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Physiological responses of three maize cultivars to drought stress and recovery

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Abstract

Water shortages and soil water losses due to environmental change and land use change are challenges to maize production. An experiment was conducted to investigate the physiological responses of the maize (*Zea mays* L.) cultivars Doge, Vero and Luce to drought conditions. Drought stress was imposed on the plants 12 days after sowing by withholding irrigation for 12 days and then rewatering for 6 days. Growth of all cultivars was retarded under drought stress conditions and regained speed during the recovery stage. RWC decreased in all cultivars by drought and reached the control values during the recovery period. Fresh and dry biomass of the cultivars significantly decreased in all cultivars. Drought affected the minimum fluorescence (F_o) of all cultivars, but a significant effect was only found in Doge. Drought also caused decreases in F_M, F_V/F_M, F_V'/F_M', Φ_{PSII} and q_L; and an increase of non-photochemical quenching (NPQ), but those returned to control values during the recovery stage in all three cultivars. Chlorophyll (chl) *a*, chl *b*, total chl (*a*+*b*) and carotenoid contents of all maize cultivars were significantly reduced under drought, but a recovery was observed following rewatering. Anthocyanin and proline contents increased in all cultivars. Although Doge was affected from drought more than the other cultivars, it could probably withstand drought with better upregulating its protective mechanisms. As a result of that Doge was classified as less drought tolerant, but others as tolerant.

Keywords: Biomass; Drought; Maize; Proline; PSII activity; Recovery

1. Introduction

Water is a major limiting factor affecting plant growth, development and yield mainly in arid and semiarid regions where plants are often exposed to periods of water deficit stress also known as drought stress. Drought is one of the major causes for crop loss worldwide, reducing average yields with 50% and over (Wang et al., 2003). Responses to drought are multiple and interconnected. It is well established that drought stress impairs numerous metabolic and physiological processes in plants (Levitt, 1980). It leads to growth reduction, reduction in the content of chlorophyll pigments and water, and changes in fluorescence parameters (Lu and Zhang, 1999; Lima et al., 2002; Colom and Vazzana, 2003; Souza et al., 2004; Zlatev and Yordanov, 2004; Ekmekçi et al., 2005; Mohsenzadeh et al., 2006; Li et al., 2006; Nayyar and Gupta, 2006; Yang et al., 2006). Nutrient uptake by plants is decreased under drought stress conditions due to reduced transpiration, impaired active transport and membrane permeability resulting in reduced root absorbing power (Tanguilig et al., 1987).

Most of the damaging effect of drought is associated with the photosynthetic process of the plant. Many studies have shown that the decrease of the photosynthetic activity under drought stress can be attributed to both stomatal and non-stomatal limitations (Shangguan et al., 1999; Yordanov et al., 2003; Zlatev and Yordanov, 2004). One of the earliest responses to drought is stomatal closure. Stomatal closure allows plants to limit transpiration, but it also limits CO_2 absorption, which leads to a decreased photosynthetic activity (Nayyar and Gupta, 2006; Yang et al., 2006). Nonetheless, limitations to CO_2 absorption imposed by stomatal closure may promote an imbalance between photochemical activity of photosystem II (PSII) and the electron requirement of the Calvin–Benson cycle, leading to an excess of absorbed excitation energy and subsequent photoinhibitory

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damage to PSII reaction centers (Foyer and Noctor, 2000; Baker and Rosenqvist, 2004). Several *in vivo* studies reported that water deficit stress resulted in damage to the PSII oxygen evolving complex (Lu and Zhang, 1999; Skotnica et al., 2000) and to inactivation of PSII reaction centers associated with degradation of D1 protein (He et al., 1995; Cornic, 2000). Thus, over reduction of photosynthetic electron chain or inactivation of oxygen evolving manganese complex, which predisposes PSII to damage caused by long-lived $P680^{+}$ or stress on the concurrent synthesis of new D1 protein may result in the formation of reactive oxygen species (ROS), which can lead to photoinhibition and oxidative damage (Asada, 1999; Hakala et al., 2005; Nishiyama et al., 2006). Plants have several protection mechanisms in order to prevent the damaging effect of ROS by an increase in dissipation of excess excitation energy or by the production of sun-screen pigments (carotenoids and anthocyanin) (Young, 1991; Sherwin and Farrant, 1998; Gould et al., 2002; Pietrini et al., 2002). In addition to that, many plants cope with drought stresses by synthesizing and accumulating some substances. They most relate osmotic adjustment (accumulation of ions and of compatible solutes), water circulation (aquaporins), reaction to oxidative stress, detoxification, protection or degradation of proteins or different cellular structures (chaperons, dehydrins, proteases and antiproteases), abscisic acid (ABA) synthesis, carbon and nitrogen metabolism and signal transduction (Hong et al., 2000; Hien et al., 2003; Pinheiro et al., 2004; Riccardi et al., 2004; Mahajan and Tuteja, 2005).

