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LIGHT-DEPENDENT CHANGES OF CHLOROPHYLL FLUORESCENCE AND XANTHOPHYLL CYCLE PIGMENTS IN MAIZE LEAVES DURING DESICCATION

CHANG CHENG XU[1,2], QI ZOU[2] and CHOON-HWAN LEE[1*]
[1]Department of Molecular Biology, Pusan National University, Pusan 609-735, Korea
[2]Department of Plant Science, Shandong Agricultural University, Taian 271018, China

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Abstract – Changes of chlorophyll fluorescence and xanthophyll cycle pigment contents in maize leaves were investigated during desiccation in darkness or in the light. In darkness, a drastic dehydration of detached maize leaves down to 50% relative water content (RWC) affected photochemical efficiency of photosystem II (Fv/Fm) and photochemical quenching (qP) only slightly. In contrast, desiccation in the light with a moderate intensity led to a pronounced reduction in Fv/Fm with a Fo quenching when RWC was greater than 70%. This reduction in Fv/Fm could be recovered in darkness under humid condition. In leaves with RWC below 70%, significant reduction in Fv/Fm was accompanied by an increase of Fo, which could not be reversed within 5 h in darkness under humid condition. The nonphotochemical quenching increased during desiccation in the light with a concomitant rise in zeaxanthin at the expense of violaxanthin. Pretreatment with dithiothreitol (DTT), an inhibitor of zeaxanthin synthesis, inhibited the development of nonphotochemical quenching and prevented the xanthophyll interconversion during desiccation in the light. These results suggest that even light with a moderate intensity becomes excessive under dehydration and zeaxanthin-associated photoprotection of photosynthetic apparatus against photodamage is involved, but the protection is not complete against severe desiccation.

INTRODUCTION

Stomatal closure associated with water stress restricts carbon assimilation due to the shortage of carbon dioxide supply. As a consequence, light energy absorbed by the pigments in leaves under water stress may be in excess than that is required to support photosynthetic electron transport for the generation of ATP and NADPH for carbon fixation. Such excess energy may result in destructive photooxidations by the formation of excited triplet-state chlorophyll and singlet oxygen.

To avoid damage by a surplus of light energy, several different mechanisms have been developed during the evolution of higher plants.[1] There is increasing evidence that the xanthophyll cycle is an important protective mechanism that dissipates excessive excitation energy in the antenna chlorophyll[1,2] and may also be involved in the dissipation process within the reaction center of photosystem II (PS II).[3] However, there is little information on the quantitative significance of xanthophyll cycle pigments and other carotenoids under water stress.[4,5]

Chlorophyll fluorescence emitted by dark-adapted leaves can be used as a probe for the primary photochemistry of photosynthesis and modulated fluorescence measurements permit the separation of fluorescence quenching into photochemical (qP) and nonphotochemical (qN) components.[6,7] A major component of qN is thought to be associated with xanthophyll cycle, and thus to be zeaxanthin- and possibly antheraxanthin-dependent[2]. Recently, there is increasing evidence that photoinhibition, manifested as a decrease in the efficiency of photosynthetic energy conversion, might actually be attributable to the processes associated with xanthophyll cycle that serve a photoprotective role in an active down-regulation of photosynthesis.[2,8]

In the present study, we investigated time courses of the changes in chlorophyll fluorescence and the interconversion of xanthophyll cycle pigments in maize leaves during desiccation in darkness or in the light. We provide evidence that under dehydration even low light becomes excessive and zeaxanthin-associated photoprotection of photosynthetic apparatus against photodamage is involved during dehydration in the light, but not in darkness, and the protection is not complete against severe desiccation.

* To whom correspondence should be addressed.

† *Abbreviations used*: DTT, dithiothreitol; NPQ, Stern-Volmer type of nonphotochemical quenching; PS II, photosystem II; qP, photochemical quenching; qN, nonphotochemical quenching; RWC, relative water content.

MATERIALS AND METHODS

Plant material and stress treatments. Plants of *Zea mays* L.

