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# CHANGES IN DROUGHT TOLERANCE IN MAIZE ASSOCIATED WITH FIFTY YEARS OF BREEDING FOR YIELD IN THE U.S. CORN BELT<sup>1</sup>

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**ABSTRACT** - Understanding the changes underlying past breeding progress may help to focus research efforts and accelerate future genetic gains. The major abiotic stress affecting maize production on a worldwide basis is drought. We addressed the improvements in drought tolerance over a 50-year period of hybrid breeding by evaluating, under targeted stress conditions, a set of 18 Pioneer-brand hybrids that had been released during the 1953-2001 period. Stress treatments were designed as overlapping windows of water deficit covering the pre-flowering to late grain filling development stages. Data were collected on grain yield, yield components and anthesis-silking interval (ASI), and were analyzed using a linear mixed model approach. Genetic gain was measured as the slope of the regression of the trait on the year of hybrid release. Significant, positive genetic gains of varying magnitude were observed for grain yield in all windows of stress evaluated. The largest genetic gains for grain yield were observed under conditions of full irrigation and severe flowering stress. ASI and barrenness, especially under stress at flowering, were significantly reduced by selection. Though flowering remains the most susceptible stage to drought in maize, selection has reduced its negative effects, and susceptibility during early grain filling is now of similar importance in many modern hybrids. Yield under drought at flowering has more than kept pace with the increase in yield potential because of the emphasis breeders have placed on improved floral synchrony.

**KEYWORDS:** Maize; Drought tolerance; Barrenness; ERA hybrids; Anthesis silking interval; Kernels per ear; Secondary traits.

## INTRODUCTION

An important goal of maize breeders has been to enhance the stability of performance of maize when exposed to stresses. These stresses may be predictable, such as those arising from higher planting densities, or they may occur randomly in timing and severity, as in the case of drought experienced during the growing season. Where stresses occur randomly and with only moderate frequency, it is important that tolerance of those stresses does not demand a yield penalty in the absence of the stress. Breeders have traditionally relied on randomly occurring stress conditions during multi-location testing to reveal susceptible and tolerant recombinants, though increasingly the focus is moving to the use of managed drought facilities where stress incidence, intensity and severity can be purposely manipulated (BARKER *et al.*, 2005).

Maize is considered more susceptible than most other cereals to drought stresses at flowering, when yield losses can be severe through barrenness or reductions in kernels per ear (BOLAÑOS and EDMÉADES, 1996). Susceptibility of maize yield to stresses at flowering has been documented in early Corn Belt germplasm (ROBINS and DOMINGO, 1953; CLAASSEN and SHAW, 1970; SHAW, 1977). These studies showed that the sensitive period extended from around 1 wk before to 2 wk after 50% silking. Yield losses per day of comparable stress, before and after flowering, were around 45 and 60%, respectively, of the peak loss at silking itself (SHAW, 1977).

Studies of more recent hybrids suggest that this window of susceptibility may have moved towards early grain filling. GRANT *et al.* (1989) grew the F1 hybrid B73/Mo17 in 44 L pots in a temperature-regulated greenhouse, and subjected plants to water stress periods in seven separate intervals during the period -3 to 37 days after silking. They reported

<sup>1</sup> Contribution to a Maydica volume honoring contributions of Dr. Donald Duvick.

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that although yields were most severely reduced (70%) by stress coinciding with silking, yields were reduced by 40-54% from stresses occurring in the period 10 to 31 days after mid-silk, and kernel number was reduced below control for stresses occurring up to 22 days after silking. A second study conducted by NE SMITH and RITCHIE (1992) imposed a moderate grain filling water stress on Pioneer hybrids P3540 and P3475. They observed that kernel numbers per plant were reduced 8-20% ( $P=0.05$ ) in P3475 when the plants were stressed in the period 18 to 31 days after silking, while weight per kernel declined by a significant 21-25%. Reductions were less in P3540. Other recent studies have shown considerable genetic variation in the response of commercial hybrids to drought stress imposed during reproductive growth (O'NEILL *et al.*, 2004), and in one study, a well-known drought tolerant hybrid, P3223, displayed no additional susceptibility to stress imposed at flowering vs. 3 wks later (BRUCE *et al.*, 2002). Therefore, it appears that kernel numbers per plant can also be affected significantly by stresses during the first half of grain filling, though less so than when stress coincides with flowering, and that these responses vary considerably among hybrids.

A characteristic of maize under drought stress at flowering is an increase in the anthesis-silking interval (ASI) (DOW *et al.*, 1984; EDMEADES *et al.*, 2000), itself an indirect indicator of ear growth rate. Relationships between ASI, partitioning of assimilates to the developing ear, and kernel number have been affected by selection. In tropical populations, recurrent selection for increased grain yield, reduced barrenness and reduced ASI under drought stress at flowering has resulted in significant improvements in all three traits (EDMEADES *et al.*, 2000). In summarizing several experiments conducted on hybrids released in Canada over a 30-year period from the mid 1960s, TOLLENAAR and WU (1999) concluded that many of these changes were due to increased stress tolerance, specifically in the ability to form an ear and fill it on a plant that resisted lodging when sown at high plant densities and grown under 150-250 kg N ha<sup>-1</sup>. CASTLEBERRY *et al.* (1984), in evaluating changes occurring over 50 years in DeKalb's breeding program, reported that gains in grain yield under drought or low fertility conditions were positive but less than under optimal conditions. DUVICK (1984) and coworkers (DUVICK *et al.*, 2004) have documented phenotypic and genetic changes in a unique set of leading Pioneer brand hybrids re-

leased from 1930 through 2001. Because these hybrids were developed from a single large breeding program, they represent a form of reciprocal recurrent selection, and offer a unique insight into changes that have occurred over time. These studies also concur with Tollenaar's conclusion that many changes over the course of breeding relate to increased stress tolerance. However, none of these studies have quantified genetic improvement under a drought stress that occurs at a specific development stage during reproductive growth.

The objectives of the present study were to address three questions:

- Has grain yield under drought stress increased with selection, and if so, has it improved at the same rate as under well-watered conditions?
- Has selection changed the stage-specific sensitivity of the flowering and grain filling periods to drought stress?
- What was the underlying mechanism of these changes?

A subset of Duvick's ERA hybrid set (DUVICK *et al.*, 2004) was chosen for a more detailed field study of these issues under managed drought stress. Data from the initial year of this study showed much reduced genetic gain for grain yield from stresses imposed during mid- to late-grain filling vs. optimal conditions (CAMPOS *et al.*, 2004). This paper reports on the combined data from two years of field evaluations.

## MATERIALS AND METHODS

### Hybrids

A subset of 18 hybrids was selected from the original 55 listed by DUVICK *et al.* (2004). Three hybrids per decade were chosen to represent successful commercial hybrids released by Pioneer Hi-Bred International, Inc. from the 1950s to the 1990s. To these were added an additional set of three hybrids released from 1999 through 2001 (Table 1). By eliminating entries developed from 1920 through 1940, we reduced hybrids under study to those that would stand well at a single intermediate density (88,900 plants ha<sup>-1</sup>). No lodging was observed in these trials in either of the years under study. Four of the hybrids were double crosses, and three were three-way crosses. The 11 hybrids released from 1975 onwards were all single crosses. All were adapted to the central U.S. Corn Belt. Seed of each hybrid was treated with Apron Plus ® fungicide prior to sowing according to manufacturer's instructions.

