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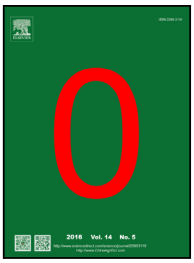

RESEARCH ARTICLE

Influence of drought hardening on the resistance physiology of potato seedlings under drought stress

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Abstract

In this paper, the influence of drought hardening on the growth, development, resistance physiology, leaf microstructure and stomatal behavior of potato seedlings under drought stress was studied, and the mechanism of drought hardening improvement of potato seedling drought resistance was elucidated. We found that drought stress had several adverse effects on potato seedlings, yet drought hardening alleviated the decrease in relative water content (RWC), net photosynthetic rate (P_n) and chlorophyll content and inhibited the increase in relative electric conductivity and malondialdehyde (MDA) content. Compared with contrast seedlings, drought-hardened seedlings also had enhanced root vigor, increased antioxidant enzyme activity and higher levels of abscisic acid (ABA), proline (Pro), soluble sugars and polyamines (PAs) under drought stress. In addition, the stomatal density of potato seedling leaves increased significantly, while the leaf area, stomatal size and stomatal aperture decreased with drought hardening treatment. These changes led to reduced leaf transpiration rate (T_r) and improved water utilization efficiency (WUE). The changes in leaf microstructure also had a positive effect on the drought resistance of the drought-hardened potato seedlings. So it can be concluded that through increasing the content of some endogenous hormones, osmotic regulatory substances and the activities of antioxidant enzymes, the resistance physiology of drought-hardened potato seedlings was enhanced.

Keywords: drought, drought hardening, potato, resistance physiology

1. Introduction

As a result of global warming, extreme weather conditions, such as drought, have widely expanded and this has resulted in a series of serious problems in many regions around the world, especially in arid and semi-arid areas (Wang *et al*. 2003; Adams *et al*. 2009). Drought is an important abiotic stress that affects cell membrane integrity, osmotic adjustment and photosynthetic ability (Bartels and Sunkar 2005; Benjamin and Nielsen 2006; Ravikumar *et al*. 2014), and thus poses a major limitation to plant growth and development. Severe drought stress reduces crop productivity and can lead to catastrophic crop failure (Sato and Yokoya 2008; Serraj *et al*. 2009; Budak *et al*. 2013; Kabira and Muthoni 2016). To cope with water deficit, plants have evolved a suite of strategies ranging from morphological or physiological adaptations to biochemical

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responses so as to survive better under drought stress conditions (Anirban *et al*. 2010).

There are four major mechanisms of plant drought resistance: drought avoidance, drought tolerance, drought escape and drought recovery (Fang and Xiong 2015). Of these, drought avoidance and drought tolerance are the two primary mechanisms for survival under drought conditions. Drought avoidance refers to the ability of plants to sustain high water status by increasing water uptake or reducing water loss in dry conditions (Yue *et al*. 2005). For example, increasing the root/shoot ratio improves the ability to uptake water, and closing stomata reduces water loss from transpiration. The main drought avoidance traits include root morphological traits and physiological traits (such as stomatal conductance and leaf relative water content (RWC)). Drought tolerance is defined as the ability of plants to maintain a certain level of physiological activity. This is accomplished through the regulation of numerous genes (for example, those related to stress signal transduction) (Zhang *et al*. 2014) and a series of metabolic pathways that reduce or repair the resulting stress damage. Drought tolerance is usually associated with physiological parameters related to osmotic adjustment (such as proline (Pro), soluble sugar and abscisic acid (ABA) content) and the alleviation of drought damage (such as the activities of protective enzymes and chlorophyll content) (Luo 2010; Fang and Xiong 2015).

In order to reduce the adverse impact of drought stress on crop production, several methods and technologies have been put to use to enhance the drought resistance of crops. Drought hardening is a convenient and feasible method and involves exposing plants to arid conditions such as reduced irrigation or partial drought during the seedling stage so as to improve the ability to adapt to subsequent serious drought (Thomas 2009; Huang *et al*. 2013). The effectiveness of drought hardening lies in the fact that young plants are malleable and are usually more able to survive under arid conditions if they have undergone a previous period of low moisture stress. It has been found that drought hardening applied in the nursery before planting improved the seedling survival rate under extreme xeric conditions (Driessche 1991). In addition, seedling hardening can improve the growth adaptability and drought resistance of mulberry (Huang *et al*. 2013). This strategy has been widely adopted in wheat, rice and other plants. (Villar-Salvador *et al*. 2004; Yang *et al*. 2015).

Potato is the fourth most important food crop in the world, with an annual production exceeding 300 million tons, and is pivotal to agricultural production and people's livelihood (http://faostat.fao.org/). Compared to other crops, potato is considered to be more sensitive to drought, and even a short period of stress may cause significant reduction in tuber yield (Loon 1981).

To date, numerous studies on potato drought resistance have been reported (Zhang *et al*. 2014; Banik *et al*. 2016; Kabira and Muthoni 2016; Cioloca *et al*. 2016). Most of them have focused on the physiological and biochemical responses to drought stress and the signal transduction pathways involved. In this study, we investigated drought resistance in both contrast seedlings and drought-hardened potato seedlings in terms of resistance physiology, growth status and leaf anatomical structure, and in this paper we discuss the connection between certain indexes with the aim of gaining a more comprehensive understanding of the mechanisms of drought resistance in potato seedlings after drought hardening.