(cv. Yedan No.2) were grown in pots under natural sunlight conditions. All the experiments were performed on the last fully expanded leaves from 50 day-old plants after sowing. Desiccation was achieved by exposing detached leaves to air of about 30% relative humidity. This treatment was done either in darkness or in the light with a photon flux density (PFD) of 250 μ mol m^{-2} s^{-1} at room temperature (25 $\pm$ 2℃). Control leaf samples were kept on moist filter paper under the same conditions. The resulting water stress was characterized by determining leaf relative water content (RWC), which was measured according to the ratio of (fresh weight–dry weight) to (water-saturated weight–dry weight). Inhibition of zeaxanthin synthesis was achieved by floating the detached leaves in a solution of 3 m*M* dithiothreitol (DTT) for 12 h in darkness prior to dehydration.

Chlorophyll fluorescence measurements. Chlorophyll fluorescence emission from the upper surfaces of leaves was measured with a pulse amplitude modulation fluorometer (Hansatech, King's Lynn, UK) as described by Schreiber *et al.*[6] The ratio of variable to maximal fluorescence (Fv/Fm) in predarkened leaves represents a measure of the photochemical efficiency of PS II.[9] Photochemical (qP) and nonphotochemical (qN) fluorescence quenching were calculated according to Schreiber *et al.*[6] Based on the observations that the energized state could be maintained for extended period after stress treatment,[10] the nonphotochemical fluorescence quenching was also calculated using Stern-Volmer equation (NPQ = Fm° /Fm' - 1) according to Gilmore and Björkman,[8] where Fm' is the maximum fluorescence yield following actinic light exposure. Fm° is the maximum fluorescence yield after 12 h dark recovery following a given period of dehydration to allow the complete relaxation of nonradiative energy dissipation.

Pigment analyses. Extraction and high performance liquid chromatography analysis of carotenoid composition were performed according to Thayer and Björkman.[11] The peak areas of the detection traces were integrated as absorbance × width at 0.5 peak height. The pigment content was calculated using the conversion factors published by Thayer and Björkman.[11] Chlorophyll was determined on the remaining extract according to Porra *et al.*[12]

RESULTS

Desiccation in air resulted in a rapid decrease in leaf RWC (Fig. 1). After 6 h of dehydration, the RWC fell down to less than 45% and 50% in light- and dark-desiccated leaves, respectively. Although we could observe a gradual dehydration during desiccation, rapid changes in PS II photochemical efficiency (Fv/Fm) and intrinsic fluorescence yield (Fo) in maize leaves were observed only when RWC dropped below a certain level (Fig. 2). The mean value of Fv/Fm in freshly-hydrated leaves was close to 0.83, which is a typical value for healthy plant leaves.

In darkness, only a slight decrease in Fv/Fm was

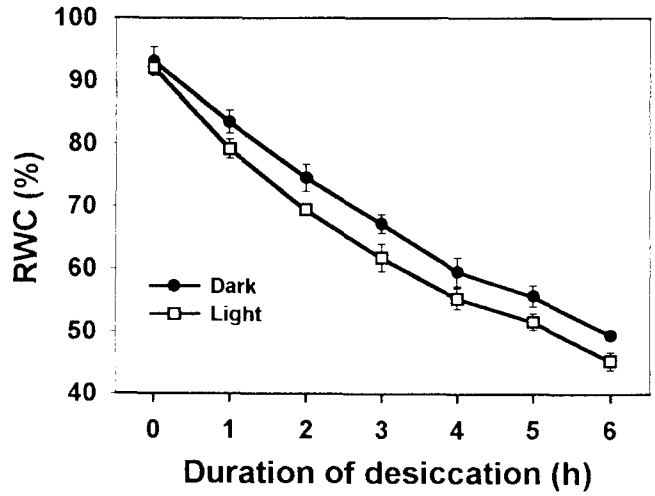

Figure 1. Time course of the changes in relative water content (RWC) of detached maize leaves during desiccation in the presence or absence of light. Vertical bars represent standard error (n=3).

observed in desiccated leaves with RWC less than 60%. However, a noticeable increase in Fo was observed in leaves with RWC less than 60%. In leaves with 50% RWC, an about 16% increase in Fo was observed, but Fv/Fm decreased only about 6%.

