

## Chlorophyll a Fluorescence Imaging Analysis in Air-Drying Stressed Sorghum (*Sorghum bicolor* L.) Leaf

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Publication Date: 7 November 2014

Article Link: <http://scientific.cloud-journals.com/index.php/IJAAS/article/view/Sci-244>



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**Abstract** The objective of this study was to find a rapid determination of the air-drying stress of sorghum (*Sorghum bicolor* L.) leaves using portable chlorophyll fluorescence imaging instrument. To assess the photosynthetic activity of sorghum leaves, an imaging analysis of the photochemical responses of sorghum was performed with chlorophyll fluorescence camera. The observed chlorophyll imaging photos were numerically transformed to the photochemical parameters on the basis of chlorophyll a fluorescence. Chlorophyll a fluorescence imaging (CFI) method showed that the rapid decrease in  $F_m$  of leaf was occurred under air-drying stress. Although  $F_v/F_m$  ratios values in air-drying stressed sorghum leaves were not changed, all other photochemical parameters such as  $F_m$ ,  $F_v$ ,  $F_p$  and  $F_t$  were lowered after air-drying stress. In air-drying stressed sorghum leaves, it showed to increase in nonphotoquenching (NPQ) and decrease in  $\Phi_{PSII}$ . Thus,  $\Phi_{PSII}$  and NPQ were available to determine nondestructively the air-drying stress of sorghum leaves. In our study, it was clearly indicated that the air-drying could be a stress in sorghum leaves. The CFI analysis and its related parameters are applicable as a rapid indicating technique for the determination of air-drying stress.

**Keywords** *Sorghum*; *Air Drying Stress*; *Chlorophyll Fluorescence Imaging*; *Nonphotoquenching*;  $\Phi_{PSII}$

### 1. Introduction

Sorghum is not only an important cereal crop but also a biofuel crop. In Korea, sorghum cultivation is front to abiotic stresses such as drought at early growth stage, wet in middle growth stage and chilling stress at heading stage. The drought and air-drying stresses are most damaging to biomass production which is most actively progressed before rainy period so called Monsoon. Therefore, a breeding endeavor for selection of air-drying tolerant sorghum mutants or lines was further needed. In

climate condition of Korea, sorghum is cultivated during dry season at least over 2 months during young vegetative stage. Therefore, the efficient selection of drought tolerant sorghum mutants or lines was important in Korean Peninsula. Biomass production and survival consequence are closely related to photosynthetic activity under dry condition. It was recently reported that the sorghum brown midrib mutant has reduced lignin content in the cell walls and vascular tissues [1]. They could potentially be advantageous for cellulosic biofuel production.

In the context of air-drying stress, chlorophyll a fluorescence transient analysis, so-called JIP-test, and chlorophyll fluorescence imaging (CFI) technique may be able to apply to investigate the energetic behaviour of photosynthetic sensory systems. The JIP-test is a tool to analyse the polyphasic rise of the chlorophyll a (Chl a) fluorescence transients (phases labelled “OJIP”). Although it corresponds to only a very small fraction of the dissipated energy from the photosynthetic apparatus of fruit surface, Chl a fluorescence is widely accepted to provide a means to a better understanding of the structure and function of the photosynthetic apparatus. At room temperature, the Chl a fluorescence of plants, algae, and cyanobacteria, in the 680–740 nm spectral regions, is emitted mainly by photosystem (PS) II, and thus it can serve as an intrinsic probe of the fate of its excitation energy. The spectra and the kinetics of Chl a fluorescence are powerful and non-invasive tools for such investigations. The primary use of fluorescence has included the estimation of chlorophyll concentration and pigment–protein interactions and studies of the stability of thylakoid membranes. However, the relationship between chlorophyll and *in vivo* fluorescence varies widely over time and space. These processes include species changes, nutrient concentrations, and incident radiation [2].

Most studies analysing the effects of heat or chilling stress on OJIP transients have been conducted on plant leaves [3, 4] but not precisely in sorghum leaves. Even these studies have been limited to apple [5-7]. Photosynthetic activities differ between leaves and fruits; for example, in the pericarp of cherry tomato, photosynthetic fixation of  $^{14}\text{CO}_2$  has been shown to occur at higher rates than in the leaves [8].