2. Materials and methods

2.1. Plant culture and stress treatments

Potato (*Solanum tuberosum* L. *cv*. cultivar Atlantic) tubers with one apical bud were germinated in pots (diameter 35 cm, height 40 cm) in a greenhouse at a temperature of (25±1)°C with a 13-h photoperiod and a photon flux density of approximately 400 μmol m^{-2} s^{-1}. All potato samples were divided randomly into two equal-sized groups. One group (regarded as the contrast) was watered normally, and soil water content was maintained at approximately 15.0% by weighing before imposing drought stress. Another group was treated the same as the contrast group before germination and in the first 24 d after germination, then drought hardening was carried out for 25 d by withholding water and maintaining the soil water content at approximately 12.5%. On the 26th day the soil water content of drought hardening groups was increased to 15%, the same water content as the contrast, for 2 d. The soil water content was measured by weighing, and a moderate amount of water was added to maintain the desired soil water content. On the 28th day drought stress was imposed by withholding water from both groups. After 7 and 14 d of the drought treatment (the first day of no watering was regarded as 0 d), the second leaves from the base and white new roots of the potato seedlings were harvested for the experiments described below.

2.2. Determination of RWC, relative electric conductivity, malondialdehyde (MDA) content and root vigor

RWC of the potato seedling leaves was calculated as follows: RWC (%)=100×(FW–DW)/FW, where, FW is the fresh weight and DW is the dry weight (Barrs and Weatherly 1962). Relative electric conductivity of the potato seedling leaves was measured according to the method of Liu *et al*.

(2014): 30 plant leaf wafers were placed into test tubes, 10 mL distilled water were added, and tubes were sealed with plastic caps and shaken for a while so as to let the leaves immersed in distilled water. The tubes were uncapped and placed under vacuum for 10 min, and then kept for 1 h at room temperature before measuring the electrical conductance value (S1). The tubes were then placed in boiling water for 20 min, then cooled and the electrical conductance value was measured (S2). The electrical conductance value of distilled water was also measured (S3). The relative electric conductivity of the leaves was calculated using the formula: Relative electric conductivity (%)=(S1–S3)/(S2–S3)×100. MDA content was determined following the method of Heath and Packer (1968) with slight modifications. One gram of experimental material was ground in a mortar, and after adding 2 mL 10% chilled trichloroacetic acid (TCA) solution and a little quartz sand, the sample was ground further. The homogenate was centrifuged for 10 min at 4 000 r min^{-1}, then 2 mL supernatant was placed into a test tube, and 2 mL distilled water was added to another test tube to serve as a standard for OD determination. To both tubes 2 mL 0.6% thiobarbituric acid (TBA) solution was added, and the tubes were placed in boiling water for 15 min. After cooling, the samples were centrifuged again. The supernatant was used for the determination of OD value at 532, 600, and 450 nm, respectively. The MDA concentration was calculated according to the formula C (μmol L^{-1})=6.45×(OD_{532}–OD_{600})–0.56×OD_{450}. Root vigor was determined according to the method of Liu *et al*. (2014).

2.3. Determination of chlorophyll content, net photosynthetic rate (P_n), transpiration rate (T_r) and water utilization efficiency (WUE)

Chlorophyll content was measured by the method of Arnon (1949). P_n and T_r were measured between 10:30 and 11:30 in the morning using a LI-6400 portable photosynthesis system (LI-Cor, USA). WUE was calculated according to the formula: WUE (%)=P_n/T_r×100

2.4. Determination of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activity

SOD activity was determined spectrophotometrically according to the method of Spychalla and Desborough (1990). CAT activity was determined according to the method described by Lin and Wang (2002). CAT activity was measured by following the consumption of H_2O_2 (extinction coefficient 39.4 mmol L^{-1} cm^{-1}) at 240 nm for 2 min. POD activity was measured following the method of Tang and Newton (2005).

2.5. Determination of polyamines (PAs), ABA, Pro and soluble sugar content

PAs extraction and HPLC analysis were conducted following the method of Flores and Galston (1982), and authentic standards of putrescine (Put), spermidine (Spd) and spermine (Spm) were benzoylated according to the procedure described by Flores and Galston (1982). Programmable liquid chromatography (Model Waters 600E, Waters Inc. USA) was applied to measure the concentration of these PAs. The solvent system consisted of methanol and water (65% v/v methanol) at a flow rate of 1 mL min^{-1}. The benzoylated extracts were eluted at room temperature through a reverse-phase column (Waters Symmetry C18, 3.9 mm×150 mm, 5 μm in particle size), and the absorbance at 254 nm was measured with a UV detector (Li *et al*. 2004). ABA content was measured according to the method of Ross *et al*. (2004). Pro was measured according to the method described by Bates *et al*. (1973). Soluble sugars were assayed by the method of Zhang *et al*. (2006).

2.6. Measurement of growth and development indexes

The main stalk height was regarded as the plant height. The stem diameter was measured at the junction between the plant and soil surface with a vernier caliper. Fibrous root number was counted visually. Root length was determined by taking the average of the length of the five longest roots. Leaf area was determined using a leaf area meter (LI-3000, Co. LI-Cor, USA). Biomass was determined by measuring the dry weight of the whole seedling after drying in an oven at 80°C for 24 h.

