

## Preliminary field screening of maize landrace germplasm from northeastern Mexico under high temperatures

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### Abstract

Northeastern Mexico has a wide variety of maize landraces that have not been characterized, and landraces varieties may be a good source of new allelic diversity for useful traits. This study evaluated 28 accessions from Tamaulipas, in Northeast Mexico, chosen by phenology and for agronomic characteristics to represent the diversity of germplasm in the collection and agro-ecosystems of this area. They were evaluated under high temperature in the field as a basis for an efficient use of this maize germplasm in a breeding program; in particular we investigated changes in agronomic characteristics affected by high temperatures in all growing seasons. Results indicated that in late planting dates air temperatures become excessively high during the flowering period and growing season; in these conditions a loss in grain yield can be sustained. In this study, the loss initially manifests itself as a reduction in grain yield (> 32%) because of fewer grains per ear (>26%). Landrace maize accessions C-3030, C-3049, C-3015, Pob. I, Pob. II, ZEM-148 and C-4050 were identified with high relative yield and yield components traits under high temperature conditions. These accessions have greater agronomic stability, and also are potential donors of genes to improve maize tolerance to high temperature.

**Keywords:** maize landraces, high temperature, planting dates, grain yield, grain number, genotypes heat tolerant; genetic resources

### Introduction

Mexico is the center of origin of maize (*Zea mays* L), which was domesticated about 6,000-10,000 years ago from tropical teosinte (*Zea mays* ssp. *Parviglumis*) (Doebley, 2004). Maize is the primary grain crop in Mexico serving, as a major staple food for human consumption. With its enormous diversity, it has played an important role in the development of modern cultivars of the world. Maize from Mexico shows a high degree of variation stemming from its history of cultivation by Mexican farmers. In agreement with Warburton et al (2008), the genetic variation of domesticated maize populations can be reduced or restructured by at least two causes, genetic drift and selection, both natural and artificial, by early farmers; this has eventually resulted in a large number of landraces adapted to the specific environmental conditions of their habitats and uses chosen by humans.

Northeastern Mexico has a wide variety of genetically diverse maize landraces that have not been characterized, and landrace varieties may be a good source of new allelic diversity for parasite, insect pest, or environmental stresses, including drought and high temperatures. These are serious threats to agriculture and the natural status of the environment (Wang et al, 2003; Barnabás et al, 2008), frequently

limiting growth and productivity of major crop species (Tester and Bacic, 2005; Mittler, 2006; Barnabás et al, 2008) such as maize in Mexico and worldwide.

Genetic diversity in maize landraces is a valuable natural resource and plays a key role in future breeding progress (Reif et al, 2004); its effective management is a critical prerequisite to allow continued improvement of agricultural productivity (Smith, 2007). Over the years, existing landraces were the bases for developing open pollinated varieties, which in turn are often used to develop hybrid cultivars, and these improved materials have often replaced landraces in the developing world (Taba et al, 2005); although worldwide about half of the non-temperate maize-growing area is still sown with landraces (particularly in Mexico), this is a decreasing trend.

Plant genetic resources are the most fundamental component of all agricultural systems, thus conservation, evaluation, and enhancement of germplasm is an essential activity (Ortiz et al, 2008). Therefore, germplasm collections as a source of genetic diversity must be well characterized for efficient management and effective exploitation. In areas where sporadic heat waves above 40°C can occur at any stage of development during the growing season (such as northeastern Mexico), landraces have evolved to

grow under these conditions. While crop management techniques such as irrigation can alleviate water stress and minimize crop losses due to drought, it is not always economically feasible, and it cannot remove all of the negative impact of heat stress (Chen et al, 2010). High temperature affects various aspects of plant growth and development (He and Huang, 2007). In maize, temperatures above optimum have detrimental effects on vegetative growth, pollination, seed set and kernel development (Wilhelm et al, 1999), but when high temperatures occur at flowering, pollination, silking and early kernel development stages, they can result in negative effects on grain yield and quality (Commuri and Jones, 1999; Wilhelm et al, 1999; Cicchino et al, 2010). Therefore, the improvement of heat tolerance in maize is an important breeding objective, because to maintain growth and productivity, plants must adapt to stress conditions and exercise specific tolerance mechanisms (Wang et al, 2003).

