Comparative analysis of drought stress response of maize genotypes using chlorophyll fluorescence measurements and leaf relative water content

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Abstract

The initial photochemical quantum efficiency of photosystem II (F_v/F_m) and performance index (PI), describing the ability of the photosynthetic apparatus to collect light energy, have been used to screen tolerance to drought stress by ten maize accessions, monitored by leaf relative water content (RWC) and soil water content (SWC). The F_v/F_m, PI, and RWC values were significantly reduced in drought-stressed plants. The analysis of chlorophyll *a* fluorescence induction rise from the basic dark-adapted fluorescence yield to the maximum (OJIP transient), distinguished accession Zea 1006 from Libya and Zea 612 from Italy, as the most tolerant and the least tolerant genotypes. The maize genotypes were classified using the Principal Component Analysis (PCA) and clustering methods, based on F_v/F_m and PI values, leaf RWC and SWC. Genotypes from Egypt and Libya were differentiated from genotypes from Europe, Russia and the USA.

Introduction

Plants display a variety of morphological, physiological, and biochemical responses to drought stress, such as deep root system, small leaves, and range of mechanisms to withstand drought, such as reduced water loss, increased water uptake, reduced transpiration, and sustained high leaf tissue water potential, and higher chlorophyll (Chl) contents and photosynthetic efficiency (Farooq *et al.* 2009, 2012; Liu *et al.* 2015). Relative water content (RWC) accurately reflects cell volume and indicates the balance between absorbed and consumed water through transpiration and is considered an appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit (Smart and Bingham 1974). A correlation between high leaf RWC of wheat cultivars and resistance to drought stress was reported by Schonfeld *et al.* (1988). Osmoregulation was proposed as one of the main mechanisms preserving turgor pressure against water loss; it allows plants to retain their metabolic activities (Gunasekera and Berkowitz 1992).

Maize (*Zea mays* L.) is well-recognized as one of the most important cereals worldwide. Maize is known to be sensitive to moderate drought particularly at the heading stage; however, shortage of soil water availability at the beginning of plant growth may also dramatically limit the biomass production and the photosynthetic ability of leaves, and thus indirectly negatively affect the formation of reproductive organs and yield parameters (Less and Galili 2008, Benešová *et al.* 2012, Anjum *et al.* 2017). Maize expresses the NADP-malic enzyme-dependent C_4-dicarboxylic acid pathway for carbon fixation. However, the C_4 pathway confers no particular ability to withstand low leaf water potential which was used as efficient reference indicators of drought tolerant triticale and maize genotypes (Grzesiak *et al.* 2006). In maize leaves exposed to drought, Chl pigments, photosynthetic rate, transpiration rate, stomatal conductance, and photochemical efficiency

are significantly reduced (Song *et al.* 2019).

Photosynthesis is the most fundamental and intricate physiological process in plants. It involves various components, including photosynthetic pigments and its mechanisms are severely affected by the stressful environments (Ashraf and Harris 2013, Bąba *et al.* 2019). Chl fluorescence (ChlF) is electromagnetic radiation emitted by the Chl in plants. ChlF analysis is used to calculate the initial quantum yield of PSII Q_A reduction (F_v/F_m) and to quantify the performance of the electron transport chain as a performance index (PI), describing the ability of the photosynthetic apparatus to collect light energy and use it for photosynthetic electron transport (Żurek *et al.* 2014, Kalaji *et al.* 2017). F_v/F_m has been widely used to describe the efficiency of PSII. This parameter is considered a sensitive indicator of plant photosynthetic performance with healthy samples typically achieving a maximum F_v/F_m value of approximately 0.83 (Björkman and Demmig 1987). Values lower than this have been observed if samples exposed to biotic or abiotic stress which reduces the capacity for photochemical quenching of energy within PSII, *e.g.*, through the process of photoinhibition (Maxwell and Johnson 2000, Strasser *et al.* 2000, 2004; Kalaji *et al.* 2016). The performance index, PI, which is derived from the first phase of fast Chl induction curves of the PSII-dependent ChlF (OJIP-test), has been developed as a fast and reliable tool for evaluating plant tolerance to abiotic stresses (Strasser *et al.* 2000, 2004; Zushi and Matsuzoe 2017).

