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Full Length Article

Photosystem II Function Response to Drought Stress in Leaves of Two Alfalfa (*Medicago sativa*) Varieties

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Abstract

The two main alfalfa varieties cultivated in northern China, *Medicago sativa* CV. Zhaodong and *Medicago sativa* CV. WL353HQ, were used to study the photosynthetic responses to drought stress. Changes in the content of chlorophyll and water in leaves during drought stress were studied using potted plants with natural drought treatment, and the effects of drought stress on the photosystem II(PSII) functions of leaves were also studied using a rapid chlorophyll fluorescence kinetics technique. Under drought stress, the chlorophyll and water content of WL353HQ leaves, although reduced, were significantly greater than those of Zhaodong leaves. On day 4 of drought stress, with the relative water content of soil at 56.3%, the maximum photochemical efficiency (F_v/F_m) of PSII and the photosynthetic performance index (PI_{ABS}) based on light energy absorption of the leaves started to decrease, and PI_{ABS} was more sensitive than F_v/F_m. The standardized OJIP curves of the two types of leaves showed that under drought stress, the values of the relative variable fluorescence of point J at 2 ms(V_J), were significantly increased. The relative variable fluorescence of point K at 0.3 ms(V_K), and point I at 30 ms(V_I), incurred only small changes, which indicated that the main reason for the drought-induced decrease of PSII photochemical activity was related to the obstruction of electron transfer from Q_A to Q_B on the acceptor side of PSII. After 10 days of drought treatment, with a relative soil water content of 29.9%, the relative variable fluorescence of point L at 0.15 ms, V_L, showed no significant changes throughout the drought process, and there was no significant difference between the two varieties, which indicated that there was no dissociation of thylakoid membranes in the leaves. However, the light energy absorption per unit reaction center (*ABS/RC*) of the two leaf types were significantly increased, thus indicating that the number of reaction centers in the leaves were greatly reduced. Drought stress also incurred a decrease in the proportion of absorbed light energy for electron transfer, while the proportion of energy dissipation was increased in both leaf types, which was an important reason for the decrease of photochemical activity in the leaves. The degree of PSII function in Zhaodong alfalfa leaves under drought stress was significantly less inhibited than those of WL353HQ, therefore, Zhaodong alfalfa should be considered an important alfalfa variety in arid regions of China. © 2018 Friends Science Publishers

Keywords: Alfalfa; Drought stress; Chlorophyll fluorescence; Photosystem II; Photoinhibition

Introduction

Because of global warming, drought is expected to occur more frequently and the global drought risk will likely be further increased (Gosling and Arnell, 2016). Drought will lead to serious reductions in grain crops and the deterioration of the ecosystems, therefore, how to deal with the adverse effects of drought has become an urgent problem. In recent years, scholars have increasingly scrutinized the physiology of drought resistance of various plants (Nichols *et al.*, 2015). Plant growth and development depend mainly on its photosynthetic physiological process; while soil water content directly affects the photosynthetic physiological processes of plants, but the process and mechanisms are very complex (Xia *et al.*, 2017). The study of the influence of soil water content on the plant photosynthetic physiological process is the basis of study on the mechanism of plant physiological changes. Photosynthetic physiological processes can be described by parameters such as photosynthesis and fluorescence (Chen *et al.*, 2012). Studies have been conducted to determine the physiological process, and the physiological status of plants has been assessed by net photosynthetic rate, water use efficiency, and PSII

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photochemical efficiency, among other indicators (Chen *et al.,* 2015; Gameiro *et al.,* 2016). Therefore, the study of the dynamic response process of plant photosynthetic efficiency under drought conditions based on the photosynthetic physiology process is critical for effectively screening drought-resistant species and improving plant drought resistance.

As a photoreaction center in plant photosynthetic apparatus, photosystem II (PSII) consists of more than 20 core protein subunits, including the chlorophyll a/b light-harvesting antenna complex (LHC), oxygen-evolving complex (OEC), and D_1 protein. PSII is mainly responsible for a series of photosynthetic physiological processes, such as light energy absorption, water photolysis, and electron transfer (Allahverdiyeva *et al.,* 2013; Chen *et al.,* 2017). Under stressful conditions, electron transfer in PSII photosynthesis is often inhibited, which leads to photoinhibition, even photooxidation and photobleaching (Sharma *et al.,* 2015). Drought is one of the important factors limiting plant growth and crop yield, drought often leads to the decrease of photosynthetic carbon assimilation capacity of plants, and even the photoinhibition of plant PSII and photosystem I (PSI). (Tariq *et al.,* 2017.), under drought stress, the maximum photochemical efficiency of the PSII (F_v/F_m) and oxidoreductive activity ($\triangle I/I_0$) decreased in leaves of sweet sorghum (*Sorghum bicolor* L. Moench) (Guo *et al.,* 2018), overreduction of the photosynthetic electron transport chain and overproduction of reactive oxygen (ROS) under drought stress (Ozkur *et al.,* 2009). Chlorophyll fluorescence kinetics is a nondestructive probe for studying the physiological status of the photosynthetic apparatus in plants, including the study of energy absorption, electron transfer, and light energy utilization in plants under stress (Müller *et al.,* 2001; Zhang *et al.,* 2016).