The main objective of this study was to characterize 28 accessions (21 landraces, one open pollinated variety, one hybrid and five advanced populations) under high temperatures in the field as a basis for an efficient use of this maize germplasm in a breeding program; in particular from these 28 accessions, we sought to identify some that do not suffer changes in agronomic characteristics that reduce yield in a high temperature environment.

## Materials and Methods

The Universidad Autonoma de Tamaulipas maize landrace collection contains over 250 accessions from northeastern Mexico (Center and South of Tamaulipas mostly), 28 of which were chosen based on phenology and for agronomic characteristics (previously characterized, unpublished) to represent the diversity of germplasm in the collection and agro-ecosystems of this area (Table 1). These were evaluated under field conditions in four environments; three planting dates with high temperature (HT) and one

location with optimum temperature (OT) as a control. The three planting dates were sown near Güemez, Tamaulipas (23°56.522'N; 99°06.179'W and 186 m from sea level) in 2006 (February 2, February 28, and March 21) and the optimal location was sown on February 14 at CERIB (Rio Bravo Experimental Field) near Rio Bravo, Tamaulipas (25°57'N; 98°01' W). The accessions were sown in a completely randomized block design with three replicates. The plants were grown in one row of 5 m length with inter and intra row spacing of 0.80 m and 0.25 m respectively. The experimental areas were fertilized at a rate of 18 kg N and 46 kg P<sub>2</sub>O<sub>5</sub> per hectare. Weed control was done with herbicide twice, at the seedling stage (second and third true leaf stage) and prior to flowering. During the growing season, two irrigations were applied, first during the floral initiation and the second eight days before full flowering. The evaluation for high temperature response of the 28 genotypes was done by comparing three planting dates (HT) with respect to CERIB (OT) plants in all traits. Significant differences identified by this comparison may result from stress and damage to plant growth and development. Air temperature and precipitation were monitored using Hobo Weather Station (Onset Computer Corp., Bourne, MA) data loggers. Measurements were collected every hour throughout the experiment.

Days to anthesis (DA) and silking (DS) were recorded from each plot. A plant was considered as having reached anthesis or silking when 50% of plants per plot extruded anthers or silk was visible. The anthesis-silking interval (ASI) was calculated as DS – DA. After the completion of male flowering, plant height (PH) and ear height (EH) were recorded as the distance between the ground surface and the node bearing the flag leaf or ear respectively. The PH and EH values were recorded on five plants per plot on average. Total and ear leaf area (LA and ELA) were determined by passing all leaves of each plant through a LI-300A leaf area meter (LI-COR, Lincoln, NE), on five plants per plot. After completion of male and female flowering, leaf area index (LAI) was calculated as the

**Table 1** - Name, origin (municipality), location (latitude and longitude), altitude and climate of 28 maize landraces accessions and populations studied to determine response to high temperature environments in northeastern of Mexico.

Accession	Origin (municipality)	Latitude/Longitude	Altitude (m)	Climate
C-3021, C-3009, C-3012, C-3014	Tula	23°00' / 99°43'	1170	Semi-warm and dry steppe
C-3015, C-3005, C-3018, C-3004 <sup>1</sup>	Padilla	24°00' / 98°47'	153	Very hot dry
C-4050, ZEM-131, ZEM-148	Ocampo	22°51' / 99°20'	325	Extreme semi-warm
C-3027, C-3030, C-3049	Hidalgo	24°15' / 99°26'	320	Extreme sub-humid and semi-hot
C-4034, C-4026	Miquihuana	23°34' / 99°45'	1770	Extreme temperate and extreme intermediate temperate
Pob. I <sup>2</sup> , Pob. II <sup>3</sup>	Germplasm pools			-
F2-H-433, F2-2744x2735	Advanced populations			-
C-3041	Llera	23°19' / 99°01'	291	Extreme semi-warm and dry steppe very hot
Barroso	Germplasm pool			-
C-3029	Villagrán	24°28' / 99°29'	380	Extreme semi-warm
C-3020	Güemez	23°55' / 99°00'	202	Dry very hot
H-437, VS-536	Commercial varieties			-
C-4020, C-4041	Bustamante	23°26' / 99°45'	1600	Sub-humid and dry semi-warm