With simple-used, hand-held devices, it is now possible to monitor the stepwise reduction of the photosynthetic electron transport chain (ETC), and its different temporal phases (Maxwell and Johnson 2000). The F_v/F_m test is designed to compare the dark-adapted leaf prephotosynthetic fluorescent state, called minimum fluorescence, or F_0, to maximum fluorescence called F_m (Murchie and Lawson 2013). The data assembled during the trajectory from F_0 to F_m can be used to quantify these reduction processes and calculate quantum efficiencies, flux rates, reaction center densities which, in turn, can be combined to the overall PI describing the ability of the photosynthetic apparatus to collect light energy in the antennae and perform electron transport beyond Q_A (Strasser *et al.* 2000, 2004; Kalaji *et al.* 2016). From the first practical utilization of F_v/F_m, as a sensitive indicator of plant photosynthetic performance, its reduction has been regarded as an indicator of the reduction of PSII efficiency known as photoinhibition. Photoinhibition of PSII occurs when the rate of photodamage of PSII exceeds the rate of repair of the photodamaged PSII. Murata *et al.* (2012) updated the views on PSII protection against photodamage by stimulating protein synthesis, with resultant repair of PSII damage and mitigation of photoinhibition. The changes in F_v/F_m and PI values have already been used to identify drought-tolerant genotypes in cereals such as Moroccan landraces; sensitive lines were indicated by decreasing PSII connectivity and inhibiting electron supply from the water-splitting system, respectively (Oukarroum *et al.* 2007, 2009). Also, the OJIP fluorescence transient analysis allowed identification of the most sensitive and the most tolerant lines of barley and sorghum (Jedmowski *et al.* 2013). The OJIP test has been recommended as a nondestructive, simple, and rapid technique to screen, characterize, evaluate, and monitor the physiological status of plants under abiotic stress conditions (Kalaji *et al.* 2016, Zushi *et al.* 2017).

Drought sensitivity indices have been used as ultimate measures of drought tolerance based on changes in dry mass or yield components under stress conditions compared to the control. Grzesiak *et al.* (2006, 2013) reported that drought susceptibility index (DSI), stress tolerance index (STI), and stress index (SI) were most useful to identify genotypes differing in their response to drought. Golbashy *et al.* (2010) found that STI and GMP (Geometric Mean Productivity) indices have a similar ability to separate drought sensitive and tolerant genotypes of maize hybrids. Recently, Grzesiak *et al.* (2019) used drought tolerance indices in PCA and clustering analyses to assess the response of 20 wheat genotypes to drought. The utility of the indices was supported by physiological markers of drought tolerance such as membrane injury, and leaf water status has been integrated with statistical methods and indicated that tolerance to drought in wheat has a common genetic background. The results of such integration allowed selection of diverse genotypes for breeders (Grzesiak *et al.* 2006, 2019). Drought tolerance in plants has been also evaluated using the PCA biplot analysis to identify parameters that best describe the tolerance to response variables. Eigenvectors generated by PCA are used to identify parameters that differentiate plant genotypes for drought tolerance. Drought tolerance in maize has been increasingly evaluated using the PCA analysis in recent years (Arisandy *et al.* 2017, Hefny *et al.* 2017).

The objectives of this study were to identify the indicators related to drought tolerance through analysis of photosynthetic traits in ten maize genotypes, particularly the maximum quantum yield of PSII (F_v/F_m) and the PI of photosynthesis. The drought tolerance was also estimated by monitoring soil water content and the determination of leaf relative water content in stressed plants and well-watered control plants of each accession.

Materials and methods

Plant material growth conditions and drought stress application: Grains of ten accessions of maize (*Zea mays* L.) were secured from the Egyptian Grain Crops Research Institute in the Agricultural Research Center and the German Gene Bank in the Leibniz Institute for Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. List of accessions and their source and origin are given in Table 1. Grains of each accession were germinated on wet filter paper and single seedlings were transplanted into $14 \times 14 \times 13$ (depth)-cm pots filled with 450 g of peat moss medium (*Stender AG*, Germany) and grown in a climate chamber under 16-h photoperiod with light intensity of 400 µmol(photon) $m^{-2} s^{-1}$, temperatures of 26°C (light)/24°C (dark), and air humidity of 70%. Soil volumetric water content (SWC) was kept at about 20%

using a moisture meter (*Delta-T Devices Ltd*., Cambridge, UK). Five morphologically similar seedlings of each accession were exposed to drought at the stage of four leaves in order to allow the successive development of an acclimation response, by withholding watering. Five other similar seedlings were watered regularly to keep SWC at 20%. The soil water potential at the end of drought treatment (17 d) was about −2.5 MPa, as measured using a *Psypro* psychrometer (*Wescor*, Utah, USA).

