MAIZE SEEDLINGS RESPONSE TO DROUGHT STRESS AND RE-WATERING: ABSCISIC ACID, A KEY REGULATOR OF PHYSIO-BIOCHEMICAL TRAITS AND GAS EXCHANGE PARAMETERS

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Abstract

A pot-culture study was conducted to estimate the role of abscisic acid (ABA) and its regulatory mechanisms in maize seedlings to adapt water deficit and re-watering conditions. The maize seedlings (*Zea mays* L.) were exposed to well-watered, water deficit and re-watering conditions at seedling stage. Results showed that ABA concentration was significantly increased 1.97-fold and 1.73-fold ($p<0.01$) under moderate and severe drought stress conditions, respectively. Moreover, drought stress significantly increased the activities of antioxidant enzymes (SOD, POD and CAT), and the concentration of MDA and O_2^-. The photosynthetic rate (Pn), stomatal conductance (g_s), and transpiration rate (E) in leaves were decreased by withholding water for 2 days. Our study demonstrated that the plants physio-biochemical traits and gas exchange parameters variation under drought probably were caused by a higher level of ABA. The shoot biomass was reduced by 34.4% and 66.1% and the root biomass was reduced by 44% and 69% under moderate and severe drought stress treatments, respectively. Results showed that the root biomass had more reduction under drought stress treatments, indicating that water deficit affected plants biomass allocation patterns. We conclude that ABA plays an important role in regulating the plant growth under drought stress and re-watering conditions, and improve the osmotic adjustment for plant better growth and development.

Key words: Adaptation mechanisms, Growth compensation, Biomass allocation, Abscisic acid (ABA), Maize seedlings.

Abbreviations used: Abscisic acid, ABA; photosynthetic rate, Pn; stomatal conductance, g_s; transpiration rate, E; days after treatments, DAT; water stress treatments, WS; re-watering treatments, RW; well watered, WW; moderate drought stress, MS; severe drought stress, SS.

Introduction

Drought stress or water deficits is one of the most important environmental constraints affecting plant survival and agricultural productivity worldwide, especially in the dry-land agricultural ecosystems (Benlloch-González *et al*., 2015; Oukarroum *et al*., 2007; Xiong *et al*., 2006). Maize is a very important cereal crops worldwide. The maize (especially the summer maize) needs more water than other crops. At seedlings stage, water deficit adversely affects the plants performance and the formation of photosynthetic organs, which directly resulted in the reduction of the biomass and grain yield. In the Loess Plateau, the arid and semi-arid areas in north-west of China, rainy season does not always coincide with the growth period of summer crops such as the maize, frequently resulting in drought stress (Turner *et al*., 2011). It is important to understand the mechanisms through which plants adapt to drought stress conditions and select maize genotypes better suited to drought.

Plants are more vulnerable to water stress (Xu *et al*., 2010). The adaptive mechanisms of plants to drought stress conditions are regulated by their photochemical and biochemical processes (Yordanov *et al*., 2000). Osmotic adjustment is a physiological trait that could improve the plants adaptive ability to drought-prone areas. Abscisic acid (ABA) is a growth regulator of plants which identified as a signal in stress-perception-response pathway such as water deficit, high and/or low temperature, and salinity stress (Cao *et al*., 2013; Sankar *et al*., 2013; Sauter *et al*., 2001). Genotypic variation in ABA accumulation is considered to

be a source for improving drought tolerance. However, the underlying mechanism for its ability to regulate ABA and its role to adapt drought stress is still obscure.

Plants adapt to different stresses by altering their physiological-biochemical metabolism, biomass allocation and morphological pattern (Zhang *et al*., 2012). In this process, ABA plays an important role in plant acclimation not only to water deficit stress but also to other abiotic stresses and induction of seed dormancy (Macková *et al*., 2013). Root-to-shoot signaling material such as ABA that induce stomatal closure to soil drying (Xiong *et al*., 2007; Fan *et al*., 2008), and re-watering conditions. Understanding the regulation of ABA and the responses of plants to re-watering is important for future drought-tolerant cultivar breeding. The main objective of this study was to evaluate the effects of ABA regulations on the maize seedlings that suffered water deficit stress and re-watering treatments at a pot-culture scale.

Materials and Methods

Plant materials and growth conditions: The pot-culture experiment was conducted in a rainout shelter (20 m long × 12 m wide × 4.5 m high) at Gansu Academy of Agricultural Sciences (GAAS), Lanzhou, China (36°6′N, 103°41′E; altitude 1,541 m). The site is representative of the semiarid climate condition in north-west of China. The average annual precipitation is 330 mm and annual evaporation is 1700 mm in this region. The drought-tolerant maize variety, Yuanhua-5 (YH-5) was used in this study with growing period of 96 days. 9 kg dry soil

was filled in each pot (The height, the top and bottom diameters is 38, 25 and 20 cm, respectively) from a nearby field site with the field capacity (FC, the percentage or the amount of soil moisture in the soil after excess water in the soil has been drained away for 48 h following saturation) of 24%. Before sowing, the fertilizers including N 0.2 g kg^{-1} (dry soil) and P$_2$O$_5$ 0.2 g kg^{-1} (dry soil) were added in each pot. Five seeds were sown per pot on 20th June, 2015 and then each pot was watered. After the expansion of 5th or 6th leaf, the seedlings were thinned to 2 in each pot.

