Evaluation of physiological characteristics as selection criteria for drought tolerance in maize inbred lines and their hybrids

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Abstract
Improvement for maize drought tolerance has always been a significant objective for breeders and plant physiologists. Nowadays, climate change sets new challenges to major crop adaptation at stressful environments. For such a purpose, the measurement of physiological traits related to maize response to drought might prove to be useful indices. The objective of the present study was to establish whether the physiological traits can be used as reliable physiological markers to evaluate the performance of parental genotypes and their hybrids under both dry and normally watered conditions, and under two densities an ultra-low density (ULD) and a normal dense stand (DS). Thirty (30) maize inbred lines and 30 single-crosses among them were evaluated across three diverse locations in Greece. The ULD was 0.74 plants/$^{m-2}$, while the DS comprised 4.44 plants m^{-2} in the water deficit regime, and 6.67 and 7.84 plants m^{-2} in the normal water treatment for lines and hybrids, respectively. There was a very good association between the physiological characteristics studied and grain yield under the ultra-low density and especially for inbred lines. It was shown that the physiological characteristics can facilitate the selection of stress-adaptive genotypes under the low-density conditions and may permit modern maize to be grown at a wider range of environments. At the normal densities such a possibility was not evidenced since physiological parameters and yield did not correlate for either parents or hybrids

KeyWords *water deficit, heterosis, environmental heterogeneity, assimilation rate, chlorophyll.*

Abbreviations : DI, deficit irrigation; DS, dense-stand; A, assimilation rate; NI, normal irrigation; PYE, plant yield efficiency; ULD, ultra-low density; WUE, water use efficiency, stomatal conductance (g_s), transpiration rate (E), intercellular CO_2 concentration (c_i).

Introduction

Water stress is one of the most important limiting factors for maize production worldwide. The economic losses in maize production due to water stress are quite significant and breeding for drought tolerance is thus one of the most important tasks maize breeders are currently confronted with. Several strategies have been used to improve drought tolerance of maize such as genomics-related tools and quantitative trait loci (QTL) (Campos et al., 2004; Parry et al., 2005; Bäzinger and Araus, 2007; Brennan and Martin, 2007; Ribaut and Ragot, 2007; Tuberosa et al., 2007; Mullet, 2009; Lawlor, 2013). Tolerance to drought through these and other modern biotechnology techniques have yet to be fulfilled (Lopes et al., 2011; Lawlor, 2013) thus classical approaches such as usage of physiological traits are still in the forefront.

Common physiological traits used to improve breeding for increased stress tolerance are gas exchange parameters (assimilation rate (A), stomatal conductance (g_s), transpiration rate (E), intercellular CO_2 concentration (c_i) and the calculated WUE as A/E, chlorophyll content, chlorophyll fluorescence, leaf water potential, and relative water content) (Di Marco et al., 1988; Schapendonk et al., 1989; Selmani and Wassom, 1991; Jamaux et al., 1997; Ober et al., 2005; Zarco-Perelló et al., 2005; O'Neill et al., 2006; Subrahmanyam et al., 2006; Khan et al., 2007; Hura et al., 2007; Živčák et al., 2008). As far as the gas exchange parameters are concerned, they have been questioned as some authors suggest for their use (Li et al., 2006; Fotovat et al., 2007; Silva et al., 2007) while others are against it (Royo et al., 2000; O'Neill et al., 2006). Nevertheless, physiological traits have showed a good correlation with tolerance to stresses and yield parameters and an adequate genetic variation in the evaluated population/genotype collection, and a high heritability and repeatability (Sayar et al., 2008; Li et al., 2006; Fotovat et al., 2007; Silva et al., 2007). Studies including physiological parameters as breeding tools aimed to determine whether any of the photosynthetic parameters can be used for screening large sets of genotypes for their tolerance to different stresses. However, usefulness of these tools to predict the performance of hybrids bred under stress conditions has not been studied with due consideration (Fracheboud et al., 1999; Betrán et al., 2003; Kościelniak et al., 2005). Qualification of such prognostic tools may assist maize breeding primary aiming to create tolerant hybrids through specific crossings.

Plant yield efficiency (PYE), that constitute a determinant element of crop yield potential, has been asserted essential for effective resource use under both favourable and stressful conditions, as well as for over season stability (Duvick, 2005; Berzsenyi and Tokatlidis, 2012; Tokatlidis, 2013). In a recent work (Tokatlidis et al., 2015), improved PYE was found contributing to maize resilience on environmental heterogeneity, desirable for coping with drought events. The PYE, fully expressed in ultra-spaced plants to preclude any interference among them for inputs, optimized heritability and was devoid of confounding crossover types of G x E interaction. Yield of space-planted environments was found to be transferred to densely seeded situations, thus PYE was suggested a criterion for dependable selection and evaluation. Since physiological traits have been associated with drought tolerance at dense stands, the correlation between widely spaced plants and common farming densities for such parameters could provide further information on whether breeding could be based on PYE. Hence, the main objective of this study was to establish whether physiological traits can be used as reliable physiological markers to evaluate the performance of parental genotypes and their hybrids under drought and well watered conditions in two different selection densities (ULD and DS).

