QTLs identified for grain yield under WW and stress conditions were different. In the WW conditions, two QTLs were identified one each on chr. 1 and 4. In contrast, four QTLs were detected for the same trait under drought stress conditions, with two on chr. 2, one on chr. 3 and one on chr. 5. The four QTLs under drought stress conditions explained nearly 19% of the phenotypic variance. Except for one QTL on chr. 5, rest of the four were from the drought-tolerant parent, Ac7643.

For ear number per plant, three QTLs were identified, one each on chr. 3 (236 cM), 5 (103 cM) and 10 (68 cM). These QTLs collectively accounted for 18.13% of phenotypic variance. Only two QTLs (one each on chr. 1 and 2) were detected for the same trait under WW conditions, together explaining only about 5% of the phenotypic variance. All the QTLs influencing ear number per plant under WW and drought stress conditions were from

Ac7643, except for one QTL on chr. 5 contributed by Ac7729.

In case of 100-kernel weight, the analysis revealed one QTL on chr. 4 (101 cM) from Ac7729 under WW conditions, and one QTL from Ac7643 on chr. 2 (166 cM) under drought stress conditions. These two QTLs accounted for about 8% and 9% of the phenotypic variance, respectively, in the two irrigation regimes.

Several QTLs were identified both under WW and stress regimes in case of kernel number per ear. The six QTLs (four from Ac7643 and two from Ac7729) identified on various chromosomes in the WW regime together accounted for nearly 25% of the phenotypic variance, while the five QTLs (all except one contributed by Ac7643) in case of stress dataset explained nearly 20% of the phenotypic variance. In the latter, three of the five QTLs were identified on chr. 2, of which two (at 170 cM and 205 cM) were in repulsion phase with one at 122 cM (Table 22). The two QTLs on chr. 10 for the same trait were in coupling phase with each other.

Further analysis of the results presented in Table 22 and Figure 11 clearly revealed that as many as 7 out of the 24 QTLs influencing the performance of the RILs under drought stress conditions were located on chr. 2, followed by 5 QTLs on chr. 3, and 4 QTLs on chr. 10. In contrast, analysis of the results from the WW conditions revealed 5 QTLs on chr. 4, followed by 3 QTLs on chr. 2.

When the QTL locations were compared across different traits, certain interesting findings emerged. For instance, a QTL located on chr. 3 (29-30 cM) flanked by the markers *umc313* and *umc50* showed significant effects on both plant height and ear height; another QTL on chr. 3, with *umc17* and *umc96* as flanking markers, influenced both grain yield and ear number per plant in the stress block. One QTL on chr. 5 (103 cM), contributed by Ac7729, showed its effect both on grain yield and ear number per plant under drought stress conditions. Similarly, another QTL on chr. 2 (166-169 cM) flanked by markers *bnl6.29* and *umc137* influencing grain yield, 100-kernel wight and kernel number per ear under drought stress conditions.

The range of $R^2$ values of the QTLs identified in this study was 0.94–11.33%, while the range of total phenotypic variance accounted by combining all the significant QTLs ranged from 4.53–24.62% in case of WW conditions, and 3.89–31.33% under stress conditions. The study was successful in identifying QTLs that could account for significant proportion of phenotypic variance for some characters like plant height (stress), ear height (control and stress), male flowering (control and stress), ASI (control), grain yield (stress), ear number per plant (stress) and kernel number per ear (control and stress).

**Analysis of the RIL Dataset from Hyderabad (2003-04):** ANOVA revealed significant differences among the genotypes for different characters analyzed under drought stress conditions (Table 20).

***Correlations among various characters:*** Pearson correlation coefficients were computed between different plant and ear characters recorded in the RIL mapping population under drought stress conditions at Hyderabad (2003-04) (Table 23). MFLW and FFLW were found to be highly correlated, while plant height and ear height did not show significant correlation with MFLW. However, the correlation between FFLW with plant height was found to be negative and significant. Correlations of ASI with MFLW and MFLW were negative and non-significant.

ASI recorded negative and significant correlations with almost all characters under study, except ear height, leaf rolling and root capacitance. Similarly, grain yield showed significant correlations with all characters, except root capacitance and leaf rolling. Similarly, 100-kernel weight showed significant correlations with all characters, except root capacitance. The correlations of grain yield with MFLW, FFLW, ASI, leaf rolling and leaf senescence were negative and significant. Ear number per plant recorded highly significant and positive correlation with grain yield, and significant correlations with many other characters, except MFLW and root capacitance.

## Table 23. Correlation coefficients among various characters in the RILs under drought stress conditions (Hyderabad 2003-04)

| Characters | EHT | MFLW | FFLW | ASI | RC | LR | TS | LS | ENO | KNO | HKW | GYLD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PHT | 0.68** | -0.08 | -0.19** | -0.23** | 0.20** | -0.14** | 0.37** | -0.16** | 0.20** | 0.32** | 0.26** | 0.38** |
| EHT | - | -0.05 | -0.07 | -0.06 | 0.16** | -0.15** | 0.34** | -0.10* | 0.10* | 0.17** | 0.17** | 0.24** |
| MFLW |  | - | 0.51** | -0.28** | -0.02 | -0.14** | -0.13** | -0.15** | 0.05 | -0.16** | 0.16** | -0.14** |
| FFLW |  |  | - | 0.38** | 0.01 | -0.06 | -0.18** | 0.03 | -0.16** | -0.31** | -0.15** | -0.28** |
| ASI |  |  |  | - | -0.02 | 0.07 | -0.12* | 0.22** | -0.35** | -0.33** | -0.48** | -0.30** |
| RC |  |  |  |  | - | -0.16** | 0.01 | 0.05 | -0.01 | 0.06 | 0.02 | 0.06 |
| LR |  |  |  |  |  | - | 0.02 | 0.32** | -0.16** | -0.05 | -0.15** | -0.08 |
| TS |  |  |  |  |  |  | - | 0.01 | 0.04** | 0.24** | 0.10** | 0.24** |
| LS |  |  |  |  |  |  |  | - | -0.46** | -0.17** | -0.41** | -0.20** |
| ENO |  |  |  |  |  |  |  |  | - | 0.41** | 0.69** | 0.43** |
| KNO |  |  |  |  |  |  |  |  |  | - | 0.37** | 0.93** |
| HKW |  |  |  |  |  |  |  |  |  |  | - | 0.42** |

*Significant at P=0.05; **Significant at P = 0.01

Abbreviations: PHT: Plant height; EHT: Ear height; MFLW: Male flowering; FFLW: Female flowering; ASI: Anthesis-silking interval; RC: Root capacitance; TS: Tassel size; LR: Leaf rolling; LS: Leaf senescence; ENO: Ear number per plant; GYLD: Grain yield; KNO: Kernel number per ear; HKW: 100-kernel weight

***QTL Mapping:*** QTL mapping was performed using adjusted means of the RIL dataset for various characters. The localization of QTLs for each trait was based on the procedures described earlier for the Karimnagar experiment. A total of 14 QTLs (as listed in Table 24) were detected in the RILs under drought stress conditions for six most important traits that determine the *per se* performance of a maize line under drought stress conditions; these were plant height, ear height, anthesis-silking interval (ASI), grain yield, ear number per plant and kernel number per ear (Figure 12). The QTLs identified for male flowering, female flowering, leaf rolling, leaf senescence, tassel size, root capacitance and 100-kernel weight were not reported due to their low level of significance (LOD<2.0).

***Plant Characters:*** Three QTLs were detected for plant height, with one each on chr. 1(183 cM), chr. 3 (230 cM) and chr. 8 (87 cM), together explaining 14.53% of total phenotypic variance for this trait under drought stress conditions. Interestingly, QTLs for ear height were identified on the same chromosomes, namely 1, 3 and 8. Except for chr. 3, the other two QTLs were flanked by the same markers (Table 24). The data indicated that all the QTLs contributing to an increase in plant height and ear height in the RIL mapping population at Hyderabad were from Ac7729. It is also important to note that the three QTLs in case of plant height accounted collectively 14.53% of phenotypic variance, while those detected for ear height had a $R^2$ value of 20.58%.

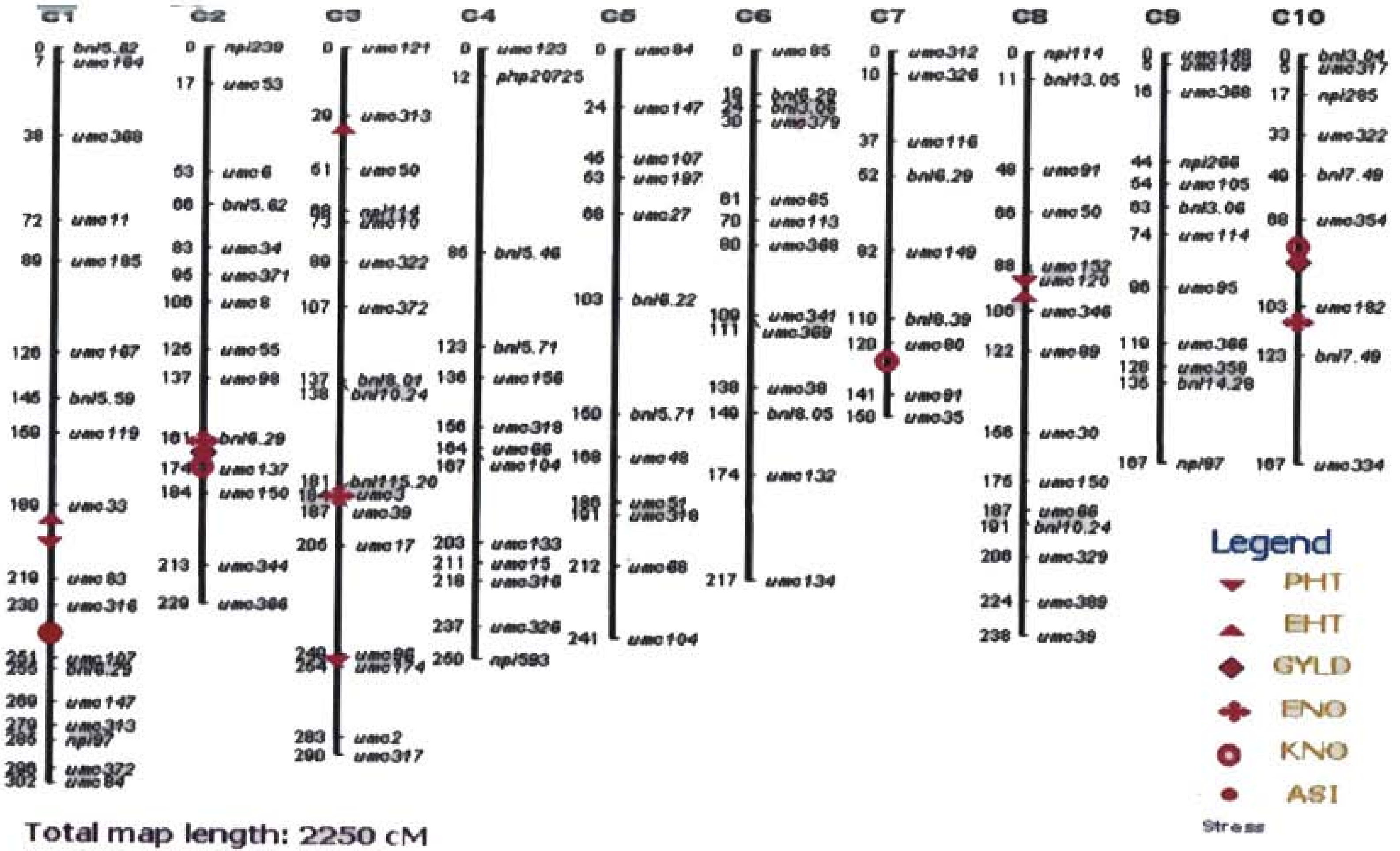
In case of ASI, only one QTLs could be identified on chr. 1 (238 cM) under drought stress conditions, explaining nearly 5% of the phenotypic variance. This specific QTL was contributed by Ac7643.

***Ear Characters:*** The QTLs identified for grain yield under WW and stress conditions were different. In the WW conditions, two QTLs were identified one each on chr. 1 and 4. In constrast, four QTLs were detected for the same trait under drought stress conditions, with two on chr. 2, one on chr. 3 and

**Table 24. QTLs detected for salient yield components under drought stress conditions at Hyderabad (2003-04; RIL dataset)**

| Chr. | cM | LR* | Markers | $R^2$ (%) | Additivity |
|---|---|---|---|---|---|
| ***Plant height*** | | | | | |
| 1 | 183 | 11.20 | *umc33-umc83* | 4.32 | -4.26 |
| 3 | 230 | 10.03 | *umc96-umc174* | 5.24 | -4.26 |
| 8 | 87 | 11.97 | *umc120-umc346* | 4.33 | -4.66 |
| | | | **Total $R^2$** | **14.53** | |
| ***Ear height*** | | | | | |
| 1 | 174 | 25.60 | *umc33-umc83* | 9.56 | -4.31 |
| 3 | 29 | 18.05 | *umc313-umc50* | 6.71 | -3.25 |
| 8 | 94 | 9.86 | *umc120-umc346* | 5.16 | -2.69 |
| | | | **Total $R^2$** | **20.58** | |
| ***Anthesis-Silking Interval*** | | | | | |
| 1 | 238 | 10.53 | *umc316-umc107* | 4.98 | 0.63 |
| | | | **Total $R^2$** | **4.98** | |
| ***Grain yield*** | | | | | |
| 2 | 166 | 11.20 | *bnl6.29-umc137* | 5.82 | 13.20 |
| 10 | 84 | 12.53 | *umc354-umc182* | 3.84 | 15.32 |
| | | | | **9.44** | |
| ***Ear number per plant*** | | | | | |
| 2 | 166 | 16.07 | *bnl6.29-umc137* | 7.34 | 0.47 |
| 3 | 170 | 11.98 | *bnl15.20-umc3* | 4.41 | 0.42 |
| 10 | 91 | 17.22 | *umc354-umc182* | 7.04 | 0.51 |
| | | | **Total $R^2$** | **19.28** | |
| ***Kernel number per ear*** | | | | | |
| 2 | 166 | 11.30 | *umc83-umc316* | 5.76 | -7.81 |
| 7 | 120 | 10.71 | *umc80-umc91* | 5.24 | 8.30 |
| | | | **Total $R^2$** | **9.77** | |

*LR: Likelihood ratio



Total map length: 2250 cM
Average density: 17 cM

Figure 12: QTLs detected for different traits in the RIL mapping population evaluated under drought stress) conditions at Hyderabad (2003-04)

PHT: Plant height; EHT: Ear height; ASI: Anthesis-silking interval; GYLD: Grain yield; ENO: Ear number per plant; HKW: 100-kernel weight; KNO: Kernel number per ear one on chr. 5. The four QTLs under drought stress conditions explained nearly 19% of the phenotypic variance.

Two QTLs, one each on chr. 2 and 10, were detected for grain yield. These two QTLs, contributed by Ac7643, showed high additivity, but accounted for only a small proportion of the phenotypic variance (9.44%). Interestingly, however, the same QTLs were also found to influence the expression of ear number per plant. In addition, ear number per plant was also affected by another QTL on chr. 3. The three QTLs for ear number per plant collectively accounted for 19.28% of the phenotypic variance.

The study also resulted in identification of two QTLs, one each on chr. 2 (166 cM) and 7 (120 cM) influencing kernel number per ear under drought stress conditions. Of these, the QTL on chr. 2 was from Ac7729, while the one on chr. 7 was from Ac7643; both these QTLs accounted for about 10% of the phenotypic variance.

Further analysis of the results presented in Table 24 and Figure 12 clearly revealed that except for one QTL on chr. 7, all the others were localized on chromosomes 1, 2, 3, 8 and 10. There was an excellent correspondence of QTL locations between different characters analyzed as explained above. The study was also successful in identifying QTLs that could account for significant proportion of phenotypic variance for plant height, ear height and ear number per plant. Of particular importance are the following QTLs: (i) chr. 1 in the marker interval of *umc33* and *umc83*; (ii) chr. 2 (166 cM) flanked by the markers *bnl6,29* and *umc137*; (iii) chr. 8, flanked by markers *umc120* and *umc346*; and (iv) chr. 10, flanked by *umc354* and *umc182*. These QTLs showed effects on diverse characters under drought stress conditions, thus highlighting their potential utility in molecular marker-assisted breeding for drought tolerance in maize.