Drought and re-watering treatments: The first 26 days after sowing (DAS), all maize plants were well watered (75 % FC) to make a good and consistent seedlings growth. The experimental duration was divided into two periods (10 days in each period). Period 1 (from 27 to 36 DAS), the amount of water watered to the plants was different to impose different levels of stress, while in period 2 (from 37 to 46 DAS), the half pots having drought stress were watered again to 75% FC and kept a well-watered condition. Water was given according to the treatments in the late afternoon (17:00–18:00 hours Beijing Standard Time (BST) and five water regimes were imposed in current study: (1) well-watered in both two sections (WW-WW), pots were watered daily to 75% FC; (2) moderate drought stress in both two sections (MS-MS), soil water content (SWC) of the pots was maintained at 55% FC by daily weighing and watering; (3) severe drought stress in both the sections (SS-SS), SWC was maintained to 35% FC by daily weighing and watering; (4) moderate drought stress in first period (11 days) and then well-watered in second period (10 days) (MS-WW); and (5) severe drought stress in first period (10 days) and then well-watered in second period (10 days) (SS-WW). There were 15 replicate per pot per treatment.

Sampling and measurements

Gas exchange parameters: The net photosynthesis rate (*P*n), stomatal conductance (*g*$_s$) and transpiration rate (*E*) were measured by an upper health and expanded leaf from each treatment between 08:30 to 10:30am using a Li-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA). From each leaf, the data was calculated by getting mean of ten observed values per replicate. After measuring the gas exchange characteristics, the chlorophyll fluorescence parameters (Fv/Fm, photosynthetic quantum yield, qP and NPQ) of leaves were measured using PAM-2500 Fluorescence detector (WALZ, Germany).

ABA measurement: ABA extraction and purification methods were modified from those described by Bollmark *et al.*, (1988) and Du *et al.*, (2012). The maize leaf samples (the upper fully-expanded leaves) were ground in liquid N$_2$ using a mortar and pestle, then the samples extracted with ice-cold 80% methanol (v/v) containing 1 mM butylated hydroxytoluence (BHT) to avoid oxidation. The extracts were put at 4°C for over night. Next day the extracts were centrifuged at 10,000 *g* for 900 s at 4°C, and the residues were suspended in the same ice-cold extraction solution and stored at 4°C for 1 h. Then the

residues were centrifuged again at 10,000 *g* for 900 s at 4°C. The supernatants were combined and passed through Chromosep C18 columns (C18 Sep-Park Cartridge, Waters, Millford, MA, USA), prewashed with 10 ml of 100% and 5 ml of 80% methanol, respectively. The efflux was collected and dried by evaporation with N$_2$. The residues were dissolved in 1.6 ml of phosphate-buffered saline (PBS) containing 0.1% (v/v) Tween 20 and 0.1% (w/v) gelatin (pH 7.5) for analysis by enzyme-linked immunosorbent assay (ELISA).

Enzyme assays: Frozen leaf segments (0.5 g) were crushed into fine powder with a mortar and pestle under liquid N$_2$. The soluble proteins were extracted by homogenizing with 10 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 15,000 rpm/min for 25 min. in a freezing centrifuge, and the supernatant was stored at 4°C and used for the total superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities assays. SOD (EC 1.15.1.1) activity was measured by monitoring the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT) using the method of Giannopolitis & Ries (1977). According to the measurement, 1 unit SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT when monitored at 560 nm. POD (EC 1.11.1.7) activity was assayed by following the method of Chance & Maehly (1955). Three ml of reaction solution contained 3ml 20m Mguaiacol, 10 μl extraction solution (50 mM potassium phosphate buffer, pH 7.0) and 10 μl 30% H$_2$O$_2$, and the enzyme activity was observed at 470 nm for 180 s. CAT (EC 1.11.1.6) activity was measured by the following methods: The reaction mixture (3 ml) contained 50 mM potassium phosphate (K$_3$PO$_4$) buffer (pH 7.0), 10 mM H$_2$O$_2$ and 200 μL enzyme extract. By adding the enzyme extract, the reaction was started, and the change in absorbance at 240 nm (extinction coefficient 39.4 M^{-1} cm^{-1}) and 25°C for 3 min was monitored (Aebi, 1984).

Biochemical traits measurements: On the same day after measuring the gas exchange and RWC measurements, totall five upper fully-expanded leaves in each treatment were selected for measuring the biochemical parameters (proline, soluble sugar and malondialdehyde (MDA) concentrations),. The leaf samples were taken between 10:30 to 11:00 am BST, and then frozen and stored at -80°C until analysis. The frozen leaves (0.5 g) were ground in liquid N$_2$ using a mortar and pestle and homogenized with 5 mL phosphate buffer (pH 7.8; 0.5 M) in centrifuge tubes. The supernatant obtained after centrifugation (25,155g for 0.25 h at 4°C) was used for the biochemical analyses. Free leaf proline was estimated according to Bates *et al.*, (1973). Lipid peroxidation, measured as malondialdehyde (MDA) concentration, was determined following the method of Dhindsa *et al.*, (1981). Soluble sugar concentration was measured using the anthrone reagent method (Jayaraman 1981).

Leaf area, plant height and biomass: At each sampling time, the shoots were cut off at soil level and three maize seedlings in each treatment were randomly selected. Plant height was measured. Meanwhile the length and width of all leaves were measured to calculate the total leaf area (Mo *et al.*, 2017):

$$\text{Leaf area (cm}^2) = \text{Leaf length (cm)} \times \text{Leaf width (cm)} \times 0.75$$

At each sampling time, the root samples were taken carefully from each pot. Firstly, the soil was carefully emptied on a plastic sheet, the soil block were crushed and the greater parts of roots were recovered, then the sieving on a 1.4 mm sieve was repeatedly done to recover the smaller parts of roots to produce a clean sample. The above- and belowground biomass were oven-dried at 105°C half an hour and then dried at 80°C to a constant weight. Meanwhile, the fresh parts were selected for measuring the biochemical traits. The samples were taken and frozen at -80°C until analysis.