2.7. Measurement of stomatal density, stomatal size and stomatal aperture

Stomatal measurements was done using the method of Chen and Gallie (2004) with slight modifications. Stomatal density was calculated from the average of 25 field of views in microscope per sample. Stomatal size was calculated by examining at least 50 closed stomatas per sample. The stomatal aperture was calculated from the width and length of at least 50 open stomatas per sample.

2.8. Measurement of anatomical structure

Anatomical structures were measured from paraffin sections: the 3rd leaf of each seedling was removed, washed with distilled water and cut into 5 mm×10 mm pieces near the midrib of the leaf. Leaf pieces were then fixed with formalin-acetic acid-alcohol (FAA) fixative for 24 h, and dyed with safranin and fast green stain. Sections were observed with

an Olympus microscope (CX22LED, Olympus, USA) and photographed. Indexes including leaf thickness, palisade tissue thickness and spongy parenchyma thickness were measured, and the thickness ratio of palisade tissue to spongy parenchyma was calculated.

2.9. Statistical analysis

All data were obtained from three or more independent replicates and were subjected to one-way analysis of variance (ANOVA). The mean differences were compared with Duncan's multiple range test (DMRT) using SPSS statistical software (ver. 17.0 SPSS, Chicago, USA). Differences at $P<0.05$ were considered significant.

3. Results

3.1. Influences of drought hardening on RWC, relative electric conductivity, MDA content and root vigor

A sharp decrease in the RWC of the contrast potato seedling leaves was observed when the drought stress lasted 14 d, but the reduction in the RWC of drought-hardened seedlings was lower relative to contrast seedlings (Fig. 1-A). Leaf relative electric conductivity and MDA content increased with drought stress, yet less of an increase was observed in the drought-hardened seedlings. For instance, the relative electric conductivity of the seedling leaves treated with drought hardening was 24.7% lower than the contrast when drought stress lasted 7 d and 6.0% lower at 14 d (Fig. 1-B). MDA content of the drought-hardened seedling leaves was 10.4 and 9.2% lower than the contrast when the stress lasted 7 and 14 d, respectively (Fig. 1-C). Root vigor of drought-hardened seedlings increased significantly and was 13.6 and 9.5% higher than the contrast when the stress lasted 7 and 14 d, respectively ($P<0.05$) (Fig. 1-D).

3.2. Influences of drought hardening on chlorophyll content, P_n, T_r and WUE

Chlorophyll content decreased with prolonged drought, but there was less of a decrease observed in drought-hardened seedlings, chlorophyll content in drought-hardened seedlings was 5.3 and 29.7% higher than the contrast when

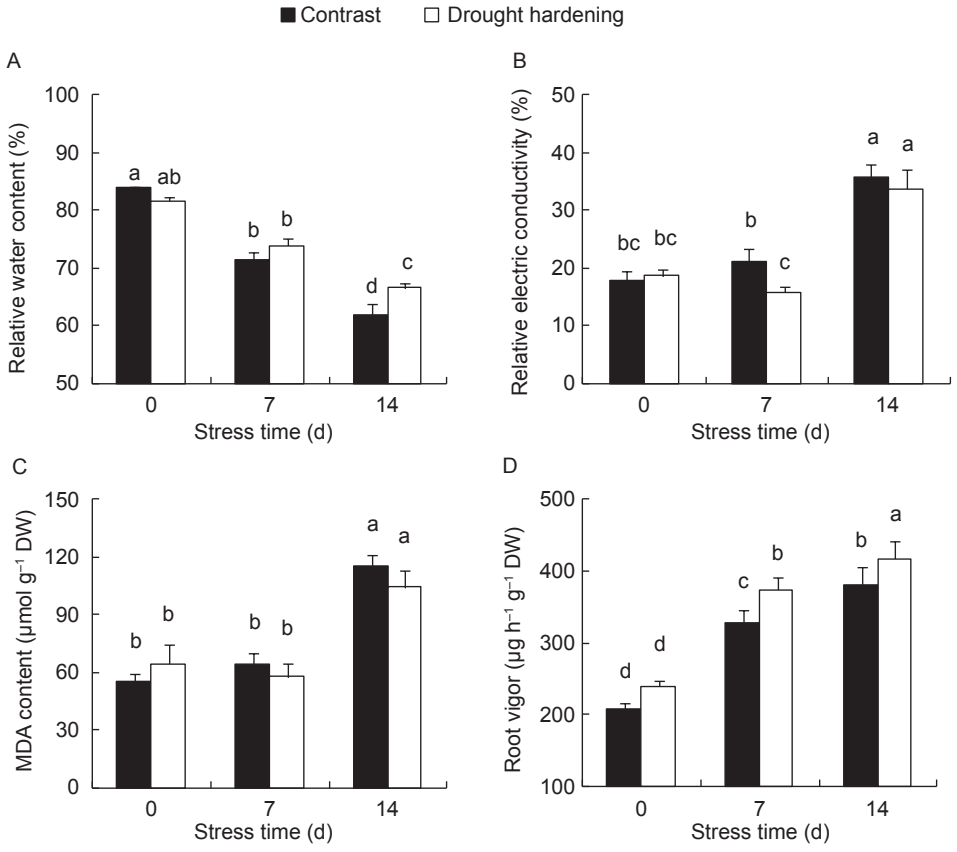

Fig. 1 Influence of drought hardening on the relative water content (RWC), relative electric conductivity and malondialdehyde (MDA) content of potato seedling leaves and potato seedling root vigor under drought stress. DW, dry weight. Bar means standand error. Different lowercase letters indicate significant differences between groups at the $P<0.05$ level.

drought stress lasted 7 and 14 d, respectively (P>0.05) (Fig. 2-A). P_n was significantly reduced when the drought stress lasted 7 and 14 d, but drought hardening treatment alleviated the reduction; P_n was 41.7 and 154.2% higher than the contrast when drought stress lasted 7 and 14 d, respectively (Fig. 2-B).

