

Changes in Chlorophyll Fluorescence in Maize Plants with Imposed Rapid Dehydration at Different Leaf Ages

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Abstract A comparison of the effects of a rapidly imposed water deficit with different leaf ages on chlorophyll *a* fluorescence and gas exchange was performed in maize (*Zea mays* L.) plants. The relationships between photosynthesis and leaf relative turgidity (RT) and ion leakage were further investigated. Leaf dehydration substantially decreased net photosynthetic rate (*A*) and stomatal conductance (G_s), particularly for older leaves. With dehydration time, F_v/F_m maintained a relatively stable level for youngest leaves but significantly decreased for the older leaves. The electron transport rate (ETR) sharply decreased with intensifying dehydration and remained at lower levels during continuous dehydration. The photochemical quenching of variable chlorophyll fluorescence (q_p) gradually decreased with dehydration intensity for the older leaves but increased for the youngest leaves, whereas dehydration did not affect the nonphotochemical chlorophyll fluorescence quenching (NPQ) for the youngest leaves but remarkably decreased it for the older leaves. The leaf RT was significantly and positively correlated with its F_v/F_m , ETR, and q_p , and the leaf ion leakage was significantly and negatively correlated with F_v/F_m and NPQ. Our results suggest that the photosynthetic systems of young

and old leaves decline at different rates when exposed to rapid dehydration.

Keywords Chlorophyll *a* fluorescence · Drought · Gas exchange · Leaf aging · Ion leakage · Photosystem II · Photosynthesis · *Zea mays* L

Introduction

Drought is a major constraint in crop productivity in many regions of the world (Boyer 1982). C_4 plants may have higher CO_2 fixation and water use efficiency (WUE) relative to C_3 plants due to their anatomical and biochemical specialization (Beale 1999; Morgan and others 2001; Niu and others 2005; Kim and others 2006; Ripley and others 2007). However, this advantage is completely lost when unwatered C_4 plants undergo severe drought (Ripley and others 2007), and actually poor productivity of maize (*Zea mays* L.) is frequently attributed to inadequate moisture (Leakey and others 2004). The studies on responses of photosynthesis of maize to environmental conditions are important for selecting varieties and managing practices to obtain high productivity in a changing climate (Lal and others 1996a; Ehleringer and others 1997; Yu and Setter 2003; Leakey and others 2004; Kim and others 2006).

Plants have many stress acclimation mechanisms such as osmotic adjustment (Nayyar 2004), antioxidant defense (Farrant and others 2004; Xu and Zhou 2006b), and synthesizing stress protein (Young and others 2001; Bernacchia and Furini 2004). Many studies have reported that stomatal regulation may play an important role in photosynthetic adaptation to drought stress (for example, Marques da Silva and Arrabaca 2004). However, under severe water stress, the fraction of nonstomatal limitation to photosynthesis may

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increase (Thiagarajah and others 1981; Paponov and Engels 2003; Galmés and others 2007). Thus, the relative role and proportion of stomatal and nonstomatal limitations in the decrease of photosynthesis induced by water stress are still being debated; different results may result from different species (Georgieva and others 2005), stress intensities (Lawlor and Cornic 2002; Grassi and Magnani 2005), leaf ages (Thiagarajah and others 1981), and development stages (Yoo and others 2003; Grassi and Magnani 2005).

The influence of water deficit on the patterns of fluorescence emission is well established (for example, Govindjee and others 1981; Ogren 1990). The gas exchange and chlorophyll fluorescence are described as good markers for plant photosynthetic physiology *in vivo*, especially when measured simultaneously using a gas-exchange system with an integrated fluorescence chamber head (Bernacchi and others 2003; Long and Bernacchi 2003; Ripley and others 2007). Thus, the simultaneous measurements of CO₂ exchange and chlorophyll fluorescence under controlled light and temperature conditions allow the researchers to obtain concurrent measurements of the photosynthetic characteristics of plants in either laboratory or field.

The acclimation of plant leaves to environmental stress is closely associated with growth phases, duration of stress (Behera and others 2003; Xu and Zhou 2007), leaf age (David and others 1998; He and others 2002), expansion (Marques da Silva and Arrabaca 2004), development (Tricker and others 2004), colors (Field and others 2002), and longevity (He and others 2002; Tricker and others 2004; Grassi and Magnani 2005). Water stress could reduce the net photosynthetic rate associated with other leaf physiologic and biochemical activities, leading to leaf senescence (Shah and Paulsen 2003; Plaut and others 2004; Xu and Zhou 2006b).

Drought may be a major constraint in plant responses to environmental factors, but studies on simultaneous changes in photosynthesis and chlorophyll *a* fluorescence under drought stress with leaf age are limited. In the present experiment we simultaneously measured gas exchange and chlorophyll *a* fluorescence to investigate the effects of rapid water stress and different leaf ages on photosynthetic and PSII activity, leaf water status, and ion leakage. We aimed to assess how gas exchange and chlorophyll fluorescence of leaves with different ages respond to rapid dehydration and their relationships with leaf water status and cell membrane damage.

