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Energy dissipation and antioxidant enzyme system protect photosystem II of sweet sorghum under drought stress

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Abstract

The effect of drought stress on energy dissipation and antioxidant enzyme system in two sweet sorghum inbred lines (M-81E and Roma) was investigated. Results showed that the germination indicator increased more in M-81E than that in Roma under rehydration. Under drought stress, both the maximal photochemical efficiency of PSII (F_v/F_m) and oxidoreductive activity ($\Delta I/I_0$) of Roma decreased more than those in M-81E. Relative to F_v/F_m, the $\Delta I/I_0$ decreased markedly, which indicated that PSI was more sensitive to drought stress than PSII. Increases in the reduction state of Q_A ($1 - q_p$), nonphotochemical quenching (NPQ) and minimal fluorescence yield of the dark-adapted state (F_0) were greater in Roma than those in M-81E; meanwhile, the H_2O_2 content was lower in M-81E than that in Roma. Our results suggested that the photoinhibition might be related to the accumulation of reactive oxygen species (ROS). The antioxidant enzyme system and energy dissipation of M-81E could respectively increase drought tolerance by eliminating ROS and excess energy more efficiently than that of Roma.

Additional key words: antioxidant enzymes; chlorophyll fluorescence; environmental stress; sorghum bicolor; water.

Introduction

Sweet sorghum (*Sorghum bicolor* L. Moench) is a high-yielding species; it is considered to have potential as an energy crop either for ethanol production obtained by fermentation of stalk sugar or for second generation biofuel acquired from the lignocellulosic biomass (Ballesteros *et al.* 2004). Sweet sorghum is primarily grown to produce sugar for syrup and is normally used as animal feed (Almodares *et al*. 2009). It is the fifth most important cereal crop in the world after wheat, rice, corn, and barley (Awika *et al*. 2004). Among widely grown crop species, sweet sorghum possesses the greatest drought tolerance (Rooney *et al.* 2007); it is able to survive in water-limited environments owing to a variety of anatomical, morphological, and physiological features (Jordan *et al.* 1983). The breeding of crop cultivars with enhanced drought tolerance is currently one of the biggest challenges for plant sciences (Ogbaga *et al.* 2014).

Drought is one of the important environmental stress factors limiting plant growth and crop yield (Terzi and Kadioglu 2006). According to available data, approximately 45% of the world's agricultural lands are prone to frequent drought, and 38% of the world's population resides in such areas (Ashraf and Foolad 2007). Unusual dry weather affects germination, growth rate of seedlings, and increases sensitivity of cell elongation to damages induced under stress conditions (Taylor *et al.* 1982, Delachiave and Pinho 2003, Hamayun *et al.* 2010). It is evident that physiological responses of plants to drought

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Abbreviations: APX – ascorbate peroxidase; AsA – ascorbic acid; Chl – chlorophyll; CAT – catalase; DM – dry mass; ETC – electron transport chain; F_0 – minimal fluorescence yield of the dark-adapted state; FM – fresh mass; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_s – steady-state fluorescence yield; F_v – variable fluorescence; F_v/F_m – maximal quantum yield of PSII photochemistry; MDA – malondialdehyde; NPQ – nonphotochemical quenching; q_p – photochemical quenching coefficient; ROS – reactive oxygen species; SOD – superoxide dismutase; Φ_{PSII} – effective quantum yield of PSII photochemistry.
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stress are extremely complicated and vary among plant species, along with the degree and duration of drought exposure (Evans *et al.* 1990). Drought-induced osmotic stress limits the availability of water to plant cells, which in turn affects the physiology of plants (Anjum *et al*. 2011). Drought stress acts by decreasing the germination rate and seedling growth rate (Delachiave and Pinho 2003). In pot marigold and tuberose plants, germination rate, seed vigor, seedling length, and dry mass all decrease with increasing drought stress (Burnett *et al.* 2005, Zavariyan *et al.* 2015). The adverse effect of water shortage on germination and seedling growth has been well reported in different crops, such as corn (Mohammadkhani and Heidari 2008, Farsiani and Ghobadi 2009, Khayatnezhad *et al.* 2010, Beiragi *et al.* 2011), wheat (Dhanda *et al.* 2004, Jajarmi 2009), sorghum (Gill *et al.* 2002), and sunflower (Ahmad *et al.* 2009).

Mannitol is a type of nontoxic plant tissue compatible solute which can be used to absorb fluids in plant tissues in order to study the physiology of water stress response, and is considered better at eliciting drought stress than polyethylene glycol (PEG) 6000. A previous study showed that inhibitory effects of mannitol were much lower than those of PEG 6000 (Slama *et al.* 2007). Adverse effects of PEG on plant development are related to inhibitory effects on root oxygen availability and/or to the presence of phytotoxic contaminants, such as heavy metal ions (Plaut and Federman 1985). Since mannitol is a macromolecular osmotic regulation substance, which that cannot pass through the cell wall, it is an ideal material to simulate soil drought.

Drought stress usually leads to oxidative stress arising from stomatal closure (Lei *et al.* 2006, Ozkur *et al.* 2009), which causes overreduction of the photosynthetic electron transport chain (ETC) (Bacelar *et al.* 2007, Ahmed *et al.* 2009) and overproduction of ROS, which results in an increase of oxidative stress (Smirnoff 1993). Stomatal closure can also reduce CO_2 availability in chloroplasts (Asada 1999, Fu and Huang 2001). In intact chloroplasts, superoxide radical and the hydrogen peroxide, which are produced by dismutation of superoxide are rapidly scavenged at their production sites and the active oxygen do not inactivate the PSI complex, the stromal enzymes, or the scavenging system itself. Thermal energy dissipation in the light-harvesting antenna complexes of PSII is measured as nonphotochemical quenching (NPQ), and it protects the photosynthetic apparatus from inactivation and damage caused by excessive excitation energy during drought stress, and thereby improving crop yield (Horton *et al.* 1994, Golding and Johnson 2003, El-Sharkawy 2016).

