

## Short-term drought responses by seedlings of three maizes from contrasting environments in Michoacán, Mexico

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### Abstract

Alterations in precipitation regimes resulting from climate change threaten countries like Mexico, where rainfed agriculture for subsistence is widespread. However, numerous local maizes are cultivated throughout the country's territory originated from a substantial environmental diversity. To investigate whether an environmental specialization exists, responses to drought by maizes from three sites (elevations of 689, 2379, and 3638 m) in the state of Michoacán, Mexico, were studied in a greenhouse experiment. Plants were watered every other day for 21 days since sowing. Watering was withheld during 26 d and followed by a 10 d recovery period during which watering was resumed. Environmental conditions were contrasting among the sites of origin, e.g., annual precipitation deficit (from an ombrotherm diagram) was 33% at the lowest site and 9% and 0.3% for the sites at higher elevations. At the greenhouse, substrate water content for the control was ca. 34% (w/w) over the course of the experiment, it decreased to 1.9% at 26 d of water withholding and recovered after resuming irrigation. Tissue water content was ca. 92% for the control and decreased by 22-33% (depending on its origin) for the droughted individuals at the peak of the drought. Dry mass was lower for droughted individuals than for the control at the end of the experiment. Leaf chlorophyll content tended to decrease with plant age, but did not respond to water withholding. In contrast, proline tended to increase for droughted individuals. The content of polyphenols, free-radical scavenging compounds, tended to decrease during the recovery period, but not during drought. Neither did the antioxidant activity of leaf tissue respond to water withholding. Germplasm that is able cope with novel environmental conditions seems to exist in Mexico. Future studies should survey yield responses to different drought intensities for various maizes from dry environments to help identify traits useful in future breeding programs.

**Keywords:** adaptation, climate change, ecophysiology, food security, mycorrhizae

### Introduction

Maize, the most widely cultivated cereal in the world, was originally domesticated over 10,000 years ago within the Balsas River basin, along the border of the current western states of Michoacán and Guerrero, Mexico (Miranda Colín, 2005). It is the food staple for some 112 million people in this country, where numerous local varieties have been developed over centuries. While tortillas, a flat bread baked from a maize dough, are the main form of consuming the cereal, over 600 distinct uses have been documented in Mexico that are predominantly specific to indigenous peoples (Turrent Fernández et al, 2013). Contributing to the international popularization of maize cultivation are the very high yields attained by improved varieties that require considerable investment in irrigation, agrochemicals, and human and mechanical labor. However, up to 80% of maize cultivation (on an area basis) in Mexico is conformed by small scale, low input, subsistence operations, for which a typical yield rarely exceeds 2 tons ha<sup>-1</sup> year<sup>-1</sup> (Servicio de Información Agroalimentaria y Pesquera, 2014).

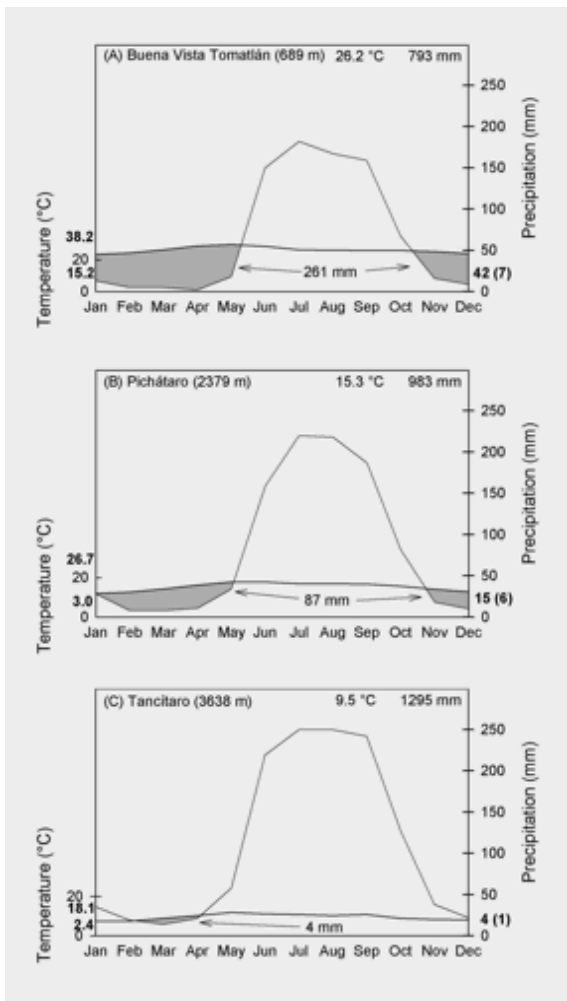
Approximately two thirds of Mexico's territory are

