

# Short-term physiological responses to drought stress in seedling of tropical and temperate maize (*Zea mays* L.) cultivars

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## Abstract

Understanding of the response of tropical and temperate maize (*Zea mays* L.) to drought is the first step for tolerant temperate maize improvement. Eight maize hybrids were used to investigate physiology responses under drought stress, four of them were tropical maize and the others were temperate maize. Results showed that there were different drought tolerances but similar trends in both tropical maize and temperate maize. Gas exchange parameters revealed different strategies of maize under the stress. In our study, most of the temperate hybrids maintained open stomata to keep a higher photosynthesis rate at the beginning of stress, while the other hybrids decreased stomatal conductance. Compared to temperate maize, the tropical maize had higher antioxidase activity and greater physiological parameter variation among hybrids. KS5731 and ZD309 had stronger drought resistance among tropical and temperate maize hybrids separately. Tolerant hybrids maintained active photosynthesis, have higher osmotic adjustment ability and antioxidase activities but lower malonaldehyde content than the sensitive ones. Our results led to a better understanding of the physiological responses of tropical and temperate maize plants to drought stress and may provide an insight of breeding for drought resistance in maize.

## Introduction

Drought is one of the major constraints for plant productivity and has become increasingly serious globally. Maize is known to be susceptible to even mild or moderate drought particularly at the beginning of plant growth, drought may also dramatically limit the biomass production and the photosynthetic ability of leaves and thus indirectly negatively affect the formation of reproductive organs and yield parameters.<sup>1-3</sup> Drought stress occurred during maize growth period may hamper the nitrogen and water use efficiencies.<sup>4</sup>

Drought-related physiological and metabolic changes might be helpful in determining the sensitivity or tolerance of a plant under water deficit conditions and can be used as stress indicators. At present, research on maize drought resistance has been carried out world widely, researchers have found many physiological indicators related to drought tolerance, such as relative water con-

tent (RWC), photosynthesis related indicators, MDA, proline, polysaccharides, antioxidant enzymes.<sup>5</sup> Efeoğlu *et al.*<sup>6</sup> conducted a 12 days of drought stress to maize seedling, the results showed that RWC, photosynthetic parameters and chlorophyll decreased in all cultivars by drought whereas anthocyanin and proline contents increased. Messina *et al.*<sup>7</sup> found that the transpiration response of maize hybrids to drought stress has genotypic diversity. Many genes that are expressed differentially in responses to drought stress affect photosynthetic systems and hormone biosynthesis. Noman *et al.*<sup>8</sup> found that drought stress could enhance the superoxide dismutase (SOD) and peroxidase (POD) activities in maize.

Due to a unique genome structure and continuous human selection for over 7000 years, maize can be divided into two main groups, tropical and temperate maize. And it is one of the most resilient plant species can grow at different altitudes and climates.<sup>9</sup> Significant differences in artificial and natural selection involved in maize domestication and improvement have contributed to its adaptation to different agro-climatic zones. Productivity of maize in the tropics is much lower than in temperate areas. When maize varieties adapted to tropical latitudes are grown in temperate environments such as the US Corn Belt, they flower later and produce little or no grain, but have higher total biomass yields compared to modern commercial maize grain hybrids. Compared with temperate maize, the tropical maize has high resistance to various of disease and insect pests and also have better tolerance to abiotic stresses.<sup>10</sup> Detailed knowledge of maize germplasm, particularly temperate and tropical types, is important not only for parental selection but also for genetic analysis and breeding.

The presented study attempts to enhance our knowledge of maize responses to drought at the early developmental stages in tropical and temperate maize, and to determine the physiological indicator traits that can discriminate maize lines for their tolerance under drought stress. The research result of germplasm drought resistance mechanism from different sources will provide a theoretical basis for future breeding practice.

## Materials and Methods

### Plant material

Eight maize hybrids were used for this work. Zhengdan 958 (ZD958), Denghai 605 (DH605), Nonghua101 (NH101), Zhengdan 309 (ZD309) are temperate maize. NS3, SW2301, SW4452, KS5731 are tropical maize and were supplied by Kasetsart University, Thailand.

### Growth conditions and drought stress treatments

The seeds were cultured with 1/2 Hoagland nutrient solution under greenhouse condition. Temperatures were between 25°C to 27°C and relative humidity was 60±5%. Maize plants were watered with nutrient solution daily until application of stress. V3 stage plants were treated with 15% PEG for three days. The drought stress samples were collected at day 0, 1, 2, 3.