Production of ROS involves the photoreduction of molecular oxygen by iron sulphur centers on the PSI acceptor side (Mehler 1951, Li *et al*. 2003) as well as by phyllosemiquinone (Kozuleva *et al.* 2011, 2014). Moreover, the plastoquinone pool (PQ-pool) is also shown to be involved in ROS production in photosynthetic electron transport chain (Mubarakshina and Ivanov 2010). An important protective mechanism is the water-water cycle, which refers to the reduction of atmospheric O_2 into water in PSI with electrons generated from water in PSII and without a net change of O_2; this has been proposed to be an effective protective mechanism under environmental stress (Nakano and Asada 1980, Asada 1999). Superoxide dismutase (SOD) is one of the key enzymes of the water-water cycle (Sui 2015); it can convert $O_2^{\bullet -}$ into H_2O_2 and O_2, and plays a vital role in defending against superoxide-derived oxidative stress in plant cells. Ascorbate peroxidase (APX) reduces H_2O_2 to water with ascorbic acid (AsA) as a specific electron donor in chloroplasts and is the most important plant peroxidase for H_2O_2 detoxification (Kanematsu and Asada 1990, Miyake and Asada 1992, Noctor and Foyer 1998).

Genetic differences in abiotic stress tolerance have been reported in sorghum under field conditions, which can be useful in recognizing genotypes better suited to sowing in semiarid areas (Igartua *et al.* 1995, Cosentino 1996, Yu *et al.* 2004). Although some water stress effects upon seed germination have been reported in sweet sorghum (Oliveira and Gomes-Filho 2009), little is known about these effects during initial embryo growth.

In this study, we determined that photoinhibition of sweet sorghum under drought was related to energy dissipation and the antioxidant enzyme system, and we investigated the protective mechanisms of energy dissipation and antioxidant enzymes in PSII.

Material and methods

Plant culture and treatments: Seeds of the two sweet sorghum lines, M-81E and Roma, used in the study were provided by Shandong Academy of Agricultural Sciences. Sweet sorghum is a variant of sorghum (*Sorghum bicolor* L. Moench). Dry seeds were stored in a refrigerator at 4°C before use.

Germination treatment: Full grain sweet sorghum seeds of uniform size and containing no worm holes were selected and disinfected for 15 min in 0.1% $HgCl_2$ solution, and then rinsed with distilled water. The seeds of M-81E and Roma were germinated in 9-cm diameter Petri dishes containing two layers of filter paper moistened with water. There were 15 seeds in each treatment with three replications. Each variety was tested under four concentrations of mannitol, which were 0, 200, 400, and 600 mM; the corresponding osmotic potentials of solutions were: 0, –0.49, –0.99, and –1.49 MPa, respectively. After 9 d of mannitol treatments, seeds were allowed to recover for 7 d. The mannitol solutions and water control were replaced daily with the same volumes. Seeds were germinated in a glasshouse under the light condition of 14-h light/10-h

dark, and light intensity of 150 μmol(photon) m^{-2} s^{-1}. The temperature in the glasshouse was 25°C during the day and 20°C at night.

Treatment: Sorghum seeds were soaked in water for 10 h. Plants were grown in an artificial climate chamber with a temperature of 28°C during the day and 20°C at night. The photoperiod was 8-h light/16-h dark, and the light intensity was about 300 ± 50 μmol(photon) m^{-2} s^{-1}. The relative humidity was 58–64% during the day and 48−54% at night.

Circular plastic pots used for planting were 16 cm high, had mouth and the bottom diameters of 17 and 9 cm, respectively. Each pot contained washed river sand and seven seeds of a given variety were planted per pot and watered with water during the germination period. Seedlings with two leaves were watered with 1/2 strength Hoagland nutrient solution. Seedlings with three leaves were watered with complete Hoagland nutrient solution until drought stress was performed. The seedlings were thinned to five seedlings per pot at the four-leaf stage, and the remaining plants were grown to eight leaves under natural drought treatment. Before the experimental treatment, the complete Hoagland nutrient solution was poured into the water collection tray. Then plants were exposed to natural condition for 7 d (the complete Hoagland nutrient solution was no longer added). After 7 d, we estimated the level of drought stress by weighing methods. The sand water content = (fresh mass − dry mass)/fresh mass × 100%. After 7-d drought stress, the sand water content reached the level of severe stress (4.2%). After 7-d drought treatment, plants were then recovered by watering with complete Hoagland nutrient solution for 7 d. Three replicates were performed for each treatment.

Germination rate: Sweet sorghum seed germination was observed every day and the seed germination rate was determined in accordance with the national seed inspection standards. The final germination rate was calculated after 9 d as follows:

Germination rate [%] = (germinated seed number/ germinated total number) × 100%

Germination potential is the index evaluating seed germination rate and germination uniformity. Germination potential was calculated as follow:

Germination potential [%] = (germinated seed number at germination peak/test seed number) × 100%

Germination index (GI) is a measure of plant germination ability and vitality. Germination index was calculated as follow:

GI = Gt/Dt

where Gt is the number of seeds germinating within 9 d; Dt is the number of the germination days.

Increase in germination rate is a measure of plant germination resilience after recovery from drought stress, and was calculated as follow:

Germination increase rate [%] = (increase in number of seeds germinating/test seed number) × 100%.

Shoot and root length were measured after the 9-d post-germination treatment and 7-d recovery period. The shoot and root lengths were measured with a ruler. Three replicates were performed for each treatment.

Chlorophyll (Chl) fluorescence of seedlings was measured using a portable fluorometer (*FMS2, Hansatech*, King's Lynn, UK) according to the protocol described by van Kooten and Snel (1990). Minimal fluorescence (F_0) with all PSII reaction centers open was determined with modulated light which was low enough to avoid induction of significant variable fluorescence (F_v). Maximal fluorescence (F_m) with all reaction centers closed was determined by irradiation of a dark-adapted leaf (15-min darkness) for 0.8 s with saturating light of 8,000 μmol(photon) m^{-2} s^{-1}. The leaf was then illuminated by an actinic light of 500 μmol(photon) m^{-2} s^{-1}. Steady-state fluorescence (F_s) was recorded when the leaf reached steady-state photosynthesis (300-s illumination is standard for induction of steady-state fluorescence, F_s) (Buschmann *et al.* 2013). A second treatment with 0.8-s saturating light of 8,000 μmol(photon) m^{-2} s^{-1} was given to determine maximal fluorescence in the light-adapted state (F_m'). Maximal photochemical efficiency (F_v/F_m) of PSII was expressed as $F_v/F_m = (F_m - F_0)/F_m$. Quantum yield of PSII electron transport was determined using $\Phi_{PSII} = (F_m' - F_s)/F_m'$. Nonphotochemical quenching (NPQ) was calculated as $NPQ = F_m/F_m' - 1$. Photochemical quenching (q_p) was calculated as $q_p = (F_m' - F_s)/(F_m - F_0')$ according to Schreiber *et al.* (1994).

