Plant Physiology and Biochemistry 136 (2019) 196–203

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Mesophyll cells' ability to maintain potassium is correlated with drought tolerance in tea (*Camellia sinensis*)

Xianchen Zhang[a,1], Honghong Wu[b,c,1], Linmu Chen[a], Ningning Wang[a], Chaoling Wei[a], Xiaochun Wan[a,*]

[a] State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, Hefei, 230036, China
[b] Department of Botany and Plant Sciences, University of California, Riverside, 92521, USA
[c] College of Plant Science & Technology, Huazhong Agricultural University, Wuhan, 430070, China

ARTICLE INFO

Keywords:
Drought
K^+ channels
K^+ fluxes
Mesophyll K^+ retention
Tea plant

ABSTRACT

Tea plant is an important economic crop and is vulnerable to drought. A good understanding of tea drought tolerance mechanisms is required for breeding robust drought tolerant tea varieties. Previous studies showed mesophyll cells' ability to maintain K^+ is associated with its stress tolerance. Here, in this study, 12 tea varieties were used to investigate the role of mesophyll K^+ retention ability towards tea drought stress tolerance. A strong and negative correlation ($R^2 = 0.8239$, $P < 0.001$) was found between PEG (mimic drought stress)-induced K^+ efflux from tea mesophyll cells and overall drought tolerance in 12 tea varieties. In agreement with this, a significantly higher retained leaf K^+ content was found in drought tolerant than the sensitive tea varieties. Furthermore, exogenous applied K^+ (5 mM) significantly alleviated drought-induced symptom in tea plants, further supporting our finding that mesophyll K^+ retention is an important component for drought tolerance mechanisms in tea plants. Moreover, pharmacological experiments showed that the contribution of K^+ outward rectifying channels and non-selective cation channels in controlling PEG-induced K^+ efflux from mesophylls cells are varied between drought tolerant and sensitive tea varieties.

1. Introduction

Drought is one of the most common abiotic stresses in the world and causes billions of dollar losses annually (Eisenstei, 2013). Tea plant is an economic crop and tea related products are popular in world-wide (Wei et al., 2018; Yang et al., 2016; Zhang et al., 2016; Liu et al., 2018). Tea plant growth is affected by multiple environmental factors e.g. drought and heavy metal (Tang et al., 2008; Li et al., 2017; Upadhyaya et al., 2013; Zhang et al., 2015; Gao et al., 2014). Among these environmental stresses, drought is one of the most important limiting factors for agricultural growth of tea plants and quality of tea products (Liu et al., 2015). Annual tea industry output was significantly reduced up to 33% by drought in tea culturing countries (Cheruiyot et al., 2010). Drought stress causes withering of the bud and youngest fully expanded leaf, resulting irreversible negative effects on tea yield and quality and thus big economic losses (Zhang et al., 2018a,b,c). For example, at 2013, about 138.6 thousand acres of tea gardens in Zhejiang province at China were suffered from severe drought stress, causing a loss of about RMB 1.72 billion (Yang et al., 2017). However, to date, improving tea plant drought stress tolerance is still impeded by insufficient information of its tolerance mechanisms. Most studies on tea plant drought tolerance are focused on morphological, physiological, and molecular levels (Ahmed et al., 2014; Upadhyaya et al., 2013; Zhou et al., 2014; Gupta et al., 2013). The information on ionic mechanisms of tea plant drought tolerance is still limited. Thus, investigating ionic mechanisms towards tea drought stress tolerance is one of the aims of this study.

Potassium is an essential marconutrient for plant growth and activities, and is recognized as a rate-limiting factor for crop yield and quality (Dreyeret and Uozumi, 2011). It compromises about 4–6% dry matter of plants. Potassium plays an important role in enzyme activation (Evans and Sorger, 1966; Wu et al., 2018a), stomatal movement, maintenance of turgor pressure and cytosolic pH homeostasis (Wang et al., 2013; Chen et al., 2015), and cell fate (Shabala et al., 2007) etc. For example, more than 80 enzymes are known requiring K^+ (Evans and Sorger, 1966; Suelter, 1970). Also, from the last decade, the importance of cell's ability to maintain potassium in plant response to abiotic stress e.g. salinity (Chen et al., 2005; Sun et al., 2009; Wu et al.,

* Corresponding author.
E-mail addresses: wanxiaochun360@163.com, xcwan@ahau.edu.cn (X. Wan).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.plaphy.2019.01.020
Received 2 October 2018; Received in revised form 15 January 2019; Accepted 15 January 2019
Available online 19 January 2019
0981-9428/

2013, 2014), and drought (Wang et al., 2013; Bahrami-Rad and Hajiboland, 2017; Zain and Ismail, 2016; Zahoor et al., 2017; Tavakol et al., 2018) has been revealed. For example, the degree of mesophyll K^+ efflux in soybean under drought is linearly correlated with stress strength (Mak et al., 2014). Also, our previous research also found that drought stress cause K^+ efflux from mesophyll cells and this drought stress induced mesophyll K^+ efflux is alleviated by re-watering treatment (Zhang et al., 2018c), suggesting that mesophyll cells' ability to retain K^+ could contribute to tea plant drought stress tolerance. However, the possible important role of mesophyll cell's ability to maintain potassium in tea drought stress tolerance has been never targeted and investigated/validated in an accessory of tea varieties varied in drought tolerance. Thus, this is the second aim of this study.

Efflux of potassium in plant cell under stress is known to be operated by K^+ channels and transporters on cell membrane. KOR (K^+ outward rectifying channels) and NSCC (non-selective cation channels) channels are the known channels mediating K^+ efflux at plasma membrane (Pottosin and Dobrovinskaya, 2014; Demidchik, 2014). Recent studies revealed the existence of tissue specificity of these two channels in controlling K^+ retention. For example, KOR channel controls NaCl-induced K^+ efflux in barley root mature zone (Chen et al., 2007), whereas NSCC channels dominate NaCl-induced K^+ efflux in barley root elongation zone (Wu, 2016). Also, in wheat, NSCC channels and KOR channels play the main role in mediating NaCl-induced K^+ efflux in mesophyll (Wu et al., 2015a) and root mature zone (Cuin et al., 2012) cells, respectively. Similar scenarios can be also existed in tea plants. Identifying the channels controlling K^+ efflux in tea plant under drought could benefit the program of breeding robust drought tolerant tea plant varieties. Thus, the third aim of this study is to identify the main channels controlling drought stress induced K^+ efflux in tea leaf mesophyll cells.

In this study, we investigated mesophyll cells' ability to maintain K^+ under drought in 12 tea plant varieties differing in drought stress tolerance and tried to link it with its antioxidant activities. Also, by applying channel inhibitors, we investigated the channels responsible for mediating K^+ efflux in mesophyll cells in tea plants under drought. Furthermore, exogenous K^+ was applied to confirm the role of potassium on tea plant drought stress tolerance.