Alfalfa (*Medicago sativa*) is a perennial leguminous plant because of its beneficial characteristics, such as high yield, good quality, tolerance to frequent cutting, and good durability; it is known as "the king of forages", and it has the longest history of cultivation and the largest planting area in the world of the leguminous forages. Alfalfa has strong resistance to drought, salinity, and low temperatures, and it is one of the main leguminous grass species for forage planting and for projects designed to return farmland to forest and grassland. Therefore, alfalfa plays an important role in animal husbandry production and ecological management (Zhang *et al.,* 2017a). However, alfalfa has a strong straight root system with high water demands, and drought stress often leads to declines in alfalfa yield and quality, and can even lead to the serious degeneration of grasslands (Hund *et al.,* 2009; Li *et al.,* 2010). Therefore, it is important to understand the photosynthetic physiological processes of different alfalfa varieties under drought stress, and to study the adaptation mechanisms of the photosynthetic apparatus to water for different alfalfa varieties. These studies could provide a theoretical basis for the rational planting and popularization of alfalfa in arid regions.

Materials and Methods

Experimental Materials and Treatments

The experiment was conducted from March to June in 2016 at the Soil Laboratory of Northeastern Agricultural University (Harbin, Heilongjiang, Peoples R China). The alfalfa varieties used, including *Medicago sativa* CV. Zhaodong and *Medicago sativa* CV. WL353HQ, are the most prominent varieties in northern China. The Crops Research Institute of the Heilongjiang Academy of Land Reclamation Sciences provided the seeds.

Matured and plump seeds with uniform sizes were selected and cultured pots 12 cm in diameter and 15 cm in height; pots had a hole in the bottom for excessive water draining. Fifteen seeds were planted in each pot and then covered by 2 cm of soil. The planting substrate was peat soil, which is highly permeable. The pots were placed in an artificial climate culture box with temperatures of 25/23^{o}C (light/dark), light intensity of 200 μmol·m^{-2}·s^{-1}, light cycle of 12/12 h (light/dark), and around 75% relative humidity. Watering and seedling management were conducted regularly. When the seedlings were about 5 cm tall, each pot was thinned to only 10 seedlings of relatively consistent size and with reasonable spacing. Watering ceased after 30 days of growth, and the natural drought treatment began. The OJIP curves of the two alfalfa leaf types were measured on days 0, 2, 4, 6, 8 and 10 after initiation of the drought treatment. The water contents of the substrate mass during the drought period were 29.6%, 25.3%, 19.3%, 14.1%, 10.8% and 10.1%, respectively, and the relative water contents (*RWC*) were 86.5%, 73.9%, 56.3%, 41.1%, 38.5%, and 29.9%, respectively. The substrate field moisture capacity, as determined by the ring shear testing method, was 28.5 ± 1%, and the bulk density was 1.25 ± 0.12 g·cm^{-3}.

Subjects and Methods of Measurement

The chlorophyll contents, water content rates, and chlorophyll fluorescence parameters in the leaves of the two alfalfa varieties were measured on day 0 (CK), 2, 4, 6, 8 and 10 after initiation of the drought treatment. Chlorophyll contents were measured using the CCM-200 chlorophyll analyzer (OPTI-SCIENCES, USA), and the SPAD value (arbitrary units) was the relative chlorophyll content; the relative water content of the leaves were weighed and the leaf relative water content was calculated as follows: leaf relative water content = (fresh weight - dry weight)/fresh weight × 100%.

The chlorophyll fluorescence fast-transient analyses (OJIP) were conducted as follows: dark adaption lasted for 0.5 h on the fully opened, functional leaves on days 0, 2, 4, 6, 8 and 10 after initiation of the drought treatment.