Statistical analysis

Three replicates for each treatment were taken in current study. Data were analysed by one way ANOVA using G_{ENSTAT} 17.0 (VSN International Ltd.). Linear regression was applied and Figures were drawn by using Origin 8.0 (Microcal Software Inc.).

Results

Effects of water deficit and re-watering on the ABA concentration of maize seedlings: From Figure 1 we can find that water deficit and re-watering treatments affected the ABA concentration in maize leaves. However, under well-watered treatment (WW-WW), there was not much variation of the leaf ABA concentration, and the mean value was 2.67 µg g^{-1} FW. Moderate drought (MS-MS) and severe drought (SS-SS) treatments significantly increased the leaf ABA concentration. In this study, the leaf ABA concentration was increased by 2.24 to 6.66 µg g^{-1} FW under MS-MS treatment, and was increased by 3.66 to 10.34 µg g^{-1} FW under SS-SS treatment (Fig. 1). Moreover, the ABA concentration under SS-SS treatment was significantly higher than that of under MS-MS treatment ($p < 0.01$). Results indicated that ABA concentration was sensitive to the soil moisture, especially under drought stress conditions. When the re-watering treatments were started (11 days after treatments, DAT), the ABA concentration in leaves under moderate drought stress-well watered treatment (MS-WW) and severe drought-well watered treatment (SS-WW) showed slight decreasing trends, while there was no significant difference in ABA concentrations in each treatment since imposing stress treatments (Fig. 1). The leaf ABA concentration decreased by 3.66 and 5.43 µg g^{-1} FW after re-watering treatment under MS-WW and SS-WW treatments, respectively which indicates that ABA concentration still maintained at higher level even the plants were re-watered. Furthermore, the ABA concentration was significantly higher under re-watering treatments (MS-WW and SS-WW) than that of well watered treatment (WW-WW) ($p < 0.05$) until the end of experiment (Fig. 1).
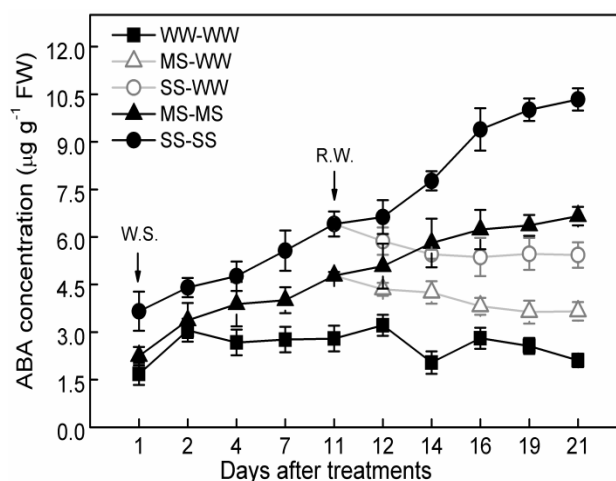

Fig. 1. Dynamics of ABA concentration of maize seedlings under drought stress and re-watering treatments. WW-WW, well watered conditions in two periods; MS-MS, moderate drought stress in two periods; SS-SS, severe drought stress in two periods in this experiment; MS-WW, moderate drought stress in the first period and well watered in the second period; and SS-WW, severe drought stress in the first period and well watered in the second period. W.S., water stress treatments; and R.W., re-watering treatments.