With prolonged drought stress, a significant decrease in the T_r was observed in both drought-hardened and contrast potato seedling leaves. When drought stress lasted 14 d, the T_r of the drought-hardened potato seedling leaves was 3.9% lower than the contrast. WUE of the drought-hardened potato leaves was much higher than the contrast when the drought stress lasted 14 d (P<0.05) (Fig. 2-C and D).

3.3. Influences of drought hardening on SOD, CAT and POD activity

The activities of SOD, POD and CAT in potato seedling leaves increased when the drought stress lasted 7 d, then decreased by 14 d in the contrast (Fig. 3A-C). Prior to drought stress (0 d), drought hardening significantly increased the activities of POD and CAT (P<0.05) (Fig. 3-B and-C). SOD activity also increased, but this increase was not significant (P>0.05) (Fig. 3-A). The activities of SOD, POD and CAT in the drought-hardened potato seedlings were lower than the contrast when the stress lasted 7 d, but higher than the contrast when the stress lasted 14 d (P>0.05) (Fig. 3-A–C).

3.4. Influences of drought hardening on PAs and ABA content

Put, Spm and Spd are the three most important PAs in plants. In the contrast, the levels of Put, Spm and Spd increased significantly when drought stress lasted 7 d and then decreased significantly when the stress lasted 14 d (P<0.05) (Fig. 4-A–C). Drought hardening treatment increased the levels of Put, Spm and Spd significantly in the leaves when the drought stress lasted 14 d (P<0.05); levels were 116.2, 69.2 and 183.6% higher than the contrast, respectively. Drought hardening increased the ABA content before drought stress (0 d), and when the stress lasted 14 d, the ABA content in the drought hardening-treated seedling leaves was still significantly higher than the contrast (P<0.05) (Fig. 4-D).

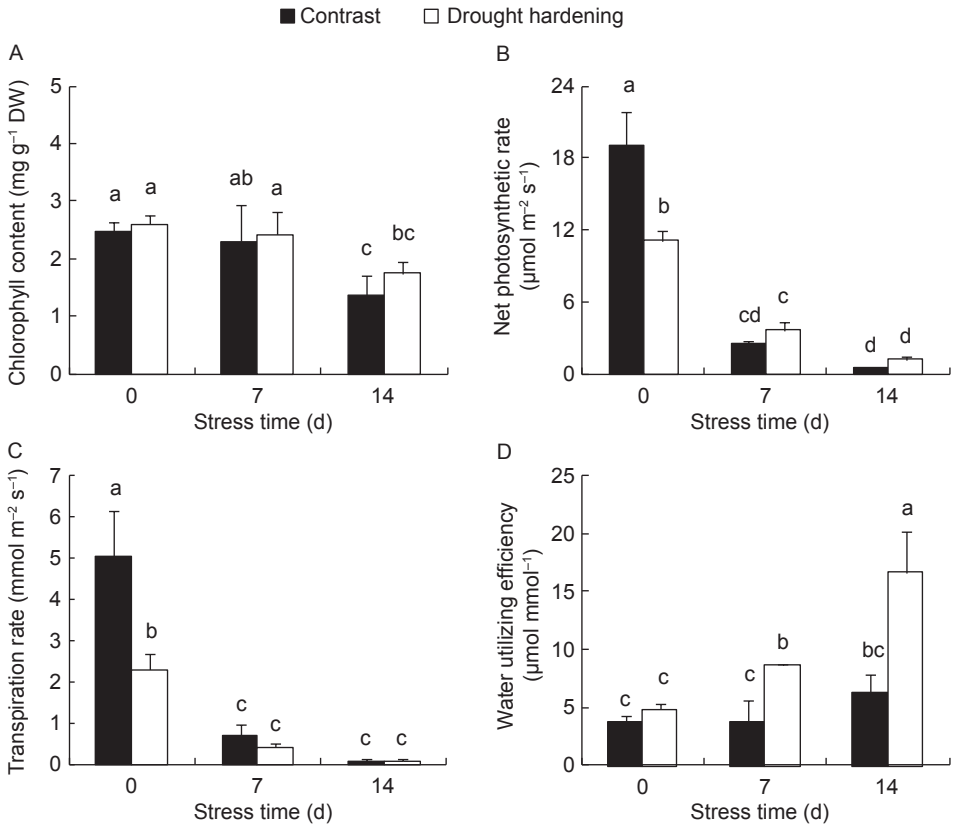

Fig. 2 Influence of drought hardening on the chlorophyll content, net photosynthetic rate (P_n), transpiration rate (T_r) and water utilization efficiency (WUE) of potato seedling leaves under drought stress. Bar means standard error. Different lowercase letters indicate significant differences between groups at the P<0.05 level.