The OJIP curves of the leaves were then measured by a fluorometer (FluorPen FP 100 Max, Czech). The OJIP curves were induced by pulsed red light at 3000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the fluorescence signal recording started at 10 μs and ended at 1 s. Each measurement was repeated five times, and the average fluorescence intensity of the five OJIP curve measurements was used to draw the OJIP curve. To determine the relative variable fluorescence V_J, V_I, V_K and V_L at the four characteristic points, J, I, K and L, respectively, the standardization of O-P, O-J and O-K curves on the OJIP curve were conducted (Zhang *et al.*, 2012), of which, points O, L, K, J, I and P corresponded to the times of 0, 0.15, 0.3, 2, 30 and 100 ms, respectively, on the OJIP curve. The relative variable fluorescence of each point on the standardized O-P curve was $V_{O\text{-}P} = (F_t\text{-}F_o)/(F_m\text{-}F_o)$; the relative variable fluorescence of each point on the standardized O-J curve was $V_{O\text{-}J} = (F_t\text{-}F_o)/(F_J\text{-}F_o)$; and the relative variable fluorescence of each point on the standardized O-K curve was $V_{O\text{-}K} = (F_t\text{-}F_o)/(F_K\text{-}F_o)$. F_t represents the fluorescence intensity at each time point, and F_O, F_K, F_J and F_m represent the relative fluorescence intensity at points O, K, J and P, respectively. The differences of the standardized curves, O-P, O-J, O-K, of the leaves of the two alfalfa varieties at different drought days compared to day 0 were calculated as follows: $\triangle V_{O\text{-}P} = V_{O\text{-}P\ (stress)} - V_{O\text{-}P\ (0\ d)}$; $\triangle V_{O\text{-}J} = V_{O\text{-}J\ (stress)} - V_{O\text{-}J\ ((0\ d)}$; and $\triangle V_{O\text{-}K} = V_{O\text{-}K\ (stress)} - V_{O\text{-}K\ (0\ d)}$. The OJIP curve was analyzed using the JIP-test to obtain the maximum photochemical efficiency of the PSII (F_v/F_m), the photosynthetic performance index based on the absorbed light energy (PI_{ABS}), the ratio of absorbed energy used for electron transfer after Q_A^- (φE_o), the maximum quantum yield of non-photochemical quenching (φD_o), the absorbed light energy per unit reaction center (ABS/RC), the absorbed light energy used for reduction of Q_A per unit reaction center (TR_o/RC), the absorbed light energy used for electron transfer per unit reaction center (ET_o/RC), and the dissipated energy per unit reaction center (DI_o/RC). The calculation followed the methods described by Strasser *et al.* (1995).

Data Processing and Statistical Analysis

The data showed in the figures were the average ± standard deviation (SD) of five repetitions. Excel and SPSS 22.0 software were used to conduct the statistical analyses. One-way ANOVA and the least significant difference (LSD) were used to compare the differences between different groups.

Results

Effect of Drought Stress on the Chlorophyll and Water Content

Both the chlorophyll and water contents in leaves of two alfalfa varieties decreased under drought stress (Fig. 1). Four days after initiation of the drought treatment, the water contents in the leaves of both varieties showed no significant changes and there was no significant difference between the two varieties. From day 6 onward, the decreasing water content of the WL353HQ alfalfa leaves was significantly greater than the leaves of Zhaodong alfalfa. At 8 and 10 days after initiation of the drought treatment, the average water contents of the Zhaodong alfalfa leaves was 11.89% ($P<0.05$) and 32% ($P<0.05$) higher, respectively, than that of the WL353HQ alfalfa leaves. Similar to the leaf water content, the SPAD values of Zhaodong alfalfa leaves were significantly higher than those of the WL353HQ alfalfa leaves 6 to10 days after initiation of the drought treatment.

Rapid Chlorophyll Fluorescence Induced Kinetic Curves under Drought Stress

The OJIP curves in leaves of the two alfalfa varieties exhibited significant changes under drought stress (Fig. 2). Compared to day 0, 2 days after initiation of the drought treatment, the relative fluorescence intensities on the OJIP curves in leaves of the two varieties displayed small increases. On days 4, 6, and 8 after initiation of the drought treatment, the relative fluorescence intensities of the leaves of both varieties at point O (0 ms) on the OJIP curves showed no significant difference, but the relative fluorescence intensities at point J (2 ms), point I (30 ms), and point P (100 ms) were significantly decreased; at point P in particular, the relative fluorescence intensities exhibited the largest decrease and indicated a significant time effect, that is, the longer the drought period, the greater the decrease. The decrease of WL353HQ alfalfa was significantly larger than that of Zhaodong alfalfa. Ten days after initiation of the drought treatment, the relative fluorescence intensities at points J, I, and P on the OJIP curves of the two alfalfa leaves all increased to different degrees compared to those at 4, 6, and 8 days after initiation of the drought treatment, and the increase of the WL353HQ alfalfa was significantly larger compared to the Zhaodong alfalfa.

Effect of Drought Stress on F_v/F_m and PI_{ABS}

From 0 to 2 days after initiation of the drought treatment, F_v/F_m and PI_{ABS} of the two alfalfa leaf types showed no significant changes (Fig. 3). Two days after initiation of the drought treatment, both F_v/F_m and PI_{ABS} exhibited a decrease, and the degree of decrease of PI_{ABS} was significantly larger than F_v/F_m. The decrease of F_v/F_m and PI_{ABS} in the Zhaodong alfalfa leaves was significantly smaller than those of the WL353HQ leaves. Ten days after initiation of the drought treatment, F_v/F_m and PI_{ABS} of Zhaodong alfalfa leaves were 12.98% ($P<0.05$) and 188.81% ($P<0.05$) higher, respectively, than those of the WL353GQ leaves.