2. Materials and methods

2.1. Plant materials and growth conditions

Twelve varieties of tea plants were used in this study. All seedlings (35–40 cm height, one year) were obtained from Anhui Agricultural University (State Key Laboratory of Tea Plant Biology and Utilization). Plants were washed with deionized water and placed in a 4 L plastic pot (five tea plants per pot) filled with nutrient solution, then transferred to an artificial climate chamber for one month with a day length of 12 h day^{-1}, temperature of 22 ± 1 °C, irradiance of 270 μM $m^{-2}s^{-1}$, and relative humidity of 45 %–50%. Plants were grown in nutrient solution for one week before treatment. The nutrient solution contained macronutrients (mM): N (1.427), P (0.1), K (0.513), Ca (0.392), and Mg (1.029), and micronutrients (μM): Zn (1.53), Cu (0.39), Mn (18.2), B (9.25), Mo (0.53), Al (0.77), and Fe (6.27) as EDTA salts for adaptation to hydroponic cultivation (Ruan et al., 2007). To mimic drought stress, the solution in pots were replaced by 10% PEG-6000 (w/v) nutrient solution (nutrient solution + 10% PEG-6000). Tea plants were then subjected to 10% PEG-6000 (w/v) nutrient solution for eight days. Tea plants grown in nutrient solution without PEG were used as control. Photographs were taken before onset of PEG (day 0) and at the end of the drought stress period (day 8, Fig. 1A) using a Canon EOS REBEL T4i digital camera (Canon, Japan). After drought stress treatment, the first and second fully expanded leaves were collected and used for further experiments (Zhang et al., 2018a,b).

2.2. Biomass measurements

During harvest, a collective sample of the first (youngest) and second fully expanded leaves from all plants in each pot was taken. Leaf fresh weight (FW) was then recorded immediately. Leaf samples were dried at 80 °C for 48 h to collect the DW (dry weight). RWC (Relative water content) was calculated based on the relative difference between the FW and the DW (Wu et al., 2014).

2.3. MDA and H_2O_2 content

MDA content were measured by following the methods described in previous publications (Wang, 2006; Zhang et al., 2018c) with some modifications. Briefly, 1 g leaves sample (the first youngest and second fully expanded leaves) were grounded with 10 mL 10% (v/v) trichloroacetic, and the supernatant was collected after centrifuging at 4000 rpm for 10 min at room temperature. The 2 mL supernatant was added into 2 mL 0.6% (m/v) thiobarbital solution and gently shaken for 15 s. Distilled water was used as the control treatment. Then the reaction mixture was boiling at 100 °C at 15 min. After cooling to the room temperature, MDA were measured by recording the 523, 600 and 450 nm absorbance. Quantitative measurement of H_2O_2 was measured using specific detection kits (Jiangsu Kemin Bioengineering Institute, China).

2.4. Screening K^+ fluxes from leaf mesophyll cells from tea plant

Net fluxes of K^+ were measured using NMT (non-invasive micro-test technique) at Nanjing Forestry University by following the method described by Wu et al. (2015c) and Zhang et al. (2018c). Briefly, the youngest fully expanded tea leaf from tea plants after eight days of 10% PEG treatment (as described above) was firstly excised to small pieces (5 mm × 8 mm) and then cut with sharp blade by a 30° angle to expose mesophyll cells. Then, these leaf samples were incubated with BSM (basic salt medium) containing 0.1 mM $CaCl_2$ and 0.5 mM KCl (pH 5.7, non-buffered) overnight (refer to Wu et al. (2015b) for details). The K^+ selective microelectrodes were provided by NMT Service Center and made prior to each NMT test to ensure their temporal resolution and ionic selectivity. Fresh reference electrode was provided by Nanjing Forestry University. After mounting the leaf samples into a measuring chamber, through the microscope, the K^+ selective microelectrode was adjusted via a micromanipulator to co-focus with the exposed mesophyll cells from the immobilized leaf samples. Then, the net fluxes of K^+ was detected by moving the K^+ selective electrode repeatedly between 5 and 35 μm in above the exposed mesophyll cells at BSM solution. Each PEG-pretreated sample was measured for 10 min. Net K^+ flux was calculated based on Fick's law of diffusion: $J = -D\ (dc/dx)$, where J equals ion flux in the x direction, dc/dx is the calcium ion concentration gradient, and D is the ion diffusion constant for a particular medium. At least eight individual samples (n = 8) were recorded for each treatment.

2.5. Measuring tea leaf K^+ content

After PEG treatment, K^+ content in leaves (collective sample of the youngest and subsequent second fully expanded leaves) was measured using Inductively coupled plasma (ICP) optical emission spectrometry (Optima 2100DV; Perkin Elmer Inc., Shelton, CT, USA) following the method described in Mekawy et al. (2015). Samples were dried in an oven at 80 °C for 48 h and grounded gently. About 0.1 g sample was added into the boiling tube with gently agitated in 5% H_2SO_4 overnight. K^+ content was then detected by ICP and calculated from K^+ standard curves.

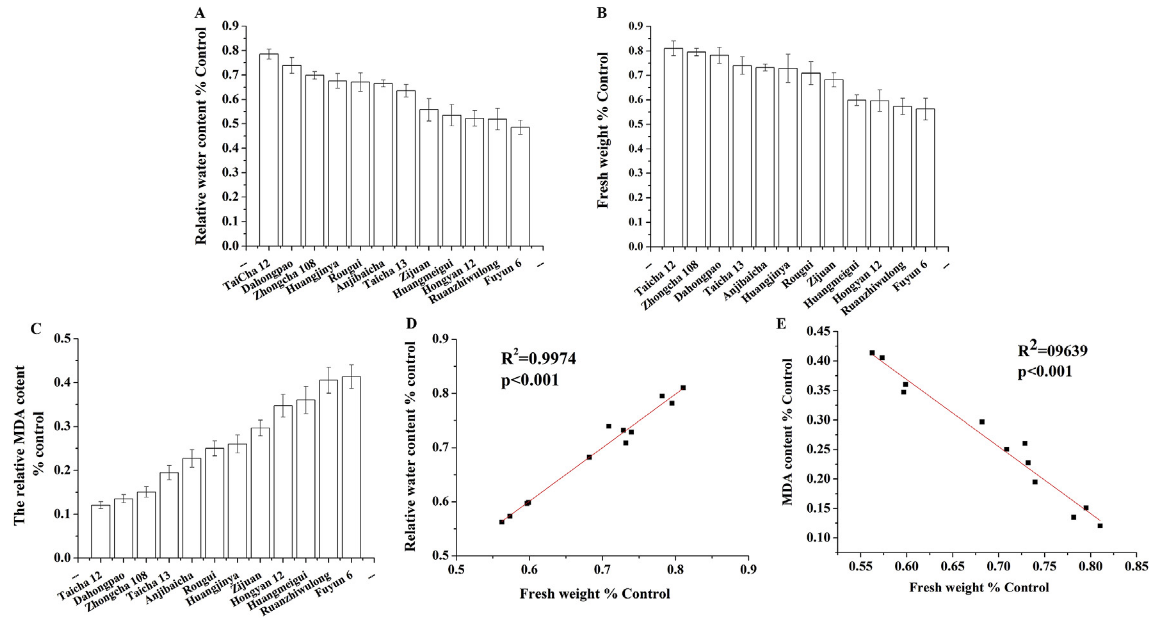

Fig. 1. Phenotypic difference of tea plant varieties in response to drought. (A) and (B) The performance of Taicha 12 under control and 8 days drought treatment, respectively. (C) and (D) The performance of Fuyun 6 under control and 8 days drought treatment, respectively.