During desiccation in the light with moderate intensity (250 μmol m^{-2} s^{-1}), slow gradual decreases in Fv/Fm were observed in leaves with RWC more than about 60%, but Fv/Fm dropped rapidly by prolonged desiccation. Significant decreases in Fv/Fm were matched with a noticeable increase in Fo.

Independent on the presence of light, significant increases in Fo were observed in leaves with RWC less than 65%. When desiccated leaves were returned to darkness, Fv/Fm recovered to near predesiccation values for leaves with RWC above 70% (Fig. 3).

Interestingly, Fo decreased slightly at the early stages of desiccation in the light and appeared to increase thereafter (Fig. 2). The decrease in Fo is probably due to the quenching of chlorophyll fluorescence from antenna complexes in thylakoid membranes.[13] A probable cause for Fo quenching is the nonradiative dissipation of excited energy by zeaxanthin and antheraxanthin.[2,13] As shown in Fig. 2, the pretreatment of 3 m*M* of DTT, a chemical known to block zeaxanthin synthesis, abolished the Fo quenching effect of desiccation in the light. In addition, the treatment of DTT caused the effect of dehydration in the presence of light more pronounced, presumably by inhibiting the protective role of xanthophyll cycle pigments. When DTT pretreated and light-dehydrated leaves were placed under darkness for 5 h, the recovery was incomplete even in the leaves subjected to mild water deficit (Fig. 3).

Changes in photochemical (qP) and nonphotochemical (qN) fluorescence quenching in maize leaves in response to desiccation are shown in Fig. 4. Both qP and

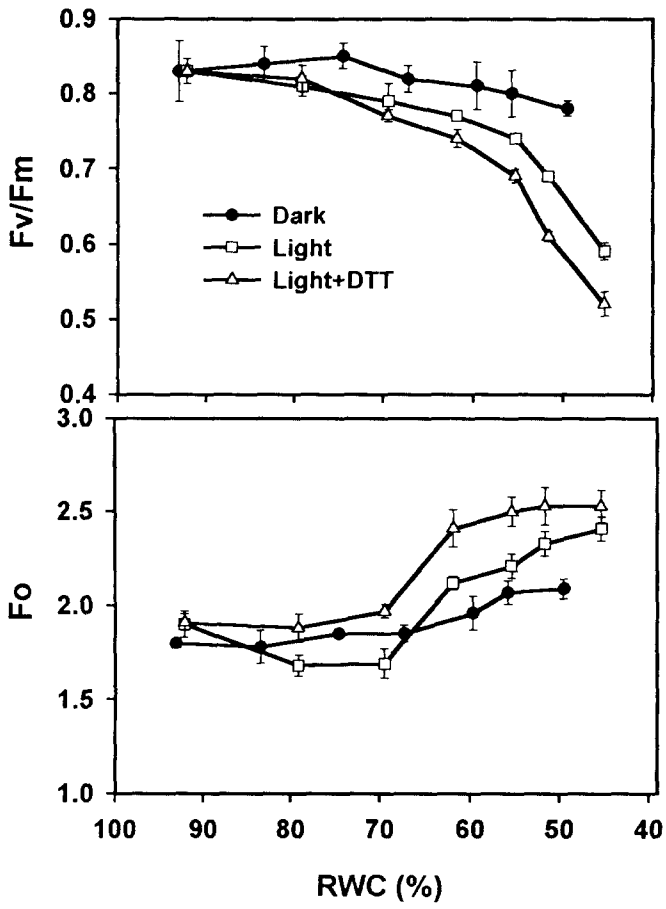

Figure 2. Changes in photochemical efficiency of PS II (Fv/Fm) and intrinsic fluorescence yield (Fo) in maize leaves during desiccation in the presence or absence of light. Leaves were floated in distilled water or distilled water with 3 m*M* of DTT for 12h in room light at 20℃ before desiccation. For light-desiccated leaves, fluorescence measurements were made after a 5-min dark adaptation. Leaves were desiccated for a certain period, and the measured values of Fv/Fm are plotted against the mean value of % RWC at the time shown in Fig. 1. Vertical bars represent standard error (n=3~8).

