Maydica

a journal devoted to maize and allied species

Maydica

VOL 60.2 - M 17

Short-term drought responses by seedlings of three maizes along an environmental gradient in Michoacán, Mexico

Zue D. Guerrero-Jiménez[1,2], Erick de la Barrera[2*]

[1]Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Ciudad Universitaria, México, DF 04510, Mexico

[2]Instituto de Investigaciones en Ecosistemas y Sustentabilidad, Universidad Nacional Autónoma de México, Morelia, Michoacán 58190, Mexico

*Corresponding author: E-mail: delabarrera@unam.mx

Abstract

Responses to drought by three maizes from Michoacán, Mexico, were studied in a greenhouse experiment. Plants were watered every other day for 21 d since sowing. Watering was withheld during 26 d, following 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% of the net annual precipitation at the lowest site and 9% and 0.3% for the sites at higher elevations. Substrate water content for the control was 34% (w/w) over the course of the experiment and it decreased to 1.9% at 26 d of water withholding; it quickly recovered after resuming irrigation. Tissue water content was 92% for the control and decreased by 22-33% depending on origin for the droughted individuals at the peak of the drought. Dry mass was lower for droughted individuals than for the control. Leaf chlorophyll content decreased with plant age, but did not respond to water withholding. In contrast, proline tended to increase for droughted individuals. The content of free-radical scavenging polyphenols decreased during the recovery period, but not during drought. Neither did the antioxidant activity of leaf tissue respond to water withholding.

Keywords: *adaptation, annual precipitation deficit, 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 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. In contrast, up to 80% of maize cultivation (area basis) in Mexico is conformed by small scale, low input, subsistence operations, for which a typical yield rarely exceeds 2 tons ha^{-1} $year^{-1}$ (Servicio de Información Agroalimentaria y Pesquera, 2015).

Approximately two thirds of Mexico's territory is 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, 2015). 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 microenvironents (Perales 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. A hypothesis was tested that an environmental specialization has occurred for the maizes considered here along an altitudinal gradient.

Materials and Methods

Seeds of locally bred maize were obtained during March 2010 directly from producers from three 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", 2,379 m; Tancítaro, at 19°25'3.66", 3,638 m). The seeds were transported to the laboratory at the Instituto de Investigaciones en Ecosistemas y Sustentabilidad, UNAM (19°42'10"N; 101°11' 32"W, 1,980 m), where they were kept in the dark (25°C, 42% relative humidity) until utilized. For each maize, 20 experimental units were prepared consisting of 3 seeds planted in black plastic nursery bags (20 cm wide × 35 cm high) that contained a 3:1 blend of sand and a commercial potting mixture (Creciroot, Sistemas Agrotec, Uruapan, Mich, Mexico). Each bag was inoculated with micorrhizae (0.5 g per bag of *Glomus intraradices* and *Glomus fasciculatum*; Biotecnología Agroindustrial, Morelia, Mich, 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.

limate 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 for each dataset in order to characterize the prevailing environmental conditions of each site of collection (Larcher, 2003), including periods of drought. 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 80ºC 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). Statistical analyses were conducted with SigmaStat (SPSS Science, Chicago, IL, USA). Differences among maizes were assessed with a 3-way ANOVA followed by pairwise Tukey tests ($p < 0.05$). Data are shown as mean ± 1 S.E. (n = sample size).

Results

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 to 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 ºC 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.

Withholding of water effectively led to a soil water environment that greatly contrasted with that of well-watered individuals (Figure 2A). 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 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 × treatment (Table 1; Figure 2A-C). The leaf dry mass for plants from Buenavista appeared to plateau at ca. 3 g leaf^{-1} (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 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^{-1} (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 rewatering 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 × time without watering was found. In 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 × time without irrigation × 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.

Total polyphenol content 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 × site of origin and of watering treatment × 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 × 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 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 their Kranz anatomy that allows the plants to achieve higher rates of CO_2 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 clorophyll 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 experimentally. 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; 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; Gill and Tuteja, 2010; Miller et al, 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 droughted plants, whose concentrations of total polyphenols actually decreased. This contrasts with what has been observed with *Salvia oficinalis*, a species with low tolerance to drought, whose phenol content increases with time after withholding water (Bettaieb et al, 2011). 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 polyphenols 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 whose consequences 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.