2.6. Pharmacological experiments

Two tea varieties contrasting in drought tolerance, Taicha 12 (tolerant) and Fuyun 6 (sensitive), were used to investigate the identity of channels involved in PEG-induced K^+ efflux from tea leaf mesophyll under drought stress for 4 days. Leaf segments prepared as described above in the section "2.4" were pre-treated for 1 h with either 20 mM tetraethy-lammoniumchloride (TEA^+; a known blocker of K^+-selective channels) or 0.1 mM gadolinium chloride (Gd^{3+}; a known blocker of NSCC channels). NMT measurement was then conducted as described above in the section "2.4" (Wu et al., 2014).

2.7. Exogenous application of K^+

Two tea varieties contrasting in drought tolerance, Taicha 12 (tolerant) and Fuyun 6 (sensitive), were grown in different solutions: 1) the nutrition solution; 2) 10% (w/v) PEG treatment; 3) 10% (w/v) PEG plus 5 mM K^+. After 8 days treatment, the K^+ content, RWC, and MDA content were measured as described above.

2.8. Quantitative real-time-PCR (qRT-PCR) analysis

The first leaf from tea varieties Taicha12 and Fuyun6 after 10% PEG6000 treatment were used for the isolation of total RNA. qRT-PCR was performed according to the protocol described previously (Feng et al., 2017). The amplification of *CsGADPH* was used as an internal control. *CsORPC599-2* and *CsORPC512-2*, are orthologous gene sequences of *AtGORK* (Wei et al., 2018). The expression levels were calculated using the relative quantification method (Feng et al., 2017). All of the primers used for qRT-PCR are listed in Table S1.

2.9. Statistical analysis

Data were presented as mean ± SD (n = 4–8 biological replicates, depends on different experiments). Statistical analysis was performed by simple *t*-test (two tailed) or one-way analysis of variance (ANOVA) with Duncan's multiple range test using the software SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Different lower case letters represent the significance level at $P < 0.05$. * means $P < 0.05$.

3. Results

3.1. Genetic variability of drought tolerance in 12 tea varieties

Compared with the significant wilting phenotype of Fuyun 6 after eight days drought, only a marginal symptom was observed in Taicha 12 (Fig. 1A). The relative leaf water content (RWC) under drought stress ranged from 78.6 ± 2.1% (Taicha 12) to 48.6 ± 2.9% (Fuyun 6) in used 12 tea varieties (Fig. 2A). Similar results were also showed in relative FW and relative MDA content, i.e. Taicha 12 had the highest relative FW (81.05 ± 3.0%, Fig. 2B) and lowest MDA content (12.04 ± 0.8%, Fig. 2C), whereas Fuyun 6 had the lowest relative FW (56.25 ± 4.4%, Fig. 2B) and highest MDA content (41.34 ± 2.7%, Fig. 2C). A strong and positive correlation ($R^2 = 0.9974$, $P < 0.001$) was found between RWC and FW (Fig. 2D). Also, the relative leaf MDA content is significantly and negatively correlated with the relative FW content in used 12 tea varieties (Fig. 2E).

3.2. K^+ retention in tea leaf mesophyll cells is correlated with drought tolerance

Fig. 3A showed the characteristics of PEG-induced (tea plants hydroponically pretreated with 10% PEG-6000 (w/v) for eight days) K^+ efflux in leaf mesophyll cells of two contrasting tea varieties (Taicha 12, drought tolerant; and Fuyun 6, drought sensitive). A 3.5-fold variability of mesophyll K^+ retention in 12 used tea varieties was showed (Fig. 3A and B). Drought tolerant Taicha 12 showed the lowest PEG-induced K^+ efflux (205.7 ± 64.32 pmol $cm^{-2}s^{-1}$) from mesophyll cells (highest mesophyll K^+ retention ability), whereas the drought sensitive Fuyun 6 showed the highest K^+ efflux (759.9 ± 59.99 pmol $cm^{-2}s^{-1}$) (Fig. 3A and B). A significant and negative correlation ($R^2 = 0.8239$, $P < 0.001$) was found between mesophyll K^+ efflux and FW under drought condition (Fig. 3C). Also, mesophyll K^+ efflux was significantly and positively correlated ($R^2 = 0.6955$, $P < 0.001$) with MDA content in used tea varieties (Fig. 3D).

Similarly, total relative leaf K^+ content (from the youngest and second fully expanded leaves) varied greatly in used 12 tea varieties under drought stress compared with the control treatment (Fig. 4A). Taicha 12, with highest mesophyll K^+ retention ability, had the highest leaf K^+ content, while Fuyun 6 had the lowest K^+ content, which was

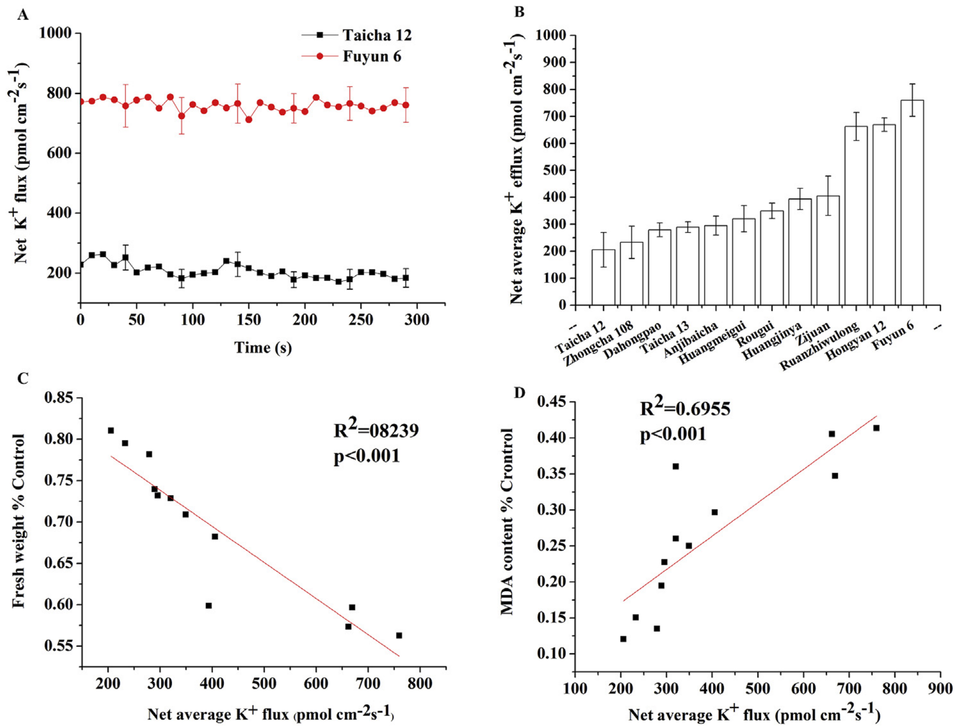

Fig. 2. Genetic variability of drought tolerance in tea plants. (A) and (B) Leaf (the collective samples of youngest and subsequent second fully expand leaves) relative water content (RWC) and relative fresh weight (FW) in 12 tea varieties under drought stress, respectively; (C) The effect of drought stress on relative MDA content in 12 tea varieties; (D) and (E) The correlation analysis between FW and RWC (D) and MDA (E) in drought stressed 12 tea varieties. Each point represents a tea separate variety.

consistent with its contrasting drought tolerance. In addition, a significant negative correlation was found between relative leaf K^+ content and mesophyll K^+ efflux ($R^2 = 0.79$, $p < 0.01$; Fig. 4B), which indicated that K^+ retention was an important competent for drought tolerance. In agreement with this, a significant correlation was found between the relative leaf K^+ content and FW ($R^2 = 0.90$, $P < 0.001$, Fig. 4C) and MDA content ($R^2 = 0.87$, $p < 0.01$ Fig. 4D).