arid or semiarid (i.e., receiving annual precipitations below 600 mm), so extensive portions of the country are considered marginally apt for agriculture (Food and Agriculture Organization, 2013). However, local maizes exist throughout the country in essentially all climates and ecosystems, originating from the millennial selection that has taken advantage of desirable plant traits and the development of cultural practices that includes creating benign microenvironments (Peralles and Golicher, 2014). Thus, the potential exists for maize germplasm to cope with the novel environmental conditions expected to develop during the present century.

By means of a greenhouse experiment, some physiological responses of seedlings from three localities from the state of Michoacán, along an altitudinal gradient, were studied in an effort to determine whether these maizes responded differently to drought.

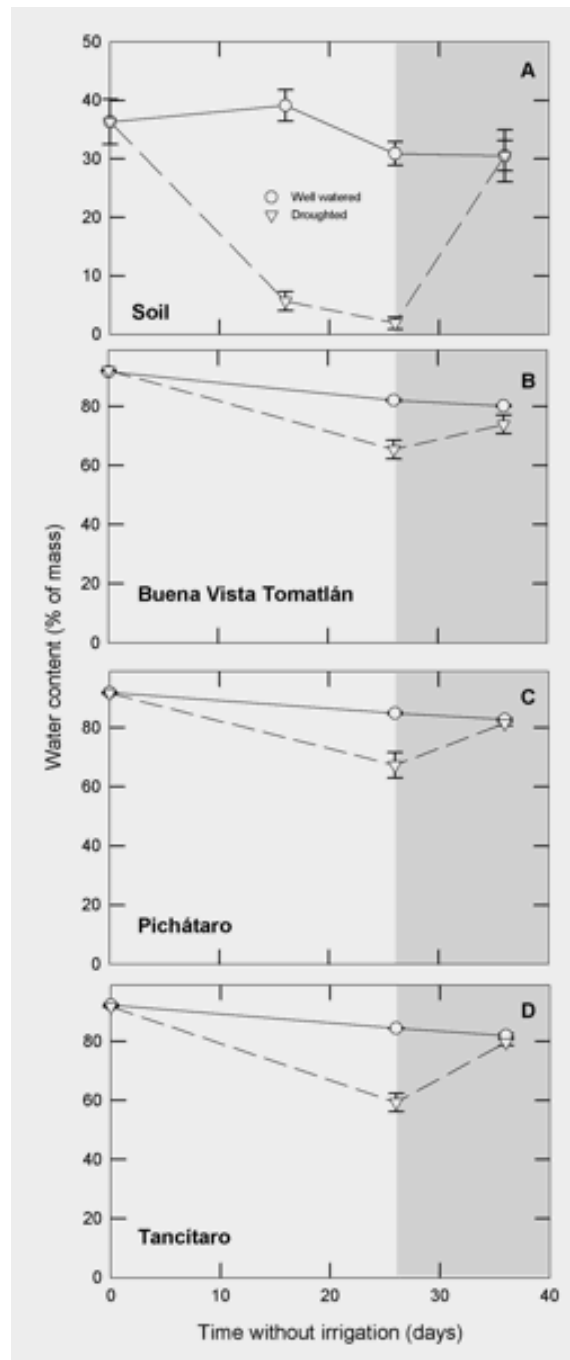
### Materials and Methods

Seeds of locally bred maize were obtained during Spring 2010 directly from producers from three



