Variability and mechanisms of drought tolerance in maize populations from Honduras

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Abstract

Maize breeding for drought tolerance requires searching mechanisms and sources of drought tolerance. We assessed drought tolerance at juvenile stages of development in a collection of open-pollinated maize populations from a dry area of Honduras. Eighteen Honduran populations were evaluated for the response of morphological, physiological and photosynthetic traits to increasing levels of water stress during germination, seedling and early growth. Water stress was imposed by using solutions of polyethylene glycol6000. The population Natual Olote Gordo faced drought by increasing root elongation; the population Langueño was able to maintain natural photosynthetic traits under drought conditions at a reasonable level; Capulin was drought tolerant at germination and early growth, and Maicito Montecristo maintained root biomass at seedling and early growth stages. At early growth, Castaño Sur, Maicito Montecristo, Negrito, Sangre de Cristo, and Tuy showed diminished conductance while there was no significant reduction in Langueño. Considering photosynthetic performance, the response to drought can be classified as resistance (Langueño) or tolerance (Capulín). Therefore, diverse mechanisms of drought adaptation are displayed by these populations depending on the feature measured and the stage of development. Different adaptive traits to drought at early stages of development could be combined by crossing the appropriate populations.

KeyWords *Zea mays L.; germplasm; water stress; photosynthesis; arid areas.*

Introduction

Drought is the main challenge of agriculture at a global scale (Boyer, 1982) and this problem is expected to worsen with climate change (Olesen et al., 2011; Chen et al., 2012). The severest effects of drought affect the dry tropical areas (Sivakumar et al., 2005), and challenges production and sustainability of farms and even the future of the local people (Douglas et al., 2015). Drought affects maize during the whole growth cycle, being critical at germination and early stages of development (Edmeades et al., 1989). In many tropical areas, maize can only be cultivated without irrigation during the humid station of the year; while the exiguous rainfall of the dry season does not allow growing maize without irrigation, which is often not available. Furthermore, large growth cycles and early sowings are avoided because they would imply severe drought stress. Breeding for drought tolerance is the most convenient approach for facing drought stress (Messmer et al., 2009). Even though drought tolerance has been increased by maize breeding in temperate areas (Cooper et al., 2014; Gaffney et al., 2015), most tropical areas have not benefited from the commercial breeding programs. Nevertheless, tropical maize has been successfully used for improving drought tolerance through classical breeding (Monneveux et al., 2006; Messmer et al., 2011).

Identifying base populations for breeding is the first step (Flint-Garcia et al., 2005), and the determination of the adaptive traits underlying drought tolerance is essential for designing the appropriate breeding approach. Maize variability for drought tolerance is limited within elite germplasm (Gouesnard et al., 2016), and tropical germplasm offers unexplored resources for breeding. Indeed, tropical maize populations were adapted to arid conditions since thousands of years ago. However, a significant amount of the autochthonous germplasm from arid areas of Central America has not been collected and studied, or is still not available in germplasm banks.

Most breeding programs for drought tolerance have

focused on yield and adult vegetative phases, while little attention has been devoted to drought tolerance at early stages of development, and drought tolerance at different stages of development is unrelated (Hall et al., 1984). Field evaluations of drought tolerance are not reliable because field conditions are unpredictable and heterogeneous; furthermore, a precise measurement of roots is not possible under field conditions (Whitmore and Whalley, 2009). Imposing water stress conditions with polyethylene glycol (PEG) is a reliable approach for screening water stress in germplasm collections, as several authors have previously demonstrated (Lu and Neumann, 1998; Ruta et al., 2010; Zhao et al., 2016; Álvarez-Iglesias et al., 2017) and the approach has been used for evaluation of drought tolerance at the juvenile phase (Khan et al., 2016; Álvarez-Iglesias et al., 2017). Based on these considerations, we have developed and validated a fast and accurate screening method for evaluating responses of maize germplasm to early drought conditions (Álvarez-Iglesias et al., 2017).

The objectives of the present work were 1) to assess drought tolerance at early stages of development (germination, seedling growth, and early growth) of a collection of open-pollinated tropical maize populations from an arid area of Honduras, and 2) to identify traits involved in maize drought tolerance at early stages of development.

Materials and Methods

Plant material

Sixteen open-pollinated maize populations (Acriollado Montecristo, Campesino, Capulín, Castaño Sur, Cuarentano, Dicta Sequía, Langueño, Maicito Montecristo, Maíz Amarillo, Natural Olote Gordo, Negrito, Olote Rojo, Quirrire Alonchano, Sangre de Cristo, Tizate, and Tuza Morada) were collected in San Lorenzo Valle (Honduras) and multiplied in isolated fields by local farmers. The 16 populations represent an exhaustive sampling of this municipality of 235 km² which climatic conditions during the maize growth period (from May to October) are relatively homogeneous, i.e. monthly average minimum temperature of 18 °C (ranging from 17.6 to 18.2), average maximum temperature of 28.5 °C (from 27.3 to 30.3), and average rainfall of 126.5 mm (from 82.3 to 177.2). However, during the preceding months, rainfall is considerably reduced, with values being 42.9 mm in April and 9.9 in March; therefore, early sowings face severe drought. These populations were adapted to the arid tropical area since ancestral times and thus were expected to show tolerance to drought.

