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Full Length Article

Effects of Water Regulation on Growth, Physiology and Chlorophyll Fluorescence of two Maize Lines

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Abstract

Changes and responses of the physiological characteristics of two maize lines H242/1523 and H14/A18 to water regulation in the field were studied. Growth, physiology and photosynthetic parameters and chlorophyll fluorescence parameters of the maize lines have been investigated under low, medium and high irrigation levels. The results showed that, under three irrigation treatments, the phenotypic traits (plant height, stem diameter, SPAD value, number of leaves and stay-green ability), antioxidant enzymes (SOD activity and POD activity), osmoregulatory substances (soluble sugar and soluble protein) and yield indicators (dry forage yield and 100-grain weight) all were found higher in H242/1523 than H14/A18. However, the plant height and the number of leaves of H242/1523 were lower than H14/A18 under low water irrigation conditions. With the increase of irrigation amount, all the above indicators showed an upward trend. For cell membrane relative permeability (EL) and membrane lipid peroxidation product (MDA), H242/1523 was lower than H14/A18, and with the increase of irrigation level, the changes of EL and MDA were contrary to the above indicators. Based on the biordered graphs of the correlation matrix, there was a certain correlation between the variables. Among them, the plant height was highly positively correlated with leaf number and hay yield. SPAD values were highly positively correlated with stem diameter and stay-green ability, but SPAD values were negatively correlated with EL and MDA. There was a highly positive correlation between EL and MDA, but a very significant negative correlation with stay-green ability and hay yield. Soluble sugar content correlated highly and positively with SOD and soluble protein. Further analysis of the clusters of correlation matrix PCA, the results revealed that H242/1523 had strong drought resistance and H14/A18 had poor drought resistance. Therefore, H242/1523 is more suitable for cultivating and planting in water-deficient areas like Yellow River Irrigation Area of Ningxia. The maize line H242/1523 can also be provided as an excellent germplasm resource for the drought-resistant breeding program and water-saving cultivation research. © 2019 Friends Science Publishers

Key words: Maize; Drought stress; Osmoregulation substance; Anti-oxygen protective enzyme; Principle components analysis; Cluster analysis

Introduction

Nowadays, drought is the most major abiotic stress confronting agricultural productivity (Wang *et al.*, 2003; Bibi *et al.*, 2010; Huang *et al.*, 2018). Until recently, a number of research works show that nearly 40% of the world's land area suffered arid or semiarid conditions. It is estimated that by 2025, world's dryland will account 48% of the total land surface (Kawai *et al.,* 2014; Huang *et al.,* 2016). In China, northwest region has been subjected under arid and semi-arid belt, with less rainfall and extremely high evaporation. Ningxia is located in the eastern part of the northwestern region, with a land area of 66,400 km^2 and an average annual precipitation of 300 mm. Among the land types of this region, 64.1% are arid lands and 27.0% are semi-arid lands. The Yellow River irrigation area covers 6.61 million mu and accounts 1% (Wang and Gao, 2010). Drought poses serious threat to local agricultural production and development through affecting the normal growth and development of plants (An *et al.,* 2011; Shan *et al.,* 2012).

The drought problem has been addressed by many researchers and in a number of ways. For example, in arid regions water conservancy construction and expansion of irrigated areas are strongly carried out. Otherwise, irrigation efficiency could be increased through the use of scientific irrigation techniques (such as sprinkler irrigation, drip irrigation, and furrow irrigation). All these methods are however, time-consuming and labor-intensive. In addition, water resources in this region are very deficient and if used improperly this could lead to a massive wastage of water resources. This in turn can exacerbate desertification in the northwest. Some studies have found that biological improvement can be used as a most effective method to solve drought problems (Glenn and Brown 1999; Zhang *et al.,* 2017; Zhang *et al.*, 2018). Basis of this was to select crop plants that can tolerate water deficit in soils. This would be a practical contribution towards addressing the problem, and it should be able to achieve good economic and ecological benefits.

Maize (*Zea mays* L.; Poaceae) is an important staple food, fodder crop and industrial raw material and are widely grown in arid and semi-arid regions (Wang and Gao 2010). The crop is moderately sensitive to drought (Fu *et al.,* 2009). According to the National Bureau of Statistics of the People's Republic of China, by 2016, among the three major cereals, the total sown area, yield, and planting structure ratio (%) of maize are the highest. This gives further emphasis to the production value and economic value of maize (Yang 2018). Some studies have shown that when maize plants are subjected to drought stress, crop yield would be reduced severely. And the problem when intensified, may cause >50% reduction in the production (Li *et al.,* 2004; Lu *et al.,* 2005). It has been found that breeding and screening maize varieties (lines) with high drought resistance can effectively reduce the yield loss brought about by drought (Li *et al.,* 2004). This method also seems most economical and effective measures to solve the drought hazard.