### Physiology measurements

The samples were cut from the middle of the 4th leaf to measure the fresh mass (FM), saturated mass (SM) were measured after the same leaf parts hydrated in the dark for 5 h. These leaves were oven-dried at 80°C for 48h to determine dry mass (DM). The RWC was calculated as  $100 \times (FM - DM) / (SM - DM)$ .<sup>11</sup>

Leaf membrane permeability was assessed by the electrolyte

leakage percentage and measured by conductivity meter. For measurement of membrane permeability, 0.2 g leaf was cut into filaments about 1 mm and soaked in 15ml distilled water for 24 h. Conductivity of the solution was determined at the end of incubation by DDSJ-308A conductivity meter, recorded as EC<sub>1</sub>. After 30 mins boiling water bath, it was measured again and recorded as EC<sub>2</sub>. Membrane permeability was calculated as percentage of EC<sub>1</sub>/EC<sub>2</sub>.<sup>12</sup>

Proline, malonaldehyde (MDA), the activities of POD, catalase (CAT) were extracted by using biochemical kit (Suzhou Comin Biotechnology Co., Ltd., Suzhou, China) and measured spectrophotometrically (Tecan infinite 200 Pro, Männedorf, Switzerland).<sup>13</sup>

The middle part of the fully expanding leaf was used for the measurements of transpiration rate (E), net photosynthesis rate (P<sub>n</sub>), stomatal conductance (G<sub>s</sub>) and intercellular CO<sub>2</sub> concentration (C<sub>i</sub>). These leaf parameters were measured *in situ* by the portable gas exchange system CIRAS-3 (PP Systems Ltd., USA).

### Data process

All data were processed by the software of Microsoft Excel 2007 and OriginPro 9.1, analyzed by SPSS19.0 with the method of ANOVA and multiple comparisons (Duncan's Multiple Range Test).

## Results

### Relative water content of leaves of drought stressed maize

The PEG treatment significantly reduces the RWC of maize. SW2301, DH605 and NH101 decreased slightly on the first day after treatment, and then decrease significantly ( $P < 0.05$ ) on the second day. KS5731 remained stable for the first two days of treatment, but fell to 85.4% on the third day. NS3 had a gradual decreasing trend from 90.3% to 65.0%. The RWC of SW4452 decreased sharply on the first day after treatment. After 3 days of drought treatment, the RWC of KS5731 and ZD309 was still above 80%, of which KS5731 was the highest; SW4452 reduced to 54.9%, the lowest among all hybrids (Figure 1).

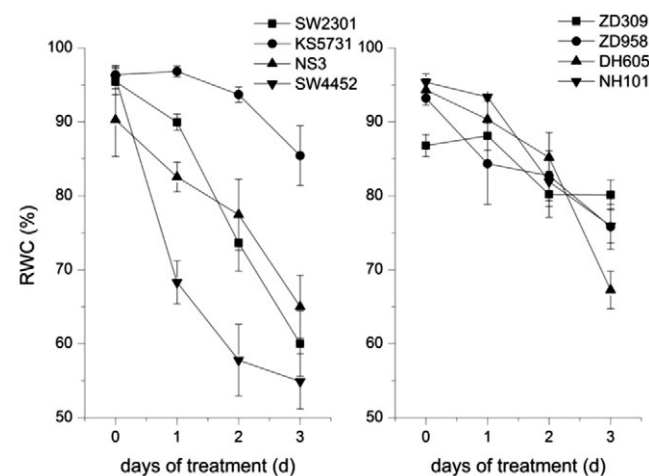


Figure 1. Relative water content of different maize hybrids under drought treatment. RWC, relative water content.