<sup>1</sup>Landraces with stay green; <sup>2</sup>Population with erect leaves; <sup>3</sup>Population with large ears.

**Table 2** - Average air temperature and precipitation measured during sowing to physiological maturity in four environments

Environment	Average Max Temp (°C)	Average Min Temp (°C)	Average Temp (°C)	Days $\geq 35^{\circ}\text{C}$		Precipitation (mm)
				Flowering Period	Growing Season	
Feb 1 (HT) <sup>1</sup>	36.9	15.7	18.6	28/29	100/137	125.0
Feb 28 (HT)	37.4	17.0	19.6	25/25	113/134	178.0
Mar 21 (HT)	38.2	18.0	20.5	20/20	119/130	187.0
Río Bravo (OT) <sup>2</sup>	30.1	19.2	24.7	0/17	9/126	56.0

<sup>1</sup>HT= High temperature; <sup>2</sup>OT= Optimum temperature

ratio of leaf area to ground surface per plant in five plants per plot. At physiological maturity (PM), grain yield per ear (GY) from five plants per plot was measured by hand-harvesting. Grain number (GN) per ear was calculated from GY and 100-kernel weight (GIW). The grain filling period (GFP) was calculated as DA-PM and grain filling rate (GFR) was calculated as GY/GFP. Relative yield (RY) was calculated as a ratio of grain yield landrace at each planting date ( $\text{RY} = \text{GY-Planting date} / \text{GY-Río Bravo} \times 100$ ) with respect to Río Bravo (OT), as suggested by [Yau and Hamblin \(1994\)](#). At each planting date, landraces having RY values  $<100$  yield less than the Río Bravo values, while landraces having RY values  $>100$  yield more. The mean relative yield (the average of each genotype across planting dates) was considered to identify the better landraces with respect the commercial variety H-437.

In each environment, the experimental design was a randomized complete block with three replications. To assess treatment effects, planting date (temperature treatment) and genotype were considered as fixed effects and replicate block was considered a random effect. Combined analyses of variance were made using the GLM procedure ([SAS Institute, 2003](#)) for all variables. Effects associated with genotype, planting date and their interactions were identified. Mean comparison was performed on the temperature treatments and genotypes using Tukey's Studentized Range (HSD) Test at the  $P = 0.05$  levels. To determine the association between grain yield and its direct components and mean relative yield, simple phenotypic correlation coefficients were used at 0.05 and 0.01 probability levels.

## Results and Discussion

Large contrasts among the three planting dates with respect to Río Bravo were observed in terms of air temperature and precipitation ([Table 2](#)). Differences between planting dates in average maximum temperature during the biological cycle were of 0.5 to 1.3°C; the difference with respect to the optimum temperature environment was between 6 and 8°C, depending on planting dates. Plants exposed to excessive heat, at least 5°C above their optimal growing conditions had a reduced growing season ( $> 4$  days) in agreement with [Porter and Moot \(1998\)](#). The three planting dates had temperatures exceeding 35°C between 73 and 92% of the days between sowing to

physiological maturity (vegetative and reproductive stages). During the flowering period, the temperatures in all planting dates exceeded 40°C on several days (data not shown). Late planting dates had reduced time to maturation (4 to 7 days) and flowering period (5 to 9 days) in response to high temperatures (temperatures  $\geq 35^{\circ}\text{C}$ ), in agreement with [Stone \(2001\)](#). Precipitation was not significant in all environments during the growing season ([Table 2](#)). Only 18 and 95 mm of precipitation were recorded between sowing and anthesis in date 2 and 3 respectively; and 43 mm in Río Bravo. The hottest sowing date was March 21 with maximum air temperature  $> 38^{\circ}\text{C}$  and 92% of days with  $\geq 35^{\circ}\text{C}$  during the growing season.