Materials and Methods

Plant Materials

Zea mays L. plants (cv. Shendan 21) at the late jointing stage, 60 days after sowing, were obtained from the field in

Jinzhou, Liaoling, China, a famous maize production belt, in late June 2004. The 60-day-old plants were 1.4 m tall and had 12 leaves per plant. The plants were excised from the roots at ground level and brought back to the lab where the dehydration treatment was applied in a controlled environment room with a 14-h photoperiod, relative humidity (RH) of 50–65%, and day/night temperatures of 28–30/18–20°C. The plants were exposed to a lower photosynthetic photon flux density (PPFD) of 280 μmol photons m⁻² s⁻¹, provided by a combination of cool-white fluorescent and incandescent lamps, to prevent excessively rapid tissue dehydration. The dehydration treatment method in a controlled environment has been widely used because of its simplicity and feasibility in investigating the physiologic metabolic response to rapid dehydration (Georgieva and others 2005; Verslues and others 2006). The leaves of each plant were separated into three age levels for the measurements: top leaves (the youngest and fully expanded leaves, about 10 days old, the 12th leaf since sowing); middle leaves (mature leaves, about 20 days old, the 9th leaf), and bottom leaves (older leaves, about 30 days old, the 6th leaf).

Leaf Relative Turgidity (RT)

The leaves were cut into small discs (about total 1 g fresh weight) and fresh weights (FW) were obtained. The leaf discs then were placed in a beaker (25 ml) filled with water overnight in the dark. They were reweighed to give a fully turgid fresh weight (TW) the next morning and dry weights (DW) after drying at 80°C for 24 h in a drying oven. Relative turgidity (RT) of the leaves is calculated by the following expression (Barrs 1968):

$$RT (\%) = [(FW - DW)/(TW - DW)] \times 100$$

Ion Leakage Determination

Ion leakage was determined according to a simplified method from García Mata and Lamattina (2001). This is commonly used as an indicator of cell membrane stability (Blum and Ebercon 1981). Leaves were cut into small discs (total 1.5 g fresh mass) and placed in beakers (50 cm³) with 40 cm³ of deionized water. After incubation at 25°C for 12 h in dark conditions, the electrical conductivity in the bathing solution was determined (initial EC) with a conductivity meter (DDSJ-308A, Shanghai Science Instrument Ltd, Shanghai, China). Then the samples were heated at 85°C for 2 h and the conductivity was read again in the bathing solution (final EC). Electrolyte leakage was defined as EC (%) = (initial EC/final EC) × 100.

Leaf Gas Exchange and Chlorophyll Fluorescence

Leaf gas exchange measurements were coupled with the measurements of chlorophyll fluorescence using an open gas exchange system (LI-6400R; LI-COR, Inc., Lincoln, NE, USA) with an integrated fluorescence chamber head (LI-6400-40 leaf chamber fluorometer; LI-COR) and data acquisition software (OPEN Software version 5.1, LI-COR). The gas exchange parameters were recorded at $380 \mu\text{mol mol}^{-1}$ of $[\text{CO}_2]$ with $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ of PPFD. At least three leaves were measured at three leaf age levels: top (youngest and fully expanded), middle (mature), and bottom (older) leaf levels of each plant, totaling three plants each time and a total of four times per plant. All leaves measured were attached to the detached plants cut directly from the field to partly avoid other effects such as wounding and regulation between source and sink organs. After a 30-min adaptation to the dark at a controlled temperature of 25°C , the minimal fluorescence yield (F_0) was measured by a modulated light that was sufficiently low ($<0.1 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) so as not to induce any significant variable fluorescence, and the maximal fluorescence yield (F_m) was determined by a 0.8-s saturating pulse at $7000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in dark-adapted leaves. The leaves were then continuously illuminated with a white actinic light at an intensity of $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (the average mid-day PPFD value for the plants) for 30 min. The steady-state value of fluorescence (F_s) was thereafter recorded and the second saturating pulse at $7000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ was imposed to determine the maximal light-adapted (F'_m) fluorescence level. The actinic light was removed and the minimal fluorescence level in the light-adapted state (F'_0) was determined after 3 s of far-red illumination. The fluorescence parameters were obtained from the following formulas (van Kooten and Snel 1990): the maximal efficiency of PSII photochemistry, $F_v/F_m = (F_m - F_0)/F_m$; the photochemical quenching of variable chlorophyll fluorescence, $q_P = (F'_m - F_s)/(F'_m - F'_0)$; the relative change of minimum chlorophyll fluorescence, $q_0 = (F_0 - F'_0)/F_0$; and the nonphotochemical chlorophyll fluorescence quenching, $\text{NPQ} = (F_m - F'_m)/F'_m$.

The electron transport rate $\text{ETR} = (F'_m - F_s)/F'_m \times fI\alpha_{\text{leaf}}$, where f is the fraction of absorbed quanta that is used by PSII and is typically assumed to be 0.4 for C_4 . I is PPFD, and α_{leaf} is effective leaf absorptance, which is assumed to be 0.85 (Maxwell and Johnson 2000).

Statistical Analysis

All statistical analyses of variance (ANOVA) were performed using SPSS v10.0 (SPSS, Chicago, IL, USA).