Effects of water deficit and re-watering on biochemical parameters of the maize seedlings: There was not much variation in MDA and O$_2^-$ concentrations under well watered treatment (WW-WW) (Fig. 2). However, drought stress significantly increased the concentrations of these two parameters. At the end of this study, the concentration of MDA was increased to 14.08 and 16.48 µml g^{-1} FW under the moderate drought (MS-MS) and severe drought stress (SS-SS) treatments, respectively. Moreover, the MDA concentration under MS-MS and SS-SS treatments was increased by 39.1% and 48.0%, compared with the WW-WW treatment, respectively. Similarly, the O$_2^-$ concentration was increased to 67.11 and 70.85 nmol g^{-1} FW at the end of this study under the MS-MS and SS-SS treatments respectively, and it was increased by 46.0% and 70.2%, compared with the WW-WW treatment, respectively. Drought stress significantly increased the activities of SOD, POD and CAT (Fig. 2). The activity of SOD was increased from 133.91 to 483.92 U g^{-1} FW and was increased from 143.15 to 472.31 U g^{-1} FW, under the MS-MS and SS-SS treatments, respectively. Moreover, the SOD activity under SS-SS treatment was significantly higher than that of MS-MS treatment from the second day to the end of this experiment. For the POD, its activity was increased from 48.22 to 151.78 µg g^{-1} FW under MS-MS treatment and increased from 58.56 to 158.78 µg g^{-1} FW under SS-SS treatment. During the whole experiment period, the POD activity under SS-SS treatment was significantly higher than that of MS-MS treatment ($p < 0.05$). Similar to SOD and POD, the activity of CAT showed an increased trend under drought stress. The values of CAT activity were 111.22 and 140.95 U g^{-1} under MS-MS and SS-SS treatments, respectively.
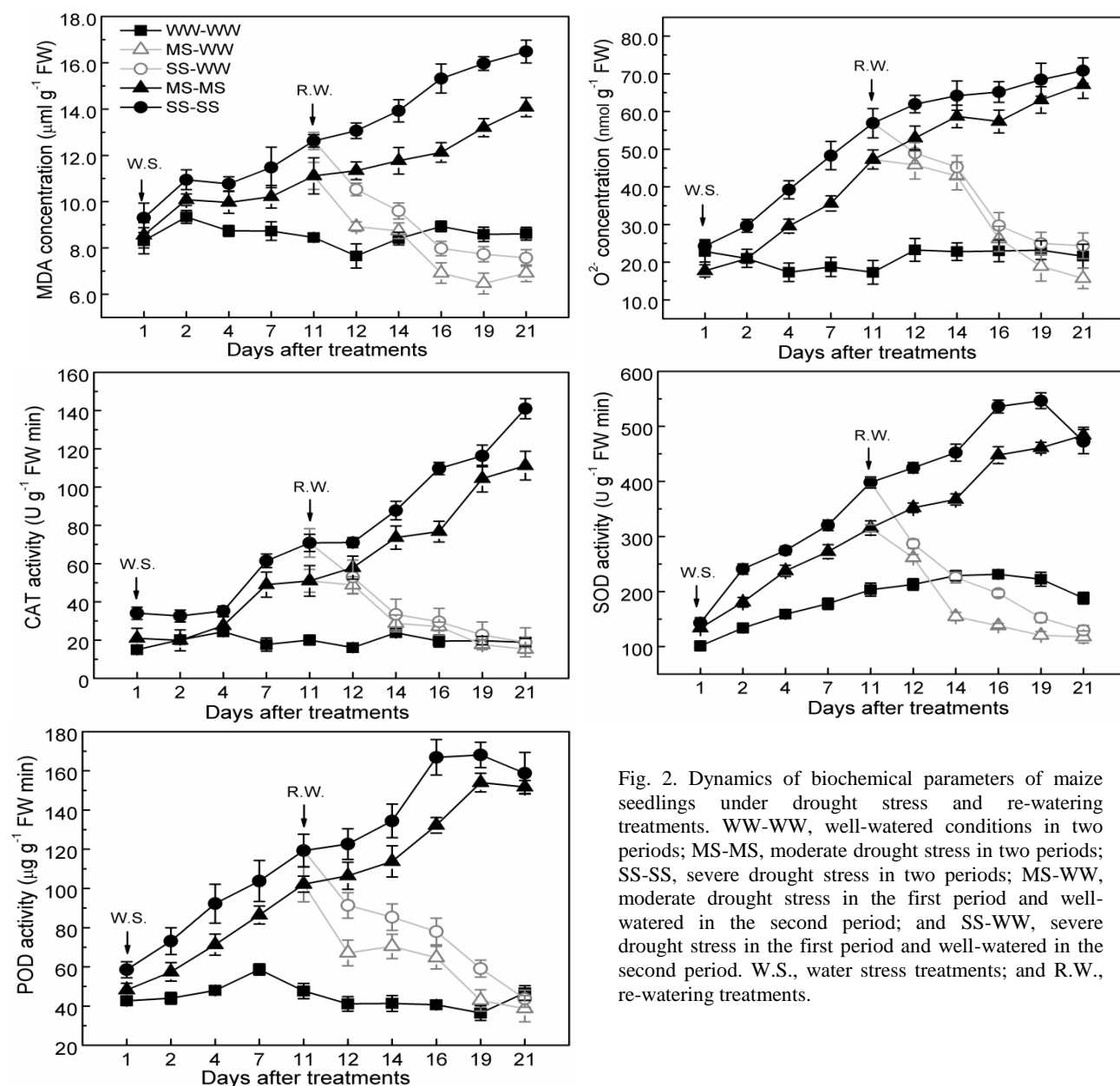
Fig. 2. Dynamics of biochemical parameters of maize seedlings under drought stress and re-watering treatments. WW-WW, well-watered conditions in two periods; MS-MS, moderate drought stress in two periods; SS-SS, severe drought stress in two periods; MS-WW, moderate drought stress in the first period and well-watered in the second period; and SS-WW, severe drought stress in the first period and well-watered in the second period. W.S., water stress treatments; and R.W., re-watering treatments.

Re-watering treatment significantly decreased the concentrations of MDA, O_2^-, and the activities of SOD, POD and CAT. The MDA concentration was significantly lower than that of well watered treatment from the two days after re-watering treatment (12 DAT), and the MDA concentration in moderate drought stress (MS-MS) was lower than that of in severe drought stress (SS-SS) treatment. At the end of this experiment, the concentrations were 6.91 and 7.57 µml g^{-1} FW in MS-MS and SS-SS treatments, respectively. The O_2^- concentration under drought stress was almost the same with WW-WW from the six days after re-watering treatment (16 DAT), while was significantly lower than that of WW-WW at the end of this experiment. There it was no significant difference of SOD activity between the re-watering and WW-WW treatments from the 2 days after re-watering (12 DAT), while it was significantly lower under re-watering treatment than that of WW-WW at the end of the experiment.

Effects of water deficit and re-watering on gas exchange parameters of the maize seedlings: Water deficit significantly reduced the photosynthetic rate (*P*n), stomatal conductance (g_s) and transpiration rate (*E*), and when the drought stress became more severe, the reduction rate was more obvious (Fig. 3). For the *P*n, the values were 7.45 and 2.84 mmol m^{-2} s^{-1} under moderate drought stress-well watered (MS-WW) and severe drought stress-well watered (SS-WW) at the end of the drought stress (S11), respectively. Obviously, re-watering treatment increased the photosynthetic rate. At the end period of this experiment, no significant difference between drought stress-well watered treatments were found (MS-WW and SS-WW) and the well watered treatment (WW-WW) (*p*>0.05). Results indicated that the *P*n can be recovered very well when the plants were rewatered. For the g_s, it was decreased by 17.2% and 33.7% under MS-MS and SS-SS, respectively. Similar to the *P*n, the g_s was also increased when the plants were at the rewatered conditions. The g_s was increased by 25.4% and 10.0% under MS-WW and