### Evaluation environment and cultural practices

Experiments were carried out in Chile in a silt loam soil with plant-available water in the upper 1.5 m of approximately 150 mm m<sup>-1</sup> and soil organic matter content of 2.0%. Weather data recorded by weather instruments (Table 2) showed that during

TABLE 1 - Hybrids selected for evaluation of drought tolerance in 2002-2004 from original ERA hybrid set. Hybrid codes are based upon their decade of release (for example, 50 and 00 stand for decades 1950-1959 and 2000-2009 respectively) and within each decade they were sequentially numbered based upon year of release.

Entry number	Hybrid code	Year of release	Type of hybrid <sup>a</sup>	CRM <sup>b</sup>
1	50-1	1953	DX	111
2	50-2	1954	DX	111
3	50-3	1958	DX	108
4	60-1	1961	DX	108
5	60-2	1963	SX	115
6	60-3	1967	3W	111
7	70-1	1971	3W	108
8	70-2	1975	3W	108
9	70-3	1975	SX	107
10	80-1	1984	SX	108
11	80-2	1988	SX	111
12	80-3	1989	SX	111
13	90-1	1991	SX	110
14	90-2	1995	SX	113
15	90-3	1997	SX	113
16	90-4	1999	SX	114
17	00-1	2001	SX	113
18	00-2	2000	SX	109

<sup>a</sup> DX = double cross hybrid; 3W = three way hybrid; SX = single cross hybrid

<sup>b</sup> Corn Relative Maturity rating.

the growing season (November to April), virtually no rainfall was received, and that daily thermal time accumulation ( $T_{opt}=30^{\circ}\text{C}$ ;  $T_{min}=10^{\circ}\text{C}$ ) averaged  $9.6^{\circ}\text{Cd}$  ( $17.3^{\circ}\text{Fd}$ ) during the main part of the growing season. Data collected by weather instruments was used to calculate ET. The temperature sensor on the weather data recorder consistently reported temperatures  $1.1^{\circ}\text{C}$  below actual in the 2002/3 season, and this was not detected and corrected until the season was completed. Female and male flowering data collected during the second year were accordingly adjusted. As a consequence, the periods of water withdrawal occurred later in crop development in the second season than the first, and the terminal stress period was only  $203^{\circ}\text{Cd}$  vs.  $\sim 300^{\circ}\text{Cd}$  for other treatments (Table 3). However, from the relative size of components of variance these modest differences in the timing of the stress windows did not significantly affect results obtained. Irrigation was supplied at a rate equal to the calculated crop water use rate (DOORENBOS and KASSAM, 1979) plus 10%, except for the treatment periods when irrigation was completely withdrawn.

The experiments were planted on 7 Nov in 2001, and on 10 Oct in 2002, and thinned to  $85,000$  plants  $\text{ha}^{-1}$ . In 2001, fertilizer was applied at the rate of  $150:92:60$  kg of  $\text{N:P}_2\text{O}_5:\text{K}_2\text{O}$   $\text{ha}^{-1}$  as granular fertilizer at planting, followed by a total of  $180$  kg N  $\text{ha}^{-1}$  applied as urea injected into the drip irrigation system on five

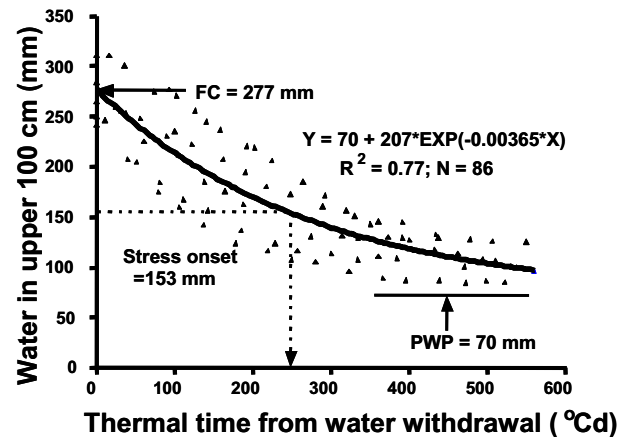


FIGURE 1 - Relationship between soil moisture content and thermal time from water withdrawal, determined from a single access tube placed in each of five water withdrawal regimes. FC = field capacity; PWP = permanent wilting point.

separate occasions during the preflowering period. In 2003, in order to offset possible deficiencies, the fertilizer rate was increased to  $175:108:71$  kg of  $\text{N:P}_2\text{O}_5:\text{K}_2\text{O}$   $\text{ha}^{-1}$  as granular fertilizer at planting, followed by  $247:100:0$  kg of  $\text{N:P}_2\text{O}_5:\text{K}_2\text{O}$   $\text{ha}^{-1}$  applied as a combination of granular fertilizer side dressing and as urea injected into the irrigation system. Weeds and insects were controlled by chemical pesticides as needed, and the incidence of diseases was negligible.

#### Water treatments

These consisted of a well-watered control (FI) and five treatments where drought was imposed at specific growth stages. Water was withdrawn over periods of  $500-550^{\circ}\text{Cd}$  (50 d) in five distinct but overlapping periods  $100-196^{\circ}\text{Cd}$  apart, starting  $256-291^{\circ}\text{Cd}$  after planting. The treatments are described in Table 3. In order to assess when plant stress actually occurred, an access tube 1 m in length was installed in one representative plot in each of the six water treatments in the 2002/3 season. A soil capacitance probe (Diviner®, www.sentek.com.au) was used for assessing volumetric soil water content at 10 cm depth intervals every 2-4 days throughout the season. These data were summed across the profile to provide total water content in the upper 1 m of soil. Using the date when irrigation ceased as zero in each of the five treatments, the total water content (mm) in the upper 1 m of soil was plotted against cumulative thermal time (TT,  $^{\circ}\text{Cd}$ ) during soil dry down. The end point was considered the re-watering date, or physiological maturity. Since there was only one tube installed per treatment and observations from that tube were restricted to a radius of 30 cm around the tube, it was considered appropriate to treat these as replicates, and a single exponential regression was fitted to all the data (Fig. 1). Soil water content at field capacity was predicted to be 277 mm, and the fitted regression predicted an asymptote of 70 mm water content, taken here to be the value of the permanent wilting point. Plant available water was the difference between these (207 mm), and stress was predicted to start when 60% of plant available soil water had been extracted (DOORENBOS and KASSAM, 1979; GRANT *et al.*, 1989) at  $250^{\circ}\text{Cd}$  after irrigation ceased. This corresponds to

TABLE 2 - Average monthly weather data for 2001/2002 and 2002/2003 seasons. *Tmin* and *Tmax* are the daily minimum and maximum temperatures; *ET<sub>0</sub>* is the potential evapotranspiration; *RH* is relative humidity; and radiation is total radiation received per day.