In the past studies, the assessment of drought resistance has been mostly performed in simulated drought environments such as potted (Zhang *et al.,* 2011) and shelters (Ming *et al.,* 2008; Zhang *et al.,* 2009). Studies on the initial stage of growth, such as seedling stage or bud stage were also carried out (Sun *et al.,* 2016). The study of the long-term real growth under drought conditions is less. However, the simulation conditions are far from the field performance. It is impossible to accurately and reliably reflect the drought resistance of plants based on simulation conditions alone. So, field experiments are needed. In recent years, the non-destructive testing of photosynthesis and fluorescence apparatus has also been applied to the study of drought resistance in plants that planted in laboratory experiments or in greenhouse conditions. The investigation about the photosynthetic and fluorescence performance of plants under field-grown conditions seems complete without some fluorescence data. And the study of chlorophyll fluorescence mainly focused on *Arabidopsis* (Gameiro *et al.,* 2016), barley (Jedmowski and 2015), tall fescue (Zhang *et al.,* 2017) and soybean (Jumrani *et al.,* 2017) and less on maize.

So considering the multifarious benefits of maize crop towards modern agriculture, it is important to carry out experiments on drought resistant lines. In this experiment, field test was carried out to identify the agronomic traits, physiological indices, photosynthetic parameters, and chlorophyll fluorescence of two different maize lines (H242/1523 and H14/A18)at the early filling stage. The intrinsic physiological mechanisms of the drought resistant maize lines were further analyzed by using methods of principal component analysis (PCA) and clustering analysis (CA). The drought resistance of the tested materials was classified and ordered. From this, the maize lines with strong drought resistance were screened out in order to provide a theoretical basis for the study of drought-resistant breeding and water-saving cultivation.

Materials and Methods

Seeds of two maize lines (H242/1523 and H14/A18) were collected in 2016 from Bai Qingyuan Grass Industry of Ningxia. The study site included two population of maize located in Tongxin, Ningxia, China (36°58′ N; 105°54′ E; Altitude 1339.3 m). This area had an annual temperature of 9.5°C and an annual precipitation of 259.8 mm.

The trial was carried out from April to September 2017 under limited irrigation conditions in Wangtuan Town, Tongxin County of the Arid Area in Central Ningxia (Fig. 1). The physical and chemical properties of the tested soil have been provided in Table 1. According to the randomized block trial design, area of the plot is 3 × 5 m. Seedling of each maize line has been planted with 3 replications, and the wide and narrow row (70 × 40 cm). The plant spacing is 0.2 m, walkway 0.8 m, single shot sowing, sowing depth of about 3 cm. Before sowing, it is necessary to select the seeds that are full and the size is basically the same. During the whole growth stage, T1 irrigation quota was 262 m^3/mu (394.3 mm), T2 irrigation quota was 334 m^3/mu (500.7 mm), T3 irrigation quota was 415 m^3/mu (622.2 mm), and rainfall during the whole growth stage was 281.7 mm.

Agronomic Traits and Biomass Determination

The height of the experimental maize crop plant was measured with the help of a steel made graduated measuring tape. The length of the plant from base to the highest tip under the natural growth state was measured in 9 replicates and the data were considered as plant height. Stay-green ability has been calculated as the ratio of the number of green leaves and the total number of leaves. This data was also measured in nine replicates.

From each plot, fresh shoots from 3 × 5 m area were collected and after being confirmed about the quality of the shoots, the organs were immediately subjected to oven drying at 80℃ until a constant weight was reached and the final value was considered as biomass. Five representative spikelets were selected to test the 100-grain weight.

Determination of Photosynthetic Parameters and Chlorophyll Fluorescence

Different photosynthetic parameters were measured on a fully expanded mature leaf of each experimental maize plant on a sunny day from 09:30–11:30 AM. The photosynthetic rate (P_n), transpiration rate (T_r), atmospheric CO_2 concentraion (C_a) and intercellular CO_2 concentration (C_i) were measured using LI-6400XT series portable photosynthesis measuring system (LI-COR Co., USA). The measured leaf surface area was 6 cm^2 and the temperature of leaf chamber varied from 27–29℃. Maximum PAR (photosynthetic active radiation) reached at surface up to 1000 mmol m^{-2} s^{-1}. The measurements were carried out on 9 replicate samples, until the data became stable and was saved as average. Leaf water use efficiency (WUE) and stomatal limitation value (Ls) were calculated using the following formulae:

$$WUE = \text{net photosynthetic rate } (P_n)/\text{transpiration rate } (T_r)$$

$$L_s = (C_a - C_i) \times 100\% / C_a$$

A Pulse Amplitude-Modulated Fluorometer (H. Walz, Effeltrich, Germany) was used to measure chlorophyll fluorescence and for that the manufacturer's instructions were followed strictly. Before measurements, all lines were dark-adapted for at least 30 min. Relative chlorophyll content (chl) of leaves was monitored non-invasive using a portable SPAD-502 meter (Minolta, Osaka, Japan). Six SPAD readings were recorded for each leaf in a relative position of 2/3 of the distance relative to the leaf base.