SS-WW treatments, compared with the WW-WW treatment, respectively. For the E, it was decreased to 0.71 mmol m^{-2} s^{-1} and to 0.32mmol m^{-2} s^{-1} under MS-MS and SS-SS treatments, respectively. While it was increased to 2.80 mmol m^{-2} s^{-1} and to 2.72mmol m^{-2} s^{-1} after the plants were rewatered under moderate and severe drought stress treatments, respectively (Fig. 3).
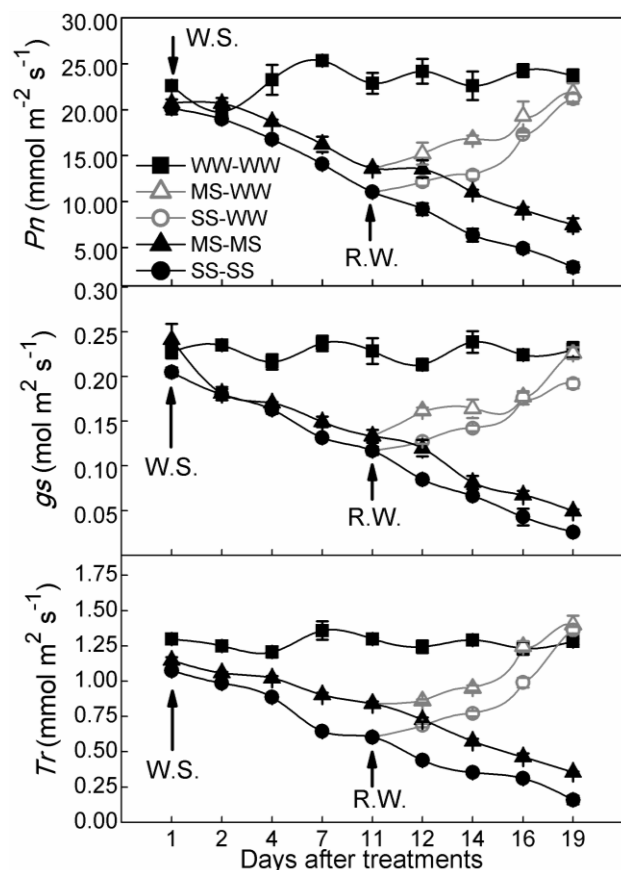

Fig. 3. Dynamics of photosynthetic parameters of maize seedlings under drought stress and re-watering treatments. WW-WW, well-watered conditions in two periods; MS-MS, moderate drought stress in two periods; SS-SS, severe drought stress in two periods; MS-WW, moderate drought stress in the first period and well-watered in the second period; and SS-WW, severe drought stress in the first period and well-watered in the second period. W.S., water stress treatments; and R.W., re-watering treatments.

Effects of water deficit and re-watering on chlorophyll fluorescence parameters of the maize seedlings: Under drought stress conditions, the Fv/Fm was decreased with the growing stage. At the 16 days after treatments (16 DAT), the Fv/Fm was decreased by 9.0% and 9.9% in moderate drought stress (MS-MS) and severe drought stress (SS-SS) treatments, compared with that at the beginning of the stage (1 DAT), respectively (Fig. 4). The Fv/Fm was decreased by 2.7% and 4.8% in MS-WW and SS-WW treatments, compared with the well watered (WW-WW) treatment, respectively. When the plants under the moderate drought stress were re-watered (MS-WW), the Fv/Fm showed an increasing trend and there was no significant difference with the WW-WW treatment. However, the Fv/Fm was decreased by 3.7% under SS-WW treatment, compared with the WW-WW

treatment. The photosynthetic quantum yield (PQ yield) showed a decreasing trend with time (Fig. 4). Generally, it was decreased by 2.3%, 34.7% and 34.7% at the end of the experimental stage in well watered, moderate drought stress and severe drought stress treatments, respectively. Furthermore, drought stress also decreased the PQ yield. The PQ yield was decreased by 20.5% and 29.3% in moderate (MS-MS) and severe drought stress (SS-SS) treatments, respectively. When the plants were rewatered, the PQ Yield was decreased by 15.2% and 24.0% in MS-WW and SS-WW treatments, respectively.

At the 6 days after re-watering treatment (16 DAT), the qP was decreased by 24.0 and 33.1% under MS-MS and SS-SS treatments, compared with that at the beginning stage (1 DAT); after the re-watering, the qP showed an increasing and then decreasing trend (Fig. 4). Compared with the CK, the qP was decreased by 11.0% and 16.2%, under MS-WW and SS-WW treatments, respectively. The NPQ showed an increasing and then decreasing trend with time in the well watered and water deficit treatments. The NPQ at the end of the experimental stage was reduced by 36.6%, compared with the beginning stage (1 DAT). The NPQ was decreased by 12.2% and 31.5% in moderate and severe water deficit treatments, respectively. The NPQ was increased by 11.2% and 12.6% under MS-WW and SS-WW treatments, compared with the WW-WW treatment, respectively (Fig. 4).

Effects of water deficit and re-watering on the biomass and morphological traits of the maize seedlings: Generally, the shoot and root biomass showed an increasing trends over time (Fig. 5). The shoot biomass of the plants under well watered treatment (WW-WW) was significantly higher than that of severe drought stress treatment (SS-SS) after the 4 days since water treatments were started (4 DAT) and were significantly higher than that of moderate drought stress (MS-MS) from the 11 days after water treatments starting (11 DAT). The shoot biomass of the plants under MS-MS treatment was significantly higher than that of SS-SS treatment from the 2 days after re-watering treatments starting (12 DAT). The shoot biomass under WW-WW treatment (7.16 g per plant) was significantly higher than that of MS-MS (4.70 g per plant) and SS-SS (2.43 g per plant) treatments, respectively. The root biomass in moderate drought stress and severe drought stress treatments was reduced by 44.0% and 69.0%, respectively. It was increased by 20.5% while decreased by 28.9% in MS-WW and SS-WW treatments, respectively (Fig. 5).