**Figure 1** - Climate diagrams for Buenavista Tomatlán (A), San Francisco Pichátaro (B), and Tancitaro (C), the localities where maize seeds were acquired for this study. The altitude, annual mean air temperature, and annual precipitation are shown in each panel. Mean monthly air temperature (solid line) is indicated in the left ordinate where the boldface temperatures are the mean maximum temperature of the highest month (usually May) or the mean minimum temperature of January, the coldest month. Monthly precipitation (dashed line) is indicated in the right ordinate, where the mean monthly precipitation deficit is indicated in boldface (number of months shown in parentheses). Said precipitation deficit occurs when the precipitation line is below the temperature line for a given month (Larcher 2004). The annual precipitation deficit (mm) is indicated at the bottom of each panel.

localities along an elevational gradient in the state of Michoacán, Mexico (Buenavista Tomatlán, at 19°17' 7.9"N;102°33'30.9"W, 689 m; San Francisco Pichátaro, at 19°34'18.34"N;101°48'40.8"W, 2,379 m; Tancitaro, at 19° 25' 3.66"W, 3,638 m). The seeds were transported to the laboratory at the Centro de Investigaciones en Ecosistemas, UNAM Campus Morelia (19°42'10"N;101°11'32"W, 1,980 m), where they were kept in the dark (25 °C, 42% relative humidity)



**Figure 2** - Soil (A) and leaf tissue gravimetric water content over the course of the water withholding experiment for well-watered (circles) and droughted (triangles) maize seedlings from Buenavista Tomatlán (A), Pichátaro (B), or Tancitaro (C). Data are shown as mean  $\pm$  S.E. (n = 20 seedlings). The gray region starting at 26 d after withholding irrigation indicates the period of recovery during which watering was resumed.

until utilized. For each maize, 20 experimental units were prepared consisting of 3 seeds planted in black plastic nursery bags (20 cm wide  $\times$  35 cm high) that contained a 3:1 blend of sand and a commercial potting mixture (Creciroot, Sistemas Agrotec, Uruapan,

Michoacán, Mexico). Each bag was inoculated with micorrhizae (0.5 g per bag of *Glomus intraradices* and *Glomus fasciculatum*; Biotecnología Agroindustrial, Morelia, Michoacán, Mexico) and placed in a greenhouse where they were watered every other day with tap water. After ten days of planting, two seedlings were removed from each bag so that only the most robust individual was utilized for the remainder of the experiment. At 21 days of planting, irrigation was suspended for 26 days for half of the plants, followed by resuming of irrigation over a 10 day recovery period.

Climate normals were obtained for each site of collection from a database kept by the Washington State University, US Forest service Moscow Forestry Sciences Laboratory (<http://forest.moscowfsl.wsu.edu/climate/>), whose data for the state of Michoacán have been carefully curated (Sáenz-Romero et al, 2010). Ombrothermic diagrams were prepared from each dataset in order to characterize the prevailing environmental conditions of each site of collection (Larcher, 2003), including periods of drought and a so called annual precipitation deficit was calculated for each site consisting of the amount of supplementary precipitation that would be required to compensate for the periods of drought.

Plant growth was monitored by periodically harvesting random individuals for which fresh and dry mass were determined gravimetrically. After harvesting, the leaves and roots were removed from the shoot. The roots were immediately washed with a solution of commercial kitchen detergent in order to dissolve soil aggregates attached to the roots, which then were thoroughly rinsed with tap water. Plant organs were weighed and dried in a gravity convection oven at 80°C until constant weight was achieved.

Colorimetric assays were utilized to measure leaf metabolites during this experiment. In particular, chlorophyll, extracted with a cold aqueous solution of acetone (80% v/v) was determined following Lichtenthaler (1987). The content of proline, an amino acid that accumulates in droughted plants, was measured via reaction with ninhydrin (Reigosa, 2001; Steubing et al, 2001). In turn, total polyphenols were quantified by the Folin-Ciocalteu method (Waterman and Mole, 1994), while the total antioxidant activity of the leaf tissue was determined from the reaction with DPPH (Brand-Williams et al, 1995; Sánchez-Moreno et al, 1998).