3.3. PEG-induced K^+ efflux in leaf mesophyll was significantly suppressed by both TEA^+ and Gd^{3+}

Fig. 5A and B showed the characteristics of PEG-induced mesophyll K^+ efflux in the presence of the channel inhibitors in Taicha 12 and Fuyun 6, respectively. Although the magnititude of PEG-induced K^+ efflux in tea mesophyll (Taicha 12) was not significantly inhibited by TEA^+ (tetraethylammonium chloride, a known blocker of K^+ selective channels), while Gd^{3+} pretreatment [gadolinium chloride, a known blocker of non-selective cation channels (NSCCs)] significantly

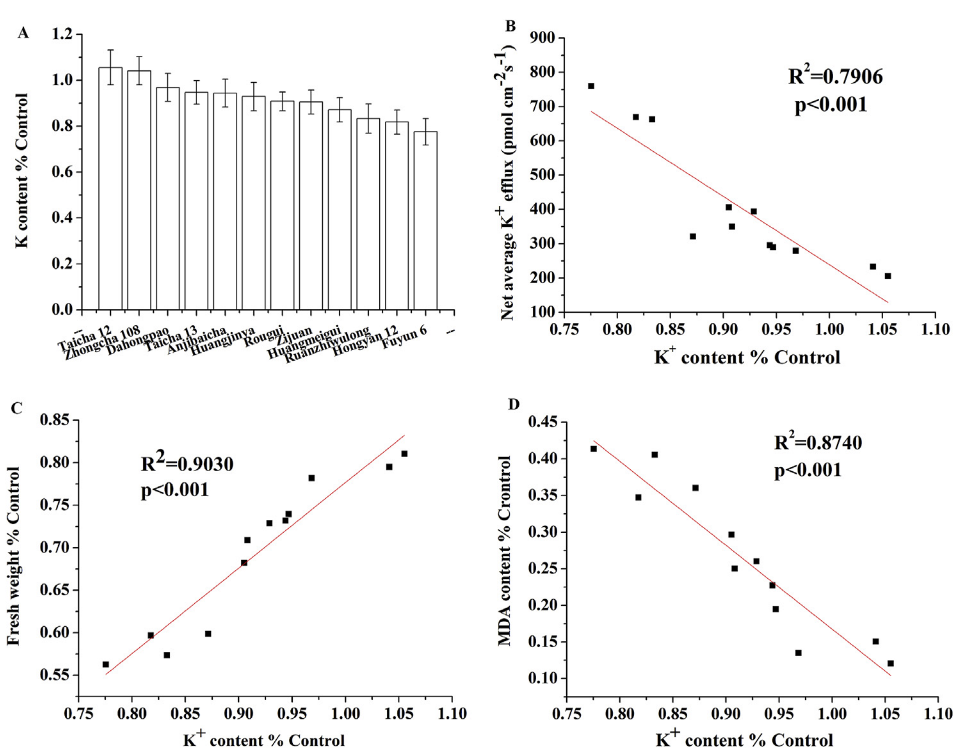

Fig. 3. PEG-induced K^+ efflux from leaf mesophyll and its correlation with drought tolerance in 12 tea varieties. (A) The kinetics of transient PEG-induced net K^+ flux from leaf mesophyll cells in Taicha 12 and Fuyun 6. Mean ± SD (n = 6); (B) Average PEG-induced K^+ efflux values from leaf mesophyll cells in 12 tea plant varieties. Mean ± SD (n = 6–9); (C) and (D) Correlation between PEG-induced net K^+ flux from tea leaf mesophyll cells and FW (C) and MDA (D). Each point represents a separate tea variety.

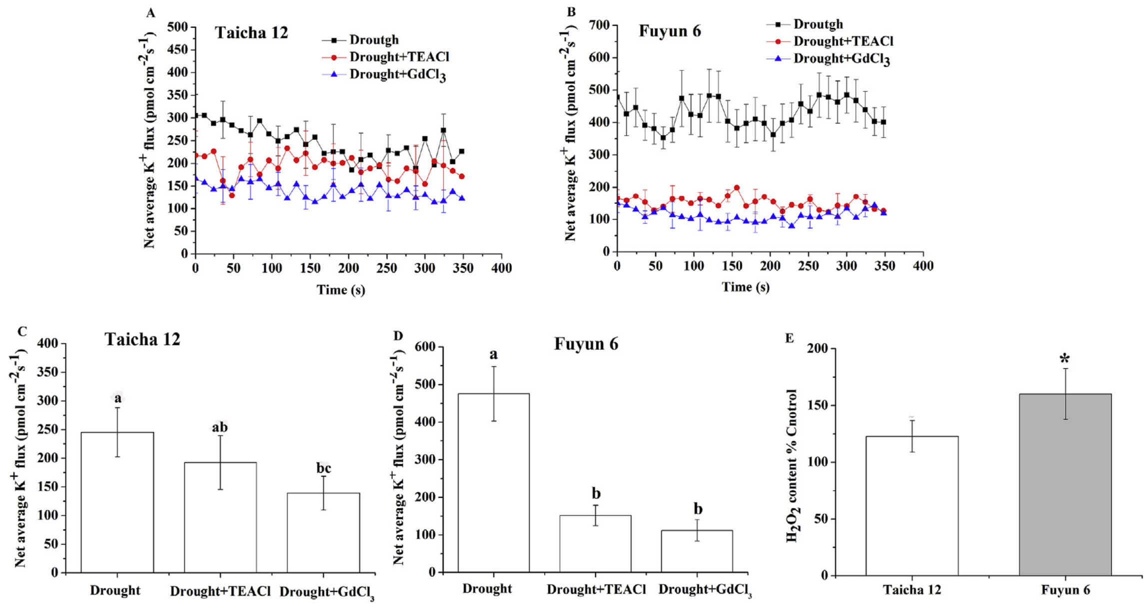

Fig. 4. Genetic variability in drought-induced changes on leaf K^+ content in 12 tea varieties. (A) Mean relative K^+ content in drought stressed 12 tea varieties compared with the control treatment. Mean ± SD (n = 7). (B), (C) and (D) Correlation between relative leaf K^+ concentration and K^+ flux (B), FW (C), and MDA (D). Each point represents a separate tea variety.

