PHYSIOLOGYCAL RESPONSES OF RICE SEEDLINGS UNDER DROUGHT STRESS

Phùng Thị Thu Hà

Faculty of Agronomy, Vietnam National University of Agriculture

Email: phungthithuha.pth@gmail.com

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TÓM TẮT

Lúa là một trong những cây lương thực quan trọng bậc nhất trên thế giới. Trong nghiên cứu này chúng tôi nghiên cứu phản ứng sinh lý của giống lúa *Oryza sativa* cv. Dongjin trong điều kiện khô hạn. Kết quả nghiên cứu cho thấy, cây lúa 3 tuần tuổi bắt đầu biểu hiện kiểu hình lá cuộn lại và khô sau 48h dừng tưới nước. Cùng với sự mất nước trong lá và giảm thế hóa nước trong thân, các gốc tự do như H_2O_2 và malondialdehyde cũng được tích lũy. Điều đó dẫn tới hiệu suất quang hợp giảm mặc dù hàm lượng chlorophyll giảm không đáng kể.

Từ khóa: Các giống lúa, Chlorophyll, enzym chống oxi hóa, MDA, stress khô hạn.

Phản ứng sinh lý của cây lúa trong điều kiện khô hạn

ABSTRACT

Rice is one of the most important crop plants over the world. Our work studied on the physiological responses of rice seedlings (*Oryza sativa* cv. Dongjin) under drought condition by withholding water. In response to drought treatment, three-week-old rice seedlings exhibited typically morphological responses as leafs rolling and drying at 48 h after exposure to water deficit. Together with the loss of water relation (relative water content and shoot water potential), reactive oxygen species (H_2O_2 and malondialdehyde level) accumulated in the leaves of rice seedlings which led to apparent reduction in photosynthetic efficiency but only slight reduction of chlorophyll content.

Keywords: Antioxidant enzyme, chlorophyll, drought stress, MDA (malondialdehyde), rice Seedlings.

1. INTRODUCTION

Rice is the most important cereal for more than three billion people, over half of the world's population. Approximately, 45 percent of the rice area in Southeast Asia is irrigated (Mutert and Fairhurst, 2002), the rest is vulnerable to drought condition.

Drought is one of the most serious abiotic stresses in plants. It is the major factor that limits crop productivity and subsequently determines the natural distribution of plant species (Saxe et al., 2001; Mpelasoka et al., 2008). Drought stress is characterized, among others, by the reduction of water content, closure of stomata and limitation of gas exchange. Much more extensive loss of water can lead to accumulation of reactive oxygen species (ROS), which disrupts metabolism and cell structure and eventually the enzyme-catalyzed reactions, and finally may result in the death of plant (Jaleel et al., 2008; Phung et al., 2011).

Our study was designed to determine the effect of drought stress on some physiological responses such as water relation, ROS accumulation, fluorescence parameters, and chlorophyll content in rice seedlings (*Oryza sativa* cv. Dongjin).

2. MATERIALS AND METHODS

2.1. Materials

The rice cultivar used in this study was *Oryza sativa* cv. Dongjin (a Korean rice cultivar). The seedlings were grown in growth chamber maintained at day/night temperature of 28°C/25°C under a 14-h-light/10-h-dark cycle (7:00 AM-9:00 PM) with a 200 mmol m^{-2} s^{-1} photosynthetic photon flux density. The humidity and air temperature of the growth chamber were maintained constant during experiments.

2.2. Methods

2.2.1. Drought treatment

Three-week-old rice seedlings were exposed to drought by withholding water for 60 h, and the youngest, fully expanded leaf tissues were sampled at 36 h (9:00 AM), 48 h (9:00 PM), 60 h (9:00 AM) after drought treatment. Control plants with sufficient water supply were harvested at the same time as the drought-treated plants.

2.2.2. Relative water content (RWC)

RWC was determined gravimetrically. The fresh weight, rehydrated weight and dry weight were measured from the same sample and RWC was calculated as follows: RWC (%) = [(Fresh weight - Dry weight)/(Rehydrated weight - Dry weight)] × 100 (Phung et al., 2011).