Drought stress significantly affects the plant height and leaf areas during the experimental stage (Fig. 6). In WW-WW treatment, the plant height was increased from 33.7 cm to 74.2 cm (from 11 DAT to 21 DAT), and the plant height was increased by 22.5% and 38.5%, compared with MS-MS and SS-SS treatments, respectively. For the leaf area, it was increased from 190.9 to 682.1 cm^2 plant^{-1}. The leaf area under MS-MS treatment was significantly higher than that of SS-SS treatment from the 15 days after water treatments starting (15 DAT). The leaf area under WW-WW treatment was increased by 33.2% and 57.9%, compared with the MS-MS and SS-SS treatments, respectively.

Re-watering treatments can increase the plant height (Fig. 6). The plant height under MS-WW treatment was significantly higher than the MS-MS treatment, from the 5 days after re-watering (15 DAT), and there was no significant difference with the WW-WW treatment (*p*>0.05). The plant height under SS-WW treatment was significantly higher than the SS-SS treatment, after 8 days re-watering (18 DAT), and the plant under SS-WW treatment was significantly lower

(60.1 cm) than that of WW-WW treatment. The leaf area under MS-WW and SS-WW treatments was significantly higher than MS-MS and SS-SS treatments from the 5th day after re-watering, respectively. There was no significant difference in the leaf area between the MS-WW and WW-WW treatments, while the leaf area under SS-WW treatment was significantly lower (607.8 cm^2 plant^{-1}) than that of WW-WW treatment (Fig. 6).
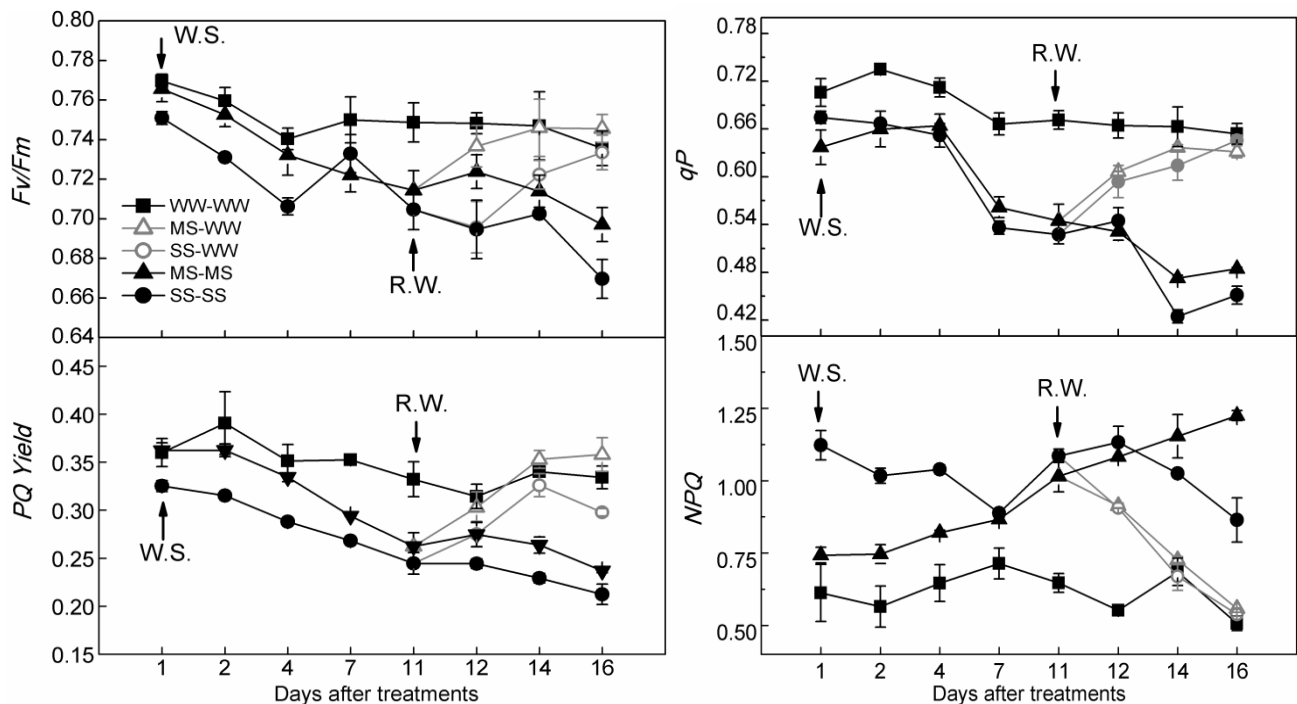