The reaction of the plants to drought differs significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and the stage of development (Chaves et al., 2003). Maize is one of the major summer crops grown in the Mediterranean region and also it is the third most important crop following wheat and barley in Turkey and raised in an approximate area of 800.000 ha (FAOSTAT, 2006). The scarce and highly variable precipitation in this region make efficient planning of water use for irrigation necessary for most summer crops. Maize has been reported to be very sensitive to drought (Farre et al., 2000). Early studies showed that genotypic differences occur in growth response of maize to drought stress. Characteristic differences in response to drought stress have also been identified for a range of morphological and physiological characteristics, including root development, stomatal activity, osmotic adjustment, abscisic acid and proline levels in the whole plant (Li and Van Staden, 1998a,b; Selmani and Wasson, 2003). In order to improve the agricultural productivity within the water limited areas, it is imperative to ensure higher crop yields against drought stress. There has only been a limited number of publications related to simultaneous comparison of photochemical efficiencies of PSII and physiological response which could be useful for identifying differences in maize cultivars under drought stress and recovery. Therefore, it may be possible to determine if there is damage to light reaction systems in photosynthetic machinery during drought and recovery by analysis of chlorophyll fluorescence which can serve as easy and rapid indicators of stress conditions in plants.

The present study was planned to identify the effect of drought stress on PSII photochemistry by analyses of fluorescence and by some physiological parameters associated with better drought tolerance among maize cultivars.

2. Materials and methods

2.1. Plant materials and stress treatments

Three maize (*Zea mays* L.) cultivars: Doge, Luce and Vero were used in this study. Seeds were surface sterilized with a 2% sodiumhypochloride (NaOCl) solution for 10 min. Then they were washed and imbibed in distilled water for 24 h. After incubation, the seeds were planted in PVC pots holding 1000 g air-dried soil. The soil had the following properties: field capacity 30%, texture clay–sand, $CaCO_3$ 1.41%, pH 7.45, EC 3020 μS/cm, organic matter 7.4% and total N 0.37%. Plants were grown under well-watered conditions. Seedlings of the cultivars were grown under a constant temperature regime of 23±1 °C for 14 h photoperiod at 40±5% relative air humidity and at a photosynthetic photon flux density (PPFD) of 250 μmol m^{-2} s^{-1} light intensity in a controlled growth room. Drought stress was imposed on plants 12 days after sowing by withholding irrigation for 12 days followed by rewatering for 6 days. Measurements were made at day 12 of the stress treatment and at day 6 following the start of rewatering. For each treatment irrigated plants were used as controls.

2.2. Growth parameters

At the end of each treatment, the seedling length (the distance from soil surface to upper end of the longest leaves) of the maize cultivars was measured (cm/plant). The fresh (g/FW) and dry (g/DW) biomass of the seedlings was also determined.

2.3. Chlorophyll a fluorescence measurements

Chlorophyll *a* fluorescence measurements were performed in a growth cabinet at 24 °C with a portable, modulated fluorescence monitoring system, (FMS-II-Hansatech, UK), on randomly selected leaves (third leaf) of the cultivars. Following 30 min dark adaptation, the minimum chlorophyll *a* fluorescence (F_o) was determined using a measuring beam of 0.2 μmol m^{-2} s^{-1} intensity. A saturating pulse (1 s white light with a PPFD of 7500 μmol m^{-2} s^{-1}) was used to obtain the maximum fluorescence (F_M) in the dark-adapted state. The quantum efficiency of open PSII reaction centers in dark-adapted plants (F_V/F_M) was calculated from $(F_M-F_o)/F_M$. Light-induced changes in chlorophyll *a* fluorescence following actinic illumination (300 μmol m^{-2} s^{-1}) were recorded prior to measurement of F_o' (minimum chlorophyll *a* fluorescence in light saturated state) and F_M' (maximum fluorescence in light saturated stage). The quantum efficiency of open PSII reaction centers in the light-adapted state, referred to as ΦPSII ($F_M'-F_S/F_M'$), was determined from F_M' and F_S values and also the quantum efficiency of excitation energy trapping of PSII, (F_V'/F_M'), was calculated according to Genty et al. (1989). After turning off the actinic light the leaves were illuminated with far-red light (7 μmol m^{-2} s^{-1}) to oxidize the PQ-pool in order to be able to determine the minimum fluorescence level of the light-adapted state (F_o'). The fraction of open PSII

