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Chlorophyll Fluorescence of C3 and C4 Species in Response to Drought Stress

Klorofil Floresen dari Spesies C3 dan C4 dalam Responnya terhadap Cekaman Kekeringan

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Abstrak

Klorofil floresen fotosintesis dari dua spesies C3 (gandum dan kale) dan dua spesies C4 (*Echinochloa cursgallii* dan *Amaranthus caudatus*) dianalisis dalam responnya terhadap cekaman kekeringan di rumah kaca. Tumbuhan ditanam dalam pot berdiameter 15 cm selama sebulan kemudian diberi perlakuan kekeringan dengan penundaan penyiraman hingga tumbuhan layu. Kuantum efisiensi maksimum dari sistem cahaya II fotosintesis (*Fv/Fm*), quenching foto kimia (*qP*) dan non-fotokimia (*qN*) dianalisis untuk mengetahui keadaan fotosintesis tumbuhan selama cekaman kekeringan. Walaupun tidak ada pola yang jelas dalam hal status air dari spesies C3 dan C4, cekaman kekeringan yang diberikan menyebabkan penurunan kadar air medium (MWC), potensial air (WP) dan potensial osmotik (OP) semua spesies. Gandum mamiliki nilai WP dan OP yang paling rendah sementara *E. crusgallii* memiliki nilai yang paling tinggi akibat cekaman kekeringan. Kekeringan menyebabkan penurunan laju fotosintesis pada semua spesies yang ditandai dengan penurunan *qP*, namun hanya kale dan *A. caudatus* yang mengalami peningkatan *qN* akibat cekaman kekeringan. Tetap stabilnya *qP* dari *E. crusgallii* pada awal cekaman mungkin berkaitan dengan mekanisme C4 yang dimiliki oleh spesies ini. Penurunan *Fv/Fm* pada *E. crusgallii* pada periode akhir cekaman menunjukkan bahwa spesies ini mengalami fotoinhibisi disebabkan cekaman kekeringan.

Kata kunci : Cekaman kekeringan, Klorofil Floresen, Spesies C3 and C4

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Introduction

Light is a critical factor for photosynthesis which determines ATP and NADPH production and therefore organic compounds (sugar) (Morishige and Dreyfuss, 1998). When some unit of light energy (photon) drive the electron transport in two photosystems of thylakoid membrane, the chemical energy (ATP and NADPH) will be created; a mechanism called as "light reaction". The ATP and NADPH then will be used to drive carbon dioxide reduction in the process known as "dark reaction" or Calvin cycle (Taiz and Zeiger, 2002). During favorable conditions, the relationship between these two reactions will be steady. However, under adverse conditions, such as drought stress, the relationship becomes imbalance, because ATP and NADPH demand decreases due to stomatal closure which limits CO_2 supply for Calvin cycle. If this is happened, the plant may undergo excess energy and consequently can result in photoinhibition and photodamage (Baker, 1993).

Chlorophyll fluorescence is a tool that has frequently been used to analyse photosynthesis of the plant in response to environmental stress (Hamim, 2004). This measurement provides data that indicate the

effects of environmental factor such as drought stress on the state of Photosystem II (PSII) in using the energy absorbed by chlorophyll and the extent to which it is damaged by excess energy (Maxwell and Johnson 2000). There are many examples of experiment using chlorophyll fluorescence which analysed response of the plant to their environment such as in *Phillyrea latifolia*, *Pistacia lentiscus* and *Quercus ilex* saplings (Filella *et al*., 1998), tomato (Haupt-Herting and Fock 2000), wheat (Lu and Zhang 2000) and C4 grass *Eragrostis curvula* (Colom and Vazzana 2003).

Some indication of chlorophyll fluorescence in response to drought stress has been reported such as decrease of photochemical (*qP*) and an increase in non-photochemical fluorescence quenching (*qN*) (Haupt-Herting and Fock 2000). Medrano *et al*., (2002) have reported that drought stress leads to increased trans thylakoid ΔpH followed by xanthophylls de-epoxidation which result in an increase of non-photochemical quenching (*qN*) through heat dissipation. The mechanism that is known as photosynthetic down regulation may protect the plant from photosynthetic- apparatus damage.