Acknowledgements

This work was funded by Consejo Nacional de Ciencia y Tecnología (CONACYT FOMIX-MICH 2009-127128) and Dirección General del Personal Académico, UNAM (PAPIIT IN224910, RN204013). ZDGJ received a graduate fellowship from CONACYT. We thank useful discussions with Drs. R. Cruz and F. Espinosa.

References

Bettaieb I, Hamrouni-Sellami I, Bourgou S, Limam F, Marzouk B, 2011. Drought effects on polyphenol composition and antioxidant activities in aerial parts of *Salvia officinalis* L. Acta Physiologiae Plantarum 33: 1103-1111

Brand-Williams W, Cuvelier ME, Berset C, 1995. Use of a free radical method to evaluate antioxidant activity. LWT Food Science and Technology 28: 25-30

Cuvelier ME, Richard H, Berset C, 1992. Comparison of the antioxidative activity of some acid-phenols: structure-activity relationship. Bioscience, Biotechnology, and Biochemistry 56: 324-325

DaCosta M, Huang B, 2009. Physiological adaptations of perennial grasses to drought stress. In: de la Barrera E, Smith WK (eds.) Perspectives in Biophysical Plan Ecophysiology: A Tribute to Park S. Nobel. Universidad Nacional Autónoma de México. Pp. 169-190.

Food and Agriculture Organization. 2015. Climate Smart Agriculture. Sourcebook. Food and Agriculture Organization. Available online at http://www.fao.org/3/a-i3325e.pdf (retrieved on 1 Februrary 2015).

Gill SS, Tuteja N, 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry 48: 909-930

González-Salvatierra C, Andrade JL, Escalante-Erosa F, García-Sosa K, Peña-Rodríguez LM, 2010. Antioxidant content in two CAM bromeliad species as a response to seasonal light changes in a tropical dry deciduous forest. Journal of Plant Physiology 167: 792-799

Granados-Ramírez R, Longar Blanco MP, 2008. Variabilidad pluvial, agricultura y marginación en el estado de Michoacán. Análisis Económico 54: 283-303

Haslam E, Cai, 1994. Plant Polyphenols (Vegetable Tannins*): Gallic Acid Metabolism. Natural Product Reports 11: 41-66

Howitt R, Medellin-Azuara J, Lund J, MacEwan D, 2014. Preliminary 2014 Drought Economic Impact Estimates in Central Valley Agriculture. Report prepared for the California Department of Food and Agriculture

Khayatnezhad M, Gholamin R, 2012. The effect of drought stress on leaf chlorophyl content and stress resistance in maize cultivars (*Zea mays*). African Journal of Microbiology Research 6: 2844-2848

Larcher, 2003. hysiological Plant Ecology: Ecophysiology and Stress Physiology of Functional Groups. Springer

Lichtenthaler HK, 1987. Chlorophylls and carotenoids: pigments of photosynthetic membranes, pp. 350-382. In: Methods in

Enzimology, Plant Membranes, vol 148. Packer L, Douce R Eds.Academic Press, San Diego, California

Magaña V, Conde C, Sánchez O, Gay C, 2000. Evaluación de escenarios regionales de clima actual y de cambio climático futuro para México, pp. 9-26. In: Mexico: una visión hacia el siglo XXI. El cambio Climático en México. Gay García C Ed. Instituto Nacional de Ecología. Universidad Nacional Autónoma de México

Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R, 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant, Cell & Environment 33: 453-467

Miranda Colín S, 2005. El origen genético y geográfico del maíz (*Zea mays* L), pp. 147–159. In: Centli Maíz. Colegio de Postgraduados. Muñoz Orozco A ed. Texcoco, Mexico

Nobel PS, 2009. Physicochemical and Environmental Plant Physiology. 4th edn. Academic Press, Elsevier

Perales H, Golicher D, 2014. Mapping the Diversity of Maize Races in Mexico. PLOS One 9:e114567

Reigosa JR, 2001. Handbook of plant ecophysiology techniques. Kluwer Academic Press

Sáenz-Romero C, Rehfeldt GE, Crookston NL, Pierre D, St-Amant R, Bealieau J, Richardson B, 2010. Spline models of contemporary, 2030, 2060, 2090 climates for Mexico and their use in understanding climate-change impacts on the vegetation. Climate Change 102:595-623

Sánchez-Moreno C, Larrauri JA, Saura-Calixto FA, 1998. F.A. procedure to measure the antirradical efficiency of polyphenols. Food Research International 76: 270-276

Servicio de Información Agroalimentaria y Pesquera, 2015. Database available online at www.siap.gob.mx (retrieved on 1 Februrary 2015)