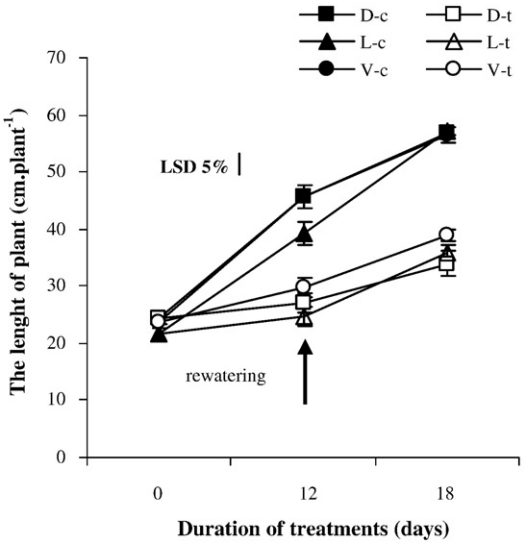

Fig. 1. Effect of drought stress and recovery on seedling length of maize cultivars. The error bars represent standard error (±SE) for 12 plants ($n=12$). Arrow indicates rewatering. D-c, Doge-control, D-t, Doge treatment (drought stress for 12 d and then recovery for 6 d), L-c, Luce-control, L-t, Luce-treatment (drought stress for 12 d and then recovery for 6 d), V-c, Vero-control, V-t, Vero-treatment (drought stress for 12 d and then recovery for 6 d).

reaction centres as $q_L=(F'_M-F_S/F'_M-F'_o)$. (F'_o/F_S) were calculated using the 'lake' model according to Kramer et al. (2004) and non-photochemical quenching $NPQ=(F'_M-F_M)/(F_M)$ were also calculated according to Bilger and Björkman (1990).

2.4. Pigment analysis

Photosynthetic pigments were extracted from leaf samples in 100% acetone. The absorbance of the extracts was measured at 470, 644.8 and 661.6 nm using a Shimadzu Mini-1240 UV–Vis spectrophotometer. The content of chlorophyll (*a* and *b*) and total carotenoids (xanthophylls and carotenes, $x+c$) was calculated using adjusted extinction coefficients (Lichtenthaler, 1987). Anthocyanins were extracted from leaf material in 1 ml of acidified methanol for 48 h at 4 °C. The absorbance was measured at 530 and 657 nm and the anthocyanin content was calculated according to Mancinelli et al. (1975). The pigment content was expressed as mg g/DW.

2.5. Relative water content

The RWC content was calculated using the standard formula [(FW−DW)/(HydW−DW)*100] previously determined by Farrant (2000), where FW, HydW and DW are the leaf fresh weight, hydrated (full turgor) and dry weights, respectively. The hydrated weight was determined by weighing the leaf after 24 h of immersion in distilled water in a sealed flask at room temperature. Dry weight was determined gravimetrically after 48 h at 70 °C in an oven.

2.6. Proline content

The proline content of drought stressed and irrigated (control) plants was determined using the method of Bates et al. (1973). Proline was extracted from leaf samples (20 mg DW) according to Weimberg (1987) with minor modifications. The absorbance of the sample extract was spectrophotometrically determined at 520 nm. The proline concentration was determined as (μmol g/DW) using a standard curve.

2.7. Data analysis

The experiments were performed in a randomized block design with three replicates. Differences among the treatments as well as between the cultivars were tested using the SPSS statistical program. Statistical variance analysis was performed using ANOVA and compared with least significant differences (LSD) at 5% level.

3. Results

3.1. Growth

Growth of the seedlings was retarded under drought stress conditions in Doge and Luce. During the recovery stage the seedlings increased their growth rate again but the length of the

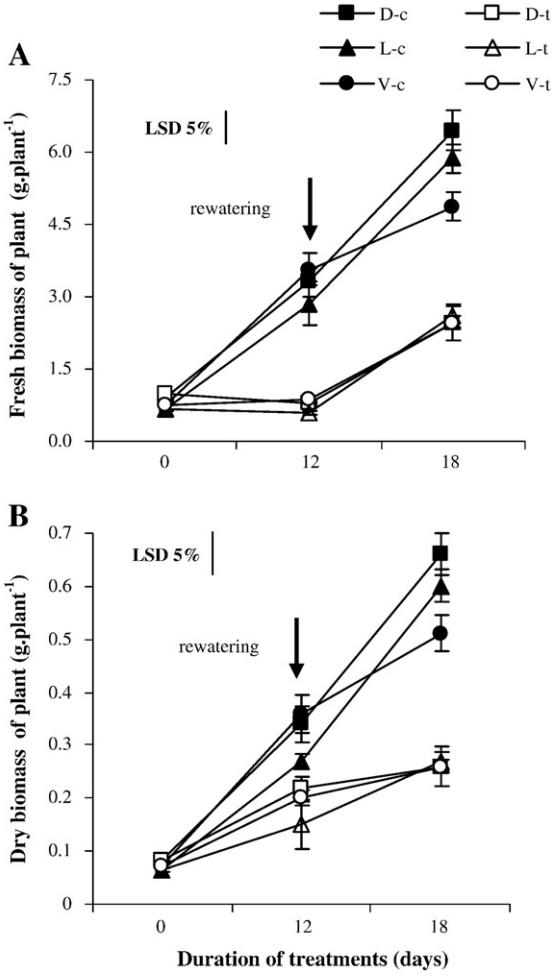

Fig. 2. Fresh (A) and dry (B) biomass of maize seedlings subjected to drought stress and recovery. The error bars represent standard error (±SE) for 6 plants ($n=6$). * See Fig. 1. for explanation of legends.