Effects of water stress, leaf age, and their interactions were analyzed using a two-way ANOVA ($p = 0.05$). Differences between the means among water stress and leaf age treatments were compared using Duncan's multiple-range tests at 0.05 probability levels. For the relationships of photosynthesis with RT and ion leakage level, linear regression analysis was also performed ($p = 0.05$).

Results

Changes in Leaf Relative Turgidity and Ion Leakage

Similar change trends of relative turgidity (RT) with dehydration were found for leaves of three ages: top (youngest and fully expanded), middle (mature), and bottom (older). Leaf RT decreased gradually from the beginning of dehydration, and the older leaves had lower RT values (Figure 1A). In contrast, the older leaves had higher levels of ion leakage, followed by the mature and the youngest leaves. Leaf ion leakage was significantly different due to leaf ages ($p < 0.05$, Figure 1B), and increased gradually with intensifying dehydration, especially for the older leaves. In the overall dehydration period, there were significant effects of dehydration, leaf age, and their interactions ($p < 0.05$) according to two-way ANOVA.

Changes in Gas-Exchange Parameters

There were lower leaf net photosynthetic rates (A) and stomatal conductances (G_s) (Figure 1C, D), but higher intercellular CO_2 concentrations (C_i) (Figure 1E) for the older leaves compared to the younger leaves, indicating that the youngest leaves exhibited higher photosynthetic capacities under dehydration stress compared to the older leaves. The values of A and G_s decreased with dehydration time, particularly for the older leaves, whereas the values of C_i remained relatively stable (Figure 1C, D). The main effects and their interactions were significant ($p < 0.05$), based on two-way ANOVA.

Changes in Chlorophyll Fluorescence Parameters

The maximal efficiency of PSII photochemistry (F_v/F_m) was not significantly affected by dehydration for the youngest leaves but significantly decreased for the older leaves after 2 days of dehydration (Figure 2A). The electron transport rate (ETR), at $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ of the PPFD, decreased with dehydration time but maintained a higher level for the youngest leaves 4 days after dehydration (Figure 2B).

Fig. 1 Effects of dehydration on leaf relative turgidity (RT) (A), ion leakage (B), net photosynthetic rate (A) (C), stomatal conductance (G_s) (D), and intercellular CO_2 concentration (C_i) (E) in the three leaf ages: top (youngest and fully expanded), middle (mature), and bottom (older) leaf levels of *Z. mays*. The gas-exchange measurement was done at a light level of PPFD of $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Vertical bars represent \pm SE of the mean ($n = 3$)

The photochemical quenching of variable chlorophyll fluorescence (q_p) decreased gradually with intensity of dehydration for the mature and the older leaves but dramatically increased for the youngest leaves at the later stage (Figure 2C). Leaf dehydration did not significantly affect the nonphotochemical chlorophyll fluorescence quenching (NPQ) of the youngest leaves except for the last two days of dehydration, but decreased it for the older leaves (Figure 2D). Leaf dehydration significantly increased the relative change of minimum chlorophyll fluorescence (q_0) for the older leaves and led to complicated changes for both mature and young leaves (Figure 2E). The effects of dehydration, leaf age, and their interactions were significant ($p < 0.05$) as the data were used for overall dehydration period.

Relationship between Chlorophyll *a* Fluorescence and Relative Turgidity (RT)

The values of leaf RT were significantly and positively linearly correlated with F_v/F_m ($y = 0.0045x + 0.395$; $R^2 = 0.60^{**}$, $p < 0.01$) (Figure 3A) and ETR ($y = 2.305x - 130.41$; $R^2 = 0.49^{**}$, $p < 0.01$) (Figure 3B). For the fluorescence quenching parameters, there was a significant and positive correlation between RT and q_p ($y = 0.0086x - 0.343$; $R^2 = 0.47^*$, $p < 0.01$) (Figure 3C). However, there was only weak correlation between RT and NPQ ($y = 0.0186x + 0.776$; $R^2 = 0.111$, $p = 0.09$; Figure 3D) and q_0 ($y = -0.0004x + 0.107$; $R^2 = 0.002$, $p = 0.83$; Figure 3E).

Relationships Between Ion Leakage and Gas Exchange and Chlorophyll *a* Fluorescence

Leaf ion leakage was significantly and negatively correlated with leaf net photosynthetic rate ($y = -0.834x + 51.031$; $R^2 = 0.49^{**}$, $p < 0.001$; Figure 4A) and stomatal conductance ($y = -0.0041x + 0.495$; $R^2 = 0.35^{**}$, $p < 0.01$) (Figure 4B) but positively correlated with intercellular CO_2 concentration ($y = 2.541x + 156.63$; $R^2 = 0.31^{**}$, $p = 0.006$) (Figure 4C).