Multiplications were made in 2014 by sowing several hundreds of plants from each population in diverse fields of the same area and harvesting more than 100 ears from the center of each field. The multiplied seeds were conserved in a dry storage facility at room temperature. Then, the 16 tropical populations were evaluated for seedling-related traits under drought conditions, along with two temperate populations (EPS5 and Tuy) used as reference as representing the base germplasm most used in our breeding programs. EPS5 is a maize synthetic representing the diversity of maize inbred lines unrelated to Iowa Stiff Stalk Synthetic; it was made by crossing 16 inbred lines (EP1, A556, EP19, A624, EA2087, A637, PB57, A652, PB60, A654, PB130, A662, A251, MS1334, A554, and W182) and recombining the resulting synthetic more than 10 times. Tuy is an open-pollinated maize population from the humid northwest of Spain. Based on previous observation, EPS5 shows a moderate tolerance to drought and Tuy is sensitive to drought but with high performance in the region where the trials were made.

Screening drought tolerance in maize at germination, seedling growth and early growth

Responses to drought were evaluated at three stages: germination (seeds), seedling development (pre-germinated seeds) and early growth (plants at the three-leaf stage). Water stress was imposed after Álvarez-Iglesias et al. (2017) by using aqueous solutions of PEG 6000 at different concentrations simulating slight, moderate and severe stress conditions (Table 1). All

Table 1. Water stress conditions simulated for the three stress levels used in the evaluation of 16 open-pollinated maize populations from Honduras, plus two checks from temperate areas.

Trial	Stress level[a]	[PEG 6000] (g/L)	Ψo (MPa) at 25 °C [b]
Germination	Slight	100	- 0.15
	Moderate	150	- 0.30
	Moderately high	200	- 0.49
	Severe	250	-0.73
Seedling growth	Slight	200	- 0.49
	Moderate	300	- 1.03
	Severe	350	- 1.37
	Moderate	300	- 1.03

[a] For each assay, there was a control treatment with Ψo = 0 MPa
[b] Ψo estimated following Michel and Kauffman (1973)

solutions were adjusted at pH = 6. Osmotic potentials were calculated following Michel and Kaufmann (1973).

Germination screening was made by placing 10 seeds from each population in 14 cm-Petri dishes with filter paper Whatman #2 imbibed with 10 ml of the corresponding solution or distilled water for the control

treatment and closed with parafilm. For each treatment, four repetitions were assigned to four shelves, respectively, of a growth chamber at 27°C and 75% relative humidity without light. The number of germinated seeds was counted every 12 h in each dish. From the data recorded, the total germination index (GT) as the percentage of germinated grains was calculated, as well as the speed of germination (S), speed of accumulated germination (AS), coefficient of rate of germination (CRG) and mean germination time (MGT), all following Chiapusio et al. (1997) and El-Siddig et al. (2004).

Seedling establishment was determined in four repetitions per treatment following the same scheme as the germination screening, by sowing 10 pre-germinated grains with similar radicle length (2-3 mm). Seeds were pre-germinated by placing 50 seeds from each population in 100 cm² boxes with filter paper Whatman #2 imbibed with distilled water. Seventy-two hours later, the shoot and main root lengths, the number of secondary roots and the fresh weight (FW) of the shoot, main root, and secondary roots were measured; finally, these fractions were separately dried 72 hours at 60 °C for recording their dry weights (DW). The total root biomass was calculated as the sum of DW of main root and secondary roots. The dry/fresh weight (DW/FW) ratio for each part and the shoot/total root DW ratio were also calculated.

The early growth assay had four repetitions per population and treatment and was performed in a greenhouse at temperatures ranging between 10 and 35 °C. The four repetitions were assigned to four successive tables of a greenhouse. Three grains per pot were sown in three one-liter pots containing a perlite/vermiculite mixture (2:1 v:v) placed in trays for irrigation. Pots were irrigated with water until coleoptile emergence and then with a half-strength Hoagland nutritive solution (H&A ½). When plants had fully developed the third leaf (V3 stage) only two plants were left in each pot. PEG 6000 was added to the nutritive solution of water stress-treated plants for reaching the intended osmotic pressure (Table 1), whereas control plants were watered with the H&A solution. Treatments were maintained for 96 h, and solutions were replaced daily from the trays in order to keep constant conditions. Every 24 hours, net assimilation of CO_2 (AN), stomatal conductance (g_s) and transpiration rate (E) were recorded in the third leaf of at least four plants per experimental unit by using a LI-6400XT Portable Photosynthesis System (Li-Cor Inc., Lincoln, NE, USA). Water use efficiency (WUE) was calculated as WUE = A_N/E. Irrigation and measurements were made daily at the same hour for limiting interactions with circadian cycles and environmental conditions (Berger et al., 2010). At the end of the assay, we recorded the FW of the roots and the aerial part, as well as the DW after drying during 72 hours at 60 °C. The third leaf of each plant was detached and weighed separately, and the leaf area (LA) was measured with a Leaf Area Meter CI-202 (CID Bio-Science Inc., Camas, WA, USA). The specific leaf area (SLA) was calculated as the ratio LA/leaf DW (Garnier et al., 2001). Relative water content (RWC) was determined on the fourth leaf of each plant according to Turner (1981).