Absorbance at 820 nm: The oxidoreductive activity ($\Delta I/I_0$) was measured at 820 nm with a *Plant Efficiency Analyzer* (*PEA Senior*, *Hansatech*, King's Lynn, UK*)* as described by Schansker *et al.* (2003), and the $\Delta I/I_0$ was measured *in vivo* after half an hour dark adaptation of sweet sorghum leaves. The first reliable measuring point for fluorescence change was at 20 μs, whereas the first measuring point for transmission change was at 400 μs. The time constant used for the transmission measurements was 100 μs. The light intensity used for the transmission measurements was 3000 μmol(photon) m^{-2} s^{-1}, and was produced by four 650 nm LEDs (light-emitting diodes). The far-red source was a *QDDH73520 LED* (*Quantum Devices Inc*., Barneveld, WI, USA) filtered at 720 ± 5 nm. The modulated (33.3 kHz) far-red measuring light was provided by an *OD820 LED* (*Opto Diode Corp*., Newbury Park, CA, USA) and filtered at 830 ± 20 nm. Command execution, such as turning on and off the LEDs, took

approximately 250 μs. Commands for activating the red light and starting the measurement were synchronized to correct for the delay; for the far-red light, there was a 250 μs delay between turning on the far-red light and initiation of measurement.

Fresh mass (FM) and dry mass (DM) of shoots and roots of seedlings: The plant material was initially cleaned with distilled water. After the water on the plant was absorbed by tissue paper, FM of the shoots and roots were determined. The DM was determined after drying the fresh material at 70°C for 4 d. Three replicates were performed for each treatment.

Antioxidant enzyme activity in seedlings: SOD (EC 1.15.1.1) activity was measured as described by Dhindsa *et al*. (1981). Samples of leaves (0.2 g) were flash frozen in liquid nitrogen to prevent proteolytic activity, followed by grinding with 5 mL of extraction buffer (0.1 M phosphate buffer, pH 7.5, containing 0.5 mM EDTA and 1 mM ascorbic acid). The homogenate was centrifuged for 20 min at 15,000 × *g* and the collection volume of enzyme solution was 3 mL. The concentration of soluble proteins in the supernatant was determined using the Bradford assay with bovine serum albumin (BSA) as the standard (Bradford 1976). The activity of SOD was estimated by recording the decrease in optical density of nitroblue tetrazolium (NBT) induced by the enzyme (Dhindsa *et al.* 1981). The reaction mixture contained 13 mM methionine, 75 μM nitroblue tetrazolium chloride, 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), 50 mM sodium carbonate, and 0.1 mL of enzyme solution. The reaction was started by adding 2 μM riboflavin. The reaction mixtures were illuminated for 20 min at 90 μmol(photon) m^{-2} s^{-1} (placing the test tubes under two 15-W fluorescent lamps). The control consisted of a complete reaction mixture without enzyme, which gave the maximal color. The reaction was stopped by switching off the light and placing the tubes in the dark. A nonirradiated complete reaction mixture served as a blank. The absorbance at 560 nm of the reaction mixture was determined by using a UV/Vis spectrophotometer (*UV-1601*, *Shimadzu*, Japan). Enzyme activity was calculated as 50% inhibition expressed in U per mg of total protein.

The activity of APX (EC 1.11.1.11) was determined according to the method of Jimenez *et al.* (1997) by monitoring the rate of ascorbate oxidation at 290 nm. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.2 mM H_2O_2, and a suitable volume of enzyme extract. Enzyme activity was calculated per mg of total protein in U, which represents the amount of enzyme needed to oxide 1 μmol of AsA in 1 min at room temperature.

H_2O_2 content was measured as described in Sairam and Srivastava (2002) and Ren *et al*. (2014). Frozen leaf samples (0.2 g) were homogenized in 3 mL of acetone. The homogenate was then centrifuged at 10,000 × *g* for 10 min at 4°C. The supernatant was collected and added to 0.3 mL of a concentrated hydrochloric acid solution (containing 0.1 mL of 20% $TiCl_4$ and 0.2 mL of concentrated ammonia). The mixture was incubated at 25°C for 10 min, and then centrifuged at 8,000 × *g* for 10 min at 4°C. The pellet was washed twice with cold acetone, and then 3 mL of 1 mol L^{-1} H_2SO_4 was added. The absorbance of the solution was measured at 410 nm (*UV-1601*, *Shimadzu*, Japan), and the amount of H_2O_2 was calculated from a standard curve prepared using known concentrations of H_2O_2.

Measurement of MDA content: MDA content was measured as described in Peever and Higgins (1989). Frozen leaf samples (0.2 g) were homogenized in 1 mL of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was treated with 1 mL of 0.1% (w/v) TCA flush and 2.5 mL of tertiary butyl alcohol (TBA). The mixture was heated at 90°C for 15 min and then quickly cooled on ice. The contents were centrifuged at 3,000 × *g* for 10 min and the absorbance of the supernatant at 532 and 600 nm was read (*UV-1601*, *Shimadzu*, Japan). The MDA content was calculated using 155 mM^{-1} cm^{-1} as the extinction coefficient.

Statistical analysis: All analyses were performed with *SPSS Version 16.0* for *Windows* (*SPSS*, Chicago, IL, USA). Multiple comparisons were performed between different environmental conditions using *Duncan*'s test at the 0.05 significance level. Figures were drawn by original data analysis and software *Sigma Plot 10.0*.

Results

Experiments in germination period

Germination rate during drought stress: The germination rate of the two sweet sorghum lines declined obviously under drought stress. After 200 mM mannitol treatment, germination rate of M-81E and Roma decreased by 7.1 and 9.1%, respectively. The germination rate of M-81E and Roma decreased by 49.9 and 43.2% after 400 mM mannitol treatment, respectively. After 600 mM mannitol treatment, germination rate of M-81E and Roma decreased by 61.8 and 79.6%, respectively (Figs. 1*A*, 2*A*). Germination recovery of M-81E was better than that of Roma after 7 d of water restoration; M-81E also had better germination ability than that of Roma following recovery from the high (600 mM) mannitol treatment (Fig. 1*B*).