Month 2001/2002	Tmax °C	Tmin °C	Thermal time °Cd	Total rain mm	ET <sub>0</sub> mm d <sup>-1</sup>	RH %	Radiation MJ m <sup>-2</sup> d <sup>-1</sup>
Nov	26.5	8.4	8.4	0.0	5.4	58	34.2
Dec	29.2	10.9	9.9	0.0	5.6	68	33.4
Jan	29.1	10.0	9.5	0.1	5.6	67	33.8
Feb	27.9	9.5	9.1	3.2	4.8	74	29.5
Mar	27.9	8.9	9.0	8.3	3.9	67	23.3
Apr	24.0	7.2	7.0	0.2	2.8	78	19.5
Mean/total	27.4	9.2	8.8	11.8	4.7	68.7	29.0
<b>2002/2003</b>							
Oct	23.9	8.7	7.1	0.0	3.5	75	23.8
Nov	26.6	9.1	8.3	0.8	4.8	72	30.9
Dec	28.5	10.8	9.5	2.3	5.3	72	32.5
Jan	30.8	12.1	10.8	5.0	5.7	68	33.8
Feb	29.9	10.1	9.9	0.0	5.4	66	31.8
Mar	27.8	10.2	9.2	0.2	3.6	79	23.0
Apr	24.2	7.0	7.0	0.5	2.3	74	15.4
Mean/total	27.4	9.7	8.8	8.8	4.4	72.3	27.3

TABLE 3 - Thermal times from planting for the cutoff of irrigation, onset of plant stress, time of rewatering and duration of stress period in the different treatments, 2001-2003. Mean anthesis date occurred at 696°Cd, and physiological maturity under optimal conditions at about 1260°Cd.

Code	Stress	2001/2002				2002/2003			
		Cut	Stress onset	Rewater	Duration	Cut	Stress onset	Rewater	Duration
		°Cd				°Cd			
FI	None	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
S1	Flowering	256	476	774	298	291	511	849	338
S2	Early fill	354	574	863	289	392	612	941	329
S3	Mid fill	448	668	954	286	503	723	1025	302
S4	Late fill	578	798	1080	282	641	861	1161	300
S5	Terminal	764	984	Harvest	276	837	1057	Harvest	203

26 d at an average daily accumulation of 9.6°Cd (Table 2). The onset of wilting in the crop was first observed about 5 d before this date. Nonetheless, the interval from this date until irrigation was reapplied was considered to be the period of stress in each of the two years (Table 3). During these intervals stress gradually increased in intensity with time until re-watering occurred. Rain-fall represented 2-3 days of potential evaporation throughout each season (Table 2), and was not considered to have any significant effect on plant performance.

### Measurements

Dates when 50% of plants had reached anthesis (AD) and silking (SD) were recorded. The Anthesis-Silking Interval (referred to as ASI hereafter) was determined for each plot. Final harvest of each treatment occurred between 28 March and 9 April, 2002, and between 6 March and 1 April, 2003, at a time when all leaves had senesced. At final harvest, the plants in the two central rows were counted and hand harvested. The number of ears bearing at least one kernel per ear was determined, and

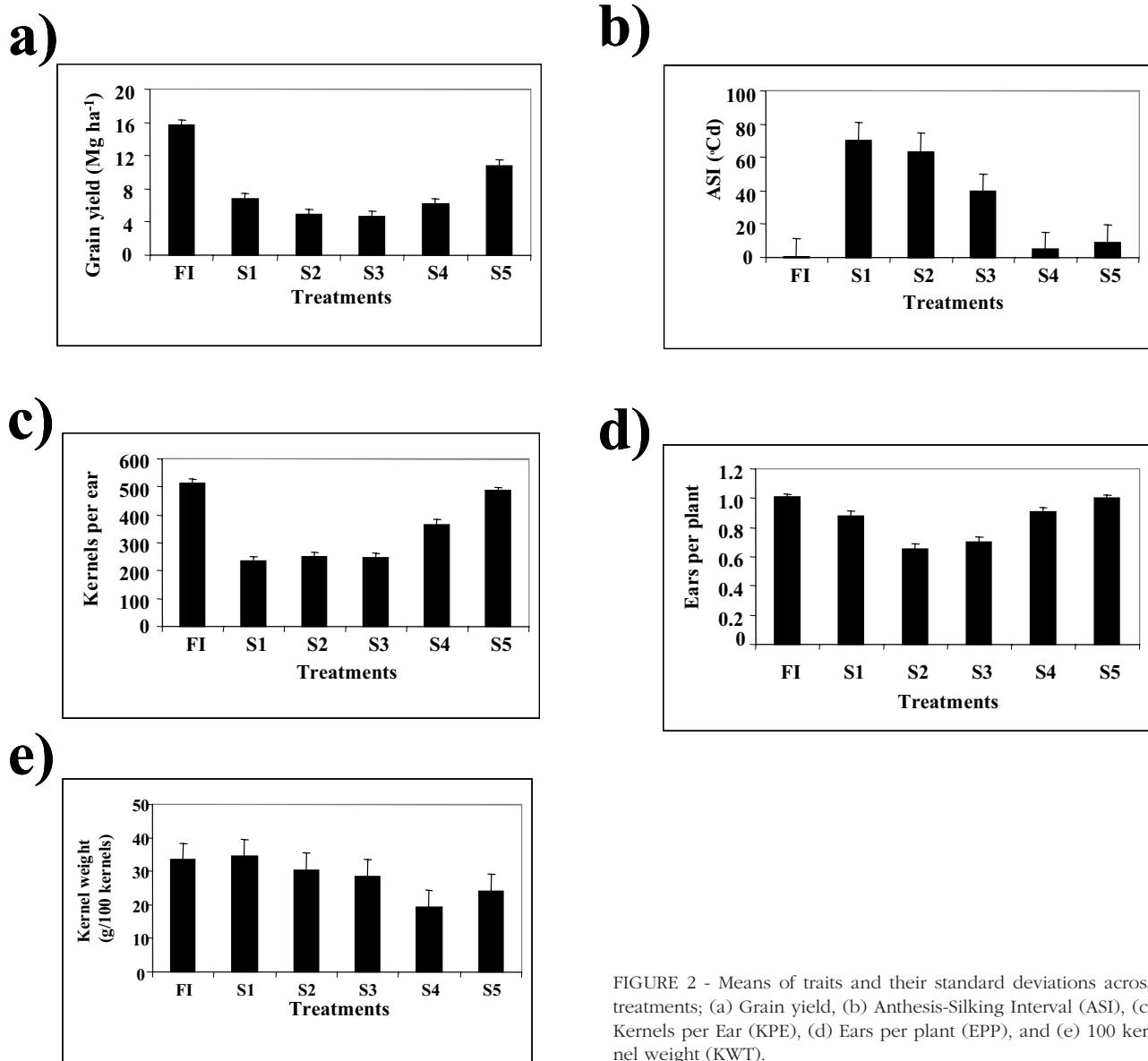


FIGURE 2 - Means of traits and their standard deviations across treatments; (a) Grain yield, (b) Anthesis-Silking Interval (ASI), (c) Kernels per Ear (KPE), (d) Ears per plant (EPP), and (e) 100 kernel weight (KWT).

ears per plant (referred to as EPP hereafter) calculated. Ears were dried at 40°C in a grain dryer, shelled, grain was weighed, and grain moisture determined by a capacitance meter. A random sample of 100 kernels was selected from the shelled bulk and immediately weighed. The weight of 100 kernels (KWT) was adjusted to 15% moisture content, and number of kernels per ear (KPE) was estimated from the other yield components.