## Gas exchange of leaves of drought stressed maize

The initial net photosynthesis rate ( $P_n$ ) of tropical maize seedlings showed decline trend after treatment. In temperate maize, the  $P_n$  raised for a short time after drought stress, and then reduce to a similar level around  $3.3 - 7.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ . After 3 days of treatment,  $P_n$  of KS5731 was still the highest of all, other tropical maize decreased by  $7.7-10.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ , while temperate maize decreased  $4.9-8.5 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Figure 2A). The stomatal conductance ( $G_s$ ) and transpiration rate ( $E$ ) of maize under drought stress showed a similar trend to the  $P_n$ . After 3 days of simulated drought stress, the  $G_s$  of KS5731 decreased by  $50.4 \text{mmol m}^{-2} \text{s}^{-1}$ , and other tropical hybrids were lower than  $25.9 \text{mmol m}^{-2} \text{s}^{-1}$ . Temperate hybrids also fell to  $12.5-28 \text{mmol m}^{-2} \text{s}^{-1}$  after treatment (Figure 2B). And transpiration rate of all hybrids had fallen below  $0.95 \text{mmol m}^{-2} \text{s}^{-1}$ , while KS5731 was still the highest. The transpiration rate of temperate hybrids has a consistent response to drought treatment, all of them decreased drastically on the second day (Figure 2C). After 3 days of drought treatment, intercellular  $\text{CO}_2$  concentration ( $C_i$ ) of most the tropical maize hybrids increased, and no significant changes in temperate maize (Figure 2D).

## Cell membrane stability and osmotic adjustment

The electrolyte leakage percentage of leaves increased under drought treatment. KS5731, NS3, ZD958, DH605 and NH101 increased significantly ( $P < 0.05$ ) after 3 days of treatment. NS3 and SW4452 leakage percentage remain under 50% (Table 1).

The MDA content of leaves under drought stress increased at the beginning and then decreased. The MDA of SW2301 and SW4452 continued to increase by 2 days after treatment, while NS3 and all temperate hybrids increased only on the first day, and then gradually declined. In tropical hybrids, SW2301 and SW4452 reached the highest MDA content in the second day as  $15.5 \text{nmol g}^{-1}$  and  $15.8 \text{nmol g}^{-1}$  respectively. Temperate hybrids ZD309 and DH605 peaked  $13.4 \text{nmol g}^{-1}$  on the first day after treatment (Figure 3A). After 3 days of treatment, the MDA of most hybrids drops to the pre-treatment level.

The content of proline showed a gradual increase trend after drought treatment. Before treatment, the proline in temperate hybrids was higher, among which ZD309 and ZD958 were the most prominent. After 3 days of treatment, SW4452 had the largest increment of  $59.9 \mu\text{g g}^{-1}$  (Figure 3B).

