

Characterizing corn hybrids (*Zea mays* L) for water shortage by principal components analysis

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Abstract

Identifying drought tolerant genotypes is very important for increasing corn yield. Thus four simple corn hybrids were submitted to periods of water shortage, at three phenological stages of the crop, to discriminate for drought tolerance by principal components analysis and identify their main differentiating characteristics under limited water conditions. Assessments were made of the leaf water potential, gas exchange, photochemical activity, grain yield and total dry matter. Analysis of variance was carried out and the mean values were compared by the Tukey test at 5% probability. Water shortage at the three phenological stages of the corn crop reduced the leaf water potential (Ψ_w), stomata conductance (g_s), photosynthesis (A_n), transpiration (E), potential quantum efficiency of the PSII (F_v/F_m), apparent rate of electron transport (ETR), photochemical dissipation coefficient (qP) and increased the non-photochemical dissipation coefficient (NPQ), mainly when there was drought at the vegetative and flowering stages. The data obtained for grain yield (GY) showed that the TOL1 hybrid was outstanding for drought tolerance at all the phenological stages of the crop, while the SENS1 hybrid demonstrated greatest sensitivity to water shortage, proving the pattern of tolerance and sensitivity of these respective hybrids to drought. The result of the principal components analysis showed that the corn hybrids could be separated into diversity groups for irrigation treatment at the vegetative stage, and that integrated photosynthesis (A_n) was considered the best characteristic to discriminate the irrigation treatments and hybrids when submitted to water shortage at this phenological stage of the crop.

Keywords: multivariate analysis, physiological parameters, drought tolerance and yield

Introduction

Modern agriculture faces great, new challenges for food production for a constantly growing population. One of these challenges is the sustainable use of water in field irrigation management because forecasts indicate that due to climatic changes, some regions will have less rainfall that will bring about the need to obtain water use efficient cultivars (Borém and Ramalho, 2011).

In this scenario, genetic breeding can contribute to mitigate food scarcity and endeavor to reduce waste of potable water used in irrigation by developing new cultivars tolerant to adverse irrigation conditions.

Whenever water availability is limiting to growth and plant development, the metabolism, biomass, photosynthesis rate and other factors that affect grain yield are damaged (Passioura, 2007). Therefore identifying or developing and using tolerant genotypes are efficient strategies to increase yield and reduce production risks in areas subject to constant periods of water shortage (Shiri et al, 2010).

However, drought tolerance is a complex characteristic. Some plants can withstand water shortage

using different mechanisms that involve morphological adaptations that allow the plants survive and produce satisfactorily (Xoconostle-Cazares et al, 2010). Thus, different characteristics have been suggested as selection criteria to improve tolerance levels to drought.

Many plant species respond differently in terms of ability to deal with drought (Silva et al, 2011). In plants considered sensitive, the physiological processes are affected because tissue hydration is reduced. In these so-called tolerant plants, the physiological and metabolic properties are maintained by the ability of the tissues to remain hydrated under water shortage conditions (Bartels and Sunkar, 2005).

Most of the areas planted with corn have constraints to plant development and diverse levels of water shortage occur during crop development. The impact of drought on food prices threatens food safety in the locations where corn is a basic food in the diet of the population. The most recent report from the American Agriculture Department (USDA) shows that the situation of this crop in the so-called Corn Belt, in the midwest of the USA, is critical because more than 1.3 thousand counties have already declared an emergency situation due to adverse irriga-

tion conditions. The prolonged impact of the drought period in the USA will cause a 3-4% increase in food prices in the supermarkets in the next year compared to the levels of 2012 (USDA, 2012).

Thus the choice of genotypes adapted to adverse irrigation conditions becomes an important stage in plant breeding programs and it is necessary to identify the most promising when in a situation of drought tolerance in the field (Pavan, 2005). Drought-tolerant genotypes can be identified by analyzing physiological characteristics helping genetic breeding (Nogueira et al, 2001), searching to understand the traits that optimize growth and confer greater tolerance to drought (Pita et al, 2005).

However, there is not a single parameter that alone can indicate drought tolerance, and the ideal is to assess parameters that can be measured at different stages of the crop, in the greenhouse and in field experiments (Pimentel and Rossiello, 1995). Selecting a drought-tolerant corn genotype with high grain yield would be most efficient if the attributes that provide these features under limited water conditions could be identified and used as selection criteria (Durães, 1997).