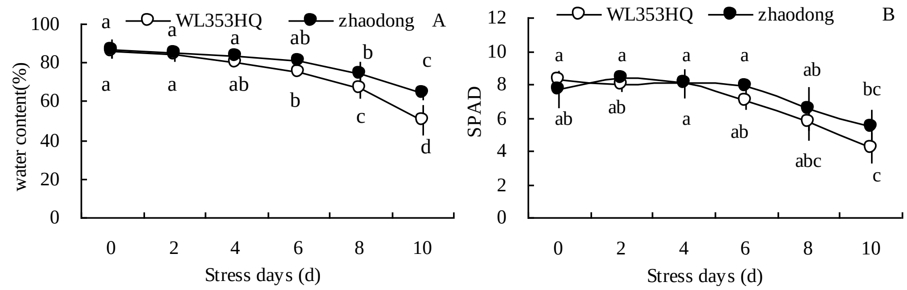

Fig. 1: Effect of drought stress on the relative water contents (A) and the SPAD values (B) in leaves of two alfalfa varieties
Note: Data in the figure are mean ±SE, values followed by different small letters denote significant difference (p<0.05)

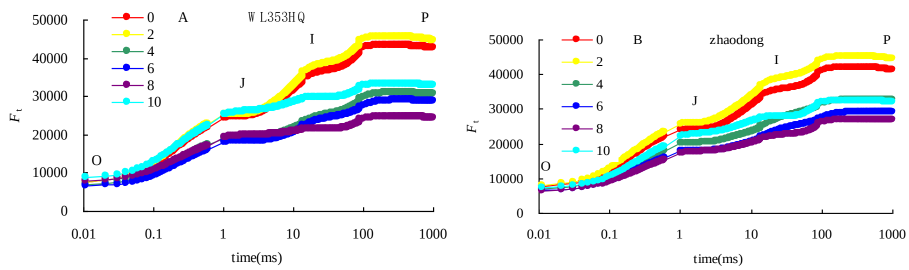

Fig. 2: Effect of drought stress on the OJIP curves in leaves of WL352HQ alfalfa (A) and Zhaodong alfalfa (B)

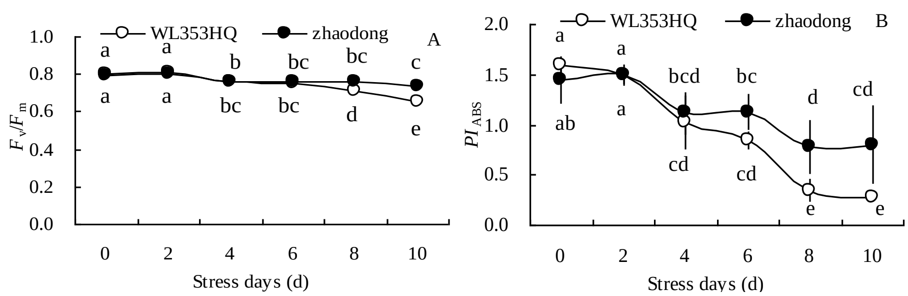

Fig. 3: Effect of drought stress on F_v/F_m (A) and PI_{ABS} (B) in leaves of the two alfalfa varieties
Note: Data in the figure are mean ±SE, values followed by different small letters denote significant difference (p<0.05)

Effect of Drought Stress on the Standardized O-P curves, V_J and V_I

The relative fluorescence intensities at points O and P were defined as 0 and 1, respectively and the OJIP curves at different days after initiation of the drought treatment were standardized. Differences between the standardized O-P curves at various days relative to day 0 were calculated (Fig. 4A and B). On days 2, 4 and 6 after initiation of the drought treatment, there were no significant differences between the standardized O-P curves and day 0. For the Zhaodong alfalfa leaves, 8 days after initiation of the drought treatment, the standardized O-P curve still showed no significant change; only at day 10 was the relative variable fluorescence at point J significantly increased. In contrast, on days 8 and 10, the relative variable fluorescence of WL353HQ alfalfa leaves at point J were significantly increased. The degree of change of the relative variable fluorescence at point I on different days of drought stress was significantly smaller compared to point J. Quantitative analysis of the changes in V_J and V_I showed that 8 and 10 days after drought stress, the extent of increase in V_J in WL353HQ alfalfa leaves was significantly larger than the leaves of Zhaodong alfalfa. On day 10 after initiation of the drought treatment, V_J of WL353HQ alfalfa leaves was 20.01% (P<0.05) higher than leaves of Zhaodong alfalfa. The difference in the values of V_I of the two alfalfa varieties was not significant under differing degrees of drought (P>0.05).