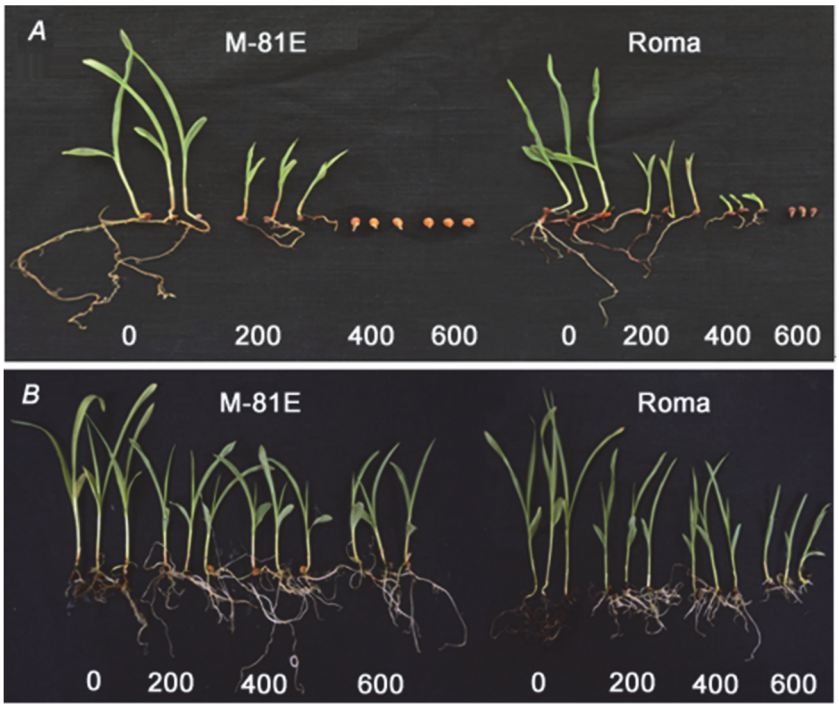

Fig. 1. Germination of two sweet sorghum (*Sorghum bicolor* L. Moench) lines M-81E and Roma under different mannitol concentration after 9 d (*A*) and the 7-d recovery (*B*).

Germination potential during drought stress: The germination potential of both sweet sorghum lines declined significantly under drought stress. After 200 mM mannitol treatment, germination potential of M-81E and Roma decreased by 28.4 and 37.2%, respectively, while the germination potential of M-81E and Roma decreased by 66.7 and 68.6% after 400 mM mannitol, and after the 600 mM mannitol treatment, the germination potential of M-81E and Roma decreased by 71.8 and 83.8%, respectively (Fig. 2*B*). These results showed that germination rate and uniformity of M-81E were higher than those of Roma.

GI during drought stress: The GI (Gt/Dt) of drought resistance decreased with increasing mannitol concentrations for both inbred sweet sorghum lines. After 200 mM mannitol treatment, the M-81E and Roma germination indices decreased by 26.1 and 32.8%, respectively. The germination indices of M-81E and Roma decreased by 65.8 and 64.0% after 400 mM mannitol treatment, respectively, while under the stress of 600 mM mannitol, the germination indices of M-81E and Roma decreased respectively by 72.2 and 86.0% (Fig. 2*C*). The results showed that germination ability and vitality of M-81E were higher under 200 and 600 mM mannitol stress than those of Roma. Under 400 mM mannitol treatment, M-81E had a lower germination ability and vitality than that of Roma.

Increase in germination rate following 7-d drought recovery: The germination rate of both sweet sorghum lines increased significantly following seven days of water restoration after mannitol-induced drought stress. The germination rate of M-81E and Roma increased by 13.3 and 11.1% after recovery from 200 mM mannitol stress, respectively (Fig. 2*D*). After recovery from 400 mM mannitol treatment, the germination rate of M-81E and Roma increased by 53.3 and 42.2%, respectively. This showed that the recovery of M-81E was better than that of Roma (Fig. 2*D*).

Shoot and root length during drought stress and recovery in germination: Shoot and root length of both sweet sorghum lines decreased significantly under mannitol-induced drought stress (Fig. 3). Under 200, 400, and 600 mM mannitol, shoot lengths of M-81E decreased by 45.3, 97.7, and 98.8%, respectively, while shoot lengths of Roma decreased by 50.1, 88.5, and 98.9%, respectively (Fig. 3*A*). Root lengths of M-81E under 200, 400, and 600 mM mannitol treatment decreased by 53.3, 95.5, and 97.7%, respectively, while root lengths in Roma similarly decreased by 52.1, 80.7, and 96.7%, respectively (Fig. 3*B*). However, after 7 d of water restoration, shoot lengths under the 200, 400, and 600 mM mannitol treatments increased by 0.5, 30.1, and 46.0 times in M-81E and 0.3, 4.3, and 39.3 times in Roma, respectively (Fig. 3*C*). Root lengths of M-81E under the 200, 400, and 600 mM mannitol treatments underwent respective increases of 0.9, 18.5, and 37.0 times, whereas root lengths of Roma underwent respective increases of 0.7, 2.1, and 13.1 times (Fig. 3*D*). Our data showed that shoot and root length of M-81E recovered better than those of Roma. Taken