Materials and Methods

Study site and crop management

A field experiment over two growing seasons (2012 and 2013) was established at three different locations in Northern Greece. Site 1 was located in Thessaloniki (40°32′N, 22°59′E, 0m) in a clay loam soil with pH (1:1 H_2O) 8.0, EC (dS m^{-1}) 1.80, bulk density (Mg m^{-3}) 1.3, field capacity (at 10 kPa, m^3 m^{-3}) 0.373, wilting point (at 1500 kPa, m^3 m^{-3}) 0.132, water holding capacity 0.241, and organic matter 12.50 g kg^{-1}. Site 2 was located in Florina (40°46′N, 21°22′E, 707m) in sandy loam soil with pH (1:1 H_2O) 6.3, and organic matter 14.0 g kg^{-1} and soil water holding capacity 0,218. Site 3 in 2012 season was in Giannitsa (40°42′N, 22°24′E, 1m) in a loam soil with pH (1:1 H_2O) 7.3, and organic matter 18.0 g kg^{-1} and soil water holding capacity 0.228 while during the 2013 a different site was used in Serres (41°01′N, 23°36′E, 15m) in a clay loam soil with pH (1:1 H_2O) 7.0, EC (dS m^{-1}) 1.60, bulk density (Mg m^{-3}) 1.3, field capacity (at 10 kPa, m3 m^{-3}) 0.312, wilting point (at 1500 kPa, m^3 m^{-3}) 0.115, water holding capacity 0.197, and organic matter 15.30 g kg^{-1}. These locations are part of the major maize belt in Greece, with the Site 2 being marginal due to the high altitude associated with cool summers and limited growing season (Tokatlidis et al., 2015). Weather data (rainfall and average temperature) were recorded daily and were reported as mean monthly data for the two years that the study was conducted for the three locations as previously described Tokatlidis et al., 2015. In all the experimental fields the previous crop was durum wheat tolerance (*Triticum turgidum subsp. durum* L.). Before seeding, the cultivation area was moldboard plowed and harrowed. Nitrogen and P fertilizer was applied at planting at the rates of 120 and 60 kg ha-1, respectively, while additional N (100 kg ha^{-1}) was top-dressed when plants reached the 50 cm height. Complete weed control was obtained by tilling and hand weeding.

Plant genotypes used in the study

During the 2012 growing season two sets of inbred lines were tested. The first set consisted of 25 inbred lines (corresponding codes in the study were 1-22,24 and 31) which according to the owner company (American Genetics Inc.) were of commercial interest including parents of cultivated hybrids. The second set comprised six experimental lines, coded 25-30, derived through selection in the absence of competition on single-plant yield (Tokatlidis et al., 1998), placing thus particular emphasis on plant yield efficiency. Thirty one hybrids, obtained from single crosses among the aforementioned lines, were tested during the 2013 season. Twenty two crosses were chosen so as to include parents from both sets, while both parents of seven and five out of the 31 crosses were from the first and the second set, respectively.

Treatments

At each site in both growing seasons two different densities were used, the ultra-low density (ULD) and the dense-stands (DS). The ultra-low density of 0.74 plants m^{-2} was achieved (hereafter low density), with individual plants occupying equidistant hills (125 cm) in a zig-zag pattern. This density was used to preclude interplant competition and allow PYE to be fully expressed. The low-density trials were composed of 40 plants from each genotype evenly and systematically allocated, according to the replicated 31-honeycomb design (Fasoula and Tokatlidis, 2012). The dense-stand plots were established in randomized complete blocks and replicated twice, comprising of two rows 4 m in length and 75 cm apart. Under normal irrigation, the in-row interplant distances were 20 and 17 cm for lines (66,666 plants ha-1) and hybrids (78,431 plants ha^{-1}), respectively, with the latter population density approximating that commonly used by farmers. In deficit irrigation treatments, the in-row distance was 30 cm (44,444 plants ha^{-1}) for both lines and hybrids. The lower density was chosen in water shortage conditions to be consistent with the fact that lower densities are required for dryland compared to irrigated maize (Norwood, 2001; Blumenthal et al., 2003; Shanahan et al., 2004; Duvick, 2005; Berzsenyi and Tokatlidis, 2012). The density treatments were overplanted and thinned after emergence to the desired stand. Planting occurred from mid-April until early May.

At each site, the low-density and dense-stand trials were established twice, corresponding to the two irrigation treatments (normal = full irrigation treatment and deficit = 50% of the normal). Up to vegetative stage V6-7, both irrigation treatments received 50 mm of water for seedling establishment and early plant growth, with different irrigation levels applied thereafter. A drip-irrigation water supply system of 4 L h^{-1} was established along every other plant row, with emitters spaced at 33-cm intervals. Irrigation scheduling was based on maize evapotranspiration (ET_c) and was applied when the crop evapotranspiration ET_c - P (rainfall) reached 50 mm. Soil water content at this level was approximately 70% of field capacity, which is considered adequate for plant growth during all stages. The ETc was calculated from climatic parameters measured daily from meteorological stations located adjacent to each experimental site and was used to calculate the reference evapotranspiration (ETo) using the Penman–Monteith method (Allen et al., 1998). The ET_c, which is the product of ET_o and the crop coefficient (K_c), was calculated using values for maize Kc adjusted to Greek conditions (K_{cini} = 0.50, K_{cmid} = 1.05, and K_{cend} = 0.15) for growth stages of 30/70/120/150 d after emergence (Georgiou et al., 2010; Lekakis et al., 2011).

Grain yield

At low density, plants were harvested individually. Thus, grain yield was recorded at the per-plant basis. At the dense-stand trials, grain yield was recorded at per area (plot) basis by harvesting the two central rows by hand in the first week of October for site 1 and site 3, while for site 2 the harvest was conducted at the end of November in both years. Drought tolerance index (DTI) was determined as a percentage of yield loss due to drought stress on the yield realized under full irrigation (Menkir et al., 2003; Derera et al., 2008) as:

DTI (%) = [(yield under well-watered – yield under drought)/(yield under well-watered)]x100

Chlorophyll measurements

Chlorophyll readings were taken with a hand-held dual-wavelength meter (SPAD 502, Chlorophyll meter, Minolta Camera Co., Ltd., Japan). For each plot the 20 youngest fully expanded leaves per plot were used when the plants were at anthesis and at physiological maturity. The instrument stored and automatically averaged these readings to generate one reading per plot.

Gas-exchange measurements

A portable photosynthesis system that measures CO_2 uptake (LI-6400 XT, Li-Cor, Lincoln, Nebraska, USA) equipped with a square (6.25 cm^2) chamber was used for determinations of CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance to water vapour (g_s), and intercellular CO_2 concentration (C_i) during anthesis and grain filling period. Leaf gas exchange was measured on the upper-most ear leaf twice, one week after silking and two weeks later during the grain-filling period. Measurements were performed on six plants from each plot from 09:00 - 12:00 in the morning to avoid high vapor-pressure deficit and photoinhibition at midday. Instantaneous water use efficiency (WUE) was obtained by dividing A by E (von Caemmeter and Farquhar 1981).