Estimation of Membrane Lipid Peroxidation Produce (MDA) and Electrolyte Leakage (EL)

Lipid peroxidation product was measured using the thiobarbituric acid method (Weng *et al.,* 2015). The 1 mL extract was mixed with 5 mL TBA (0.5% TBA prepared in 5% trichloroacetic acid), and the mixture was boiled at 100°C for 10 min. After cooling in an ice bath, samples were centrifuged at 3000×g for 15 min and the resulting supernatant was read at 450, 532 and 600 nm. The MDA content was calculated with the formula:

$$MDA\ (mmol\ g^{-1}\ FW) = [6.452\times(A_{532}-A_{600})-0.559*A_{450}]\times Vt/(Vs*FW)$$

To quantify the EL, 0.2 g of treated leaves were washed three times with deionized water. The leaves were cut into 0.5 cm long pieces and put into test tubes filled with 20 mL deionized water. The tubes were placed in a vacuum pump, and then exhausted for 10 min at 25°C and the initial electrical conductivity (L_1) was measured by a conductivity meter (DDS-307). Subsequently, the leaves were boiled at 100°C for 5 min to release the electrolytes of the tissue completely. The final conductivity (L_2) was measured after the solution had been cooled to room temperature. The relative EL was calculated using the formula:

$$EL\ (\%) = (L_1/L_2) * 100\%.$$

Determination of Antioxidant Enzyme Activities (SOD and POD)

Crude enzyme extraction: For crude enzyme extraction, 0.5 g of fresh leaf tissue with liquid nitrogen was extracted in 7 mL phosphate buffer (50 mM, pH 7.8) using a pre-chilled mortar and pestle. Then, the homogenate was centrifuged at 10000× g at 4°C for 20 min. Finally, the supernatant was collected and stored at 4°C to determine biochemical analysis of malodialdehyde (MDA), SOD and POD activity.

The SOD activity was measured by using the method of Askari and Ehsanzadeh (2015). Adding an assay mixture containing 50 mM phosphate buffer (pH 7.8), 130 mM methionine, 0.1 mM EDTA, 200 μM riboflavin, 750 μM NBT, and 0.1 mL enzyme extract was incubated under 4000 lux fluorescent lights for 15 min. Optical density was read at 560 nm and one unit of SOD activity was defined as the inhibition of nitroblue tetrazolium (NBT) reduction by 50%.

The POD activity was measured with guaiacol method (Guan *et al*., 2015). In brief, the 0.50 mL enzyme extract was added into 1.00 mL 0.18% H_2O_2, 1.00 mL guaiacol, 0.20 mL metaphosphoric acid. Then we recorded the absorbance changes at 470 nm per minute for 3 min. One unit of POD activity is defined as the increase in absorbance per minute.

Estimation of Soluble Sugar and Soluble Protein

Soluble sugar content was measured using the method of Anthrone-sulfuric acid colorimetry (Zou 2008). Soluble protein content was determined by the method of Coomassie brilliant blue G-250 (Fu *et al*., 2016), and followed by absorbance readings at 595 nm using bovine serum albumin as standard. Soluble protein was expressed as mg protein g^{-1} FW. For the purpose, 0.2 g fresh plant material was used.

Statistical Analysis

The data collation was done firstly, by using Microsoft Excel 2007. Secondly, one-way Analysis of Variance (ANOVA) and Duncan's multiple range tests were conducted using SPSS statistical 17.0 software and 5% level of probability was used to test the significant effects of treatments. The graphs were produced by Origin 8.0 Program. Finally, principal component analysis (PCA) and cluster analysis (CA) were done using the R Programming Language software. All values are expressed as "mean values ±SE (standard error)".

Results

Soil Moisture Content

For the whole growth period, there is a difference in soil moisture content between different water treatments (Fig. 2). The overall performance in the amount of water used in irrigation followed, high irrigation>medium irrigation>low irrigation. Among them, the soil water content, under the three irrigation gradients measured on July 8, was significantly different ($P < 0.05$). The water content of the high irrigation amount measured on August 5 was significantly higher than that of the low irrigation amount ($P < 0.05$). The medium irrigation amount remained between the high irrigation amount and the low irrigation amount, and the difference was not significant ($P > 0.05$). The water content of the high irrigation amount measured on September 9, was significantly higher than that of the medium and low irrigation amount ($P < 0.05$). On the other hand, the difference between the low irrigation amount and the medium irrigation amount was not significant and there was no significant difference ($P > 0.05$).

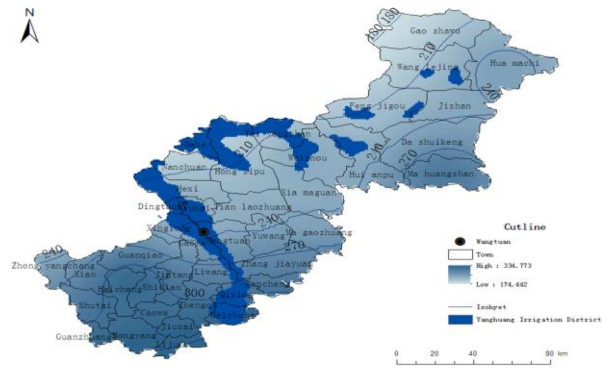

Fig. 1: The isohyet and test site in Yanghuang Irrigation District

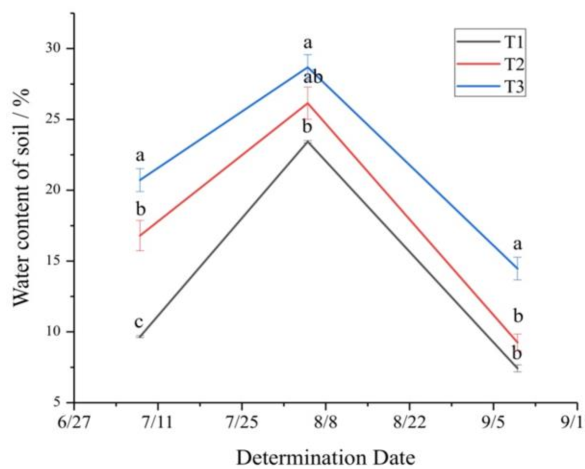

Fig. 2: Changes in soil moisture content over time under different levels of irrigation