Since several physiological characteristics may need be assessed, a statistical methodology should be used to indicate the traits that most discriminate the genotype under drought tolerance conditions (Shiri et al, 2010). The principal components analysis (PCA) is a multivariate statistics technique that allows analysis among several quantitative variables, measured in many treatments (Bailey, 1974). This analysis involves mathematically the transformation or reduction of a number of variables correlated with a smaller number of non-correlated variables. The principal components analysis has been widely used in plant research to reduce variables and group genotypes.

Therefore, the objectives of the present study were to characterize the response of four contrasting corn hybrids to drought tolerance, submitted to water shortage at three phenological stages and to interpret the data with multivariate statistics to identify the main traits that differentiate the hybrids under limited water conditions and prove the pattern of drought tolerance and sensitivity.

Materials and Methods

Plant material

Four simple corn hybrids were sown directly on soil in a greenhouse in April 2011. The experiment was carried out at the Fazenda Santa Elisa Experimental Center – Campinas Agronomic Institute (IAC), in Campinas, São Paulo, Brazil. The hybrids used in the study were DAS2B707 and DAS2B710, considered drought tolerant, due to good performance and yield when submitted to water shortage conditions; and two commercial hybrids that were considered sensitive when the results were analyzed of regional corn trials for assessment and VCU (cropping and

use value) purposes in the state of São Paulo.

The hybrids were called TOL1, TOL2, SENS1 and SENS2, respectively, for tolerance and sensitivity to drought.

Growing conditions

Drip irrigation was used (3.75 L h⁻¹) with nozzles spaced at 0.5 m. Irrigation was applied when the soil water potential was around -0.10 MPa, except during the periods of low water availability in the soil. Water potential was monitored by tensiometers placed in the experimental plots. Water shortage was imposed by withholding water at three phenological plant stages: when the plants presented five completely opened leaves (V5); at flowering when 50% of the plants presented style-stigma (R1); grain swelling when the corn ears presented grains with a milky appearance (R3). In the control treatment, the plants were irrigated during the whole cycle of the crop.

The plants under to drought were rehydrated when they presented visual symptoms of accentuated wilting in the early morning. The water was withheld for 40 days at V5, 75 days at R1 and 52 days at the R3 stage.

Leaf Water Retention

The leaf water potential (Ψ_w) was measured with a pressure chamber, model 3005 (SoilMoisture, USA) on leaves of the plants in the plots submitted to the four water regimens. The measurements were taken

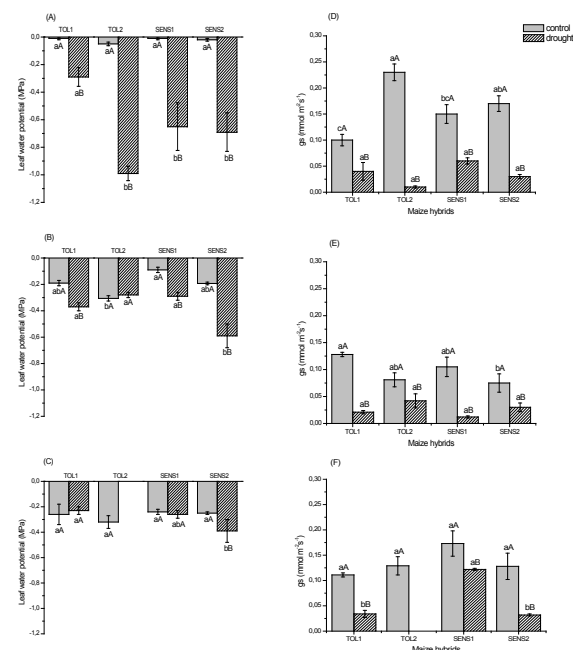


Figure 1 - The mean leaf water potential (Ψ_w) and stomata conductance (gs) assessed on the day of maximum water shortage at the vegetative, flowering and grain swelling stage. Means with lowercase letters in the graph differ by the Tukey test ($P < 0.05$) for genotype and means with uppercase letters differ by the Tukey test ($P < 0.05$) for irrigation treatments.