Leaf ion leakage was significantly and negatively correlated with F_v/F_m ($y = -0.006x + 1.002$; $R^2 = 0.42^{**}$,

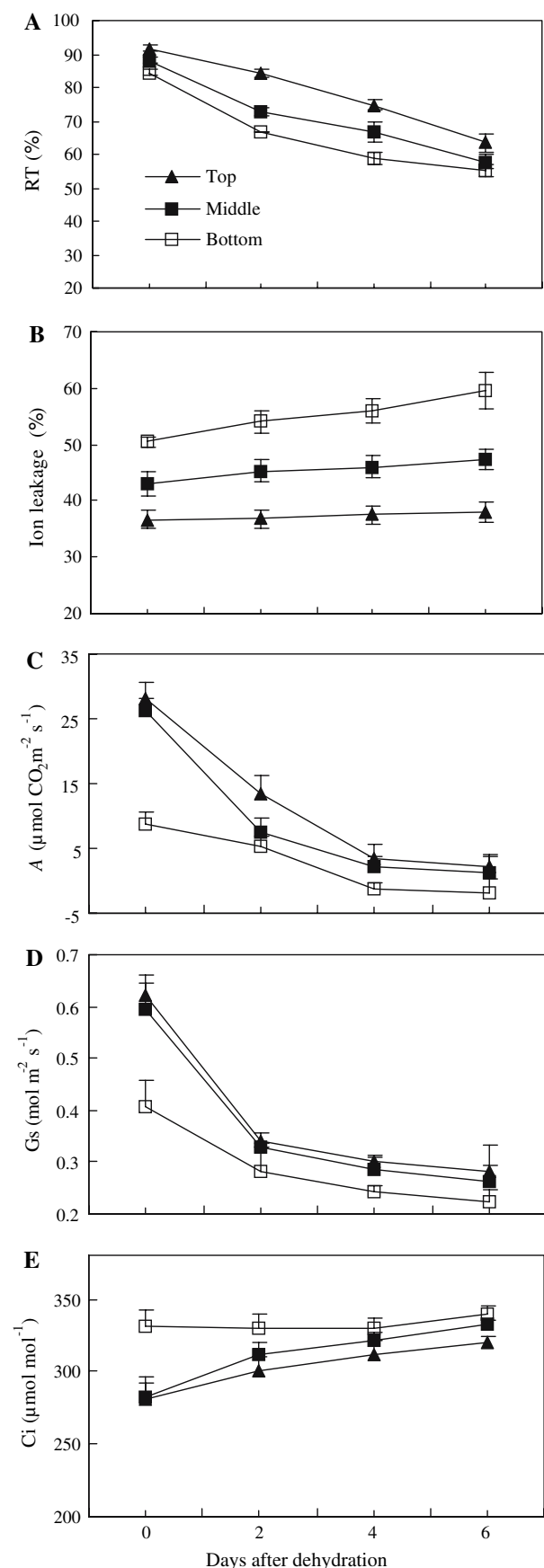


Fig. 2 Effects of dehydration on the maximal efficiency of PSII photochemistry (F_v/F_m) (A), the electron transport rate (ETR) (B), the photochemical quenching of variable chlorophyll fluorescence (q_p) (C), the nonphotochemical chlorophyll fluorescence quenching (NPQ) (D), and the relative change of minimum chlorophyll fluorescence (q_0) (E) in the three leaf ages: top (youngest and fully expanded), middle (mature), and bottom (older) leaf levels of *Z. mays*. The measurement light level was PPFD of 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Vertical bars represent \pm SE of the mean ($n = 3$)

$p < 0.01$) (Figure 5A) but not significantly with ETR ($y = -0.922x + 80.554$; $R^2 = 0.05$, $p = 0.31$) (Figure 5B). Leaf ion leakage did not have a close correlation with q_p ($y = 0.0013x + 0.250$; $R^2 = 0.004$, $p = 0.767$) (Figure 5C) but was significantly and negatively correlated with NPQ ($y = -0.058x + 4.882$; $R^2 = 0.46^{**}$, $p < 0.001$) (Figure 5D) and significantly and positively correlated with q_0 ($y = 0.005x - 0.168$; $R^2 = 0.23^*$, $p = 0.022$) (Figure 5E).

There were significantly positive linear relationships of A with F_v/F_m ($y = 0.0041x + 0.673$; $R^2 = 0.27^*$, $p = 0.011$) and with ETR ($y = 1.514x + 18.774$; $R^2 = 0.19^*$, $p = 0.038$; Figure 6), indicating that the decrease in photosynthesis was partly explained by the decline in PSII function.

Discussion

The underlying mechanism of the photosynthetic response of plants, particularly C_4 plants, to drought is still being studied in both lab and field experiments (Lawlor and Cornic 2002; Marques da Silva and Arrabaca 2004; Ripley and others 2007). In the present study, the plants excised from the roots at ground level rapidly underwent dehydration in a controlled-environment room, which may assess a rapid response to dehydration to understand underlying physiologic mechanisms (Blum and Ebercon 1981; Georgieva and others 2005; Verslues and others 2006). The present study determines how gas exchange and chlorophyll fluorescence of leaves of different ages respond concurrently to rapid dehydration, and their relationships with leaf water status and cell membrane damage. The results suggested that the photosynthetic systems of young and old leaves might decrease at different rates when exposed to rapid dehydration, which is associated with cell membrane damage.