Chlorophyll fluorescence

The minimum Chl fluorescence (F0) and the maximum Chl fluorescence (Fm) were measured also *in situ* with the portable Z995 FluorPen PAR (Qubit Biology Inc. Kingston, Ontario, Canada). The maximum quantum efficiency of photosystem (PS) II was calculated as Fv/Fm (Fv = Fm – F0).

Heterosis indices

Average heterosis for grain yield was determined as the difference between F1 value and the mid-parent value (Hallauer and Miranda, 1988). Mid-parent heterosis (MPH) for individual crosses was calculated as:

MPH (%) = (F1-MP) x 100/MP

where, F1 is the mean of the hybrid performance and MP = (P1 + P2)/2 in which P1 and P2 are the means of the inbred parents, respectively.

Also, better-parent heterosis (BPH), that is, heterobeltiosis, for individual crosses was calculated as:

BPH (%) = (F1-BP) x 100/BP

where BP is the better parent.

Statistics

The experiments were performed into two consecutive years 2012 and 2013 at three locations. Analyses were performed according to Steel et al. (1997) using the statistical program SPSS™ (SPSS Inc., IL, USA). A combined analysis of variance (ANOVA) was used on the three-factor pattern and for all the parameters that were determined. The analysis was based on the linear model and involved three fixed effect factors: locations as main plots, water regimes as subplots and genotypes as sub-subplots. For all statistical analyses, a probability level of 0.05 was used as a baseline for significance. In addition, the LSD (P = 0.05) test was used to find significant differences among means. Pearson correlation analyses across years were done with SPSS.

Results

Grain yield of the inbred lines and also of their respective hybrids was affected by genotype, irrigation, and location and also their interactions in ULD and DS plots (Tables 1 and 2). Gas exchange parameters (A, E, C_i, and gs) were affected by the genotype, irrigation, and location in both densities (ULD and DS) for the inbred lines and their hybrids. The interaction between genotype and location was significant in most characteristics except for the WUE and chlorophyll fluorescence in ULD and A, Ci, WUE and chlorophyll fluorescence in the DS. Furthermore, interaction between irrigation and location was significant in most parameters except for chlorophyll content in ULD and in E, chlorophyll content and chlorophyll fluorescence in the DS. However, in most characteristics there was no interaction between genotype and irrigation and also there was no interaction between genotype, location, and irrigation (Tables 1 and 2).

In ULD conditions grain yield of the inbred lines ranged from 152.9 g plant^{-1} for line 31 up to 826.6 g plant^{-1} for line 26 under control conditions. In contrast, under drought conditions grain yield was reduced and ranged from 90.2 g plant^{-1} for line 31 up to 666 g plant^{-1} for line 26 (Table 3). DTI ranged from negative values -25.7 % up to 41.02 %. Similar trend was found under DS as under well watered conditions the lowest gain yield was found at line 14 and the highest in line 27. Under drought the grain yield was in the range of 3.71 Mg ha^{-1} for line 31 up to 11.19 Mg ha^{-1} for line 26. DTI also ranged from negative values -42.94% as was not affected significantly by the drought stress in some lines (4, 8, 14, 19, and 26) up to 63.15% in line 27. On average, grain yield of inbred lines under drought was

Table 1. Analysis of variance of various parameters measured in inbred lines under ultra-low density (ULD) and dense-stand (DS) affected by Location (L), Irrigation (Irr), and Genotype (G).

Parameters	Location (L)	Irrigation (Irr)	Genotype (G)	G x L	Irr x L	G x Irr	G x L x Irr
df	2	1	29	58	2	29	58
			ULD				
Grain yield	***	***	***	***	***	***	***
Assimilation rate (A)	***	**	***	***	*	NS	NS
Transpiration rate (E)	***	NS	***	***	***	NS	NS
Stomatal conductance (gs)	***	**	***	***	***	NS	NS
CO2 concentration (Ci)	NS	**	***	***	***	NS	NS
WUE	***	**	**	NS	*	NS	NS
Chlorophyll	***	NS	***	***	NS	NS	NS
Chlorophyll Fluorescence	***	***	NS	NS		NS	NS
			DS				
Grain yield	***	**	***	***	**	***	***
Assimilation rate (A)	***	**	NS	NS	**	*	NS
Transpiration rate (E)	***	***	***	***	NS	NS	NS
Stomatal conductance (gs)	***	***	***	***	*	*	NS
CO2 concentration (Ci)	***	NS	NS	NS	***	NS	NS
WUE	***	***	NS	NS	*	NS	NS
Chlorophyll	***	NS	***	*	NS	NS	NS
Chlorophyll Fluorescence	***	NS	NS	NS	NS	NS	NS

* Significant at the 0.05 level of probability, ** Significant at the 0.01 level of probability, *** Significant at the 0.001 level of probability, NS nonsignificant

Table 2. Analysis of variance of various parameters measured in hybrids under ultra-low density (ULD) and dense-stand (DS) affected by Location (L), Irrigation (Irr) and Genotype (G).