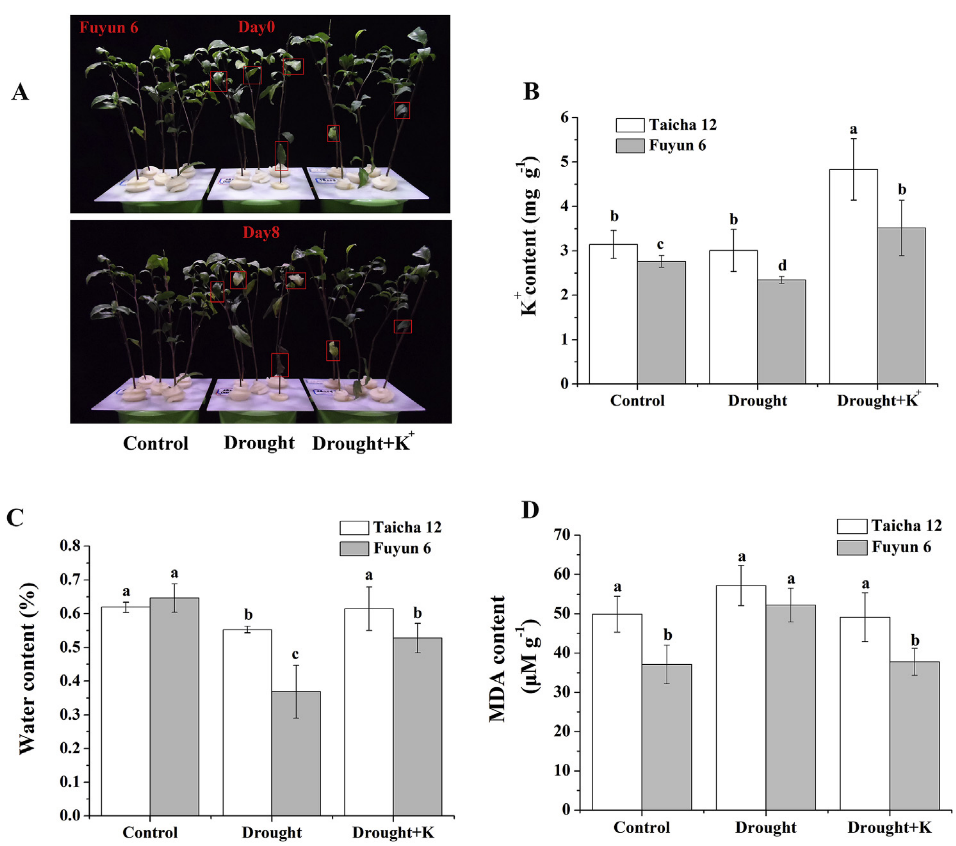

Fig. 5. Effect of channel inhibitors on PEG-induced net K^+ efflux from tea leaf mesophyll cells. (A) and (B) Kinetics of PEG-induced net K^+ fluxes from TEA and Gd^{3+} pretreated tea leaf mesophyll cells, Taicha 12 (A) and Fuyun 6 (B); (C) and (D) Calculated net PEG-induced K^+ flux measured from leaf mesophyll cells pretreated by TEA and Gd^{3+}; Different lower case letters represent significance at $P < 0.05$; (E) The effect of drought on leaf (the collective samples of youngest and subsequent second fully expand leaves) H_2O_2 content in the two contrasting tea varieties, Taicha 12 and Fuyun 6. * means $P < 0.05$.

inhibited PEG-induced mesophyll K^+ efflux in drought tolerant Taicha 12 by 43.24% (Fig. 5A and C). In drought sensitive tea variety Fuyun 6, TEA pretreatment inhibited 68.1% ($P < 0.001$) of PEG-induced K^+ efflux, and Gd^{3+} pretreatment blocked 76.41% ($P < 0.001$) of PEG-induced K^+ efflux (Fig. 5B and D). To further illustrate the role of the K^+ outward rectifying channel on the drought stress, relative gene expression analysis was conducted. The expression of *CsORPC599-2* and *CsORPC512-2* (the plasma membrane located K^+ outward potassium channel in tea plant) in the first leaf of tea plants was significantly lower in Taicha12 than Fuyun6 under drought (Fig. S3). Furthermore, drought tolerant Taicha 12 showed significant less leaf H_2O_2 content than the sensitive tea variety Fuyun 6 (Fig. 5E).

3.4. Applying K^+-alleviated drought stress symptom on tea plants

Adding 5 mM K^+ into hydroponic solution significantly alleviated

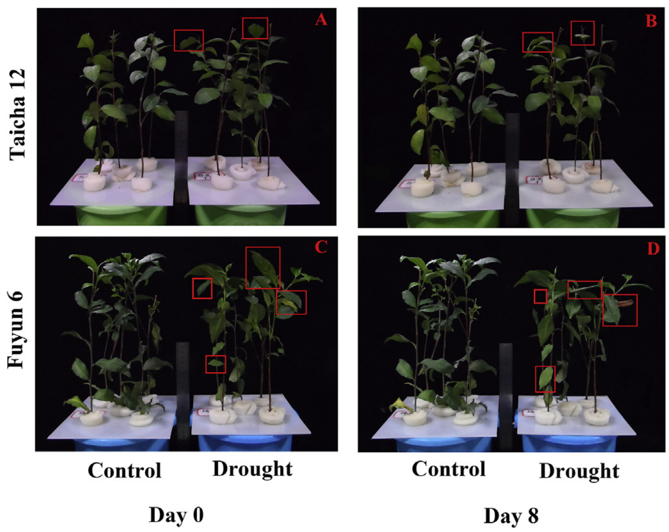

Fig. 6. The effect of exogenous K^+ on drought tolerance in tea plants.
(A) The representative images of phenotypic performance of drought sensitive varieties under control, drought and subsequent "drought + K^+" treatment; (B), (C) and (D) Leaf K^+ content (B), RWC (C) and MDA (D) in contrasting tea varieties, Taicha 12 and Fuyun 6, under control, drought and subsequent "drought + K^+" treatment.

drought-induced injuries (Fig. 6A and Fig. S1). With the application of exogenous K^+, leaf K^+ content was significantly increased in drought stressed tea plants compared with the one without exogenous K^+ application (Fig. 6B). Also, leaf MDA content was significantly reduced in drought stressed plants with exogenous K^+ application (Fig. 6C). Furthermore, exogenously applied K^+ significantly improved water content by 11.09% and 42.72% in tea varieties Taicha 12 and Fuyun 6 under drought stress than the one without adding extra K^+ (Fig. 6D).