The photosynthetic apparatus is the most sensitive component in evaluating the degree of temperature-related stress damage [9]. CFI technique has been mainly used as effective tools in order to study the damage and activity of the electron transport chain in the photosynthetic apparatus under various environmental stresses. CFI as a rapid and non-destructive technique has quickly progressed, and has been used successful in evaluating plant photosynthetic activity [10]. CFI incorporates advancements in the technology of light emission, imaging detectors, and rapid data handling [11]. This study was performed to evaluate the validity of CF technology to determine the degree of air-drying stress in sorghum leaves.

## 2. Materials and Methods

### 2.1. Growth Condition of Sorghum Seedling

Seeds of sorghum (*Sorghum bicolor* L.) mutants, M2P1064 bmr36, were obtained from USDA, Texas. Seeds were sown in pots mixed with basal fertilizer N-P-K (12-5-8 kg/10a). When the third leaves were occurred, the pots were transfer to growth chamber. The rapid air-drying was performed for 24 hours with 12 hours  $2,500 \mu\text{mol m}^{-2} \text{s}^{-1}$  illumination. All of the treatments were prior to exposure to air-drying condition. Sorghum seedlings were placed in a chamber and kept at room temperature. The growth chamber was set to 50 % relative humidity. All treatments were performed and each treatment was carried out in triplicate.

## 2.2. Measurement of Chlorophyll Fluorescence Imaging

The sorghum seedlings were measured separately for each treatment at 7 days after treatment (DAH) of the air-drying stress. A CFI FluorCam (Handy FluorCam FC 1000-H, PS I, Czech Republic) was used to measure the fluorescence images of the leaves.

The source of actinic light was orange LED at an intensity of  $200 \text{ m}^{-2}\text{s}^{-1}$ . The source of saturating light was a halogen lamp with an intensity of  $2,500 \text{ m}^{-2}\text{s}^{-1}$ . The fluorescence parameters maximum quantum efficiency of PS II ( $F_v/F_m$ ), PS II operating efficiency ( $\Phi_{\text{PS II}} = F'_q/F'_m$ ), and non-photochemical quenching (NPQ) were monitored by quenching kinetics analysis [12-14]. The data were calculated according to the parameters of the CFI FluorCam, which measured quenching kinetic. Light conditions were: actinic light, red LED,  $200 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ; saturating light, moderate light,  $1,250 \mu\text{mol m}^{-2} \text{ s}^{-1}$ .

## 2.3. Chlorophyll Fluorescence Parameters

Chlorophyll fluorescence parameters were defined as follows [15]:

$F_0$ : Minimal chlorophyll fluorescence intensity measured in the dark-adapted state, when all PS II RCs are open

$F_m$ : Maximal chlorophyll fluorescence intensity measured in the dark-adapted state during the application of a saturating pulse of light

$F_p$ : Maximum fluorescence value under saturating illumination when all reaction centers are closed or in reduced state

$F_v$ : Variable chlorophyll fluorescence ( $F_m - F_0$ ) measured in the dark-adapted state, when non photochemical processes are minimum

$F_t$ : Fluorescence at time  $t$  after onset of actinic illumination

$F_v/F_m$ : Maximum quantum yield

$\Phi_{\text{PSII}}$ : Effective quantum yield of photochemical energy conversion in PS II ( $F'_q/F'_m$ )

NPQ: Non-photochemical quenching

Rfd: Fluorescence decrease ratio

## 2.4. Data Analysis

The measured data were analyzed with the CFI software (FluorCam Software 7.0, <http://www.psi.cz/products/fluorcams/>). All statistical analyses were carried out in Microsoft Excel and SAS program (Version 9.02).

## 3. Results

### 3.1. Chlorophyll Fluorescence Imaging (CFI) Analysis

Sorghum seedlings were apparently similar. Thus, it was not ease to determine the damage on eyes before 18 hours after air-drying (Figure 1).

In air-drying stressed sorghum leaves, dark adapted fluorescence value (red color) at  $F_0$  was lower than in control at 24 hours after treatment (HAT).  $F_m$  value of 1,420 was about three fold lower in control than in air-drying stressed leaves of 560 (Figure 2).