**Identification of 'consensus QTLs':** A comparison of the QTL information obtained based on analyses of the Karimnagar and Hyderabad datasets (Tables 22 and 24, respectively) revealed the correspondence of QTLs in the RILs influencing specific characters under drought stress conditions in both locations. These were: (i) for plant height, chr. 1 QTL (183-188 cM) and chr. 8 QTL (87-88 cM); (ii) for ear height, chr. 3 (29-30 cM) and chr. 8 (94-96 cM); (iii) for grain yield, chr. 2 (166-169 cM).

Significant is the fact that the same QTLs were found to be important in drought mapping experiments undertaken by CIMMYT (Ribaut *et al.*, 2002, 2003; Prasanna and Ribaut, 2004) using the same set of RILs at Mexico, Kenya and Zimbabwe. Such 'universal drought QTLs' could be of great value in molecular marker-assisted breeding for drought tolerance in maize, besides genomics.

# Discussion

Food production in the tropics is influenced by two major forces affecting supply and demand: (i) declining availability of good quality agricultural land and water; and (ii) an annual population increase of around 80 million people. Water deficit is now recognized as a major threat to food security, sustainability of production systems, and the well being of people living in drought-prone areas. It adversely affects the lives of 2.6 billion people (43% of the world population) that are engaged in agriculture (Edmeades *et al.*, 2000).

Drought is not uncommon in developing countries and represents, after low soil fertility, the second most important cause of yield loss for maize (around 17% annually) [Edmeades *et al.*, 1992]. In India, development of drought tolerance in crop plants like maize assumes particular significance considering the fact that nearly 75 per cent of the maize acreage is under rainfed conditions (Singh *et al.*, 2004). Maize is thought to be more susceptible than other rainfed crops because of its near-synchronous development of florets which are usually borne on a single ear and single stem, and its physical separation of male and female flowers.

Genetic improvement for tolerance to drought stress is an important component in stabilizing food crop production in the tropics. Breeders, agronomists, physiologists, molders, and molecular geneticists must forge new alliances to accelerate the search for unique drought adaptive mechanisms using full range of genetic diversity available. This will require development of efficient screening techniques which expose genetic variability for such traits, critical field evaluations to determine their true worth (Boyer, 1996), identification of promising genotypes, and rapid transfer of drought tolerance into elite germplasm.

The present investigation was, therefore, aimed at (i) analyzing the genetic variability in the maize germplasm for drought stress tolerance and identifying promising maize inbred lines and experimental hybrids with desirable agronomic performance; (ii) analyzing the molecular polymorphisms in a selected set of drought tolerant and susceptible maize inbred lines, and (iii) mapping of QTLs for various characters influencing the performance of maize genotypes under drought stress conditions.

## Analysis of Genetic Variability in the Maize Inbred Lines for Drought Tolerance

To evaluate the genetic variability in maize germplasm for tolerance to drought stress at the flowering stage, two experiments were undertaken in the present study at Maize Winter Nursery, Hyderabad during 2001-02 and 2003-04. Experiment I comprised 18 inbred lines, including 9 lines developed by the maize research centers in India, and 4 lines derived from the CIMMYT maize populations, and 5 lines from CIMMYT, Mexico. In Experiment II, a selected set of lines developed by the CIMMYT Maize Programme, specifically for drought stress tolerance, were evaluated. These included 39 DTPY lines (yellow kernel type) and 19 DTPW lines (white kernel type).

For most plant breeders, drought tolerance has a meaning only if it confers greater yield or stability under stress. Grain yield itself has limitations as an index for stress tolerance, as grain yield in a particular drought environment is influenced not only by genetic differences in drought tolerance, but also by differences in time to flowering (drought escape) and differences in yield potential. Selection for grain yield under drought has also often been considered inefficient because of the increase in environmental variance relative to genetic variance, which decreases yield heritability as yield decreases.

Under these conditions, selection for secondary traits which are correlated to grain yield and have relatively high heritability may increase selection efficiency (Bolanos *et al.*, 1993). CIMMYT physiologists have evaluated many secondary traits for their value in a drought breeding programme. Grain yield, ears per plant, ASI, leaf senescence, tassel size and leaf rolling were recommended for use in estimating drought tolerance index of the genotypes (Edmeades *et al.*, 2000; Banziger *et al.*, 2000).

Putative drought tolerance traits have been reviewed extensively (Blum, 1988; Ludlow and Muchow, 1990; Fukai and Cooper, 1995; Subbarao *et al.*, 19996), although very few have proven useful in drought breeding programmes. Ideally, a secondary trait should be: (i) genetically associated with grain yield under drought; (ii) highly heritable; (iii) genetically variable; (iv) cheap and fast to measure; (v) stable over the measurement period; (vi) observed at or before flowering, so that undesirable parents are not crossed; (vii) an estimator of yield potential before final harvest; and (viii) not associated with yield loss under unstressed conditions (Edmeades *et al.*, 2000).

When drought stress occurs just before and during the flowering period, a delay in silking is observed resulting in an increase in the length of the anthesis-silking interval (ASI) (Hall *et al.*, 1982; Westgate and Bassetti, 1990; Bolanos and Edmeades, 1993) This asynchrony between male and female flowering was found to be strongly associated with grain yield decrease under drought (Du Plessis and Dijkhuis, 1967; Hall *et al.*, 1981; Westgate and Boyer, 1986; Bolanos and Edmeades, 1993).

ASI meets most of the requirements of a useful secondary trait for selection purposes, and has found a leading place among the plethora of traits that are putatively linked to stress tolerance and reproductive fitness in maize (Edmeades *et al.*, 2000). The benefits of selection for reduced ASI under drought spill over as tolerance to several other stresses that exert their influence during the flowering period (Banziger *et al.*, 2000).

In the present study, ASI was found to vary from 0.0 to 12 days in Experiment I and from -0.5 to 5.1 days in the DTPY lines and from -0.1 to 6.5 days in the DTPW lines. It should be mentioned that the range of ASI values in Experiment II were relatively lesser than those recorded in Experiment I. This could be because in Experiment II, all the 39 DTPY and 19 DTPW lines were selected from populations specifically developed for drought tolerance in a breeding programme undertaken at CIMMYT. Some of the DTP lines showed low ASI values, and in some cases negative ASI; for example, DTPY199 among DTPY lines and DTPW208 among DTPW lines with -0.5

days and -0.1 days. In contrast, CIM500, IPA34, CML247 and Ac7729 recorded larger ASI in drought stress conditions; these lines were also drought susceptible. Ac7729 is one of the parental lines of the RIL mapping population utilized in the present study, and showed an ASI value of 8.6 days in Experiment I. In contrast, the drought tolerant parent, Ac7643 showed lower ASI in this experiment. Though in majority of the cases, larger ASI resulted in lesser grain yield and huge yield penalty (in comparison to the control/unstressed condition), some lines with low ASI values were also drought susceptible. This indicates that there are several other factors, apart from ASI, that determine the response of a maize genotype to drought stress at the flowering stage.

Ear number per plant plays an important role in determining the yield under drought stress conditions. In the present study, this particular trait was considered as an indicator of barrenness rather than prolificacy. In Experiment I, greater number of ears per plant was recorded in some highly promising inbred lines, namely FSA17, IPA29B and CM140. These lines were also promising on the basis of grain yield in both unstressed and stress conditions. The number of ears per plant is generally determined by the supply of C and N near flowering. Determination of the number of kernels per ear occurs during the lag phase between pollination and the onset of the liner phase of grain biomass is constant with time (Tollenaar, 1977). Maize is characterized by a greater number of potential ears per plant and kernels per ear than are observed at maturity (Fischer and Palmer, 1983) and both of these yield components are particularly sensitive to environmental stresses.

Leaf rolling ratings have been used to visually assess W because leaf rolling reduces radiation interception, most breeders consider it deleterious for production. Genotypic differences have been reported in several species (e.g., Tumer *et al.*, 1986) and trait is highly heritable (Biolanos and Edmeades. 1996) leaf rolling may, however, also indicate structural characteristics of leaves that have no obvious relationship to W.

Delayed leaf senescence may indicate access to a larger W staygreen in sorghum is probably controlled by a single major gene (Walulu *et al.*,

1994) and is therefore easily modified. Heterosis also appears to delay leaf death under drought (Wan Oosterom *et al.*, 1996). Since N cannot be taken up readily from a dry soil a small sink may also be the cause of apparent staygreen. We estimate that an increase in grain yield under drought from 2.0 to 2.8 that will increase N demand by developing kernels by an amount equal N contained in 30% of the leaf biomass. There is also evidence that leaves in maize exhibiting staygreen may not always be metabolically active (Smart *et al.*, 1995).

Reduction in tassel size could lead to increased ear growth and improved ear fertility under stress. Lack of major changes in leaf rolling, tassel size and leaf senescence in the control/unstressed vs. stressed conditions in the present study reflects their lack of adaptive value under drought and consequently, the small weighting they received for formulating the DTI values of the genotypes under study. Similar observations were made by Bolanos and Edmeades (1996).

Genetic variation in ear growth characteristics appears to be important in stress environments. Drought tolerance in advanced selection cycles of the tropical maize population 'Tuxpeno sequia was associated with increased ear biomass at anthesis, reduced number of florets per ear, and reduced silk delay and reduced barrenness under drought stress (Edmeades *et al.*, 1993). Drought tolerant selections also developed a greater number of kernels per ear under stressed and unstressed conditions, though prolificacy under well-watered conditions remained unchanged (Bolanos and Edmeades, 1993a).

An analysis of the *per se* performance of the genotypes under both control and stress conditions, in conjunction with % yield penalty over control (Table 7) in Experiment I, revealed that most of the genotypes were severely affected by drought stress at flowering stage. It is important to note that elite maize lines such as CM139, CM207 and Bio-5 (derivative of LM5) from the Indian maize breeding programme, as well as several inbred lines from CIMMYT maize populations developed for unstressed conditions (CML247, Ac7729, K64R, CML444, CIM493, CIM495, CIM500 and CIM506) were found to be highly vulnerable to drought stress at the flowering stage.

Taking into account both drought tolerance index (DTI), which is based on several important secondary traits besides yield, and % yield penalty over control, the present study revealed the potential of IPA29B, FSA17, Ac7643 and CM140. FSA17 and IPA29B were developed by the Maize Breeding Unit, Division of Genetics, IARI, under the rainfed maize research programme, while Ac7643 is a drought tolerant line developed from Population 43 (La Posta Sequia) at CIMMYT, Mexico. CM140, not developed purposely for drought tolerance, incidentally has favourable genes conferring moderate drought tolerance under Indian conditions. CM140 is the male parent of a popular single cross hybrid, Parkash, developed at Punjab Agricultural University, Ludhiana (Dhillon and Prasanna, 2001). Parkash was also reported to exhibit moderate drought tolerance (N.S. Malhi, personal communication). Since the female parent of Parkash, CM139 is drought susceptible, it appears that the moderate drought tolerance of Parkash is due to CM140.

A set of DTPY and DTPW lines from CIMMYT were analyzed for their drought stress tolerance through Experiment II. The drought tolerant source germplasm from which these DTPY and DTPW lines were derived, were developed by combining drought tolerant sources from Mexico, USA, Africa, and Thailand. Changes in biomass, GY, and harvest index with selection in these populations have been reported (Edmeades *et al.*, 1999). Although these lines were ranked highly in experiments carried out at CIMMYT, many of the lines did not respond favourably under drought stress conditions at Hyderabad.

A comparison of DTI values for the DTPY lines (Table 9) indicated that most of the genotypes (23 out of 39 DTPY lines) were severely affected by drought stress at the flowering stage, thereby recording negative DTI values; the check (CM140) recorded a DTI value of 10.00. In this experiment, DTPY199 (19.2), DTPY100 (18.9), DTPY165 (18.0), DTPY116 (15.9), DTPY149 (13.7), DTPY5 (13.1) and DTPY138 (11.3) with high DTI values (indicated in parentheses) were found to be highly promising among the inbred lines evaluated. The trend was similar in the DTPW lines, where 11 out of the 19 DTPW lines recorded negative DTI values, indicating their susceptibility to drought stress at the flowering stage, whereas the check (CM140) recorded a DTI value of 9.05. Nevertheless, the study resulted in identification of three highly promising lines, DTPW51 (17.65), DTPW195 (15.34), DTPW1 (11.90), which recorded very high DTI values and were significantly superior to the CM140 (check).

## Evaluation of Experimental Hybrids for Drought Stress Tolerance

Hybrid maize technology led to significant increases in production and productivity of maize in both the developed and developing countries. Commercial cultivation of single-cross hybrids in particular has revolutionized maize cultivation by breaking the yield barriers. In India, research and development on single-cross maize hybrids has gained momentum in the last 10 years, although a number of double-cross and three-way cross hybrids have been developed and released by several public sector institutions (Dhillon and Prasanna, 2001), under the All-India Coordinated Maize Improvement Project (AICMIP). However, most of the hybrids being developed under AICMIP are targeted for favourable conditions, and not specifically under drought stress. Therefore, there is a distinct need to utilize proven sources of drought tolerance in developing hybrids that can perform reasonably well under both normal and drought stress conditions.

In this experiment, drought stress responses of 17 experimental hybrids were evaluated under two different regimes: well-watered (control) and severe water stress condition at the flowering stage during winter/dry season at Hyderabad in 2002-03. ASI was found to vary from 2.7 days (CM140 x CM123-4) to 8.5 days (K64R x Bio-5) under stress condition, whereas under unstressed/well-watered conditions, ASI ranged from 1.35 (Ac7729 x CML247) to 3.45 (CM207 x IPA21A).

Taking into account both the DTI and the % yield penalty over control, the present study led to the identification of three most promising experimental hybrids, namely CM140 x CM123, FSA17 x IPA34 and CM140 x CM207.Among the crosses, CM140 x CM123 was found to be most promising with high DTI value, maximum grain yield under drought stress, lower % yield penalty, maximum number of ears per plant, less leaf rolling, moderate leaf senescence and tassel size. FSA17 was drought tolerant, CM140 was moderately tolerant, and CM123, CM207 and IPA34 were highly susceptible to drought stress at flowering stage.

## Molecular Characterization and Genetic Diversity Analysis of Selected Maize Lines

Molecular markers have been utilized for a variety of purposes including DNA fingerprinting of genetic resources, examining genetic relationships between individuals, construction of linkage maps, mapping genes/QTLs and marker-assisted selection. DNA-based markers are considered not only as valuable tools for plant breeding programmes but also for studies related to phylogenetics, evolution and biodiversity conservation. Among the various DNA-based markers, the microsatellite or simple sequence repeat (SSRs) markers, have become highly popular and 'the marker system of choice' in diverse crop plants, including maize, owing to their abundance in the genome, robustness, reproducibility, hypervariability and codominance (Powell *et al.*, 1996; Prasanna and Hoisington, 2003).

Application of SSR markers to genetic analysis in maize began with an initial study by Senior and Heun (1993). Since then, the power and potential of SSR markers for a wide range of applications in genetics and breeding of maize has been well-demonstrated by several researchers (e.g., Taramino and Tingey, 1996; Smith *et al.*, 1997; Pushpavalli *et al.*, 2001, 2002; Kassahun and Prasanna, 2003; Prasanna and Hoisington, 2003), as substantial numbers of maize microsatellites are available in the public databases. Microsatellite genotypic data from a number of loci have the potential to provide unique allelic profiles or DNA fingerprints for establishing genotype identity.