ChlF: The fast ChlF induction curves (Strasser and Strasser 1995) of the fifth leaf of control and drought-stressed plants, were measured with a *Pocket PEA* (*Hansatech*, King's Lynn, UK), following 6 h of dark adaptation. Ten measurements were taken per accession and treatment twice a week starting after 3 d from the date of withholding watering of the drought-stressed plants. For comparison of the induction curves of drought-stressed (D) and control plants (C), fluorescence curves (F_t *vs.* log t) were double normalized to obtain $V_{(t)}$ *vs*. log t curves with $V_{(t)} = (F_t - F_0)/(F_m - F_0)$ and averaged per line and treatment per day. Then, differential curves of the average curves were calculated according to $\Delta V_{(t)} = V_{(t)DS} - V_{(t)C}$ (Jedmowski *et al.* 2013).

SWC and leaf RWC: The drought-stress treatment was stopped when permanent wilting was evident for two successive days on the leaves of the most sensitive accession (Zea 612) after 17 d of withholding watering. At the end of the drought-stress treatment, SWC was measured using a moisture meter (*Delta-T Devices Ltd*.), as described above. At the end of the drought application period, five measurements for five control plants and five drought-stressed plants were taken regularly for calculating the RWC as described by Smart and Bingham (1974) using the equation RWC [%] = [(FM – DM)/(TM – DM)] × 100, where FM, TM, and DM are the fresh, turgid, and dry masses, respectively. Three leaf discs for each accession plants exposed to drought and corresponding control plants were cut and immediately weighted (FM), then saturated to turgidity by immersing in cold water overnight, briefly dried, and weighted (TM), and oven-dried at 80°C for 24 h and weighted (DM).

Data analyses and presentation: The measurements of SWC, RWC, F_v/F_m, and PI were subjected to analysis of variance (*ANOVA*) using *GenStat* for *Windows ver. 18* (*VSN International*) and analyzed as a complete block design with five replicates. Probability of significance in *ANOVA* (*P*<0.05) was used to indicate significant differences between the accessions. Means were separated according to the *Fisher*'s Least Significant Difference (LSD) at 0.05 levels of probability. The values of RWC, F_v/F_m, and PI for five plants of each genotype grown under drought stress and control plants were also used to calculate a drought sensitivity index (SI) for each accession as $X_1/X_2 \times 100$, where X_1 is the value obtained under drought stress conditions and X_2 is the value obtained under the control well-watered conditions (Bouslama and Schapaugh 1984).

The mean values for RWC, F_v/F_m, and PI for plants of all accessions grown under drought stress and nonstressed conditions were used to compare genotypes based on their response to drought, by the neighbour joining (NJ) method (Saitou and Nei 1987), which clusters the accessions by joining them based on distance using the software *PAST ver. 3.22* (https://folk.uio.no/ohammer/past/). The same means plus the values of SWC were used as variables to construct a PCA biplot using the PCA analysis. The PCA was applied to assign the variables to genotypes and to classify which of them were more sensitive or tolerant to drought stress. The PCA utilizes orthogonal transformation to convert a set of possibly correlated variables into a set of linearly uncorrelated variables called principal components. This transformation is defined in such a way that the first principal component has the largest possible variance. PCA is sensitive to the relative scaling of the original variables in the PCA scatter plotting visualization. The *XLSTAT* software was used to perform the PCA data analysis and biplot visualization. Eigenvectors generated by PCA were used to rank the accessions for their drought tolerance (Hefny e*t al*. 2017).

Results and discussion

Impact of drought stress on RWC, F_v/F_m, PI, and SI: The measured values of SWC, leaf RWC as well as the F_v/F_m and PI for control plants and drought-stressed plants of the examined genotypes of maize are given in Table 1. The SWC was reduced from control values ranging between 27.6% for accession Eg 75 to 29.2% for accession Zea 3445 to values ranging between 4.5% for accession Zea 1006 and 9.2% for Zea 3550 under drought conditions. Under control conditions, the values of RWC ranged between 95.3% for accession Eg 75 to 93.4% for accession Zea 3244. However, remarkable reductions in RWC were recorded for all genotypes under drought stress compared to the control, ranging between 83.8% for genotypes Zea 1006 from Libya to only 57.8% for Zea 612 from Italy. Significant differences were also found between the genotypes Eg 75, Eg 85, Eg 237, Eg 247 from Egypt and the IPK genotypes Zea 3244, Zea 3400, Zea 3445, and Zea 3550, from Germany, Georgia, Russia, and the USA, respectively (Table 1). The above results are further supported by the SI value of the maize genotypes to drought (Fig. 1). The lowest SI was scored for the accession Zea 612 (68.72) and the highest SI was scored for accession Zea 1006 (91.06). As indicated in Fig. 1, the Egyptian genotypes Eg 75, Eg 85, Eg 237, Eg 247 generally have higher SI values (81.22 to 84.38) compared to the IPK accessions Zea 3244, Zea 3400, Zea 3445, and Zea 3550 from Germany, Georgia, Russia, and the USA, respectively (74.42 to 77.18). The results indicate genetic variation between maize genotypes in the degree of drought tolerance which is better manifested under severe conditions of water deficit in the soil. The data on RWC and SWC physiologically mean that the accession Zea 1006 from Libya can extract water much better from the soil than accession Zea 612 from Italy but does not release it to the atmosphere, in contrast to