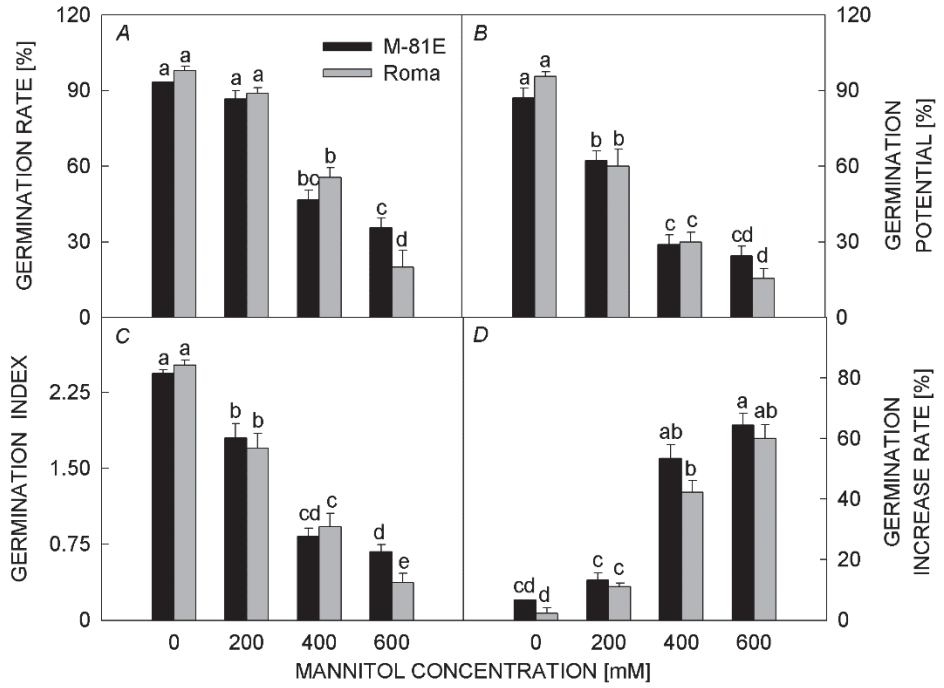

Fig. 2. Germination rate (*A*), germination potential (*B*), germination index (*C*), and germination increase rate (*D*) of two different sweet sorghum lines (*Sorghum bicolor* L. Moench) under different mannitol concentration. Each point represents the means ± SD of five measurements on each of five plants (n = 5). Bars with different *lowercase letters* show significant differences at the P<0.05 level.

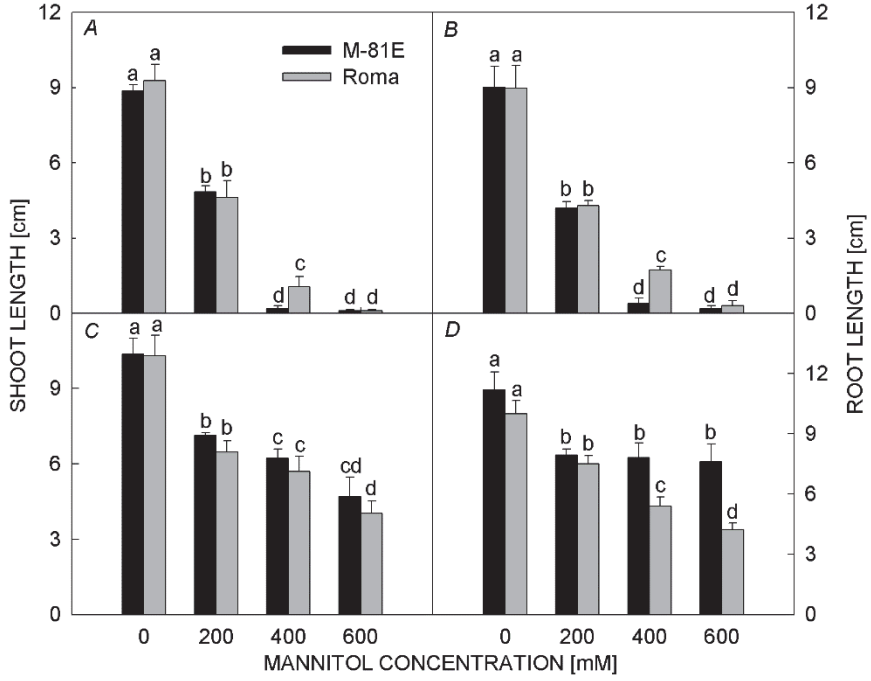

Fig. 3. Shoot and root length of two different sweet sorghum (*Sorghum bicolor* L. Moench) lines under different mannitol concentration (*A*, *B*) and rewatering (*C*, *D*). Values are means ± SD of five measurements for each of five plants (n = 5). Bars with different *lowercase letters* show significant differences at the P<0.05 level.

together, these results showed that M-81E had higher germination rate and recovery ability than those of Roma during the germination period.

Experiments in the seedling period

Photoinhibition and energy dissipation under natural drought stress and rewatering: Photoinhibition of PSII and PSI was estimated by measuring F_v/F_m and $\Delta I/I_0$, respectively. As shown in Fig. 4, the F_v/F_m and $\Delta I/I_0$ decreased in leaves of both sweet sorghum lines under drought stress; however, Roma showed greater decreases of F_v/F_m and $\Delta I/I_0$ than those of M-81E. During drought stress, the respective decreases in F_v/F_m of M-81E and Roma were 4.8 and 12.0%; similarly, the $\Delta I/I_0$ of M-81E and Roma decreased by 18.9 and 36.1%, respectively. The $\Delta I/I_0$ declined markedly relative to the F_v/F_m in the two sweet sorghum lines under drought stress, which indicated that PSI was more sensitive to drought stress than PSII. After 7-d recovery, the F_v/F_m and $\Delta I/I_0$ both returned to normal levels in M-81E, but not in Roma.

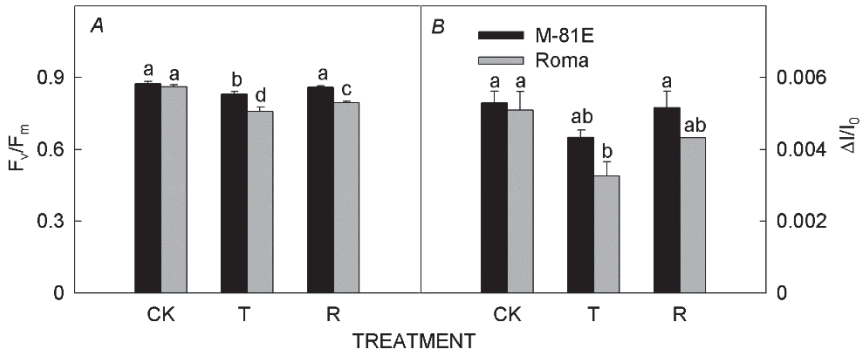

Fig. 4. The maximal photochemical efficiency of PSII (F_v/F_m) and the oxidoreductive activity ($\Delta I/I_0$) in two sweet sorghum (*Sorghum bicolor* L. Moench) lines under natural drought treatment and after withholding water (CK – control, T – natural drought treatment for 7 d, R – rewatering for 7 d, the same below). Values are means ± SD of five measurements for each of five plants (n = 5). Bars with different *lowercase letters* show significant differences at the P<0.05 level.