Parameters	Location (L)	Irrigation (Irr)	Genotype (G)	G x L	Irr x L	G x Irr	G x L x Irr
df	**2**	**1**	**30**	**60**	**2**	**30**	**60**
			ULD				
Grain yield	**	***	***	***	**	***	***
Assimilation rate (A)	***	***	NS	***	***	NS	NS
Transpiration rate (E)	***	***	NS	***	***	NS	NS
Stomatal conductance (gs)	***	***	NS	***	***	NS	NS
CO2 concentration (Ci)	***	***	NS	***	***	NS	NS
WUE	***	***	NS	***	***	NS	NS
Chlorophyll	***	***	**	***	***	NS	NS
Chlorophyll Fluorescence	***	***	NS	***	***	NS	NS
			DS				
Grain yield	**	***	***	***	**	***	***
Assimilation rate (A)	**	***	NS	***	**	NS	NS
Transpiration rate (E)	**	***	NS	***	**	NS	NS
Stomatal conductance (gs)	**	***	NS	***	**	NS	NS
CO2 concentration (Ci)	**	***	NS	***	**	NS	NS
WUE	**	***	NS	***	**	NS	NS
Chlorophyll	**	***	***	***	**	NS	NS
Chlorophyll Fluorescence	**	***	NS	***	**	NS	NS

* Significant at the 0.05 level of probability, ** Significant at the 0.01 level of probability, *** Significant at the 0.001 level of probability.
NS, nonsignificant

11.16% and 16.64 % lower of the yield obtained under well watered conditions in ULD and DS respectively (Table 3).

Similar trend with the inbred lines was found in hybrids as there was also significant effect of drought stress in grain yield in both ULD and DS. The highest grain yield was found in the 26 x 29 hybrid (1622.2 g plant^{-1}) under control conditions and in 29 x 9 hybrid (1147.8 g plant^{-1}) under stress conditions (Table 4). While under DS and normal irrigation the highest grain yield was found at the 26 x 30 hybrid (16.35 Mg ha^{-1}) and the lowest at the 13 x 22 (11.2 Mg ha^{-1}). In drought conditions the commercial hybrid had the highest grain yield while the lowest was found in the 25x2 hybrid which had the least grain yield reduction (6.41%) under drought and ULD whereas had much higher 41.64% yield reduction under DS. On average, grain yield of hybrids under drought was 19.89 and 34.66 % of the yield obtained under well watered conditions in ULD and DS respectively (Table 4). Of the 31 hybrids used in this study, only three had DTI below 10% 25 x 2, 7 x 29, and 15 x 12 under ULD conditions. However, under DS conditions the average DTI was much higher and the hybrids with the lowest index was 14 x 20 and the commercial.

The assimilation rate (A) was affected by genotype, irrigation treatment, and location in inbred lines and also in their hybrids (Tables 1 and 2). Mean assimilation rate was in the range of 23.93-29.04 μmol CO_2 m^{-2} s^{-1} and 17.27-23.42 μmol CO_2 m^{-2} s^{-1} for the control conditions and the water stressed conditions at ULD respectively. Under the DS conditions assimilation rate ranged from 20.10-26.52 μmol CO_2 m^{-2} s^{-1} and between 12.96-21.37 μmol CO_2 m^{-2} s^{-1} under control and water stressed conditions. There was an agreement in most cases with grain yield as the reduction in A was lower in tolerant lines and also in their hybrids. The maximum assimilation rate under control conditions in the ULD was found at the inbred line 31 and the minimum assimilation at the inbred line 20. However, under water stressed conditions the maximum assimilation rate under ULD was found at the 17 inbred line and the lowest at the line 14. There was much higher reduction under DS in A compared with the ULD conditions due to water stress in both inbred lines and in hybrids (Tables 5). Under DS conditions the situation was quite different as the highest A at the control conditions was found at the inbred line 28 and the lowest at line 6 while under stressed conditions the highest and the lowest A was at the lines 26 and 10 respectively.

Correlation between the physiological and agronomic characteristics

There were significant correlation coefficients among grain yield and A, chlorophyll fluorescence, WUE, and chlorophyll content under control conditions for the inbred lines under ULD (Table 6). Similar trend was found under stressed conditions as there was also strong correlation between grain yield and A, chlorophyll fluorescence, WUE, and chlorophyll content. In addition, under both control and stressed conditions there was also correlation between A, and all the physiological

Table 3. Mean grain yield of 30 lines at ultra low density (ULD) and dense stand (DS) conditions, across two irrigation treatments and three sites. The average (Avg) mean yield from the three environments as well as the least significant difference (LSD) for comparisons among individual lines within each column (P ≤ 0.05), are also given.

	ULD (g plant^{-1})			DS (Mg ha^{-1})		
Inbred lines	**Control**	**Stressed**	**DTI(%)**	**Control**	**Stressed**	**DTI(%)**
1	166.8	154.3	7.48	4.19	3.81	9.11
2	166.2	175.7	-5.76	5.63	4.43	21.36
3	228.0	191.6	15.96	6.35	5.12	19.39
4	197.5	182.4	7.66	4.31	4.77	-10.67
5	236.6	223.4	5.57	9.72	6.14	36.85
6	302.4	284.5	5.92	9.53	9.26	2.80
7	269.5	268.1	0.53	10.60	8.38	20.89
8	197.3	203.4	-3.06	5.53	7.90	-42.94
9	277.6	257.0	7.42	9.13	4.70	48.54
10	366.1	298.4	18.50	8.61	6.96	19.21
11	176.0	155.1	11.89	8.09	5.75	28.94
12	229.7	166.8	27.39	5.36	5.13	4.37
13	223.1	206.2	7.58	7.55	5.10	32.39
14	174.7	186.9	-6.99	3.25	6.11	-87.82
15	212.6	190.2	10.56	6.21	5.72	7.80
16	256.3	194.1	24.30	9.26	8.25	10.88
17	264.7	212.5	19.73	9.18	4.46	51.40
18	188.6	191.8	-1.71	6.67	4.60	31.00
19	208.1	203.8	2.06	7.30	8.95	-22.57
20	186.9	162.8	12.93	4.82	5.08	-5.58
21	239.5	301.2	-25.72	5,43	4,80	11.59
22	231.4	214.6	7.26	6.47	6.08	6.08
24	226.7	176.9	21.94	7.83	5.46	30.25
25	682.3	496.7	27.20	14.65	9.65	34.10
26	826.6	666.0	19.43	10.92	11.19	-2.44
27	278.9	244.9	12.20	12.24	4.51	63.15
28	590.8	462.4	21.73	8.61	8.06	6.46
29	597.8	436.4	26.98	10.49	7.36	29.91
30	475.1	404.6	14.84	8.01	5.41	32.46
31	152.9	90.2	41.02	4.87	3.71	23.78
Average	294.4	253.4	11.26	7.71	6.28	16.64
LSD	25.5	28.7	1.65	1.12	1.24	3.21

parameters that were measured (g_s, c_i, E, chlorophyll fluorescence, chlorophyll content, and WUE).