Table 1. List of maize accessions and their source and origin and the mean values of soil water content (SWC) for control plants (C-SWC) and drought stressed plants (D-SWC) and the values of leaf RWC for control plants (C-RWC) and drought stressed plants (D-RWC) as well as the F_v/F_m for control plants (C-F_v/F_m) and drought stressed plants (D-F_v/F_m) and ChlF performance index (PI) for control plants (C-PI) and drought stressed plants (D-PI) for the examined genotypes of maize. Based on these data, Zea 1006 and Zea 612 have been regarded as the most tolerant and the most sensitive genotypes, respectively. *Identical letters* in each column (treat) indicate no significant difference at $P{\leq}0.05$ according to the LSD test.

| Accession code	Accessions source and origin	Soil water content		Leaf water content		Chlorophyll fluorescence		Photosynthesis PI	
		C-SWC	D-SWC	C-RWC	D-RWC	C-F_v/F_m	D-F_v/F_m	C-PI	D-PI
Eg 75	ARC, Giza, Egypt	27.62[a]	8.225[bc]	95.25[a]	77.00[cd]	0.815[a]	0.664[a]	6.02[bc]	5.48[bc]
Eg 85	ARC, Giza, Egypt	28.07[a]	8.922[c]	93.49[a]	73.00[c]	0.813[a]	0.646[a]	6.52[c]	5.61[bc]
Eg 237	ARC, Giza, Egypt	29.10[a]	7.405[bc]	94.65[a]	74.75[c]	0.830[a]	0.696[a]	5.68[abc]	5.12[bc]
Eg 247	ARC, Giza, Egypt	27.66[a]	8.405[bc]	94.12[a]	76.00[c]	0.811[a]	0.660[a]	6.68[ab]	4.61[ab]
Zea 612	IPK, Italy	28.57[a]	6.143[ab]	94.39[a]	57.75[a]	0.802[a]	0.568[b]	6.54[c]	4.85[abc]
Zea 1006	IPK, Libya	27.71[a]	4.478[a]	94.86[a]	83.75[d]	0.810[a]	0.725[c]	6.07[bc]	5.79[c]
Zea 3244	IPK, Germany	28.13[a]	7.463[bc]	93.40[a]	61.25[b]	0.784[a]	0.625[a]	6.49[c]	5.51[bc]
Zea 3400	IPL, Georgia	27.81[a]	7.125[bc]	94.03[a]	62.75[b]	0.781[a]	0.607[a]	4.68[a]	3.93[a]
Zea 3445	IPL, Russia	29.18[a]	9.180[c]	94.73[a]	59.00[a]	0.792[a]	0.620[a]	6.61[c]	5.47[bc]
Zea 3550	IPK, USA	28.19[a]	8.763[c]	95.41[a]	64.25[ab]	0.780[a]	0.624[a]	6.52[c]	5.49[bc]
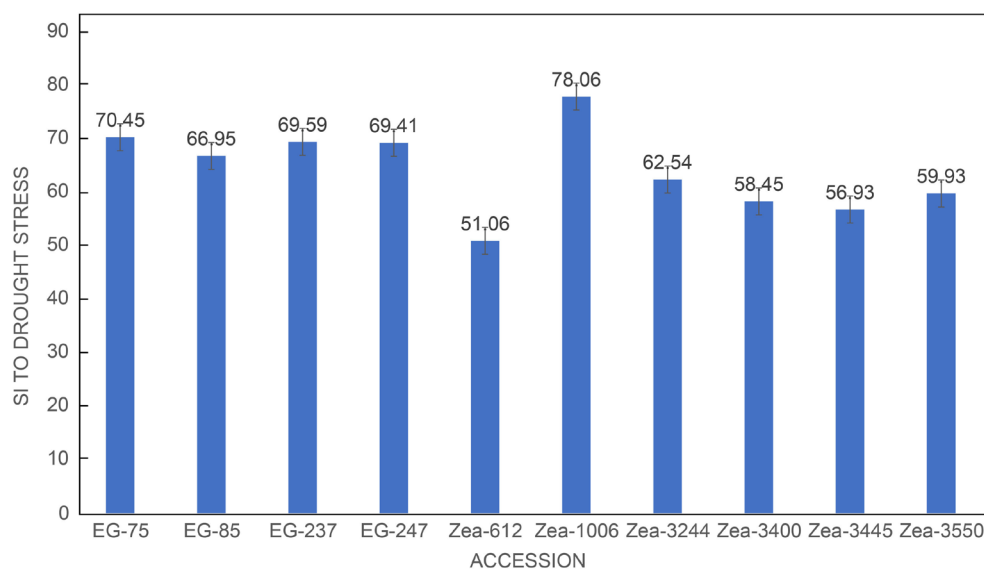
Fig. 1. Histogram illustrating variation between the examined ten maize accessions in the drought sensitivity index (SI) based on the sum average of leaf relative water content, in the initial photochemical quantum efficiency of photosystem II and performance index calculated as percentage of their value in plants grown under drought divided by their corresponding values in control plants.