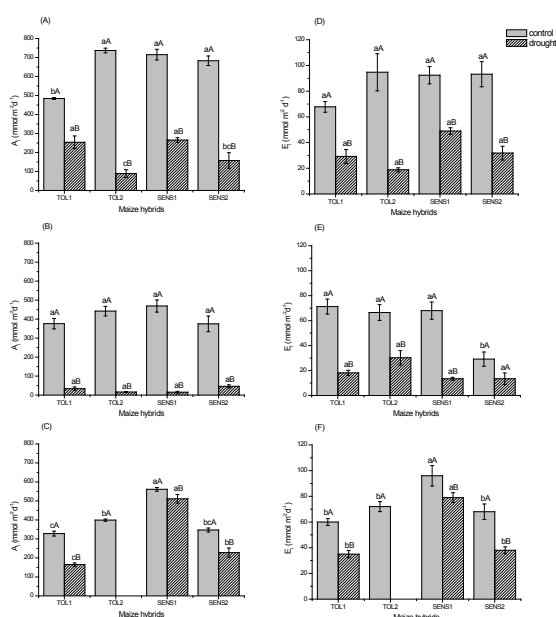


Figure 2 - Mean integrated photosynthesis (A) and integrated transpiration (E) assessed on the day of maximum water shortage at the vegetative, flowering and grain swelling stage. Means with lowercase letters in the graph differ by the Tukey test ($P < 0.05$) for genotype and means with uppercase letters differ by the Tukey test ($P < 0.05$) for irrigation.

in the early morning (5:20 AM) on the days of maximum water shortage.

Gas exchanges and photochemical activity

The leaf gas exchanges were assessed with an infrared radiation gas analyzer model Li-6400F (Licor, USA). The variables studied were: stomata conductance (gs), photosynthesis (A) and transpiration (E).

Fluorescence emission from chlorophyll a was measured with a modulated fluorometer (6400-40 LCF) coupled to the Li-6400F. The leaves were previously adapted to the dark for 30 min to determine minimum fluorescence in tissues adapted to the dark (F_0), then a pulse of saturating light ($\lambda = 630$ nm, $Q \sim 6000 \mu\text{mol m}^{-2} \text{s}^{-1}$, 0.8 s) was applied to determine the maximum fluorescence in tissues adapted to the dark (F_m).

The fluorescence in the dark ($F_v = F_m - F_0$) variable was calculated from the F_0 and F_m , and then the potential quantum efficiency of photosystem II (F_v/F_m) was determined. Instantaneous (F_s) and maximum (F_m') fluorescence in tissues adapted to luminosity were also assessed by the saturation pulse method. The minimum fluorescence in tissues adapted to luminosity (F_0') was assessed after exciting photosystem I with distant red radiation ($\lambda = 740$ nm, $Q \sim 5 \mu\text{mol m}^{-2} \text{s}^{-1}$, 2.0 s). The following were also assessed: the coefficients of photochemical extinction [$\text{qP} = (F_m' - F_s)/(F_m' - F_0')$] and non-photochemical [NPQ = $(F_m - F_m')/F_m'$] extinction of the fluorescence and apparent electron transport [ETR = $Q_x \Delta F/F_m' \times 0.5 \times 0.84$]

(Rohacek, 2002). In the ETR calculation it was taken that the leaf absorbed 0.84 Q (Demmig and Björkman, 1987) and that there was identical energy partition between the first systems, i.e., 0.5.

The photosynthesis (A) and transpiration (E) data were integrated during the assessment day at the three phenological stages of the crop. These measurements were taken at 8:30 AM, 10 AM, 1:30 PM, and 4:30 PM, while the other physiological traits were assessed at the time of high atmospheric demand (1:30 PM) on the day of maximum water shortage at the three phenological stages studied.

Biometric assessments

At 160 days after planting, the grain yield (GY) and plant total dry matter (TDM) were assessed. The GY was assessed after shucking the ears from seven plants in each plot. The TDM value was determined after weighing the leaf, stem and ear fractions of seven plants per plot. The fractions were collected and dehydrated in a forced air circulation chamber at 60°C to obtain constant dry matter.

The values of the biometric variables of the hybrids submitted to drought were converted to the relative tolerance value (RTV), where the values of each characteristic assessed under water shortage were divided by the values of the irrigated condition (Souza et al, 2011).

Experimental design and data analysis

A split plots design was used with three replications. The plots consisted of the four water regimens (control, drought in vegetative stage, drought in flowering stage and drought in grain swelling stage), while the subplots consisted of the four hybrids (TOL1, TOL2, SENS1 and SENS2). Data from each of the four watering regimens was averaged and compared to the control treatment, giving two levels of irrigation, drought and well watered. The data were submitted to analysis of variance (ANOVA) and the mean of the values ($n = 3$) compared by the Tukey test ($p \leq 0.05$) when there was significant difference.