Effect of Drought Stress on the Standardized O-J Curves, O-K Curves, V_K and V_L

The relative fluorescence intensities at point O was defined as 0, and the relative fluorescence intensities at points J and K were defined as 1; the OJIP curves of the two alfalfa leaves at different days of drought stress were standardized for O-J (Fig. 5A) and O-K (Fig. 5B).

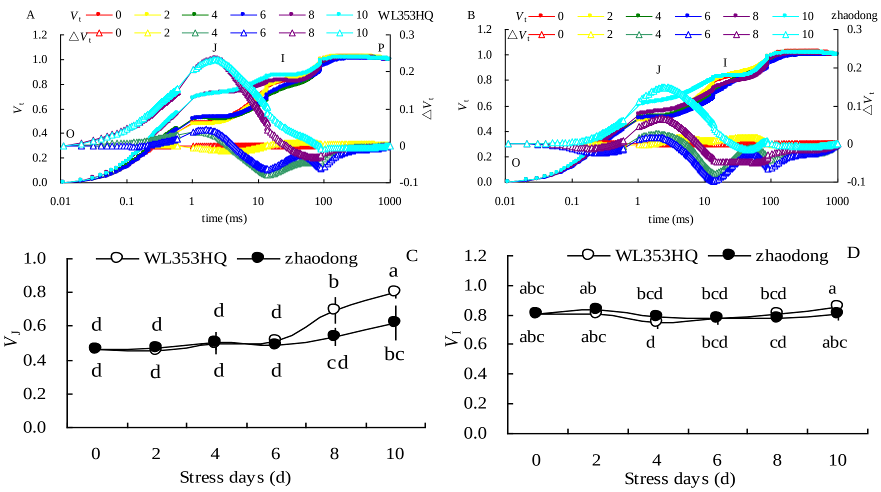

Fig. 4: Effect of drought stress on the standardized O-P curves (A and B), V_J (C) and V_I (D) in leaves of the two alfalfa varieties
Note: Data in the figure are mean ±SE, values followed by different small letters denote significant difference (p<0.05)

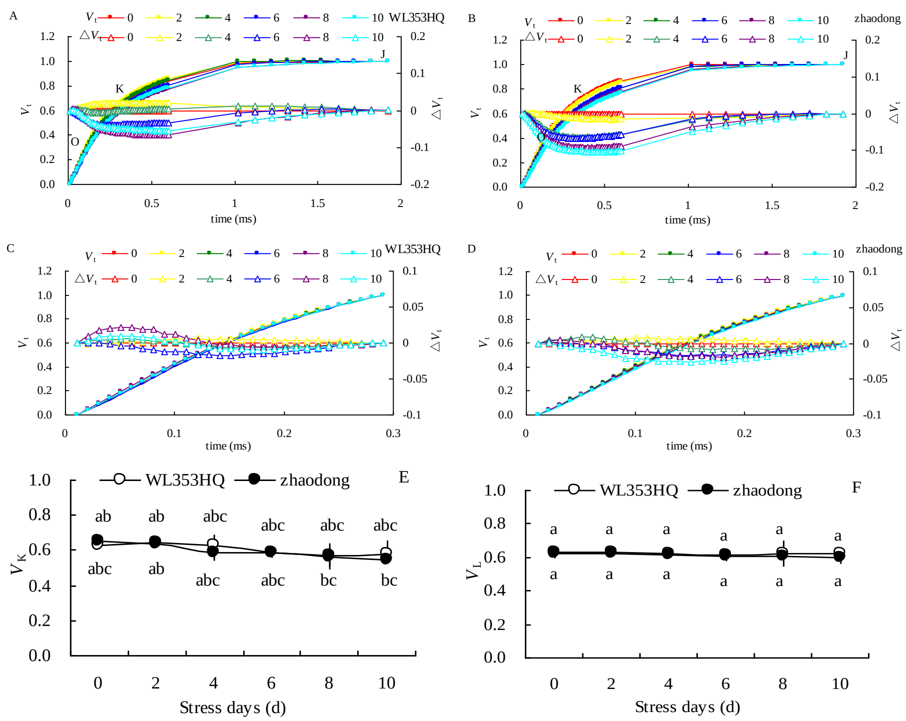

Fig. 5: Effect of drought stress on standardized O-J curves (A and B), O-K curves (C and D), and their V_K (E) and V_L (F) in leaves of the two alfalfa varieties
Note: Data in the figure are mean ±SE, values followed by different small letters denote significant difference (p<0.05)

On day 2 after initiation of the drought treatment, there was only a small difference between the standardized O-J curve compared to day 0. From day 4 to day 10, the relative variable fluorescence at each point on the standardized O-J curves showed differing degrees of decrease. However, the quantitative analysis of the relative variable fluorescence of point K at 0.3 ms on the standardized O-J curve showed (Fig. 5E) that compared to day 0, there was no significant difference in V_K of the leaves of the two alfalfa varieties. The changes of the standardized O-K curves were relatively small and the relative variable fluorescence of point L at 0.15 ms showed no significant change (Fig. 5F).