4. Discussion

4.1. K^+ retention in mesophyll cells: a component for drought tolerance in tea plants

Drought stress significantly restrained the yield and quality of tea products in main tea-growing countries, which further aggravated economic loss (Wang et al., 2016). Although growing more evidences showed that the morphological, physiological and molecular changes occurred in tea plants under drought stress (Upadhyaya and Panda, 2013; Liu et al., 2015; Zhou et al., 2014), but no studies was conducted to investigate the role of mesophyll cell ability to maintain K^+ in tea plant drought tolerance, at least at the level of an accessory of tea varieties varied in drought tolerance. In this study, we screened drought-induced K^+ efflux from 12 tea varieties differed in drought stress tolerance. Our results found that the most drought tolerant variety Taicha 12 used in this study showed the lowest net K^+ efflux from mesophyll cells under drought stress, whereas Fuyun 6 (the most drought sensitive variety used in this study) showed the highest mesophyll K^+ efflux (Fig. 3B). This mesophyll K^+ retention ability in screened 12 tea varieties is significantly and positively ($R^2 = 0.8239$, $P < 0.001$) correlated with its biomass under drought (Fig. 3C), suggesting a role of mesophyll K^+ retention in tea plant drought tolerance. As mentioned early, the importance of mesophyll K^+ retention in plant abiotic stress tolerance e.g. salinity stress tolerance (Wu et al., 2013, 2018b) is demonstrated. This is in agreement with previous studies about the role of K^+ in plant drought stress response. For example, drought-tolerant barely variety retained more potassium and showed higher water content and chlorophyll content in the flag leaf under drought stress than the sensitive variety (Hosseini et al., 2016). Similarly, drought tolerant Tibetan wild barley XZ5 and cv Tadmor retained more K^+ in leaves under drought stress than the drought-sensitive barely cv. ZJU9 (Feng et al., 2016). We further confirmed the role of mesophyll K^+ retention in tea plant drought tolerance by measuring the relative K^+ content after drought. Indeed, drought tolerant species showed significant higher maintained K^+ than the sensitive varieties (Fig. 5A), suggesting this more maintained K^+ in drought tolerant tea varieties could be attributed by its higher mesophyll K^+ retention. Previous studies also found that plants possess better mesophyll K^+ retention ability have higher leaf K^+ content than the one without superior mesophyll K^+ retention ability (Wu et al., 2014, 2015b).

Moreover, we further applied exogenous K^+ to drought stressed tea plants to investigate its effect on leaf K^+ content and drought stress performance. Our results showed that regardless of the genotypic difference of two tea varieties (Taicha 12 and Fuyun 6) contrasting in drought stress tolerance, applying K^+ significant increased leaf K^+ content and mitigated drought stress symptom (Fig. 6A and Fig. S2), showing a beneficial role of exogenous K^+ in tea plant drought tolerance. This is in accordance with previous studies showing applying K^+ can alleviate drought stress in plants. Zain and Ismail (2016) reported that the application of potassium alleviated the negative effect of drought on rice. Similar results were also found in cotton (Zahoor et al., 2017) and maize plants (Martineau et al., 2017) under drought stress. This result (Fig. 6) further supported our claim about the importance of mesophyll K^+ retention in tea plant drought stress tolerance. Also, supplying K^+ can be a feasible way to mitigate the detrimental effects of drought on tea plant performance.

4.2. Identity of channels conferring K^+ retention in tea leaf mesophyll

As mentioned early, KOR and NSCC channels are the main channels located on plasma membrane controlling K^+ efflux. To better understand the mechanisms beyond mesophyll K^+ retention in tea plants, we used channels inhibitors TEA (a known blocker of KOR and KIR channels) and blockers Gd^{3+} (blockers of NSCC channels) (Chen et al., 2007; Wu et al., 2014) to investigate the identity of channels controlling mesophyll K^+ efflux in drought stressed tea plants. Our results showed that compared with K^+ outward rectifying channel, NSCCs played the main role on controlling K^+ efflux in the drought tolerant tea variety Taicha 12, whereas both K^+ outward rectifying channel and NSCCs played a role in regulating K^+ efflux in drought sensitive tea variety Fuyun 6 (Fig. 5A and B). These results suggest a specificity of actual mechanisms controlling drought induced K^+ efflux in mesophyll cells between tolerant and sensitive tea plants. Cell and tissue specificity of channels controlling K^+ efflux was found across many plant species, e.g. wheat (Cuin et al., 2012; Wu et al., 2014), barley (Chen et al., 2007; Wu et al., 2013) and pea (Shabala et al., 2007). Furthermore, this result (Fig. 5B) further suggested that targeting K^+ outward rectifying channel to reduce its activity could potentially modulate mesophyll K^+ retention ability in drought sensitive tea plants and thus benefit the program of breeding drought tolerant tea plants.

ROS accumulation is very common in plants under stress (Zhu, 2016; Wu et al., 2017). KOR channels are known to be activated by both membrane depolarization and ROS (Demidchik and Maathuis, 2007). ROS-activated NSCC channel are also known to play an important role in mesophyll K^+ retention (Wu et al., 2015a, 2018b). In our study, we found that drought tolerant tea varieties have lower MDA content than the sensitive varieties (Fig. 2C), suggesting a link between K^+ retention in tea leaf mesophyll cells and its redox status. The MDA represented the degree of lipid-membrane oxidation (Zhang et al., 2018a). Also, more importantly, drought tolerant tea variety Taicha 12 showed significant lower leaf H_2O_2 content than sensitive variety Fuyun 6 (Fig. 5E). These results further support our finding that drought tolerant tea varieties have superior mesophyll K^+ retention ability than sensitive varieties via modulating the activities of membrane located

KOR and NSCC channels.

Overall, our results showed that mesophyll K^+ retention is a component involved in tea plant drought stress tolerance. Also, targeting the K^+ outward rectifying channel could be an option for improving drought tolerance in sensitive tea plants since the identity of ROS-activated NSCC channels still need to be studied.

5. Conclusion

Our results strongly suggest that mesophyll K^+ retention is an important mechanism involved in tea plant drought tolerance. Applying exogenous K^+ also helps to mitigate the drought stress of tea plants. In addition, the role of the K^+ outward rectifying channel in K^+ retention in tea mesophyll is relatively small in drought tolerant tea variety Taicha12, while ROS-activated NSCCs may be most likely the main pathways for tea mesophyll K^+ leak under drought conditions.

Author contribution

Zhang, X.,C., Wu, H.,H., Chen, L.,M., and Wan, X.,C., conceived and designed the study. Zhang, X.,C., Chen, L.,M., and Wang N.,N., conducted the experiments. Zhang, X.,C., Chen, L.,M., and Wu, H.,H., analyzed the data. Zhang, X.,C., and Wu, H.,H., wrote the paper.

Acknowledgments

This work was supported by the Open Fund of State Key Laboratory of Tea Plant Biology and Utilization at Anhui Agricultural University (SKLTOF20170112) to H.W. and X.Z., and supported by the Science Foundation for Anhui Province (KJ2017A126) to X.Z. In addition, this work was also supported by the National Natural Science Foundation of China (grant number 31800583) grant to W.X.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.01.020.

References

Ahmed, S., Stepp, J.R., Orians, C., Griffin, T., Matyas, C., Robbat, A., Cash, S., Xue, D.,Y., Long, C.,L., Unachukwu, U., Buckley, S., Small, D., Edward Kennelly, E., 2014. Effects of extreme climate events on tea (*Camellia sinensis*) functional quality validate indigenous farmer knowledge and sensory preferences in tropicalchina. PLoS One 9, e109126.