Principal components analysis (PCA) was carried out with the first two principal components accounting for over 80% of the total variability. The principal components analysis was performed according to Cruz and Regazzi (1994), using the GENES program (Cruz, 2007).

Results and Discussion

The leaf water potential (Ψ_w) is one of the traits most used to measure water shortage intensity in plants (Flexas et al, 2004) and when it is assessed in the early morning it shows the water condition of the plant in balance with the soil (Bergonci et al, 2000). Generally, the Ψ_w was reduced because watering was suspended at all the phenological stages assessed, so that it could be inferred that this trait is a good indicator of water shortage. A similar result was

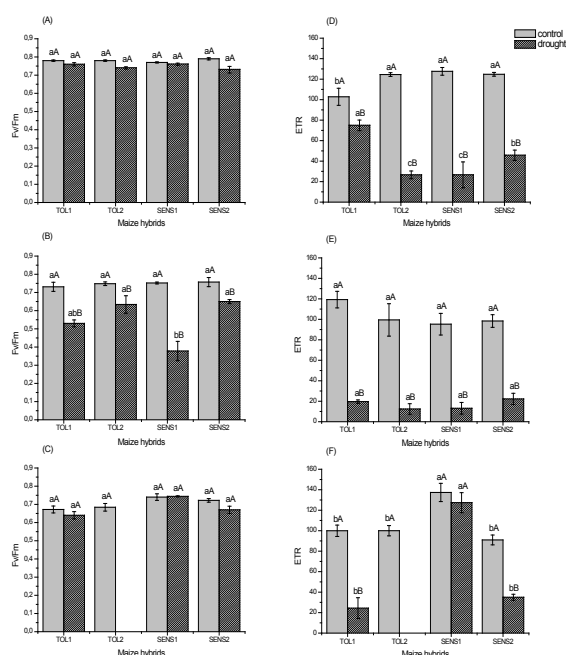


Figure 3 - The mean potential quantum efficiency values of PSII (Fv/Fm) and the apparent electron transport rate (ETR) assessed on the day of maximum water shortage at the vegetative, flowering and grain swelling stage. Means with lowercase letters in the graph differ by the Tukey test ($P < 0.05$) for genotype and means with uppercase letters differ by the Tukey test ($P < 0.05$) for irrigation.

reported by Bergonci et al (2000) who assessed the Ψ_w in corn hybrids submitted to three irrigation levels and reported that the Ψ_w in the non-irrigated plants were lower than in the irrigated plants.

Absence of irrigation affected the hybrids differently, with Ψ_w ranging from -0.30 (TOL1) to -0.99 MPa (TOL2); from -0.28 (TOL2) to -0.60 MPa (SENS2) and from -0.23 (TOL1) to -0.40 MPa (SENS2), respectively at the vegetative stage (Figure 1A), flowering (Figure 1B) and grain swelling (Figure 1C) stages. The SENS2 hybrid presented low Ψ_w values after water shortage in all the phenological stages assessed while the TOL1 hybrid presented the highest Ψ_w values when there was drought in the phenological stages assessed. The high Ψ_w value observed in the TOL1 hybrid under water shortage was related to partial stomata closure. Furthermore, the better hydration of this hybrid was certainly associated to some other characteristic, such as increase in root density and depth that ensured greater soil exploitation.

Analysis of the effect of water shortage showed that when available water was restricted in the soil at the three development stages there was a fall in the stomata conductance values (gs) in all the hybrids (Figure 1D-F). This result showed that under severe stress one of the first responses of these hybrids was stomata closure to minimize water loss and also reduce the net photosynthesis rate (Flexas et al, 2004; Larcher, 2004). Control of stomata opening and closing is essential to regulate the balance between wa-

ter loss through transpiration (E) and CO_2 assimilation (A) by the plant (Pimentel, 2004). Indeed, in the present study, the water shortage imposed implied gs reduction and consequently it reduced E_i and A_i in all the hybrids compared with the plants that were kept irrigated. Significant difference was not detected among the hybrids used under drought conditions in the vegetative and flowering stages. The TOL1 and SENS2 hybrids presented greater reductions in gs at the grain swelling stage. The SENS1 hybrid presented the greatest gs when submitted to drought at this stage, that consequently led to greater photosynthesis and respiration under the same condition. Decrease in stomata conductance and photosynthesis were also observed by Xu et al (2009) in a Chinese corn variety and by Ghannoum et al (2003) in four different grass species under water shortage.