It has been postulated that the maximum efficiency of PSII determined in dark adapted plants (*Fv/Fm*) is usually not affected due to drought stress suggesting the resistance of photosynthetic apparatus to drought stress (Cornic 2000). However, a few experiments have observed a decrease of *Fv/Fm* due to severe drought stress such as in *Quercus ilex* (Scarascia-Mugnozza *et al*., 1996), *Leucaena leucocephala* (Liang *et al*., 1997), an epiphytic orchid (Stancato *et al*., 2001) and in the C4 grasses, *Eragrostis curvula* (Colom and Vazzana 2003). These conflicting reports may be due to differences in drought severity and plant sensitivity to photoinhibition (Hamim, 2004). Under severe drought damage may occur in PSI and PSII, even though under mild drought PSII photochemistry may be not affected (Genty *et al*., 1987).

In this experiment chlorophyll fluorescence of two C3 (wheat and kale) and two C4 species *(Echinochloa crusgallii* and *Amaranthus caudatus*) in response to drought stress were examined in the glasshouse. These two types of species are compared because the different characteristic and response of them to drought stress. Some experiment suggested that photosynthesis of C4 species is less affected than that of C3 species under mild drought (Long, 1999; Hamim, 2003). However, the different response of chlorophyll fluorescence of these two types species to drought stress is still rarely analyzed.

Materials and Methods

The experiment was performed in John Tabor Laboratory, University of Essex, Colchester, UK from March – September 2002. In this experiment, two C3 (spring wheat [*Triticum aestivum* var. IMP] and kale [*Brassica oleraceae* L. var. Kestrel]) and two C4 species (*Echinochloa crusgallii* and *Amaranthus caudatus*) were used. Wheat and *E. crusgallii* represented monocotyledonous species, while kale and *A. caudatus* represented dicotyledonous species.

The seeds were sown in a cabinet using a mixed medium of compost and perlite (1:1 v/v) until germination and then transferred to 15 cm (D) pots in the glasshouse. The pots were placed on benches with additional light (high pressure sodium vapour 400 W, Thorn, UK) upon the top of each bench to provide the minimum light intensity of 150 μmol $m^{-2}s^{-1}$ at the pot level (the maximum was 750 μmol $m^{-2}s^{-1}$ near the light) and to increase day length during winter season. The day length was set to 14 hours per day from 06:00 to 20:00. The minimum temperature was approximately 10ºC at night and maximum temperature was 25ºC during the summer on a sunny midday.

Each species was divided into two groups, one droughted and the other the well-watered control. Plants were watered daily and fertilized by Hoagland solution twice a week; on the first occasion the fertilizer was given at half strength followed by the full strength solution for all the remaining times. Three weeks after planting, the drought was given by withholding water until the plants severely wilted, whereas the control plants were watered daily.

Water status measurement

Plant water status was analyzed by measuring medium water content, leaf relative water content, leaf water potential and leaf osmotic potential at two or three day intervals during the drought period and two days after rewatering. Medium water content was measured on a fresh weight basis by drying the medium samples in the 80°C oven for three days. The samples were taken randomly from three locations at 3-10 cm depth with approximately 20-30 g per sample.

Leaf water potential was measured with pressure chamber (SKPM 1400, Skye Instruments Ltd, Powys, UK). The youngest fully expanded leaf was cut and loaded into the chamber and the pressure increased slowly until water and bubbles were observed on the cut xylem surface under a magnifying glass.