Bahrami-Rad, S., Hajiboland, R., 2017. Effect of potassium application in drought-stressed tobacco (*Nicotiana rustica L.*) plants: comparison of root with foliar application. Ann. Agric. Sci. 62, 121–130.

Chen, Z., Newman, I., Zhou, M., Mendham, N., Zhang, G., Shabala, S., 2005. Screening plants for salt tolerance by measuring K^+ flux: a case study for barley. Plant Cell Environ. 28, 1230–1246.

Chen, Z.H., Pottosin, I.I., Cuin, T.A., Fuglsang, A.T., Tester, M., Jha, D., Zepeda-Jazo, I., Zhou, M.X., Palmgren, M.G., Newman, I.A., Shabala, S., 2007. Root plasma membrane transporters controlling K^+/Na^+ homeostasis in salt-stressed barley. Plant Physiol. 145, 1714–1725.

Chen, G., Feng, H.M., Hu, Q.D., Qu, H.Y., Chen, A.Q., Yu, L., Xu, G.H., 2015. Improving rice tolerance to potassium deficiency by enhancing OsHAK16p:WOX11-controlled root development. Plant Biotechnol. J. 13 (6), 833–848.

Cheruiyot, E.K., Mumera, L.M., Ngetich, W.K., Hassanali, A., Wachira, F.N., 2010. High fertilizer rates increase susceptibility of tea to water stress. J. Plant Nutr. 33, 115–129.

Cuin, T.A., Zhou, M., Parsons, D., Shabala, S., 2012. Genetic behaviour of Physiological traits conferring K^+/Na^+ homeostasis in wheat. Plant Biol. 14, 438–446.

Demidchik, V., 2014. Mechanisms and physiological roles of K^+ efflux from root cells. J. Plant Physiol. 171, 696–707.

Demidchik, V., Maathuis, F.J.M., 2007. Physiological roles of nonselective cation channels in plants: from salt stress to signalling and development. New Phytol. 175, 387–404.

Dreyer, I., Uozumi, N., 2011. Potassium channels in plant cells. FEBS J. 278, 4293–4303.

Eisenstei, M., 2013. Plant breeding: discovery in a dry spell. Nature 501, 7–9.

Evans, H.J., Sorger, G.J., 1966. Role of mineral elements with emphasison the univalent cations. Annu. Rev. Plant Physiol. 17, 47–76.

Feng, X., Liu, W.X., Zeng, F.R., Chen, Z.H., Zhang, G.P., Wu, F.B., 2016. K^+ Uptake, H^+-ATPase pumping activity and Ca^{2+} efflux mechanism are involved in drought tolerance of barley. Environ. Exp. Bot. 129, 57–66.

Feng, L., Yang, T.Y., Zhang, Z.L., Li, F.D., Chen, Q., Sun, J., Shi, C.Y., Deng, W.W., Tao, M.M., Tai, Y.L., Yang, H., Cao, Q., Wan, X.C., 2017. Identification and characterization of cationic amino acid transporters (CATs) in tea plant (*Camellia sinensis*). Plant Growth Regul. 84, 1–13.

Gao, H.J., Zhao, Q., Zhang, X.C., Wan, X.C., Mao, J.D., 2014. Localization of fluoride and aluminum in subcellular fractions of tea leaves and roots. J. Agric. Food Chem. 62 (10), 2313–2319.

Gupta, S., Bharalee, R., Bhorali, P., Das, S.K., Bhagawati, P., Bandyopadhyay, T., Gohain, B., Agarwal, N., Ahmed, P., Borchetia, S., Kalita, M.C., Handique, A.K., Das, S., 2013. Molecular analysis of drought tolerance in tea by cDNA-AFLP based transcript profiling. Mol. Biotechnol. 53, 237–248.

Hosseini, S.A., Hajirezaei, M.R., Seiler, C., Sreenivasulu, N., Wiren, N., 2016. A potential role of flag lead potassium in conferring tolerance to drought-induced leaf senescence in barley. Front. Plant Sci. 7, 206.

Li, Q.S., Lin, X.M., Qiao, R.Y., Zheng, X.Q., Lu, J.L., Ye, J.H., Liang, Y.R., 2017. Effect of fluoride treatment on gene expression in tea plant (*Camellia sinensis*). Sci. Rep. 7, 9847.

Liu, S.C., Yao, M.Z., Ma, C.L., Jin, J.Q., Li, C.F., Chen, L., 2015. Physiological changes and differential gene expression of tea plant under dehydration and rehydration conditions. Sci. Hortic. 184, 129–141.

Liu, S.R., An, Y.L., Li, F.D., Li, S.J., Liu, L.L., Zhou, Q.Y., Zhao, S.Q., Wei, C.L., 2018. Genome-wide identification of simple sequence repeats and development of polymorphic SSR markers for genetic studies in tea plant (*Camellia sinensis*). Mol. Breed. 38, 59.

Mak, M., Babla, M., Xu, S.C., O'Carrigana, A., Liu, X.H., Gong, Y.M., Holford, P., Chen, Z.H., 2014. Leaf mesophyll K^+, H^+ and Ca^{2+} fluxes are involved in drought-induced decrease in photosynthesis and stomatal closure in soybean. Environ. Exp. Bot. 98, 1–12.

Martineau, E., Domec, J.C., Bosc, A., Denoroy, P., Fandino Jr., V.A., J, L., Jordan-Meille, L., 2017. The effects of potassium nutrition on water use in field-grown maize (*Zea mays* L.). Environ. Exp. Bot. 134, 62–71.

Mekawy, A.M.M., Dekoum, V.M., Yahagi, A.H., Tada, Y., Ueda, A., Saneoka, H., 2015. Growth, physiological adaptation, and gene expression analysis of two Egyptian rice cultivars under salt stress. Plant Physiol. Biochem. 87, 17–25.

Pottosin, I., Dobrovinskaya, O., 2014. Non-selective cation channels in plasma and vacuolar membranes and their contribution to K^+ transport. J. Plant Physiol. 171, 732–742.

Ruan, J.Y., Gerendas, J., Hardter, R., Sattelmacher, B., 2007. Effect of root zone pH and form and concentration of nitrogen on accumulation of quality-related components in green tea. J. Sci. Food Agric. 87, 1505–1516.

Shabala, S., Cuin, T.A., Pottosin, I., 2007. Polyamines prevent NaCl-induced K^+ efflux from pea mesophyll by blocking non-selective cation channels. FEBS Lett. 581, 1993–1999.

Suelter, C.H., 1970. Enzymes activated by monovalent cations. Science 168, 789–795.

Sun, J., Dai, S., Wang, R., Chen, S., Li, N., Zhou, X., Lu, C., Shen, X., Zheng, Z., Hu, Z., Zhang, Z., Song, J., Xu, Y., 2009. Calcium mediates root K^+/Na^+ homeostasis in poplar species differing in salt tolerance. Tree Physiol. 29, 1175–1186.

Tang, Q., Li, X.L., Zhu, X.Y., Huang, Y., Yang, H., 2008. Effects of plumbum and chromium stress on the growth of tea plants, Southwest China. J. Agric. Sci. 21, 156–162.