When the plants were submitted to adverse irrigation systems in the vegetative stage, the TOL2 hybrid presented greater reduction in integrated photosynthesis (A) (Figure 2A). At the flowering stage significant difference was not detected among the hybrids under drought conditions, there was only significant difference between irrigation treatments (Figure 2B). The TOL2 hybrid after 52 days without irrigation at the grain swelling stage no longer presented green leaves, so only the results of the physiological variables of this hybrid and its irrigated conditions are presented (Figure 2C). Generally, the drought periods at the vegetative and flowering stages were the most critical for the hybrids assessed for photosynthesis.

Water shortages in the plants at the vegetative and flowering stages reduced the E_i values (Figure 2D and E, respectively). However, at these stages significant differences were not observed among hybrids. Nevertheless there was significant difference among the water regimens. Water shortage in the grain swelling stage caused stomata closure in the TOL1 and SENS2 hybrids (Figure 2F). This tendency coincided with the reductions in the stomata conductance (gs) observed over the experimental period, showing differences among the hybrids assessed. The direct relation between transpiration and stomata conductance was expected, bearing in mind the decrease in the water vapor flow to the atmosphere and consequently in transpiration, as these stomata closed. For corn, stomata can close within a leaf water potential range of -1.7 and -2.0 MPa (Bergonci and Pereira, 2002). However, Bergonci et al (2000) observed decrease in stomata conductance in corn when the leaf water potential reached -1.5 MPa.

The Fv/Fm variable is an estimate of the maximum quantum efficiency of the photochemical activity of photosystem II, when all the PSII reaction centers are open (Baker and Rosenqvist, 2004). This ratio has frequently been used to detect disturbance in the photosynthesis system caused by environmental and biotic stress, because its reduction indicates photochemical activity inhibition with probable damage to

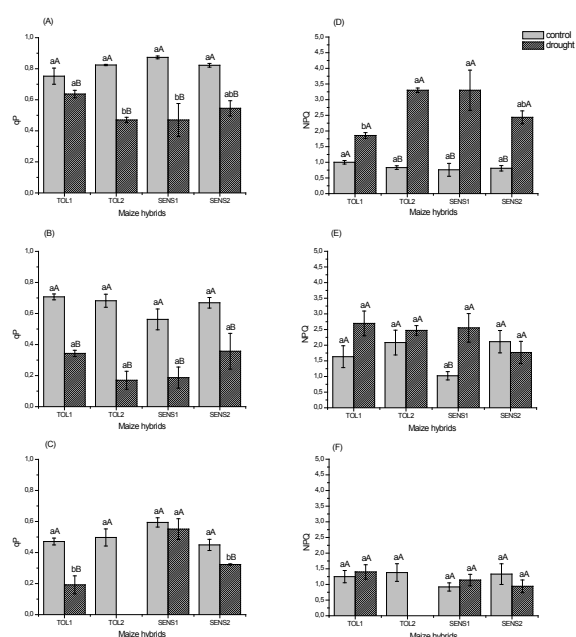


Figure 4 - Mean photochemical dissipation coefficient (qP) and non-photochemical dissipation coefficient (NPQ) assessed on the day of maximum water shortage at the vegetative, flowering and grain swelling stage. Means with lowercase letters in the graph differ by the Tukey test ($P < 0.05$) for genotype and means with uppercase letters differ by the Tukey test ($P < 0.05$) for irrigation.

CO₂ assimilation.

When water shortage was imposed at the vegetative stage, no significant differences were observed among the hybrids or irrigation treatments (Figure 3A). This capacity to maintain similar Fv/Fm values under water shortage in relation to the control treatment indicated the high resistance of the photochemical activity under limiting conditions (Silva et al, 2007). When water shortage occurred at the flowering stage the Fv/Fm value decreased in all the hybrids, showing that suspending irrigation damaged the PSII function, causing damage to the thylakoid structure caused by stress (Glynn et al, 2003). The SENS1 hybrid was most affected by water shortage when the Fv/Fm was considered (Figure 3B). Drought at grain swelling also caused significant differences among the hybrids assessed, and SENS1 obtained the greatest Fv/Fm compared to the other hybrids assessed (Figure 3C).