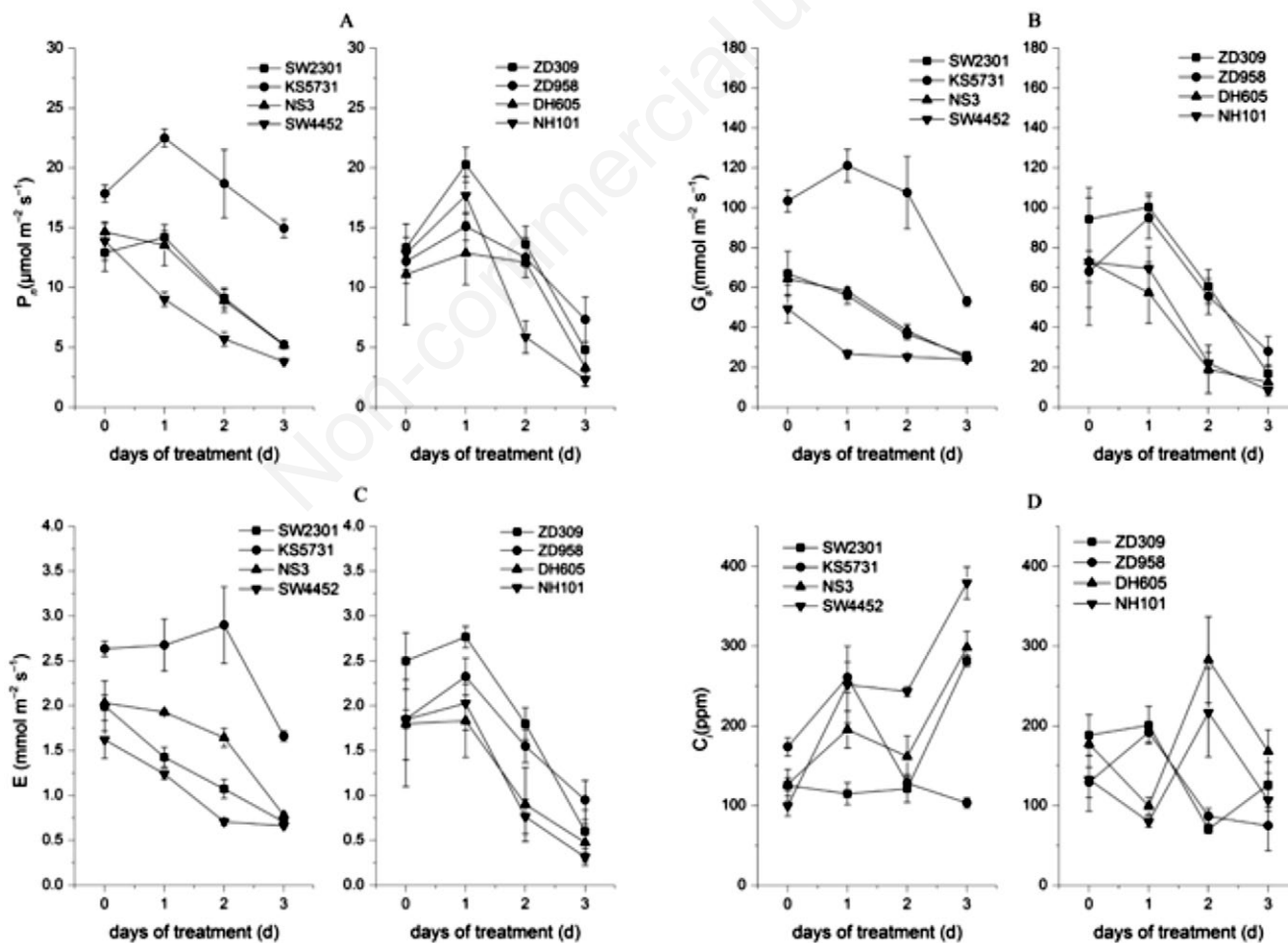


Figure 2. (A) The net photosynthetic rate ( $P_n$ ); (B) stomatal conductance ( $G_s$ ); (C) net transpiration rate ( $E$ ); (D) intercellular  $\text{CO}_2$  concentration ( $C_i$ ) in the leaves of drought stressed maize.

## Antioxidase activities in the leaves of drought stressed maize

The CAT activity in the leaves of maize seedlings increased rapidly after drought treatment, while ZD309 was an exception. This growth was a short-term effect. The CAT activity of most hybrids (except KS5731) declined on the third day of treatment but still higher than that before treatment. The CAT activity of tropical hybrids responded to drought stress more obviously, and the highest activities could be 1842.6-2477.7 U g<sup>-1</sup>. The response of temperate hybrids was not obvious. The highest activity of ZD958 was only 1675.8 U g<sup>-1</sup> (Figure 4A). The response of POD activity of different hybrids to drought varied greatly. The POD activity of most hybrids increased after treatment, but the rate of increase was less than that of CAT. The POD activity of KS5731, SW4452 and NH101 decreased in the first day of treatment, and then gradually increased. The initial POD activity of tropical hybrids was between 3365-4083 U g<sup>-1</sup> and higher than that of most temperate hybrids. SW2301, NS3 and SW4452 had relatively high POD activity after 3 days of treatment (Figure 4B).

## Discussion

In our study, drought could significantly ( $P < 0.05$ ) reduce the maize RWC. Many studies had shown that the RWC of plant leaves decreased after PEG induced drought stress. Siddique *et al.*<sup>14</sup> found that drought stress significantly reduced RWC for 43% during wheat development, similar results had been confirmed in barley, rice, peanut and other crops.<sup>15-17</sup> The RWC of KS5731 and ZD309 declined slowly, suggesting that the hybrids in both tropical and temperate maize with stronger drought resistance had a strong water retention capacity.

In gas metabolism such as carbon assimilation, respiration and transpiration, stomata control the fluxes of gases between the atmosphere and the plant interior.<sup>18</sup> The net photosynthesis rate, transpiration rate and stomatal conductance of maize seedlings had same change trend after treatment and all decreased at 3<sup>rd</sup> day, indicating that drought caused stomatal closure, and absorption of CO<sub>2</sub>