The present study identified some drought tolerant DTPY and DTPW lines, besides CM140, an Indian maize inbred line. These selected inbred lines, along with a few elite but drought susceptible lines such as CM139 and

Ac7729 have been further analyzed for microsatellite polymorphisms and to ascertain the genetic relationships among these lines. The study included 12 genotypes, namely DTPW195, DTPW51, DTPW105, DTPW183, DTPY65, DTPY116, DTPY108, DTPY194, CM139, CM140, Ac7729 and Ac7643. Among these, DTPW51, DTPW195, DTPY65, DTPY116, Ac7643 and CM140 are drought tolerant, while DTPW105, DTPW183, DTPY108, DTPY194, Ac7729 and CM139 are drought susceptible. CM139 and CM140 also served as 'reference lines' for molecular analysis since these lines were characterized in detail by the Maize Genetics Unit at IARI under the Asian Maize Biotechnology Network (AMBIONET) programme. While carrying out SSR profiling, due consideration was given to stratified sampling of polymorphic SSR loci covering bin locations on various chromosomes (Table 2).

The study revealed a total of 331 SSR alleles across the 71 polymorphic loci, at an average of 4.69 SSR alleles per locus, with the maximum number of alleles (seven) in *phi037* (1.08), *bnlg1064* (2.03), *dup21* (2.05), *bnlg121* (2.06), *bnlg1520* (2.09), *bnlg1272* (9.00) and a minimum of two in *bnlg1347* (1.10). The PIC values ranged from 0.42 for *phi097* (1.01) to 0.93 for *bnlg594* (10.06). The average PIC value across all the SSR loci analyzed was 0.68. It is notable that 9 SSR markers (*bnlg1556, bnlg1064, dup21, bnlg121, bnlg1520, bnlg1182, phi080, bnlg1518* and *bnlg594*) recorded PIC values equal to or more than 0.80 are particularly worth mentioning. The PIC values recorded in this study are comparable with those reported by several earlier reports on molecular analyses of inbred lines in maize (e.g., Smith *et al.*, 1997; Senior *et al.*, 1998; Kassahun and Prasanna, 2003; Prasanna and Hoisington, 2003), although the germplasm and the SSR loci analyzed in these studies were different.

A number of reports indicated that SSR loci with dinucleotide repeats are the most frequent polymorphic class of microsatellites among the plant species examined to date (Morgante and Olivieri, 1993; Wang *et al.*, 1994). Fifty-four of the 71 SSR loci analyzed in this study have di-repeats. Except *phi080* (8.08-8.09), which has a penta-repeat, all the other eight loci with PIC values equal to or exceeding 0.80 have di-repeats. Such SSR markers with high PIC values are extremely valuable in genotypic identification as well as for QTL mapping experiments.

Genotypic differences with respect to SSR variation is often attributed to stepwise mutations at the SSR loci leading to several alleles (Robinson and Harris, 1999) as well as possible errors during DNA replication (Moxon and Willis, 1999; Jarne and Lagoda, 1996) due to "slippage" of DNA polymerase when copying the repeat region. Larger changes in repeat number at a specific SSR locus, leading to high level of polymorphism, are also considered to be the result of processes such as unequal crossing over (Strand *et al.*, 1993). Mutations in regions flanking microssatellite repeats can make the evolution of microsatellite loci even more complex. Recently, researchers reported that interspecific and intraspecific size variation at microsatellite loci could also be caused by 'indels' (insertions-deletions) in the flanking region and not necessarily only at the microsatellite repeat region (Angers and Bernatchez, 1997; Grimaldi and Crouau-Roy, 1997).

The inbred lines analyzed in this study can be considered as highly homozygous and 'genetically pure', considering the extremely low frequencies of the heterozygous SSR loci (Table 18). The SSR heterozygosity could be mainly due to residual heterozygosity (Smith *et al.*, 1997) or presence of active transposable elements leading to allelic diversity (Prasanna and Sarkar, 1997). Alternatively, mutations could have occurred in a parental stock at a specific SSR locus, leading to two different alleles on homologous chromosomes. Prasanna and Sarkar (1997) demonstrated the presence of active transposable elements such as *Ubiquitous* (*Uq*) in some of the Indian maize inbred lines, such as CM111, that are commonly used in Indian maize breeding programmes. Since the heterozygosity was restricted to highly limited number of SSR loci, the possibility of DNA/seed contamination can be ruled out.

'Null' alleles were noticed at a few of SSR loci in some of the maize inbred lines analyzed in the study (Table 18). Such 'null' alleles might occur due to mutations in the binding region of one or both of the microsatellite primers thereby inhibiting primer annealing (Callen *et al.*, 1993). Occurrence of 'null alleles' has been often observed by researchers working with SSR loci in various crop species, including maize, and the null alleles problem is not amenable to troubleshooting, since there is no direct way to turn these (partially) dominant loci into codominant ones to give consistent effects (Robinson and Harris, 1999).

Ninety-six out of 331 polymorphic SSR alleles were found to be 'unique' or 'rare', as these were found to occur in only one out of the 12 inbred lines in each case. This reflects the genetic divergence among the lines. Such unique or rare SSR alleles are also valuable in DNA fingerprinting for effective discrimination of the inbred lines. DTPW195 showed the maximum number of such rare SSR alleles (13), followed by CM139 (11), DTPW183 and DTPY116 (10 each), while DTPY194, DTPW105 and CM140 showed 9 rare alleles each.

The genetic similarity matrix (Table 19) and the dendrogram depicting the genetic relationships among the 12 inbred lines (Figure 8) highlight the high genetic divergence among these genotypes. The highest GS value was between DTP194Y and DTP195W, whereas the lowest GS value was between DTP183W and CM139. On the base of average of similarity of inbred lines DTP194Y (0.20) and DTP108Y (0.145) showed maximum and minimum genetic similarity among the 12 inbred lines under study. The high genetic divergence between CM139 and CM140, as well as CM139 with rest of the inbred lines under study reconfirmed the earlier observations by Pushpavalli *et al.* (2001) and Prasanna and Hoisington (2003). This is the first report of the analysis of the genetic relationships among the drought tolerant and susceptible lines, including the DTPY and DTPW lines.

There were primarily four major clusters: Group I included DTP195W and DTP65Y. Group II has two sub-groups: IIA including DTP51W and DTP183W, and IIB comprising DTP183W and DTP108Y. Group III included two sub-groups: IIIA including DTP116 and DTP194Y and IIIB comprising CM140 and Ac7643. Group IV which includes CM139 and Ac7729 was found to be genetically distinct from the other three groups.

Bootstrap analysis was carried out to ascertain the statistical strength of each of the nodes identified by cluster analysis. The analysis was carried out under high stringency (1000 permutations). Of the six nodes in the dendrogram, one was supported by large bootstrap values (more than 50%) and the others had moderate support (30-50%) (Figure 8). Bootstrap values with just above 30% at certain nodes within a major group/sub-group suggest the requirement of additional data for finer differentiation of the genetic relationships. The analysis clearly indicates that the SSR dataset generated in this study was, in general, robust and reliable enough to decipher the genetic relationships among the inbred lines analyzed.

To complement the cluster analysis in terms of understanding the relationships among the entries under study, Principal Component Analysis (PCA) was carried out. PCA further aided in depicting the relationships among the various entries in a three-dimensional mode (Figure 9). The analysis once again highlighted the distinctness of CM139 and Ac7729 from the rest of the inbred lines, and the close genetic similarity between the DTPW lines as compared to other lines. It was interesting to note that all the DTPY inbred lines are grouped with drought tolerant lines Ac7643 and CM140.

Thus, the present investigation not only highlighted the genetic divergence among the different drought tolerant and drought susceptible inbred lines from India and CIMMYT, but also provides potentially useful information for objective selection of parental lines in developing mapping populations as well as for breeding studies.

## Phenotyping of RILs and Mapping of QTLs for Drought Stress Tolerance in Maize

Whole-plant response to drought stress is a complex process conditioned by a number of component responses that both interact and differ in their individual responses to the intensity and duration of water deficits, making it difficult to isolate individual traits with major effects on tolerance. In recent years, developments in molecular marker technologies and their use in QTL analysis have provided effective new opportunities for the study of plant responses to the environment (e.g., Prioul *et al.*, 1997; Ribaut *et al.*, 2002, 2003; Prasanna and Ribaut, 2004). Molecular markers provide opportunities not only to identify QTLs (and their functions) that determine complex phenotypes such as drought tolerance, but also to improve greatly the efficiency of genetic improvement by facilitating the introgression of desirable traits through the us of linked markers (Ribaut *et al.*, 2002, 2004).

In the present study, the responses of the same set of 210 RILs, along with parental lines, to drought stress were recorded for several characters. These experiments were undertaken during the winter/dry season at Karimnagar (2002-02) and Hyderabad (2003-04).

**Correlations among various characters under drought stress:** Genetic correlations among various characters influencing the *per se* performance of the RIL mapping population under drought stress conditions were analyzed in the present study. Bolanos and Edmeades (1996), through an analyses of correlations between grain yield (GY) under severe drought stress and secondary traits, obtained from trials of inbred progenies from maize populations, showed a strong dependence of GY on ears per plant (r = 0.90) and grains per ear (r = 0.71). A moderately strong correlation (r= -0.60) was reported between GY and ASI, while genetic correlations between GY and leaf rolling, stay green, leaf angle, canopy temperature, tassel branch number, leaf chlorophyll concentration, and plant height were generally less than 0.20. Similar results were reported for two other maize populations by Guei and Wasson (1992).

In the present study, ASI was found to be negatively correlated with the grain yield under drought stress, confirming the usefulness of lower ASI as the secondary trait in drought tolerance (Edmeades *et al.*, 2000). A short ASI is usually associated with good kernel set and high grain yield. ASI has a heritability that on average is slightly greater than that for grain yield under stress (Bolanos and Edmeades. 1996). An advantage of using ASI in breeding is that it is easily measured in the field, on either a plant or row basis, and data are available at flowering. Thus, it could be used to direct crossing in an accompanying well-watered nursery that was planted a few weeks later.

Lesser leaf rolling and delayed leaf senescence are additional secondary traits that are often considered by maize researchers in breeding for drought tolerance (Banziger *et al.*, 2000). In the present study, negative correlation between leaf rolling, tassel size and leaf senescence with grain yield were found, indicating their value in breeding for drought tolerance. However, in several instances, lower value for leaf rolling or leaf senescence did not necessarily result in higher grain yield.

Measuring roots is difficult and selection difficult to apply in a breeding programme. Use of root capacitance meters could facilitate the selection for desirable root characteristics in genotypes adapted to drought environments. This method offers advantages over other methods in the portability of the instrument and the speed with which large numbers of plants may be screened in the field. On average, it would take a researcher approximately 20 h to obtain root fresh mass for the same 20 plants. Thus, capacitance meters have a potential to become a valuable tool in enhancing our understanding of the root systems, particularly in cultivar comparisons across uniform soils at or near field capacity (Van Beem *et al.*, 1998). Several researchers have used electrical capacitance measurements for rapid assessment of root dimensions (reviewed by Van Beem *et al.*, 1998). Van Beem *et al.* (1998) reported a positive correlation between root electrical capacitance and total root volume; however, the capacitance measurement cannot assess the root distribution. In the present study, the correlation between root capacitance and GY of the genotypes under drought stress conditions was found to be non-significant. Van Beem *et al.* (1998) reported similar results for full-vigour genotypes. A number of factors appear to affect accurate measurement of root capacitance under field conditions, including the level of soil moisture and the essential requirement of a continuous path between the meter, electrodes, plant roots, and soil (Dalton, 1995). Careful thought must be given to experimental design, as the biggest limitation of the capacitance method is its sensitivity to water content in the soil. Consequently, capacitance readings obtained from root bound, full-vigor genotypes might not accurately estimate root fresh mass, leading to non-significant or poor correlations with other important traits affecting the performance of the maize genotypes under drought stress conditions. Therefore, there is a distinct need to optimize the assays for analysis of root capacitance before their routine use in breeding for drought tolerance.

In the present study, GY was also highly correlated with kernel number per plant under drought stress conditions at both Karimnagar and Hyderabad, confirming the observations made earlier by Bolanos and Edmeades (1996). Grain number per plant in water-deficient maize appears to depend directly on the flux of current photosynthate during the two weeks bracketing flowering (Schussler and Westgate, 1995), implying that the reserves of pre-flowering assimilates are either scarce or are not attracted to the ear. Kernels on drought-affected plants frequently abort in the few days following successful pollination (Westgate and Byer, 1986). Furthermore, acid invertase activity in ovaries of water-stressed plants is much reduced, impairing sink strength (Westgate, 1977). Once kernels enter the linear phase of biomass accumulation, they develop the sink strength needed to remobilize the C reserves. In the present study, the correlation coefficients between most of the important secondary traits and GY were found to be high.

**QTL Analyses:** The RIL datasets from Karimnagar and Hyderabad were analyzed separately using a powerful QTL mapping software that makes use of different Composite Interval Mapping (CIM) Models (Zeng, 1994). The use of molecular markers to study drought tolerance in a set of RILs is a powerful approach for dissecting the genetics of this complex trait. RILs permit replications of genotypes under stressed and unstressed conditions in a specific trial as well as undertaking trials at several target locations; this facilitates, in turn, better analyses of QTL positions and effects, and consequently better understanding of the genetic basis of a complex trait like drought tolerance.

QTL identification was achieved by successively running four models and comparing different outputs. Model 1 uses a simple interval mapping (SIM) approach similar to Mapmaker-QTL (Lander and Botstein, 1989), which does not include any marker as a cofactor. In Model 2, preselected markers closely linked to significant QTL peaks identified through SIM (by Model 1), were used as cofactors to reduce the residual variation throughout the genome. In Models 3 and 4, markers flanking the tested interval were used as cofactors to block the effects of possible QTL that were close to the interval of interest; two different "window" sizes were successively tested (30 cM and 20 cM, respectively. At each significant QTL, additive effects, as applicable to the RIL population were estimated. The presence of a QTL was declared significant if the LOD threshold value was more than 2.5 when analyzing data from a single environment.

In the Karimnagar dataset, two QTLs were identified for expression of ASI on chr. 2 and 10 with total phenotypic variance of 13.12% in normal condition and one QTL on chromosome 10 with close to 4% phenotypic variance in stress condition. Agrama and Moussa (1996) identified QTL associated with ASI under drought on chr. 1, 3, and 6, accounting for 38% of the phenotypic variance. In a different cross, six putative QTL have been identified for ASI under drought on chromosomes 1, 2, 5, 6, 8, and 10, accounting for 47% of the phenotypic variance (Ribaut *et al.*, 1996). At one important position, the allele contributing to a reduction in ASI also contributed to a decrease in GY, and for this reason a marker-assisted selection (MAS) strategy for drought tolerance should be based on an index of best QTL for both traits (Ribaut *et al.*, 1996; Beck *et al.*, 1996).

Flowering parameters in maize were studied earlier by some researchers under both well-watered and stress regimes, and QTLs for MFLW and FFLW identified (Phillips *et al.*, 1994; Beavis *et al.*, 1994; Veldboom *et al.*, 1994; Ribaut *et al.*, 1996). Considering the four studies mentioned above and our results under normal/unstressed conditions, the

QTLs influencing the expression of MFLW and FFLW were identified on all the 10 chromosomes, particularly on chr. 1, 3, 4, 8, and 9 for MFLW and on chr. 1, 8 and 9 for FFLW. The source of parental lines, the size of progeny, genotype-by-environment interaction effects and the different types of maps used, are most likely responsible for differences in QTL localization (Beavis *et al.*, 1994).

In the Karimnagar dataset, under normal conditions, two QTLs were detected for grain yield on chr. 1 and 4, accounting for about 7% of phenotypic variance, whereas for expression of this important character in stress condition, four QTLs were detected on chr. 2 (two QTLs), 3 and 5 collectively accounting for 19% of phenotypic variance. In the Hyderabad dataset, two QTLs were identified on chr. 2 and 10 with total phenotypic variance of 9.44%. On chr. 2, a clear tendency for localization of grain yield QTL was detected in Hyderabd corresponding to the genomic location of grain yield QTL detected in the Karimnagar dataset.