Under mild-to-moderate water stress, about two thirds of the decline in the net photosynthetic rate (A) is attributable to stomatal limitation (Grassi and Magnani 2005). However, with heightened water stress intensity, nonstomatal limitation increases progressively, even playing a major role, for example, through inhibiting biochemical metabolism and adversely affecting chlorophyll *a* fluorescence

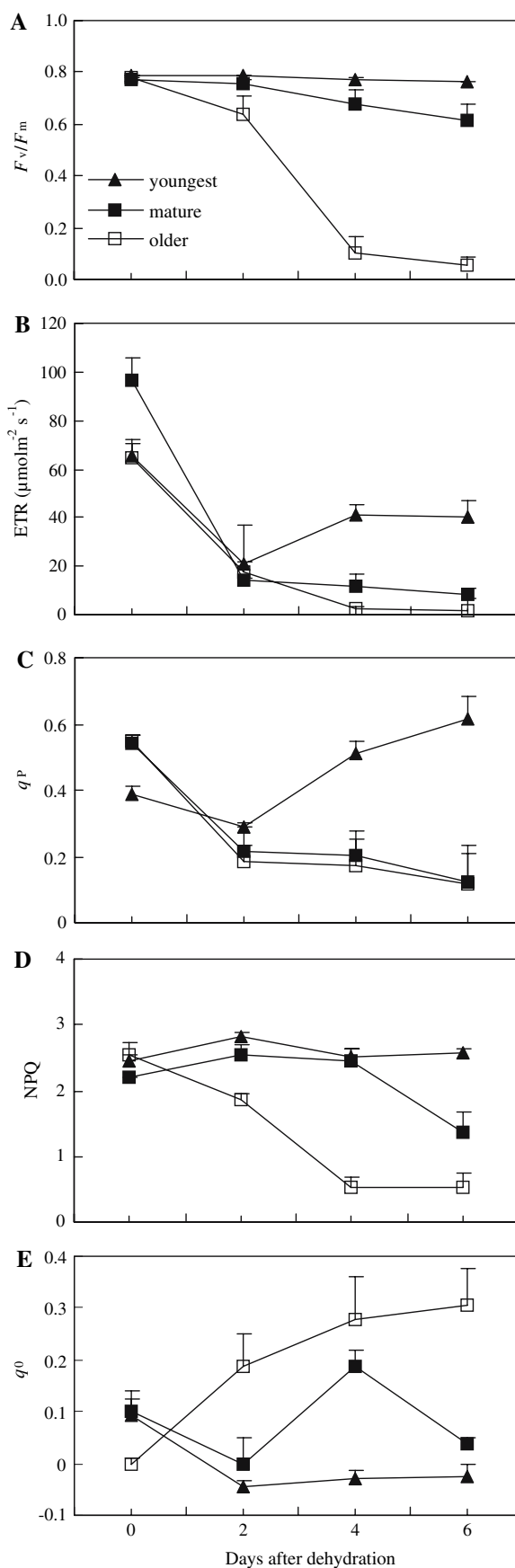
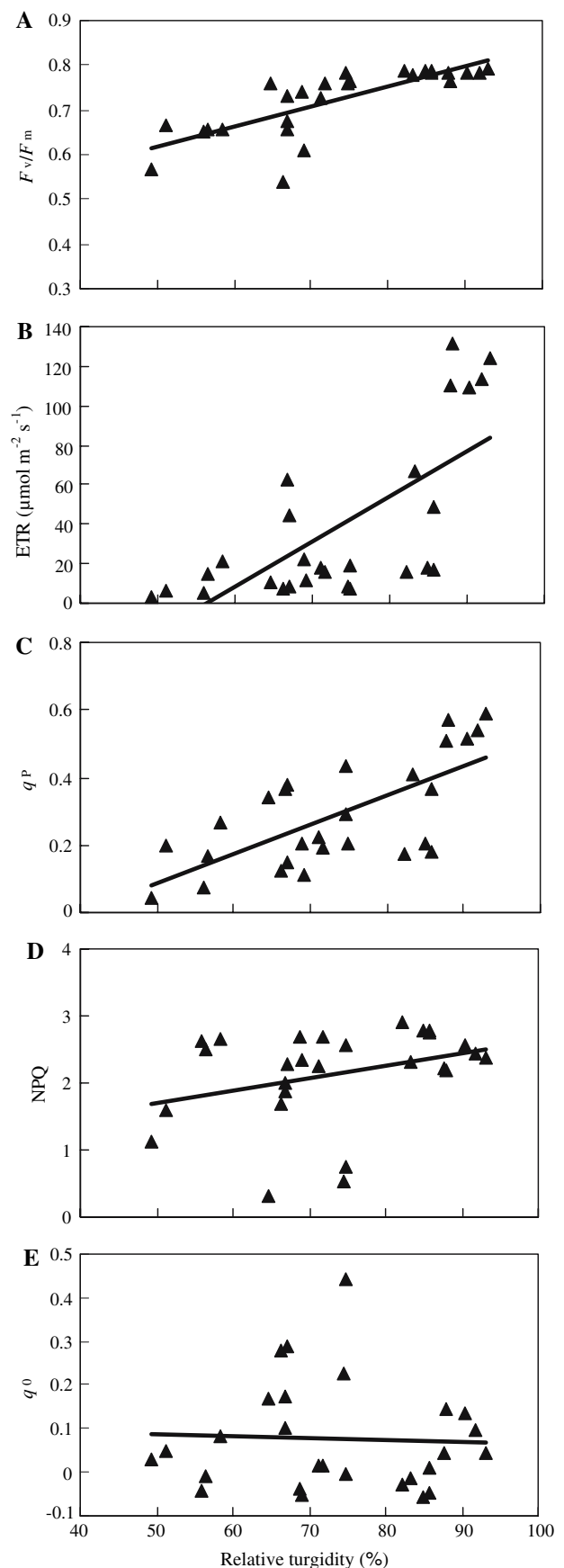