#### Statistical analysis

**Plot and experimental design.** Plot size was four rows 4.5 m in length and 0.75 m apart. Alley width was maintained at 0.5 m. Each water treatment was considered an environment, and designed as an independent, resolvable, alpha lattice design with three replications (PATTERSON and WILLIAMS, 1976). In such an experimental design, each complete replicate is divided into sets of

smaller blocks to adjust for environmental heterogeneity among these blocks (BARRETO *et al.*, 1997; BARKER *et al.*, 2005). The order across the field of the water treatments was randomized between years.

Analyses of variance were carried out using the ASREML software (GILMOUR *et al.*, 2002). ASREML fits linear mixed models using Residual Maximum Likelihood (REML). Throughout the paper, interactions between sources of variation are expressed as Stress.Hybrid for the interaction between stress and hybrid, for example. Following the theoretical arguments and empirical recommendations of SMITH *et al.* (2002a,b) hybrids, and the environmental interactions with hybrids, were treated as random terms in the mixed model. Consequently, hybrid trait performance within each water treatment was evaluated as Best Linear Unbiased Predictors (BLUPs). Following COOPER *et al.* (1999), a linear



mixed model was used with Year, Stress and Stress.Year treated as fixed effects and Rep.Stress.Year, Hybrid, Year.Hybrid, Stress.Hybrid and Stress.Year.Hybrid considered as random effects. The phenotypic observation  $x_{ijkl}$  on hybrid  $i$ , in replicate  $l$  of stress  $j$  and year  $k$  was modeled as:

$$x_{ijkl} = \mu + y_k + s_j + (sy)_{jk} + (r/sy)_{ljk} + g_i + (gy)_{ik} + (gs)_{ij} + (gsy)_{ijk} + \epsilon_{ijkl} \quad (1)$$

where  $\mu$  is the grand mean;  $y_k$  the fixed effect of year  $k$ ;  $s_j$  the fixed effect of stress  $j$ ;  $(sy)_{jk}$  the fixed effect of the interaction between stress  $j$  and year  $k$ ;  $(r/sy)_{ljk}$  the random effect of replicate  $l$  within the stress  $j$  and year  $k$  and is  $\sim \text{NID}(0, \sigma^2_p)$ ;  $g_i$  the random hybrid (genotypic) effect of hybrid  $i$  and is  $\sim \text{NID}(0, \sigma^2_g)$ ;  $(gy)_{ik}$  the random interaction effect between hybrid  $i$  and year  $k$  and is  $\sim \text{NID}(0, \sigma^2_{gy})$ ;  $(gs)_{ij}$  the random interaction effect between hybrid  $i$  and stress  $j$  and is  $\sim \text{NID}(0, \sigma^2_{gs})$ ;  $(gsy)_{ijk}$  the random interaction effect between hybrid  $i$ , stress  $j$  and year  $k$  and is  $\sim \text{NID}(0, \sigma^2_{gsy})$ ; and  $\epsilon_{ijkl}$  the random residual effect for hybrid  $i$  in replicate  $l$  of stress-year combination  $j-k$  (experimental error) and is  $\sim \text{NID}(0, \sigma^2_e)$ . The experimental error was further investigated to adjust for spatial field trends within treatments following the methodology given by SMITH *et al.* (2002a,b) and following the procedures applied to maize experiments by BARKER *et al.* (2005). A slightly different version of the mixed model given in equation (1) was used to analyze EPP.

Evidence for a significant component of variance was declared when the ratio of the variance component over its standard error (Comp/SE ratio) was greater than 2. Likelihood Ratio Tests are usually found to be significant when the Comp/SE ratio is greater than 1.5 (CADENA *et al.*, 2000).

Applying equation (1), ASREML was used to calculate components of variance, the approximate standard errors for random terms included in the model and BLUPs for hybrid trait performance across water treatments and for hybrid trait performance within water treatments. Genetic gain for individual traits was estimated by regression of predicted values from the ASREML procedure on year of release of the hybrids. Phenotypic correlations among traits averaged over years were computed within and across water treatments.

### **Biplot construction**

GGE biplots were constructed following the procedures described by LÖFFLER *et al.* (2005) for hybrid-by-stress environment combinations using BLUPs as predictors of hybrid mean performance in different stress environments.

## **RESULTS AND DISCUSSION**

### **Stress treatments**

The stress periods imposed were approximately 280°Cd (or 29 d) in duration, and overlapped by approximately 180°Cd (Table 3). Although the original intent was to provide treatments with distinctly different windows of stress, the relatively slow development of stress in this environment did not allow for this. Thus, S1 and S2 clearly increased ASI, but so also did S3, even though it was causing only mild leaf rolling at silking. Since stress intensity in-

creased with time, the major effect of S3 should have occurred in the first 50% of the grain filling period, yet kernel and ear numbers were still reduced by this treatment. The possibility clearly exists, however, that the sensitivity of kernel numbers per plant extends further into the grain filling period, as suggested by other data from more recent germplasm (GRANT *et al.*, 1989; NESMITH and RITCHIE, 1992). However, since ASI and kernel numbers were all affected by S1, S2 and S3, these have been grouped as flowering stresses in much of the subsequent study. The stresses S4 and S5 were clearly grain filling stresses, and S5, especially in 2002/3, was of reduced duration compared with the other treatments. Nonetheless, S4 and S5 can reasonably be combined as late grain filling or terminal drought stresses in subsequent discussion.

### **Grain yield**

The average grain yield across environments was 8.1 Mg ha<sup>-1</sup>. Grain yields ranged from 4.7 Mg ha<sup>-1</sup> under a mild flowering and major mid grain filling stress, to 15.7 Mg ha<sup>-1</sup> under well watered conditions (Fig. 2a). The diverse drought stresses imposed were effective in decreasing grain yield to different degrees, and no significant effects were attributed to the different types (three way, double- or single cross) of hybrid cultivars evaluated. Averaged yields of the five stress treatments, namely S1 through S5 were 43%, 31%, 29%, 39% and 69% of the irrigated control, respectively. Such levels of stress fall within the range of stress levels reported in other studies aimed at improving drought tolerance in maize (BOLAÑOS and EDMEADES, 1993; BETRÁN *et al.*, 2003; ZAIDI *et al.*, 2004). Even though flowering remains a growth stage sensitive to drought in maize, treatments such as S3, with a moderate stress at flowering and severe during the first stages of the grain filling period, exerted a significant, negative impact upon grain yield. Furthermore, the late grain filling and terminal stress treatments (S4-S5) resulted in lesser yield reductions.