Ribaut *et al.* (1997) identified four QTLs for grain yield in normal condition on chr. 1, 2, 8 and 10 with 20% phenotypic variance, whereas in intermediate stress condition they found five QTLs on chr. 1 (two QTLs), 4, 7 and 10 with phenotypic variance of 26.3%. No QTL with major effect was identified under our study, which was in congruence with the observations made by Ribaut *et al.* (1997).

In the Karimnagar dataset, under unstressed conditions, two QTLs were detected for ear number per plant on chr. 1 and 2, accounting for about 5% of phenotypic variance, whereas for expression of this secondary trait in stress condition, three QTLs were detected on chr. 3, 5 and 10, collectively explaining 18.13% of phenotypic variance. Ribaut *et al.* (1997) identified seven QTLs for ear number per plant in normal conditions on chr. 1 (two QTLs), 2, 4, 7, 8 and 9 with 39.8% phenotypic variance, whereas in stress condition, four QTLs were found on chr. 1, 3, 6 and 9 with phenotypic variance of 17.2%. Our analysis revealed that a QTL detected under unstressed condition on chr. 2 was same as that identified by Ribaut *et al.* (1997).

In the dataset from the normal irrigation regime at Karimnagar, six QTLs were identified for kernel number per ear on chr. 1, 2, 3, 4 and 10 (two QTLs) accounting for about 25% of phenotypic variance, whereas for expression of the same trait under stress condition, five QTLs were detected on chr. 2 (three QTLs) and 10 (two QTLs), which collectively accounted for 19.21% of phenotypic variance. In the Hyderabad dataset, two QTLs were identified on chr. 2 and 10 with 9.77% phenotypic variance. Ribaut *et al.* (1997) identified three QTLs for kernel number per ear in normal condition on chr. 1 (two QTLs) and 7 with 17.5% phenotypic variance, whereas in stress condition they found four QTLs on chr. 1, 3, 4 and 9 with phenotypic variance of 20.7%. Our results revealed that a QTL on chr. 10 corresponded with the QTL identified for the same trait by Ribaut *et al.* (1997).

The results of QTL mapping based on Karimnagar dataset presented in Table 22 and Figure 11 clearly revealed that as many as 7 out of the 24 QTLs influencing the performance of the RILs under drought stress conditions were located on chr. 2, followed by 5 QTLs on chr. 3, and 4 QTLs on chr. 10. In contrast, analysis of the results from the WW conditions revealed 5 QTLs on chr. 4, followed by 3 QTLs on chr. 2. Similarly, the results of QTL mapping based on Hyderabad dataset presented in Table 24 and Figure 12 revealed that except for one QTL on chr. 7, all the others were localized on chromosomes 1, 2, 3, 8 and 10.

In the present study, the QTLs detected for yield components were in general not stable, in terms of their genomic locations across different water regimes in a location (Karimnagar) or across different locations (Karimnagar vs. Hyderabad), with some major exceptions. This is largely in agreement with the summary of QTL mapping experiments carried out at CIMMYT over the last 10 years (Ribaut *et al.*, 2003, 2004). From the genetic point of view, it is logical to image that a large number of genes are involved in the determination of grain yield. Grain yield is really the final product of plant development, and each physiological mechanism involved in plant development should affect yield to varying degrees. The large number of major phenotypic and physiological changes (such as female flowering, ASI, plant height, water content, cell growth or hormone content), demonstrates the complexity of the plants' response to water stress, and it is expected that the number of QTLs involved in the expression of yield will be large. Of course the stress level is very important, and the role of facultative traits (like ASI), depends on the stress intensity, affecting the identification of new sets of QTLs. This in turn might reduce the chance of identifying major QTLs across water regimes or locations, and make yield-QTL detection above a given threshold of probability dependent on specific environmental conditions. In this respect, Beavis (1994) explained the inconsistency of yield QTLs across experiments using progenies derived from the same cross, by the fact that yield should be under the control of a large number of small-effect QTLs segregating in the genome. Such loci may be important for the expression of yield only under certain environment conditions. This would be consistent with the large environmental interaction generally associated with yield in most crop species. If environmental interaction explains these results, selection for the loci should not be a priority unless it can be shown that they are particularly important in the target environment for this crop. Moreover, the QTL inconsistencies demonstrate that only a few yield-QTLs will be helpful in a MAS strategy and that the use of QTLs involved only in the expression of yield components in a MAS scheme will not result in rapid gains in yield under drought.

Although most of the QTLs for various traits identified in this study showed very low $R^2$ values, the present study was successful in identifying QTLs that could account for significant proportion of phenotypic variance for some characters like plant height (stress), ear height (control and stress), male flowering (control and stress), ASI (control), grain yield (stress), ear number per plant (stress) and kernel number per ear (control and stress) in the Karimnagar dataset, and for plant height, ear height and ear number per plant in the Hyderabad dataset.

When the QTL locations were compared across different traits, certain interesting findings emerged. Several QTLs mapped to identical or immediately adjacent intervals under control and stress conditions in both

Karimnagar and Hyderabad QTL datasets (Figures 11 & 12). For instance, a QTL located on chr. 3 (29-30 cM) flanked by the markers *umc313* and *umc50* showed significant effects on both plant height and ear height; another QTL on chr. 3, with *umc17* and *umc96* as flanking markers, influenced both grain yield and ear number per plant in the stress block. One QTL on chr. 5 (103 cM), contributed by Ac7729, showed its effect both on grain yield and ear number per plant under drought stress conditions. Similarly, another QTL on chr. 2 (166-169 cM) flanked by markers *bnl6.29* and *umc137* influencing grain yield, 100-kernel wight and kernel number per ear under drought stress conditions. This co-mapping was consistent with the correlations obtained previously between these GY component traits.

In summary, a number of QTLs associated with the drought tolerance of maize in terms of grain yield and its component traits have been identified based on evaluation of RIL mapping population at two locations in India (Karimnagar and Hyderabad). This is the first study on QTL mapping for drought tolerance of maize in India, and would provide a new opportunity in breeding programmes for water-limited environments, if their incorporation into otherwise adapted genotypes can be shown to improve yield under stress.

Of particular importance are the following QTLs: (i) chr. 1 in the marker interval of *umc33* and *umc83*; (ii) chr. 2 (166 cM) flanked by the markers *bnl6,29* and *umc137*; (iii) chr. 8, flanked by markers *umc120* and *umc346*; and (iv) chr. 10, flanked by *umc354* and *umc182*. These QTLs showed effects on diverse characters under drought stress conditions, thus highlighting their potential utility in molecular marker-assisted breeding for drought tolerance in maize. Whether these associations of QTLs with diverse traits are due to pleiotropic effects of the individual QTLs, or whether there were specific QTLs for each trait that were tightly linked, was difficult to establish in this study due to the relatively small number of mapping progenies that were phenotyped. Fine mapping of these genomic regions should help further resolve this issue.

## Potential Utility of the Present Study in Breeding for Drought Tolerance of Maize in India

The objective of plant improvement can be defined as the accumulation in a plant genome of the maximum number of favorable alleles involved in the expression of target traits. This task can be achieved through several approaches and selection schemes, including phenotypic observations and/or DNA marker technology. The emergence of molecular genetics and associated technologies represents a major new breeding tool; the current challenge is to integrate this tool and the information it generates into breeding schemes to further the development of efficient marker-assisted selection (MAS) strategies.

The present study gives a signal about high vulnerability of important breeding materials, particularly elite lines that are often used by maize breeders across the country. At the same time, identification of highly consistent and stable source of tolerant from CIMMYT offers an important opportunity to launch a focused programme on developing elite germplasm for drought tolerance. The drought tolerant lines such as FSA17, IPA29B, CM140, Ac7643, DTPY199, DTPY100, DTPY65, DTPY116, DTPW195, DTPW51 and DTPW1, identified in this study could be valuable for this purpose.

Marker-assisted selection (MAS) strategies should be integrated with conventional breeding for drought tolerance according to the target environment and the germplasm available. The cumulative knowledge based on the results available in maize from earlier studies, particularly those carried out by CIMMYT in countries such as Mexico, Kenya and Zimbabwe, coupled with these from the present study carried out under the Asian Maize Biotechnology Network (AMBIONET), would facilitate formulation of an integrated strategy that can lead to improvement of Indian maize lines for drought tolerance.

The recent focus of CIMMYT with respect to molecular breeding for drought stress tolerance in maize has been to initiate a MAS experiment based on "universal drought genomic regions" identified on a maize consensus map. Such a map is being based on: (1) QTL information for yield components, morphological traits and physiological parameters; (2) positions of candidate genes; and (3) gene expression information. The success of this MAS experiment would depend heavily on (i) location of key genes involved in drought response at the same genomic positions, independent of the genetic background, and (ii) phenotypic differences across the germplasm attributable to the nature/quality of the alleles at those genes (Ribaut *et al.*, 2003; Prasanna and Ribaut, 2004).

There are now exciting opportunities to combine information related to drought tolerance emerging at three different levels: phenotypic characterization of the plant, QTL data, and changes in expression of genes involved in specific physiological pathways (Ribaut *et al.*, 2002, 2003). A collaborative programme on functional genomics for drought stress tolerance in maize has been initiated at CIMMYT to optimize the relevant parameters for identification of the most informative changes in gene expression, and to evaluate the associated phenotypes. Once the characterization of the key genes involved in drought responses is achieved, MAS could be effectively employed for screening of the elite germplasm and promising gene bank accessions to evaluate the allelic value at those genes based on field performance.

# Summary

In India, development of drought tolerance in crop plants like maize assumes particular significance considering the fact that nearly 75 per cent of the maize acreage is under rainfed conditions. Genetic improvement for tolerance to drought stress warrants development of efficient screening techniques which expose genetic variability for such traits, critical field evaluations to determine their true worth, identification of promising genotypes, and rapid transfer of drought tolerance into elite germplasm. The present investigation was, therefore, aimed at (i) analyzing the genetic variability in the maize germplasm for drought stress tolerance and identifying promising maize inbred lines and experimental hybrids with desirable agronomic performance; (ii) analyzing the molecular polymorphisms in a selected set of drought tolerant and susceptible maize inbred lines, and (iii) mapping of QTLs for various characters influencing the performance of maize genotypes under drought stress conditions.

To evaluate the genetic variability in maize germplasm for tolerance to drought stress at the flowering stage, two experiments were undertaken in the present study at Maize Winter Nursery, Hyderabad during 2001-02 and 2003-04. Experiment I comprised 18 inbred lines, including 9 lines developed by the maize research centers in India, and 4 lines derived from the CIMMYT maize populations, and 5 lines from CIMMYT, Mexico. In Experiment II, a selected set of lines developed by the CIMMYT Maize Programme, specifically for drought stress tolerance, were evaluated. These included 39 DTPY lines (yellow kernel type) and 19 DTPW lines (white kernel type). Measurements were taken on the following plant and ear characters: (i) plant stand; (ii) plant height; (iii) ear height; (iv) days to 50% male flowering or anthesis date; (v) days to 50% female flowering or silking date; (vi) anthesis-silking interval; (vii) tassel size; (viii) number of ears per plant; (ix) leaf senescence; (x) leaf rolling; (xi) grain yield; (xii) ear length; (xiii) ear diameter; (xiv) number of kernels per ear; and (xv) 100-kernel weight (HKW).

An analysis of the *per se* performance of the genotypes under both control and stress conditions, in conjunction with % yield penalty over control in Experiment I, revealed that most of the genotypes, including elite maize lines such as CM139, CM207 and Bio-5 (derivative of LM5) from the Indian maize breeding programme and several inbred lines from CIMMYT (CML247, Ac7729, K64R, CML444, CIM493, CIM495, CIM500 and CIM506) were severely affected by drought stress at flowering stage. Nevertheless, taking into account both drought tolerance index (DTI), which is based on several important secondary traits besides yield, and % yield penalty over control, the present study revealed the potential of IPA29B, FSA17, Ac7643 and CM140.

A set of DTPY and DTPW lines from CIMMYT were analyzed for their drought stress tolerance through Experiment II. The drought tolerant source germplasm from which these DTPY and DTPW lines were derived, were developed by combining drought tolerant sources from Mexico, USA, Africa, and Thailand. Although these lines were ranked highly in experiments carried out at CIMMYT, many of the lines did not respond favourably under drought stress conditions at Hyderabad. A comparison of DTI values for the DTPY lines (Table 9) indicated that most of the genotypes (23 out of 39 DTPY lines) were severely affected by drought stress at the flowering stage, thereby recording negative DTI values; the check (CM140) recorded a DTI value of 10.00. In this experiment, DTPY199 (19.2), DTPY100 (18.9), DTPY165 (18.0), DTPY116 (15.9), DTPY149 (13.7), DTPY5 (13.1) and DTPY138 (11.3) with high DTI values (indicated in parentheses) were found to be highly promising among the inbred lines evaluated.

The trend was similar in the DTPW lines, where 11 out of the 19 DTPW lines recorded negative DTI values, indicating their susceptibility to drought stress at the flowering stage, whereas the check (CM140) recorded a DTI value of 9.05. Nevertheless, the study resulted in identification of three highly promising lines, DTPW51 (17.65), DTPW195 (15.34), DTPW1 (11.90), which recorded very high DTI values and were significantly superior to the CM140 (check).

In another experiment, drought stress responses of 17 experimental hybrids were evaluated under two different regimes: well-watered (control) and severe water stress condition at the flowering stage during winter/dry season at Hyderabad in 2002-03. Taking into account both the DTI and the % yield penalty over control, the study led to the identification of three most promising experimental hybrids, namely CM140 x CM123, FSA17 x IPA34 and CM140 x CM207.Among the crosses, CM140 x CM123 was found to be most promising with high DTI value, maximum grain yield under drought stress, lower % yield penalty, maximum number of ears per plant, less leaf rolling, moderate leaf senescence and tassel size.

Selected drought tolerant inbred lines, along with a few elite but drought susceptible lines (such as CM139 and Ac7729) were further analyzed in the present study for microsatellite polymorphisms and to ascertain the genetic relationships among these lines. The study included 12 genotypes, namely DTPW195, DTPW51, DTPW105, DTPW183, DTPY65, DTPY116, DTPY108, DTPY194, CM139, CM140, Ac7729 and Ac7643. Among these, DTPW51, DTPW195, DTPY65, DTPY116, Ac7643 and CM140 are drought tolerant, while DTPW105, DTPW183, DTPY108, DTPY194, Ac7729 and CM139 are drought susceptible. CM139 and CM140 also served as 'reference lines' for molecular analysis.

The study revealed a total of 331 SSR alleles across the 71 polymorphic loci, at an average of 4.69 SSR alleles per locus, with the maximum number of alleles (seven) in *phi037* (1.08), *bnlg1064* (2.03), *dup21* (2.05), *bnlg121* (2.06), *bnlg1520* (2.09), *bnlg1272* (9.00) and a minimum of two in *bnlg1347* (1.10). The PIC values ranged from 0.42 for *phi097* (1.01) to 0.93 for *bnlg594* (10.06). The average PIC value across all the SSR loci analyzed was 0.68. It is notable that 9 SSR markers (*bnlg1556, bnlg1064, dup21, bnlg121, bnlg1520, bnlg1182, phi080, bnlg1518* and *bnlg594*) recorded PIC values equal to or more than 0.80 are particularly worth mentioning.

The inbred lines analyzed in this study can be considered as highly homozygous and 'genetically pure', considering the extremely low frequencies of the heterozygous SSR loci detected. Ninety-six out of 331 polymorphic SSR alleles were found to be 'unique' or 'rare', as these were found to occur in only one out of the 12 inbred lines in each case. This reflected the genetic divergence among the lines. Also, such unique or rare SSR alleles are valuable in DNA fingerprinting for effective discrimination of the inbred lines.

Cluster analysis of the SSR dataset revealed primarily four major clusters: Group I included DTP195W and DTP65Y. Group II has two sub-groups: IIA including DTP51W and DTP183W, and IIB comprising DTP183W and DTP108Y. Group III included two sub-groups: IIIA including DTP116 and DTP194Y and IIIB comprising CM140 and Ac7643. Group IV which includes CM139 and Ac7729 was found to be genetically distinct from the other three groups. Bootstrap analysis was carried out to ascertain the statistical strength of each of the nodes identified by cluster analysis. The analysis clearly indicates that the SSR dataset generated in this study was, in general, robust and reliable enough to decipher the genetic relationships among the inbred lines analyzed.