Fig. 3 The relationships between fluorescence parameters and relative turgidity (RT) for the leaves of *Z. mays*. **A** F_v/F_m , **B** ETR, **C** q_p , **D** NPQ, **E** q_0 . The measurement light level was PPFD of 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ($n = 36$)

(Schulze 1986; Lu and Zhang 1999; Grassi and Magnani 2005; Xu and Zhou 2006a; Ripley and others 2007). Saccardy and others (1996) and Marques da Silva and Arrabaca (2004) indicated that stomatal limitation is a major factor in decreased photosynthesis of water-stressed C_4 plants, whereas with maize leaf aging and senescence, the intercellular CO_2 concentration (C_i) increases, indicating that the activity of the carboxylating system is more decreased than stomatal conductance (Thiagarajah and others 1981; Paponov and Engels 2003). The A of dehydrated maize leaves declines rapidly as the RT decreases from 100% to 60%, whereas maximal efficiency of photosystem II (PSII) photochemistry (F_v/F_m) remains relatively constant, declining only when RT is less than 60% (Figure 2A). No reduction in F_v/F_m under moderate drought was also reported in maize leaves by Lu and Zhang (1999), but it declined in some C_4 grasses (Ghannoum and others 2003) and C_3 *Quercus pubescens* (Gallé and others 2007). The present study showed that A , stomatal conductance (G_s), and F_v/F_m were reduced together by severe dehydration for the older leaves. However, although it is noted that downregulation of photochemistry occurs in response to stomatal limitation, this does not mean that photosynthesis impose additional nonstomatal limitations. Compared with old leaves, the younger leaves may have greater plasticity to acclimate to dehydration, and their nonstomatal limitations are more profound with continuous water stress (Lal and others 1996b). Nevertheless, stomatal limitation's relative role still needs to be reassessed.

The relationship between A and PSII efficiency can assess the energy efficiency of CO_2 fixation; for example, that less CO_2 is fixed per transported electron suggests that there is an increase in electron transport to a process such as photorespiration, O_2 reduction, and nitrogen assimilation rather than to CO_2 acclimation (Watling and others 2000; Siebke and others 2003). Saccardy and others (1998) reported that A gradually decreased with dehydration, but ETR decreased until below 60% of maize leaf RT. The results of Ghannoum and others (2003), and Ripley and others (2007) showed that A , ETR, and quantum yield of PSII of four C_4 grasses decreased rapidly with increasing drought. The present experiment showed that the positively close correlation of A with F_v/F_m and ETR (Figure 6) indicates that a reduction in photosynthesis may partly be explained by PSII function. Furthermore, in our experiment, in young leaves A and ETR declined during the first 2 days of dehydration. After 2 days of dehydration, although A declined with decreasing G_s , ETR increased