In most of the hybrids, the apparent electron transport rate (ETR) was affected by water shortage at the three phenological stages of the crop (Figure 3D-F), except for SENS1 during water shortage at the grain swelling stage, that presented the highest ETR value compared to the other hybrids assessed, regardless of the water conditions (Figure 3F). This reduction in ETR due to water shortage indicated that stress inhibited electron transport in photosystem II, damaging the carbon metabolism in these hybrids.

Water shortage reduced the photochemical extinction coefficient (qP) in function of the reduction

in electrons exported from PSII, because enzymes involved in the carbon metabolism and the stomata opening were inactivated. This variable represents the proportion of energy of the photons captured by the PSII reaction centers opened and dissipated by electron transport (Juneau et al, 2005), reflecting the degree of oxidation and reduction in the PSII primary quinone electron acceptor (QA). Absence of irrigation at the vegetative stage showed that TOL1 presented greater qP compared to the other hybrids (Figure 4A). There was no significant difference in this variable when the plants were submitted to water shortage at the flowering stage (Figure 4B), while at the grain swelling stage SENS1 was the only hybrid that did not show reduction in qP due to water shortage (Figure 4C).

Generally, water shortage increased non-photochemical extinction (NPQ) at the vegetative (Figure 4D) and flowering stages (Figure 4E), increasing the proportion of energy used to generate the transthylakoidal proton gradient (Baker and Rosenqvst, 2004). This increase in NPQ indicated that these plants dissipated more of the luminous energy absorbed by heat formation in the PSII in detriment to the photochemical activity, that is, chemical energy production in the form of ATP and NADPH, a process considered to be photoprotective. There was no significant difference in plants under water shortage at the grain swelling stage among the hybrids and among the irrigation regimens (Figure 4F).

Corn is extremely sensitive and yield decreases if drought occurs at the reproductive stage as a result of physiological processes linked to zygote formation and the start of grain swelling (Zinselmeier et al, 1995). However, periods of drought at the reproductive stage did not affect the yield of the TOL1 and TOL2 hybrids considered tolerant to water shortage regarding grain swelling nor yield of the SENS2 hybrid, considered sensitive to drought. The three hybrids were outstanding for RTV for grain yields (GY) in practically all the phenological stages of the crop (Figure 5A). The SENS1 hybrid productivity was damaged when it was submitted to drought at the vegeta-

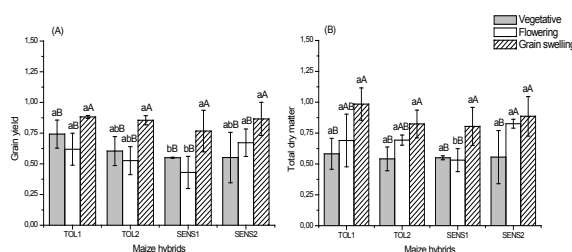


Figure 5 - Mean of the relative tolerance value (RTV) of grain yield (GY) and total dry matter (TDM) assessed on the day of maximum water shortage at the vegetative, flowering and grain swelling stage. Means with lowercase letters in the graph differ by the Tukey test ($P < 0.05$) for genotype and means with uppercase letters differ by the Tukey test ($P < 0.05$) for irrigation.

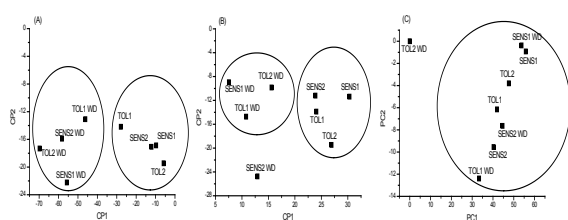


Figure 6 - Graphic dispersion of four corn hybrids submitted to drought at the vegetative (A), flowering (B) and grain swelling (C) stages, considering the principal components analysis (CP1 and CP2) reference to eight physiological traits. WD-water deficit.

tive and flowering stages of the crop.

There were no significant differences among the hybrids at the grain swelling stage when irrigation was suspended, indicating that in the present study, this stage was not considered critical for the productivity of these hybrids.

There was significant difference among the hybrids for total dry matter (TDM) only when there was water shortage at the flowering stage, and the SENS1 hybrid suffered most with water shortage at this stage (Figure 5B).

Generally, the four hybrids assessed suffered greater reductions in GY and TDM when there was water shortage at the vegetative and flowering stages, indicating that they are the most critical stages of the corn crop when under adverse irrigation conditions.