To complement the cluster analysis in terms of understanding the relationships among the entries under study, Principal Component Analysis (PCA) was carried out. PCA further aided in depicting the relationships among the various entries in a three-dimensional mode (Figure 9). The analysis once again highlighted the distinctness of CM139 and Ac7729 from the rest of the inbred lines, and the close genetic similarity between the DTPW lines as compared to other lines.

Thus, the present investigation not only highlighted the genetic divergence among different the drought tolerant and drought susceptible inbred lines from India and CIMMYT, but also provides potentially useful information for objective selection of parental lines in developing mapping populations as well as for breeding studies.

For mapping of Quantitative Trait Loci (QTLs) conferring drought tolerance in maize, the responses of the same set of 210 Recombinant Inbred Lines (RILs) developed at CIMMYT using Ac7643 and Ac7729 as drought tolerant and drought susceptible parents, respectively, were analyzed at experiments undertaken during the winter/dry season at Karimnagar (2002-03) and Hyderabad (2003-04).

Genetic correlations among various characters influencing the *per se* performance of the RIL mapping population under drought stress conditions were analyzed. ASI was found to be negatively correlated with the grain yield under drought stress, confirming the usefulness of lower ASI as the secondary trait in drought tolerance. Also, negative correlation between leaf rolling, tassel size or leaf senescence with grain yield were found, indicating their value in breeding for drought tolerance. However, the correlation between root capacitance and grain yield of the genotypes under drought stress conditions was found to be non-significant. In contrast, grain yield was highly correlated with kernel number per plant under drought stress conditions at both Karimnagar and Hyderabad.

The RIL datasets from Karimnagar and Hyderabad were analyzed separately using a powerful QTL mapping software, that makes use of different Composite Interval Mapping (CIM) Models (Zeng, 1994). QTL identification was achieved by successively running four models and comparing different outputs. A total of 52 QTLs, including 22 QTLs under the control/well-watered condition and 30 QTLs under drought stress conditions based on analysis of the RIL dataset from Karimnagar. These QTLs were distributed on almost all chromosomes, except chr. 7. A total of 14 QTLs were detected based on analysis of the RIL dataset from drought stress conditions for five most important traits that determine the *per se* performance of maize under drought stress conditions; these were plant height, ear height, ASI, grain yield, ear number per plant and kernel number per ear.

A comparison of the QTL information obtained based on analyses of the Karimnagar and Hyderabad datasets revealed the correspondence of QTLs in the RILs influencing specific characters under drought stress conditions at both locations. These were: (i) for plant height, chr. 1 QTL (183-188 cM) and chr. 8 QTL (87-88 cM); (ii) for ear height, chr. 3 (29-30 cM) and chr. 8 (94-96 cM); (iii) for grain yield, chr. 2 (166-169 cM). Such QTLs, which expressed across different locations/environments, are of particular value in both molecular marker-assisted breeding as well as for basic studies related to identification of candidate genes influencing drought tolerance of maize genotypes. Similarly, the study was successful identifying some QTLs that influenced diverse characters. Of particular importance among these are: (i) a QTL on chr. 1 in the marker interval of *umc33* and *umc83*; (ii) a QTL on chr. 2 (166 cM) flanked by the markers *bnl6,29* and *umc137*; (iii) a QTL on chr. 8, flanked by markers *umc120* and *umc346*; and (iv) a QTL on chr. 10, flanked by *umc354* and *umc182*. Significant is the fact that the same QTLs were found to be important in drought mapping experiments undertaken by CIMMYT (Ribaut *et al.*, 2002, 2003; Prasanna and Ribaut, 2004) using the same set of RILs at Mexico, Kenya and Zimbabwe. Such 'universal drought QTLs' could be of great value in molecular marker-assisted breeding for drought tolerance in maize, besides genomics.

Thus, the present study is the first in India on QTL mapping for drought tolerance of maize, and would open up exciting avenues in breeding maize for water-limited environments.

# References

Agrama, H.A.S. and Moussa, M.E. (1996). Mapping QTLs in breeding for drought tolerance in maize (*Zea mays L.*). *Euphytica* **91**: 89-97.

Angers, B. and Bernatchez, L. (1997). Complete evolution of a salmonoid microsatellite locus and its consequences in inferring allelic divergence from size information. *Mol. Biol. Evol.* **14**: 230-238.

Atlin, G.N. and Lafitte, H.R. (2002). Marker assisted breeding versus direct selection for drought tolerance in rice. In: Saxena N.P. and John C. O'Toole (eds.). Field Screening for Drought Tolerance in Crop Plants with Emphasis on Rice. ICRISAT, Hyderabad.

Austin, D.F., Lee, M. (1998). Detection of quantitative trait loci for grain yield and yield components in maize across generations in stress and non stress environments. *Crop Sci.* **38**: 1296-1308.

Baker, RJ. (1986). *Selection Indices in Plant Breeding.* Boca Raton: CRC Press Inc.

Banziger, M. and Cooper, M.E. (2002). Breeding for low-input conditions and consequences for participatory plant breeding - examples from tropical maize and wheat. *Euphytica* **122**:503-519.

Banziger, M. and Lafitte, H.R. (1997). Efficiency of secondary traits for improving maize for low-nitrogen target environments. *Crop Sci.* **37**:1110-1117.

Banziger, M., Edmeades, G.O. and Lafitte, H.R. (1999). Selection for drought tolerance increases maize yields over a range of N levels. *Crop Sci.* **39**:1035-1040.

Banziger, M., Edmeades, G.O. and Quarrie, S. (1996). Drought stress at seedling stage: Are there genetic solutions? In: Edmeades *et al* (eds.) Developing Drought and Low N- Tolerant Maize: Proceeding of a Symposium. CIMMYT, Mexico.

Banziger, M., Edmeades, G.O., Beck, D. and Bellon, M. (2000). Breeding for Drought and Nitrogen Stress Tolerance in Maize: From Theory to Practice. CIMYYT, Mexico.

Banziger, M., Lafitte, H.R. and Edmeades, G.O. (1995). Intergenotypic competition during evaluation of maize progenies under limited and adequate N supply. *Field Crops Res.* **44**: 25-31.

Banziger, M., Mickelson, H.R. and Pena-Valdivia, C.B. (eds.) (1996). *Developing Drought and Low N- Tolerant Maize. Proceedings* of a *Symposium, March* 25-29, 1996, CIMMYT, Mexico.

Banziger, M., Mugo, S. and Edmeades, G.O. (2000a). Breeding for drought tolerance in tropical maize conventional approaches and challenges to molecular approaches. In: J.M. Ribaut and D. Poland (eds.) Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water Limited Environments. Proceedings of a Symposium, June 21-25, 1999, CIMMYT, El Batan, Mexico, pp. 69-72.

Banziger, M., Pixley, K.V., Vivek, B. and Zambezi, B.T. (2000). Characterization of elite maize germplasm grown in eastern and southern Africa: Results of the 1999 regional trials conducted by CIMMYT and the

Maize and Wheat Improvement Research Network for SADC (MWIRNET). CIMMYT, Harare, Zimbabwe, pp. 1-44.

Banziger, M., Setimela, P.S., Hodson, D. and Vivek, B. (2004). Breeding approaches to develop drought tolerant maize hybrids. In: D. Poland, M. Sawkins, J.-M. Ribaut and D. Hoisington (eds.) Resilient Crops for Water Limited Environments : Proceedings of a Workshop held at Cuernavaca, Mexico, 24-28 May 2004. Mexico, D.F.: CIMMYT, pp. 237-238.

Beavis, W.D. and Keim, P. (1996) Identification of quantitative trait loci that are affected by environment. In: Kang, M.S. and Gauch, H.G. (eds.) Genotype -by-environment interaction, CRC Press, Boca Raton, Florida, pp. 123-149.

Beavis, W.D., Smith, O.S., Grant, D. and Fincher, R. (1994) Identification of quantitative trait loci using a small sample of topcrosses and $F_4$ progeny from maize. *Crop Sci.* **34**: 882-896.

Beck, D., Betran, E.J., Banziger, M., Willcox, M. and Edmeades, G.O. (1997). From landrace to hybrid: strategies for the use of source populations and lines in the development of drought tolerant cultivars. In: Edmeades, G.O. *et al.* (eds.), *Developing Drought and Low N- Tolerant Maize. Proceedings* of a *Symposium, March* 25-29, 1996, CIMMYT, Mexico, pp. 235-240.

Beck, D., Betran, J., Banziger, M., Ribaut, J.-M., Willcox, M., Vasal, S.K. and Ortega, A. (1996). Progress in developing drought and low soil nitrogen tolerance in maize. In: Wilkinson, D.B. (ed.) Proc. 51st Annual Corn & Sorghum Research Conference, ASTA, Washington, pp 85-111.

Below, E.E. (1997). Growth and productivity of maize under nitrogen stress. In: Edmeades, G.O. *et al.* (eds.), *Developing Drought and Low N-Tolerant Maize. Proceedings* of a *Symposium, March* 25-29, 1996, CIMMYT, Mexico.

Bennetzen, J.I., and Freeling, M. (1997). The unified grass genome: synergy in synteny. *Genome Res.* **7**:301-306.

Betran, F.J., Banziger, M. and Beck, D.L. (1997). Relationship between line and topcross performance under drought and non-stressed conditions in tropical maize. In: Edmeades, G.D. *et al.* (as above), pp. 383-386.

Betran, F.J., Beck, D., Banziger, M. and Edmeades, G.O. (2003). Genetic analysis of inbred and hybrid grain yield under stress and non-stress environments in tropical maize. *Crop. Sci.* **43:** 807-817.

Betrán, F.J., Ribaut, J. M., Beck, D. and Gonzalez de León, D. (2002).Genetic diversity, specific combining ability, and heterosis in tropical maize under stress and non-stress environments *Crop Sci.* **43**: 797-806.

Bidinger, F.R. (2002). Field screening and genetic improvement of drought in other crops. In: N. P. Saxena and John C. O'Toole (eds.). Field Screening for Drought Tolerance in Crop Plants with emphasis on Rice. ICRISAT, Hyderabad.

Bidinger, F.R., Mahalakshmi, V., Talukdar, B.S. and Alagarswamy, G. (1982). Improvement of drought resistance in millet. In: Drought Resistance in Crop Plants with Emphasis on Rice, IRRI, Philippines, pp. 357-376.

Bidinger, F.R., Mahalaxmi, V. and Rao, GDP. (1987). Assesment of drought resistance in pearl millet [*Pennisetum americanum* (L.) Leeke]. I. Factors affecting yield under strss. *Aust. J. Agric. Res.* **38:** 37-48.

Blum, A. (1988). Plant Breeding for Stress Environments. CRC Press, Boca Raton, Florida.

Blum, A. (1997).Constitutive traits affecting plant performance under stress. In: Edmeades, G.O., Banziger, M., Mickelson, H.R. and Pefia-Valdivia, CB. (eds.) Developing Drought and Low-N Tolerant Maize. CIMMYT, Mexico, pp 131-135.

Blum, A. (2002). Drought tolerance: Is it a complex trait? In: N. P.Saxena and John C O'Toole (eds.). Field Screening for Drought Tolerance in Crop Plants with Emphasis on Rice. ICRISAT, Hyderabad.

Bohnert, H., Fischer, R., Kawasaki, S., Michalowski, C., Wang, H., Yale, J. and Zepeda, G. (2000). Cataloging stress-inducible genes and pathways leading to stress tolerance. In: Ribaut, J.-M. and Poland, D. (eds.) Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-Limited Environments. A Strategic Planning Workshop held at CIMMYT, Mexico, June 21-25, 1999, CIMMYT, Mexico, pp. 156-161.

Bohnert, H.J., Nelson, D.E. and Jensen, R.G. (1995). Adaptations to environmental stresses. *Plant Cell* **7**:1099-1111.

Bolanos, J., and Edmeades. G.O. (1991). Value of selection for osmotic potential in tropical maize. *Agron. J.*. **83**: 948-956.

Bolanos, J., and Edmeades G.O. (1993a). Eight cycles of selection for drought tolerance in lowland tropical maize. I. Responses in grain yield, biomass, and radiation utilization. *Field Crops Res.* **31**:233-252.

Bolanos, J., and Edmeades, G.O. (1993b). Eight cycles of selection for drought tolerance in lowland tropical maize. II: Responses in reproductive behavior. *Field Crops Res.* **31:** 253-268.

Bolanos, J. and Edmeades, G.O. (1996). The importance of the anthesis-silking interval in breeding for drought tolerance in tropical maize. *Field Crops Res.* **48**:65-80.

Bolanos, J., Edmeades, G.O. and Martinez, L. (1993). Eight cycles of selection for drought tolerance in lowland tropical maize. III. Responses in drought-adaptive physiological and morphological traits. *Field Crops Res.* **31:** 269-286.

Bonierbale, M.W., Plaisted, R.L. and Tanksley, S.D. (1988). RFLP maps based on a set of common clones reveals modes of chromosomal evolution in potato and tomato. *Genetics* **120**:1095-1103.

Borrell, A.K. (2000). Physiological basis, QTL and MAS of the stay green drought resistance trait in sorghum. In: Ribaut, J.-M. and D. Poland (eds.). Proceedings on Workshop for 'Molecular Approaches for Genetic Improvement of Cereals for Stable Production in Water Limited Environments'. CIMMYT, Mexico, pp. 142-146.

Borrell, A.K., Tao, Y. and MacIntyre, C.L. (2000). Physiological basis QTL and MAS of stay green drought resistance trait in grain sorghum. In: Ribaut, J.M. and Poland, D. (eds.) Molecular approaches for genetic improvement of cereals for stable production in water-limited environments. A strategies planning workshop held at CIMMYT, Mexico, June 21-25, 1999, CIMMYT, Mexico, pp. 142-146.

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphism. *Am. J. Human Genet.* **32:** 314-331.

Boyer, J.S. (1996). Advances in drought tolerance in plants. *Adv. Agron.* **56:** 187-218.

Burr, B. and Burr, F. A. (1991). Recombinant inbreds for molecular mapping in maize: theoretical and practical consideration. *Trends Genet.* **7**:55-60.

Burr, B., Burr, F.A., Thompson, K., Albertson, M. and Stuber, C.W. (1988). Gene mapping with recombinant inbreds in maize. *Genetics* **118:** 519 - 526.

Byrne, P.F., and McMullen, M.D. (1996). Defining genes for agricultural traits: QTL and the candidate gene approach. *Probe* **7**:24-27.

Byrne, P.F., Bolanos, J. Edmeades, G.O. and Eaton, D.L. (1995). Gains from selection under drought versus multilocation testing in related tropical maize populations. *Crop Sci.* **35**: 63-69.

Callen, D.F., Thompson, A.D., Shen, Y., Phillips. H.A., Richards, R.I., Mulley, J.C., and Sutherland, G.R. (1993). Incidence and origin of "null" alleles in the (AC)n microsatellite markers. *Am. J. Human Genet.* **52**: 922-927.

Campbell S.A., and Close, T.J. (1997). Dehydrins: genes, proteins, and associations with phenotypic traits. *New Phytol.* **137**: 61-74.

Castleberry, R.M. (1986). Border height effects on two row corn plot yields. In: D. Wilkinson (ed.), Proc. 41st Annual Corn and Sorghum Research Conference, ASTA, Washington D.C., pp.171-179.

Castleberry, R.M., Lerette, R.J. (1979). Latente, a new type of drought tolerance? In: H. Loden and D. Wilkinson (eds). Proc. 34th Annual Corn and Sorghum Res. Conf., Chicago, Dec 11-13, 1979. ASTA, Washington, pp 46-56.

Chambers, G.K. and MacAvoy, E.S. (2000). Microsatellites: consensus and controversy. *Comparative Biochemistry and Physiology, Part B* **126:** 455-476.

Chandra, S. and Bidinger, F. R. (2002). Phenotyping for QTL Mapping. In: Saxena N.P. and John, C O'Toole (eds.). Field Screening for Drought Tolerance in Crop Plants with Emphasis on Rice. ICRISAT, Hyderabad.