Statistical analyses

For each population, germination and seedling growth data were expressed as a percentage with respect to the corresponding control in order to allow a standardized comparison among varieties, thus removing differences in quality of the seed and potential growth rates. For germination and seedling growth characters, the best-fit equation based on the coefficient of determination (R^2) was selected from the linear, logarithmic, inverse, quadratic, cubic, potential, growth and exponential models for each dose-response curve. The IC_{50} and IC_{80} values (concentrations required to reduce the expression of each trait 50 or 80% respectively from the control) were calculated from the generated equations. These statistical analyses were conducted by using the IBM SPSS Statistics 19.0 software package (IBM SPSS Inc., Chicago, IL).

Analyses of variance were made with stress and populations as sources of variation along with the corresponding interactions. Repetitions and their interactions were considered random effects, while populations were considered fixed effects. For the early growth assay, analyses of variance were made also for each population in order to test statistical differences among treatments. Mean comparisons were made with the Fisher's protected LSD at p= 0.05 for germination, seedling growth and early growth traits.

A repeated-measure analysis was performed to test differences in photosynthetic traits by using the PROC MIXED procedure of SAS software (2008) (Littell et al., 2006). First-order autoregressive covariance structure (AR-1) was chosen in the within-subject correlation. Differences among genotypes and among treatments using least square (LS) means adjusted by the initial traits' valued were tested at each sampling time. Additionally, linear and quadratic coefficients of regression of the photosynthetic traits over time were obtained for each treatment-genotype combination. Within each genotype, the comparison of the photosynthetic curves of two treatments was performed by making orthogonal contrasts between the two treatments regression pa-

rameters (intercept, linear and quadratic components, respectively) (p ≤ 0.05) (Littell et al., 1996; Littell et al., 2006).

Finally, principal component analyses (PCA) were performed with standardized data from the PEG 6000 screening assays, in order to synthesize all the measured variables into a limited number of principal components (PC). PCA analysis allows organizing populations based on their performance under water stress conditions. PCA was made with the PRINCOMP procedure of SAS. Four separated PCA were carried out for 1) germination traits, 2) seedling growth traits, 3) early growth traits, and 4) photosynthesis-related traits, respectively.

Results

Germination assays

The combined over treatments analyses of variance showed that population × treatment interactions were significant for all germination-related traits (data not shown). Under slight, moderate and severe stress conditions, populations were significantly different for all traits except GT at slight stress conditions.

A significant quadratic regression for GT over increasing water stress pressure was detected for all populations, with R^2 ranging from 0.459 for the Honduran population Capulín to 0.937 for the local check Tuy (Supplementary Table 1). Inhibitory indexes IC_{50} and IC_{80} were above the range of conditions included in this study for four Honduran populations indicat-

ing that the maximum of drought tolerance of these populations exceeds the range imposed in our assay. Conversely, the checks along with some populations reached the IC_{80} within the range of water stress conditions evaluated.

The effect of increasing water stress conditions on the indexes S, AS, CRG and MGT fitted to a quadratic model that was significant except for S index for the population Dicta. Excepting S index for Capulín, R^2 values were above 0.5 (Supplementary Table 2). Values of IC_{50} and IC_{80} for CRG and IC_{80} for MGT were above the detectable range of the assay for all populations (excepting three Honduran populations for MGT).

The first principal component for germination (PC1g) accounts for 93% of the variability (Fig. 1). For germination, the most discriminant stress level was moderately high (-0.49 MPa), wherein the most tolerant populations are Natural Olote Gordo, Olote Rojo, Maíz Amarillo, Cuarentano and Sangre de Cristo. Furthermore, Maíz Amarillo has the best performance under severe stress levels (-0.73 MPa) although this population cannot be considered as tolerant because its performance is reduced to 10% of the control value. Eight populations improved their germination under low-stress levels, but all populations reduced their performance as stress increased.