According to variance analysis, it was found that there were significant differences ( $P \leq 0.001$ ) in temperatures among environments (planting dates and location), and genotypes differences ( $P \leq 0.001$ ) for all traits, indicating a great genetic variability between the evaluated germplasm. This variability is desirable in our genetic breeding program (and others) to improve the maize heat tolerance. Other studies ([Reif et al, 2004](#); [Xia et al, 2004](#); [Reif et al, 2006](#)) suggest that the traditional farmer's landrace varieties, such as our collections, may be good sources of new allelic diversity for improving the different inbred lines.

Planting dates with respect to optimum temperature offered three distinctive conditions in terms of

**Table 3** - Comparison of high temperature (HT) and optimum temperature (OT) on various phenology, growth and yield traits of maize landraces.

Trait	OT	HT	HSD
Days to anthesis (d)	69.8	74.2	0.653 ***
Days to silking (d)	71.8	79.9	0.894 ***
Total leaf area (cm <sup>2</sup> plant <sup>-1</sup> )	4840.2	3090.3	247.79 ***
Leaf area index	2.42	1.55	0.12 ***
Anthesis-silking interval (d)	1.95	5.8	0.71 ***
Plant height (cm)	230.7	135.2	21.88 ***
Ear height (cm)	112.2	55.9	4.74 ***
Grain number per ear	321.3	227.0	33.27 ***
Grain individual weight (g)	0.21	0.21	NS
Grain yield (Mg ha <sup>-1</sup> )	3.43	2.27	0.264 ***
Number of leaves above ear	6.20	4.94	0.15 ***
Leaf area of leave ear (cm <sup>2</sup> )	547.33	394.90	43.30 ***
Physiological maturity (d)	95.5	106.3	1.19 ***
Grain filling period (d)	23.7	26.4	0.29 ***
Grain filling rate (g d <sup>-1</sup> )	2.92	1.73	0.21 ***

\*\*\* significant at  $P \leq 0.001$ ; NS: Non significant  $P > 0.05$ ; HSD: Tukey Studentized Range ( $P = 0.05$ )

**Table 4** - Maize grain yield, direct yield components, yield reduction and plant parameters as affected by three planting dates with respect to Rio Bravo, averaged across 28 genotypes during 2006.

Env.	Total leaf area (cm <sup>2</sup> )	Anthesis silking interval (days)	Leaf area index	Plant height (cm)	Ear height (cm)	Grain number per ear (g)	Grain individual weight	Grain yield (Mg ha <sup>-1</sup> )	Yield reduction (%)
Feb 1	2758.9d	6.33a	1.39d	132.4b	48.9c	215.4b	0.22a	2.33b	32
Feb 28	3081.2c	5.45b	1.55c	138.2b	60.2b	238.3b	0.19b	2.23b	35
Mar 21	3430.8b	5.62b	1.71b	134.9b	58.7b	224.3b	0.19b	2.23b	35
Rio Bravo	4840.2a	1.95c	2.42a	230.7a	112.2a	321.3a	0.22a	3.43a	

\* Means within a plant parameter not followed by the same letter are significantly different at  $P \leq 0.05$  according to Tukey's multiple range test.

weather for conducting the research and the opportunity to identify landraces with the capacity to tolerate high temperatures, as indicated by Wang et al (2003).