Osmotic potential was estimated by a thermocouple psychrometer, (Tru-psy SC10X, Decagon Devices, Inc., Pullman, Washington) kept in a near constant temperature regime. To provide sample sap, the leaves were excised and put inside a fiber-glass tube and immediumtely frozen by liquid nitrogen and kept in the -60°C freezer until required. To collect the sample sap, the frozen samples were firstly thawed at room temperature for 30 minutes. The samples were then put in a silicone rubber tube and hand pressed, and the sap was then collected in a small Eppendorf tube and immediumtely placed into a psychrometer cup. Before the samples were added, the cups were placed in the psychrometer chamber to reach a temperature equilibrium for 25-35 minutes. Then the samples were loaded into the cups and were equilibrated for 30 minute before taking the reading.

Fluorescence Measurement

To study the effect of drought on the efficiency of photosystem II, chlorophyll fluorescence from three samples of each species were measured with a modulated chlorophyll fluorimeter (PAM-2000, Heinz Walz GmbH, Germany). To determine the maximum quantum efficiency of PSII photochemistry (*Fv/Fm*), chlorophyll fluorescence was measured after 20-25 minutes of dark adaptation during the day. The steady state chlorophyll fluorescence was measured in saturated light with PAR of 650 μmol m^{-2} s^{-1} to determine F_o, Fm', F_v', F_τ and F_o' from which the quantum yield of PSII photochemistry, Fq'/Fm', the photochemical yield of open PSII centres, Fv'/Fm', and the photochemical quenching (*qP)* and non-photochemical fluorescence quenching coefficient (*qN)* could be calculated (Genty *et al*., 1989).

Results and Discussion

Plant water status during the drought and after re-watering

The drought treatment reduced medium water content (MWC) of all droughted plants to less than 25 %, while the MWC of control plants was maintained at approximately 55-70 % by daily watering (Figure 1). Wheat and *E. crusgallii* were wilted after nine days of drought, while *A. caudatus* and kale were wilted after six and eight days. All species had recovered well two days after rewatering except *E. crusgallii* where some leaves became necrotic and were damaged during the drought and consequently did not recover.

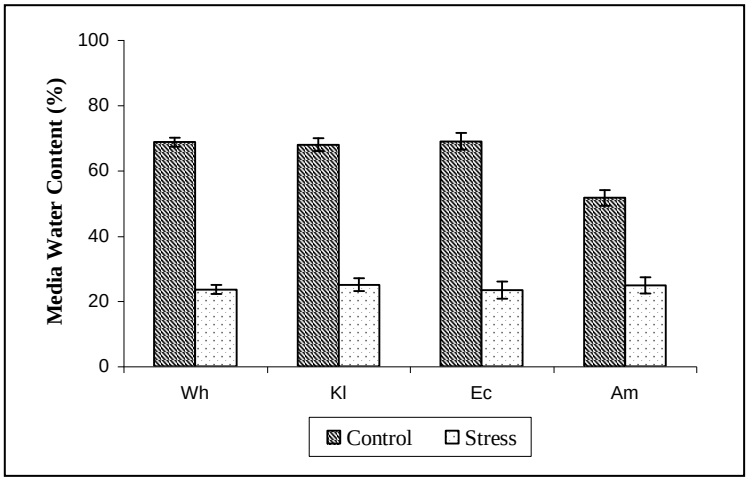

Figure 1. Medium water content (MWC) of wheat (Wh), kale (Kl), *E. crusgallii* (Ec) and *A. caudatus* (Am) under well watered (control) and at the last stage of drought cycle (stress)

The reduction of medium water content in the drought treatment caused a decrease in leaf water content, leaf water potential (WP) and osmotic potential (OP) of all species (Figures 2 and 3). Even though all species were wilted at the last stage of the drought cycle, wheat had the lowest OP and WP (-3.0 and -3.5 MPa) followed by kale and *A. caudatus* (-1.7 and -2.0 MPa), while *E. crusgallii* had the highest values (-1.2 and -1.4 MPa). The lower value of WP than of OP at the last stage of the drought cycle indicated that turgor pressure of all species at that time was zero, which resulted in plant wilting (Figures 2 and 3; Frensch, 1997).