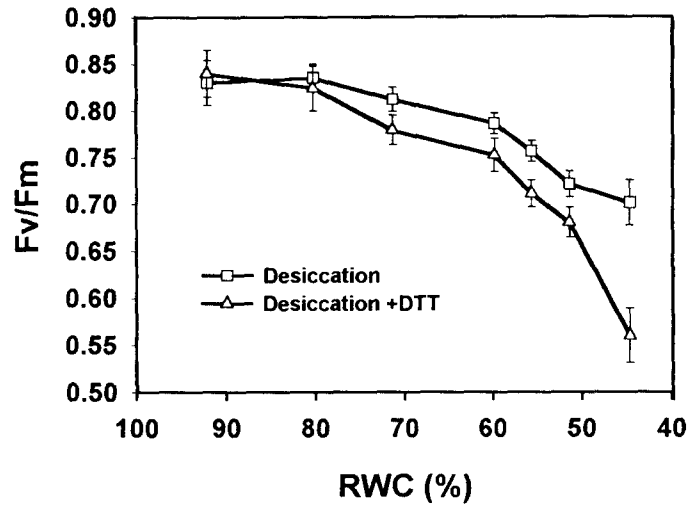

Figure 3. Recovery of Fv/Fm in maize leaves following desiccation in the light. After desiccation for given periods, leaves were kept on moist filter paper at 20℃ in darkness for 5 h of recovery period. Leaves were pretreated with 3 m*M* of DTT as described in Fig. 2. All fluorescence measurement was made after 5-min dark adaptation. The measured values of Fv/Fm were plotted against the mean value of % RWC corresponding to the time of desiccation in Fig. 1. Vertical bars represent standard error (n=5).

qN were little altered during desiccation in darkness. Conversely, desiccation in combination with moderate irradiance caused a significant decrease in qP and a dramatic increase in qN.

During dehydration, we observed a significant increase of qN (Fig. 4). Although the increase of qN is known to be correlated with the xanthophyll cycle pigments, the Stern-Volmer type of nonphotochemical quenching (NPQ) is believed to be proportional to the zeaxanthin content.[5,13] The NPQ increased in response to desiccation in the light, and the increase of NPQ was blocked by the treatment of DTT (Fig. 5). A similar blockage of NPQ development by DTT was reported by Eickmeier *et al.*[5] in *Selaglnella lepidophylla*. The Fo, Fv/Fm and other fluorescence parameters for hydrated control leaves, kept under the same experimental conditions except for desiccation, remained stable (data not shown).

To test the possible changes in the xanthophyll cycle pigments during desiccation in the light, the contents of chlorophylls and carotenoids were measured. In dark-desiccated leaves, pigment compositions remained unchanged throughout the experimental periods for 4 h (Table 1). The amounts of zeaxanthin and violaxnthin were very low and were not altered significantly during desiccation in darkness. In light-desiccated leaves, zeaxanthin contents increased at the expence of violaxanthin (Table 2). The decrease in violaxanthin was sufficient to account for the increase in zeaxanthin, resulting in a constant level of xanthophyll cycle pool (zeaxanthin + antheraxanthin + violaxanthin) throughout the desiccation treatment for 6 h. The contents of β-carotene and chlorophyll decreased slightly, while lutein and neoxanthin remained largely unchanged during desiccation in the light for 6 h (Table 2). Pretreatment with 3 m*M* of DTT almost completely eliminated zeaxanthin synthesis in leaves desiccated in the moderate light (data not shown).