Zea 612. It may be concluded that the most tolerant accession Zea 1006 has better stomatal conductance control.

The physiological reasons for differences between sensitive and tolerant genotypes may be efficient protection of tissue water status in tolerant genotypes that may be associated with leaf water potential and gas exchange as efficient reference indicators of drought tolerance (Grzesiak *et al*. 2006). Physiological markers of drought tolerance such as leaf water status complemented by integrated statistical methods indicated that tolerance to drought in maize like wheat has a common genetic background (Grzesiak *et al*. 2006, 2019). Evaluation of

drought tolerance in maize was made based on the relative reduction of seeds produced under drought conditions compared to normal conditions and an observation of various morphology and physiology variables related to drought tolerance (Bänziger *et al*. 2000). Direct selection of drought-tolerant maize hybrids by yield only has a low selection effectivity because of more environmental influences than the genetic influence (Shiri *et al*. 2010). Plant characters that show a correlation under drought conditions expressed as drought sensitivity indices have proved useful as selection characters of drought-tolerant maize hybrids (Arisandy *et al*. 2017) and genotypes
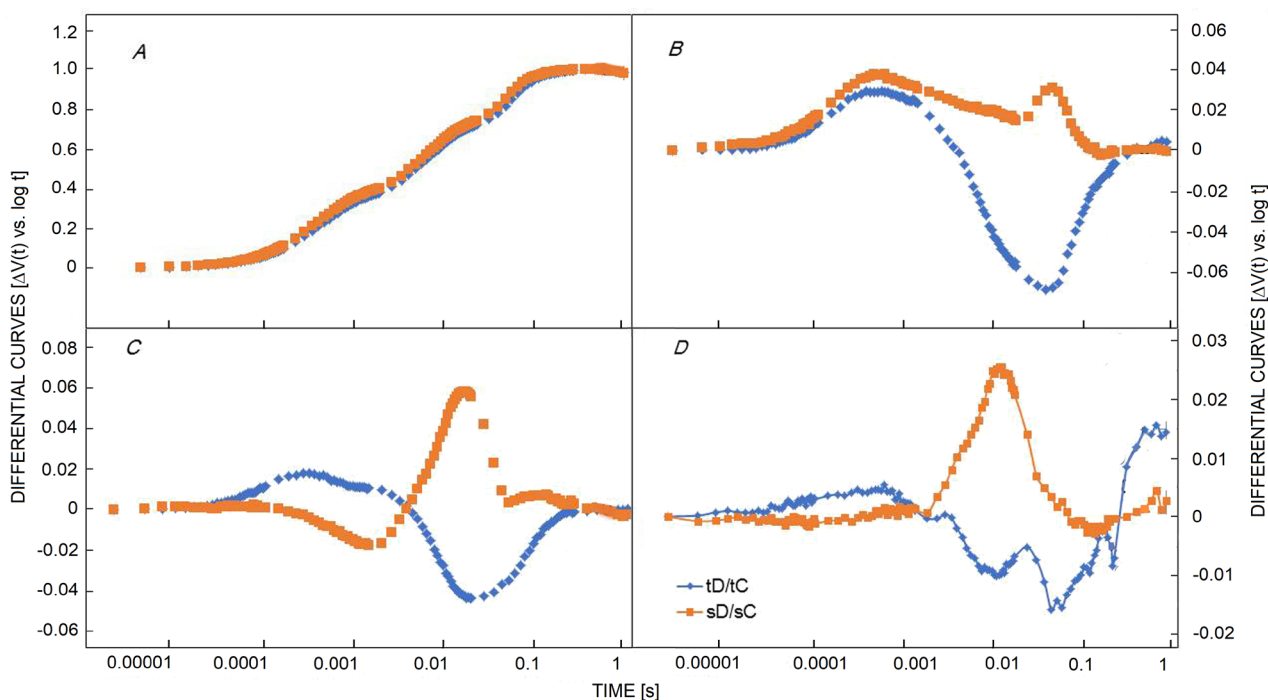
Fig. 2. Double normalized original OJIP transients (*A*) and differential (*i.e.*, $\Delta V_{(t)} = V_{(t)DS} - V_{(t)C}$) chlorophyll fluorescence induction curves (*B–D*) of dark-adapted leaves of the drought tolerant maize accession Zea 1006 and the drought sensitive accession Zea 612 under drought stress as compared to the controls, after drought exposure for 3 (*A*), 11 (*B*), 14 (*C*), and 17 d (*D*). *Blue curves* are for Zea 1006 and *red curves* are for Zea 612.