While in both irrigation treatments under ULD there was a significant correlation among some physiological parameters and grain yield, under DS there was not a correlation between grain yield and most of the physiological characteristics that were determined with the only exception being chlorophyll content under control conditions (Table 7). In contrast under stressed conditions there was significant correlation between A, c_i, WUE and SPAD whereas under control conditions there was no difference. Also, under both irrigation treatments there was significant correlation between g_s, c_i, E whereas under control conditions there was correlation between g_s, chlorophyll fluorescence, and under stressed conditions there was correlation between g_s and SPAD.

Under ULD in hybrids the trend was quite different compared with the inbred lines as there was no correlation between grain yield and the physiological characteristics measured under control conditions (Table 8). However, under stressed conditions in ULD there was correlation between grain yield, A, g_s, and chlorophyll fluorescence. MPH and HPH were not correlated with any of the parameters that were determined. However, average heterosis was correlated with grain yield and the other heterosis indices under both irrigation treatments. Also all the heterosis indices were correlated between them in both densities (Tables 8 and 9). In addition, there was correlation of A with most of the characteristics that were studied and also with the MPH and HPH (Tables 8 and 9).

Table 4. Mean grain yield of 31 hybrids at ultra-low density (ULD) and dense stand (DS) conditions across two irrigation treatments and three sites. The average (Avg) mean yield from the three environments as well as the least significant difference (LSD) for comparisons among individual hybrids within each column (P ≤ 0.05), are also given.

	ULD (g plant^{-1})			DS (Mg ha^{-1})		
Hybrids	**Control**	**Stressed**	**DTI(%)**	**Control**	**Stressed**	**DTI(%)**
25 x 7	847.9	697.6	17.72	12.71	8.11	32.41
25 x 30	967.5	813.3	15.93	12.06	7.85	29.13
7 x 29	769.7	711.8	7.52	12.75	6.90	35.51
10 x 30	1069.8	884.4	17.33	13.89	6.81	44.42
6 x 15	700.6	589.9	15.80	11.46	6.24	33.21
25 x 2	701.5	656.5	6.41	12.72	6.19	41.64
25 x 5	1002.3	838.8	16.32	12.14	7.74	25.61
25 x 9	944.5	811.8	14.06	15.39	7.89	41.17
25 x 17	986.8	750.5	23.95	13.62	7.81	35.90
28 x 8	1006.1	848.0	15.71	12.66	8.30	29.31
29 x 9	1606.8	1147.8	28.57	16.26	7.53	42.31
29 x 16	1326.6	969.3	26.94	14.39	8.77	27.74
26 x 12	1128.6	897.7	20.47	16.23	9.17	35.16
26 x 18	867.1	706.2	18.55	13.00	8.60	23.87
26 x 22	1119.7	908.2	18.89	14.51	9.01	33.59
26 x 27	1614.1	1096.5	32.07	14.46	9.11	33.84
13 x 22	705.2	619.3	12.18	11.20	7.36	27.38
2 x 15	880.9	649.4	26.28	14.95	10.05	31.60
22 x 30	933.0	752.1	19.39	12.25	7.27	35.84
26 x 3	915.4	742.0	18.95	14.94	9.05	42.95
26 x 29	1622.2	1058.4	34.75	12.89	8.73	34.91
26 x 30	1033.7	880.4	14.84	16.35	9.15	46.65
26 x 17	1014.3	771.0	23.99	12.53	7.49	39.45
28 x 22	1086.2	926.7	14.69	12.19	7.55	54.26
3 x 30	886.8	749.4	15.50	14.45	9.27	35.93
28 x 18	974.1	836.2	14.16	13.40	10.24	23.60
15 x 12	661.1	635.2	3.92	12.37	8.18	42.77
17 x 20	859.8	717.1	16.61	13.90	8.80	49.76
24 x 20	909.0	659.1	27.49	14.11	9.86	33.63
14 x 20	1001.4	809.3	19.18	15.34	12.07	16.05
Commercial hybrid	1077.6	876.5	18.66	16.22	13.69	14.78
Average	1007.1	806.8	19.89	13.72	8.54	34,66
LSD	123.4	110.41	2.54	1.21	1.65	3.25

Discussion

From the present study it is obvious that there was significant variation among inbred lines and hybrids for grain yield and also the physiological characteristics that were studied under drought and control conditions. The presence of significant genetic variation among the inbred lines implies that significant progress could be made from the selection for improved grain yield and the development of productive maize hybrids for drought prone and optimal growing environments. Similar results were found by others using different inbred lines and their hybrids (Rosielle and Hamblin, 1981; Badu-Apraku et al., 2011b; Badu-Apraku and Oyekunle, 2012). The grain yield reduction expressed as DTI was up to 41% and 63 % at ULD and DS respectively among inbred lines and up to 34% and 54 % in hybrids for ULD and DS respectively. The DTI values indicated that the levels of drought stress imposed were severe enough to elucidate the differences in response to drought among the inbred lines and their hybrids under both plant densities.

The levels of yield reduction due to water shortage in the present study fell within the range reported by other authors (Rosielle and Hamblin, 1981; NeSmith and Ritchie, 1992; Badu-Apraku et al., 2011b). The relatively low yield reduction observed in some inbred lines in both plant densities suggested that these lines may carry drought-tolerant genes. These lines exhibited high grain yield and A at the water deficit regime. Hybrids 25 x 5, 28 x 8, 29 x 16, 28 x 18, 14 x 20 likewise the commercial hybrid were identified as the most outstanding in performance under drought and well-watered conditions. The tolerance of the hybrids that were derived from specific crosses did not follow a particular trend as there were hybrids that were tolerant and others were not. In particular, some of the tolerant

Table 5. Mean assimilation rate (A) of 30 inbred lines and their hybrids at ultra-low density ULD) and dense-stand (DS) conditions across two irrigation treatments and three sites. The average (Avg) mean yield from the three sites as well as the least significant difference (LSD) for comparisons among individual lines within each column (P ≤ 0.05), are also given.