As shown in Fig. 6, a linear correlation of the changes in zeaxanthin content with the changes in Stern-Volmer NPQ of maize leaves during desiccation was observed over the entire range of pigment changes induced by desiccation in the light, suggesting that nonphotochemical quenching of excitation energy may be closely related to zeaxanthin content. A similar result has been reported by Demmig *et al.*[4,14] using *Zerium oleander* and by Eickmeier *et al.*[5]

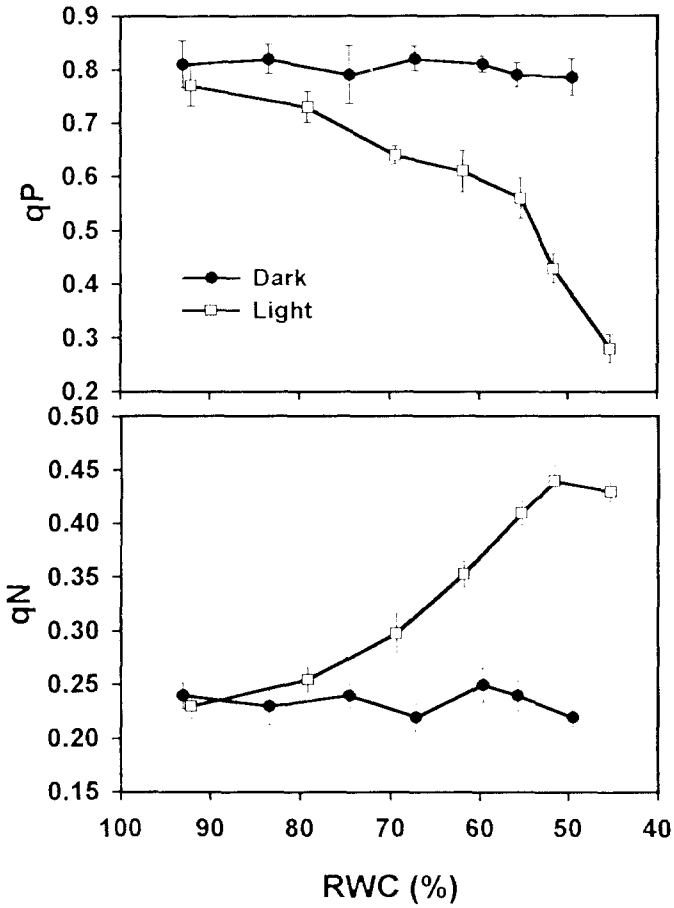

Figure 4. Changes of photochemical fluorescence quenching (qP) and nonphotochemical fluorescence quenching (qN) in maize leaves during desiccation in the presence or absence of light. Leaves were desiccated for a certain period, and the measured values of Fv/Fm are plotted against the mean value of % RWC at the time shown in Fig. 1. Vertical bars represent standard error (n=3).

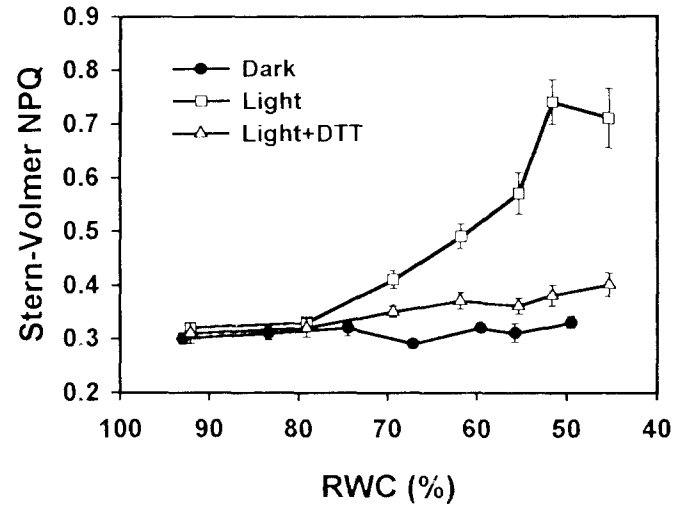

Figure 5. Changes of Stern-Volmer nonphotochemical fluorescence quenching (NPQ) in maize leaves during desiccation in the presence or absence of light. Leaves were pretreated with 3 mM of DTT as described in Fig. 2. Leaves were desiccated for a certain period, and the measured values of Fv/Fm are plotted against the mean value of % RWC at the time shown in Fig. 1. Vertical bars represent standard error (n=3).