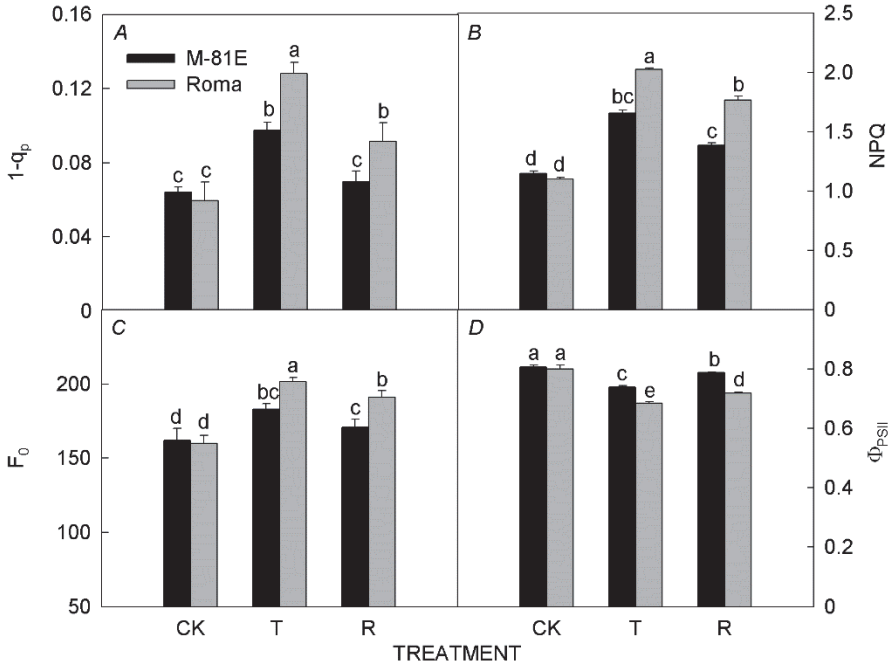

Fig. 5. Changes of the reduction state of Q_A ($1 - q_p$), nonphotochemical quenching (NPQ), minimum fluorescence (F_0), and Φ_{PSII} in two sweet sorghum (*Sorghum bicolor* L. Moench) lines under natural drought treatment and after withholding water. Values are means ± SD of five measurements for each of five plants (n = 5). Bars with different *lowercase letters* show significant differences at the P<0.05 level.

The $1 - q_p$, NPQ, and F_0 in leaves of both sweet sorghum lines increased under drought stress, but to a greater extent in Roma (Fig. 5). The $1 - q_p$, NPQ, and F_0 increased by 51.9, 43.8, and 12.9% in M-81E, and by 114.7, 84.1, and 26.1% in Roma, respectively. During drought stress, Φ_{PSII} of M-81E and Roma decreased by 8.1 and 14.5%, respectively. After 7-d recovery, the $1 - q_p$, NPQ, and F_0 of M-81E increased relative to the untreated plants by 8.8, 20.4, and 5.3%, while they increased in Roma by 53.6, 60.7, and 19.4%, respectively. The Φ_{PSII} of M-81E and Roma decreased by 2.2 and 10.2%, relative to control group, respectively. These data showed that the recovery of M-81E was better than that of Roma.

Effect of drought stress on FM and DM at the seedling stage: The degrees of drought tolerance and recovery capability during the seedling stage were better in M-81E than those in Roma. FM and DM of shoots and roots were significantly reduced for both sweet sorghum lines grown under natural drought conditions. Shoot FM of M-81E and Roma under drought stress decreased by 26.9 and 33.6%, respectively (Fig. 6*A*). Shoot DM of M-81E and Roma under natural drought decreased by 21.7 and 31.8%, respectively (Fig. 6*B*). Root FM of M-81E and Roma under drought decreased by 21.4 and 52.2%, respectively (Fig. 6*C*). Root DM of M-81E and Roma under natural drought stress decreased by 16.7 and 52.2%, respectively (Fig. 6*D*). After rewatering for 7 d, FM and DM of shoots and roots increased compared to those of plants under natural drought stress; the respective values for shoot FM of M-81E and Roma increased by 33.2 and 9.6% (Fig. 6*A*), respectively; shoot DM of M-81E and Roma increased by 22.2 and 20.0% (Fig. 6*B*), respectively; root FM of M-81E and Roma increased by 23.1 and 15.5% (Fig. 6*C*) and root

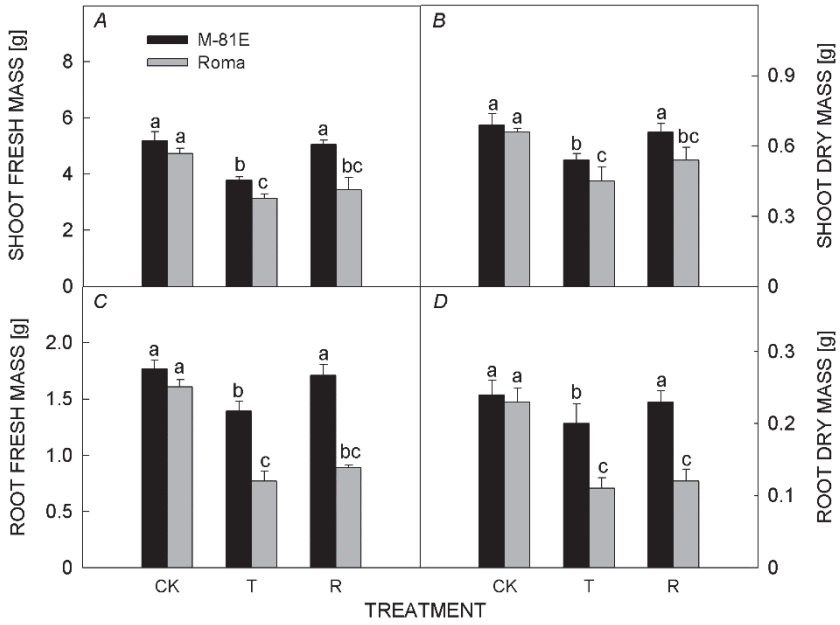

Fig. 6. Shoot and root fresh mass and dry mass in two sweet sorghum (*Sorghum bicolor* L. Moench) lines under natural drought treatment and after withholding water. Values are means ± SD of five measurements for each of five plants (n = 5). Bars with different *lowercase letters* show significant differences at the P<0.05 level.