2.2.3. Shoot water potential Ψ_W

Shoot water potential was evaluated immediately as the plant stem xylem-pressure potential by using a pressure chamber (PMS Instrument Co., Corvallis, OR, USA).

2.2.4. In vivo detection of H_2O_2 in plant

H_2O_2 was visually detected in the leaves of plants by using 3,3-diaminobenzidine as the substrate (DAB) (Thordal-Christensen et al., 1997).

2.2.5. Lipid peroxidation

Lipid peroxidation was estimated by the level of MDA production using a slight modification of the thiobarbituric acid method described by Buege and Aust (1978).

2.2.6. Chlorophyll content

Chlorophyll concentration was extracted and measured spectrophotometrically following the method of Lichtenthaler (1987).

2.2.7. Photosynthesis activity measurement

Fluorescence parameters variable (Fv = Fm - Fo), minimal (Fo) and maximal (Fm) of rice leaves were measured using chlorophyll fluorometer (Handy PEA chlorophyll fluorometer, Handsatech instrument, England) according to the manufacturer's instruction.

Microsoft Excel was used for data analysis. The data represents the mean ± S.E. of three replicates.

3. RESULTS AND DISCUSSION

3.1. Dehydration symptom in response to drought stress

Three-week-old rice seedlings were drought treated by withholding irrigation for 60 h. The dehydration symptoms were observed and pictures were taken at 0 h, 36 h, 48 h and 60h after non-watering. Rice seedlings started to exhibit dehydration symptom at 48 h after withholding water as leaf rolling. At 60 h after drought treatment, the dehydration symptom was more severe, the leaves lost more water (Fig. 1).

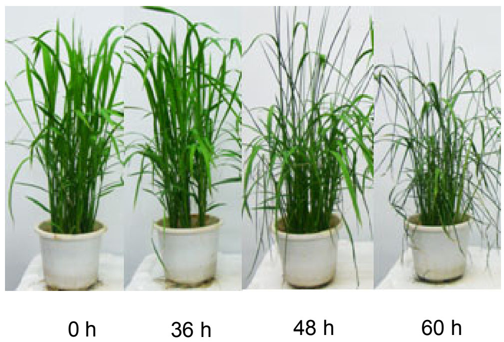

Figure 1. Phenotypes of rice seedlings before and after water withholding for 36h, 48h and 60h

3.2. Effect on water relation

Relative water content (RWC) in the leaves and water potential in the shoots of rice seedlings were determined during drought treatment. The results showed that RWC (Fig. 2A) and water potential (Fig. 2B) decreased in response to drought condition with a greater at 60 h as compared to at 48 h after treatment. Those data correlated well with dehydration symptom of rice seedlings during non-watering time (Fig. 1).

Our results are in accordance with previous studies, water relation decreased in all plant species in response to drought condition such as Wheat (Siddique et al., 2000), *Hibiscus rosa-sinensis* (Egilla et al., 2005), Bentgrass species (Dacosta and Huang, 2007), *Oryza sativa* L. (Faroog et al., 2009), *Plantago ovata* and *Plantago psyllium (*Rahimi et al., 2010) and chickpea (Rahbarian et al., 2011)...

3.3. Effect on oxidative metabolism

Drought tress usually leads to accumulation of reactive oxygen species (ROS) due to stomatal closure. Excessive ROS production can cause oxidative stress, which damages plants by oxidizing photosynthetic pigments, membrane lipids, proteins and nucleic acids (Cruze de Carvalho, 2008; Phung et al., 2011). H_2O_2 is one kind of ROS expressing