## Results

### Climate at the regions of origin

The climates of the localities from which the seeds were obtained differed among each other (Figure 1). In particular, for Buenavista (Figure 1A), at the lowest altitude of 689 m, the mean annual temperature was 26.2°C and an annual precipitation of 793 mm was seasonally distributed, with 91% occurring in 5 months. In turn, the high temperatures observed over the remaining seven months of the year led to an annual precipitation deficit of 261 mm, equivalent of 33% of the annual precipitation. For the case of Pichátaro (Figure 1B), at 2,397 m, a lower mean annual temperature of 15.3 °C and a higher annual precipitation of 983 mm led to a precipitation deficit of only 9% of the annual precipitation, which was also seasonally distributed, 88% occurring over six months. Finally, for Tancítaro (Figure 1C), at the highest elevation of 3,638 m, the lowest mean annual temperature of 9.5°C and the highest annual precipitation of 1,295 mm were recorded. At this site, despite the fact that precipitation was also highly seasonal, with 84% occurring in 5 months, the precipitation deficit amounted to a mere 0.3 % of the annual precipitation and was only observed in March.

### Soil water status and leaf water content

Withholding of water effectively led to a soil water environment that greatly contrasted with that of well-watered individuals. In particular, the gravimetric soil water content for well-watered plants averaged 33.8 ± 1.7% over the course of the experiment, while that for the droughted experimental units decreased to 1.9 ± 1.0% at 26 d of drought and quickly recovered to well-watered levels once irrigation was resumed.

Leaf water content (Figure 2B-D) significantly differed among the maizes over the course of the experiment and responded to the watering regime (Table 1). The interactions of time of drought × site of origin and of time of drought × treatment were also significant. In turn, no significant interaction was found for site of origin × treatment. Regardless of the site of origin, leaf water content was very similar between droughted and well watered plants at the start of water withholding. It decreased for the droughted individuals at 26 d of withholding water. The contrast was greater for the individuals from Tancítaro, the site with the highest precipitation, than for the maizes from the other two localities (Figure 1D). Once irrigation was resumed, droughted individuals recovered to tissue

Table 1 - Generalized linear model for maize responses to water withholding.

	d.f.	Leaf tissue water content		Biomass						Chl		Proline		Phenols		Antioxydant activity	
		F	P	Root		Shoot		Leaf		F	P	F	P	F	P	F	P
				F	P	F	P	F	P								
Site of origin	2	3.592	0.029	16.32	< 0.001	0.01	.991	4.92	0.008	6.95	0.001	196.71	< 0.001	5.21	0.006	32.44	< 0.001
Time without irrigation	2	194.95	< 0.001	110.09	< 0.001	58.54	< 0.001	81.59	< 0.001	24.48	< 0.001	204.22	< 0.001	113.31	< 0.001	59.38	< 0.001
Treatment	1	105.20	< 0.001	46.50	< 0.001	53.54	< 0.001	39.81	< 0.001	0.081	0.776	282.26	< 0.001	10.57	0.001	0.010	0.919
Site × Time	4	0.03	0.032	4.15	0.003	2.90	0.022	0.76	0.551	4.26	0.002	223.38	< 0.001	1.87	0.116	37.47	< 0.001
Site × Treatment	2	0.27	0.273	4.23	0.015	0.14	0.866	2.89	0.057	0.75	0.473	195.96	< 0.001	3.75	0.025	1.60	0.203
Time × Treatment	2	64.21	< 0.001	16.04	< 0.001	17.10	< 0.001	12.56	< 0.001	0.45	0.641	204.94	< 0.001	13.35	< 0.001	0.10	0.901
Site × Time × Treatment	4	2.101	0.080	1.54	0.191	1.89	0.113	2.16	0.074	1.90	0.110	223.56	< 0.001	1.19	0.317	0.79	0.531