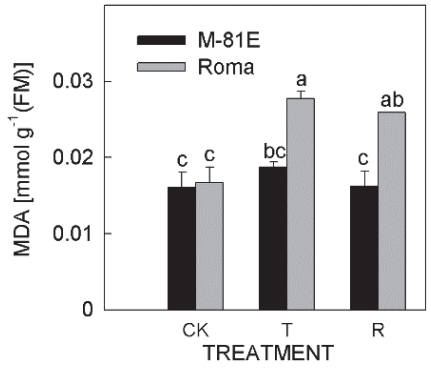

Fig. 7. Changes of MDA content in two sweet sorghum (*Sorghum bicolor* L. Moench) lines under natural drought stress and rewatering. Values are means ± SD of five measurements for each of five plants (n = 5). Bars with different *lowercase letters* show significant differences at the P<0.05 level.

DM of M-81E and Roma increased by 15.0 and 9.1%, respectively (Fig. 6*D*).

Effect of drought stress on antioxidant enzyme activities and H_2O_2 content: SOD is one of the key enzymes for scavenging toxic ROS. After 7-d treatment with drought stress, SOD activities of M-81E and Roma increased 1.6 and 1.1 times relative to their respective controls (Fig. 7*A*). After 7-d rewatering, SOD activity of M-81E and Roma both decreased, but to a greater extent in Roma. These results showed that M-81 had higher antioxidant enzyme activity than that of Roma.

Under drought conditions, APX activity increased in M-81E and Roma by 1.7 and 0.9 times, respectively (Fig. 7*B*). After 7-d rewatering, APX activity of M-81E recovered to normal levels, while that of Roma recovered slightly relative to the untreated plants. These data showed that M-81E had stronger scavenging ability than Roma.

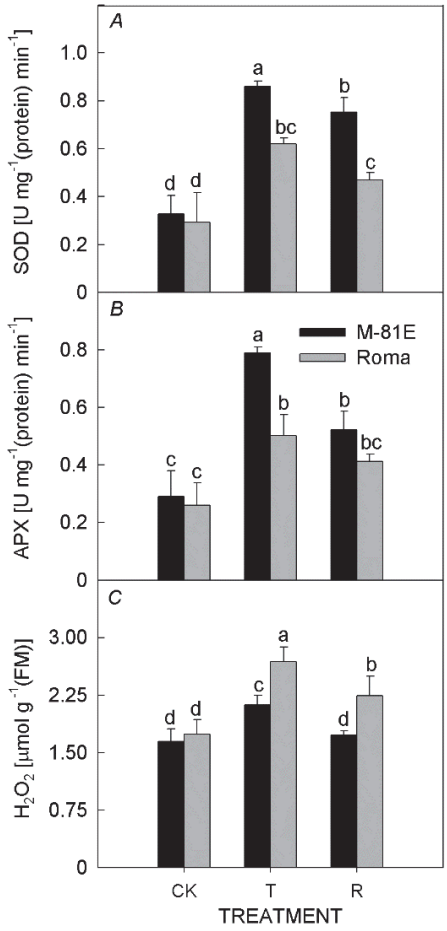

Fig. 8. Changes in the activities of SOD (*A*) and APX (*B*), the content of H_2O_2 (*C*) in two sweet sorghum (*Sorghum bicolor* L. Moench) lines under natural drought treatment and after withholding water. Values are means ± SD of five measurements for each of five plants (n = 5). Bars with different *lowercase letters* show significant differences at the P<0.05 level.

Content of H_2O_2 increased under drought stress and then decreased after rewatering (Fig. 7*C*), and the respective increases of drought-induced H_2O_2 content of M-81E and Roma were 28.9 and 54.9%. After 7-d recovery, the H_2O_2 of M-81E decreased to normal content, while those of Roma remained higher than in the untreated control.

Effect of drought stress on MDA content at seedling stage: Under drought stress, the MDA content of M-81E and Roma increased by 16.8 and 65.8%, respectively; after 7-d recovery, the MDA content of M-81E decreased to normal level, while that of Roma remained high (Fig. 8). These results showed that damage of cell membranes in M-81E was less severe than that in Roma.

Discussion

Drought stress often adversely affects plant growth and development, resulting in severe crop losses. With fresh water resources in shortage, drought has become a serious problem in agricultural production worldwide; thus, the research has focused on understanding the mechanisms of drought stress in plants and the selection of drought-resistant varieties. In this study, we investigated the effects of drought stress on seed germination, the antioxidant enzyme systems, and energy dissipation-mediated photoprotection of two inbred sweet sorghum lines.

Germination rate and germination indices under drought stress are predictive of drought tolerance in sweet sorghum. Germination potential is an index for evaluation of the seed germination rate and germination uniformity; it measures plant germination ability and vitality. Our results showed obvious declines in the germination rate, germination potential, and germination index for the two sweet sorghum lines with increasing mannitol concentrations. The germination rate, germination potential, and germination index of M-81E decreased less than those of Roma under 200 and 600 mM mannitol stress (Fig. 2*A–C*), which showed the better germination rate and stability for M-81E than that for Roma. The germination rate and germination index of M-81E decreased more than those of Roma under 400 mM mannitol, which showed that M-81E adapted poorly to the stress of 400 mM mannitol, and this could represent a transitional phase; at higher mannitol concentrations, M-81E began to adapt to the stress-inducing mannitol. After 7 d of water restoration, germination percentage increase of M-81E was higher than that of Roma (Fig. 2*D*), suggesting that M-81E had a stronger ability to recover. Shoot and root length of both sweet sorghum lines decreased significantly with increased mannitol concentrations; however, M-81E shoot length decreased less than that of Roma under stresses of 200 and 600 mM mannitol (Fig. 3*A*), but M-81E root length decreased more than that of Roma (Fig. 3*B*). After 7 d of water restoration, shoot and root length of M-81E increased more than that of Roma (Fig. 3*C*,*D*); this demonstrated that the stronger ability of M-81E to recover might be related to the powerful osmotic adjustment ability to ensure establishment of seeds and seedling under different drought stresses. In order to further illustrate the drought resistances of the two sweet sorghum inbred lines, we also performed the experiment during the seedling stage.