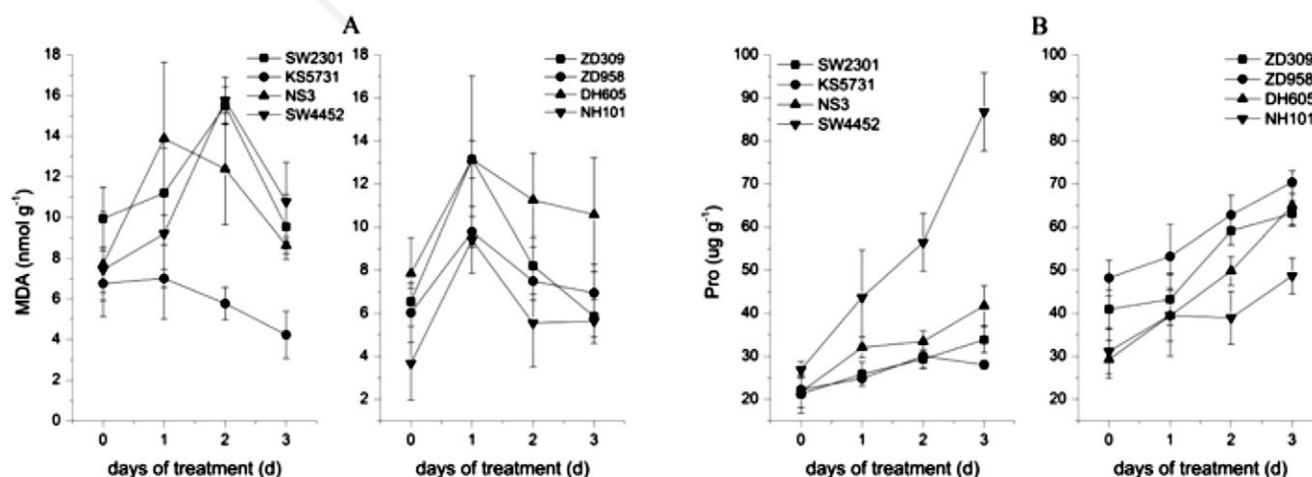
was also inhibited. The deleterious effect of drought maybe targeted photosynthesis.<sup>19,20</sup> Most of the temperate maize hybrids maintained open stomatal and efficient transpiration. This situation may lead to a greater water loss but maintained efficient photosynthesis. It's a risky strategy of maintaining stomata open under drought conditions, however it would be beneficial under a short time, as the plant would be able to retain a relatively normal growth capacity.<sup>1</sup> The gas exchange index of KS5731 and ZD958 indicate that they have good resistance under drought conditions.

Both electrolyte leakage percentage and MDA can reflect the damage degree of cell membrane, usually the lower index value represents the stronger drought resistance. In terms of electrolyte leakage, most of the tropical maize hybrids, KS5731, NS3 and SW4452 have good cell membrane stability under drought stress, suggesting that tropical maize has strong cell membrane stability. MDA could represent the degree of lipid peroxidation.<sup>21</sup> The MDA content of KS5731, ZD309, ZD958 and NH101 was reduced after drought treatment, suggesting that they had strong lipid antioxidant and self-healing capacity under stress. The proline of SW4452, ZD309, ZD958 and DH605 increased after treatment, which may

**Table 1. Electrolyte leakage of different maize hybrids under drought treatment.**

Hybrids	Electrolyte leakage (%)		
	0d	2d	3d
SW2301	53.4±9.8 <sup>a</sup>	63.8±14.8 <sup>a</sup>	63.3±4.2 <sup>a</sup>
KS5731	22±2.5 <sup>a</sup>	26.7±8.4 <sup>a</sup>	50.1±1.3 <sup>b</sup>
NS3	28.9±4 <sup>a</sup>	43.3±7.9 <sup>ab</sup>	47.8±10.6 <sup>b</sup>
SW4452	41.5±6.1 <sup>a</sup>	46.2±4.4 <sup>a</sup>	49.5±5.7 <sup>a</sup>
ZD309	43.2±5.8 <sup>a</sup>	52.9±2.4 <sup>a</sup>	50.2±8 <sup>a</sup>
ZD958	39.9±4.3 <sup>a</sup>	53.7±9 <sup>a</sup>	77.9±10 <sup>b</sup>
DH605	41.2±14.3 <sup>a</sup>	46.7±1.9 <sup>ab</sup>	65.9±5 <sup>b</sup>
NH101	44.3±5.1 <sup>a</sup>	55.4±9.5 <sup>ab</sup>	63.8±11.9 <sup>b</sup>

a, b represent significant differences in the same hybrid ( $P < 0.05$ ).