Skerget M, Kotnik P, Hadolin M, Rizner Hras A, Simonic M, Knez Z, 2005. Phenols, proanthocyenidins, flavones and flavonols in some plant materiasl and their antioxidant activities. Food Chemistry 89: 191-198

Steubing L, Godoy R, Alberdi M, 2001. Métodos de ecología vegetal. Editorial Universitaria, Chile

Subramanian KS, Charest C, Dwyer LM, Hamilton RI, 1997. Effects of arbuscular mycorrhizae on leaf water potential, sugar content, and P content during drought and recovery of maize. Canadian Journal of Botany 75: 1582-1591

Taiz L, Zeiger E, 2002. Plant Physiology. 3rd ed, Sinauer Press

Tenopala J, González FJ, de la Barrera E, 2012. Physiological responses of the green manure, *Vicia sativa*, to drought. Botanical Sciences 90: 305-311

Turrent Fernández F, Wise TA, Garvey E, 2013. Achieving Mexico's Maize Potential. Food Sovereignity: A Critical Dialague. Conference Paper # 10, Yale University

Valliyodan B, Nguyen HT, 2006. Understanding regulatory networks and engineering for enhanced drought tolerance in plants. Current Opinion in Plant Biology 9: 189-195

Waterman G, Mole S, 1994. Analysis of phenolic plant Metabolites. Oxford University Press

Zaefyzadeh M, Quiliyev RA, Babayeva SM, Abbasov MA, 2009. The effect of the interaction between genotypes and drought stress on the superoxide dismutase and chlorophyll content in durum wheat land races. Turkish Journa of Biology 33: 1-7

Table 1. ANOVAs for maize responses to water withholding.

	d. f.	Leaf tissue water content		Biomass						Chl		Proline		Phenols		Antioxydant activity	
				Root		Shoot		Leaf									
		F	P	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	0.991	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

Figure 1 - Climate diagrams for Buenavista Tomatlán (A), San Francisco Pichátaro (B), and Tancítaro (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). 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.