seedlings was significantly shorter than the length of the controls. The growth of the seedlings continued under irrigated and drought stress conditions in all genotypes but growth was significantly less in drought stressed plants during recovery than in control plants for the cultivar Vero. While the seedling length was not significantly different among cultivars with respect to the control seedlings, Vero had the longest seedlings both during the stress treatment and during recovery (Fig. 1).

Maize cultivars exposed to drought had a lower fresh and dry biomass than their controls due to a significant drought-induced reduction in growth (Fig. 2). Fresh biomass of cultivars was significantly reduced under drought stress conditions but it was significantly increased during recovery. There was no significant difference among cultivars neither during the stress treatment nor during recovery (Fig. 2A). In addition, dry biomass was significantly decreased under drought stress and recovery conditions in all genotypes compared to their controls. There was no significant difference among cultivars at the beginning of stress, but Doge had the highest dry biomass after 12 and 18 days of growth under control conditions (Fig. 2B).

3.2. Relative water content

RWC was significantly reduced under drought stress conditions, but it significantly increased during recovery. Whereas Luce had the highest RWC following Doge and Vero at control, there was no significant difference among cultivars at stress and recovery stage (Fig. 3). These low RWC (approx. 45%) values of leaves of all cultivars rapidly recovered following rewatering and reached control levels after 6 d of rewatering. Moreover, leaves of the maize cultivars showed wilt symptoms and leaf rolling throughout the drought stress treatment.

3.3. Chlorophyll a fluorescence measurements

Drought tolerance of three maize cultivars was evaluated on the basis of their response to 12 days of stress and 6 days of rewatering analysing several fluorescence parameters determined under dark-adapted and steady state conditions (Fig. 4). There was no significant change in F_o under irrigated and drought stress conditions in Luce and Vero, but drought caused a significant increase in F_o in Doge. There was no significant difference in F_o between recovery and control in all cultivars (Fig. 4A). Further a significant reduction in F_M in Doge and Luce exposed to drought was observed (Fig. 4B). In control leaves, the quantum efficiency of open PSII reaction centers in the dark-adapted state (F_V/F_M) was approximately 0.81–0.82 (Fig. 4C). This parameter decreased significantly in response to drought stress in all cultivars. Following rewatering a full recovery of the F_V/F_M-value was observed (Fig. 4C). Additionally, drought stress induced reduction in the quantum yield of PSII electron transport, ϕ_{PSII}, in all cultivars. The reduced ϕ_{PSII} was a result of the decrease in the efficiency of excitation energy trapping of PSII reaction centers, F_V'/F_M' (Fig. 4D, E). A significant decrease in q_L was also observed in all drought stressed cultivars (Fig. 4F) indicating that the balance between excitation rate and electron transfer rate has changed leading to a more reduced state of the PSII reaction centers. The effect on the q_L was larger than the effect on the F_V'/F_M' in Vero and Luce. On the other hand, increase of *NPQ* in drought conditions reflecting the non-photochemical energy dissipation in all cultivars was determined significant compared to their controls (Fig. 4G). This value was highest in Doge followed by Vero and Luce. At end of 6 days rewatering, all fluorescence parameters in leaves of all cultivars returned to their control values.

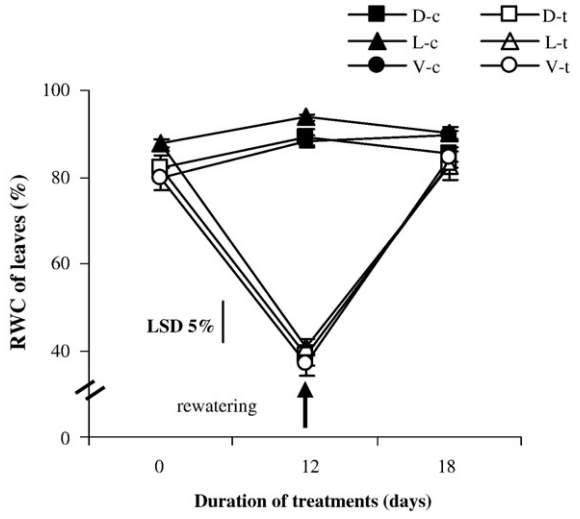

Fig. 3. Effect of drought stress and recovery on relative water content of leaves. The error bars represent standard error (±SE) for 6 plants ($n=6$). * *See* Fig. 1. for explanation of legends.