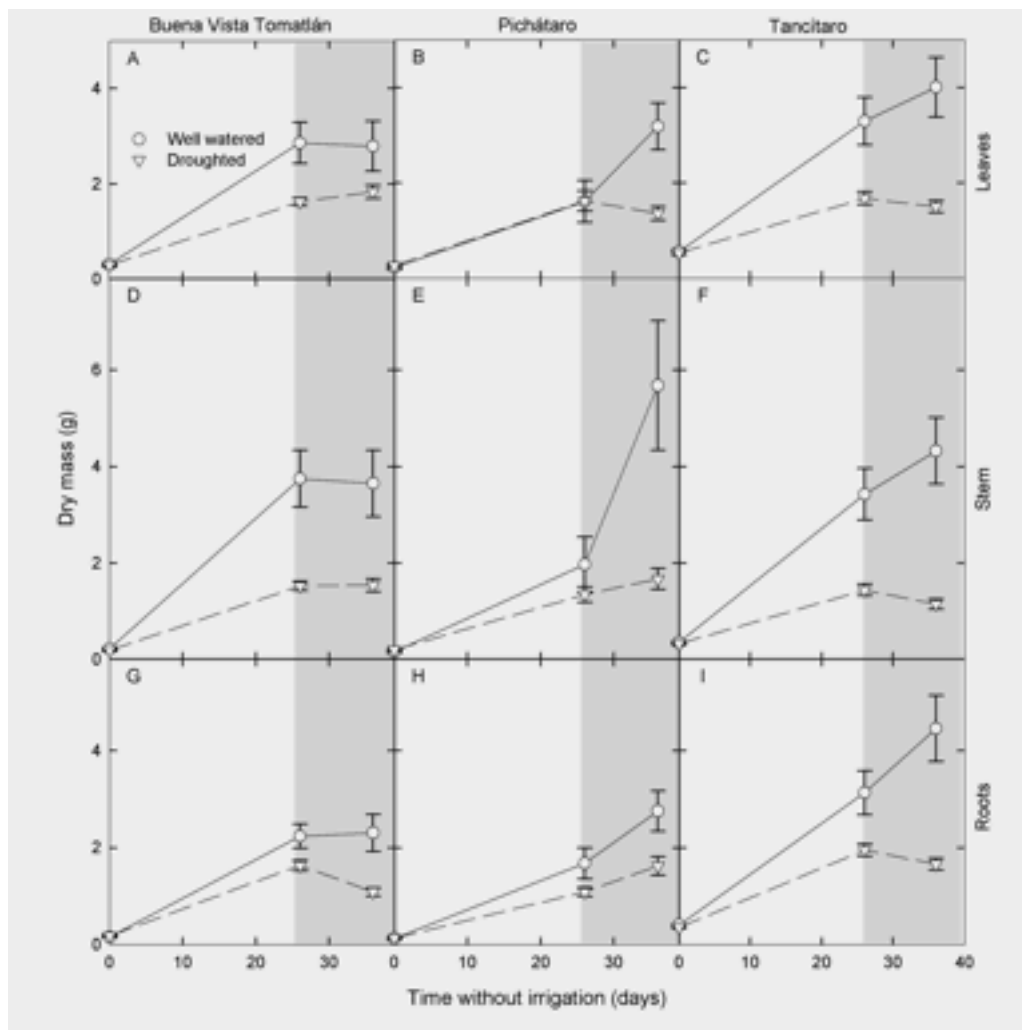
water contents similar to those of the control.

Dry mass accumulation for leaves was reduced by drought, while a significant interaction was found for time  $\times$  treatment (Table 1; Figure 2A-C). The leaf dry mass for plants from Buenavista appeared to plateau at ca. 3 g/leaf (Figure 2A). In contrast, leaf dry mass accumulation for the plants from Pichátaro appeared to accelerate once irrigation was resumed (Figure 2B). In turn, leaf dry mass appeared to increase at a constant rate for well watered plants from Tancítaro, while the droughted plants remained stunted even after watering was resumed (Figure 2C). Dry mass accumulation by shoots varied significantly over the course of the experiment between the well-watered and droughted individuals regardless of their site of origin (Table 1; Figure 2D-F).