Seedling growth assays

For seedling growth assays, the combined over treatments analyses of variance showed that population × treatment interactions were significant for root

Figure 1 - Principal component analysis of germination-related traits (GT, S, AS, CRG, MGT) measured on 16 maize populations from an arid tropical Honduran region, plus two temperate checks evaluated in vitro under four increasing levels of water stress pressure (obtained with PEG 6000) plus a control. Samples were distributed according to the scores of the **principal component 1 for germination (PC1g) and scores**.
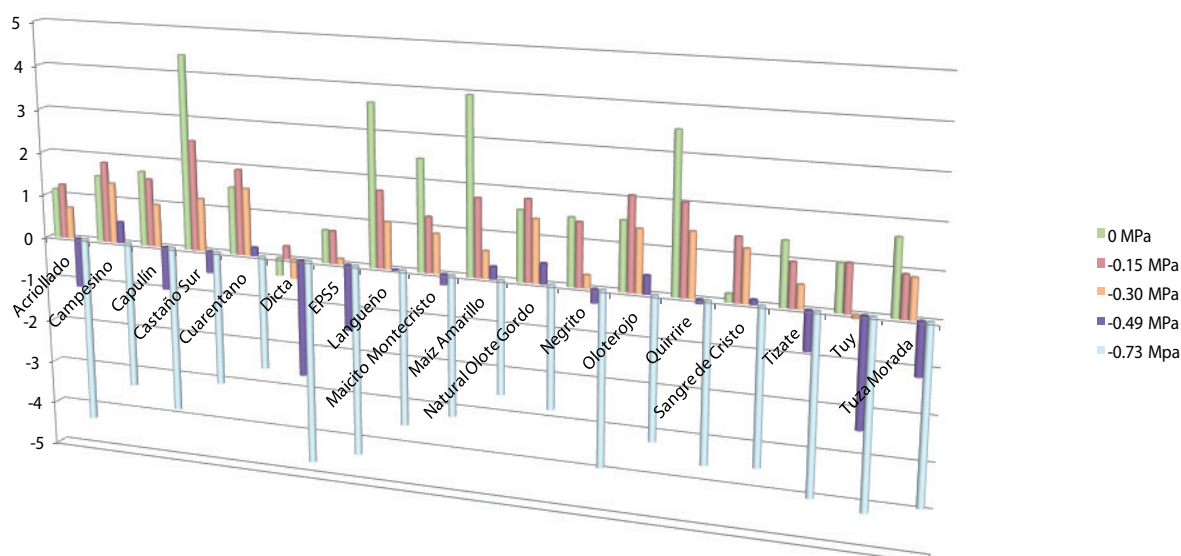
DW/FW, number of secondary roots, DW of secondary roots, and shoot DW/FW; and the interaction was not significant for root length, root DW, DW/FW of secondary roots, shoot length and shoot DW. The analyses of variance for control conditions showed that populations were not significantly different for any trait. Under slight stress, populations were significantly different for root DW/FW, DW of secondary roots, and shoot DW/FW; and under moderate and severe stress conditions, populations were significantly different for all traits.

The response of most root growth-related traits to increasing water stress conditions showed large variations among parameters and populations. Root length (for all populations), root DW and number of secondary roots (for some populations) fitted to a cubic model due to significant hormetic effects observed at slight stress conditions, whereas DW of secondary roots always fitted to a quadratic model according to a stress intensity-dependent inhibition of early growth (Supplementary Table 3). Except for DW of secondary roots, IC_{80} values were above the evaluated range of stress conditions in most cases. When significant effects on the regression model were found, R^2 values ranged between 0.306 and 0.992, being above 0.8 in most cases. The effect of water stress on shoot growth-related parameters (shoot length and DW) followed a significant quadratic model in all cases, with all IC values within the ranges evaluated and R^2 values above 0.9. However, these parameters were largely affected by water stress conditions and were not as discriminant as root-related traits. All seedling growth ratios (root, secondary roots and shoot DW/FW, and Shoot/Root ratio) were significantly and

notably affected by increasing water stress levels in all populations, fitting to a quadratic model with high R_2 values (excepting DW/FW of secondary roots for population EPS5) (Supplementary Table 4). Values of IC_{50} and IC_{80} were within the evaluated range of stress conditions in all cases.

The first principal component for seedling growth traits (PC1s) explains the majority of variance with positive loadings for all length and weight parameters. As high values for PC1s implies better seedling growth, the PC1s can be considered as a general index of resistance to drought at this stage (Fig. 2). A second principal component (PC2s) can be taken into account, as in this case positive loadings for root growth-related traits and negative loadings for shoot-related traits are found. The populations with high scores in PC1s had also high scores in PC2s, indicating that populations with tolerance to drought had reduced shoot length compared to root length (data not shown). The most discriminative stress level for seedling growth was moderate (-1.03 MPa). The most outstanding populations at this stress level were Maíz Amarillo, Cuarentano, and Tuza Morada, which were also classified as tolerant to drought at germination. Furthermore, Olote Rojo has also tolerance to higher stress levels. Only Olote Rojo and Tuza Morada reached values close to control at low-stress conditions.