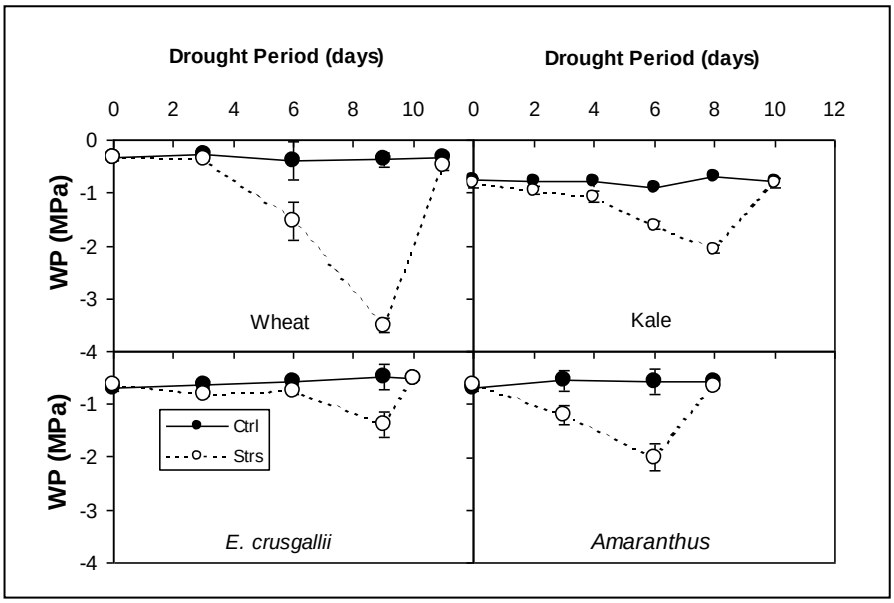

Figure 2. Water potential (WP) of wheat, kale, *E. crusgallii* and *A. caudatus* during drought stress and recovery. (The arrow indicates the time of rewatering)

Figures 2 and 3 indicate that the plants tend to maintain its turgor by reduce osmotic potential lower than water potential, because turgor is very important to sustain metabolic processes (Clifford *et al*., 1998). Some times, this process involves active accumulation of ionic compounds or organic compounds known as osmotic adjustment (Morgan, 1984; Kramer and Boyer, 1995; Zhang *et al*., 1999). However, under severe drought, when WP decreases to the value lower than OP, the plants will be wilted. In this experiment, at the last cycle of the drought treatment, the plants underwent stress due to severe drought indicated by leaf wilting started from the morning (Figures 2 and 3). Meanwhile, the plants was still alive and recovered well two days after re-watering.