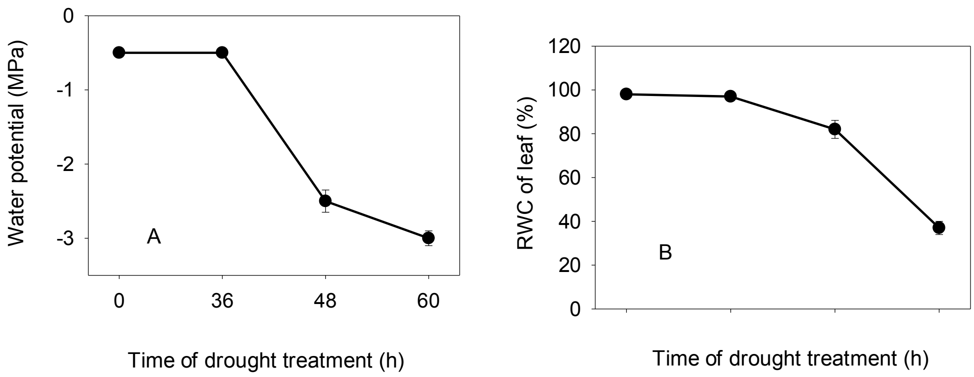

Figure 2. Effect of drought stress on (A) relative water content and (B) water potential in rice seedlings. The data represents the mean ± S.E. of three replicates

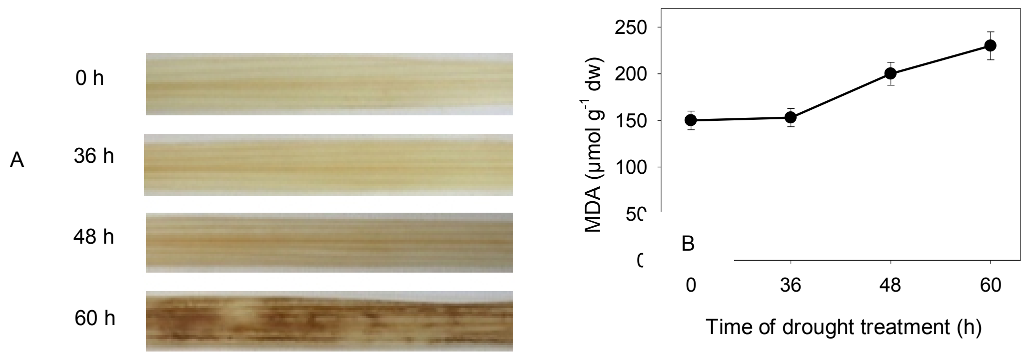

Figure 3. Effect of drought stress on oxidative metabolism in rice seedling leaves, (A) *in vivo* H_2O_2 production, (B) malondialdehyde (MDA) production. Each data point is the mean ± S.E. of three replicates

by brown color after DAB staining. In our study, the presence of H_2O_2 in the leaves of rice seedlings at 48 and 60 h indicated the presence of ROS as a result from drought causing oxidative stress. The magnitude of brown color increased at 60 h as compared to at 48 h after drought treatment (Fig. 3A). This data correlated well with dehydration symptoms (Fig. 1), relative water content and water potential data (Fig. 2).

Malondialdehyde (MDA) production is an index of peroxidation of unsaturated membrane lipids. The formation of MDA radical also indicates the present of ROS in drought-treated leaves. MDA level increased in the leaves of rice seedlings at 48 h after drought condition and continuously increased at 60 h after non-watering when plants exhibited severe dehydration symptoms (Fig. 3B). The level of MDA correlated well with brown color after DAB staining in drought-treated leaves of rice seedlings (Fig. 3). Together, they indicated the damage of membrane lipid in leaves of rice seedlings after drought treatment.

The present of excess ROS can oxidize multiple cellular components like proteins and lipids which will ultimately cause cell death and it also acts as secondary messenger that triggers acclamatory/defense responses by specific signal transduction pathways (Cruze de Carvalho, 2008). In responding to drought stress, H_2O_2 and MDA content also increased in the leaves of *Oryza sativa* L. (Faroog et al., 2009); MDA level increased in root and shoot of two Canola (*Brassica napus* L.) cultivars (Mirzaee et al., 2013) and in the leaves of Bentgrass species (Dacosta and Huang, 2007).