Tavakol, E., Jákli, B., Cakmak, I., Dittert, K., Karlovsky, P., Pfoh, l K., Senbayram, M., 2018. Optimized potassium nutrition improves plant-water-relations of barley under PEG-induced osmotic stress. Plant Soil 430, 23–35.

Upadhyaya, H., Panda, S.K., 2013. Abiotic stress responses in tea [*Camellia sinensis L (O) Kuntze*]: an overview. Rev. Agric. Sci. 1, 1–10.

Upadhyaya, H., Dutta, B.K., Panda, S.K., 2013. Zinc modulates drought-induced biochemical damages in tea [*Camellia sinensis (L) O Kuntze*]. J. Agric. Food Chem. 61, 6660–6670.

Wang, X.K., 2006. The Principle and Technology of Plant Physiology and Biochemistry Experiment. Higher Education Press, Beijing.

Wang, M., Zheng, Q.S., Shen, Q.R., Guo, S.W., 2013. The critical role of potassium in plant stress response. Int. J. Mol. Sci. 14, 7370–7390.

Wang, W.D., Xin, H.H., Wang, M.L., Ma, Q.P., Wang, L., Kaleri, N.A., Wang, Y.H., Li, X.H., 2016. Transcriptomic analysis reveals the molecular mechanisms of drought stress-induced decreases in Camellia sinensis leaf quality. Front. Plant Sci. 7, 385.

Wei, C.,L., Yang, H., Wang, S.,B., et al., 2018. Draft genome sequence of Camellia sinensis var. sinensis provides insights into the evolution of the tea genome and tea quality. P Natl Acad Sci USA 115, E4151–E4158.

Wu, H., 2016. Tissue Specificity of Cytosolic K^+ Retention, Na^+ Extrusion, and Vacuolar Na^+ Sequestration Traits in the Context of Differential Salinity Stress Tolerance in Barley and Wheat. University of Tasmania, Australia, Hobart.

Wu, H., Shabala, L., Barry, K., Zhou, M., Shabala, S., 2013. Ability of leaf mesophyll to retain potassium correlates with salinity tolerance in wheat and barley. Physiol. Plantarum 149, 515–527.

Wu, H., Shabala, L., Zhou, M., Shabala, S., 2014. Durum and bread wheat differ in their ability to retain potassium in leaf mesophyll: implications for salinity stress tolerance. Plant Cell Physiol. 55, 1749–1762.

Wu, H., Shabala, L., Zhou, M., Shabala, S., 2015a. Chloroplast generated ROS dominate NaCl-induced K^+ efflux in wheat leaf mesophyll. Plant Signal. Behav. 10, e1013793.

Wu, H., Shabala, L., Zhou, M., Shabala, S., 2015b. MIFE technique based screening for mesophyll K^+ retention for crop breeding for salinity tolerance. Bio-Protocol 5, e1446.

Wu, H., Zhu, M., Shabala, L., Zhou, M., Shabala, S., 2015c. K^+ retention in leaf mesophyll, an overlooked component of salinity tolerance mechanism: a case study for barley. J. Integr. Plant Biol. 57, 171–185.

Wu, H., Tito, N., Giraldo, J.P., 2017. Anionic cerium oxide nanoparticles protect plant photosynthesis from abiotic stress by scavenging reactive oxygen species. ACS Nano 11, 11283–11297.
Wu, H., Shabala, L., Shabala, S., Giraldo, J.P., 2018a. Hydroxyl radical scavenging by cerium oxide nanoparticles improves *Arabidopsis* salinity tolerance by enhancing leaf mesophyll potassium retention. Environ. Sci. Nano 5, 1567–1583.
Wu, H., Zhang, X., Giraldo, J.P., Shabala, S., 2018b. It is not all about sodium: revealing tissue specificity and signaling roles of potassium in plant responses to salt stress. Plant Soil 431, 1–17.
Yang, D.,D., Ling, J., Wang, Y.,S., Sun, F., Tao, H., Xu, Q., Zhang, L., Zhang, Z.,Z., Ho, C.,T., Wan, X.,C., 2016. Tea waste: an effective and economic substrate for oyster mushroom cultivation. J. Sci. Food Agric. 96, 680–684.
Yang, F., Li, P.P., He, Z.Y., 2017. The effect of high temperature and drought on tea plants growth and quality. Jiangsu Agric. Sci. 45, 10–13 (In Chinese with English abstract).
Zahoor, R., Dong, H.R., Abid, M., Zhao, W.Q., Wang, Y.H., Zhou, Z.G., 2017. Potassium fertilizer improves drought stress alleviation potential in cotton by enhancing photosynthesis and carbohydrate metabolism. Environ. Exp. Bot. 137, 73–83.
Zain, N.A.M., Ismail, M.R., 2016. Effects of potassium rates and types on growth, leaf gas exchange and biochemical changes in rice (Oryza sativa) planted under cyclic water stress. Agric. Water Manag. 164, 83–90.
Zhang, X.C., Gao, H.J., Wu, H.H., Yang, T.Y., Wang, Y.M., Wan, X.C., 2015. Ca^{2+} and CaM are involved in Al^{3+} pretreatment-promoted fluoride accumulation in tea plants. Plant Physoil. Bioch. 96, 288–295.
Zhang, X.C., Gao, H.J., Yang, T.Y., Wu, H.H., Wang, Y.M., Zhang, X.C., Wan, X.C., 2016. Al^{3+}-promoted fluoride accumulation in tea plants (Camellia sinensis) was inhibited by an anion channel inhibitor DIDS. J. Sci. Food Agric. 96 (12), 4224–4230 2016.
Zhang, X.C., Wu, H.H., Chen, L.M., Li, Y.Y., Wan, X.C., 2018a. Efficient iron plaque formation on tea (*Camellia sinensis*) roots contributes to acidic stress tolerance. J. Inter. Plant Biol. https://doi.org/10.1111/jipb.12702.
Zhang, X.C., Chen, L.M., Wu, H.H., Liu, L.L., Wan, X.C., 2018b. Root plasma membrane H^{+}-ATPase is involved in low pH-inhibited nitrogen accumulation in tea plants (*Camellia sinensis L.*). Plant Growth Regul. https://doi.org/10.1007/s10725-018-0440-2.
Zhang, X.C., Wu, H.H., Chen, L.M., Liu, L.L., Wan, X.C., 2018c. Maintenance of mesophyll potassium and regulation of plasma membrane H^{+}-ATPase are associated with physiological responses of tea plants to drought and subsequent rehydration. Crop J. https://doi.org/10.1016/j.cj.2018.06.001.
Zhou, L., Xu, H., Mischke, S., Meinhardt, L.W., Zhang, D., Zhu, X., Zhu, X.J., Li, X.H., Fang, W.P., 2014. Exogenous abscisic acid significantly affects proteome in tea plant (*Camellia sinensis*) exposed to drought stress. Hortic. Res. 1, 14029.
Zhu, J.K., 2016. Abiotic stress signaling and responses in plants. Cell 167, 313–324.