**Figure 3. The malonaldehyde (A) and proline (B) content in the leaves of drought stressed maize.**

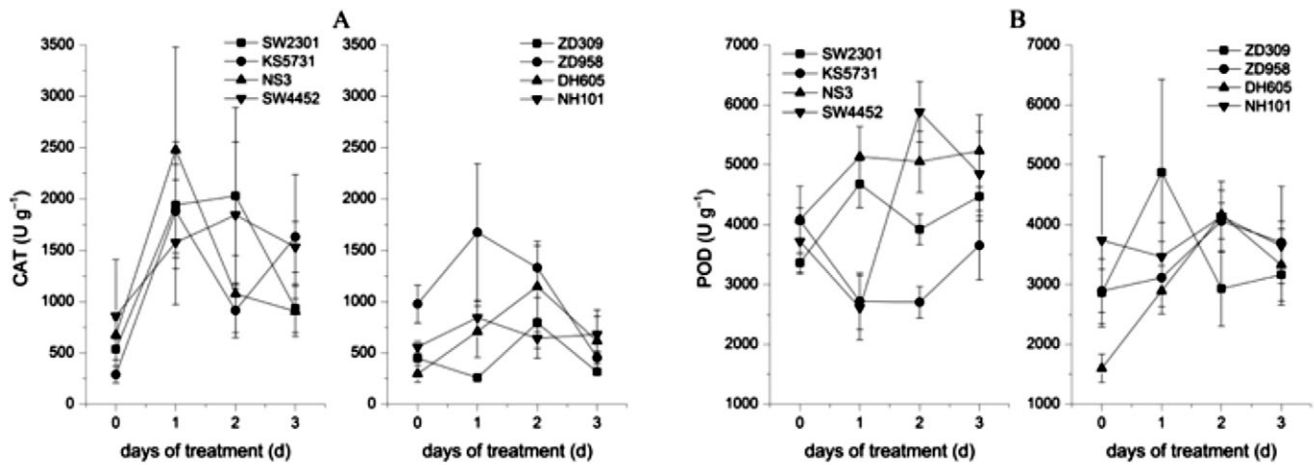


Figure 4. The activities of antioxidant enzymes in the leaves of drought stressed maize.

suggest these four hybrids have advantages in osmotic regulation. Some researchers speculated the accumulation of proline may be the adaptation of maize to drought.<sup>22,23</sup> Our results showed that the proline level had a negative trend with injury parameters (MDA and electrolyte leakage) under drought stress, indicating proline is an important resistance factor in the early stage of drought.

Studies had shown that drought stress induced an increase in reactive oxygen of plant leaves, accompanied by the antioxidant system activation.<sup>24–26</sup> Wang *et al.*<sup>27</sup> found that activities of POD and CAT were increased under light drought stress, while decreased under higher level drought stress. Our results also confirmed this phenomenon, activities of both enzymes in most maize seedlings shortly increased after drought stress, but decreased as the drought duration prolong. Most antioxidant activity of the tropical maize was higher than temperate maize after three days of treatment, indicating the antioxidant system of tropical maize could be rapidly activated by drought stress and maintained for a period of time. KS5731 and SW4452 could maintain high CAT activity over a relatively long period of drought stress, suggesting that these two cultivars had some potential on drought resistance.

Temperate maize has the advantage of high yield. Tropical maize has abundant stress related genes, especially drought related genes. Tropical germplasm is a key resource to improve the genetic resiliency of temperate maize.<sup>28,29</sup> Physiological parameters in different tropical maize hybrids showed great difference under drought stress, while the temperate maize showed a relatively consistent performance, which suggested that tropical maize had greater genetic diversity than temperate maize. Lai *et al.*<sup>30</sup> found tropical maize had more unique non-redundant TEs (NRTEs) than temperate maize. The drought-related genes (such as *dhn1* and *olc1*) in tropical maize have potential roles in drought tolerance.<sup>31</sup> As the global climate change and water shortage, it is very urgent to screen drought resistance traits from tropical maize germplasm resources.

## Conclusions

The determination of drought related physiological parameters of maize hybrids showed that there were different drought tol-

erances but similar responses in both tropical maize and temperate maize. The tropical maize may have greater genetic diversity than temperate maize. KS5731 and SW2301, ZD309 and DH605 have stronger drought resistance in tropical and temperate maize hybrids separately. Different strategies were used to tolerate drought stress in maize. In our study, most of the temperate hybrids maintained open stomata to keep a higher photosynthesis rate at the beginning of stress. Compared with sensitive hybrids, tolerant hybrids maintained active photosynthesis rate, had higher osmotic adjustment ability and antioxidant activities but lower MDA content. Our results led to a better understanding of the physiological responses of tropical and temperate maize plants to drought stress and may provide an insight of breeding for drought resistance in maize.

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