In the high temperature trials ( $\geq 35^{\circ}\text{C}$ ), as a result of the high temperature environment, a significant reduction ( $P \leq 0.001$ ) of the grain yield ( $> 34\%$ ) was observed, caused mainly by a significant reduction ( $P \leq 0.001$ ) of the grain number per ear ( $> 29\%$ ); in addition, significant increase ( $P \leq 0.001$ ) were observed in days to anthesis ( $> 4$  days), days to silking ( $> 8$  days), anthesis-silking interval ( $> 3$  days), physiological maturity ( $> 10$  days) and grain filling period ( $> 2$  days); significant reductions ( $P \leq 0.001$ ) were seen in plant growth, total leaf area ( $> 36\%$ ), leaf area index ( $> 35\%$ ), plant height ( $> 41\%$ ), ear height ( $> 50\%$ ), number of leaves above ear ( $> 20\%$ ), leaf area leave ear ( $> 27\%$ ) and grain filling rate ( $> 1$  day); all these depending on the environment (Table 3) when compared to the environmental control (optimal temperature).

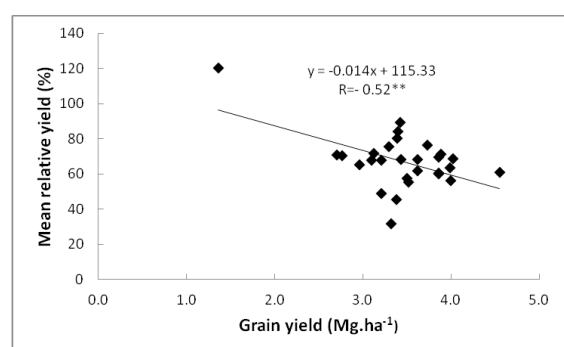
It has been reported that the reduction in the development of maize seed occurs at temperatures higher than  $38^{\circ}\text{C}$  mainly because of a reduction in pollen germination ability and pollen tube elongation (Stone, 2001). In addition, lateral ear heating (by  $4.5^{\circ}\text{C}$  above the air temperature in the heated zone) prior to silk emergence has been reported to reduce kernel number per ear (Cárcova and Otegui, 2001); these effects are more drastic during the flowering stage (Saini and Westgate, 2000; Boyer and Westgate, 2004). These mechanisms may be responsible for reducing yield up to 32% in our study, as well as grain number per ear and grain individual weight (Table 4). Grain yield was positively correlated to grain number per ear ( $r = 0.83$ ;  $P = 0.01$ ) under optimal temperature, and also was negatively correlated to mean relative yield ( $r = -0.52$ ;  $P = 0.01$ ) (Figure 1). This correlation is strongly influenced by one data point however and in the absence of this data point, the correlation is not significant.

The yield reduction can be explained, by the effect on the components in Table 4, by a reduction in grain number per ear ( $> 25\%$ ) and grain individual weight ( $> 13\%$ ); in addition to reductions in the total

leaf area ( $> 29\%$ ), the leaf area index ( $> 29\%$ ), the plant height ( $> 40\%$ ), the ear height ( $> 46\%$ ) and an increase of the anthesis-silking interval ( $> 3$  days).

Relative yield facilitates comprehension of agronomic stability (Yau and Hamblin, 1994) or genotype-by-environment interaction; based on relative yield stability, the better genotypes are C-3030 and C-3049 (from Hidalgo); C-3015 (from Padilla); ZEM-148 and C-4050 (from Ocampo); Pob I and Pob II (Table 5). Sixty-one percent of the genotypes equal or surpass the control variety H-437 in yield under high heat and optimal temperature conditions. For example, under optimum conditions the genotype C-3004 was higher than H-437 in grain yield by 42%; on the hottest planting date the C-3004 genotype exhibited a better performance in grain yield of 73% caused by higher grain number per ear (50%) and higher grain individual weight (21%). The superior populations have greater agronomic stability and are from diverse geographical areas, climate and altitude within the state of Tamaulipas (Table 1).