Agronomic Traits (Morphological Indicators)

The effect of water regulation on plant height, stem diameter, SPAD values and the number of leaves of maize lines are presented in Table 2. As can be seen from Table 2, plant height, stem diameter, SPAD value and leaf number of maize line H242/1523 were higher than H14/A18 under three irrigation regimes. However, the plant height and leaf number of H242/1523 were lower than H14/A18. In addition, the indicators of agronomic traits of all lines showed a downward trend with the decrease of irrigation levels. Under low water irrigation, the plant height and the number of leaves of H242/1523 were lower than H14/A18. But the stem diameter and SPAD values of H242/1523 were higher than H14/A18. There was a significant difference in SPAD values and the number of leaves between these two lines ($P < 0.05$). Under medium water irrigation, the plant height, stem diameter, SPAD values and leaf number of H242/1523 were higher than H14/A18. Compared to H242/1523, the plant height and SPAD values of H14/A18 decreased significantly by 26.68 and 21.61%, respectively ($P < 0.05$). Under high water irrigation, the plant height of H14/A18 was significantly lower than H242/1523 by 8.92% ($P < 0.05$). The results indicated that the level of irrigation could significantly improve the growth and development of maize. Under drought stress, H242/1523 decreased the plant height and the number of leaves to reduce the loss of leaf water, and enhanced its drought tolerance ability in order to adapt drought stress.

Electrolyte Leakage (EL) and Membrane Lipid Peroxidation Produce (MDA)

As can be seen from Fig. 3, the relative permeability of cell membrane (EL) of H242/1523 was lower than that of H14/A18 under the three irrigation levels, but there were no obvious difference among the two maize lines ($P > 0.05$). With the increase of irrigation amount, the EL between two maize lines showed a decreasing trend. Under low, medium and high irrigation levels, the EL of H242/1523 was lower than that of H14/A18 (13.52, 16.76 and 13.12%, respectively) but not significant ($P > 0.05$). For H242/1523, the EL of medium and high irrigation level was lower than low irrigation amount by 12.25 and 16.83%, respectively, and the difference was significant ($P < 0.05$). While for H14/A18, the results showed that the medium and high irrigation levels was lower than low irrigation level by 8.84 and 17.22%, respectively, and the difference was not significant ($P > 0.05$). With the increase of irrigation amount, membrane lipid peroxidation production (MDA content) decreased in two maize lines. The MDA content of H242/1523 was lower than that of H14/A18 at three irrigation levels, and was significantly different ($P < 0.05$) between the two maize lines. Under low, medium and high irrigation levels, MDA content of H242/1523 was 54.81, 35.22 and 65.20% lower than that of H14/A18, respectively ($P < 0.05$). For H242/1523, the MDA content of medium and high irrigation level was lower than low irrigation amount by 16.07 and 59.43%, respectively ($P < 0.05$).

Table 1: Showing the soil physicochemical parameters of the experimental fields ready for spring sowing

Parameters	Values
pH	8.84
Soil organic matter (g/kg)	8.048
Total phosphorus (g/kg)	0.559
Total nitrogen (g/kg)	0.469
Total potassium (g/kg)	16.807
Alkaline hydrolytic nitrogen (mg/kg)	34.348
Available phosphorus (mg/kg)	135.058
Available potassium (mg/kg)	124.734
Top soil salt (g/kg)	0.150

Table 2: Effects of water regulation on plant height (cm), stem diameter (mm), SPAD values and the number of leaves in maize lines. Data presented are the means ± SE of three independent experiment. Different uppercase letters indicate that there is significant difference between the different lines of the same treatment (*P* < 0.05), and different lowercase letters indicate that there is significant difference among the different treatments of the same lines (*P* < 0.05)

Lines	Treatment	Plant height/cm	Stem diameter/mm	SPAD values	The number of leaves
H242/1523	LW	93.000±4.583Ac	22.577±1.522Aa	35.467±1.658Ac	9.667±0.333Bb
	MW	173.667±5.044Ab	25.250±1.787Aa	42.733±1.073Ab	16.000±0.000Aa
	HW	272.667±1.333Aa	31.177±2.554Aa	54.400±1.570Aa	17.333±1.202Aa
H14/A18	LW	105.333±5.783Ab	19.173±0.214Ab	21.933±0.584Bc	12.000±0.577Ac
	MW	127.333±4.667Bb	25.173±0.214Aab	33.500±1.079Bb	13.333±0.667Ab
	HW	248.333±4.410Ba	27.437±3.279Aa	46.567±1.147Aa	17.000±0.577Aa