Assimilation rate (A) (μmol CO_2 m^{-2} s^{-1})									
	ULD		DS			ULD		DS	
Inbred lines	Control	Stressed	Control	Stressed	Hybrids	Control	Stressed	Control	Stressed
1	21.61	19.17	21.63	15.43	25 x 7	28.00	20.12	22.85	13.99
2	22.60	19.84	24.64	17.17	25 x 30	25.09	20.49	23.25	14.74
3	24.63	21.18	19.98	19.86	7 x 29	24.16	20.35	24.87	14.62
4	18.81	16.11	19.29	16.82	10 x 30	29.01	20.11	25.52	16.97
5	23.15	17.88	22.00	17.21	6 x 15	25.79	22.10	21.74	14.53
6	20.20	18.52	16.89	20.81	25 x 2	24.46	20.72	20.10	13.03
7	22.23	21.75	21.82	22.60	25 x 5	23.92	21.31	26.31	16.82
8	21.64	17.79	21.42	20.70	25 x 9	27.93	18.76	25.40	15.97
9	23.86	20.07	22.89	22.55	25 x 17	25.50	21.43	21.88	13.36
10	22.39	17.74	24.62	12.91	28 x 8	25.32	20.09	22.80	12.96
11	19.87	17.90	19.56	21.41	29 x 9	28.96	20.74	25.12	15.36
12	20.86	21.86	18.38	18.10	29 x 16	25.89	18.57	22.94	14.06
13	21.69	18.76	20.46	21.40	26 x 12	24.71	20.86	26.16	15.28
14	22.95	18.02	22.70	18.27	26 x 18	26.78	21.42	21.03	13.62
15	23.39	19.51	18.72	19.12	26 x 22	24.32	17.21	23.10	15.20
16	23.23	19.52	21.50	20.26	26 x 27	24.05	19.14	23.29	15.56
17	25.51	22.99	22.83	20.07	13 x 22	22.49	19.92	24.49	15.12
18	23.79	19.48	23.46	17.98	2 x 15	25.99	20.44	25.41	14.62
19	22.15	20.34	23.46	20.27	22 x 30	22.67	18.71	22.99	16.72
20	16.92	17.38	21.77	17.74	26 x 3	26.41	19.64	23.12	15.09
21	24.54	20.92	19.32	20.25	26 x 29	25.98	19.79	20.97	12.15
22	21.98	21.48	18.56	15.43	26 x 30	26.55	19.80	23.82	16.86
24	21.67	21.71	18.56	22.20	26 x 17	23.44	20.24	21.78	16.52
25	24.08	21.79	20.22	21.31	28 x 22	23.88	19.11	23.96	15.09
26	24.48	20.61	21.25	25.73	3 x 30	25.50	22.99	21.17	15.04
27	21.71	18.50	19.68	20.65	28 x 18	25.57	21.82	22.00	13.72
28	24.11	19.64	26.92	18.21	15 x 12	29.04	18.84	26.52	18.41
29	22.00	16.76	23.55	22.37	17 x 20	25.35	21.16	25.62	16.30
30	22.54	19.95	24.53	22.41	24 x 20	25.17	23.44	21.94	16.47
31	26.05	22.70	17.97	21.75	14 x 20	24.04	20.52	23.92	21.37
					commercial	25.89	19.16	25.99	20.42
Average	22.49	19.66	21.38	19.85		25.54	20.29	23.55	15.48
LSD	2.45	2.86	2.12	2.64		2.87	2.13	2.98	2.21

hybrids were from the tolerant lines (28 x 8, 26 x 22 and 14 x 20), others from the sensitive lines (29x16) and others from the lines that were tolerant and sensitive (26 x 30, 28 x 18) indicating that the response to drought is a quite complex characteristic and cannot be predicted from the behavior of the parental inbred lines. Other researchers also tried to use drought tolerant inbred lines and to produce tolerant hybrids but they couldn't find any tolerant hybrids and therefore the tolerance could be transferred to their hybrids (Badu-Apraku et al., 2011a,b). This emphasizes the difficulty to produce drought tolerant hybrids from specific inbred lines and the need to concentrate also in the physiological basis of the tolerance of the inbred lines and their hybrids in order to be able to produce tolerant hybrids. Nevertheless, in the majority of the above hybrids one of their parents was experimental line developed for improved PYE (Tokatlidis et al., 1998), an agronomic trait documented as essential for high productivity of rainfed maize cultivation (Tokatlidis et al., 2015), as well as to adapt the crop to the climate change and alleviate the food insecurity problem especially in drought prone areas (Duvick, 2005; Berzsenyi and Tokatlidis, 2012; Tokatlidis, 2013).

The effect of drought on plant growth and development has been studied extensively in different levels, whole plant, molecular, and biochemical (Campos et al., 2004, Parry et al., 2005; Bäzinger and Araus, 2007; Brennan and Martin, 2007; Ribaut and Ragot, 2007; Tuberosa et al., 2007; Mullet, 2009; Tokatlidis, 2013). The decrease in photosynthetic efficiency is a well-known symptom of drought-induced stress and has been shown in many plant species (Di Marco et al., 1988; Schapendonk et al., 1989; Selmani and Wassom, 1991;

Table 6. Correlation coefficients of grain yield and the physiological parameters measured under ultra-low density (ULD) conditions for inbred lines over the three sites under control and water stressed conditions.