Chang, T.T., Armento-Soto, J. L., Mao, C. X., Peiris, R. and Loresto, G.C. (1986). Genetic studies on the components of drought tolerance in rice .In: Rice Genetics. IRRI, Philippines, pp. 387-398.

Chapman, S.C. and Edmeades. G.O. (1999). Selection improves drought tolerance in tropical maize populations: direct and correlated responses among secondary traits. *Crop Sci.* **39:**1315-1324.

Chapman, S.C., Barreto, H.J., Cooper, M. and Hammer, G.L. (1997). Simulation models and statistical methods to assist in targeting and breeding for drought tolerance. In: Edmeades *et al.* (eds.) Developing Drought and Low-N Tolerant Maize (as above).

Chen, Z. and Gallie, D.(2004 ).The ascorbic acid redox state controls guard cell signaling and stomatal movement. *The Plant Cell Preview,* www.aspb.org. American Society of Plant Biologists.

Chetti, B. M. (2002). Global scenario of drought research: past, present and future. In: Chetti, B.M., Hiremath, S.M. and Kalpana, M. (eds.). Physiological Approaches for Enhancing Productivity Potential under Drought Condition. University of Agricultural Sciences, Dhawad, Karnatacka.

Chin E.C.L., Senior, M.L., Shu, H. and Smith, J.S.C. (1996). Maize simple repetitive DNA sequences: abundance and allele variation. *Genome* **39:**866-873.

CIMMYT (1994). 1993/94 World maize facts and trends. Maize seed industry revisited: Emerging roles of the public and private sectors. CIMMYT, Mexico.

CIMMYT (2000). CIMMYT Annual Report. CIMMYT, Mexico.

Claassen, M.M. and Shaw, R.H. (1970). Water deficit effects on corn: II. Grain components. *Agron. J.* **62**: 652-655.

Coe, F.H., Neuffer, M.G. and Hoisington, D.A. (1988). The genetics of corn. In: Sprague, G.F. and Dudley, J.W. (eds.) Corn and Corn Improvement, 3rd edition, Agron. Monogr. No. 18. ASA, CSSA, and SSSA, Madison, WI, pp. 81-258.

Curry, R.B., Jones, J., Boote, K.J., Peart, R.M., Allen, L.H. and Pickering, N.G. (1995). Responses of soybean to predicted climate change in the USA. In: Climatic Change in Agriculture: Analysis of Potential International Impact. ASA Special Publication No. **59,** ASA, Madison, WI, USA, pp. 163-182.

De Vienne, D., Leonardi, A., Damerval, C. and Zivy, M. (1999). Genetics of proteome variation for QTL characterization: application to drought stress responses in maize. *J. Exp. Bot.*, **50**: 303-*309.*

Dekkers, J.C.M. and Hospital, F. (2002). The use of molecular genetics in the improvement of agricultural population. *Nature Rev.* **3**: 22-23.

Denmead, O.T., and Shaw, R.H. (1960). The effects of soil moisture stress at different stages of growth on the development and yield of corn. *Agron. J.* **52**: 272-274.

Devine, T. E. (1982). Genetic fitting of crops to problem soil. In: Christiansen, M.N. and Lewis C.F. (eds.) Breeding Plants for Less Favorable Environment. John Wiley, USA, pp.143-173.

Dhillon, B.S. and Prasanna, B.M. (2001) Maize. In: V.L. Chopra (ed.) *Breeding Field Crops.* Oxford & IBH, New Delhi, India, pp. 149-185.

DMR (2003). Annual Report, Directorate of Maize Research (DMR), 51st Annual Maize Workshop, Directorate of Maize Research, held at CSS-HAU, Uchani, Karnal, Haryana, India, April 8-11, 2003.

Doebley, J., Stec, A. and Gustus, C. (1995). *Teosinte branched1* and the origin of maize: evidence for epistasis and the evolution of dominance. *Genetics* **141:**333-346.

Doorenbos, J., and Pruitt, W.O. (1984). *Guidelines for Predicting Crop Water Requirements.* FAO Irrigation and Drainage Paper 24. Rome: FAO.

Dudley, J.W. (1984). A method of identifying lines for use in improving parents of a single cross. *Crop Sci.* **24**:355-357.

DuPlessis, D.P. and Dijkhuis, F.J. (1967). The influence of time lag between pollen shedding and silking on the yield of maize. *South African J. Agric. Sci.* **10**: 667-674.

Edmeades, G.O., Bolanos, J. Banziger, M.. Ribaut, J.-M., White, J.W., Reynolds, M.P. and Lafitte, H.R. (1998). Improving crop yields under water deficits in the tropics. In: Chopra, V.L., Singh, R.B. and Varma, A. (eds.), Crop Productivity and Sustainability - Shaping the Future. Proc. 2nd Int. Crop Science Congress, 437451. New Delhi: Oxford and IBH, pp. 437-451.

Edmeades, G.O., Bolanos, J., Banziger, M., Chapman, S.C., Ortega, A., Lafitte, H.R., Fischer, K.S. and Pandey, S. (1997a). Recurrent selection under managed drought stress improves grain yields in tropical maize. In: Edmeades, G.D., Banziger, M., Mickelson, H.R. and Pefia-Valdivia, C.B. (eds.). *Developing Drought and Low N- Tolerant Maize. Proceedings of* a *Symposium, March* 25-29, 1996, CIMMYT, Mexico, pp. 415-425.

Edmeades, G.O., Bolanos, J. and Chapman, S.C. (1997b). Value of secondary traits in selecting for drought tolerance in tropical maize. In: Edmeades, G.O., Banziger, M., Mickelson, H.R. and Pefia-Valdivia, C.B. (eds.). *Developing Drought and Low N Tolerant Maize. Proceedings of* a *Symposium, March* 25-29, 1996, CIMMYT, Mexico, pp. 222-234.

Edmeades, G.O., Bolanos, J. and Lafitte H.R. (1992). Progress in breeding of drought tolerance in maize. Proc. 57th Annual Corn and Sorghum Industry Research Conference, pp. 93-111.

Edmeades, G.O., Bolanos, J., Hernandez, M. and Bello, S. (1993). Causes for silk delay in a lowland tropical maize population. *Crop Sci.* **33**:1029-1035.

Edmeades, G.O., Bolanos, J., Chapman, S.C., Lafitte, H.R. and Banziger, M. (1999). Selection improves drought tolerance in tropical maize populations: I. Gains in biomass, grain yield, and harvest index. *Crop Sci.* **39:** 1306-1315.

Edmeades, G.O., Bolanos, L., Elings, A. Ribaut, J.M., Banziger, M. and Westgate, M.E. (2000). The role and regulation of the anthesis-silking interval in maize. In: Westgate, M.E. and Boote, KJ. (eds.) Physiology and Modeling Kernel Set in Maize. CSSA Special Publication No. 29. CSSA, Madison, WI, pp. 43-73.

Edmeades, G.O. and Daynard, T.B. (1979). The relationship between final yield and photosynthesis at flowering in individual maize plants. *Can. J. Plant Sci.* **59:** 585-601.

Edward, K.J. and Mogg, R. (2001). Plant genotyping by analysis of single nucleotide polymorphisms. In: Henry, R.J. (ed.) Plant genotyping: The DNA Fingerprinting of Plants. CABI Publishing, Wallingford, UK, pp. 1-13.

Edwards, M.D. Helentjaris, T., Wright, S. and Stuber, C.W. (1992). Molecular marker facilitated investigations of quantitative trait loci in maize 4. Analysis based on genome saturation with isozyme and restriction fragment length polymorphism markers. *Theor. Appl. Genet.* **83**:765-774.

Edwards, M.D., Stuber, C.W. and Wendel, J.F. (1987). Molecular marker facilitated investigation of quantitative trait loci in maize. I. Number, genomics distribution and types of gene action. *Genetics* **116**:113-125.

Efron, B. (1979). Bootstrap methods: Another look at the jackknife. *Ann. Stat.* **7**: 1-26.

Epstein, E. and Rains D. W. (1987). Advances in salt tolerance. *Pl. Soil* **99**:17-29.

Falconer, D.S. (1989). Introduction to Quantitative Genetics, 3rd Edition. London: Longman.

FAO (1998). Food and Agriculture Organization of the United Nations. Annual Report. FAO, Rome, Italy.

FAO Agrostat. (1992). PC Files of Food Balance Sheets. FAO, Rome, Italy.

FAO/WHO/UN (1985). Expert Consultation. WHO Technical report Series No. 724. World Health Organization, Geneva.

Fatokun, C.A., Menancio-Hautea, D.I., Danesh, D. and Young, N.D. (1992). Evidence for orthologous seed weight genes in cowpea and mungbean based on RFLP mapping. *Genetics* **132**:841-846.

Felsentein, J. (1985). Confidence limits on phylogenesis: An approach using the bootstrap. *Evolution* **38**: 789-791

Fischer, K.S., Edmeades, G.O. and Johnson, E.C. (1989). Selection for the improvement of maize yield under moisture-deficit. *Field Crops Res.* **22**:227-243.

Fischer, K.S., Lafitte, R., Atlin, G. and Hardy, B. (eds.). (2003). Breeding Rice for Drought-Prone Environments. IRRI, Philippines.

Fischer, K.S. and Palmer, A.F.E. (1983). Maize.. In: Proc. Symp. on Potential Productivity of Field Crops under Different Environments. IRRI, Los Banos, Phillippines, 22-26 Sept. 1980. IRRI, Phillippines, pp. 155-180.

Fischer, R.A. and Maurer, R. (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. *Aust. J. Agric. Res.* **29**: 897-912.

Fracheboud, Y., Ribaut, J.-M., Vargas, M., Messmer, R. and Stamp, P. (2002). Identification of quantitative trait loci for cold-tolerance of photosynthesis in maize. *J. Exp. Bot.* **53**: 1967-1977.

Frei, O.M., Stuber, C.W. and Goodman, M.M. (1986). Use of allozymes as genetic markers for predicting performance in maize single-cross hybrids. *Crop Sci.* **26**: 37-42

Frova, C,, Krajewski, P., Di Fonzo, N., Villa, M. and Sari-Gorla, M. (1999).Genetic analysis of drought tolerance in maize by molecular markers. I. Yield components. *Theor. Appl. Genet.* **99**: 280-288.

Fukai, S., and Cooper, M. (1995). Development of drought-resistant cultivars using physio-morphological traits in rice. *Field Crops Res.* **40**:67-86.

Fussell, L.K., Bidinger, F.R. and Bieler, P. (1991). Crop physiology and breeding for drought tolerance: research and development. *Field Crops Res.* **27**: 183-199.

Gardner, C.O. (1961). An evaluation of effects of mass selection and seed irradiation with thermal neutrons on yield of corn. *Crop Sci.* **1**:241-245.

Geadelmann, J. (1984). Using exotic germplasm to improve northern corn. In: Wilkinson, D. (ed.) Proc. 3rd Annual Corn & Sorghum Research Conference, ASTA, Washington, D.C., pp. 98-110.

Gelderman, H., (1975). Investigation on inheritance of quantitative characters in animals by gene markers. I Methods. *Theor. Appl. Genet.* **46**:319-330.

Gethi, J.G., Labate, J.A., Lamkey, K.R., Smith, M.E. and Kresovich, S. (2002). SSR variation in important US maize inbred lines. *Crop Sci.* **42:** 951-957.

Gilles, P.N., Wu, D.J., Foster, C.B., Dillon, P.J. and Chanock, S.J. (1999). Single nucleotide polymorphic discrimination by an electronic dot blot assay on semiconductor microchips. *Nature Biotech.* **17**:365-370.

Gilmour, A.R., Cullis, B.R., Welham, S.J. and Thompson, R. (1998). ASREML, NSW Agriculture, Orange, 2800, Australia.

Grant, R.F., Jackson, B.S. Kinry, J.R. and. Arkin, G.F. (1989). Water deficit timing effects on yield components in maize. *Agron. J.* **81**:61-65.

Grimaldi, M.C. and Crouau-Roy, B. (1997). Microsatellite alleic homoplasy due to variable flanking sequences. *J. Mol. Evol.* **44**: 336-340.

Gupta, P.K., Balyan, H.S., Sharma, P.C. and Ramesh, B. (1996). Micosatellites in plants: a new class of molecular markers. *Curr. Sci.* **70:** 45-54.

Guei, R.G. and Wassom, C.E. (1992). Inheritance of some drought adaptive traits in maize: I. Interrelationships between yield, flowering and ears per plant. *Maydica* **37:** 157-164.

Hall, A.E., Richards, R.A., Condon, A.G., Wright, G.C. and G.D. Farquhar. (1994). Carbon isotope discrimination and plant breeding. *Plant Breed. Rev.* **12**: 81 113.

Hall, A.J. and Trapani J.H. (1981).Water stress before and during flowering in maize and its effects on yield, its components, and their determinants. *Maydica* **26:**19-38.

Hall, A.J., Vilella, F., Trapani, N. and Chimenti, C. (1982). The effects of water stress and genotype on the dynamics of pollen-shedding and silking in maize. *Field Crops Res.* **5**:349-363.

Hallauer, A.R, and Eberhart, S.A. (1970). Reciprocal full-sib selection. *Crop Sci.* **10**:315-316.

Hallauer, A.R. (1973). Hybrid development and population improvement in maize by reciprocal full-sib selections. *Egyptian J. Genet. Cytol.* **2:** 84-101.

Hallauer, A.R. and Miranda, J.B. (1988). Quantitative Genetics in Maize Breeding. 2nd edition, Iowa State Univ. Press, Ames.

Hallauer, A.R., Russell, W.A. and Lamkey, K.R. (1988). Corn breeding. In: Corn and Corn Improvement (eds. G.F. Sprague and J.W. Dudley), 3rd edition, Agron. Monogr. 18. ASA, CSSA, and SSSA, Madison, WI, pp. 463-564.

Hammada, H.M., Petrino, E.S. and Kanunnaga, T. (1982). A novel repeated element with Z-DNA-forming potential is widely found in evolutionarily diverse eukaryotic genomes. *Proc. Nat. Acad. Sci. USA*, **79**: 6465-6469.

Heisey. P.W. and Edmeades, G.O. (1999). Part 1. Maize production in drought stressed environments: technical options and research resource allocation. In: World Maize Facts and Trends 1997/98. CIMMYT, Mexico, pp. 1-36.

Helentjaris, T., Slocum, M., Wright, S. Schaefer, A. and Nienhuis, J. (1986). Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. *Theor. Appl. Genet.* **72**:761-769.

Hittalmani, S., Parco A., Mew, T.V., Zeigler, R.S and Huang, N. (2000).Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. *Theor. Appl. Genet.* **100**: 1121-1128.

Hsiao, TC. (1973). Plant responses to water stress. *Annu. Rev. Plant Physiol.* 24:519-570.

Ito, T. (1999). Direct and correlated responses among secondary traits. *Crop Sci.* **39**: 1315-1324.

Jaccard, P. (1908). Nouvelles researches sur la distribution florale. *Bull. Soc. Vaudoise Sci. Nat.* **44:** 223-270.

Jarne, P., and Lagoda, P.J.L. (1996). Microsatellites, from molecules to populations and back. *Trends Eco. Evol.* **11**: 424-429.

Jiang, C. and Zeng, Z.B. (1995). Multiple trait analysis of genetic mapping for quantitative trait loci. *Genetics* **140**: 1111-1127

Johnson, G.R., Mumm, R.H. (1996). Marker assisted maize breeding. In: Proc. 51st Annual Corn and Sorghum Res. Conf., Chicago. Dec. 11-12, 1996. ASTA, Washington. DC, pp. 75-84.

Jordan, W.R. and Miller, F.R. (1980). Genetic variability in sorghum root systems: Implication for drought tolerance. In: Turner, N. C. and Karamer, P. J. (eds.) Adaptation of Plants to Water and High Temperature Stress. Wiley Interscience, New York.

Kassahun, B. and Prasanna, B.M. (2003). Simple sequence repeat polymorphism in Quality Protein Maize (QPM) lines. *Euphytica* **129**: 337-344.