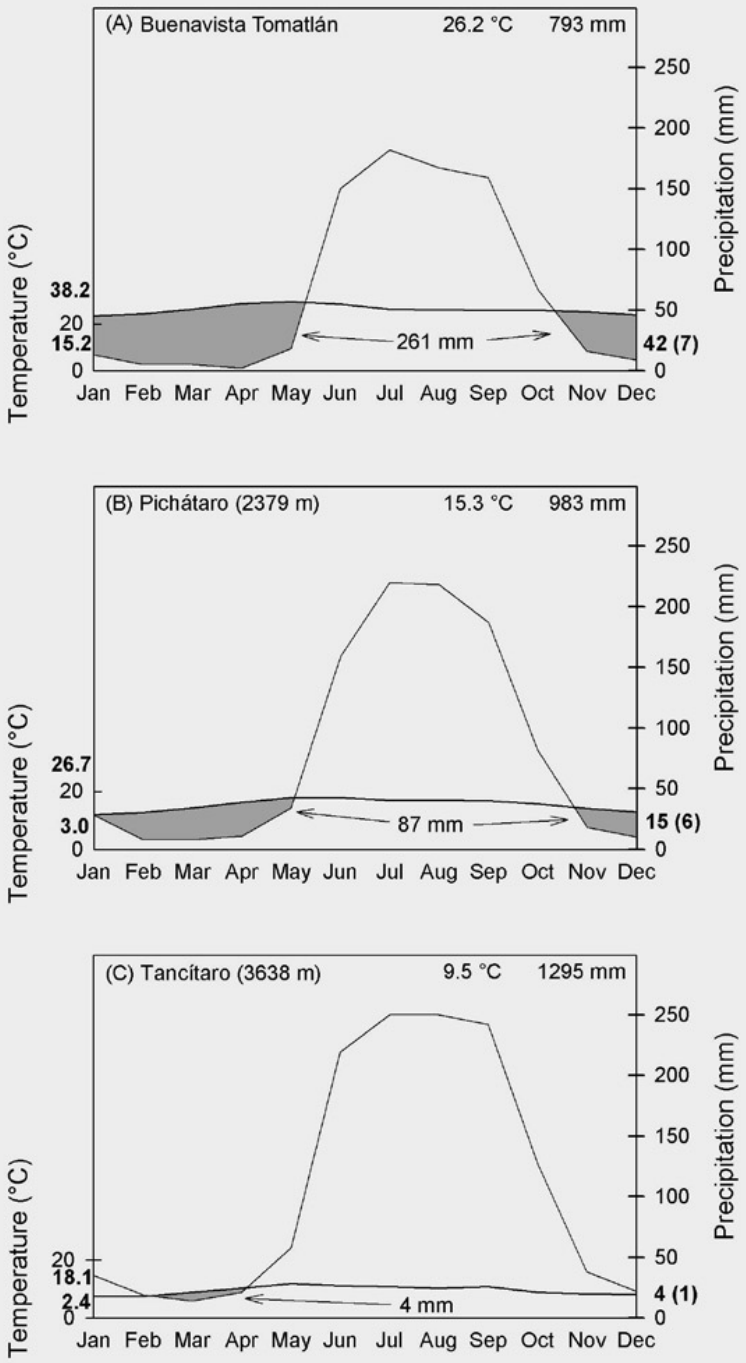

Figure 2 - Soil (A) and leaf tissue gravimetric water content over the course of the water withholding experiment for well-watered (circles) and droughted (triangles) maize seedlings from Buenavista (A), Pichátaro (B), or Tancítaro (C). Data are shown as mean ± S.E. (n = 20 seedlings). The gray region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

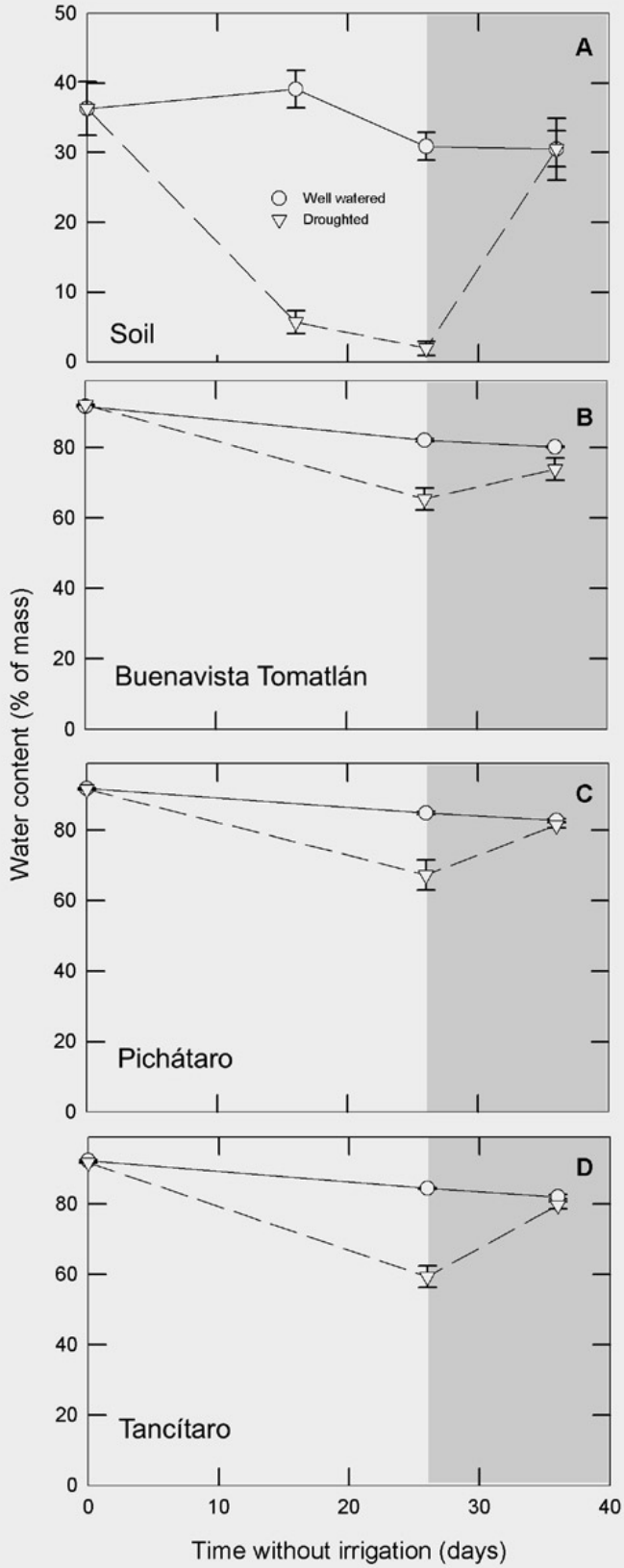

Figure 3 - Dry mass accumulation by leaves (A-C), shoots (D-F), and roots (G-I) of well-watered (circles) and droughted (triangles) maize seedlings from Buenavista (A, D, G), Pichátaro (B, E, H), or Tancítaro (C, F, I) over the course of the water withholding experiment. Data are shown as mean ± S.E.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