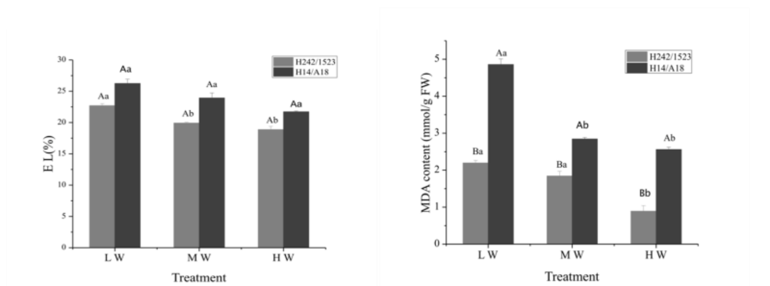

Fig. 3: Effects of water regulation on electrolyte leakage (EL) (%) and MDA content (mmol •g −1 FW) in maize lines. Data presented are the means ± SE of three independent experiment. Different uppercase letters indicate that there is significant difference between the different lines of the same treatment (*P* < 0.05), and different lowercase letters indicate that there is significant difference among the different treatments of the same lines (*P* < 0.05)

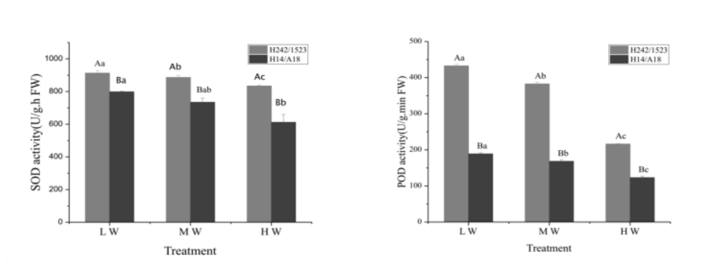

Fig. 4: Effects of water regulation on SOD acivity (U/g • h FW) and POD activity (U/g•min FW) in two maize lines. Data presented are the means ± SE of three independent experiment. Different uppercase letters indicate that there is significant difference between the different lines of the same treatment (*P* < 0.05), and different lowercase letters indicate that there is significant difference among the different treatments of the same lines (*P* < 0.05)

However, the same relationship for line of H14/A18 showed 41.45% (*P* > 0.05) and 47.31% (*P* < 0.05), respectively. Under low irrigation level, the results showed that the degree of damage to the plasma membrane of H242/1523 was lower, and the MDA content was lower, which showed the maize line H242/1523 had high drought resistance capability.

Antioxidant Enzyme Activity

The activity of anti-oxygen protective enzyme in maize plants under the three irrigation regimes was illustrated in Fig. 4. Results show that the activity of superoxide dismutase (SOD) and peroxidase (POD) of H242/1523 were significantly higher than that of H14/A18 ($P < 0.05$), and the SOD and POD of the two maize lines increased significantly with the decrease in irrigation levels ($P < 0.05$). Under low, medium and high irrigation levels, the SOD activity of H14/A18 was lower than that of H242/1523 by 12.54, 17.11 and 26.63%, respectively. And the difference was significant ($P < 0.05$). But the differences of POD activity between the two maize lines were 56.31, 56.00 and 43.02%, respectively ($P < 0.05$). For two maize lines (H242/1523 and H14/A18) the SOD activities of medium and high irrigation amount were lower than low irrigation amount by 2.94 and 8.64% and 8.01and 23.36%, respectively, while the POD activities were 11.54 and 50.02% and 10.93 and 34.82%, respectively. The results showed that the SOD and POD activity were more sensitive to drought stress, and they were the main antioxidant protective enzyme in response to drought stress.

Accumulation of Soluble Sugar and Soluble Protein

As can be seen from Fig. 5, the soluble sugar content and soluble protein content of maize leaves were decreased with the increase of irrigation levels. The soluble sugar content and soluble protein content of H242/1523 were higher than those of H14/A18, and the soluble sugar content of the two maize lines was significantly different at each irrigation level ($P < 0.05$). Under the condition of low, medium and high water irrigation amount, the soluble sugar content of H14/A18 was 14.32, 12.34 and 38.07%, respectively which is significantly lower than that of H242/1523 ($P < 0.05$). The difference of soluble protein content between the two maize lines manifested as 23.21, 13.04 and 66.67%, respectively. But in this case, the difference was not significant ($P > 0.05$). For two maize lines H242/1523 and H14/A18, the soluble sugars content of medium and high irrigation amount were lower than low irrigation amount by 10.34 and 15.39% and 8.27 and 38.85%, respectively. While the soluble protein content showed 17.86 and 46.43% and 6.98 and 76.74%, respectively. The results obtained highlight that under drought stress; maize can improve the balance of osmotic stress by increasing the content of soluble sugar, soluble protein and other osmoregulatory substances, so as to maintain the normal water requirement of plants.

Biomass

The results of Table 3 showed that the stay-green ability, hay yield and hundred-grain weight of H242/1523 were all higher than H14/A18 under all the three water treatments. This indicated that H242/1523 grew better than H14/A18. With the increase of irrigation a, both the stay-green ability and hay yield of the two maize lines increased gradually, while the hundred grain weight decreased. Low water irrigation had a serious impact on the stay-green ability, hay yield and hundred grain weight of maize lines. The stay-green ability, hay yield and hundred grain weight of H14/A18 were reduced by 15.50, 45.60 and 44.70%, respectively. There are significant differences between the two lines ($P < 0.05$). Medium irrigation increased the stay-green ability and hundred grain weight of H242/1523 by 21.30 and 48.84%, respectively compared with H14/A18 ($P < 0.05$). Under high water irrigation, the stay-green ability, hay yield and hundred grain weight of H242/1523 were higher than H14/A18, but insignificant ($P > 0.05$). The results suggested that the amount of irrigation seriously affects the normal growth and yield of maize plants.