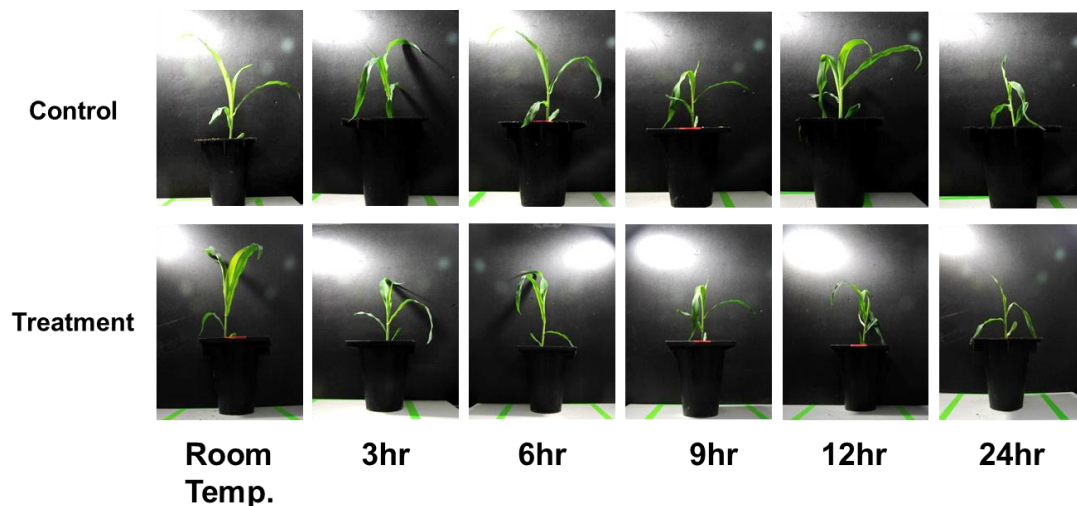
### 3.2. Maximum Quantum Yield ( $F_v/F_m$ ) and Other Parameters

The maximum quantum yield ( $F_v/F_m$ ) was 0.82 before air-drying stress. It was not changed until 24 HAT. Although the  $F_v/F_m$  ratios in air-drying stress sorghum leaves were almost normal,  $F_m$  and  $F_v$

values were far lower than in control seedling (Figure 3A, B). Also, maximum fluorescence value ( $F_p$ ) under saturating illumination when all reaction centers are closed or in reduced state was extremely lowered by air-drying stress.  $F_t$  values were considerably reduced already at 6 HAT. In air-drying stressed leaves, all measured fluorescence parameters were declined to over halves comparing control seedling.

### 3.3. Effective Quantum Yield of Photochemical Energy Conversion ( $\Phi_{PS II}$ )

In air-drying stressed sorghum leaves, the  $\Phi_{PS II}$  has decreased from 0.52 to 0.36 at 24 HAT, indicating that the effective quantum yield of photochemical conversion in PSII was inactivated (Figure 3B).



**Figure 1:** Sorghum Seedlings were grown in Growth Chamber Controlled with  $23\pm 2^\circ\text{C}$  Temperature (Control) and 40% Relative Humidity (Air-Drying)

### 3.4. Non-photochemical Quenching (NPQ)

In air-drying stressed leaves, the NPQ value has steeply increased from 0.33 to 0.70 at 6 HAT (Figure 3B). Finally, the value was enhanced up to 0.70 at 24 HAT.

### 3.5. Fluorescence Decrease Ratio (Rfd)

The Rfd value was considerably increased from 0.53 to 0.80 at 6 HAT, and then decreased to 0.48 at 12 HAT. At 24 HAT, the Rfd value was again increased to 0.73.