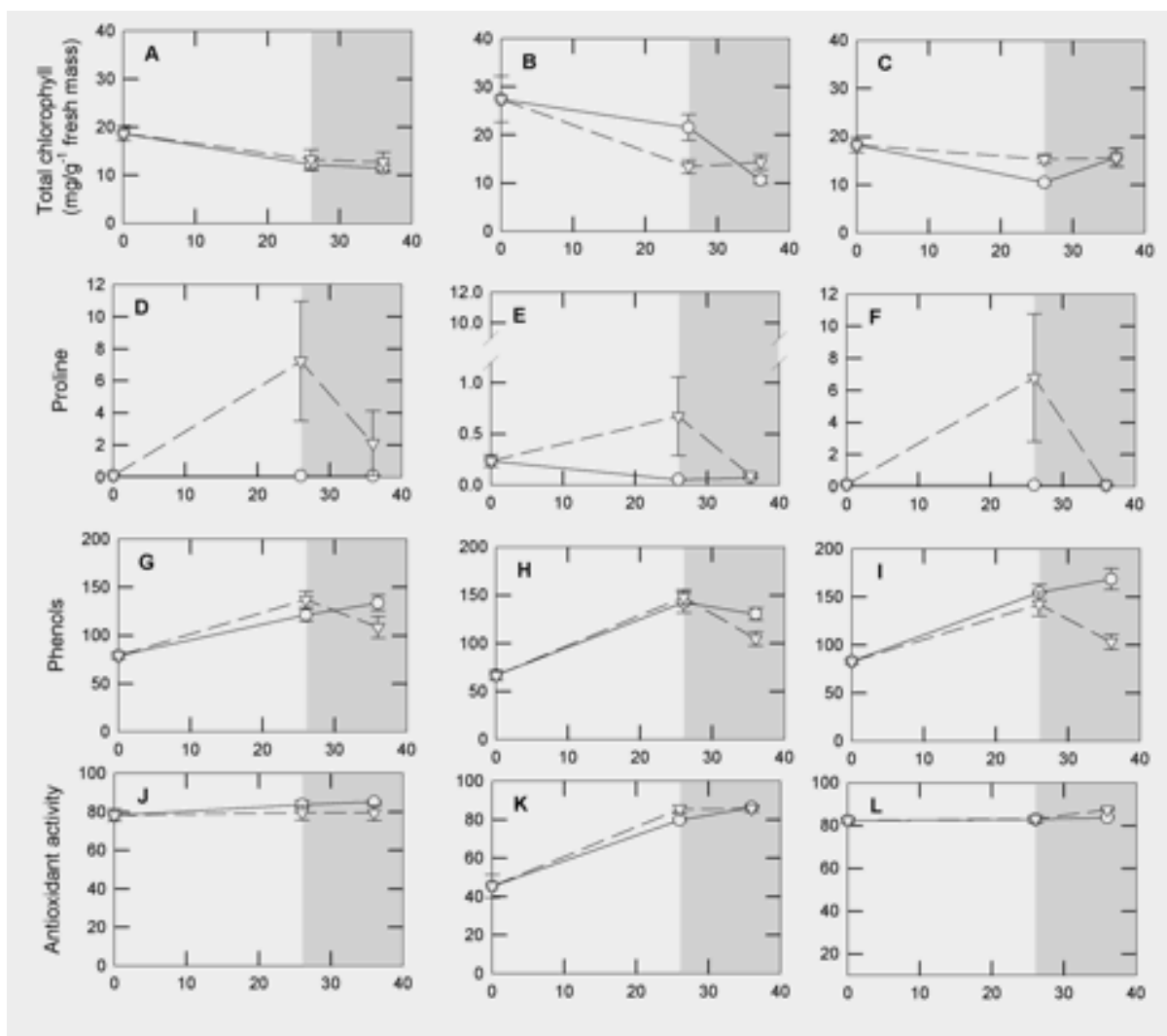
Root dry mass accumulation significantly differed among the maizes over the course of the experiment and between the watering regimes (Table 1; Figure

2G-I). In addition, all the pairwise interactions were significant, but this was not the case for the three-factor interaction. The roots of well-watered plants from Buenavista were the lightest and their dry mass appeared to plateau at ca. 2 g plant<sup>-1</sup> (Figure 2G). In contrast, the roots of well-watered plants from Pichátaro (Figure 2H) and Tancítaro (Figure 2I) appeared to grow at constant rates over the course of the experiment. In all cases, water withholding reduced biomass accumulation for droughted individuals in comparison to their well-watered counterparts. A significant increase of root dry mass following re-watering was only observed for the plants from Pichátaro (Figure 2H).