Kempthorne, O. (1957). An Introduction to Genetic Statistics. John Wiley and Sons Inc., New York.

Khavkin, E. and Coe, E. (1997). Mapped genomic locations for developmental functions and QTLs reflect concerted groups in maize (*Zea mays* L). *Theor Appl. Genet.* **95**: 343-352.

Kiesselbach, T.A. (1949). The Structure and Reproduction of Corn. Nebraska *Agr. Exp. Stn. Res. Bul.* 161p.

Kim, J.Y., Mahe, A., Brangeon, J. and Prioul, J.L. (2000). A maize vacuolar invertase, IVR2 is induced by water stress with organ/tissue specificity and diurnal modulation of expression. *Plant Physiol.* **124**: 71 84.

Kiniry, J. (1991). Maize phasic development. In: *Modeling Plant and Soil Systems*. Agronomy Monograph No. 13. Madison, Wisconsin: ASA-CSSA-SSSA, pp. 55-70.

Kowalski, S.P., Lan, T.H., Feldmann, K.A. and Paterson, A.H. (1994). Comparative mapping of *Arabidopsis thaliana* and *Brassica oleracea* chromosomes reveals islands of conserved organization. *Genetics* 138:495-510

Lafitte, H.R, and Banziger, M. (1997). Maize population improvement for low soil N: selection gains and the identification of secondary traits. In: Edmeades, G.O., Banziger, M., Mickelson, H.R and Peria-Valdivia C.B.

(eds.). *Developing Drought and Low N- Tolerant Maize. Proceedings of a Symposium, March* 25-29, 1996, CIMMYT, Mexico, pp. 485-489.

Lafitte, H.R, and Edmeades, G.O. (1994a). Improvement for tolerance to low soil nitrogen in tropical maize. I. Selection criteria. *Field Crops Res.* **39**:1-14.

Lafitte, H.R., and Edmeades, G.O. (1994b). Improvement for tolerance to low soil nitrogen in tropical maize. II. Grain yield, biomass production, and N accumulation. *Field Crops Res.* **39:**15-25.

Lafitte, H.R., and Edmeades, G.O. (1995). Stress tolerance in tropical maize is linked to constitutive changes in ear growth characteristics. *Crop Sci.* **35**:820-826.

Lafitte, H.R., Banziger, M. Bell, M.A. and Edmeades, G.O. (1997). Addressing soil variability in low nitrogen breeding programs. In: Edmeades, G.O., Banziger, M., Mickelson, H.R. and Pena-Valdivia, C.B. (eds.). *Developing Drought and Low N- Tolerant Maize. Proceedings of* a *Symposium, March* 25-29, 1996, CIMMYT, Mexico, pp. 537-540.

Lander, E.S. and Botstein, D. (1989). Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **121**:185-199.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E. and Newburg, L. (1987). MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* **1**: 174-181.

Langridge, P., Dong, C., Whitford, R., Sutton, T., Wolters, P., Rafalski, A., Morgante, M., Gumaelius, L., Uhlmann, N. and Tingey S. (2002). Early meiotic genes and the PH2 region of wheat, Abstract W176, Proceeding of the Plant, Animal and Microbe genome, January 12-16, 2002, San Diego, USA, pp. 50.

Lebreton, C., Lazic-Jancic, V., Steed, A., Pekic, S. and Quarrie S.A. (1995). Identification of QTL for drought responses in maize and their use in testing causal relationships between traits. *J. Exp. Bot.* **46**:853-865.

Lee, M. (1995). DNA markers and plant breeding programs. *Adv. Agron.* **55**: 265-344.

Levitt, J. (1972). Response of Plants to Environmental Stresses. Vol. II. Water, Radiation, Salt and Other Stresses. Academic Press, New York, USA.

Levitt, J. (1980). Response of Plants to Environmental Stresses. Vol. II. Water, Radiation, Salt and Other Stresses (2nd edition). Academic Press, New York, USA.

Lewin, L.G. and Sparrow, D.H. (1975). The genetic and physiology of resistance to stress. Proc. 3rd International Barley Genetics Symposium, Garching, pp. 486-501.

Lin, C.Y. (1978). Index selection for genetic improvement of quantitative characters. *Theor. Appl. Genet.* **52**:49-56.

Liu, B.H. (2002). Statistical Genomics: Linkage, Mapping and QTL analysis. CRC Press, USA.

Loresto, G.C. and Chang, T.T. (1976). Field evaluation and breeding for drought resistance. *Philipp. J. Crop Sci.***1**:36-39.

Ludlow, M.M. and Muchow, R.C. (1990). A critical evaluation of traits for improving crop yields in water-limited environments. *Adv. Agron.* **43**: 107-153.

Mahalakshmi, V., Bidinger, F.R. and Rao, G.D.P. (1990). Line-source vs. irrigated and non-irrigated treatments for evaluation of genotype drought response. *Agron. J.,* **82**: 841-844.

McCouch, S.R., Kochert, G., Yu Z.H., Wang, Z.Y. and Khush, G.S. (1988). Molecular mapping of rice chromosomes. *Theor. Appl. Genet.* **76**:815-829.

Mogg, R., Hanley, S. and Edvards, K.J.(1999).Generation of maize allele-specific oligonucleotides from the flanking regions of microsatellite markers. Plant and Animal Genome VII Conference Abstracts, pp.491.

Mohammadi, S.A. and Prasanna, B.M. (2003). Analysis of genetic diversity in crop plants-salient statistical tools and considerations. *Crop Sci.* **43:**1235-1248.

Mohan, M., Naill, S., Bhagwat, A., Krishna, T.G., Yano, M. Bhatia, C.R. and Sasaki, T. (1997). Genome mapping, molecular markers and marker-assisted selection in crop plants. *Mol. Breed.* **3**: 87-103.

Morgante, M. and Olivieri, A.M. (1993). PCR amplified microsatellites as markers in plant genetics. *Plant J.* **3**: 175–182.

Moss, G.I. and Downey, L.A. (1971). Influence of drought stress in female gametophyte development in corn (*Zea mays* L.) and subsequent grain yield. *Crop Sci.* **11:** 368-372.

Moxon, E.R. and Wills, C. (1999). DNA microsatellites: agents of evolution? *Sci. Amer.* Jan. Issue, pp. 72-77.

Mugo, S.N., Banziger, M. and Edmeades, G.O.(2000). Prospects of using ABA in selection for drought tolerance in cereal crops. In: Ribaut, J.M. and Poland, D. (eds.). Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-Limited Environments. A Strategic Planning Workshop held at CIMMYT, El Batan, Mexico, June 21-25, 1999, CIMMYT, Mexico, D.F., pp. 73-78.

Nair, S.K. (2003). Genetic and molecular marker analysis of sorghum downy mildew resistance in maize. Ph.D. Thesis, Division of Genetics, IARI, New Delhi.

NeSmith, D.S. and Ritchie, J.T. (1992). Effects of soil water-deficits during tassel emergence on development and yield components of maize *(Zea mays* L). *Field Crops Res.* **28:**251-256.

Nguyen, H.T., Babu, R.C. and Blum, A. (1997). Breeding for drought resistance in rice: physiology and molecular genetics considerations. *Crop Sci.* **37**: 1426-1434.

Paterson, A.H., Lander, E.S., Hewitt, J.D. Peterson, S., Lincoln, S.E. and Tanksley, S.D. (1988). Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nature* **335:**721-726.

Paterson, A.H., Lin, Y.R., Li, Z., Schertz, K.F., Doebley, J.F. Pinson, S.R.M., Liu, S.C., Stansel, J.W. and Irvine, J.E. (1995). Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. *Science* **269:**1714-1717.

Patil, S. A. (2002).Drought as a Challenge for the Plant Breeder. In: Chetti, B.M., Hiremath, S.M. and Kalpana, M. (eds.) Physiological Approaches for Enhancing Productivity Potential under Drought Condition. University of Agricultural Sciences Dhawad, Karnataka,

Pelleschi, S. *et al.* (1999). *Ivr2*, a candidate gene for a QTL of vaculoar invertase activity in maize leaves: gene-specific expression under water stress. *Plant Mol. Biol.* **39**: 373-380.

Phillips, R.L., Kim, T.S., Kaeppler, S.M., Parentoni, S.N., Shaver, L., Stucker, R.E. and Openshaw, S.J. (1992) Genetic dissection of maturity using RFLPs. In: Wilkinson D (ed) Proc. 47th Annual Corn & Sorghum Research Conference, ASTA, Washington, pp. 135-150.

Powell, W., Machray, G.C. and Provan, I. (1996). Polymorphism revealed by simple sequence repeats. *Trends Plant Sci* **1:** 215-221.

Prasanna, B.M. and Hoisington, D. (2003). Moleçular breeding for maize improvement: An overview. *Indian J. Biotech.* **2:** 85-98.

Prasanna, B.M. and Ribaut, J.M. (2004). Molecular approaches for improving drought tolerance in tropical maize. In: Zaidi, P.H., Harrington, K., de Meyer, J. and Singh, N.N. (eds.) Improving Maize Productivity under Abiotic Stresses. ICAR and CIMMYT, India, pp. 123-134.

Prasanna, B.M. and Sarkar K.R. (1997). Active transposable elements in Indian maize germplasm and assorted genetic testers. *Curr. Sci.* **73:** 464-469.

Prasanna, B.M., Mohammadi, S.A., Sudan, C., Nair, S.K., Garg, A., Rathore, R.S., Setty, T.A.S., Kumar, R., Zaidi, P.H. and Singh, N.N. (2004). Application of molecular marker technologies for maize improvement in India - present status and prospects. In: G. Srinvasan, P.H. Zaidi, B.M. Prasanna, F. Gonzalez and K. Lesnick (eds). Proc. 8th Asian Regional Maize Workshop, Bangkok, Thailand, August 5-8, 2002, pp. 73-81.

Prioul, J.L., Pelleschi, S. Sene, M., thevenot, C., Causse, M., de Vienne, D. and Leonardi, A. (1999). From QTLs for enzyme activity to candidate genes in maize. *J. Exp. Bot.* **337**:1281-1288.

Pushpavalli, S.N.C.V.L., Sudan, C. Mohammadi, S.A. Nair, S.K., Prasanna, B.M., Gadag, R.N. and Singh, N. N. (2002). Analysis of simple sequence repeat (SSR) polymorphism in Indian maize inbred lines. *J. Genet. Breed.* **56:** 202-212.

Quarrie, S.A., Stojanovic, J. and Pekic, S. (1999). Improving drought resistance in small grained cereals: a case study, progress and prospect. *Plant Growth Reg.* **29:** 1-21.

Quisenbury, J.E. (1982). Breeding for drought resistance and water use efficiency. In: Breeding Plants for Less Favorable Environments (eds., M. N. Christiansen and C.F. Lewis). John Wiley, USA, pp. 193-212.

Ragot, M., Gay, Biasiolli, M., Delbut, M.F., Dellorco, A., Malgarini, L., Thevenin, P., Vemoy, J., Vivant, J., Zimmelmann, R. and Gay, G. (1995). Marker-assisted backcrossing: a practical example. In: Techniques et utilisations des marqueurs moleculaires, (Les Colloques, No. 72), INRA, Paris, pp. 45-56.

Ragot, M., Gay, G., Muller, J.P. and Duroway, I. (2000). Efficient selection for adaptation to the environment through QTL mapping in manipulation in maize. In: Ribaut, J.-M. and Poland, D. (eds.) Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-Limited Environments. A Strategic Planning Workshop held at CIMMYT, Mexico, June 21-25, 1999, CIMMYT, Mexico, pp. 128-130.

Reynolds, M.P., Skovmand, B., Trethowan, R. and Pfeiffer, W. (2000). Evaluating a conceptual model for drought tolerance. In: J.-M. Ribaut and D. Poland (eds.) Molecular Approaches for Genetic Improvement of Cereals for Stable Production in Water-Limited Environments'. CIMMYT, Mexico.

Ribaut, J.M. and Betran, J. (1999). Single large-scale marker-assisted selection (SLS-MAS). *Mol. Breed.* **5**: 531-541.

Ribaut, J.M. and Hoisington, D. (1998). Marker-assisted selection: new tools and strategies. *Trends Plant Sci.* **3**: 236-239.

Ribaut, J.M. and Poland, D. (eds.) (2000). Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-Limited Environments. A Strategic Planning Workshop held at CIMMYT, El Batan, Mexico, June 21-25 June, CIMMYT, Mexico.

Ribaut, J.M., Banziger, M., Betran, J., Hang, C., Edmeades, G.O., Dreher, K. and Hoisington D. (2002). Use of molecular markers in plant breeding: drought tolerance improvement in tropical maize. In: M.S. Kang (ed.) Quantitative Genetics, Genomics, and Plant Breeding, CABI Publishing, Wallingford, UK.

Ribaut, J.M., Banziger, M., Setter, T., Edmeades, G.O. and Hoisington, D. (2003).Genetic dissection of drought tolerance in maize: A case study. In: Nguyen, H. and Blum, A. (eds.). Physiology and Biotechnology Integration for Plant Breeding. Marcel Dekker, Inc., New York,

Ribaut, J.M., Jiang, C., Gonzalez-de-Leon, D., Edmeades, G.O. and Hoisington, D.A. (1997a). Identification of quantitative trait loci under drought conditions in tropical maize: II. Yield components and marker-assisted selection strategies. *Theor. Appl. Genet.* **94**:887-896.

Ribaut, J.M., Gonzalez-de-Leon, D., Jiang, C., Edmeades, G.O. and D. Hoisington. (1997b). Identification and transfer of ASI quanitative trait loci (OTL): A strategy to improve drought tolerance in maize lines and populations. In: G.D. Edmeades, M. Banziger, H.R. Mickelson, and C.B. Pena-Valdivia (eds.), *Developing Drought and Low N-Tolerant Maize. Proceedings of* a *Symposium, March* 25-29, 1996, CIMMYT, Mexico, pp. 396-400.

Ribaut, J.M., Hoisington, D.A., Deutsch, J.A., Jiang, C. and Gonzalez-de-Leon, D. (1996). Identification of quantitative trait loci under drought conditions in tropical maize: 1. Flowering parameters and the anthesis-silking interval. *Theor. Appl. Genet.* **92**:905-914.

Rincon, F., Johnson B., Crossa J. and Taba, S. (1996). Cluster analysis, an approach to sampling variability in maize accessions. *Maydica* **41**: 307-316.

Robinson, J.P., and Harris, S.A. 1999. Amplified fragment length polymorphisms and microsatellites: A phylogenetic perspective. In: Gillet, E.M. (ed.). Which DNA Marker for Which Purpose? Final Compendium of the Research Project Development, optimisation and validation of molecular tools for assessment of biodiversity in forest trees in the European Union DGXII Biotechnology FW IV Research Programme Molecular Tools for Biodiversity. URL http://webdoc.sub.gwdg.de/ebook/y/1999/whichmarker/index.html.

Rohlf, F.J. (1992). NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System). Version 2.02. Exeter Publ., Setauket, NY.

Rosielle, AA, and J. Hamblin. (1981). Theoretical aspects of selection for yield in stress and non-stress environments. *Crop Sci.* **21**:943-946.

Ruan, Y., Gilmore, J. and Conner, T. (1998). Towards Arabidopsis genome analysis: Monitoring expression profiles of 1400 genes using cDNA microarrays. *Plant J.* **15**: 821-833.

Saghai-Maroof, M.A., Soliman, K.M, Jorgenson, R., and Allard, R.W. (1984). Ribosomal DNA space length polymorphisms in barley: Mendelian inheritance, chromosomal locations and population dynamics. *Proc. Natl. Acad. Sci. USA* **81:** 8014-8018.

Saini, H.S., Westgate, M.E., (2000). Reproductive development in grain crops during drought. *Adv. Agron.* **68:** 59-96.

Sanguineti, M.C. *et al.* (1999). QTL analysis of drought related traits and grain yield in relation to genetic variation for leaf abscisic acid concentration in field-grown maize. *J. Exp. Bot.* **50**: 1289-1297.