## 4. Discussion

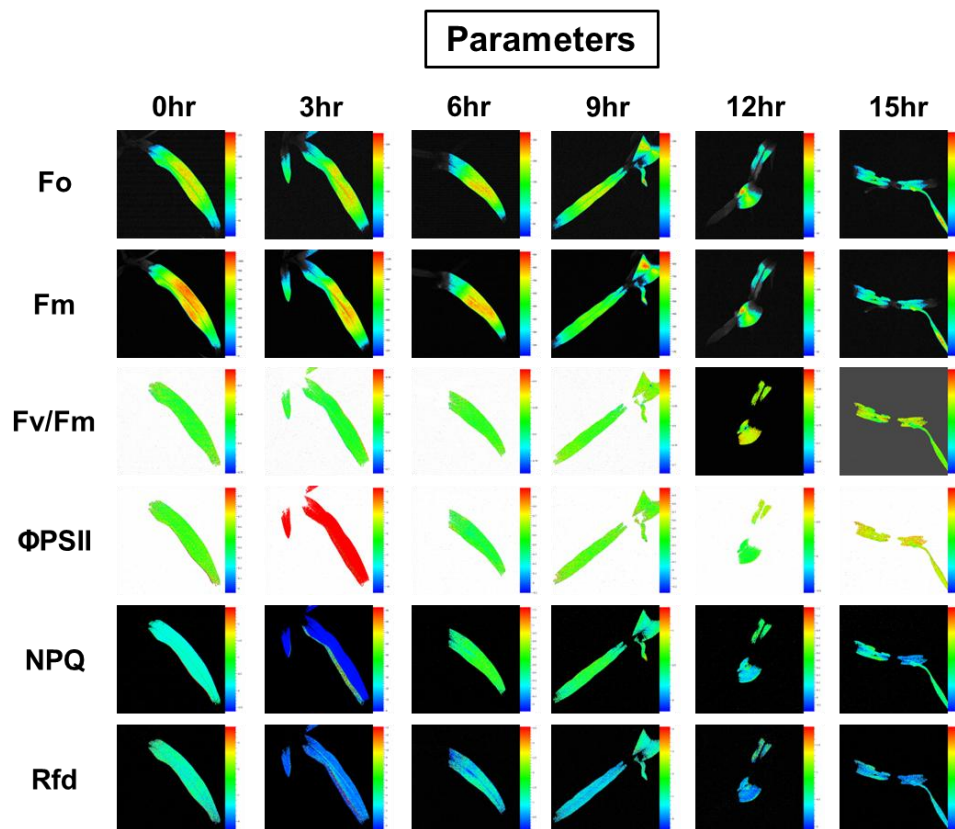
### 4.1. CFI Technique

CFI was initiated to investigate whether it can be used as a reliable indicator to evaluate the air-drying stress of sorghum seedlings in order to study the light stress-specific difference that may be involved in the photosynthetic apparatus. CFI has been applied for different purposes in the postharvest life of fruits [6, 10], but the main focus has been on detecting factors that can increase or decrease product quality [10]. Moreover, the technique has been used for various objectives both in pre- and postharvest conditions for the detection of biotic or abiotic stresses in plants and plant products.