The high temperature environment resulted in grain yield and grain number per ear reductions in all genotypes, except C-4050 (from Ocampo) for the February 1 and February 28 planting dates (Table 5). This planting dates produced significantly increased grain yield in C-4050 suggesting that this accession may be adapted to high temperature environments; however this depends on the planting date, because



**Figure 1** - Relationship between grain yield and mean relative yield for 28 maize genotypes grown under high temperature treatments.

**Table 5** - Maize grain yield and direct components and relative yield for the best nine landraces genotypes and the control variety H-437 with respect to Río Bravo (optimal location), affected by high temperature.

Genotype	Environment	Grain number per ear	Grain individual weight (g)	Grain yield (Mg ha <sup>-1</sup> )	Relative yield (%)
C-3030	Río Bravo	288.3	0.21	3.42	100
	Feb 1	269.7	0.25	3.29	96.4
	Feb 28	277.0	0.21	2.96	86.4
	Mar 21	259.4	0.23	2.92	85.4
C-3049	Río Bravo	360.7	0.19	3.30	100
	Feb 1	231.0	0.23	2.67	80.8
	Feb 28	237.6	0.22	2.56	77.6
	Mar 21	244.5	0.18	2.25	68.0
C-3015	Río Bravo	348.3	0.22	3.86	100
	Feb 1	261.9	0.20	2.62	67.9
	Feb 28	232.0	0.21	2.54	62.7
	Mar 21	250.9	0.23	2.91	75.4
C-3005	Río Bravo	411.7	0.20	3.99	100
	Feb 1	227.0	0.25	2.69	67.4
	Feb 28	264.0	0.18	2.53	63.4
	Mar 21	292.3	0.16	2.39	60.0
C-3004	Río Bravo	387.0	0.24	4.55	100
	Feb 1	273.9	0.20	2.70	59.4
	Feb 28	218.3	0.19	2.03	44.5
	Mar 21	310.7	0.23	3.57	78.4
Pob. I	Río Bravo	300.0	0.23	3.39	100
	Feb 1	269.5	0.24	3.14	92.6
	Feb 28	268.0	0.19	2.53	74.7
	Mar 21	269.4	0.18	2.47	72.9
Pob. II	Río Bravo	335.7	0.22	3.74	100
	Feb 1	239.7	0.26	3.02	80.8
	Feb 28	242.4	0.21	2.56	68.6
	Mar 21	262.1	0.22	2.96	79.2
ZEM-148	Río Bravo	332.7	0.24	4.02	100
	Feb 1	239.3	0.25	2.94	73.2
	Feb 28	295.3	0.20	2.89	72.0
	Mar 21	242.8	0.20	2.42	60.2
C-4050	Río Bravo	131.7	0.21	1.36	100
	Feb 1	229.6	0.21	2.38	175.3
	Feb 28	155.9	0.22	1.67	123.2
	Mar 21	130.4	0.14	0.84	61.8
H-437	Río Bravo	329.0	0.19	3.21	100
	Feb 1	238.8	0.22	2.57	79.9
	Feb 28	175.6	0.21	1.88	58.6
	Mar 21	207.0	0.19	2.07	64.4

on a late date this response was very different (reduced 38%); C-4050 is not an improved maize variety, but may still contain untapped allelic variation useful for future breeding progress to moderately high temperatures, as suggested by Warburton et al (2008).

In conclusion, in late planting dates, air temperatures become excessively high during the flowering period and growing season, when a loss in grain yield can be sustained. The loss initially manifests itself as a reduction in grains per ear. Maize landrace accessions were found to have high relative yield and yield components traits under high temperature conditions. These accessions have greater agronomic

stability, and also are potential donors of genes to improve maize tolerance to high temperature.

### Acknowledgements

The study was partially supported by the Consejo Nacional de Ciencia y Tecnología (CONACYT) through project SEP 2003-CO2-44713 (México).



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