(Hefny *et al*. 2017).

The values of F_v/F_m showed slight variations between genotypes under control conditions ranging between 0.815 for accession Eg 75 to 0.78 for Zea 3550. Under drought stress, the value of F_v/F_m was considerably reduced for all accessions, the maximum value (0.725) was recorded for accession Zea 1006 from Libya and the minimum value (0.568) was scored for Zea 612 from Italy. The value of PI under control conditions ranged between 6.68 for accession Eg 247 from Egypt to 4.68 for accession Zea 3400 from Georgia and showed significant differences between genotypes. Under drought stress, the PI value was reduced in all genotypes with maximum reduction in accession Zea 612 down to PI of 4.85 and minimum reduction in accession Zea 1006 down to PI of 5.79. Based on these data, accession Zea 1006 from Libya and accession Zea 612 from Italy were regarded as the most tolerant and the most sensitive genotypes, respectively. Using F_v/F_m and PI values as useful parameters for drought tolerance in maize genotypes at early stage of stress may be compared to earlier findings in barley and sorghum (Jedmowski *et al*. 2013). It was demonstrated by Strasser *et al*. (2004) and Oukarroum *et al*. (2009) that the maximum fluorescence and the maximum number of reaction centers are reduced or closed by a saturating light source. In general, the greater the plant stress, the fewer open reaction centers are available and the F_v/F_m ratio is lowered. It has been also found that the rise to maximum fluorescence from minimum fluorescence in the OJIP transient possesses

intermediate peaks and dips that can be used for testing plant stress susceptibility, summarized in PI (Kalaji *et al*. 2016, Grzesiak *et al*. 2019). The measurements and analysis of the ChlF by the examined ten maize genotypes under stress indicated that this variable fluorescence parameter can be used to measure the level of plant stress and support the view of Kalaji *et al*. (2016) that it is useful for screening genotypes for drought tolerance.

However, the underlying sophisticated mechanisms that differentiate tolerant from sensitive genotypes from susceptibility within a plant species, especially water-saving and drought-resistant crops to cope with the water shortage is more complicated. It is well known that photosynthesis is strongly influenced by environmental conditions (Blankenship 2014) and is governed mainly by stomata for CO_2/water exchange and photosynthetic activity in the mesophyll cells of the leaves. Drought affects not only the light reactions, but also the assimilation efficiency of the dark reactions, thereby reducing the contents of the photosynthetic products. Being a C_4 species, maize utilizes moisture and sunlight efficiently to produce high yield and total dry matter (Bell 2017). However, compared to other plants, such as wheat and barley, maize is not drought tolerant, due to its fast growth rate to produce much higher biomass in a short life cycle compared to other cereals. Photosynthesis-related processes are the most sensitive to water deficit of the many biological processes activated when plants encounter environmental stresses (Huo *et al*. 2016, Kalaji *et al*. 2018).

Comparative analysis of the OJIP induction curves in tolerant and sensitive genotypes: Fig. 2 illustrates the detailed changes in the OJIP transient induction curves, of dark-adapted leaves, of the most tolerant accession Zea 1006, the most sensitive accession Zea 612 under drought stress for up to 17 d by calculating differential curves [$\Delta V_{(t)}$ *vs.* log t] between those obtained for drought-exposed plants relative to the control plants. Initially, after 3 d of drought stress, the double normalized curves for both genotypes were still nearly indistinguishable, showed a steady rise and reached a maximum of 1.0 on the *y*-scale in 0.1 s on the *x*-scale. As the time of exposure to stress increased and both SWC and leaf RWC decreased, the rise of the curves was retarded, and the differential curves developed evident peaks for the drought-sensitive accession Zea 612 (sD/sC) and dips for the drought-tolerant accession Zea 1006 (tD/tC). After 11 d, the initial rise of the curve to F_m was faster in both lines, exposing a peak at 1 ms, while between 10 and 100 ms a strong dip for the accession Zea 1006 (tD/tC) was observed. After 14–17 d of drought treatment, the peak for the sensitive accession Zea 612 (sD/sC) was delayed to approximately 20 ms and the dip for the tolerant accession 1006 (tD/tC) became smaller (*i.e.*, from –0.06 at day 11 to –0.015 at day 17) (Fig. 2).