Effect of Drought Stress on φE_o and φD_o

Fig. 6 shows that drought stress significantly decreased φE_o of the leaves of the two alfalfa varieties, while φD_o showed an increasing trend as drought stress intensified.

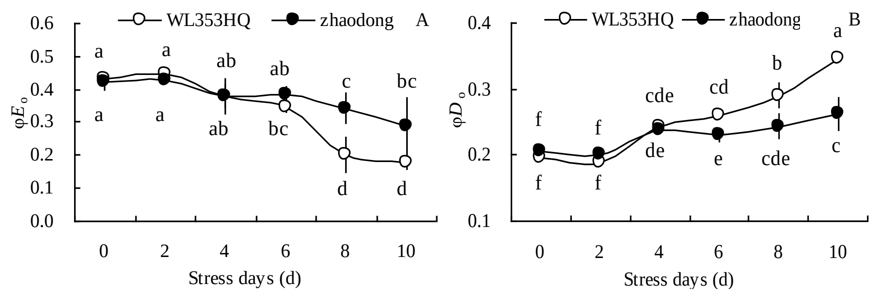

Fig. 6: Effect of drought stress on φE_o (A) and φD_o (B) in leaves of two alfalfa varieties
Note: Data in the figure are mean ±SE, values followed by different small letters denote significant difference (p<0.05)

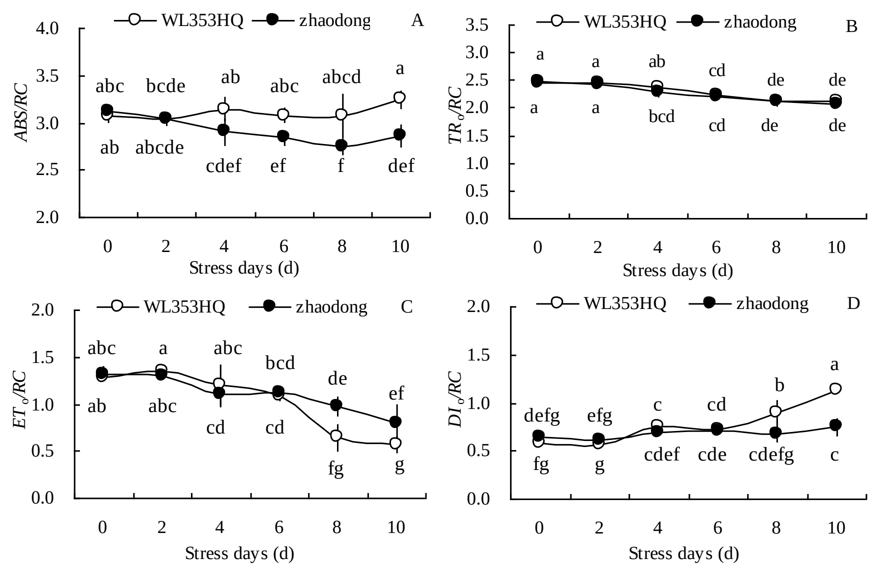

Fig. 7: Effect of drought stress on *ABS* /*RC* (A), TR_o /*RC* (B), ET_o /*RC* (C), and DI_o /*RC* (D) in leaves of two alfalfa varieties
Note: Data in the figure are mean ±SE, values followed by different small letters denote significant difference (p<0.05)

During the first 4 days after initiation of the drought treatment, φE_o and φD_o showed no significant differences, but after 6 days, the degree of decrease in φE_o of WL353HQ alfalfa leaves was significantly greater compared to Zhaodong alfalfa. After 8 and 10 days, φE_o of the Zhaodong alfalfa leaves were 70.06% (P< 0.5) and 63.70% (P<0.05) higher, respectively, than WL353HQ alfalfa leaves, while φD_o was 15.95% (P<0.05) and 24.42% (P<0.05) lower, respectively compared to WL353HQ alfalfa leaves.