Early growth assays

The combined over treatments analyses of variance showed that population × treatment interactions were not significant for plant height, leaf 3 DW, root DW, LA,

Figure 2 - Principal component analysis of seedling growth-related traits measured on 16 maize populations from an arid tropical Honduran region, plus two temperate checks evaluated in vitro under three increasing levels of water stress pressure (obtained with PEG 6000) plus a control. Samples were distributed according to the scores of the **principal component 1 for seedling growth (PC1s).**
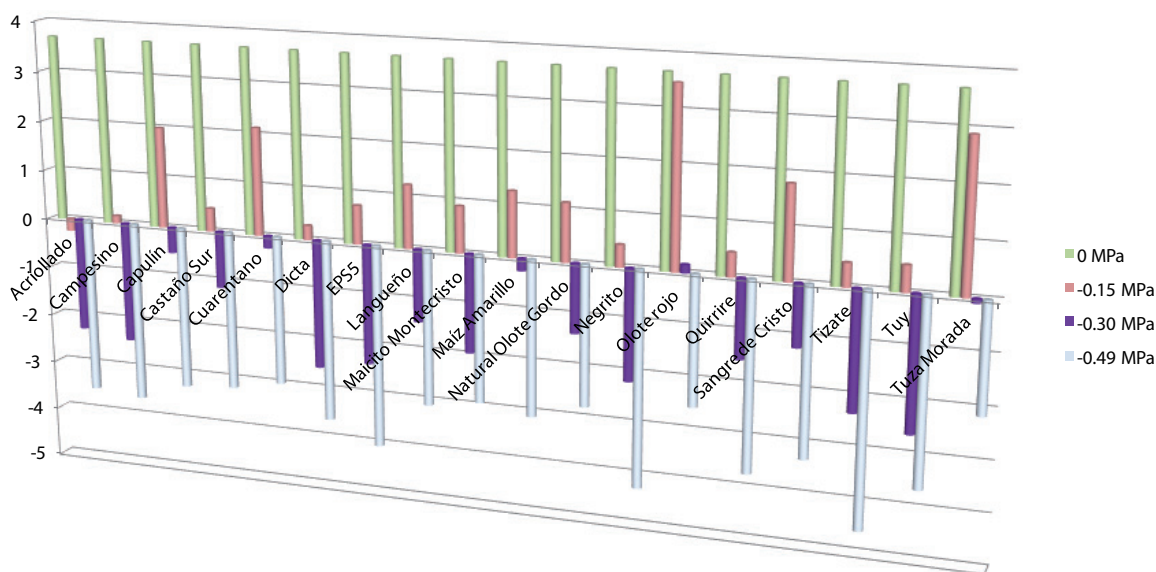
RWC and aerial biomass (data not shown). Maíz Amarillo had plants with lower height under water stress than under control conditions. Plants were taller for Spanish checks Tuy and EPS5 than for Honduran populations, both under water stress and control conditions (Supplementary Table 5). Under water-stress, eleven populations showed reduced leaf 3 DW when compared to control conditions, but three of them were not significantly different from the population with the lowest dry matter under both water stress and control conditions. Minor differences among populations were found for LA, as most populations were not significantly different from EPS5 or Dicta, which were the populations with largest areas under water stress and control conditions, respectively. Furthermore, LA was not significantly different between water stress and control conditions. RWC was significantly lower under water stress than under control conditions for three populations. Root DW was lower under water stress than under control conditions for most populations.

The first principal component for early growth (PC1e) accounted for 56% of the variability. Given the contributions of drought-related traits to PC1e, it can be considered as an indicator of biomass production, with positive loadings for all traits excepting leaf 3 FW and root FW. Values for PC1e decreased under stress conditions except for Cuarentano that had very low performance under control conditions (Fig. 3). The temperate checks EPS5 and Tuy had the largest coefficients both under stress and control, and Acriollado is also considered as tolerant because this population showed good performance under both control and water stress conditions.

Photosynthesis in the early growth trial

Population × treatment interactions were never significant for A_N, g_s, E or WUE. The repeated-measure analyses for photosynthetic traits showed that differences among populations were significant for all traits overtime under water stress, but they were not significant under control conditions (Supplementary Table 6). In five populations, gs was dramatically reduced along the water stress period. Only in Langueño, the effect of water stress on stomatal conductance was not significantly different (at P value > 0.9) under both water stress and control conditions. In opposition, three populations reduced their gs along the water stress period when compared to the control. Among these populations, Capulin also reduced its A_N and E values while significantly increased WUE along the water stress period. Other three populations also reduced A_N and E values, but these populations decreased their WUE through the water stress period, which is a symptom of susceptibility to drought. Furthermore, the reduction of A_N in those populations and the reduction of WUE in EPS5 and Sangre de Cristo over the water stress period were significantly higher than under control conditions, indicating that these populations were susceptible to drought. For Maicito Montecristo none of the reductions of A_N, E or WUE was significantly larger under water stress than under control conditions; suggesting that this population might have an intermediate performance under drought.