Gas Exchange Parameters

As can be seen from Fig. 6, the water use efficiency (WUE) of two maize lines under low irrigation amount was significantly higher than under high irrigation amount ($P < 0.05$). The WUE of H242/1523 and H14/A18 under low water irrigation amount were significantly ($P < 0.05$) higher by 28.15 and 23.72% than high irrigation amount, respectively. The stomatal limitation value (Ls) of the two maize lines was higher in low irrigation level and medium irrigation level than in high irrigation level. The Ls of H242/1523 and H14/A18 under low irrigation were higher than those in high irrigation by 25.50 and 17.76%, respectively. The difference between the two treatments of H242/1523 was significant ($P < 0.05$). The above data analysis showed that, WUE objectively reflect the plant's utilization of water thereby reflecting directly the plant's ability to adapt to the environment.

Chlorophyll Fluorescence

Performance indexes (PIabs) is used to quantify the PSII behavior. It represents the comprehensive performance of the plant and relates with PSII behavior directly. In this experiment, Fig. 7 showed that the PIabs of H14/A18 were 26.73, 53.01 and 31.15% lower than those of H242/1523 under low, medium and high irrigation levels, respectively. PIabs showed significant differences in the two maize lines under the same water regulation. In the high irrigation level, the PIabs of H242/1523 and H14/A18 were lower than low irrigation amount and medium irrigation amount by 10.33 and 10.32%, and 38.79 and 4.58%, respectively.

Principal Component Analysis (PCA) and Cluster Analysis (CA) Of Water Regulation on Maize Plants

Now we interpret the Biplot of Fig. 8: The cumulative variance explained by the first two axes of functional traits was 80.49%. Such a high interpretation rate is enough for us

Table 3: Effects of water regulation on stay-green ability (%), Dry forage yield (t/ha), and hundred-grain weight (g) in maize lines. Data presented are the means ± SE of three independent experiment. Different uppercase letters indicate that there is significant difference between the different lines of the same treatment ($P < 0.05$), and different lowercase letters indicate that there is significant difference among the different treatments of the same lines ($P < 0.05$)

Lines	Treatment	Stay-green ability/ %	Dry forage yield/ t/ha	hundred-grain weight/ g
H242/1523	LW	74.510±1.961Ab	14.717±0.050Ab	41.500±1.500Aa
	MW	82.066±2.144Aab	15.901±0.556Ab	19.350±0.650Ab
	HW	87.427±4.533Aa	28.951±1.457Aa	9.250±1.050Ac
H14/A18	LW	62.963±1.852Bb	8.006±0.433Bc	22.950±0.550Ba
	MW	64.583±4.167Bb	13.249±0.181Ab	9.900±0.100Bb
	HW	80.828±3.723Aa	22.979±0.866Aa	5.750±0.450Ac

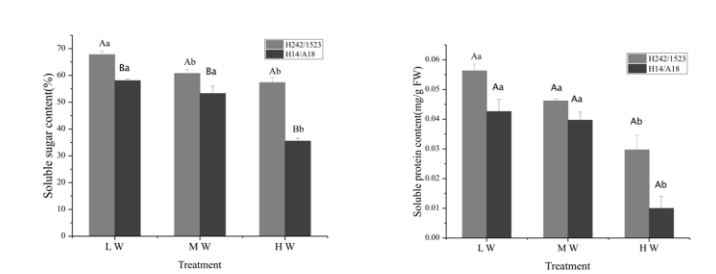

Fig. 5: Effects of water regulation on souble sugar content (%) and souble protein content (mg/g FW) in two maize lines. Data presented are the means ± SE of three independent experiment. Different uppercase letters indicate that there is significant difference between the different lines of the same treatment ($P < 0.05$), and different lowercase letters indicate that there is significant difference among the different treatments of the same lines ($P < 0.05$)

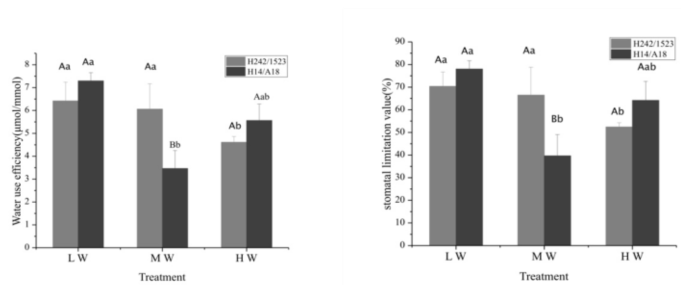

Fig. 6: Effects of water regulation on water use efficiency (μmol/mmol) and stomatal limitation value (%) in two maize lines. Data presented are the means ± SE of three independent experiment. Different uppercase letters indicate that there is significant difference between the different lines of the same treatment ($P < 0.05$), and different lowercase letters indicate that there is significant difference among the different treatments of the same lines ($P < 0.05$)

to believe that the first two axes can express most of the data structure information.