3.4. Pigment analyses

The chlorophyll content (*a*, *b*, *a*+*b*) of all maize cultivars was significantly reduced under stress, but it increased and reached the control values during recovery (Table 1). The strong drought-induced decrease of the chl *a* content indicates that the drought stress induced a strong loss of photosynthetic reaction centers (PSI and PSII). At the same time the chl *a*/*b* ratio increased during the treatment both in the control and treated plants indicating a decrease in the antenna size as the seedlings develop. However, the calculated values were too variable to observe any clear drought stress-related trends. The carotenoid content decreased in parallel with the chlorophyll content. However, calculating the ratio between the two parameters there are considerably more carotenoids per chlorophyll molecule in drought stressed Doge-plants. In Vero, on the other hand, the drought stress treatment did not increase the carotenoid to chlorophyll ratio relative to the control plants.

The anthocyanin content of all cultivars significantly increased under drought stress conditions compared to the control plants; it significantly decreased in Doge and Vero during recovery. Table 1 shows that the plants with the lowest control level of anthocyanins (Vero) showed the strongest drought-induced increase. On the other hand, the plants with the highest control level of anthocyanin (Luce) had the lowest drought-induced increase.

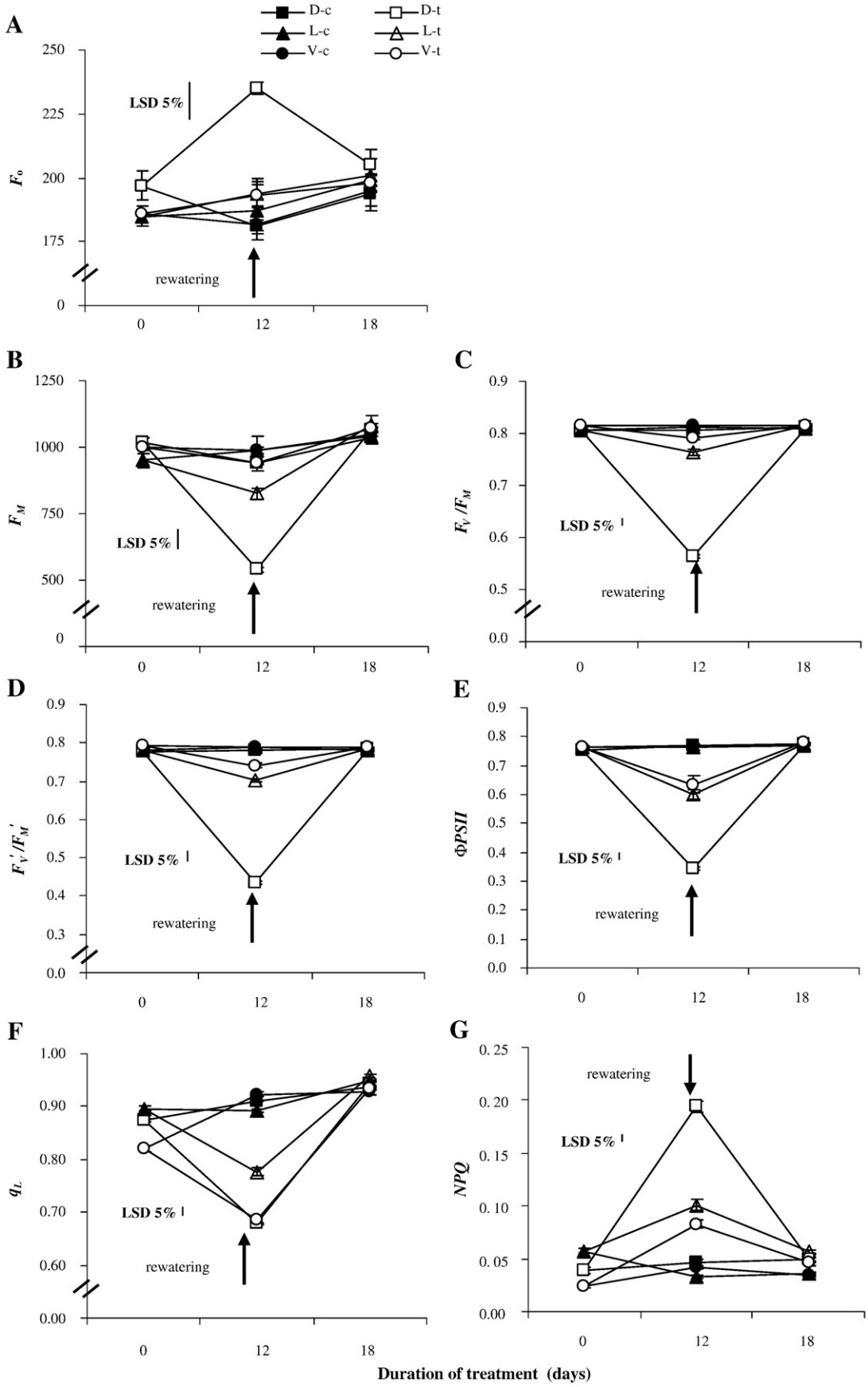

Fig. 4. Chlorophyll fluorescence responses of maize cultivars to imposed drought stress and recovery. (A) F_o, minimum fluorescence (B) F_M maximum fluorescence (C) F_V/F_M, the quantum efficiency of PSII in dark-adapted state (D) F_V'/F_M', the quantum efficiency of excitation energy trapping of PSII in light-adapted state (E)ΦPSII, the quantum efficiency of PSII in light adapted state (F) q_L, the fraction of open PSII reaction centres (G) NPQ, non-photochemical quenching. The error bars represent standard error (±SE) for 3 plants (n=3). * See Fig. 1. for explanation of legends.

Table 1