The first principal component for photosynthesis-related traits (PC1p) accounted for 67% of the variability (Fig. 4). Traits with the highest positive loadings were A_N and g_s. The population with the most favorable perfor-

Figure 3 - Principal component analysis of early growth-related traits measured on 16 maize populations from an arid tropical Honduran region, plus two temperate checks evaluated in a greenhouse under moderate water stress (obtained with PEG 6000) and control conditions. Samples were distributed according to the scores of the **principal component 1 for early growth (PC1e)**.
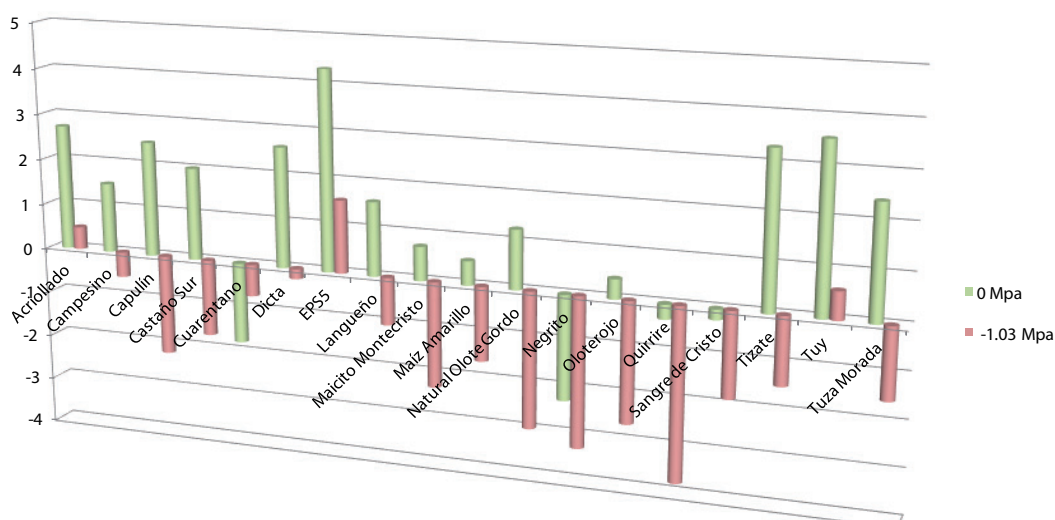
Figure 4 - Principal component analysis of early growth-related traits measured on 16 maize populations from an arid tropical Honduran region, plus two temperate checks evaluated in a greenhouse under moderate water stress (obtained with PEG 6000) and control conditions. Samples were distributed according to the scores of the **principal component 1 for early growth (PC1e).**
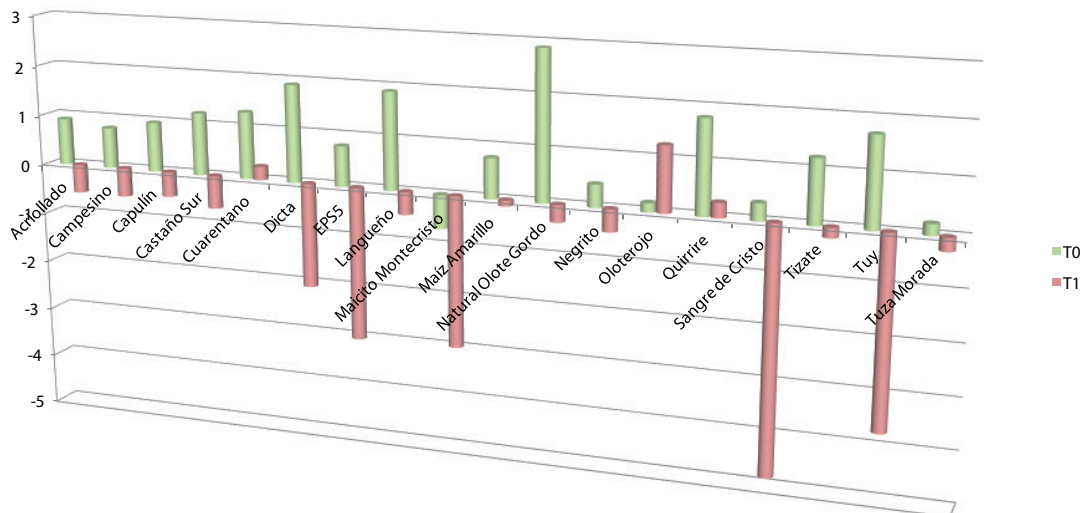
mance under stress conditions is Olote Rojo, although its WUE was not high. A second principal component (PC2p), accounting for 24% of the total variability, could be considered. This component (data not shown) had positive loadings for A_N and WUE but negative loadings for g_s and E, thus discriminating populations less efficiently in the use of water under stress conditions due to a poor control of stomatal opening, such as Natural Olote Gordo.