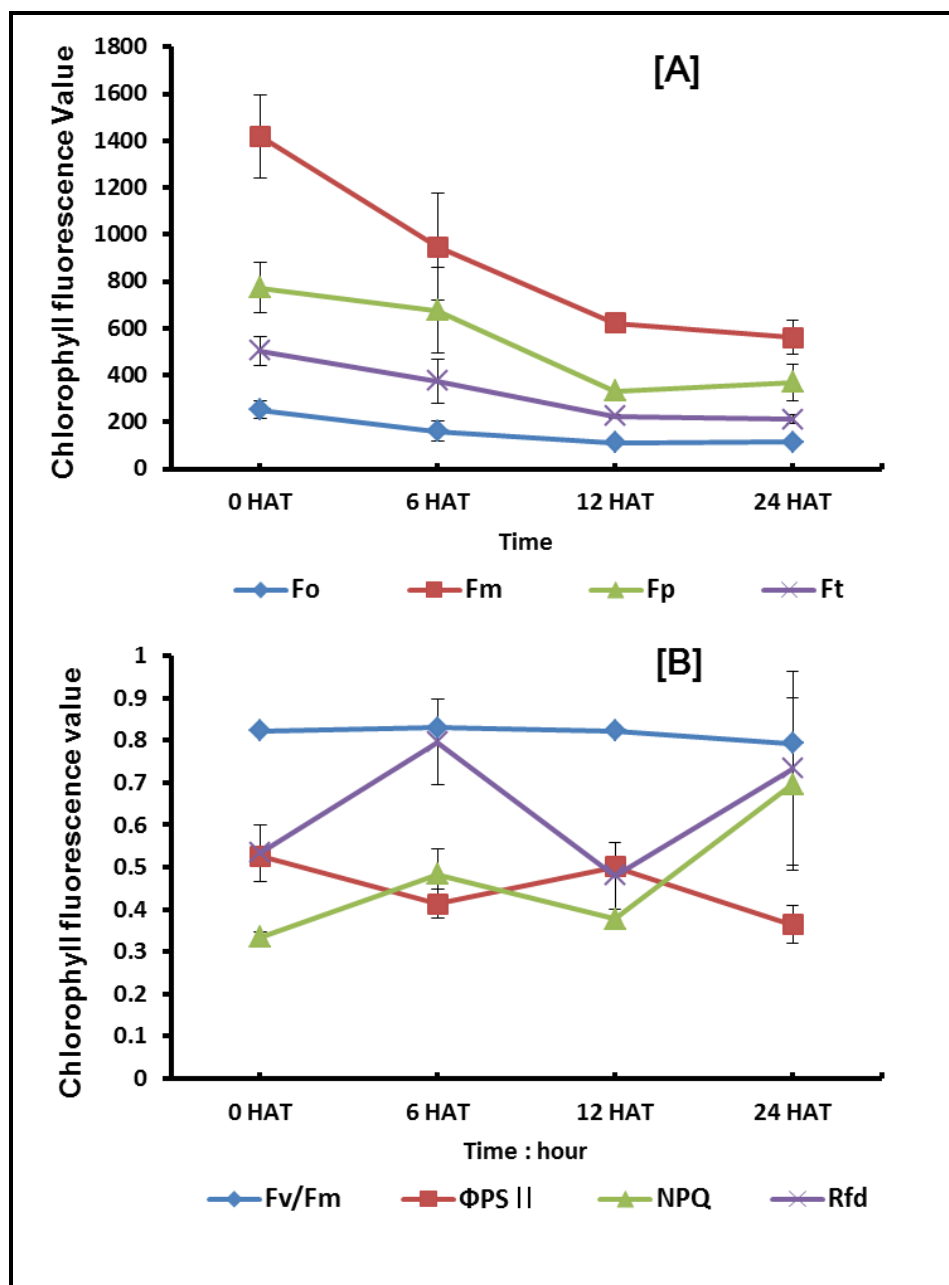
In comparison to the application of CFI for detection of abiotic stresses in different crops [6, 15], this study focused on the photochemical responses to air-drying stress condition. At least, CFI technique is applicable to determine the air-drying stress in numeric transformation of direct measured fluorescence values (Figure 2).

#### 4.2. $F_v/F_m$ Ratios

At 24 HAT,  $F_v/F_m$  value was not changed from 0.82 to 0.79 under air-drying stress. It may mean that the sorghum leaves are still photosynthetically active. Therefore, this photochemical parameter can be not good indicator for the determination of air-drying stress. In general, healthy plants have a very conservative  $F_v/F_m$  value of about 0.8 [16]. In air-drying stress condition,  $F_v/F_m$  has greatly retained compared to control. Björkman and Demmig [17] and Johnson et al. [18] also reported optimal values of  $F_v/F_m$  around 0.8 for most plant species, and values lower than this are observed in plants exposed to stress, indicating in particular the phenomenon of photoinhibition. However, it is still unclear whether air-drying stress is the case.