Pigment contents of maize cultivars exposed to drought stress and recovery, where "0 day" treatment corresponds to 12 days-old maize seedlings before drought

Cultivars	Treatments	Chl *a* content (mg g DW^{-1})	Chl *b* content (mg g DW^{-1})	Chl *a*+*b* content (mg g DW^{-1})	Chl *a*/*b*	Carotenoid content (mg g DW^{-1})	Anthocyanin content (mg g DW^{-1})
Doge	0 day	17.34±1.55	4.82±0.23	22.17±1.72	3.59±0.25	3.40±0.23	0.042±0.003
	12 days control	31.45±4.41	7.36±0.80	38.81±5.13	4.26±0.24	7.23±1.08	0.055±0.003
	12 days drought	10.94±1.21	2.32±0.28	13.26±1.46	4.80±0.29	3.03±0.42	0.092±0.018
	18 days control	31.00±3.32	6.66±0.87	37.66±4.18	4.74±0.16	6.25±0.29	0.054±0.005
	12 days drought+6days recovery	22.19±1.43	5.19±0.30	27.39±1.79	4.28±0.10	5.51±0.33	0.059±0.005
Luce	0 day	17.27±2.03	7.47±1.03	36.90±2.96	3.94±0.30	5.70±0.63	0.051±0.006
	12 days control	32.72±1.54	7.37±0.56	39.91±2.02	4.39±0.19	7.75±0.33	0.048±0.007
	12 days drought	13.07±0.86	2.69±0.22	14.34±0.99	4.43±0.26	3.06±0.23	0.079±0.011
	18 days control	24.98±1.39	6.28±0.66	37.75±2.00	5.05±0.26	7.23±0.34	0.060±0.005
	12 days drought+6days recovery	24.36±2.67	6.35±0.68	34.29±3.33	4.40±0.15	6.68±0.63	0.089±0.017
Vero	0 day	29.43±4.99	5.89±1.16	23.17±6.12	3.16±0.17	3.47±0.90	0.036±0.003
	12 days control	32.54±3.58	7.20±0.60	39.92±4.12	4.61±0.23	7.96±0.89	0.049±0.004
	12 days drought	11.65±0.79	3.29±0.29	16.36±1.05	4.02±0.21	3.31±0.23	0.100±0.018
	18 days control	31.47±3.50	6.00±0.79	30.97±4.29	4.30±0.08	5.85±0.82	0.044±0.005
	12 days drought+6days recovery	27.95±1.68	5.22±0.33	29.58±2.00	4.73±0.08	6.17±0.39	0.028±0.003
LSD 5%		**6.26**	**1.68**	**8.31**	**0.54**	**1.50**	**0.021**

Each value represents the mean of six replicates (n=6) and its standard errors (±SE).

3.5. Proline content

Proline content was significantly increased under drought stress conditions and significantly decreased during recovery in all genotypes examined. Doge and Luce had the highest drought stress-induced proline contents (Fig. 5). During recovery the proline content fell back again to the levels observed before the treatment in all three cultivars.