Effect of Drought Stress on *ABS*/*RC*, TR_o/*RC*, ET_o/*RC*, and DI_o/*RC* of the Leaves

From day 0 to day 8 after initiation of the drought treatment, *ABS*/*RC* of the two alfalfa varieties showed a decreasing trend (Fig. 7A). After 10 days, *ABS*/*RC* of the two alfalfa varieties increased slightly. However, from day 4 to day 10, the values of *ABS*/*RC* of WL353HQ alfalfa leaves were significantly higher than those of Zhaodong alfalfa. Drought stress decreased TR_o/*RC* and ET_o/*RC* of the two varieties (Fig. 7B and C), while DI_o /*RC* showed an increasing trend as drought stress continued (Fig. 7D). During the drought stress process, TR_o/*RC* exhibited no significant difference between the two alfalfa varieties. On days 8 and 10 after initiation of the drought treatment, ET_o/*RC* of Zhaodong alfalfa leaves were 51.95% (P<0.05) and 71.68% (P<0.05) higher, respectively, than WL353HQ alfalfa leaves, while DI_o/*RC* was 44% (P<0.05) and 33.27% (P<0.05) lower, respectively than WL353HQ alfalfa leaves.

Discussion

Light energy absorption, transfer and conversion in plants depend on chlorophyll content, which directly affects the absorption and utilization of light energy by plants. Stress conditions can inhibit the synthesis of chlorophyll or accelerate its degradation rate (Ludewig and Sonnewald, 2000). In our study, the chlorophyll contents of the leaves of the two alfalfa varieties decreased when the relative water content in the soil decreased. WL353HQ showed a significant decreasing trend when the relative soil water content dropped to 56.3%, but for Zhaodong alfalfa the decreasing trend did not initiate until the relative soil water content was 41.1%, which indicated that the chlorophyll degradation rate or its stability in the leaves of Zhaodong alfalfa was significantly higher than WL353HQ alfalfa leaves. In addition, at the later stages of drought stress, the relative water content of the Zhaodong alfalfa leaves was also significantly higher than WL353HQ alfalfa leaves; a higher leaf water content provides is required for the normal operation of leaf photosynthesis.

Under drought stress, it is difficult for plant roots to absorb water, which leads to the decrease of water content in leaves and the destruction of the photosynthetic apparatus. Many studies have shown that under stress damaged sites of the photosynthetic apparatus often occurred in PSII, thus maintaining relatively high PSII photochemical activity under stress is the characteristic that reflects strong drought resistance in plants. The decrease of the maximum photochemical efficiency F_v/F_m is an important manifestation of photoinhibition in plants (Nultsch *et al.*, 2015), while PI_{ABS} can not only reflect the capture of light energy in the PSII reaction center, but it also reflects the capacity for photosynthetic electron transfer between the two photo systems (Sun *et al.*, 2008). In this study, during the first 6 days after initiation of the drought treatment, there were no significant differences in F_v/F_m and PI_{ABS} of the leaves of the two alfalfa varieties; however, on day 8 and day 10 of drought stress, when the relative soil water content was reduced to 38.5% and 29.9%, respectively, F_v/F_m and PI_{ABS} were significantly decreased, with PI_{ABS} being more sensitive than F_v/F_m. At the later stages of drought stress, both F_v/F_m and PI_{ABS} of Zhaodong alfalfa leaves were significantly higher than WL353HQ alfalfa leaves, which indicated that the PSII photochemical activity in Zhaodong alfalfa leaves had stronger drought tolerance.

The decrease in the photochemical activity of the PSII reaction center in plants under stress is mainly related to the obstruction of electron transfer. To analyze the causes of the decreased PSII photochemical activity, the OJIP curves were standardized. Changes in the relative variable fluorescence V_J at point J on the standardized OJIP curve reflect the characteristics of electron transfer from Q_A to Q_B on the electron acceptor side of PSII, while the changes of the relative variable fluorescence V_I at point I reflects the heterogeneity of the PQ pool (Li *et al.*, 2005). In this experiment, beginning 8 days after initiation of the drought treatment, when the relative soil water content was decreased to 29.9%, V_J of the leaves of the two alfalfa varieties started to increase rapidly, and the degree of increase of WL353HQ leaves was significantly greater than Zhaodong alfalfa leaves. During the entire drought stress period, V_I of the leaves of the two alfalfa varieties showed no significant changes, which suggested that the reason that the decreased photochemical activity of the PSII in the leaves is related to the blockage of electron transfer from Q_A to Q_B on the electron acceptor side of the PSII, and the sensitivity of WL353HQ alfalfa was significantly higher compared to Zhaodong alfalfa. Research has determined that the destruction of PSII usually occurred at the core subunit D_1 protein of the PSII reaction center. The His residues of D_1 protein at positions 215 and 272 are the main binding site for the ligand formed with non-heme Fe-Q_A-Q_B complexes; therefore, the degradation of the D_1 protein can cause compounds, such Q_B and Q_A, to fall off the D_1 protein, which can lead to the inhibition of the electron transfer on the acceptor side, thus, the D_1 protein is considered to be critical for normal electron transfer in PSII (Nadia *et al.*, 2006; Faraloni and Torzillo, 2010). Our experiment showed that changes of V_J were in accordance with the changes of F_V/F_m and PI_{ABS}, which indicated that the reason the drought stress induced decreased photochemical activity of PSII may be related to the degradation of the D_1 protein.