Using optimum plant densities gives a more realistic measurement of genetic gain, as compared with measurements at any single plant density, because each hybrid is evaluated at a reasonably close approximation of the plant density at which it was bred and tested (DUVICK *et al.*, 2004). Because of the size of this experiment, however, a single intermediate density was selected. Older hybrids were planted at a density exceeding that used during their development, and might have been expected to lodge.

TABLE 4 - Estimates of components of variance ( $\pm$  standard errors) and LSD values ( $P < 0.05$ ) for the analysis of variance of: (a) grain yield ( $\text{Mg ha}^{-1}$ ), ASI ( $^{\circ}\text{Cd}$ ), KPE and weight of 100 kernels (KWT), and (b) Ears per plant (EPP), measured on 18 ERA hybrids grown under six water regimes, 2001-2003.

(a)

Source	Yield		ASI		KPE		KWT	
	Component $\pm$ SE	Comp/SE	Component $\pm$ SE	Comp/SE	Component $\pm$ SE	Comp/SE	Component $\pm$ SE	Comp/SE
Rep.Stress.Year	0.55 $\pm$ 0.22	2.5	53.9 $\pm$ 24.5	2.2	391.374 $\pm$ 170.2	2.3	0.21 $\pm$ 0.11	1.8
Hybrid	3.52 $\pm$ 1.34	2.6	1497.6 $\pm$ 534.9	2.8	716.5 $\pm$ 325.7	2.2	6.3 $\pm$ 2.4	2.6
Year.Hybrid	0.08 $\pm$ 0.06	1.3	22.4 $\pm$ 13.2	1.7	74.7 $\pm$ 70.5	1.1	0.18 $\pm$ 0.21	0.9
Stress.Hybrid	1.69 $\pm$ 0.31	5.4	217.5 $\pm$ 46.3	4.7	750.4 $\pm$ 184.4	4.1	3.3 $\pm$ 0.72	4.6
Stress.Year.Hybrid	0.28 $\pm$ 0.1	2.9	18.2 $\pm$ 14	1.3	141.8 $\pm$ 118.2	1.2	1.4 $\pm$ 0.38	3.7
Residual	1.69		656.3		2395.3		3.60	
LSD ( $P < 0.05$ )	1.4		15.1		34.8		1.6	

(b)

Source	EPP	
	Component $\pm$ SE	Comp/SE
Block.Rep.Stress.Year	4.9 $\pm$ 2.1	2.35
Hybrid	0.44 $\pm$ 0.24	1.85
Year.Hybrid	4.48 $\pm$ 0.91	4.9
Stress.Hybrid	0.04 $\pm$ 0.13	0.34
Residual	3.19 $\pm$ 0.74	
LSD ( $P < 0.05$ )	0.07	

The average number of lodged plants per plot for the nine oldest hybrids under treatments (S4-S5) and FI control were 2.2 and 1.1%, respectively, whereas in all other treatments lodging was essentially zero. Differential lodging, therefore, was not a complicating factor in this assessment of hybrids derived from different eras of maize breeding.

Significant components of variance for grain yield were identified for the sources Hybrid, Stress.Hybrid and Stress.Year.Hybrid (Table 4). The dominant source of variance components was Hybrid followed by Stress.Hybrid. Furthermore, Year.Hybrid and Stress.Hybrid.Year were small and the Year.Hybrid component was not significant. Because of this limited interaction with years, all discussion is focused on means across the two years. This suggests that even though stress treatments exerted a significant effect, from a biological perspective the most relevant components were Hybrid and Stress.Hybrid, reflecting the differential response of hybrids bred in different eras to precisely timed drought stresses. The residual component of variance was approximately half the genotypic (Hybrid) component of variance for grain yield, suggesting

that these trials are suitable for studying differences among hybrids.

Large differences in genetic gains measured under specific water regimes were observed (Fig. 3a). The largest gain was under FI ( $0.196 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) followed by  $0.120 \text{ Mg ha}^{-1} \text{ yr}^{-1}$  for treatment S1, coinciding with flowering. They reflect the effectiveness of Pioneer's breeding programs to sustain large genetic gains under unstressed conditions, and when stress coincides with flowering.

Although these gains are generally larger than those previously reported for maize (for instance, a  $+77 \text{ kg ha}^{-1} \text{ yr}^{-1}$  genetic gain for central Iowa) (Duvick *et al.*, 2004), it is important to note that the latter estimate likely is confounded by the mixture of environmental conditions corn is grown under in central Iowa over the years, whereas the  $+0.196 \text{ Mg ha}^{-1} \text{ year}^{-1}$  rate reported here was achieved under optimal growing conditions in a long, cool, radiation-rich environment in absence of pests and under high levels of applied N. Genetic gains fell off under stress treatments S4 and S5 that directly affected grain filling, reaching  $0.050$  and  $0.070 \text{ Mg ha}^{-1} \text{ year}^{-1}$ , respectively. Therefore, in terms of grain