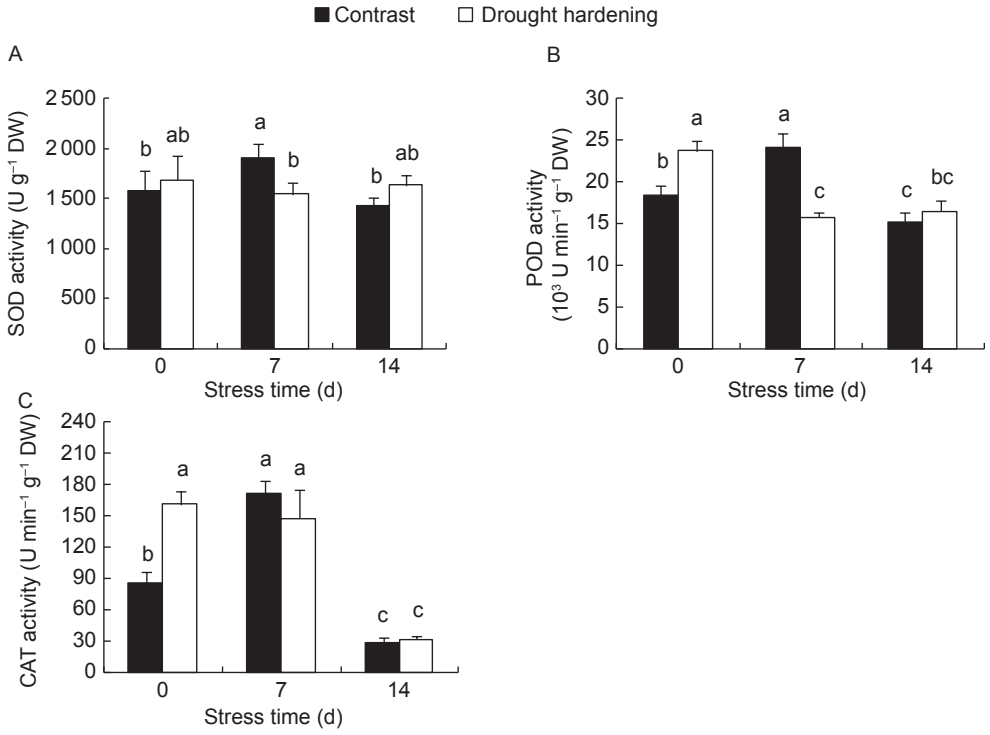

Fig. 3 Influence of drought hardening on the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in potato seedling leaves under drought stress. Bar means standard error. Different lowercase letters indicate significant differences between groups at the $P<0.05$ level.

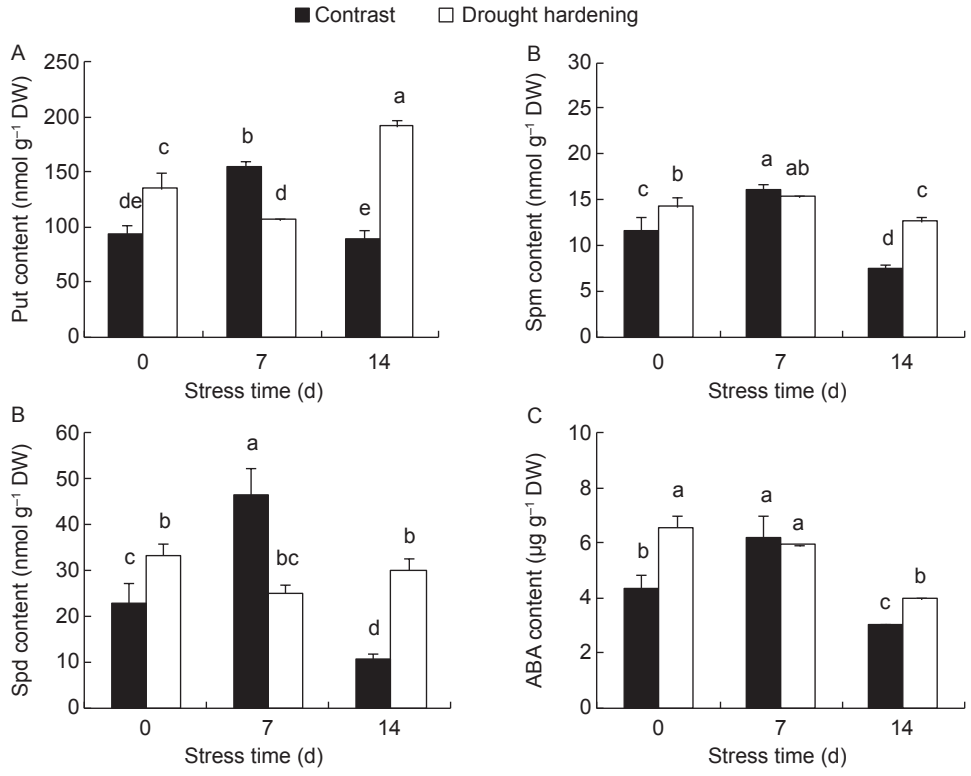

Fig. 4 Influence of drought hardening on the levels of polyamines (PAs) and abscisic acid (ABA) in potato seedling leaves under drought stress. Put, putrescine; Spd, spermidine; Spm, spermine. Bar means standard error. Different lowercase letters indicate significant differences between groups at the $P<0.05$ level.