DISCUSSION

Chlorophyll fluorescence emitted by dark-adapted leaves may reveal either disorders in PS II or the onset of protective mechanisms against excess light energy.[5] A decline in optimal PS II photochemical efficiency (Fv/Fm) should be observed in both cases. But in the latter case, these decreases should be reversible as soon as the light stress is alleviated. Furthermore, net decreases in Fo have been suggested to be associated with energy dissipation processes, particularly with a dissipation process within the chlorophyll pigment bed. Sustained increases in Fo, however, is thought to be linked with photodamage[13,15] or dissociation of the LHC II from reaction center proteins.[16] In *Ceratodon purpureus* under photoinhibitory conditions, a good correlation between the rise in the Fo level and the loss of the D1 protein from thylakoid membranes was observed by Rintamäki *et al.*[17] In maize leaves desiccated in the light, the initial decrease in the Fv/Fm ratio was acompanied by a decrease of Fo (Fig. 2), and the dark-recovery to pretreatment values was relatively rapid through a range of tissue-desiccation values to an RWC of about 70% (Fig.3), suggesting photoprotective mechanisms are working at the early stage of dehydration. As the leaves continued to dry beyond this point, Fo rose and Fv/Fm could not be recovered rapidly in darkness, indicating that severe water deficit shifted the balance in the photoinhibition from a photoprotective down-regulated state to a photoinhibitory damaged state.

Additional data support the conclusion that photoprotection may be involved in minimizing PS II damage during desiccation in maize leaves under irradiance condition. Desiccation in the light caused an increase in the nonphotochemical quenching which was accompanied by an increase in xanthophyll pigment zeaxanthin (Figs. 4, and 5 and Table 2). This response to water deficit is similar to that found with leaves of *Zerium oleander*[4] and with resurrection plant *Selaginella lepidophyll*[7]. However, contrary to the results with *Nerium oleader* and *Selagilla lepidophylla*, in which the increase in the pool size of the xanthophyll cycle components (zeaxanthin + antheraxanthin + violaxanthin) was observed, the pool size of xanthophyll cycle components stayed constant throughout the desiccation treatment for 6 h in maize leaves (Table 2). Pretreatment with dithiothreitol (DTT) substantially decreased nonphotochemical fluorescence quenching (Fig. 5) and prevented the synthesis of zeaxanthin (date not shown) in leaves desiccated in the light. These results indicate that zeaxanthin-related energy dissipation is involved in the protection of PS II against excess light stress induced by water deficit. This conclusion is supported by the observation that treatment with DTT before desiccation promoted a more pronounced reductions in

Table 1. Changes of chloroplast pigment contents in detached maize leaves during desiccation in darkness. Leaves were excised at the end of the 12 h dark period and subjected to desiccation immediately. The data represent the mean of one to three independent experiments. Chl, chlorophyll; V, violaxanthin; L, lutein; N, neoxanthin; β-C, β-carotene.

Desiccation Time (h)	Chl a	Chl b	V	L	N	β-C
	(μ mol (g dry weight)$^{-1}$)					
0	5.2	1.8	0.33	0.89	0.24	0.69
1	5.3	1.9	0.31	0.87	0.26	0.68
2	5.1	1.7	0.32	0.89	0.23	0.67
3	5.0	1.9	0.33	0.90	0.24	0.65
4	5.0	1.8	0.32	0.88	0.23	0.66

PS II photochemical efficiency (Fig. 2).