According to the grouping of biordered graphs, the maize line of H242/1523 can be found to have strong drought resistance. During low water irrigation, H242/1523 can accumulate many osmotic adjustment substances (soluble sugar and soluble protein) and antioxidant protection substances e.g., superoxide dismutase (SOD) and peroxidase (POD). All these were used to adapt to drought stress, thereby increasing water use efficiency (WUE) and hundred grain weight. Under medium irrigation conditions, H242/1523 is mainly characterized by high PIabs and good comprehensive performance. Under high water irrigation conditions, H242/1523 exhibited higher stem diameter, better SPAD value and stay-green ability, and finally obtained high biomass. However, the drought resistance of H14/A18 was poor. Under low irrigation and medium irrigation conditions, the plants were exposed to drought stress. This was mainly reflected in the sensitivity to cell membrane which caused substantial enhancement in the membrane lipid peroxidation product (MDA) and cell membrane relative permeability (EL) and therefore, the normal growth and development of plants are hindered. When the amount of irrigation is increased to reach a high irrigation level, the number of leaves and plant height of H14/A18 increases significantly.

The biordered graphs based on the correlation matrix are more intuitive than the correlation coefficient matrix

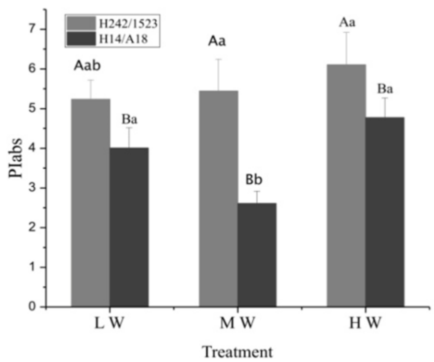

Fig. 7: Effects of water regulation on PIabs in two maize lines. Data presented are the means ± SE of three independent experiment. Different uppercase letters indicate that there is significant difference between the different lines of the same treatment (*P* < 0.05), and different lowercase letters indicate that there is significant difference among the different treatments of the same lines (*P*< 0.05)

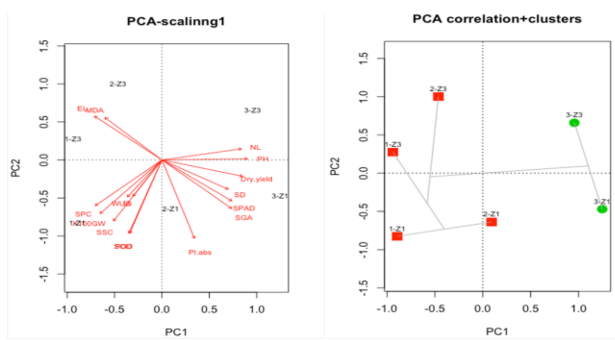

Fig. 8: The PCA Analysis Binary Sequence Diagram was generated by using the biplot function, and PCA clusters (based on correlation matrix)

between the variables. The experimental results use the angles between the arrows in the biordered graphs to determine the correlation between variables. Among them, plant height, leaf number and hay yield showed highly positive correlation. SPAD values correlated positively and highly with stem diameter and stay-green ability, but negatively with EL and MDA. High positive correlation also prevailed between EL and MDA. But highly significant negative correlation existed between stay-green ability and hay yield. Soluble sugar content is highly and positively correlated with SOD and soluble protein content.

According to the cluster analysis based on the correlation matrix PCA, different symbol shapes and colors represent different clusters. Under low water irrigation and middle water irrigation conditions, the maize lines of H242/1523 and H14/A18 belong to the same category, and they can be classified as one type of drought stress. But under high water irrigation, the two maize lines belong to another category, and they could be classified as second type, that grows under insufficient moisture.

Discussion

When plants are subjected to drought stress, there is a series of changes in the cell structure and physiological characteristics of the plants, and resulting in changes in the external morphology (Ali *et al.*, 2009; Kin *et al.*, 2018). For example, the plant height, stem diameter and the number of leaves will be changed. These changes are the instincts of plant self-protection, and also the autonomous response of plants to drought stress. Therefore, these morphological indicators can be used as indirect screening for plant drought resistance (Denmead and Shaw, 1960; Dhindsa *et al.,* 1981; Morgan 1984; Dedove and Wattiaux, 1996). This study found that at low irrigation levels, the plant height and the number of leaves in maize lines with stronger drought resistance are lower. It happens mainly because drought stress may be caused when the irrigation amount is low, and the plant itself will reduce plant height to adapt to the drought stress damage. As a result, the number of leaves is reduced in order to reduce the transpiration of water. The finding is quite consistent with the results of Fischer *et al.* (1989). The experiment also found that when the soil water deficit results, maize stalks will shrink slightly, and in the high moisture state, the stem diameter regains. So the change of stem diameter can also be used to monitor the water status of the plant body.