Stress may inhibit the activity of the oxygen-evolving complex on the donor side of the PSII reaction center in the leaves (Allakhverdiev *et al.*, 2001; Park *et al.*, 2004), however, some studies have also suggested that stress may cause the up-regulation of expression of oxygen-evolving complex proteins in leaves, as an adaptation to the stresses (Pang *et al.*, 2010, Zhang *et al.*, 2016). The increase in the relative variable fluorescence V_K at point K at 0.3 ms on the standardized O-J curve was considered as a sign of damage to the activity of the oxygen-evolving complex on the electron donor side of PSII (Zhang *et al.*, 2012). However, the V_K change is not only affected by the injury on the PSII donor side, but also affected by the injury on the PSII acceptor side. When the injury level on the acceptor side is greater than that of the donor side, V_K will no longer increase significantly (Jin *et al.*, 2015). In our experiment, the variation of V_K in the leaves of the two alfalfa varieties was small and slightly decreased, and there was no significant difference between the two varieties, however, this does not mean that drought had no effect on the activity of the oxygen-evolving complex on the PSII electron donor side of the two alfalfa varieties. Combined with the changes in V_J, it further explained that the main reason for the decrease of the PSII photochemical activity in the leaves of the two alfalfa varieties caused by drought was the damage on the acceptor side of PSII.

The increase of the intracellular ROS concentration is one of the direct causes of photoinhibition in plants. When electron transfer in plant leaves is blocked, the excess electrons attack the internal free O_2 in the chloroplasts and they produce superoxide anions and other active oxygen species (Murata *et al.*, 2007). The outbreak of active oxygen species can aggravate the chloroplast membrane peroxidation, resulting in the dissociation of the thylakoid membrane and the destruction of the PSII apparatus (Zhang *et al.*, 2017b). An increase in the relative variable fluorescence at point L (V_L) on the standardized O-K curve is a specific sign of thylakoid dissociation (Mlinarić *et al.*, 2017). In this experiment, the V_L of the leaves of the two alfalfa varieties did not change significantly under drought stress, and there was no significant difference between the two varieties, which indicated that when the relative soil water content decreased to 29.9% at day 10 after initiation of the drought treatment, the thylakoid membrane dissociation of the alfalfa leaves had not induced any obvious photoinhibition occurred in PSII. This may be related to the increase in the activity of antioxidant enzymes or in the content of the antioxidant enzymes, and it also indicated that the photosynthetic membrane structure of alfalfa leaves has a strong resistance to drought.

Reasonable energy absorption and distribution of PSII is the basis for ensuring the normal physiological function of PSII in plants. At the early stages of drought stress, *ABS*/*RC* of the two alfalfa leaves displayed a decreasing trend; however, 10 days after initiation of the drought treatment, the *ABS*/*RC* of the leaves increased slightly, which suggested that the light absorption per unit reaction center was increased. The reason may be related to the degradation or inactivation of the reaction centers in the leaves of the two alfalfa varieties under severe drought stress, which could result in an increase in the efficiency of the remaining active centers (Zhang *et al.,* 2011). As drought stress increased, the decreased ability of PSII to separate charges and transfer electrons resulted in decreases in φE_o, TR_o/RC, and ET_o/RC, while φD_o and DI_o/RC increased. Excessive thermal dissipation competes for energy in the chain of the photosynthetic electron transfer, which would lead to a decreased assimilation capacity and result in energy shortages in the photosynthetic electron transfer chain; this shortage would lead to decreased accumulation of assimilation power (ATP and NADPH), and the decreased assimilation capacity of the photosynthetic carbon resulted in decreased drought resistance of the alfalfa plants. However, the energy ratio of photosynthetic electron transfer in the Zhaodong alfalfa leaves was significantly higher compared to WL353HQ alfalfa leaves under drought stress; therefore, relatively more energy was supplied to Zhaodong alfalfa under drought stress, thus providing energy for its drought resistance.

Conclusion

Under drought stress, the chlorophyll content and the water content in leaves of the two alfalfa varieties decreased, and the PSII photochemical activities of the leaves were significantly inhibited. The reason for the decreased PSII photochemical activity of the alfalfa leaves under drought stress was mainly related to the obstruction of electron transfer from Q_A to Q_B on the electron acceptor side of PSII. Drought also caused changes in the absorption and utilization of light energy by the leaves of the two alfalfa varieties, which was mainly manifested by the decreased proportion of light energy absorption for electron transfer and the increase of the proportion for heat dissipation. Drought resistance of PSII in Zhaodong alfalfa leaves was significantly higher than that of WL353HQ alfalfa leaves.

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