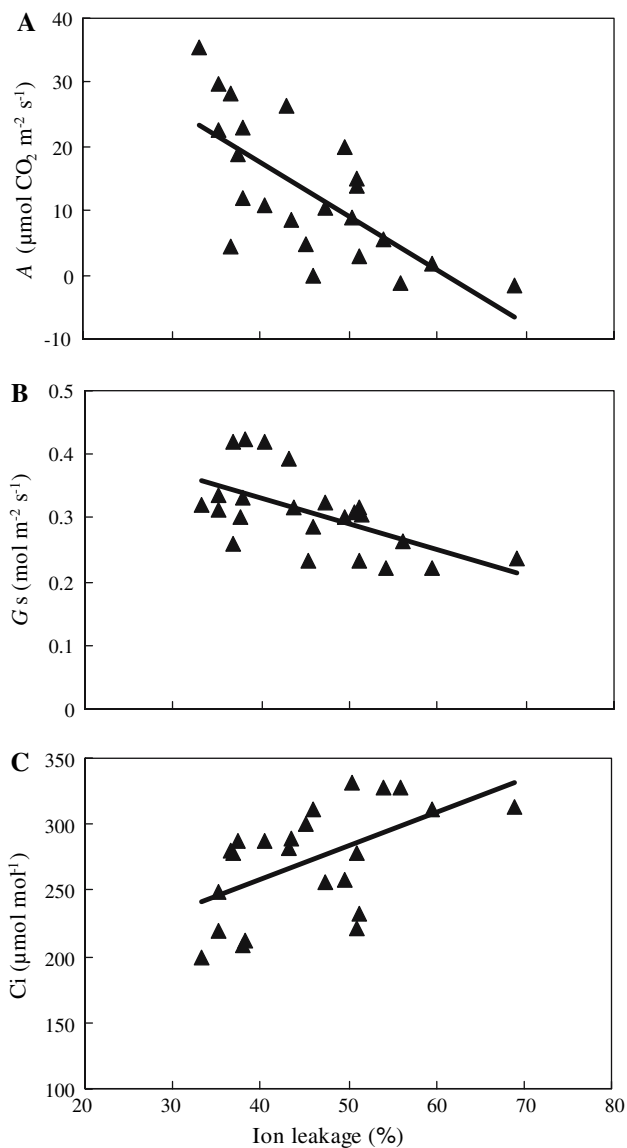


Fig. 4 The relationships between gas exchange parameters and ion leakage in leaves of *Z. mays*. **A** Net photosynthetic rate (*A*), **B** G_s , **C** C_i . The measurement light level was PPFD of 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ($n = 36$)

(Figures 1C, D, 2B), suggesting that severe dehydration triggers the functioning of an alternative flux of electrons, such as O_2 reduction, which acts as a protection mechanism for PSII from photoinactivation and damage (Siebke and others 2003; Farage and others 2006; Ripley and others 2007).

In the present study, no net CO_2 fixation was observed in the last 2 days of dehydration (Figure 1C), indicating that the net photosynthetic rate may equate with respiration rate under severe water stress (Liu and Li 2005). The two fundamental processes are important to plant performance (Ryan 1991; Turnbull and others 2002); in particular, they can result in the transfer of ATP and the reduction of power

between mitochondria and chloroplasts (Maurousset and others 1992; Lawlor and Cornic 2002). This is closely correlated with cell membrane damage, using ion leakage as the index (García Mata and Lamattina 2001; Stevens and others 2006). In the current study, ion leakage increase induced by dehydration may indicate the adverse effect on photosynthesis (Figures 1B, C, 4A). Furthermore, the association of PSII function with cell membrane stability depends on genotype (Georgieva and others 2005), and that issue requires further investigation.

A decrease in photochemical quenching of variable chlorophyll fluorescence (q_p) was observed under water stress in leaves of *Sorghum bicolor* (Loreto and others 1995), *Setaria sphacelata* (Marques da Silva and Arrabaca 2004), and *Quercus pubescens* (Gallé and others 2007), although the extent of inhibition may depend on water-stress tolerance of the species (Georgieva and others 2005) and water-stress intensity (Marques da Silva and Arrabaca 2004). The present result showed that q_p increased gradually with intensification of dehydration for the youngest leaves but decreased for the old leaves (Figure 2C), which is in contrast with changes in *A*. It is suggested that the decrease in photosynthesis may partially result from stomatal limitation during the first 2 days of dehydration, whereas an increase in q_p after 2 days of dehydration in young leaves (Figure 2C) may result from the operation of an alternative sink to CO_2 assimilation for photosynthetic reducing equivalents, possibly oxygen reduction via a Mehler reaction (Farage and others 2006). However, more studies are needed to distinguish the differences.

The nonphotochemical quenching of chlorophyll fluorescence (NPQ) increased with moderate water stress but decreased under severe water stress (Marques da Silva and Arrabaca 2004). In most drought studies, at RWC below approximately 75%, a large NPQ cannot be fully reversed by increased C_i , indicating that factors other than CO_2 supply limit *A* (Lawlor and Cornic 2002), partly leading to a decrease in the ability to dissipate excess energy indicated by NPQ (Golan and others 2006). In our study, leaf dehydration did not affect the NPQ for the youngest leaves but remarkably reduced it for the older leaves (Figure 2D), also indicating a lower ability to regulate excess energy for the latter. Furthermore, there were significant and negative relationships between NPQ and ion leakage. It is suggested that the older leaves may be close to the maximal capacity of heat dissipation under severe dehydration, and dehydration may trigger damage to dissipating mechanisms as a result of impaired pumping of protons or membrane leakage. The relative change of minimum chlorophyll fluorescence (q_0) can quantify the thermal dissipation processes within PSII complexes and may be connected with the electron flow-regulating mechanisms, inactivation of PSII reaction centers (photoinhibition and