3.5. Influences of drought hardening on Pro and soluble sugar content

There was a significant increase in the levels of Pro and soluble sugars with prolonged drought stress, and the drought hardening treatment markedly enhanced the levels of Pro and soluble sugars compared to the contrast (P<0.05). For instance, when the stress lasted 14 d, the levels of Pro and soluble sugars in the drought-hardened potato seedling leaves were 24.4 and 11.7% higher, respectively, than the contrast (Fig. 5-A and B).

3.6. Influences of drought hardening on growth and development indexes

Plant height, stem diameter, fibrous root number, root length and leaf area in both the contrast and drought-hardened potato seedlings increased gradually over time. Drought hardening increased the stem diameter, fibrous root number and root length relative to the contrast when the stress lasted 7 and 14 d, whereas the plant height and leaf area indices were reduced. For instance, fibrous root number and root length were 13.0 and 13.7% higher than the contrast, respectively, when the stress lasted 14 d, while leaf area was 14.7% lower than the contrast. When the drought stress lasted 14 d, the biomass and the root-shoot ratio of the drought hardening treated potato seedlings were significantly higher than the contrast (P<0.05) (Table 1).

3.7. Influences of drought hardening on stomatal density, stomatal size and stomatal aperture

The stomatal density of potato seedling leaves increased while stomatal size and stomatal aperture decreased significantly during drought stress. Drought hardening treatment reinforced this trend. For instance, when the stress lasted 0, 7 and 14 d the stomatal aperture width of drought-hardened seedlings was reduced by 2.2, 9.9 and 22.0%, respectively, compared with the contrast (Table 2).

3.8. Influences of drought hardening on anatomical structure

In the contrast seedling leaves, there was a slight decrease in leaf thickness as the drought stress progressed, and there was a significant decrease in the thickness of the spongy parenchyma and a significant increase in the thickness of the palisade tissue and the palisade tissue to spongy parenchyma ratio (P<0.05). Drought hardening treatment also significantly increased the thickness of palisade tissue (P<0.05) compared with the contrast. The palisade tissue to spongy parenchyma ratio also increased compared with the contrast, but this increase was not significant (P>0.05) (Table 3).

4. Discussion

When plants are grown under adverse stress, free radicals or reactive oxygen species (ROS), such as H_2O_2, $O_2^{-}\cdot$ and HO· may accumulate. Under drought stress, plants have a limited capacity for C fixation, stomatal closure restricts the assimilation of CO_2 and leads to surplus electron flux to O_2 and overproduction of ROS (Chutipaijit 2016). When a certain threshold value is reached, ROS are toxic and capable of causing lipid peroxidation, oxidative damage to the DNA and cellular proteins and the leakage of intracellular electrolytes, ultimately damaging the structure and function of the cell membrane (Henricks and Nijkamp 2001; Siedlinski *et al*. 2009; Gill and Tuteja 2010). However, plants have evolved an effective ROS scavenging and signaling system,

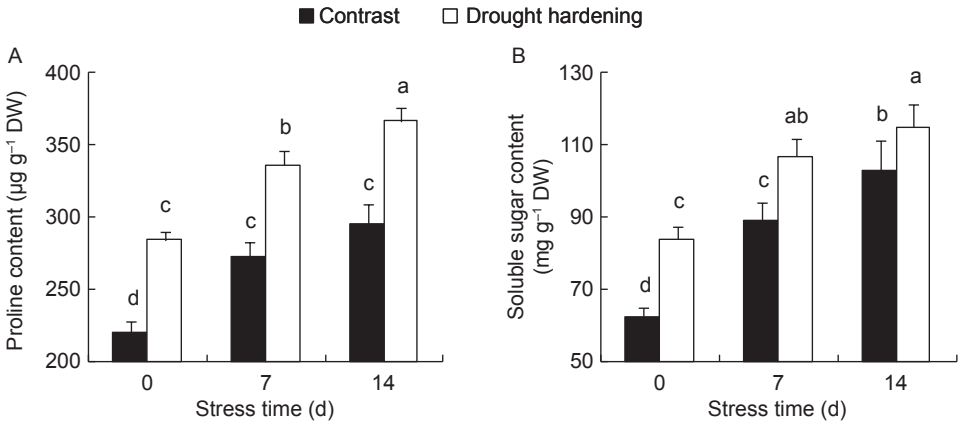

Fig. 5 Influence of drought hardening on the levels of proline (Pro) and soluble sugars in potato seedling leaves under drought stress. Bar means standard error. Different lowercase letters indicate significant differences between groups at the P<0.05 level.