While peaks in the ΔV curves denote a problem in electron transport at the particular point of time, which can often be attributed to certain components of the electron transport chain, a dip means that at this point of time electron transport is relatively favored in the stressed plant as compare to the control plant. However, if a dip follows a peak, it may also mean that at the given point of time the stressed plants retain electron flux again (Strasser *et al.* 2000, 2004; Oukarroum *et al.* 2009, Kalaji *et al.* 2016). The difference in the behavior of photosynthetic parameters under the same stress factor between the sensitive accession Zea 612 and the tolerant accession Zea 1006 may represent different mechanisms of tolerance and strategies for the conversion of light energy into chemical energy for both genotypes. The data indicate that in the early phase (*i.e.*, around 1 ms) problems in electron transport arise in the tolerant line, Zea 1006, which are then overcome after approximately 20 ms. They may be due to a relative inefficiency of Q_A reduction in the DS plants (*cf.* Oukarroum *et al.* 2007, Kalaji *et al.* 2016). In the sensitive line, the same effect occurs, but later (at day 17) and may have been overturned by hampered electron transport around 20 ms, *i.e.*, in the reduction of intersystem carriers and/or electron transport to PSI (Strasser *et al.* 2000, 2004).

The PI is the most widely used parameter from the ChlF OJIP transient, which provides quantitative information about the general state of plants and their vitality. According to Strasser *et al.* (2004), PI is the product of three independent characteristics: the concentration of reaction centers per Chl, primary photochemistry, and electron transport. Therefore, PI has been found sensitive to changes in either antenna properties, trapping efficiency or electron transport beyond Q_A in some plants. For example, the drought tolerance of wheat genotypes estimated from PI values correlated well with the drought tolerance assessed by grain yield. It was also closely related to the drought factor index (DFI), which represents the relative drought-induced reduction of PI during defined time of drought stress (Živčák *et al.* 2008, Kalaji *et al.* 2016). In barley and sorghum, the most tolerant and the most sensitive genotypes were identified using the PI parameter and the ChlF fast induction curve (Jedmowski *et al.* 2013); the drought-tolerant and drought-sensitive cultivars have been differentiated at the level of PSII. An increase of ABS/RC ratio under drought stress has also been observed possibly due to inactivation of some PSII RCs or an increase in antenna size (Maxwell and Johnson 2000, Strasser *et al.* 2000, Kalaji *et al.* 2016).

Classification of maize genotypes based on their response to drought: The grouping of the examined genotypes of maize, based on their response to drought stress, clustered by the NJ method is illustrated in Fig. 3. The ten genotypes are divided into two groups, one comprised of the accession Zea 1006 from Libya and the Egyptian accessions Eg 75, Eg 85, Eg 237, and Eg 247 and the other comprised of the accessions Zea 612, Zea 3400, Zea 3445, Zea 3244, and Zea 3550. However, the two accessions Zea 1006 in the first group and Zea 612 in the second group are clearly distinguished from other genotypes. The distinction of these two accessions is congruent with the indications of the leaf RWC, F_v/F_m and PI measurements (Table 1). The scattering of the genotypes in the PCA biplot is displayed in Fig. 4. Eigenvectors generated by PCA were used to rank the tested genotypes for their drought tolerance and the biplot is constructed by plotting PC1 and PC2 which account for maximum variability of the parameters measured and used to group the genotypes in the PCA biplot. The PCA biplot displays higher impact of the measured parameters under drought stress compared to control conditions. From Fig. 4, it is evident that soil water content under drought (DSWC) and relative water content under drought (DRWC) imposed
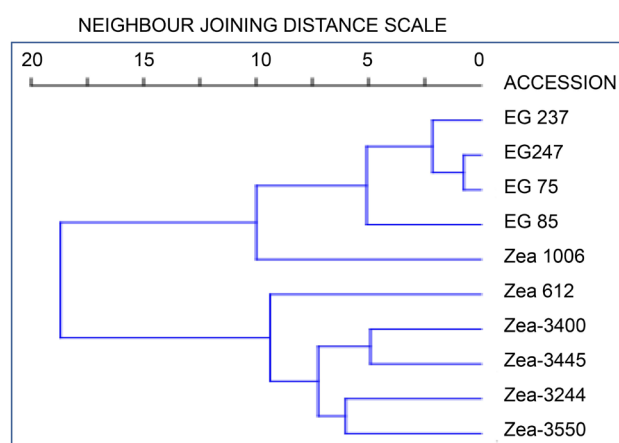