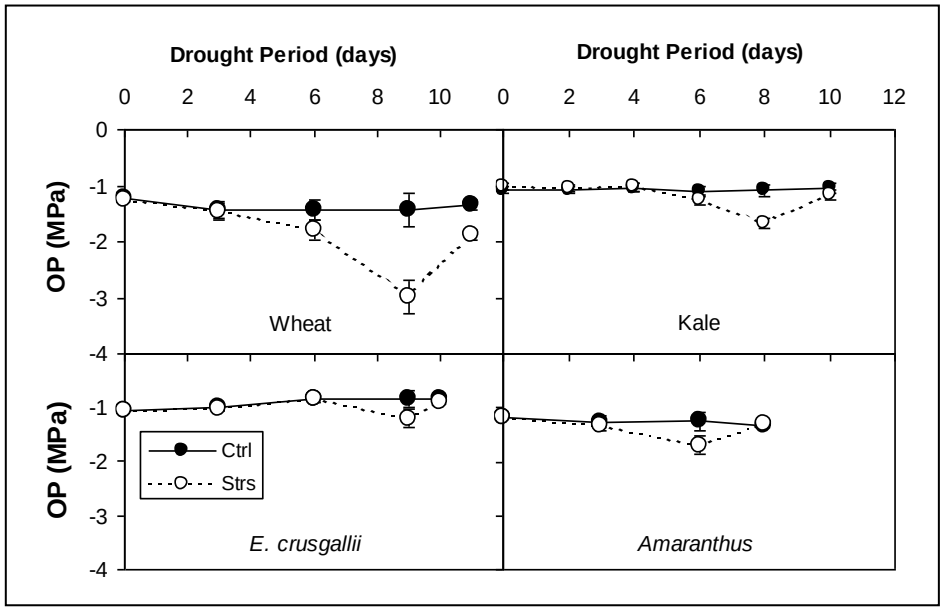

Figure 3. Osmotic potential (OP) of wheat, kale, *E. crusgallii* and *A. caudatus* during drought stress and recovery. (The arrow indicates the time of rewatering)

Even though there was no special pattern of C3 and C4 in term of water status during the drought, the monocot C4 species (*E. crusgallii*) tends to maintain higher WP and OP. This phenomenon may be related to the character of C4 species which have lower stomatal conductance than C3 species (Long, 1999). In addition, *E crusgalli* is a species adapted to condition with higher water (flooding) because the original habitat of this species is a wet and it may not tolerate a lower WP. This species is one of the major weeds in wet rice cultivation and has been considered a waterlogging tolerant species (McDonald *et al*., 2001).

Chlorophyll fluorescence measurements

An analysis of chlorophyll fluorescence was conducted to determine the maximum quantum yield of PSII photochemistry (*Fv/Fm*), photochemical fluorescence quenching (*qP*) and non-photochemical fluorescence quenching of photosynthesis (*qN*) (Genty *et al*., 1987; Figure 4, 5 and 6). This measurement was performed to analyse light utilization in photosystem II (PSII) of photosynthesis by comparing the light absorbed by chlorophyll, used for biochemical reaction, or re-emitted through heat dissipation (Ott *et al*., 1999). The *Fv/Fm* measured under dark adaptation be a sign of the potential quantum efficiency of PSII photosynthesis that indicates photosynthetic performance of the plant (Maxwell and Johnson, 2000). The *qP* is associated with the proportion of the light absorbed by PSII photosynthesis that is used in photochemical reaction, while *qN* is associated with the proportion of the light absorbed by

PSII that is dissipated as non-photochemical reaction usually as heat (Maxwell and Johnson, 2000; Baker, 1993).

The *Fv/Fm* of well watered plants after 30 minutes dark adaptation varied slightly between species, and was between 0.77 to 0.82 except in *A. caudatus* in which the *Fv/Fm* was approximately 0.70-0.75. Drought stress did not affect *Fv/Fm* of wheat, kale (C3) and *A. caudatus* (C4), but it reduced *Fv/Fm* of *E. crusgallii* significantly (Figure 4). The sustain of *Fv/Fm* of wheat, kale and *A. caudatus* in response to drought stress (Figure 4) suggesting that PSII fotosintesis is resistant to drought stress (Genty *et al.*, 1987; Cornic *et al*., 1989). In this experiment, decrease of *Fv/Fm* under severe drought stress in *E. crusgallii* indicates that metabolic limitation may reduce photosynthesis under severe drought in this species (Berkowitz, 1998) which results in photoinhibition. A different range of reduction of *Fv/Fm* under drought stress has also been reported in several species such as in *Leucaena leucocephala* which reduced only from 0.85 to 0.83 when the WP dropped to - 2.5 MPa (Liang *et al*., 1997) and in *Quercus ilex* (Scaracia-Mugnozza *et al*., 1996), in the C4 grass, *Eragrostis curvula* (Colom and Vazzana, 2003) or even in the CAM epiphytic orchid (Stancato *et al*., 2001) which reduced dramatically from approximately 0.7 to 0.4 respectively.