	A	g_s	Ci	E	Chl. Fl.	WUE	SPAD
			Control				
Grain Yield	0.393*	0.262	0.112	0.322	0.371*	0.381*	0.447*
A		0.924**	0.813**	0.979**	0.864**	0.897**	0.880**
g_s			0.842**	0.935**	0.723**	0.718**	0.781**
Ci				0.862**	0.833**	0.780**	0.786**
E					0.849**	0.848**	0.881**
Chl. Fl.						0.925**	0.838**
WUE							0.863**
SPAD							
			Stressed				
Grain Yield	0.384*	0.186	0.201	0.294	0.533**	0.467**	0.456**
A		0.885**	0.901**	0.961**	0.854**	0.807**	0.890**
g_s			0.819**	0.916**	0.609**	0.504**	0.710**
Ci				0.864**	0.821**	0.800**	0.840**
E					0.780**	0.653**	0.825**
Chl. Fl.						0.908**	0.866**
WUE							0.848**
SPAD							

* Significant at the 0.05 level of probability, ** Significant at the 0.01 level of probability, *** Significant at the 0.001 level of probability

Jamaux et al., 1997; Ober et al., 2005; Zarco-Perelló et al., 2005; O'Neill et al., 2006; Subrahmanyam et al., 2006; Khan et al., 2007; Hura et al., 2007; Živčák et al., 2008). In the present study a reduction of the assimilation rate was observed in lines and their respective hybrids after exposure to drought. This reduction was usually accompanied with a decrease in g_s and E (data not shown). However, there were genotypes that did not show any changes of stomatal function or even displayed an increased g_s under drought compared with the nonstressed plants and in this case there was also an increase in E and A. Under water stress stomatal closure occurs which affects E and also reduces A. But as the water stress persists there is a greater reduction in

Table 7. Correlation coefficients of grain yield and the physiological parameters measured under dense-stand (DS) conditions for inbred lines over the three sites under control and water stressed conditions.

	A	g_s	Ci	E	Chl. Fl.	WUE	SPAD
			Control				
Grain Yield	0.335	-0.103	0.234	-0.012	0.310	0.082	0.438*
A		-0.046	0.200	0.124	0.289	0.164	0.082
g_s			-0.652**	0.952**	-0.521**	-0.840**	-0.007
Ci				-0.552**	0.441*	0.507**	-0.089
E					-0.479**	-0.883**	0.173
Chl. Fl.						0.625**	0.012
WUE							-0.213
SPAD							
			Stressed				
Grain Yield	0.336	0.264	-0.027	0.273	-0.108	0.127	0.180
A		0.863**	0.089	0.834**	-0.164	0.484**	0.460*
g_s			0.525**	0.893**	-0.093	0.150	0.440*
Ci				0.367*	0.003	-0.440*	0.190
E					-0.140	-0.074	0.393*
Chl. Fl.						-0.070	-0.312
WUE							0.199
SPAD							

* Significant at the 0.05 level of probability, ** Significant at the 0.01 level of probability, *** Significant at the 0.001 level of probability

Table 8. Correlation coefficients of grain yield and the physiological parameters measured under ultra-low density (ULD) for hybrids over the three sites under control and water stressed conditions.

	A	g_s	Ci	E	Chl. Fl.	WUE	SPAD	MPH	HPH	Aver. Het
					Control					
Grain Yield	0.043	-0.093	-0.121	0.046	-0.103	0.006	0.322	0.193	0.064	0.873**
A		0.425*	0.159	0.877**	-0.174	0.463**	0.197	-0.097	-0.093	0.016
g_s			0.400*	0.556**	0.049	-0.148	0.180	-0.012	-0.012	-0.105
Ci				0.319	0.275	-0.269	0.088	-0.050	-0.133	-0.099
E					-0.080	-0.018	0.173	0.020	-0.013	0.062
Chl. Fl.						-0.223	-0.023	0.103	0.045	-0.064
WUE							0.082	-0.256	-0.181	-0.089
SPAD								0.214	0.102	0.426
MPH									0.960***	0.620*
HPH										0.489*
					Stressed					
Grain Yield	-0.356*	-0.392*	-0.232	-0.319	-0.422*	-0.085	0.138	0.044	-0.103	0.757**
A		0.563**	0.053	0.886**	-0.289	0.263	0.117	-0.008	0.062	-0.233
g_s			0.483**	0.514**	-0.005	0.108	0.161	-0.279	-0.148	-0.434*
Ci				0.146	0.116	-0.207	0.287	-0.201	0.022	-0.190
E					-0.369*	-0.214	0.080	0.026	0.085	-0.179
Chl. Fl.						0.148	0.089	-0.298	0.069	-0.426*
WUE							0.066	-0.071	-0.038	-0.130
SPAD								0.188	0.191	0.289
MPH									0.590**	0.418*
HPH										0.634**

* Significant at the 0.05 level of probability, ** Significant at the 0.01 level of probability, *** Significant at the 0.001 level of probability

A (Chaves et al., 2002; Chaves and Oliveira, 2004). It is generally accepted the model about the "stomatal control" which proposes that stomatal closure and the decrease of g_s are the main causes for the reduction of A under water stress (Chaves et al., 2002, 2009; Lawlor, 2002; Reddy et al., 2004; Christensen and Feldman, 2007; Lawlor and Tezara, 2009).

The maximum quantum efficiency of PSII photochemistry was affected by genotype and water stress, in agreement with previous studies (Di Marco et al., 1988; Selmani and Wassom, 1991; O'Neill et al., 2006). In addition, primary photosynthetic processes such as photosynthetic electron transport are considered to be rather resilient to water deficit, and reduction in photosynthetic electron transport efficiency occurs after there is an imbalance between the generation of NADPH and its utilization in the photosynthetic carbon reduction cycle (Cornic and Fresneau, 2002; Baker and Rosenquist, 2004). Under severe drought stress it was found that there is an increased generation of reactive oxygen species leading to photooxidation and the degradation of photosynthetic membrane proteins (particularly D1, D2 and CP43 proteins of PSII) and associated pigments and lipids (Cornic and Fresneau, 2002; Reddy et al., 2004). A close relationship between A and chlorophyll fluorescence was found only in the cases of lines in ULD but not at DS while it was absolutely absent in hybrids. Therefore, the lack of such a relationship suggests that the net photosynthesis in drought-stressed plants was not limited by the efficiency of PSII or the amount of chlorophylls or carotenoids but rather by the functioning of stomata.