Leaf chlorophyll content significantly differed among the varieties utilized over the course of the experiment (Table 1). Although no response to the watering regime was observed, a significant interaction of site of origin  $\times$  time without watering was found. In



**Figure 3** - Dry mass accumulation by leaves (A-C), shoots (D-F), and roots (G-I) of well-watered (circles) and droughted (triangles) maize seedlings from Buenavista Tomatlán (A, D, G), Pichátaro (B, E, H), or Tancítaro (C, F, I) over the course of the water withholding experiment. Data are shown as mean  $\pm$  S.E. ( $n = 20$  seedlings). The gray region starting at 26 d after withholding irrigation indicates the period of recovery during which watering was resumed.



**Figure 4** - Leaf tissue concentration of total chlorophyll (A-C), proline (D-F), and total phenols (G-I), as well as antioxidant activity (J-L), for well-watered (circles) and droughted (triangles) maize seedlings from Buenavista Tomatlán (A, D, G, J), Pichátaro (B, E, H, K), or Tancítaro (C, F, I, L) over the course of the water withholding experiment. Data are shown as mean  $\pm$  S.E. ( $n = 20$  seedlings). The gray region starting at 26 d after withholding irrigation indicates the period of recovery during which watering was resumed.

general, the chlorophyll concentration tended to decrease over the course of the experiment, especially for the plants from Pichátaro (Figure 4B).

For the leaf content of proline, the three-way interaction of site of origin  $\times$  time without irrigation  $\times$  watering regime was significant (Table 1). However, some patterns could be observed (Figure 4D-F). For instance, no significant change in the very low proline concentration was observed for leaves of well-watered plants over the course of the experiment. In addition, the tissue concentration of proline appeared to increase for the three maizes at 26 d of withholding water, although the change was not significantly greater than for the well watered individuals. Finally, once irrigation was resumed, the proline concentration returned to be indistinguishable between the leaves of droughted plants and those of the well-

watered control.

The content of total polyphenols in leaves also varied among the maizes considered in the present work over the course of the experiment and responded to water withholding (Table 1; Figure 4G-I). In this case, the interactions of watering treatment  $\times$  site of origin and of watering treatment  $\times$  time without irrigation were statistically significant. In turn, phenol content did not depend on the time without irrigation. The leaf content of total phenols tended to increase over the course of the experiment for the well watered individuals. In contrast, resuming irrigation led to a decrease of the leaf content of total phenols for the droughted plants, both relative to the levels measured at the end of the drought period and, especially, after resuming irrigation relative to the well watered individuals.

The antioxidant activity of the maizes considered

in the present work did not respond to water withholding, but varied among the maizes and changed over the course of the experiment (Table 1; Figure 4J-L). However, the fact that the interaction of site of origin  $\times$  time was statistically significant probably reflects the distinct behavior of plants from Pichátaro, for which the antioxidant activity was about half than that measured for the other two maizes at the onset of the experiment.

## Discussion

Short-term drought responses were studied for three maizes from localities with contrasting environments. Sensitivity to water withholding was generally greater for the plants from sites at higher elevations, i.e., with higher precipitations and lower air temperatures, in comparison with those from lower elevations. Moreover, the prevailing environmental conditions of low water availability and high air temperature over the course of the experiment were similar to those found, at least during part of the year, in Buenavista and Pichátaro, but not in Tancítaro.

It was not surprising that the maize from Buenavista, the lowest site, was able to maintain a higher water content after 26 days of water withholding than its counterparts from the more mesic localities. A common characteristic of C4 grasses, such as maize, is a high water use efficiency resulting from its Kranz anatomy that allows the plants to achieve higher rates of CO<sub>2</sub> uptake at lower stomatal conductances than those measured for C3 species (Nobel, 2009). However, certain plant traits such as stomatal frequency, leaf surface area, or, in the case for the current study, leaf dry mass, have also been observed to respond to water availability over the course of plant development (Subramanian et al, 1997; DaCosta and Huang, 2009).