Fig. 4. Dynamics of chlorophyll fluorescence parameters of maize seedlings under drought stress and re-watering treatments. WW-WW, well-watered conditions in two periods; MS-MS, moderate drought stress in two periods; SS-SS, severe drought stress in two periods; MS-WW, moderate drought stress in the first period and well-watered in the second period; and SS-WW, severe drought stress in the first period and well-watered in the second period. W.S., water stress treatments; and R.W., re-watering treatments.
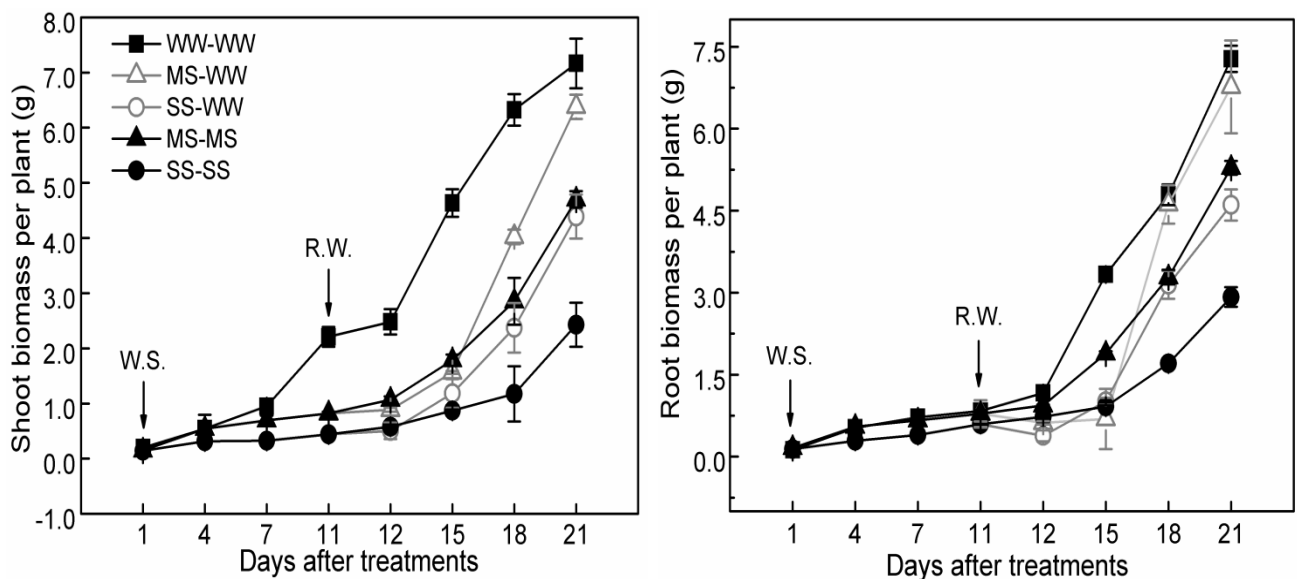

Fig. 5. Dynamics of shoots and root biomass of maize seedlings under drought stress and re-watering treatments. WW-WW, well-watered conditions in two periods; MS-MS, moderate drought stress in two periods; SS-SS, severe drought stress in two periods; MS-WW, moderate drought stress in the first period and well-watered in the second period; and SS-WW, severe drought stress in the first period and well-watered in the second period. W.S., water stress treatments; and R.W., re-watering treatments.
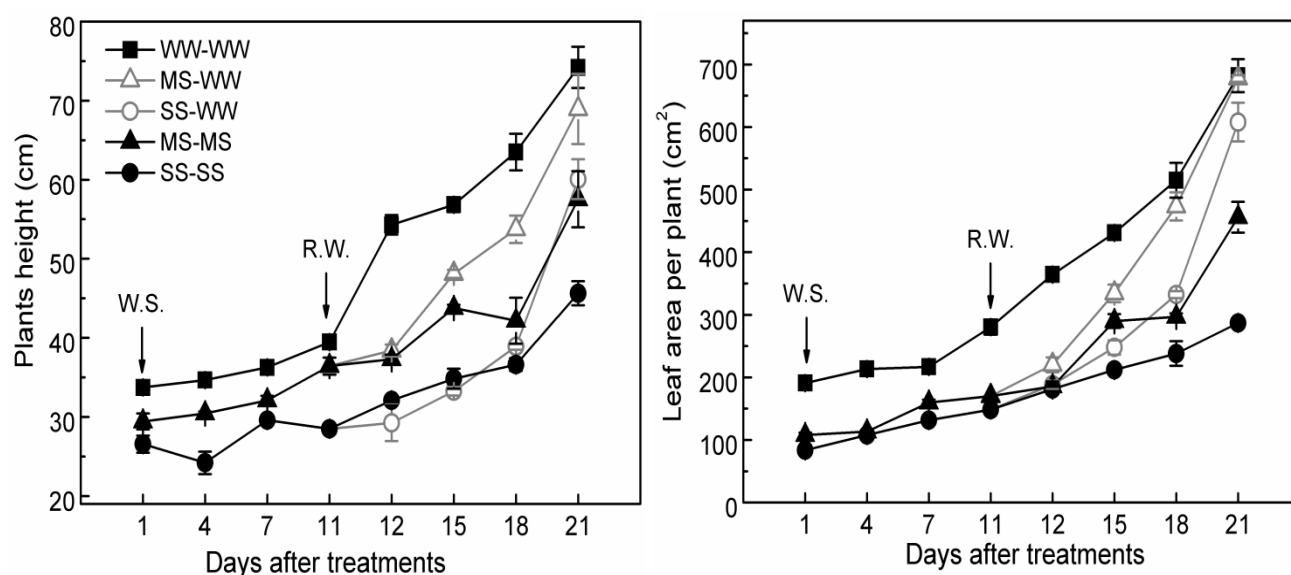
Fig. 6. Dynamics of plant height and leaf area of maize seedlings under drought stress and re-watering treatments. WW-WW, well-watered conditions in two periods; MS-MS, moderate drought stress in two periods; SS-SS, severe drought stress in two periods; MS-WW, moderate drought stress in the first period and well-watered in the second period; and SS-WW, severe drought stress in the first period and well-watered in the second period. W.S., water stress treatments; and R.W., re-watering treatments.

Discussions

Drought stress severely affected maize biomass allocation, photosynthetic rate and plants growth (Wang *et al*., 2017). We have characterized the biochemical, physiological and morphological traits of maize seedlings in response to drought stress and re-watering. The response of plants to water deficit mainly depends on several factors such as growth stages, severity and duration of stress and cultivar genetics (Beltrano & Ronco, 2008). The ability to cope with water deficit stress of plants may depend on different adaptive mechanisms, such as the capacity to maintain a high level of ABA, and morphological and physiological trait changes (Wang *et al*., 2017). Understanding adaptive mechanisms of plants to water deficit and the ABA regulations in this process is very useful for improving crop drought tolerance and increasing the yield production in the future.