3.4. Effect on Chlorophyll content and Photosynthesis efficiency

Chlorophyll is one of the important pigments of photosynthetic apparatus which absorb light and transfer light energy to the reaction center of the photosystem. In our experiment, chlorophyll content in drought-treated leaves was not significantly altered in response to drought treatment (Fig. 4A.). Chlorophyll was produced from tetrapyrrole pathway. Together with chlorophyll intermediates, they are photosensitive molecules; their excess amount will lead to photo-oxidative damage in plant cells (Tanaka and Tanaka, 2007). The slow reduction of chlorophyll content in response to drought stress may contribute to severe drought symptom in rice seedlings.

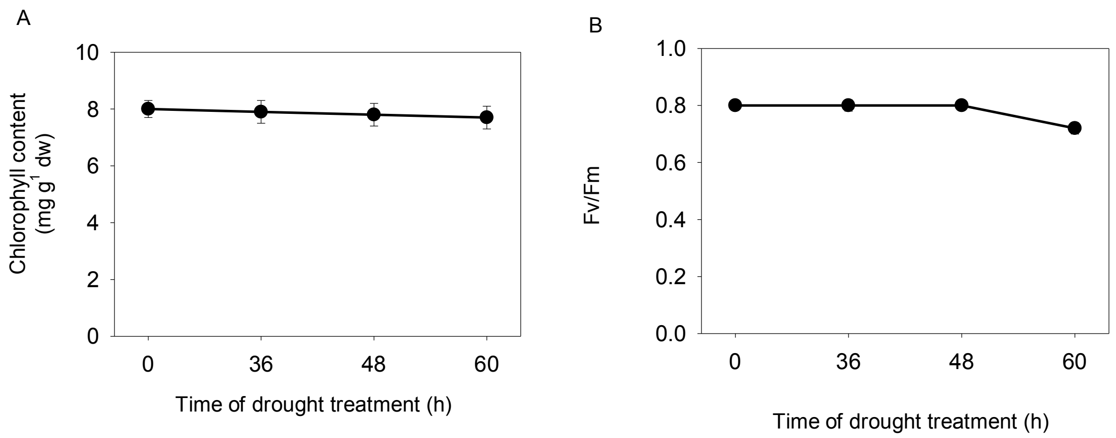

Fig. 4. Effect of drought stress on chlorophyll content and photosynthetic efficiency, (A) Chlorophyll content, (B) Photosynthesis efficiency, the maximum potential quantum efficiency of PS II (Fv/Fm) was determined in rice seedlings before and after withholding. Each data point is the mean ± S.E. of three replicates

A measurement of chlorophyll fluorescence parameters provides useful information about stress-induced perturbations in the photosynthetic apparatus (Shangguan et al., 2000). After withholding water, the maximum potential quantum efficiency of PS II slightly decreased until 48 h but greatly reduced at 60 h when the leaves of rice seedlings exhibited severe drought stress symptom (Fig. 4B). It indicated that the present of ROS caused damage to photosystem.

Responses of plants to drought depend on plant species, stage of plant development, and the duration and intensity of drought stress. Schelmmer et al. (2005) reported that drought stress had no significant effect on chlorophyll content of maize leaves. Other recent publications reported that chlorophyll reduced in *Plantago ovata* and *Plantago psyllium* (Rahimi et al., 2010) and photosynthetic efficiency decreased in Bentgrass species (Dacosta and Huang, 2007) and Chickpea (Rahbarian et al., 2011) under drought condition.

4. CONCLUSIONS

In conclusion, rice seedlings (*Oryza sativa* cv. Dongji) exhibited dehydration symptom at 48 h after drought treatment by withholding irrigation and the symptoms were more and more severe after 60 h. The magnitude of dehydration symptoms correlated with the loss of water content in the leaves, the reduction of water potential in the shoot of rice seedlings, the increase of ROS accumulation in the leaves of rice seedlings, and the decrease of chlorophyll content and photosynthetic efficiency. The findings provide a view on physiological responses of rice seedlings under drought condition. They also contribute to ongoing studies on molecular responses of rice seedlings under drought stress.

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