It is interesting to note that PS II is quite resistant to desiccation itself. Drastic dehydration treatments resulting in a leaf RWC as low as 60% did not significantly impair the PS II functioning in dark-desiccated leaf samples (Fig. 2). Furthermore, no interconversion of violaxanthin to zeaxanthin occurred during dehydration in darkness (Table 1). These are in sharp contrast to the results obtained when leaves were desiccated in the presence of moderate light (Fig. 2, Table 2). On this point, our data are in agreement with the recent notion that the photosynthetic machinery can tolerate high levels of water deficit.[18]

During desiccation in the light, leaves seem to tolerate the light stress via accumulation of zeaxanthin, which could be assumed from the Fo quenching in leaves with RWC greater than 70% (Fig. 2) and supported by the observation of the accumulation of zeaxanthin (Table 2). However, when RWC was less than 60%, photochemical efficiency of PS II dropped significantly, although

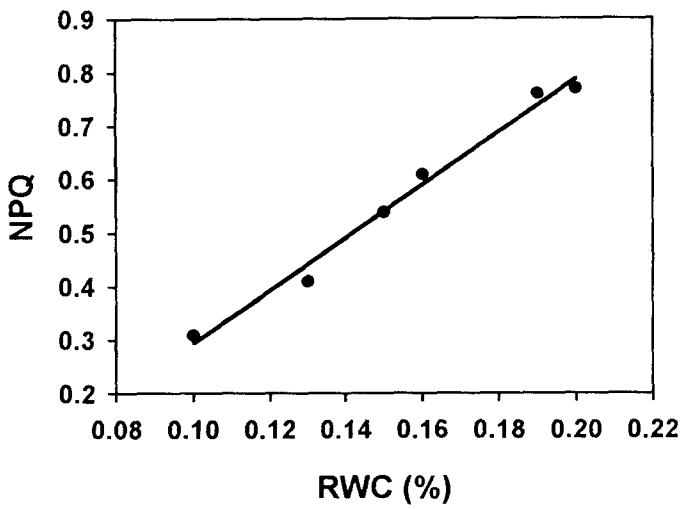

Figure 6. Relationship between the Stern-Volmer nonphotochemical quenching (NPQ) and zeaxanthin content of maize leaves during desiccation in the light. The estimated correlation coefficient (r) was 0.993.

Table 2. Changes of chloroplast pigment contents in detached maize leaves during desiccation in the light. Leaves were collected at the end of the 12 h dark period and subjected to disiccation immediately. The data represent the mean of one to three independent experiments. Chl, chlorophyll; V, violaxanthin; L, lutein; N, neoxanthin; A, anthraxanthin;Z, zeaxanthin; β-C, β-carotene.

Desiccation Time (h)	Chl a	Chl b	V	L	N	A	Z	β-C	V+A+Z
	(μ mol (g dry weight)$^{-1}$)								
0	5.4	2.0	0.32	0.87	0.20	0.08	0.10	0.68	0.51
2	5.3	1.9	0.29	0.86	0.21	0.10	0.13	0.68	0.52
3	5.3	1.8	0.27	0.87	0.18	0.09	0.15	0.66	0.50
4	5.4	1.9	0.23	0.85	0.19	0.11	0.16	0.65	0.50
5	5.2	1.7	0.21	0.86	0.18	0.10	0.20	0.62	0.51
6	5.0	1.7	0.21	0.85	0.19	0.10	0.19	0.57	0.50

zeaxanthin kept accumulated until RWC reached about 50% (Table 2, Fig. 1). Under this severe stress, Fo increased significantly (Fig. 2), and the reduced photochemical efficiency could not be recovered within 5 h in darkness (Fig. 3), suggesting that the accumulation of zeaxanthin cannot completely protect PS II from photodamage under severe desiccation in the light. Such a limitation has been also observed by Demmig-Adams *et al.*[14] and Demmig *et al.*[4]. Under mild desiccation in the light, photoinhibition or downregulation of PS II is a regulatory response to bring electron transport capacity into balance with carbon metabolism, reducing the probablity of photodamage to PS II, and maintaining a high oxidative state of primary electron acceptors of PS II[2,10,19].

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