Physio-biochemical adaptations to water deficit and re-watering: Our results showed that under water stress conditions SOD, POD and CAT activities and the concentration of MDA and O_2^- were increased in maize seedlings. The drought tolerant ability of maize plants were improved by increasing these elements, and this elements appears to contribute substantially to osmotic adjustment in plants. This was agreed with Mahouachi *et al*., (2006) who reported similar results in papaya (*Carica papaya* L.).Most of physiological and biochemical elements variations were detected 1 day after re-watering to well watered conditions, indicating that effects of water deficit on maize seedlings were reversible. Maize plants showed fast recovery of gas exchange parameters and biochemical parameters suggesting a good tolerance to water stress (Zhang *et al*., 2012). However, in this study, re-watering treatment did not fully recover the gas exchange parameters such as photosynthetic rate in maize seedlings which were subjected to water deficit and re-hydration treatments indicating that water deficiency injured leaf tissues of the maize plants. Similar results were reported by Mahouachi *et al*., (2006) on papaya seedlings (*Carica papaya* L.). Nonetheless, recovery after re-watering treatment was faster and more complete in moderate than severe drought stress plants. This was agreed with Miyashita *et al*., (2005) on kidney bean (*Phaseolus vulgaris* L.).

Under drought stress conditions, the ABA can promote the stomatal closure. On the other hand, it decreased the biochemical substances concentration, which alleviated the effects of water deficient on plants, and suggested that ABA was the determinant in these substances biosynthesis in maize seedlings. The physiological and biochemical traits is expected to directly influence the abilities of water and nutrient uptake, which are important for plant growth and biomass accumulation. Hence, a physiological and biochemical regulation is a possible mechanism of plants to adapt to drought stress conditions.

Morphological adaptations to drought stress and re-watering: Plant height, leaf area and biomass are the primary processes to be affected by water deficit (Sapeta *et al*., 2013). Drought stress resulted in a reduction of total leaf area and plant height which reduced the canopy size (Fig. 6). In our study, the growth reduction of maize seedlings were observed under moderate and severe water deficit stress conditions. Results indicated that the soil water availability even became low, but the maize plants were able to grow. Our results agreed with the findings of Wang *et al*., (2017) and Nuccio *et al*., (2015) who observed same phenomenon in wheat and maize crops. From the allometric theory and phenotypic plasticity perspectives, the reduction of plants height and leaf area will result in a minimum transpiration area, which is a water conservation mechanism to adapt the water deficit conditions (Boyer, 1970; Passioura, 2012).

The biomass allocation to the plant shoot and root parts was further analyzed. The shoot biomass was reduced by 34.4%–66.1%, while the root biomass was reduced by 44.0%–69.0% under drought stress conditions, respectively. Results showed that the root biomass had more reduction than that of shoot biomass under drought stress conditions, indicating that biomass partitioning patterns have changed due to the variation of the environments. After the re-watering treatment starting (11 DAT), a relative higher level of ABA concentration maybe directly or indirectly affected the photosynthesis, which is useful for carbohydrates accumulation. Furthermore, on re-watering stage, root to shoot ratio was decreased (data were not shown), indicating that less biomass were allocated into the root organs, our results agreed with Azhiri-Sigari *et al.*, (2000) who reported the same in rice crops.

ABA regulations on plants growth and biomass accumulation: ABA is one of the most important hormones for plants to resist water deficit and other abiotic stresses (Sankar *et al.*, 2016; Zhao *et al.*, 2016), and it is referred to the regulation of many life activities processes, such as stomatal closure, dormancy and osmotic adjustment (Macková *et al.*, 2013). Moreover, as a conduction signal, ABA probably played a positive role in the regulation of metabolism, water relations and plant growth by controlling g_s and photosynthetic activity (Zhang *et al.*, 2012). Previous studies found that after ABA addition, plants growth and leaf area were significantly decreased (Zhang *et al.*, 2005). The reduction in gas exchange parameters induced by water deficit was correlated with an increase in leaf ABA concentrations, suggesting that this hormone could be involved in the regulation of stomatal closure under water deficit conditions in maize (Xiong *et al.*, 2007; Mahouachi *et al.*, 2007). When the re-watering treatment was finished, the ABA concentration showed a decrease trends (Fig. 1), result indicated that ABA elements in leaves could actually act as a signal that promoting plant growth, such as increasing the canopy size (plant height and leaf area) and the biomass accumulation (Fig. 6).

Previous researches shown that ABA promotes plants senescence by closing the stomatal conductance and producing ethylene element (Zhao *et al.*, 2016). However, the results in our study showed that the plants with higher ABA concentration had significantly higher photosynthetic rate under both moderated and severe drought stress (Figs. 1 and 3). The results demonstrated that ABA can regulate the gas exchange parameters and biochemical parameters, and improved the osmotic adjustments process. However, some of our results are not similar with other studies, probably due to the difference in drought stress levels, and the magnitude of ABA concentration in plants. As Kamoshita (2004) mentioned, during the plants growth stages, especially when plants suffered drought stress, ABA regulation played an important role for plant survival and growth and it was a possible mechanism to adapt the water stress and re-watering conditions. However, this hypothesis needs to be further investigated in maize seedlings under water deficit conditions. Overall, our data showed that ABA in maize seedlings under water deficit condition has a positive effect on biochemical parameters for maintaining plant growth and increasing the total dry matter.

Conclusion

In the well watered treatment, there was no great variation in ABA concentration. Drought stress reduced the canopy size such as the plant height and leaf area. On the other hand, the root biomass had a larger reduction, while shoot biomass had a smaller reduction, the root to shoot ratio was decreased under drought stress conditions, indicating that plants changed their biomass allocation patterns to adapt to the water deficits. The maize seedlings increased their drought tolerance by increasing the ABA and other biochemical substances concentrations, as well as by reducing their canopy sizes to reduce the water loss.

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