4. Discussion

Drought, like other environmental stresses, affects many physiological and metabolic processes within plants. Growth was unaffected at mild stress levels (−0.4 MPa) but largely inhibited in plants root and leaves of wheat and maize at moderate and high stress levels. Leaf growth was inhibited relatively more than root growth in both the plant types (Nayyar and Gupta, 2006). In this study, growth of seedlings was retarded under drought stress conditions and regained during recovery stage in all cultivars (Fig. 1). All maize cultivars exposed to drought stress, had lower fresh and dry biomass compared to control plants, as happens in many species exposed to drought stress (Fig. 2A, B). The relative water content in leaves of drought stressed cultivars decreased significantly (Fig. 3). Many investigations have shown that when leaves are subjected to drought leaves exhibit large reductions in relative water content and water potential (Kyparissis et al., 1995; Scarascia-Mugnozza et al., 1996; Li and Van Staden, 1998a,b; Decov et al., 2000; Nayyar and Gupta, 2006). It is known that dehydration is often reversible. This is compatible with this study that RWC was significantly reduced under drought, but it significantly increased at recovery stage comparing to drought conditions in all cultivars (Fig. 3).

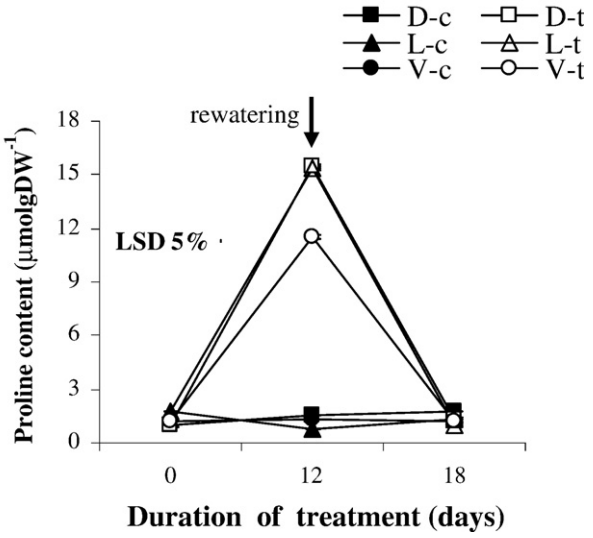

Fig. 5. Leaf proline content in maize cultivars exposed to drought stress and recovery. The error bars represent standard error (±SE) for 3 plants (n=3), LSD 5%=0.13. * See Fig. 1. for explanation of legends.

Stomatal closure is one of the first responses to drought stress. It causes primarily a decline in the rate of photosynthesis (Mahajan and Tuteja, 2005). The measurement of chlorophyll fluorescence emitted by intact, attached leaves is thought to be a reliable, non-invasive method for monitoring photosynthetic events and for judging the physiological status of the plant. Fluorescence induction patterns and derived indices have been used as empirical diagnostic tools in plant physiological studies (Strasser et al., 2000; Baker and Rosenqvist, 2004; Kocheva et al., 2004). In the C_4 species maize the fluorescence quenching parameters in light are directly reflecting the photosynthetic activity during the drought stress, without being obscured by the buffering effect of photorespiration during the initial stomatal closure, which is the case in C_3 species as Baker and Rosenqvist (2004 and references therein) emphasized in their paper. Of the cultivars tested only Doge showed an increase of F_o (Fig. 4A). An F_o-increase may have a variety of causes. It has been associated with a dissociation of light harvesting complex (LHC) II from the reaction centers, it may due to the presence of photoinhibited reaction centers, however also a more reduced

PQ-pool in dark-adapted leaves will lead to an increase of the measured F_o. It may even represent an artefact, if the PSI content would increase relative to the PSII content leading a relatively larger contribution of PSI-fluorescence to F_o. It is interesting that despite the measured strong decrease of the chlorophyll content no decrease of the F_o was observed. It suggests that the modulated measuring equipment simply probes somewhat deeper in the stressed plants monitoring the same number of reaction centers as in the control plants. Changes in light scattering related to changes in the RWC seem to have had little impact on the fluorescence measurements. Zlatev and Yordanov (2004) reported that drought stress induced in their bean plants always an increase in F_o accompanied by a decrease in F_M. In Doge the drought stress treatment led to a drastic decrease of F_M (Fig. 4B) associated with a F_o increase (Fig. 4A) leading to a strong decrease of the F_V/F_M value. A sustained decrease of the F_V/F_M may indicate the occurrence of photoinhibitory damage (Maxwell and Johnson, 2000; Colom and Vazzana, 2003). Normally, photoinhibited reaction centers are quickly de-assembled and replaced by new ones (Aro et al., 1993). The data presented for Doge only make sense if it is assumed that the drought stress treatment led to an inhibition of the repair cycle accompanied by an accumulation of damaged reaction centers (Nishiyama et al., 2006). An inactivation mechanism for PSII favoured recently is an inactivation of the oxygen evolving complex, which predisposes PSII to damage caused by long-lived $P680^+$ or stress on the concurrent synthesis of new D1 protein (Hakala et al., 2005; Ohnishi et al., 2005). The decrease of the quantum efficiency of open reaction centers in the light (F_V'/F_M') observed in Fig. 4D can be interpreted to represent an *NPQ*-related increase of the probability of heat emission lowering the trapping efficiency of open reaction centers (Lu and Zhang, 2000). Increases in *NPQ* in all cultivars under drought conditions were indeed detected (Fig. 4G). The quantum yield of electron transfer at PSII (ϕ_{PSII}) is the product of the efficiency of the open reaction centers (F_V'/F_M') and the photochemical quenching (q_P) (Genty et al., 1989; Lu and Zhang, 1999; Sinsawat et al., 2004). In all cultivars a decrease of the q_L was observed in response to the drought stress treatment, indicating that a larger percentage of the PSII reaction centers was closed at any time, which in turn indicates that the balance between excitation rate and electron transfer rate had changed.