Discussion

The highly significant differences among populations found in the analyses of variance under different levels of water stress indicate that variability for drought tolerance exists in this germplasm. According to Grzesiak et al. (2013), total germination index has been the most used trait when screening for drought tolerance, and according to Abdel-Ghani et al. (2015) and Aslam et al. (2015), high maize kernel germination is directly associated with high post-germination performance. However, drought tolerance at different growth stages is not necessarily related (Hall et al., 1984). In our case, the response of all the germination indexes to the increasing levels of water stress varied among populations, but always fitted to a quadratic model according to a dose-dependent inhibition of germination caused by water stress. Moreover, G_T was more stable across water stress pressures for some Honduran populations than for checks. The complementary germination indexes S, AS, CRG, and MGT have been used by several authors (Chiapusio et al., 1997; Anjum and Bajwa, 2005) and they were used previously for screening for drought tolerance in maize

by Álvarez-Iglesias et al. (2017). According to Chiapusio et al. (1997), none of the germination indexes alone (G_T, S, AS, CRG, and MGT) can efficiently describe the germination process, so the use of several indexes is more convenient for identifying drought-tolerant genotypes. Indeed, the PC1g grouped all germination indexes, thus behaving as an overall index of resistance to drought at germination with positive loadings for GT, S, AS, and CRG, and negative loading for MGT. Individually, S and AS allowed to detect significant delays in the germination process, with IC_{50} and IC_{80} values revealing differences among populations, even in cases where no effects were observed on GT. On the contrary, calculation of IC values for CRG and MGT was poorly discriminative for these populations under the different stress levels.

The lack of significant population × treatment interactions for root length, root DW, DW/FW of secondary roots, shoot length and shoot DW indicates that the populations with higher performance under control conditions have also favorable performance under water stress conditions. Our results agree with those of Messmer et al. (2009) who found that genotype × environment interactions were significant when analyses were combined over control and drought environments, but those interactions were not significant when the analyses were combined over drought levels.

The analysis of seedling-related traits, especially the hormetic effects and the high IC_{50} and IC_{80} values for root-related traits, together with the strategies revealed by PC1s and PC2s, demonstrates that most of the populations evaluated are able to maintain

an adequate root development (although reducing their shoot growth), an adaptation strategy of capital importance under drought conditions. Under this strategy, we found at least three different ways to cope with drought among these populations, i.e., Natural Olote Gordo and Tuza Morada were able to bear increasing levels of drought while maintaining root length and biomass, as well as the number of secondary roots; conversely, root length was reduced in Cuarentano and Olote Rojo but these populations were able to compensate that drawback by maintaining the biomass of secondary roots. On the other hand, Castaño Sur maintained the development of the main root instead of the secondary roots. In a previous study with maize hybrids, Álvarez-Iglesias et al. (2017) found that drought imposed at a seedling development stage inhibited plant growth of the main root and coleoptile and also secondary roots, but this effect was smaller in tolerant cultivars. A large main root would increase the ability for finding new water sources, while secondary roots would allow the absorption of superficial water. Previous records have also shown that under drought conditions, roots tend to grow more than shoots because seedlings with greater root development are more likely to succeed (Sharp and Davies, 1989, Weerathaworn et al.1992a, 1992b). However, Bruce et al. (2002) reported that improved genotypes with poorer early root development reached higher yields and, according to Messmer et al. (2009), the relationship between root traits and drought tolerance is still unclear. Otherwise, Bänziger and Araus (2007) concluded that selection under drought stresses in tropical maize was associated with variation of root length but not with changes in biomass. These results suggest that having high root biomass is more effective for drought tolerance than increasing the length of the main root.

The evaluated populations had similar behavior for the calculated seedling ratios; particularly, all populations showed a significant reduction of shoot/root ratio as osmotic pressure increased. This ratio is genetically regulated but also has significant environmental effects (Caloin et al., 1990). Our results confirm that genetic variability exists among these populations for morphological responses to drought, particularly for root-related traits, but less so in shoot-related traits. Under optimal conditions coleoptile length is associated with seedling biomass (Bruce et al., 2002, Rebetzke et al., 2006) but coleoptile development implies decreasing root development. Under drought conditions, plants tend to develop their root system at the expense of shoot, so that tolerant genotypes had lower shoot/root development ratio (Grzesiak et al.1997).

Our results show that root-related traits are more discriminant for drought tolerance than shoot-related traits. Assessing root response to drought is a challenge because field trials do not allow a precise measurement of roots, whereas hydroponic trials do not reproduce real field conditions. Therefore, given the difficulties of studying the roots under reliable conditions, many evaluations of drought performance focus on the aerial part (Whitmore and Whalley, 2009). Nevertheless, some authors have emphasized that *in vitro* evaluations of the first stages of development under simulated drought produced with PEG are a convenient approach for studying the whole plant without restrictions (Ruta et al., 2010; Hasanuzzaman et al., 2016; Álvarez-Iglesias et al., 2017). Furthermore, the response of a plant to drought varies at different stages of development, indicating that drought should be evaluated at different developmental stages independently. Although it is currently assumed that there are diverse responses to different stress levels among plants (Larcher, 2003), some populations might have some common mechanism for tolerance at successive stages of development.