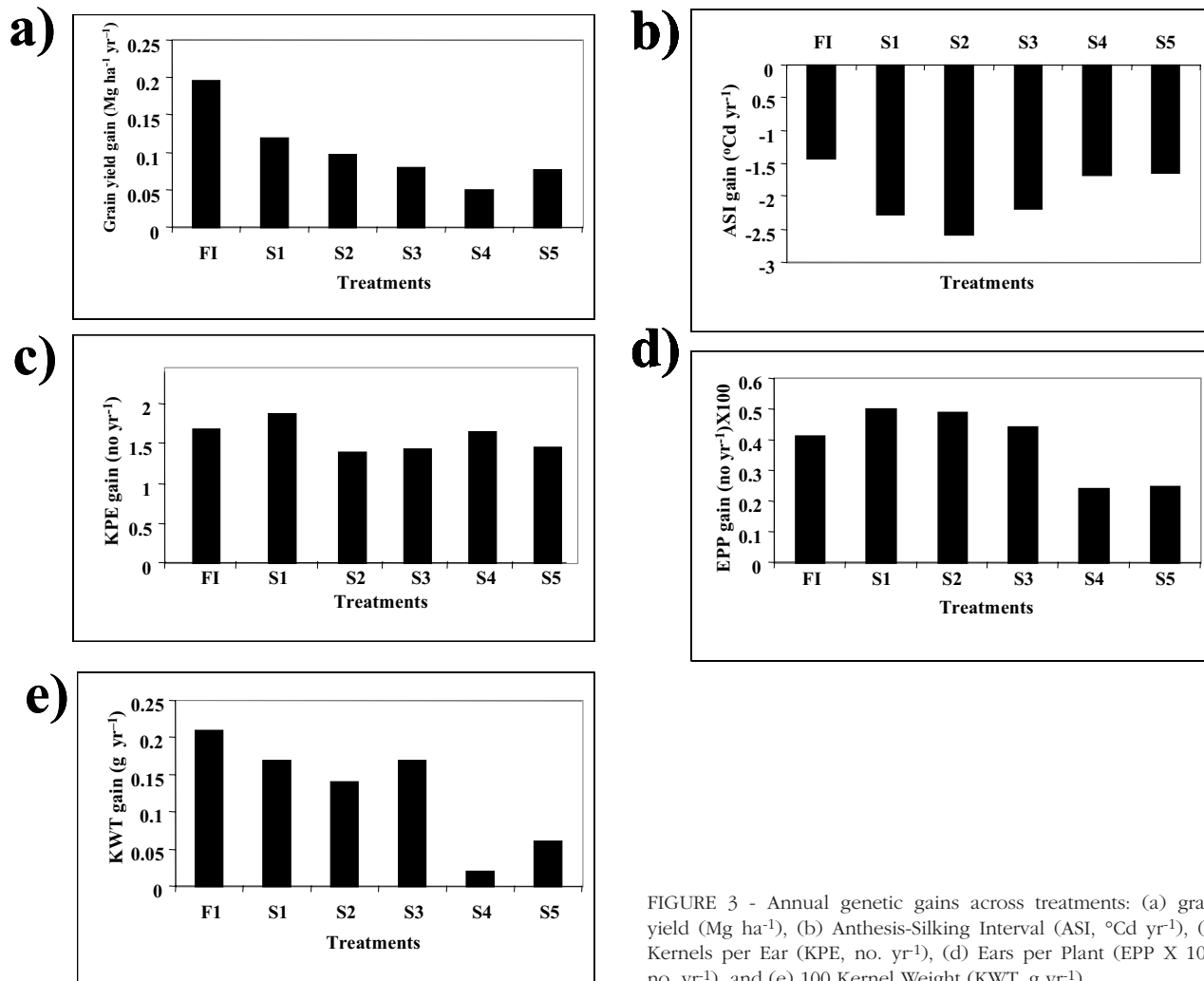


FIGURE 3 - Annual genetic gains across treatments: (a) grain yield (Mg ha<sup>-1</sup>), (b) Anthesis-Silking Interval (ASI, °Cd yr<sup>-1</sup>), (c) Kernels per Ear (KPE, no. yr<sup>-1</sup>), (d) Ears per Plant (EPP X 100, no. yr<sup>-1</sup>), and (e) 100 Kernel Weight (KWT, g yr<sup>-1</sup>).

yield improvements a consistent trend was observed, with absolute genetic gains in the decreasing order FI>S1>S2>S3>S5>S4. This trend suggests that genetic gains can be achieved not only during normal growth conditions but also during stressful seasons, although they are of lesser magnitude. Genetic variance and heritability of grain yield, and thus genetic progress during the development of maize inbreds, are generally lower under stress than under optimal conditions (BOLAÑOS and EDMANDES, 1996). Nevertheless, although the results reflect the effectiveness of traditional multilocation testing to achieve genetic progress, improvement under grain filling stresses could be accelerated by using managed stress environments to expose genetic variability at that developmental stage. The gains under different stresses reported here may provide a more

realistic assessment of the genetic progress achieved in the central Midwest of the United States, where the occurrence of drought stress is unpredictable and selection for drought stress is hampered by natural climatic variation. Nevertheless, they are consistent with trends observed by CASTLEBERRY *et al.* (1984) and DUVICK *et al.* (2004) towards greater rates of improvement in high vs. low yielding environments.

The low phenotypic correlations between grain yield and anthesis date across stresses encompassing flowering (S1 through S3) (Table 5) suggests genetic tolerance to flowering stress was being fairly assessed, unencumbered by any effect that flowering date may have had in escaping intense stress at flowering. Grain yield under stress expressed as a percentage of yield under optimal conditions is a

TABLE 5 - Pearson's phenotypic correlation coefficients calculated among grain yield, yield components, ASI and thermal time to anthesis for 18 ERA hybrids grown under: (a) all water regimes, (b) no stress (FI), or regimes that imposed stress at (c) flowering (S1, S2 and S3) or (d) only during grain filling (S4, S5).

(a) All water regimes (106 df,  $r < 0.18$ ,  $P < 0.05$ ;  $r < 0.24$ ,  $P < 0.01$ )

	Yield	KPE	EPP	ASI	KWT
KPE	0.85				
EPP	0.81	0.76			
ASI	-0.63	-0.65	-0.66		
KWT	0.35	-0.14	0.05	0.05	
Anthesis	-0.05	-0.26	-0.08	-0.1	0.42

(b) Treatment FI (16 df,  $r < 0.46$ ,  $P < 0.05$ ;  $r < 0.59$ ,  $P < 0.01$ )

	Yield	KPE	EPP	ASI	KWT
KPE	0.67				
EPP	0.86	0.47			
ASI	-0.86	-0.59	-0.83		
KWT	0.92	0.4	0.73	-0.75	
Anthesis	0.45	-0.02	0.51	-0.52	0.55

(c) Treatments S1, S2, S3 (52 df,  $r < 0.26$ ,  $P < 0.05$ ;  $r < 0.34$ ,  $P < 0.01$ )

	Yield	KPE	EPP	ASI	KWT
KPE	0.74				
EPP	0.84	0.38			
ASI	-0.65	-0.73	-0.44		
KWT	0.66	0.17	0.69	-0.31	
Anthesis	0.36	0.25	0.22	-0.32	0.54

(d) Treatments S4, S5 (34 df,  $r < 0.32$ ,  $P < 0.05$ ;  $r < 0.42$ ,  $P < 0.01$ )

	Yield	KPE	EPP	ASI	KWT
KPE	0.95				
EPP	0.85	0.81			
ASI	-0.28	-0.2	-0.51		
KWT	0.88	0.75	0.64	-0.12	
Anthesis	-0.01	-0.06	0.15	-0.41	0.07

valuable measure of stress tolerance, even though ultimately, absolute yield under stress is what counts to the grower. For stresses imposed from flowering until 260°Cd later (or the first 45% of the grain filling period), this ratio has increased steadily with time ( $0.21\% \text{ yr}^{-1}$ ;  $R^2 = 0.21$  ( $P < 0.05$ ), and suggests a steady improvement in true tolerance to drought that coincides with the flowering period. Developing hybrids that escape drought through

earlier flowering is possible but not useful in many cases. For example, the U.S. Corn Belt has well-defined maize maturity requirements to optimize yield potential while minimizing the risk of early frost damage, and the timing of drought stress in the target environment is unpredictable.

Consistent with the genetic gains observed, newer hybrids outperformed older hybrids under non-as well as diverse drought stress conditions. In comparison, O'NEILL *et al.* (2004), analyzing a subset of 12 hybrids evaluated under drought and low N conditions, concluded that newer hybrids possess lesser stress tolerance in terms of grain yield than older hybrids. Nevertheless, hybrids selected for their study only spanned around 20 years of breeding, a period not extended enough to detect the trends discussed here. Moreover, the large range of maturity included in their work (CRM 106 through 118) may have confounded drought stress tolerance with length of developmental cycle.