MDA is one of the main products of lipid peroxidation, the level of its content reflects the level of lipid peroxidation and the degree of damage of membrane structure to a certain extent. In this experiment, when two maize lines were grown under low irrigation conditions, the membrane structure of the leaves was damaged due to drought stress. This had increased the relative permeability of the cell membrane and the electrolyte penetrated outward. Under this condition, the relative conductivity increased triggering an increase in the MDA content. However, the degree of damage to cell membrane of H242/1523 was stronger than that of H14/A18 and the accumulation of membrane lipid peroxidation product was relatively less. Studies have also shown that electrolyte permeability is highly sensitive and has a significantly negative correlation with drought resistance (Wang *et al.,* 1990; Fan *et al.,* 2007). Therefore, it can be used as one of the indirect indicators to identify drought resistance of maize. When plant encounters adverse effects, a large amount of reactive oxygen species (ROS) is rapidly produced in the body, and to a certain extent, the accumulation of ROS can cause the plant growth be damaged (Jabs 1999; Prasad *et al.,* 1994). How to clear ROS? Plants scavenge superoxide radicals through the use of anti-oxygen protective enzymes (SOD and POD) and maintain the balance of active oxygen free radical production and elimination systems. The present study also found that SOD and POD activities are more sensitive to drought stress and they are the main antioxidant enzymes for maize to adapt to drought stress (Vranova *et al.,* 2002). Some studies have also shown that SOD and POD are the most important protective enzymes for plant cells against reactive oxygen species damage (Mallick and Mohn 2000). The accumulation of these enzymes, provide the plants' ability to clear ROS and the resistance of plants is thus improved. In addition to anti-oxygen protective enzymes, osmotic adjustment substances also play an important role in drought stress. In this study, maize plant increased the balance of osmotic stress by increasing soluble sugars, soluble proteins, and other important osmotic protective substances under drought stress, so as to maintain the normal water needed in the plant, and it was also a manifestation of maize plant's adaptation to drought.

The dry weight of the above-ground biomass is a commonly used quantitative indicator for evaluating the stress resistance of plants. Stay-green ability is the capacity to postpone leaf senescence. It is an important factor affecting yield, and chlorophyll is one of the common indicators of drought resistance of plants. The study revealed that low irrigation levels (water scarcity) made the 100-grain weight of maize to be high. The more the irrigation amount, the lighter of the 100-grain weight. This may be due to the fact that the excess water causes plant growth and keeps it in a vegetative state. The time to the reproductive growth phase is later and the growth period be delayed. Therefore, if it is mainly grain harvesting, it can be achieved by reducing the amount of irrigation. If the harvest of the whole plant is meant for silage corn, it is necessary to increase the amount of irrigation to increase the yield of hay. But the specific amount of irrigation needs further research. In this study, under the three irrigation treatments, the stay-green ability and relative chlorophyll content of the H242/1523 with higher drought resistance were higher than that of the H14/A18. In this, the premature senescence was caused by water shortage in plants with low irrigation. Therefore, we must strictly control the amount of irrigation in the entire growth period of maize in order to achieve high yield.

Water use efficiency (WUE) depends on CO_2 net assimilation efficiency and transpiration efficiency. It is not only affected by the characteristics of plant tissue structure, but also has a close relationship with an array of environmental factors. One of the reasons for the decrease of photosynthesis in plants with water deficit (or stress) is the reduction of stomata and the decrease of CO_2 entering into the stomata. This situation cannot meet the requirements of photosynthesis, namely the stomatal limitation of photosynthesis, and with the stomatal limit value (Ls) to represent. In this study, the WUE of the two maize lines was lower under the condition of high irrigation and the WUE was higher under the treatment of the lower irrigation. The main reason is that the WUE was increased due to the decrease of the T_r of the leaves when the maize was subjected to drought stress. This is also a self-protection mechanism that plants have evolved over a long period of time in the absence of water stress. This study finds that the Ls at low irrigation is higher than high irrigation, mainly because low irrigation may make the maize under drought stress, but under drought stress, Gs is smaller, due to less CO_2 entering the mesophyll cells through the stomata. So that Ls is higher, this is consistent with the study of Li *et al.* (2017). In recent years, the chlorophyll fluorescence kinetics of plants is a new technique that has been developed in the study of mechanisms of photosynthesis to detect physiological conditions of photosynthesis in plants. This study found that PIabs are sensitive to changes in environmental moisture and are important indicators for evaluating comprehensive performance of maize. Fv/Fm, also known as maximal photochemical efficiency of PSII can reflect the original light energy conversion efficiency of PSII. Its variation is minimal under normal conditions, but it is significantly reduced in adversity conditions. The Fv/Fm value of healthy leaves can reach 0.8–0.9, and the stress is about 0.3–0.7. In this experiment, the results of Fv/Fm are all about 0.75, indicating that the degree of drought stress on the plants is less.

Conclusion

Through the PCA and CA it has been found that the two tested maize lines are subjected to different drought resistance under different irrigation treatments. Under low water irrigation and middle water irrigation conditions, the maize lines of H242/1523 and H14/A18 belong to the same group, and they can be classified as one type of drought stress. But, under high irrigation, the two maize lines belong to another category, and they were classified as another type that grown in sufficient moisture condition. Based on the above analysis, maize plants may cause drought stress under low water irrigation amount and medium water irrigation levels. Under this condition the drought-resistant maize lines will accumulate osmotic adjustment substances and antioxidant protection substances by themselves to reduce the physiological drought stress. While for drought-sensitive maize lines, the self-regulatory ability is weak. The cell membrane is first damaged when plants suffer from drought stress, and then a large amount of MDA is accumulated in the body, which ultimately affects the normal growth and development of the maize line. Drought-resistant maize lines had good comprehensive performance at high irrigation amount, not only in morphological indicators such as plant height, stem diameter and stay-green ability, but also in yield indicators.

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