Chlorophyll content in inbred lines was also affected by location, genotype and the interaction between genotype and location. In hybrids, chlorophyll content was affected by location, irrigation, genotype, and interaction of GxL, IrrxL and GxIrr in both plant densities. Chlorophyll content has been proposed as a good indicator of green color and the stay green characteristic (Li et al., 2006; Fotovat et al., 2007). Chlorophyll content was correlated with most of the physiological parameters measured and also with grain yield under both ULD and DS conditions. In the inbred lines, it was correlated only with A, g_s, and E but not with grain yield in both water regimes. In hybrids, however, the trend was quite different as chlorophyll content showed low correlation at ULD and DS conditions. These results indicate that chlorophyll content couldn't be a very good index for the selection of tolerant hybrids.

The use of physiological traits in breeding can help in the improvement of plant tolerance but has to fulfill several criteria such as the possibility of relatively simple and fast measurements of the respective parameter in many samples, its good correlation with the tolerance/sensitivity to the target stress factor, and an adequate intraspecific genetic variation (Brennan

Table 9. Correlation coefficients of grain yield and the physiological parameters measured under dense-stand (DS) for hybrids over the three sites under control and water stressed conditions.

	A	g_s	Ci	E.	Chl. Fl.	WUE	SPAD	MPH	HPH	Aver. Het
					Control					
Grain Yield	0.201	0.301	0.200	0.231	-0.155	-0.126	-0.048	0.344	0.329	0.478*
A		0.577**	0.330	0.836**	0.154	-0.053	0.117	0.300	0.389*	0.301
g_s			0.642**	0.658**	0.047	-0.332	-0.040	0.196	0.199	0.262
Ci				0.300	-0.028	-0.037	0.149	0.102	0.015	0.224
E					0.101	-0.588**	0.122	0.209	0.300	0.216
Chl. Fl.						0.061	0.074	0.006	0.118	-0.080
WUE							-0.058	0.049	0.018	0.033
SPAD								0.386**	0.291	0.445*
MPH									0.940**	0.941**
HPH										0.845**
					Stressed					
Grain Yield	0.142	-0.052	0.270	0.184	-0.055	-0.128	0.197	0.554**	0.491**	0.444**
A		0.774**	0.070	0.911**	0.341	0.127	0.351	0.522**	0.556**	0.481**
g_s			0.132	0.656**	0.323	0.209	0.355	0.337	0.340	0.345
Ci				0.230	-0.210	-0.390*	0.136	0.020	-0.054	0.009
E					0.186	-0.293	0.443*	0.558**	0.523**	0.522**
Chl. Fl.						0.335	0.091	0.314	0.293	0.304
WUE							-0.259	-0.149	0.001	-0.141
SPAD								0.411**	0.373	0.341
MPH									0.886**	0.890**
HPH										0.660**

* Significant at the 0.05 level of probability, ** Significant at the 0.01 level of probability, *** Significant at the 0.001 level of probability

and Martin, 2007; Sayar et al., 2008). The physiological parameters examined in our study certainly satisfy the first condition (particularly the Chl fluorescence measurements) and more-or-less meet also the second condition (based on the presence of positive correlations between Chl fluorescence parameters and the drought-induced changes in plant morphology and water status). In other studies it was found a good association between maize drought tolerance and Chl fluorescence excitation spectra (Grzesiak et al., 2007a) or Chl content (Grzesiak et al., 2007b). From this point of view, the measurement of A seems to be the least suitable among the three categories of photosynthetic parameters examined, as it is rather time-consuming and the relationship between A and drought-induced changes in plant morphology and development is not unequivocal (Grzesiak et al., 2006).

The significant intraspecific variability in physiological characteristics used in the present study were evidenced in numerous studies (Rao et al., 1978; Monma and Tsunoda, 1979; Baer and Schrader, 1985; Csapó et al., 1991; Crafts-Brandner and Poneleit, 1992; Mehta et al., 1992; Dolstra et al., 1994; Krebs et al., 1996). Therefore, these parameters can be used in breeding programs for finding maize drought tolerant genotypes. However, these characteristics should have high heritability (Sayar et al., 2008). From the present study it is obvious that the heritability of most of the characteristics was low and also quite complex results that are in agreement with other studies (Baer and Schrader, 1985; Mehta et al., 1992).

The weak correlations between grain yield and physiological traits that was found in hybrids in the present study emphasizes the need to evaluate hybrids under drought stress to identify superior hybrids for stress environments. The positive and significant correlation observed between mid-parent heterosis and the other heterosis indices and grain yield in this study are consistent with the findings of others (Betrán et al., 2003; Makumbi et al., 2011). Furthermore, the presence of strong associations between grain yield and some physiological characteristics under stress and control conditions demonstrated that some of these traits could be utilized as secondary traits for indirect selection for improved grain yield under stress and control conditions especially under ULD conditions. These results imply that drought stress significantly affected these traits, indicating the potential of the traits for predicting drought tolerance in maize.

Conclusions

We can thus conclude that although the determination of physiological characteristics can be used for a simple assessment of drought tolerance in collections of maize stressed conditions, the practical usability of such parameters in maize breeding programs is quite limited, because their measurement in parental genotypes subjected to water stress cannot provide any information on the progeny performance under such conditions.

Acknowledgement

Work co-financed by the European Union (European Regional Development Fund–ERDF) and Greek national funds through the Operational Program "Competitiveness and Entrepreneurship" of the National Strategic Reference Framework (NSRF)-Research Funding Program: Synergasia2009. Action I. Cooperative small- and mid-scale projects, program code 09 SYN-22-604. We are grateful to Dr. Pantazis Georgiou for helping of the ET calculations and to Dr. George Menexes for helping us with the statistical analysis.

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