Table 1 Influence of drought hardening on the growth and development of potato seedlings under drought stress

Growth indexes	0 d		7 d		14 d	
	Contrast	Drought hardening	Contrast	Drought hardening	Contrast	Drought hardening
Plant height (cm)	36.83±0.76 b	35.33±2.31 b	37.33±0.58 ab	36.75±1.59 ab	38.33±6.66 ab	37.72±1.25 ab
Stem diameter (cm)	0.77±0.09 a	0.76±0.08 a	0.80±0.07 a	0.81±0.18 a	0.81±0.14 a	0.82±0.09 a
Fibrous root number	30.00±7.23 c	32.00±1.73 c	41.00±6.56 b	47.00±4.51 ab	46.00±3.00 ab	52.00±2.08 a
Root length (cm)	35.87±3.79 a	39.73±11.49 a	42.67±8.10 a	48.73±3.48 a	43.75±9.95 a	49.73±9.09 a
Leaf area (cm^2)	50.41±1.85 c	46.89±2.60 c	85.20±3.15 b	83.19±4.22 b	102.99±4.50 a	87.90±2.28 b
Root-shoot ratio	0.80±0.07 c	0.96±0.01 b	1.09±0.01 b	1.31±0.15 a	1.13±0.02 b	1.39±0.07 a
Biomass (g DW)	31.65±1.59 c	27.24±6.32 d	35.89±6.50 b	36.32±3.40 b	36.64±4.59 b	38.75±2.76 a

Different lowercase letters indicate significant differences among groups at the $P<0.05$ level.

Table 2 Influence of drought hardening on the stomatal density, stomatal size and stomatal aperture of potato seedling leaves under drought stress

Treatment	Stomatal density	Stomatal size (μm)		Stomatal aperture (μm)	
		Length	Width	Length	Width
Contrast					
0 d	25.67±2.08 d	30.33±0.85 a	22.22±0.69 a	17.96±0.56 a	5.06±0.09 a
7 d	33.67±3.06 c	28.33±0.36 b	20.75±0.51 ab	15.27±0.86 b	4.05±0.08 b
14 d	42.33±2.52 b	25.31±1.24 cd	18.21±1.68 c	12.13±1.91 c	3.09±0.04 c
Drought hardening					
0 d	34.67±3.51 c	29.92±0.42 a	21.82±0.33 ab	17.58±0.93 a	4.95±0.12 a
7 d	45.00±2.00 b	26.20±0.39 c	20.20±0.75 b	13.68±0.79 bc	3.65±0.53 b
14 d	50.00±2.00 a	24.15±1.22 d	16.91±1.38 c	10.09±0.61 d	2.41±0.22 d

Different lowercase letters indicate significant differences among groups at the $P<0.05$ level.

Table 3 Influence of drought hardening on the anatomical structure of potato seedling leaves under drought stress

Parameter	0 d		7 d		14 d	
	Contrast	Drought hardening	Contrast	Drought hardening	Contrast	Drought hardening
Leaf thickness (μm)	127.17±1.72 bc	140.82±1.69 a	125.22±1.46 c	139.34±3.08 a	118.89±1.17 d	129.47±1.02 b
Thickness of palisade tissue (μm)	42.28±0.61 e	48.09±1.73 d	47.64±2.98 d	54.355±1.73 c	61.26±0.94 b	65.04±0.11 a
Thickness of spongy tissue (μm)	55.78±1.17 b	60.65±1.26 a	52.04±1.84 c	53.91±0.74 bc	34.46±1.07 d	36.03±0.59 d
Palisade tissue to spongy tissue ratio (%)	0.75±0.02 d	0.79±0.01 d	0.92±0.09 c	1.01±0.03 b	1.78±0.08 a	1.82±0.05 a

Different lowercase letters indicate significant differences among groups at the $P<0.05$ level.

which consists of a series of enzymatic and non-enzymatic antioxidants to protect the cells from toxic substances so as to maintain intracellular redox state homeostasis (Langebartels *et al*. 2002; Foyer and Noctor 2005; Kubiś 2005; Fang and Xiong 2015). Recently studies have shown that there is a correlation between drought tolerance and the level of antioxidant enzyme activity, which indicates that activation of these enzymes is an important mechanism to protect against drought stress in plants (Uzilday *et al*. 2012). SOD, POD and CAT are key antioxidant enzymes that defend against the increase in ROS and free radicals caused by drought stress (Apel and Hirt 2004).

MDA is a by-product of lipid peroxidation and is often used as an index of oxidative stress (Oral *et al*. 2006; Göbel *et al*. 2009). In this paper, we showed that the change in relative electric conductivity and MDA content followed similar trends under drought stress and both indices were comparatively lower in drought-hardened than contrast samples (Fig. 1-B and-C). Consistent with this, antioxidant enzyme activity in drought-hardened potato seedling leaves was markedly higher than contrast seedlings prior to drought stress (Fig. 3A-C). After 7 to 14 d of drought stress, drought hardening reduced the decline in the activity of three antioxidant enzymes (SOD, POD, CAT) compared to the contrast. These results indicate that drought-hardened potato seedlings have a higher ability to reduce ROS accumulation and thus maintain the stability of the membrane system and alleviate the damage induced by drought stress. We concluded that drought hardening enhanced drought resistance in potato seedling leaves via

improving the activity of the antioxidant enzymes.