Fig. 3. Cluster diagram illustrating the classification of the examined genotypes of maize based on their response to drought stress, calculated by the Neighbour joining (NJ) method.
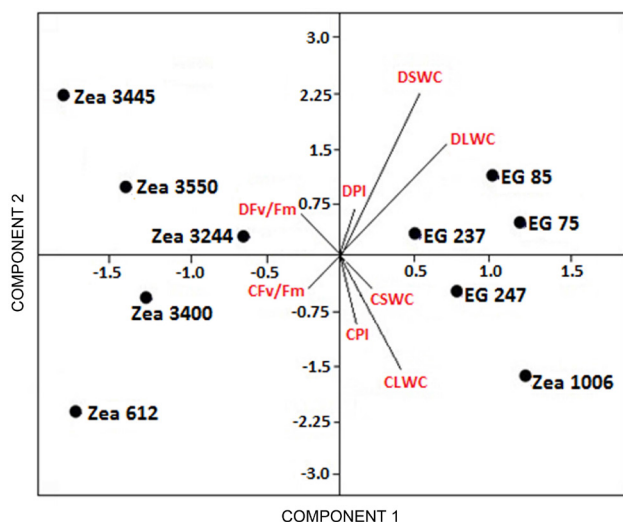
Fig. 4. Principal Component Analysis biplot display of the examined genotypes of maize as impacted by measurements of soil water content (SWC) for control plants (CSWC) and drought-stressed plants (DSWC), leaf relative water content for control plants (CLWC) and drought-stressed plants (DLWC) as well as the initial photochemical quantum efficiency of photosystem II for control plants (CF$_v$/F$_m$) and drought-stressed plants (DF$_v$/F$_m$) and chlorophyll fluorescence performance index for control plants (CPI) and drought-stressed plants (DPI), as parameters reflecting drought tolerance.

higher impact on the grouping of accessions compared to the F$_v$/F$_m$ and PI. The cluster diagram and the PCA display of the examined genotypes confirm that the Libyan accession Zea 1006 is the most tolerant genotype and the Italian accession Zea 612 is the most sensitive genotype of the examined accessions. Both the NJ clustering (Fig. 3) and the PCA biplot (Fig. 4) show closer distance between the Egyptian genotypes Eg 75, Eg 85, Eg 237, and Eg 247 compared to the distances between the accessions form different countries.

The PCA biplot describes the drought tolerance in response to variables and assigned variables to genotypes. The PCA is sensitive to the relative scaling of the original variables in the PCA biplot, the values on the *x*- and *y*-axis of the biplot indicate as displayed on the first and second principal components. The classification of maize genotypes based on their response to drought stress imposed by reduced soil water content and the drought stress indicators leaf RWC, F$_v$/F$_m$, and PI clearly support using the PCA biplot analysis for screening drought tolerance of maize genotypes as it has been proposed in the literature (Ahmadi *et al.* 2000, Shiri *et al.* 2010, Arisandy *et al.* 2017). Golabadi *et al.* (2006) also reported similar results on selection of drought-tolerant genotypes of durum wheat, based on the combination of indices using the biplot method; thereby this method is better than using one index alone to identify superior genotypes for drought conditions. Water-use efficiency (WUE) for drought-tolerant hybrids was significantly greater than that for drought-sensitive hybrids (Adee *et al.* 2016). Hefny

et al. (2017) evaluated 21 genotypes of maize comprising six inbred lines and their 15 F1 crosses for their drought tolerance. Maize hybrids response to a range of macro- and microenvironmental stresses were characterized in terms of WUE, grain yield, and environmental index. However, the use of ChlF is a fast and nondestructive reliable method for screening germplasm for drought tolerance at vegetative stages.

In conclusion, under drought stress for 17 d, the initial quantum yield of PSII Q$_A$ reduction (F$_v$/F$_m$), as well as the PI were reduced in drought-stressed plants compared to the control plants and indicated differences in drought tolerance in maize genotypes. Comparative analysis of the OJIP fluorescence transient curves for the most sensitive and the most tolerant lines recommend the use of OJIP fluorescence transient analysis as a nondestructive, simple, and rapid technique to screen, evaluate and monitor the physiological status of plants under abiotic stress conditions. The reduction in F$_v$/F$_m$ and PI was accompanied by reduction in leaf RWC and SI with remarkable differences between genotypes in these variables. The PCA and clustering methods classified the genotypes based on their response to drought stress based on F$_v$/F$_m$ and PI, leaf RWC and soil water content. In general, genotypes from Egypt and Libya which showed higher values of F$_v$/F$_m$, PI, and RWC were differentiated from genotypes from Europe, Russia and the USA.

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