The environment standardized GGE biplot for grain yield (Fig. 4) further illustrates the relationships existing between the experimental environments. It also includes data from ERA experiments carried out in U.S. Corn-Belt environments and shows the correlation among the managed-stress environments and Corn-Belt performance. The first two components accounted for 93% of the standardized grain yield variation, and all the environments created showed excellent discrimination among the hybrids under study. Vectors show a separation between stresses that coincided fully or in part with flowering (S1, S2 and S3) and those occurring at mid- to terminal grain fill (S4, S5), and the correlation of FI, S2 and S3 with the majority of the Corn-Belt environments. This is consistent with the contrasting performance observed with secondary traits associated with drought tolerance in maize under flowering and grain filling stresses, to be discussed later. This suggests that the mechanisms of yield determination and the subsequent strategies for improving drought tolerance also differ between these two groups of environments.

### Secondary traits

The use of surrogate, or secondary traits, when selecting for improved drought tolerance has been an active area of research, in part because the heritability of the primary trait (grain yield) falls in stressed environments and reduces genetic gain. Heritability declines because genetic variances decrease more rapidly than the environmental vari-

ance among plots with increasing stress. If secondary traits can be identified whose genetic variances increase under stress or are reduced less than that of yield, these can be used with yield to increase selection efficiency (BOLAÑOS and EDMÉADES, 1996). This condition has been demonstrated for traits associated with drought, such as ASI and barrenness by BETRÁN *et al.* (2003) and for ASI, barrenness and stay-green under low N conditions by BÄNZIGER and LAFITTE (1997).

### ***Anthesis-Silking Interval***

An almost universal response of maize to drought stress at flowering is a delay in silk appearance relative to pollen shed. Delayed silk emergence, reflected as an increase in ASI, has been associated with reduced ear growth under stress (EDMÉADES *et al.*, 2000). ASI ranged from 70.1°Cd under a severe flowering stress to 0.8°Cd under well-watered conditions (Fig. 2b). In treatments that overlapped with flowering (Table 3), ASI increased to 60-70°Cd, and it also increased by 40°Cd by treatment S3, indicating that at least in the more susceptible, older hybrids this treatment that imposed only moderate stress at flowering was also effective at delaying silk emergence (Fig. 2b). As expected, treatments that experienced no stress at flowering (FI, S4 and S5) showed little or no silk delay, except among the older hybrids, and recent hybrid releases displayed a negative ASI under these conditions. Hybrid differences dominated the variance components for ASI, although the Stress.Hybrid variance component for ASI was also significant (Table 4), showing the importance of this differential response of ASI among hybrids from different eras. The largest genetic gain for ASI was observed in treatment S2 (-2.6°Cd yr<sup>-1</sup>) (Fig. 3b), a treatment designed to expose genetic variation for this trait. Smaller but highly significant gains of -1.5 to -1.7°Cd yr<sup>-1</sup> were also observed where stress did not coincide with flowering. DUVICK *et al.* (2004) reported a reduction in ASI due to selection of -1.3°Cd yr<sup>-1</sup> in central Iowa environments, a value similar to that observed under FI. The greater gain (or genetic variation) observed under a stress imposed at flowering implies that the use of managed stress environments could be used effectively to accelerate progress in ASI. In studies of the effects of recurrent selection for drought tolerance in tropical maize, CIMMYT scientists (EDMÉADES *et al.*, 2000) also observed that improved yield under stresses occurring at flowering was as-

sociated with marked reductions in ASI and improved ear growth rates, and effectively utilized managed stress environments to expose genetic variation for these traits.

The importance of ASI as a predictor of kernel set, as proposed by others (e.g., BOLAÑOS and EDMÉADES, 1996; EDMÉADES *et al.*, 2000) is reinforced by the phenotypic correlations between ASI and grain yield or its components KPE and EPP ( $r = -0.63$  to  $-0.66$ ,  $P < 0.001$ ) (Table 5a), and between grain yield and KPE or EPP ( $r = 0.81$  to  $0.85$ ;  $P < 0.001$ ). On the other hand, the correlation between grain yield and KWT, although significant, was comparatively weak ( $r = 0.35$ ;  $P < 0.01$ ), suggesting that the major cause of variation in yield across water regimes was kernel number rather than kernel weight, even though stress treatments were evenly distributed throughout the grain filling period. Examination of the relationships between ASI and yield and its components under specific stresses, however, shows little effect on the magnitude of the correlations. This is a result of the underlying structure to this dataset, in which the progress due to selection over years generated strong relationships among these variables, even under optimal conditions (Table 5b), and these dominated the kernel number and weight changes that were generated by the different stress treatments (Tables 5c,d).

ASI can also be thought of as an external indicator of increased partitioning of assimilates to the growing ear. When combined with data on barrenness, ASI is a useful selection tool for improving partitioning (EDMÉADES *et al.*, 2000). DUVICK *et al.* (2004), analyzing a large set of ERA maize hybrids, reported a significant shift in partitioning away from tassel development and toward increased ear growth, as defined by decreases in ASI and barrenness. These changes have also resulted in a greater tolerance to high plant densities (DOW *et al.*, 1984; EDMÉADES *et al.*, 2003). Additional factors related to the flowering dynamics of maize under flowering stress are silk elongation and senescence, since these can also directly impact kernel set and yield stability (ANDERSON *et al.*, 2004).

The importance of dry matter partitioning to the ear, indirectly observed as a reduced ASI in newer US hybrids in the current study, as a mechanism conferring improved performance in newer Argentinean hybrids, has been discussed by ECHARTE *et al.* (2004), who found that newer hybrids are able to set more kernels per unit of ear growth rate around silking.

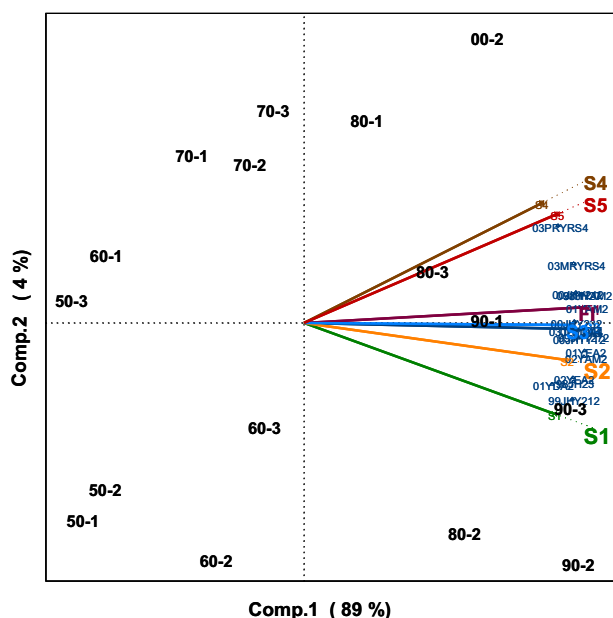


FIGURE 4 - Environment-standardized GGE biplot of grain yield of 18 ERA maize hybrids grown in six environments over two years. Percent of the total GGE variation explained by the main two principal components are in parentheses. Hybrids 90-4 and 00-1 were not included in the biplot due to insufficient data. Vector names correspond to treatments and US locations are shown in blue.