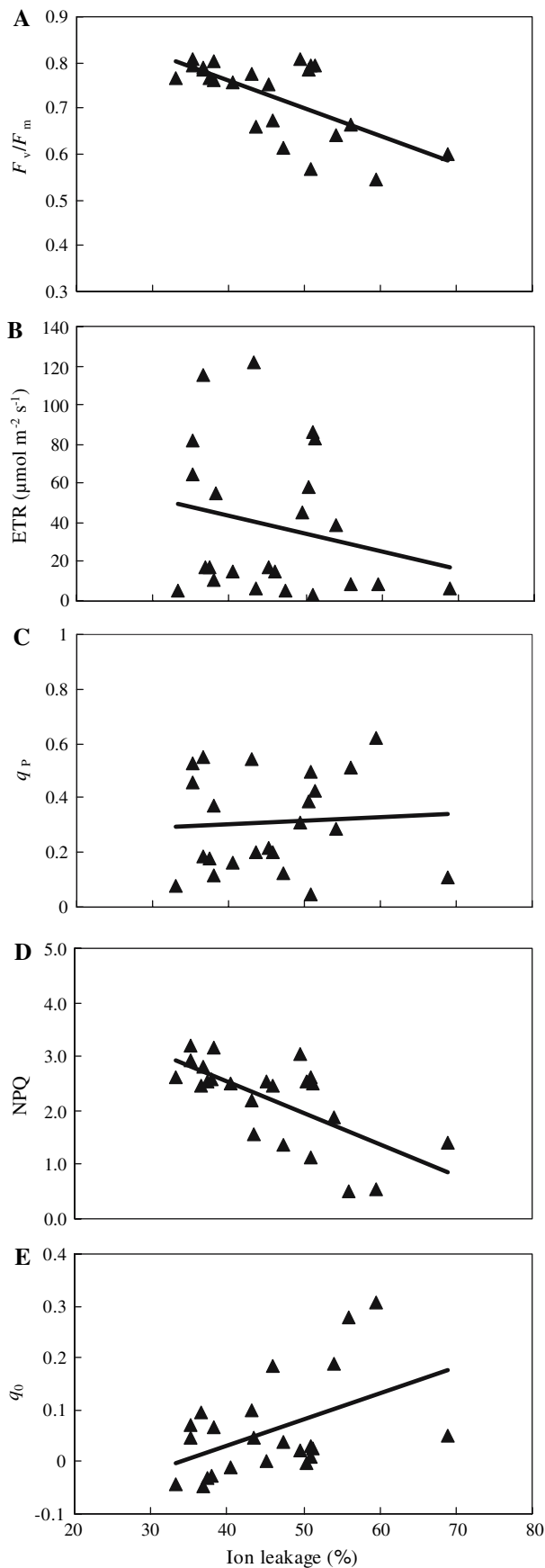


Fig. 5 The relationships between fluorescence parameters and ion leakage in leaves of *Z. mays*. **A** F_v/F_m , **B** ETR, **C** q_p , **D** NPQ, **E** q_0 . The measurement light level was PPFD of $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ($n = 36$)

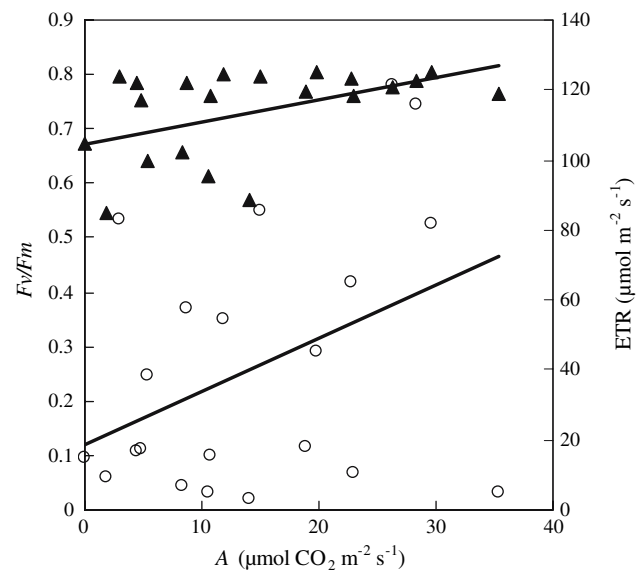


Fig. 6 The relationships between net photosynthetic rate (A) and F_v/F_m (closed triangle) and ETR (open circle) in leaves of *Z. mays*. The measurement light level was PPFD of $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ($n = 36$)

photodestruction), violaxanthin de-epoxidation, and conformation changes within pigment-protein complexes (Šiffel and Vácha 1998; Roháček 2002). Our results showed that dehydration significantly increased q_0 for the older leaves (Figure 2E), also indicating a more adverse effect of dehydration on the thermal dissipation processes for the older leaves relative to the young.

The most characteristic effects of water stress on plant growth are ascribed to the decrease in leaf expansion and photosynthetic rate (Marques da Silva and Arrabaca 2004; Xu and Zhou 2005). Water stress could reduce A , thus accelerating senescence (Shah and Paulsen 2003; Plaut and others 2004) and consequently reducing the regulating capacity to tolerate drought (Xu and others 1995; Chaves and others 2003). Thiagarajah and others (1981) reported that the biochemical limitation proportion increases with maize leaf senescence. Chlorophyll content and the chlorophyll/carotenoid ratio decrease during leaf senescence (Lu and Zhang 1998; He and others 2002), which is closely associated with leaf age (David and others 1998), suggesting that leaf aging/senescing may be responsible for a decrease in heat dissipation, a photoprotective mechanism. Thus, photosynthetic response to dehydration may depend on leaf aging/senescing.

Our results show that the effect of dehydration on photosynthesis and the activity of PSII varies with leaf age,

both in stomatal and biochemical limitations, suggesting that photosynthetic plasticity to rapid water stress may depend on leaf growth stage. The result is significant in view of global climate change because water stress often occurs at later C₄ plant growth stages in semiarid regions (Marques da Silva and Arrabaca 2004; Kim and others 2006). Improvement in senescence tolerance by genetic methods and/or field water management practices would enable the C₄ plant to adapt to drought.

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