Plants respond to water stress by closing stomata, which reduces CO_2 availability in the chloroplasts, progressively decreasing photosynthesis and photosynthetic capacity (Lawlor 1995, Reddy *et al*. 2004, Ashraf 2013). The activities of PSII and PSI appeared to be inhibited in response to drought stress in plants such as *Tuberaria major* (Cistaceae) (Osório 2013), and this occurred to a greater extent in Roma compared to M-81E (Fig. 4). We also demonstrated that PSI of Roma was more sensitive to drought stress than PSII (Fig. 4). After 7 d of water restoration, recovery ability of M-81E was better than that of Roma. PSII is thought to perform a vital function in the response of leaf photosynthesis to environmental stresses (Baker 1991). The lower utilization of light energy inevitably results in an excess of light energy, and PSII consequently becomes overexcited. Our results showed that excess energy produced in Roma under drought stress resulted in accumulation of electrons at the PSI acceptor side (Inoue *et al*. 1989).

PSII photoinhibition is closely related to the redox state of Q_A under a range of stress conditions (Havaux *et al*. 1991). The relative redox state of Q_A *in vivo* can be estimated as $1 - q_p$ (Qin *et al*. 2011). Results showed that $1 - q_p$ in both sweet sorghum lines increased under drought stress, with a greater increase in Roma (Fig. 5*A*). This suggested that the extent of Q_A reduction in Roma was more severe under drought stress. The increase of $1 - q_p$ was accompanied by an increase in NPQ (Fig. 5*B*), suggesting that the extent of PSII photoinhibition correlated closely with the redox state of Q_A (Xu *et al*. 1999). Changes of F_0 depend on the dominant factor between the energy dissipation and damage to PSII. We showed that the F_0 of M-81E increased less than that of Roma under drought treatment (Fig. 5*C*), which indicated that damage to PSII of Roma was greater than that of M-81E. We found that Φ_{PSII} of M-81E decreased less than that of Roma (Fig. 5*D*), which suggested that M-81E also had a higher photosynthetic capacity. After 7 d of water restoration, M-81E had a greater capacity for recovery than Roma; this suggested that under drought stress, the PSII of M-81E experienced less photoinhibition than that of Roma, and energy dissipation was effective at preventing damage to the M-81E PSII reaction center.

In this study, we observed higher shoot and root FM and DM in M-81E than in Roma under drought stress, and M-81E could recover quickly relative to Roma after rewatering for 7 d (Fig. 6). If excess energy in plants could not be dissipated and CO_2 assimilation was blocked, PSII reaction centers were completely reduced to produce

triplet Chl; it could easily react with singlet oxygen to generate ROS in normal metabolic processes (Asada 1992, 1999), especially under environmental stress. The resulting deficit in removal of ROS in plant cells leads to excessive accumulation of free radicals, which triggers or aggravates membrane lipid peroxidation of cells, causing damage to the plant (Fridovich 1975, Smirnoff 1993, Mehdy 1994). Meanwhile, plants contain various non-enzymatic antioxidants, such as ascorbate, glutathione, flavonoids, and carotenoids; they also contain enzymatic antioxidants, such as SOD, APX, and catalase (CAT) (Song *et al*. 2005). These all function to properly scavenge the toxic ROS and protect the plants from ROS damage (Noctor and Foyer 1998). Under drought stress, the activities of SOD, APX, and CAT are positively correlated with drought resistance (Jiménez and Pick 1993, Scandalios 1993). Drought-resistant varieties could maintain higher SOD, APX, and CAT activity than varieties with weaker drought resistances. SOD activity is crucial to enzymatic scavenging of active oxygen. When exposed to drought stress, the antioxidative enzyme system seemed to function better in M-81E than in Roma (Fig. 7). During drought stress, the ETC tends to form $O_2^{\bullet -}$, which could be metabolized by SOD to form H_2O_2. When exposed to drought stress, the SOD activity of M-81E was higher than that of Roma (Fig. 7*A*). APX, which eliminates peroxides by converting AsA to dehydroascorbate, is one of the most important enzymes for eliminating toxic H_2O_2 in plants (Foyer *et al*. 1994). Activities of APX increased in both sweet sorghum lines under drought stress (Fig. 7*B*); however, the APX activity of M-81E increased more than that of Roma under drought stress. The H_2O_2 contents of M-81E were consistently lower than those of Roma under drought stress, and it seems that H_2O_2 could not be scavenged efficiently in Roma under drought stress with the relatively lower SOD and APX activities (Fig. 7*C*). The higher H_2O_2 contents of Roma also enhanced photoinhibition of PSII and PSI (Fig. 4), and even damaged the PSII reaction centers. After rewatering for 7 d, H_2O_2 contents decreased more quickly in M-81E than in Roma (Fig. 7*C*); this showed that when environmental conditions improve, the growth and physiological parameters of M-81E could recover more quickly than those of Roma.

Changes of cell membrane permeability in plants under stress conditions can reflect the extent of damage (Agarie *et al*. 1998). The strongly cytotoxic MDA is one of the main products of membrane lipid peroxidation, and the degree of damage to plants positively correlates with MDA accumulation under stress conditions, but is negatively related to drought resistance (Wang and Luo 1990, Bai *et al*. 2011). Antioxidant enzyme activity and MDA content could be used to identify and select drought-resistant plants (Li *et al*. 2008). In this study, the leaf MDA content of M-81E was lower than that of Roma under natural drought stress. After rewatering for 7 d, leaf MDA content of M-81E decreased more significantly than that of Roma (Fig. 8). This showed that the stress-related damage to Roma was more serious, and M-81E had stronger drought resistance.

In conclusion, all the results showed that under same drought stress, M-81E had stronger recovery ability during the germination period. Compared to Roma at the seedling stage, M-81E showed stronger osmotic adjustment and more powerful antioxidative enzyme system, which ensured establishment of seeds and seedlings during drought conditions.

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