Leaf chlorophyll content for the three maizes considered in the present work tended to decrease with plant age, not in response to drought. This is contrasting with observations for durum wheat, a C3 species, for which a decrease in photosynthetic pigments follows week-long droughts (Zaefyzadeh et al, 2009). In any case, different susceptibility has been observed to occur for maize leaf chlorophyll during drought depending on the cultivar (Khayatnezhad and Gholamin, 2012). An early response mediated by abscisic acid could contribute to pigment persistence (Subramanian et al, 1997; Tenopala et al, 2012). As discussed above, precipitation in the state of Michoacán has a markedly seasonal precipitation, which ultimately determines the agricultural calendar. However, even within the wet season periods without precipitation can last up to a few weeks and create effective short-term droughts (Granados-Ramírez and Longar Blanco, 2008). It is likely that these local landraces of crops have been selected to be able to cope with intermittent periods of high irradiance and lower soil water availability, which should be confirmed experi-

mentally. This could inform future breeding programs considering the reduction in precipitation that is expected to occur during the current century.

Proline accumulation, along with that of trehalose and other organic compounds, is a well known indicator of drought sensing and tolerance in plants (Valliyodan and Nguyen, 2006; Krasensky and Jonak, 2012; Tenopala et al, 2012). In the present study, the droughted individuals tended to accumulate substantially more proline than the well-watered control. However, no significant differences from the control were detected, given the very high dispersal of the data for droughted individuals. This could be a result of a relatively high genetic diversity of landraces. Indeed, from preliminary observations conducted during the setup of the analytical technique, it was found that the dispersal of the proline content was minimal for a hybrid maize in comparison with a local landrace.

Drought, like other environmental stresses, favors the production of reactive oxygen species, which can lead to cell damage (Larcher, 2003; Miller et al, 2010; Gill and Tuteja, 2010). Polyphenols are among numerous leaf compounds with antioxidant activity (Cuvelier et al, 1992; Skerget et al, 2005; Gill and Tuteja, 2010). In the present study, total polyphenols tended to increase over the course of the experiment for well watered individuals in contrast with the plants that had water withheld, whose concentrations of total polyphenols actually decreased. This contrasts with what has been observed with *Salvia officinalis*, a species with low tolerance to drought, whose phenol content increases with time after withholding water (Bettaieb et al, 2001). Our observations could reflect the involvement of constitutive polyphenols in chemical reactions that ultimately protect the plants against oxidation. In turn, the fact that the difference among treatments was greatest (the rewatered plants had much less phenols than the well watered control) could be an indication of phenols being utilized for synthesizing new compounds utilized for drought recovery. Indeed, plant phenols can give origin to numerous compounds (Haslam and Cai, 1994).

Similar to the lack of accumulation of phenols during drought, measurements of the antioxidant activity of aqueous extracts of the maizes considered in the present work suggest that the imposed short-term drought did not induce oxidative stress on the plants (Larcher, 2003). Indeed, the antioxidant activity remained stable during the experiment. A most notable exception was the initial measurement for plants from Pichátaro, whose antioxidant activity was about half of the values observed for the rest of measurements and landraces. This maize also had an initial chlorophyll concentration that was ca. 50% higher than that of the other two landraces. In this respect, a most generous source of free radicals is a saturated photosynthetic machinery (Taiz and Zeiger, 2002; Larcher, 2003; González-Salvatierra et al, 2010). For the case

of the maize from Pichátaro, the initial absence of antioxidant activity may have been compensated by an initial greater capacity to absorb and assimilate more radiation than at later parts of the experiment.

Climate change projected to occur during the present century within the inter tropical region involves changes in precipitation patterns that are likely to be more severe than those expected from a mere increase of air temperature (Magaña et al, 2000; Sáenz-Romero et al, 2010). Prolonged drought, thus, is a real and severe risk for agriculture, even at sites that, like the state of California in the USA, usually have access to irrigation (Howitt et al, 2014). Such risk is greater for countries or regions where rain fed agriculture is predominant, such as Mexico (Turrent Fernández et al, 2013). Whether subsistence agriculture will be able to cope with the risks of climate change will depend both on the adaptation capacity of producers and on the selection for tolerance in different crops to the future adverse conditions.

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