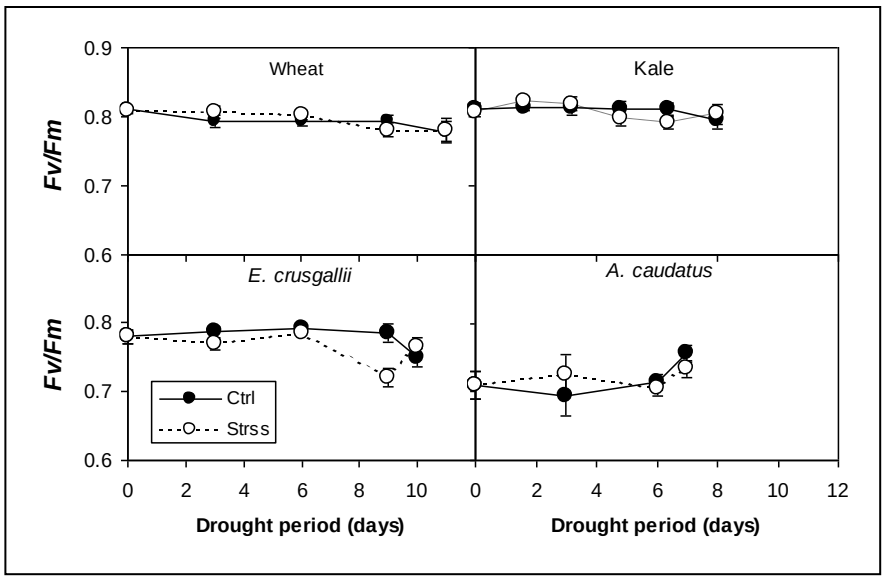

Figure 4. The maximum quantum yield of PS II photochemistry (*Fv/Fm*) of wheat, kale, *E. crusgallii* and *A. caudatus* during the drought and recovery (The arrow indicates the time of rewatering)

The photochemical quenching coefficient, *qP* reduced slightly during the experiment even in well watered plants (Figure 5). Drought stress reduced *qP* of all species with maximum reduction at the last period of drought stress, and *qP* completely recovered after rewatering (Figure 5). Interestingly, in C3 species the *qP* decreased progressively during the drought, while in C4 species (*E. crusgallii*) it only decreased at the last period of drought. This might be associated with the reduction of photosynthetic CO_2 assimilation rate, *Pn*, of the C3 species which is more susceptible to drought stress than that of C4 species (Knapp and Medina, 1999) so that under mild drought *Pn* of C3 species is decreased while that of C4 is still sustained (Long, 1999).

Decrease of *qP* during the drought also suggests that drought caused photosynthetic down regulation of all species. The reduced *Pn* of C3 species under mild drought (six days drought) caused the plants to experience excess of energy, and photosynthetic down regulation is the consequence of energy dissipation to protect the plant from photoinhibition (Baker, 1993).

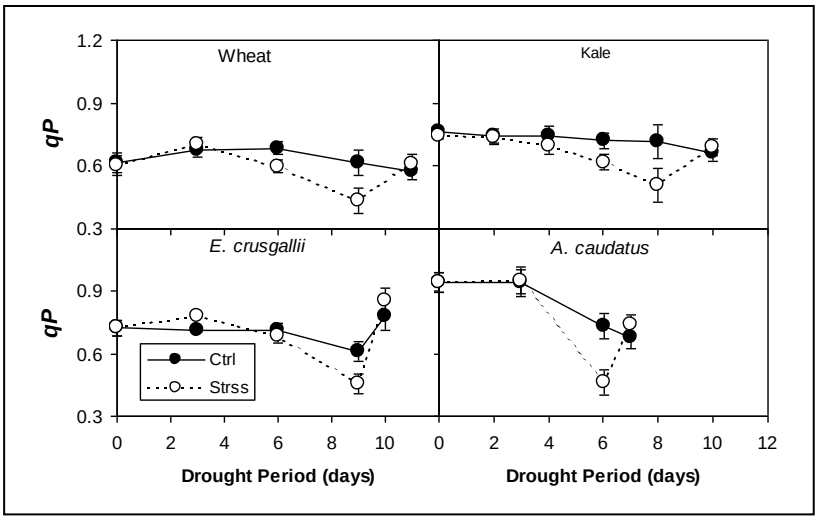

Figure 5. The photochemical fluorescence quenching coefficient (*qP*) of photosynthesis of wheat and kale, *E. crusgallii* and *A. caudatus* during drought stress and at recovery. (The arrow indicates the time of rewatering)