The early growth assay did not show significant variability for most traits, and the population × treatment interactions were not significant for most traits. Possible explanations for the limited effects of drought response detected in the greenhouse are that the stress pressure has not been applied long enough for detecting differences, as the water status of maize leaves decreases quite slowly in response to decreasing water availability (Messmer et al., 2009). Nevertheless, Acriollado, Langueño, Maicito Montecristo, Maíz Amarillo, and Negrito were not significantly affected by drought for leaf 3 DW, and Cuarentano, Maicito Montecristo, Maíz Amarillo, Negrito, Olote Rojo, Quirrire, and Tuy were not affected for root DW. The large vigor of Tuy under optimal conditions could have masked the effect of drought, as reported by Messmer et al. (2009) for CML444. Maicito Montecristo was the most drought-tolerant population based on morphological traits at early growth; this population was able to maintain root biomass both at seedling and early growth stages. RWC was not very discriminative among these populations indicating that probably the stress level applied at early growth was not severe enough for these populations, as previous reports have shown that RWC was able to discriminate genotypes according to their drought tolerance only under severe stress conditions (Álvarez-Iglesias et al., 2017).

Population × treatment interactions were not significant for photosynthetic traits indicating that populations with

high photosynthetic capacity under control conditions were also efficient under drought conditions. There were two different reactions to drought concerning stomatal conductance, i.e., some populations reduced gs, while there was no significant reduction in Langueño. The central role of photosynthesis in maize response to drought is regulated by stomatal opening, a complex process affected by many factors (Comstock, 2000). The best indication of drought can be obtained by monitoring gas interchange (Berger et al., 2010). Genotypes reduce stomatal conductance in order to limit water loss, but stomatal closing also limits CO_2 availability. Stomatal opening in Langueño could imply that this population was not affected by drought considering its performance under drought conditions for A_N, E and WUE was not significantly different from the most tolerant populations. Genotypes that reduce transpiration under drought conditions increase yields when drought occurred at flowering and during grain filling (Messina et al., 2015). Among the populations that reduced conductance, Capulín also reduced A_N and E but increased WUE. Therefore, Langueño was resistant to this level of drought pressure, while Capulin behaved as tolerant to drought. Previous authors have also shown that there were differences in the ability of maize genotypes to respond to drought at different stress levels (Chaves et al., 2009). Stomatal closure is a genetically variable trait that can reduce water loss and minimize the impact of drought, but also reduces photosynthesis (Tardieu, 2013, Zinselmeier et al., 1999). A comprehensive comparison of these populations, based on principal component analyses, indicate that the most tolerant populations at germination are Natural Olote Gorde, Olote Rojo, Cuarentano, and Sangre de Cristo, while Maíz Amarillo has an outstanding performance under severe stress, though all populations reduce performance as stress increases. At seedling growth, the populations with most favorable performance under drought conditions were Maíz Amarillo, Cuarentano, and Tuza Morada, Olote Rojo showing tolerance at high stress. For early growth, the temperate populations EPS5 and Tuy had the largest coefficients both under stress and control conditions, and Acriollado is also tolerant to drought conditions. Concerning photosynthesis-related traits, the population with most favorable performance under stress conditions is Olote Rojo. Therefore, some populations showed drought tolerance at diverse growth stages, and only Olote Rojo was among the most tolerant populations at all stages of development. We identified different populations with drought tolerance at juvenile growth stages: Campesino, Castaño Sur, Dicta, and Quirrire at final germination,

and Dicta and Capulín at germination speed; Natual Olote Gordo and Tuza Morada for seedling root; Maicito Montecristo for morphological traits at early growth; and based on photosynthetic traits, Langueño was resistant, while Capulin behaved as tolerant to drought. Only Capulin was identified as tolerant at two different stages of development: germination and early growth. Maicito Montecristo was able to maintain root biomass both at seedling and early growth stages. We also identified different mechanisms of response to drought at each stage: first, at seedling growth, there were three different strategies for facing drought among these populations: 1) maintaining root length, dry weight, and biomass as well as the number of secondary roots, 2) reducing root length but compensate it by maintaining dry weight of secondary roots, and 3) maintaining the development of the main root instead of the secondary roots. Regarding early growth, root-traits are more discriminant for drought-tolerance than shoot-traits. And second, for early growth, there were two different reactions to drought concerning conductance as the populations Castaño Sur, Maicito Montecristo, Negrito, Sangre de Cristo and Tuy reduced conductance while there was no significant reduction in Langueño. Finally, different mechanisms of response to drought at early stages of development could be combined by crossing the appropriate populations.

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