PAs, primarily consisting of Spd, Spm and their diamine precursor, Put, are low-molecular-weight aliphatic polycations that are widespread and found in almost all living organisms (Bohra *et al*. 2015). Being positively charged at physiological pH, they can interact with various cellular macromolecules, such as nucleic acids, proteins, membrane phospholipids and thus regulate many fundamental cellular processes (Martin-Tanguy 2001; Shi *et al*. 2010; Fiscaletti *et al*. 2013). It has been reported that PAs frequently accumulate in response to biotic and abiotic stresses (Chattopadhayay *et al*. 2002; Kubiś 2008; Kusano *et al*. 2008; Alcázar *et al*. 2010) and could act as signaling molecules, resulting in the alleviation of negative drought effects (Kubiś *et al*. 2014). When encountering environmental stresses, such as water deficit, plants accumulate a large number of amine substances to promote the activities of scavenging enzymes, slow down the process of lipid peroxidation, and maintain plasma membrane integrity (Shen *et al*. 2000; Liu *et al*. 2007). In this study we found that the levels of Put, Spm and Spd increased after 7 d drought stress then decreased after 14 d in the contrast seedlings (Fig. 4-A–C). Drought hardening treatment prevented the decrease in PAs content and markedly increased PAs content compared to the contrast at 14 d (P<0.05) (Fig. 4-A–C), and thus improved the drought resistance of the potato seedlings.

Among the phytohormones, ABA plays a major role in the response of plants to environmental stresses, such as water stress and extreme temperature stress (Sauter *et al*. 2001; Bray 2002; Finkelstein *et al*. 2002), and it is one of the key factors regulating stomatal behavior. ABA curtails transpirational water loss by promoting stomatal closure, which limits gas and water exchange between the interior of leaves and the atmosphere, and thus prevents water loss (Bhargava and Sawant 2013; Hernández *et al*. 2013; Koffler *et al*. 2014). Thus higher ABA concentrations during drought stress help to maintain water status in plants (Duan *et al*. 2008; Zhang *et al*. 2010). Endogenous ABA content increases rapidly even up to 30-fold during drought stress and improves drought tolerance by enhancing osmotic adjustment and inducing stomatal closure (Acharya and Assmann 2009; Peng *et al*. 2012; Bhargava and Sawant 2013). We showed that drought hardening increased ABA content in the potato seedling leaves before drought stress, and after 14 d of drought stress the ABA content in the leaves of drought-hardened plants was still 30.6% higher than the contrast (P<0.05) (Fig. 4-D). Similarly, the T_r was sharply reduced at 14 d (Fig. 2-C). The result suggests that, the increase in ABA content after drought hardening enhance the ability of potato seedlings to adapt to drought stress by inducing stomatal closure to reduce water loss via transpiration.

Smaller stomata can open and close more rapidly and their general association with high stomatal densities provides the capacity for rapid increase in leaf stomatal conductance and maximizes CO_2 diffusion into the leaf (Aasamaa *et al*. 2001). In this paper, drought hardening treatment increased stomatal density and reduced stomatal size and aperture (Table 2), and thus reduced leaf transpiration rate and improved WUE (Fig. 2-C and-D). This should have a positive effect on potato seedlings growth and development. Consistent with this, drought hardening increased biomass (Table 1) and root vigor (Fig. 1-D) relative to the contrast.

Leaves are the action centers of photosynthesis in higher plants and among plant organs have the largest area exposed to the environment (Liu *et al*. 2016). The long-term influence of arid environments alters leaf morphology (Gao *et al*. 2003). Plants have evolved many leaf structural adaptations to protect against and minimize water loss under limited moisture conditions (Hameed *et al*. 2012). Thicker plant leaves result in a higher water storage capacity, which prevents excessive transpiration and guarantees a higher WUE. Palisade tissue provides mechanical support for leaves and can prevent moisture loss, whereas spongy parenchyma can store a lot of water and efficiently avoid leaf blight caused by drought. To survive under long-term water shortages, the thickness of palisade tissue increased and the thickness of spongy parenchyma decreased (Chartzoulakis *et al*. 2002). We found a significant increase in the thickness of the palisade tissue and in the ratio of palisade tissue to spongy parenchyma during drought stress, and these increases were even higher after drought hardening treatment (Table 3). These results indicate that better-developed potato leaf palisade tissue might have less mechanical damage under drought stress conditions.

Plants have evolved several mechanisms to manage the damaging effects of abiotic stresses, and there exists a positive compensation mechanism in plants under drought stress. Photosynthetically active radiation is absorbed by chlorophyll, and a higher chlorophyll content under water stress conditions is believed to result in more efficient use of light energy (Guo *et al*. 2008). We observed that, compared with contrast seedlings, drought hardening increased the RWC, chlorophyll content and P_n of potato seedling leaves under drought stress (Fig. 1-A and Fig. 2-A and B). High leaf WUE is a water-saving strategy allowing plants to maintain strong drought tolerance (Wu and Bao 2012). It is generally believed that reduced transpiration and increased photosynthesis jointly lead to improvement in leaf WUE. In our experiment, when the stress lasted 14 d, the T_r of the drought-hardened potato seedling leaves was 3.9% lower than the contrast, while the P_n was about 154.2% higher than

the contrast on the same day (Fig. 2-B and C), thus resulting in an evident increase in leaf WUE (Fig. 2-D).

5. Conclusion

With drought hardening treatment, leaf area, stomatal size, stomatal aperture as well as the T_r were reduced, so the reduction of water content caused by drought stress was alleviated. Both P_n and WUE were improved; these changes may be beneficial for plant growth. Furthermore, drought hardening treatment enhanced the levels of PAs, ABA, Pro and soluble sugars and the activities of antioxidant enzymes in seedling leaves, thus improving the resistance physiology of potato seedlings. In a word, drought hardening improves the drought resistance of potato seedlings by impacting aspects of leaf microstructure and anatomical structure, the levels of endogenous hormones, osmotic adjustment and antioxidant enzyme activity.

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