An increase of non-photochemical quenching fluorescence, *qN* is one of the protective mechanisms to dissipate excessive energy through thermal dissipation (Baker, 1993). However, in wheat and *E. crusgallii* there was no effect of stress on non-photochemical fluorescence quenching, *qN* but there was a slight increase for kale and *A. caudatus* (Figure 6). Two days after rewatering the *qN* of kale and *A. caudatus* had recovered to the control level (Figure 6). In this experiment, only kale and *A. caudatus* showed an increased *qN* (Figure 6) perhaps caused by the leaf shape and orientation causing excess light. Wheat may not suffer excess light due to orientation and leaf rolling, or dissipate its energy through other mechanisms, such as photorespiration. Photorespiration and the Mehler reaction have also been suggested as mechanisms for energy dissipation (Asada, 1999; Niyogi, 1999).

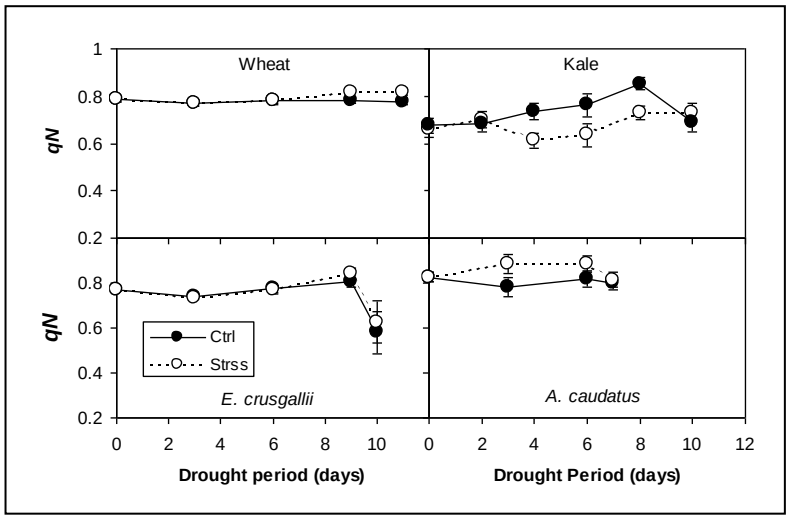

Figure 6. The non-photochemical fluorescence quenching (*qN*) of photo-synthesis of wheat and kale, *E. crusgallii* and *A. caudatus* during drought stress and at recovery. (The arrow indicates the time of rewatering)

In contrast, in *E. crusgallii*, the decrease of *Fv/Fm* (Figure 4) may indicate that it experienced photoinhibition under severe drought stress, because the reduction of *qP* due to drought stress occurred without any increase in the *qN* (Figure 6). It means that excess energy as a consequence of decreased photochemical quenching was not dissipated safely through thermal dissipation. *E. crusgallii* is a C4 species, which have a lower photorespiration, even under drought stress (Knapp and Medina, 1999). Because photorespiration is one of another alternative for excess energy dissipation especially in C3 species (Asada 1999; Niyogi 1999), the absent of photorespiration may have caused *E. crusgallii* undergo photoinhibition.

Conclusion

Drought decreased water potential (WP) and osmotic potential (OP) of all plant, with wheat had the lowest and *E. crusgallii* the highest. Drought stress caused photosynthetic down regulation in all species through decrease of photochemical (*qP*), but increase of non-photochemical fluorescence quenching coefficient (*qN*) was only observed in kale and *A. caudatus*. In *E. crusgallii*, severe drought stress caused a decrease in the maximum photochemical efficiency photosynthesis *Fv/Fm* suggesting that non-stomatal limitation of photosynthesis occurred in this species.

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