Sari-Gorla, M., Krajewski, P., Di Fonzo, N., Villa, M. and Frova, C. (1999). Genetic analysis of drought tolerance in maize by molecular markers. II. Plant height and flowering. *Theor. Appl. Genet.* **99**: 289 295.

Schena, M., Shalon, D., Davis, R.W., Brown, P.O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* **270**: 467–470

Schena, M., Shalon, E., Helier, R., Chai, A., Brown, P.O. and Davis, R.W. (1996). Parallel human genome analysis: Microarray-based expression monitoring of 1000 genes. *Proc. Natl. Acad. Sci. USA* **93**:10614-10619.

Schlotterer, C. and Tautz, D. (1992). Slippage synthesis of simple sequence DNA. *Nucleic Acids Res.* **20:** 211-215.

Schussler, J.R. and Westgate, M.E. (1995). Assimilate flux determines kernel set at low water potential in maize. *Crop Sci.* **35**:1074-1080.

Seki, M., Narusaka, M., Abe, H., Kasuga, M., Yamaguchi-Shinozak,i K., Carninci, P., Hayashizaki, Y. and Shinozaki, K. (2001). Monitoring the expression pattern of 1300 Arabidopsis genes under drought and cold stresses by using a full-length cDNA microarray. *The Plant Cell* **13**:61- 72.

Seki, M., Narusaka, M., Ishida, J., Nanjo, T., Fujita, M., Oono, Y., Kamiya, A., Nakajima, M., Enju, A., Sakurai, T., Satou, M., Akiyama, K., Taji,T., Yamaguchi-Shinozaki, K., Caminci, P., Kawai, J, Hayashizaki, Y. and Shinozaki, K. (2002). Monitoring the expression profiles of 7000 Arabidopsis genes under drought. cold and high-salinity stresses using a full-length cDNA microarray. *The Plant* 131: 279-292.

Senior, M.L, Murphy, J.P., Goodman, M.M. and Stuber, C.W. (1998). Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. *Crop Sci.* **38:** 1088-1098.

Senior, M.L. and Heun, M. (1993). Mapping maize microsatelites and polymerase chain reaction confirmation of the target repeats using a CT primer. *Genome* **36:** 884-889.

Setter, T. and Flannigan, B.A. (2001). Water deficit inhibits cell division and expression of transcripts involved in cell proliferation and endoreduplication in maize endosperm. *Exp. Bot.* **52**: 1401-1408.

Shannon, M.C. (2001). Adaptation of plants to salinity. *Adv. Agron.* **60**:75-120.

Sharma, J.R. (1998). Statistical and Biometrical Techniques in Plant Breeding. New Age International Limited, India.

Shen, L., Courtois, B., McNally, K.L., Robin, S. and Li, Z. (1998). Evaluation of near-isogenic lines of rice introgressed with QTLs for root depth through marker-assisted selection. *Theor. Appl. Genet.* **103:**75-83.

Singh, D.P. (2002). Breeding for Resistance to Abiotic Stresses. IBDC, Lucknow, India.

Singh, N.N., Zaidi, P.H., Mehta, M. and Yadav, P. (2004). Abiotic stresses - the major constraint limiting maize production and productivity in south and south-east Asia. In: Improving Maize Productivity under Abiotic Stresses (eds. Zaidi, P.H, Harrington, K., Meyer, J. de. and Singh N.N.), ICAR and CIMMYT, New Delhi.

Smart, C.M., Hosken, S.E., Thomas, H., Graves, J., Blair, B.G. and Schuch, W. (1995). The timing of maize leaf senescence and characterization of senescence-related cDNAs. *Physiol. Plant.* **93:** 673-682.

Smiciklas, K.D. and Below, F.E. (1990). Influence of heterotic pattern on nitrogen use and yield of maize. *Maydica* **35:** 209-213.

Smith, J.S.C. and Helentjaris, T. (1996). Applications of SSRs in plant identification and variety protection. In: A.H. Patterson (ed.) Genome Mapping in Plants, R.G. Landes Co., USA, pp. 96-110.

Smith, J.S.C., Chin, E,C.L., Shu, H., Smith, O.S., Wall, SJ. Senior, M.L., Mitchell, S.E., Kresovich, S. and Ziegle, J. (1997). An evaluation of the utility of SSR loci as molecular markers in maize *(Zea mays L.):* Comparisons with data from RFLPs and pedigree. *Theor. Appl. Genet.* **95:** 163-173.

Smith, O.S., Smith, J.S. C, Bowen, S.L., and Tenborg, R.A. (1991). Numbers of RFLP probes necessary to show associations between lines. *Maize Genet. Coop. Newsletter.* **65:** 66.

Sneath, P.H.A. and Sokal, R.R. (1973). Numerical Taxonomy. W.H. Freeman & Co., San Francisco.

Strand, M., Prolla, T.A., Liskay, and R.M. and Petes, T.D. (1993). Destabilisation of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. *Nature* **365**: 274-276.

Stuber, C. W., Edwards, M.D. and Wendel, J.F. (1987). Molecular marker facilitated investigation of quantitative trait loci in maize. II. Factors influencing yield and its component traits. *Crop Sci.* **27:** 639-648.

Stuber, C.W. (1989a). Marker-based selection foe quantitative traits. In: Proc. XII Congress of EUCARPIA. *Vortr. Pflanzenzucht.* **16:** 31-49

Stuber, C.W. (1989b). Molecular markers in the manipulation of quantitative characters. In: Brown A.H.D., Hahler, M.T. and Weir, B.S. (eds.). Plant Population Genetics, Breeding and Genetic Resources. Sinaur Associates, Inc., Sunderland, MA, USA.

Subbarao, G.V., Johansen, C., Slinkard, A.E., Nageswara Rao, R.C., Saxena, N.P. and Chauhan, Y.S. (1995). Strategies for improving drought resistance in grain legumes. *Crit. Rev. Plant Sci.* **14:** 469-523.

Tansley, S.D (1993). Mapping polygenes. *Annu. Rev. Genet.* **27**: 205-234.

Tanksley, S.D., Grandillo, S., Fulton, T.M., Zamir, D., Eshed, Y., Petiard, V., Lopez, J. and Beck-Bunn, T. (1996). Advanced backcross QTI analysis in a cross between an elite processing line of tomato and its wild relative *L pimpinellifolium. Theor. Appl. Genet.* **92**: 213-224.

Tanner, C.B., and Sinclair, T.R. (1983). Efficient water use in crop production: research or re-search? In: H.M. Taylor, W.R. Jordan and T.R. Sinclair (eds.), *Limitations to Efficient Water Use in Crop Production,* ASA-CSSA-SSSA.

Taramino, G. and Tingey, S. (1996). Simple sequence repeats for germplasm analysis and mapping in maize. *Genome* **39:** 277-287.

Troyer, A.F. and Brown. W.L. (1976). Selection for early flowering in corn: Seven late syntheitics. *Crop Sci.* **16:** 767-772.

Troyer, A.F. and Larkins, J.R. (1985). Selection for early flowering in corn: 10 late synthetics. *Crop Sci.* **25:** 695-697.

Tautz, D. (1989). Hypervariability of simple sequences as a general source of polymorphic DNA markers. *Nucl. Acids Res.* **17:** 6463-6471.

Thomas, H, and Smart C.M. (1993). Crops that stay-green. *Ann. Appl. Biol.* **123**: 193-219.

Thomas, H. and Howarth, C.I. (2000). Five ways to stay green. *J. Exp. Bot.* **51:** 329-337.

Thomas, M.R. and Scott, W.S. (1993). Microsatellite repeats in grapevine reveal DNA polymorphisms when analyzed as sequence tagged sites. *Theor. Appl. Genet.* **86:** 985-990.

Tollenaar, M. (1977). Sink-source relationships during reproductive development in maize. A review. *Maydica* **22:** 49-75.

Tollenaar, M., Dwyer, L.M. and Stewart, D.W. (1992). Ear and kernel formation in maize hybrids representing three decades of grain yield improvement in Ontario. *Crop Sci.* **32:** 432-438.

Tuberosa, R., Salvi, S., Sanguineti, M.C., Landi, P. Macafferi, M. and Conti, S. (2002). Mapping QTLs regulating morpho-physiological traits and yield: Case studies, shortcomings and perspectives in drought-stressed maize. *Ann. Bot.* **89**: 941-963.

Tuberosa, R., Sanguineti, M.C., Landi, P., Salvi, S. and Casarini, C.S. (1998). RFLP mapping of quantitative trait loci controlling abscissic acid concentration in leaves of drought-stressed maize (Zea mays L). *Theor. Appl. Genet.* **97:** 744-755.

Tuinstra, M.R., Grote, E.M., Goldbrough, P.B. and Ejeta, G. (1997). Genetic analysis of post-flowering drought tolerance and components of grain development in *Sorghum bicolor* Moench. *Mol. Breed.* **3:** 439-448.

Turner, N.C. (1979). Drought resistance and adaptation to water deficits in crop plants. *In:* Stress Physiology in Crop Plants (eds., H.W. Mussell and R.C. Staples). Wiley Interscience, New York.

Turner, N.C. (1981). Techniques and experimental approaches for the measurement of plant water status. *Plant Soil* **58**:339-366.

Turner, N.C. (1982). The role of shoot characteristics in drought resistance of crop plants. *In:* Drought Resistance in Crops with Emphasis on Rice. IRRI, Philippines, pp. 115-134.

Turner, N.C. (1986). Crop water deficits: A decade of progress. *Adv. Agron.* **39**: 1-5l.

Turner, N.C., O'Toole, J.C., Cruz, R.T., Namuco, O.S. and Ahmed, S. (1986). Responses in seven diverse rice cultivars to water deficit. I. Stress development, canopy temperature, leaf rolling and growth. *Field Crops Res.* **13:** 257-271.

Van Beem, J, Smith, M.E. and Zobel, R.W. (1998). Estimating root mass in maize using a portable capacitance meter. *Agron. J.* **190**: 566-570.

Van Oosterom, E.J., Jayachandran, R. and Bidinger, F.R. (1996). Diallel analysis of stay green trait and its components in sorghum. *Crop Sci.* **36:** 549-555.

Vasal, S.K., Cordova, H.S., Beck, D.L. and Edmeades, G.O. (1997). Choices among breeding procedures and strategies for developing stress-tolerant maize germplasm. In: G.O. Edmeades, M. Banziger, H.R. Mickelson, and C.B. PenaValdivia (eds.), *Developing Drought and Low N- Tolerant Maize. Proceedings of* a *Symposium, March* 25-29, 1996, CIMMYT, Mexico, pp. 336-347.

Veldboom, L. R., Lee, M. and Woodman, W. L. (1994). Molecular marker-facilitated studies in an elite maize population: I. Linkage analysis and determination of QTL for morphological traits. *Theor. Appl. Genet.* **88**:7-16.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee van de, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kupier, M., and Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucl. Acids Res.* **23**: 4407-4414.

Vuylsteke, M., Mank, R., Antonise, R., Bastiaans, E., Senior, M.L., Stuber, C.W., Melchinger, A.E., Lubberstedt, T., Xia, X.C., Stam, P. and Kuiper, M. (1999). Two high-density AFLP linkage maps of *Zea mays* L.: Analysis of distribution of AFLP markers. *Theor. Appl. Genet.* **99:** 921-935.

Vuylsteke, M., Mank, R., Brugmans, B., Stam, P. and Kuiper, M. (2000). Further characterization of AFLP data as a tool in genetic diversity assessments among maize (*Zea mays* L.) inbred lines. *Mol. Breed.* **6:** 265-276

Walulu, R.S., Rosenow, D.T., Webster, D.B. and Nguyen, H.T. (1994). Inheritance of stay green trait in sorghum. *Crop Sci.* **34:** 970-972.

Wang, Z., Weber, J.L., Zhong, G. and Tanksley, S.D. (1994). Survey of plant short tandem DNA repeats. *Theor. Appl. Genet.* **88:** 1-6.

Weber, J.L. and May, P.E (1989). Abundant class of human DNA polymorphisms, which can be typed using the polymerase chain reaction. *Am. J. Hum. Genet.* **44**:388-339.

Weir, B.S. (1996). Genetic Data Analysis II. 2nd edition. Sinauer Associates Inc. Sunderland.

Westgate, M.E. (1997). Physiology of flowering in maize: identifying avenues to improve kernel set during drought. In: G.O. Edmeades, M. Banziger, H.R. Mickelson, and C.B. Pefia Valdivia (eds.) *Developing Drought and*

*Low N- Tolerant Maize. Proceedings* of a *Symposium, March* 25-29, 1996, CIMMYT, Mexico, pp. 136-141.

Westgate, M.E. and Boyer, J.S. (1985). Carbohydrate reserves and reproductive development at low leaf water potentials in maize. *Crop Sci.* **25**:762-769.

Westgate, M.E, and Boyer, J.S. (1986). Reproduction at low silk and pollen water potentials in maize. *Crop Sci.* **26**:951-956.

Westgate, M.E and Bassetti, B. (1990). Heat and drought stress in corn: what really happens to the corn plant at pollination? In: D. Wilkinson (ed). Proc. 45th Annual Corn and Sorghum Research Conference, ASTA, Washington.pp12-28.

Westgate, M.E., and Bassetti, P. (1991). Heat and drought stress in com: what really happens to the corn plant at pollination? In: D. Wilkinson (ed.), Proc. 45th Annual Corn and Sorghum Res. Conf. ASTA, Washington D.C.

Yadav, R., Courtois, B., Huang, N. and McLaren, G. (1997). Mapping genes controlling root morphology and root distribution in a double haploid population of rice. *Theor. Appl. Genet.* **94:** 619-632.

Young, N.D. (1999). A cautiously optimistic vision for marker-assisted breeding. *Mol. Breed.* **5**: 505-510.

Yu, L.X. and Setter, T.L. (2002). Comparative transcriptional profiling of developing maize kernels in response to water deficit. Proc. Annual Meeting of the American Society of Plant Biologists, August 3-7, 2002. Denver, CO.

Zeng, Z.B. (1994) Precision mapping of quantitative trait loci. *Genetics* **136**:1457-1468.

Zhang, J., U. Schurr, and W.J. Davies. 1987. Control of stomatal behaviour by abscisic acid which apparently originates in the roots. *J. Exp. Bot.* **38**: 1174-1181.

Zhang, J., Zheng, H.G., Aarti, A., Pantuwan, G., Nguyen, T.T, Tripathy, I.N., Sarial, A.K., Robin, S., Babu, R.C., Nguyen, B.D., Sarkarung, S., Blum, A., Nguyen, H.T. (2001). Locating genomic regions associated with components of drought resistance in rice: comparative mapping within and across species. *Theor. Appl. Genet.* **103**:19-29.

Zhang, L., Nguyen, H.T., Blum, A. (1999).Genetic analysis of osmotic adjustment in crop plants. *J. Exp. Bot.* **50**:291-302.

Zhao, X. and Kochert, G. (1993). Phylogenetic distribution and genetic mapping of a (GCG)n microsatellite from rice *(Oryza saliva* L.). *Plant Mol. Biol.* **21**: 607-614.

Zinselmeier, C., Habben, I.E., Westgate, M.E., Boyer, I.S. (2000). Carbohydrate metabolism in setting and aborting maize ovaries. In: Westgate, M.E. and Boote, K.J. (eds.) Physiology and Modeling Kernel Set in Maize. CSSA Special Publication No. 29. CSSA, Madison, WI, pp. 113-120.

Zinselmeier, C., Jeong, B.R. and Boyer, I.S. (1999). Starch and the control of kernel number in maize at low water potentials. *Plant Physiol.* **121**: 25-35.

Zinselmeier, C., Schussler, J.R., Westgate, M.E. and Jones, R.J. (1995). Low water potential disrupts carbohydrate metabolism in maize ovaries. *Plant Physiol.* **107**:385-391.

Zinselmeier, C., Sun, Y., Helentjaris, B.M., Yang, S., Smith, H, and Habben, J. (2002). The use of gene expression profiling to dissect the stress sensitivity of reproductive development in maize. *Field Crop Res.* **75**:111-121.