Drought stress leads to a strong reduction of the uptake of CO_2 and therefore decreases also the demand for the products of photochemistry: ATP and NADPH. In that respect a reduction of the reaction center content would make sense for the plant from a regulatory point of view. The rapid recovery of the plants following rewatering also suggests that the reaction center loss may have played a regulatory role and did not just represent damage (Table 1). Leaf rolling of cultivars during drought has been proposed to limit the absorption of light by the photosynthetic antenna systems. In addition, leaf rolling may have limited the loss of water via transpiration. Previous studies indicated that drought-tolerant genotypes were able to maintain a higher chlorophyll content than susceptible genotypes. Chandrasekar et al. (2000) reported this phenomenon for drought resistant and susceptible wheat cultivars. Çiçek and Çakırlar (2008) reported for soya bean that salt stress affected the chl *a*/*b* ratio in several cultivars. Some of the cultivars seemed to adapt to the salt stress by reducing their chl *a*/*b* ratio (indicating a larger antenna size). In the maize cultivars studied here this effect was not so strong. During the experiment, the chl *a*/*b* ratio increased both in the control and the treated plants. As shown in Table 1, the changes in the chl *a*/*b* ratio in the control (in both directions) were quite substantial making it e.g. difficult to judge the significance of the relatively high chl *a*/*b* ratio found in the cultivar Doge at the end of the drought treatment. Mullineaux and Emlyn-Jones (2005) have suggested that the rate of PSII photo damage will be reduced by minimizing PSII antenna size and thereby reducing the absorptive cross section of the reaction centers.

Plants contain substantial amounts of carotenoids that serve as non-enzymatic scavengers of active oxygen species (Jung et al., 2000). Carotenoids are responsible for scavenging of singlet oxygen hence comparatively high carotenoid levels in genotypes have been suggested to be a measure of their tolerance (Chandrasekar et al., 2000). Drought stress caused a strong loss of photosynthetic reaction centers (loss of chl *a*, see Table 1). Since carotenoids are mainly found in association with photosynthetic reaction centers, the observed carotenoid-loss was to be expected. However, if the carotenoid content is determined on a chlorophyll basis, drought-induced an increase in the cultivar Doge. This could give this cultivar a means to dissipate more excitation energy. In this respect we note that Doge was also the cultivar with the highest *NPQ*-value following drought stress. Anthocyanins are located in the vacuoles. They also have the potential to scavenge active oxygen species like for example long-lived hydrogen peroxide (Gould et al., 2002; Pietrini et al., 2002). The anthocyanin responses differed quite strongly between Doge and Vero on the one hand and Luce on the other hand. In Luce the anthocyanin levels were quite high independent of the presence of a stress. In Doge and Vero there was a quite strong drought stress induced increase but before and after the stress the measured levels were low. The inducibility of the response by drought stress in Doge and Vero suggests that anthocyanin levels play a role in the defence of plants to drought stress.

The synthesis of osmolytes including proline is widely used by plants to stabilize membranes and maintain the conformation of proteins at low leaf water potentials. The synthesis and accumulation of osmolytes varies among plant species as well as among different cultivars of the same species. Proline is also known to be involved in reducing the photo damage in the thylakoid membranes by scavenging and/or reducing the production of $^1O_2$ (Reddy et al., 2004). Increase in proline accumulation observed under drought stress in Doge, Luce and Vero (Fig. 5) was in accordance with earlier observations made on maize (Chandrasekar et al., 2000).

In conclusion, the three maize cultivars investigated showed similar responses against drought stress. Although Doge was the most affected cultivar in terms of some chlorophyll fluorescence parameters such as F_o, F_M, F_V/F_M; all cultivars recovered after 6 days of rewatering. Doge may have coped with stress by upregulating protective mechanisms such as

increasing *NPQ*, chl *a*/*b* ratio (smaller antenna size), anthocyanin and proline content, decreasing F_V'/F_M' compare to other two cultivars. Results of the study suggest that Doge is less drought tolerant than the other two cultivars.

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