### Kernels per Ear

Drought stress sharply reduced KPE from 513 under unstressed conditions to 237 under flowering stress (S1) (Fig. 2c). Unlike grain yield and ASI, the components of variance associated with KPE corresponding to factors Hybrid and Stress.Hybrid were equally important, and both were small relative to the residual component of variance (Table 4). When maize plants are exposed to stress at flowering, grain yield per plant is closely associated with kernels per plant. Usually plants will become barren at the same time as kernel number per fertile ear (KPE) also declines (CHAPMAN and EDMANES (1999). When assimilate source increases during the critical flowering period, KPE also increased (SCHOPER, 1982). Across all environments we observed a correlation of 0.85 ( $P < 0.01$ ) between KPE and grain yield, increasing from 0.67 ( $P < 0.01$ ) under unstressed conditions to 0.74 ( $P < 0.01$ ) under stress at flowering, and to 0.95 ( $P < 0.01$ ) for stresses during grain filling (Table 5). BOLAÑOS and EDMANES (1996) reported a genetic correlation of 0.71 to 0.78 between maize grain yield and KPE under a range of

water regimes, similar to findings by OTEGUI (1995). CHAPMAN and EDMANES (1999) reported a correlation of 0.79 ( $P < 0.01$ ) between maize grain yield and grains per ear under drought stress.

Genetic gains for KPE were quite similar across all water regimes, ranging from 1.19 to 1.55  $\text{yr}^{-1}$ . This suggests that the underlying genetic mechanism for increasing KPE through selection was relatively independent of water stress at any specific stage (Fig. 3c). All were positive for all treatments studied, even though DUVICK *et al.* (2004) reported a negative ( $-1.1 \text{ yr}^{-1}$ ) change per year in the full set of ERA hybrids under Corn Belt conditions.

As with ASI, KPE relates to ear growth rates, and is therefore sensitive to their dynamics near flowering. A close relationship exists between ASI and KPE under flowering stress ( $r = -0.73$ ,  $P < 0.001$ ), similar to that reported by CHAPMAN and EDMANES (1999). This suggests that the steady increase in KPE with selection observed under all water regimes reflects a progressive increase in partitioning to the ear with selection. In a study of correlated responses among secondary traits in tropical maize subjected to drought stress, CHAPMAN and EDMANES (1999) also reported an increase in KPE, even though ASI, and not KPE, was the subject of direct selection.

### Ears per Plant

There is a clear dependency of grain yield on kernels per plant and this is mediated through EPP and less through KPE. Drought induces barrenness, and a symptom of impending barrenness is an increase in ASI (EDMANES *et al.*, 1997). EPP was reduced from 1.01 under non-stressed conditions to 0.65 under flowering stress (S2) (Fig. 2d). Terminal episodes of drought did not affect EPP significantly. Components of variance Block.Stress.Rep.Year and Year.Hybrid were of equal importance, and were greater than the Hybrid effect for this trait (Table 4). Flowering stress can significantly reduce EPP even in drought tolerant maize hybrid progenies, although this effect is much greater in hybrids developed under optimal input conditions (ZAIDI *et al.*, 2004).

A strong negative association was observed between ASI and EPP (Table 5). This is consistent with previous reports that a short ASI under flowering stress conditions is associated with increased partitioning to developing ears at the reproductive phase (EDMANES *et al.*, 1993). Significant genetic gains have clearly been achieved in EPP under conditions of flowering stress. DUVICK *et al.* (2004) reported a genetic gain of 0.0036 ears  $\text{yr}^{-1}$ , similar to that ob-

served under FI conditions. Genetic gains through flowering stresses S1-S3 are of equal or higher magnitude than those achieved under FI conditions (Fig. 3d), reflecting the selection pressures imposed by breeders over breeding cycles. The improvement under flowering conditions observed suggests that selection has significantly enhanced the sink strength of ears in Corn Belt germplasm.

### Kernel Weight

Once the number of kernels per ear has been set, stress conditions during grain filling normally reduce 100 kernel weight (KWT). Drought stress at flowering had no effect on KWT (Fig. 2e), whereas stresses affecting grain filling significantly depressed KWT by 41% (S4) and by 27% (S5) compared with the unstressed control. Correlations between KWT and grain yield were highest under unstressed conditions ( $r=0.92$ ,  $P<0.001$ ) and under late grain filling stresses ( $r=0.88$ ,  $P<0.001$ ), and least under flowering stress conditions ( $P=0.66$ ,  $P<0.01$ ) (Table 5). Correlations between KWT and KPE were either weakly positive or non-significant. The Hybrid component of variance was approximately two and four times larger than the Stress.Hybrid and Stress.Year. Hybrid components, respectively, for KWT (Table 4). The largest genetic gains for this trait were observed in FI ( $0.21 \text{ g yr}^{-1}$ ) and flowering stress treatments ( $0.16 \text{ g yr}^{-1}$ ), and the least in grain filling stresses ( $0.02$  (NS) to  $0.06 \text{ g yr}^{-1}$ ) (Fig. 3e).

The inability of recent hybrids under grain filling stress to fill kernels to their potential size as observed under unstressed conditions may relate to improvements achieved in assimilate partitioning towards the stalks to maintain late season health. This suggests that considerable potential for increases in yield under late grain filling stress exists, provided assimilation can be maintained or stored assimilate remobilized (EDMEADES *et al.*, 2003; CAMPOS *et al.*, 2004).

### CONCLUSIONS

We conclude that:

1) There has been a steady improvement of maize performance under stress, measured as grain yield under drought, over the past 50 years. Breeding programs have been particularly successful at increasing the ability of maize to withstand drought occurring at flowering, as shown by a decrease in ASI, and increases in EPP and KPE. This has been

achieved without affecting significantly crop duration and at no expense to yield potential.

2) Flowering stress remains the most sensitive stage of maize growth to drought stress. Nevertheless, the post-flowering window of sensitivity in kernel number in modern hybrids may extend longer than the 2 weeks post silking period identified in older hybrids (e.g., SHAW, 1977).

3) Under optimal growth conditions, genetic gains of  $196 \text{ kg ha}^{-1} \text{ yr}^{-1}$  were observed, reflecting the increasing yield potential of newer hybrids. Although genetic gains under drought stress were of lesser magnitude, yields under flowering stress especially have increased with selection.

4) Newer hybrids showed a marked ability to set more ears and kernels under stress, and potential kernel weight has increased significantly with selection. These advantages decreased when stress coincided with mid- to late grain filling.

5) The deployment of long-term studies, such as the Duvick's ERA hybrid experiments, represents a valuable opportunity to gain insight into the underlying changes due to genetic improvement of U.S. corn-belt maize in the long term. Such retrospective analyses would enhance our chances to further improve maize over the years, and therefore to increase the availability of quality food and feedstuff for mankind, a central objective of plant breeding.

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