By using the principal components analysis parameters can be discarded that contribute little to

the differentiation of the hybrids assessed or that are redundant because they are highly correlated with other characteristics, so that the analysis of the data is more objective (Cruz and Regazzi, 1994).

At the vegetative stage, the first two principal components explained, respectively, 98.2% and 1.3% of the variability therefore accounting for 99.5% of the total variance which allowed the graphic analysis of the dispersion of these two variables in a bi-dimensional space (Table 1). Two diversity groups were formed, the first consisting of the hybrids under adequate irrigation conditions and the second group formed by the hybrids submitted to water shortage. It was verified that in the group of the hybrids submitted to water shortage, the SENS1 hybrid was most damaged regarding its physiological parameters assessed under stress (Figure 6A). The variables of greatest importance for diversity were A_i and ETR, because they presented a higher weighted coefficient in the components of greater eigenvalue.

For the flowering phenological stage, the first two principal components explained, respectively, 60.0% and 25.0% of the variability therefore accounting for 85.1% of the total variance (Table 1). Three groups were formed, the first by the hybrids submitted to water shortage, TOL1 WD, TOL2 WD, and SENS1 WD. The second group consisted of the same hybrids under adequate irrigation whilst the SENS2 WD hybrid performed differently from the other hybrids when submitted to the same stress conditions and was separated into a third diversity group (Figure 6B). The variables of greatest importance for separating the hybrids were A_i and Ψ_w . (Table 1).

Table 1 - Estimates of variance of the components associated to the principal components, relative accumulated importance (root %), and importance of the eigenvectors characteristics regarding the eight physiological traits assessed in four corn genotypes submitted to drought in three phenological stages of the crop.

Components	Variance	Root (%)	% Accumulated	Importance of the characteristics
Vegetative				
PC1	2,108.972	98.21	98.21	A_i
PC2	131.587	1.31	99.52	ETR
PC3	13.279	0.34	99.86	gs
PC4	0.125	0.08	99.94	ETR
PC5	0.005	0.06	100	qP
Flowering				
PC1	1,811.394	60.05	60.05	A_i
PC2	68.180	25.03	85.08	Ψ_w
PC3	45.294	10.97	96.05	ETR
PC4	0.189	2.08	98.13	E_i
PC5	0.004	1.87	100	gs
Grain swelling				
PC1	557.203	91.48	91.48	A_i
PC2	51.431	7.04	98.52	qP
PC3	10.513	0.66	99.18	ETR
PC4	0.049	0.51	99.69	qP
PC5	0.005	0.41	100	ETR

A_i : integrated photosynthesis, E_i : integrated transpiration, stomata conductance (gs), photochemical dissipation coefficient (qP), non-photochemical dissipation coefficient (NPQ), potential quantum efficiency of the PSII (Fv/Fm), apparent electron transport rate (ETR) and leaf water potential (Ψ_w).

At the grain swelling stage, the first two principal components explained, respectively, 91.5% and 7.0% of the variability therefore accounting for over 98.5% of the total variance (Table 1). The hybrids submitted to water shortage and those under adequate irrigation conditions remained in the same diversity group, except for TOL2 WD that under stress conditions no longer presented tissue suitable for physiological analysis at 42 days after suspending irrigation (Figure 6C). The variables of greatest importance, that present most of the total variation available, were A_1 and qP .

Conclusions

Generally, periods of water shortage at the three phenological stages of the corn crop reduced the leaf water potential (Ψ_w), stomata conductance (g_s), photosynthesis (A_1), transpiration (E_i), PSII potential quantum efficiency (F_v/F_m), apparent electron transport rate (ETR), photochemical dissipation coefficient (qP) and increased the non-photochemical dissipation coefficient (NPQ), especially when there was drought at the vegetative and flowering stages.

The productivity data showed that the TOL1 hybrid was outstanding for drought tolerance in all the phenological stages of the crop, while the SENS1 hybrid showed the greatest sensitivity to water shortage, proving the tolerance and sensitivity pattern of these respective hybrids for drought.

The results of the principal components analysis showed that it was possible to separate the corn hybrids into diversity groups for irrigation treatment used at the vegetative stage, that indicated that the SENS1 hybrid was the most damaged regarding its physiological parameters under stress, and integrated photosynthesis (A_1) was considered the best characteristic to discriminate the irrigation treatments used in the present study.

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