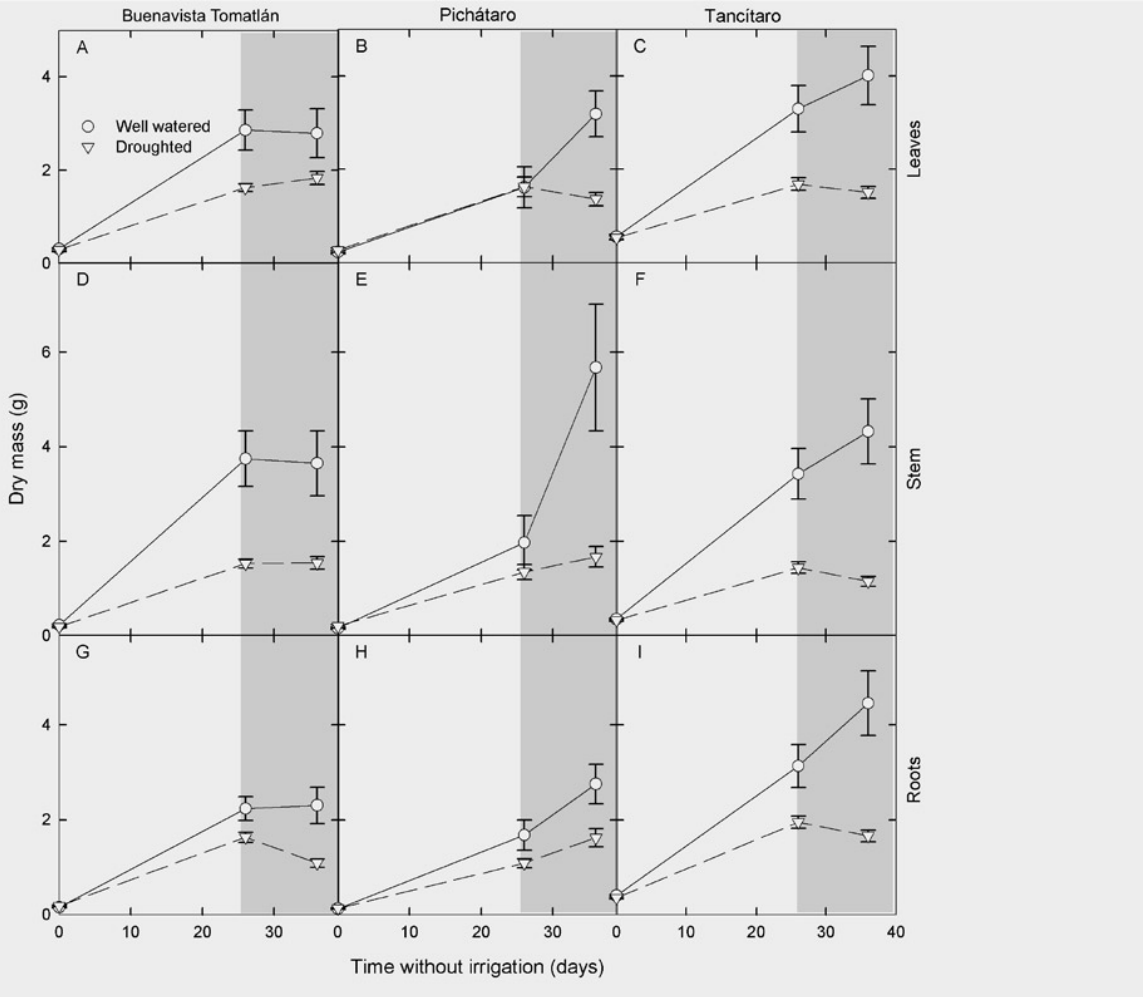

Figure 4 - Leaf tissue concentration of total chlorophyll (A-C), proline (D-F), and total phenols (G-I), as well as antioxidant activity (J-L), for well-watered (circles) and droughted (triangles) maize seedlings from Buenavista (A, D, G, J), Pichátaro (B, E, H, K), or Tancítaro (C, F, I, L) over the course of the water withholding experiment. Data are shown as mean ± 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.