**Figure 2:** Changes in Chlorophyll Fluorescence Imaging ( $F_o$ ,  $F_m$ ,  $F_v/F_m$ ,  $\Phi PSII$ , NPQ, and Rfd) of Sorghum Seedlings Grown in Growth Chamber Controlled with Temperature ( $23 \pm 2^\circ C$ ) and 40 Relative Humidity for 24 Hours



**Figure 3:** Changes in Chlorophyll Fluorescence Imaging ( $F_o$ ,  $F_m$ ,  $F_v/F_m$ ,  $\Phi$ PSII, NPQ, and Rfd) of Sorghum Seedlings Grown in Growth Chamber Controlled with Temperature ( $23\pm 2^\circ\text{C}$ ) and 40 Relative Humidity for 24 Hours

#### 4.3. $\Phi$ PSII

In air-drying stressed sorghum leaves,  $\Phi$ PSII was gradually declined from 0.53 to 0.36 within 24 HAT (Figure 3). It has implied that they were photosynthetically inactive. The lower  $\Phi$ PSII values in air-drying stressed sorghum leaves were assumed to be low chlorophyll a fluorescence in steady state, i.e. under continuous light pulse. In general, the chlorophyll fluorescence near at  $25^\circ\text{C}$  is most sensitive as the temperature in the growth chamber used in this study [16, 19, 20]. However, it was thought that this lower effective quantum yield of photochemical energy conversion in PS II was related to lower electron transport activity to  $Q_A$  resulting in severe stress on electron-transport from PSII to PSI.



#### 4.4. NPQ

The NPQ values in air-drying stressed leaves have gradually increased from 0.33 to 0.70 at 24 HAT. In general, the increase in NPQ means inefficiency and energy dissipation in photosynthetic apparatus [14, 18, 21]. It may imply that the NPQ parameter is most sensitive to air-drying stress. Because the higher value of NPQ indicates low possibility of photosynthetic electron transport resulting in an increase in inactive chlorophyll of sorghum leaves [13, 21]. Thus, these NPQ values of sorghum leaves can be an available indicator parameter.

#### 4.5. Rfd

In earlier report [22], the decrease of fluorescence decrease ratio (Rfd) in tomatoes under the sprinkler irrigation could be explained as photoinhibitory effect on the Calvin cycle by changes in the environmental conditions such as temperature, oxygen, light and physiological status of plants. There is no report until now on Rfd value under air-drying stress. Although the increase in Rfd seemed to be air-drying stress as shown in Figure 3B, the stressed sorghum leaves did not accompany with the increase of  $\Phi$ PSII. Thus, it could be assumed that the stressed leaves were not recovered to normal status. When this parameter uses as a stress indicator, therefore, it should be carefully applied.

#### 4.6. Comparison among Photochemical Parameters

The air-drying condition seemed to cause severe damage to the photosynthetic apparatus, resulting in changes in photosynthetic activity of sorghum leaves. The measurement of photochemical responses may be able to determine the photosynthetic stress of sorghum leaves under air-drying.

This study has shown that CFI can be used as a reliable tool to evaluate the healthy condition of sorghum leaves.

In sorghum leaves exposed to continuous air-drying stress condition for night, NPQ values increased gradually until 24 HAT. NPQ values in steady state have nonphotoquenching characteristics in dark-adapted state [16]. Although changes in NPQ are nonlinearly related to higher values than  $\Phi$ PSII in leaves as earlier suggestion [16, 23], the  $\Phi$ PSII seemed to be applicable to determine the stress under air-drying condition.

The air-drying stress causes severe damage to the photosynthetic apparatus, resulting in changes in appearing viability of leaves. This study has clearly shown that CFI can be used as a reliable tool to evaluate the air-drying stress of sorghum leaves.

### 5. Conclusion

The fluorescence imaging and the numeric data of  $F_v/F_m$ ,  $\Phi$ PSII, and NPQ showed that different responses occurred under air-drying stress condition. This practical study of the CFI technique has shown that the numeric values in  $F_v/F_m$ ,  $\Phi$ PSII, and NPQ could be applied to determine the light stress. Especially,  $F_m$  and  $\Phi$ PSII values were declined under air-drying condition.  $F_v/F_m$  values retained almost 0.8. On the basis of the results of this study, the photochemical numeric parameters obtained from CFI were useful for an evaluation of air-drying stressed sorghum leaves. The numeric data derived from CFI analysis can be a rapid method for air-drying stress determination.

### Acknowledgements

This work was supported by a grant from the Next-Generation BioGreen 21 program (No. PJ009101), Rural Development Administration, Republic of Korea.

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