Effects of exogenous calcium on the drought response of the tea plant (*Camellia sinensis* (L.) Kuntze)

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**ABSTRACT**

**Background:** Drought is one of the major factors reducing the yield of many crops worldwide, including the tea crop (*Camellia sinensis* (L.) Kuntze). Calcium participates in most of cellular signaling processes, and its important role in stress detection and triggering a response has been shown in many crops. The aim of this study was to evaluate possible effects of calcium on the tea plant response to drought.

**Methods:** Experiments were conducted using 3-year-old potted tea plants of the best local cultivar Kolkhida. Application of ammonium nitrate (control treatment) or calcium nitrate (Ca treatment) to the soil was performed before drought induction. Next, a 7-day drought was induced in both groups of plants. The following physiological parameters were measured: relative electrical conductivity, pH of cell sap, and concentrations of cations, sugars, and amino acids. In addition, relative expression levels of 40 stress-related and crop quality-related genes were analyzed.

**Results:** Under drought stress, leaf electrolyte leakage differed significantly, indicating greater damage to cell membranes in control plants than in Ca-treated plants. Calcium application resulted in greater pH of cell sap; higher accumulation of tyrosine, methionine, and valine; and a greater Mg²⁺ content as compared to control plants. Drought stress downregulated most of the quality-related genes in both groups of tea plants. By contrast, significant upregulation of some genes was observed, namely CRK45, NAC26, TPS11, LOX1, LOX6, Hydrolase22, DREB26, SWEET2, GS, ADC, DHN2, GOLS1, GOLS3, and RHL41. Among them, three genes (LOX1, RHL41, and GOLS1) showed 2–3 times greater expression in Ca-treated plants than in control plants. Based on these results, it can be speculated that calcium affects galactinol biosynthesis and participates in the regulation of stomatal aperture not only through activation of abscisic-acid signaling but also through jasmonic-acid pathway activation. These findings clarify calcium-mediated mechanisms of drought defense in tree crops. Thus, calcium improves the drought response in the tea tree.
INTRODUCTION

High yields under unfavorable environmental conditions are important for sustainable crop production. Drought is a major environmental constraint reducing the yield of many economically important crops, and climate aridization has been increasing worldwide. One negative effect of drought stress is oxidative damage leading to disturbances of physiological and biochemical processes causing significant losses in tea quality and yields (Upadhyaya & Panda, 2004; Marciniška et al., 2013; Maritim et al., 2015). During earlier research, many genetic and physiological mechanisms of drought tolerance have been clarified in various crops including the tea crop (Bhagat, Baruah & Cacigue, 2010; Damayanthi, Mohotti & Nissanka, 2010; Man et al., 2011; Baruah & Bhagat, 2012; Maritim et al., 2015; Zhu, 2016; Fleta-Soriano & Munné-Bosch, 2016; Malyukova et al., 2020, 2021; Samarina et al., 2020). It has been shown that plants reorganize their osmoregulatory and antioxidant systems and secondary-metabolite production in response to drought stress (Fleta-Soriano & Munné-Bosch, 2016; Zhu, 2016). Many transcription factors and metabolite-related genes are involved in the drought response in the tea plant. For example, key cold response regulators ICE, CBF, and DHN participate in the drought response too (Liu et al., 2016a; Yin et al., 2016; Ban et al., 2017; Hu et al., 2020). Transcriptional data on the tea plant have revealed 12 transcription factor families (AP2/EREBP, bHLH, bZIP, HD-ZIP, HSF, MYB, NAC, WRKY, zinc-finger protein transcription factors, SCL, ARR, and SPL) performing crucial functions in tea drought responses via both abscisic acid (ABA)-dependent and ABA-independent pathways (Liu et al., 2016a; Yue et al., 2015; Wang et al., 2016a; Cui et al., 2018; Chen et al., 2018; Ma et al., 2019; Samarina et al., 2020).

Although many mechanisms of tea drought responses have been revealed, the topic of exogenous regulation of drought tolerance by chemical and biological compounds is still not elucidated sufficiently. Some studies indicate enhancement of drought tolerance by hormone treatments (Man et al., 2011; Njoloma, 2012; Upadhyaya & Panda, 2004). On the other hand, external application of mineral nutrients to increase drought tolerance still has not been studied well. Among a wide range of biogenic macro- and microelements, calcium is of particular interest because it participates in signal transduction under unfavorable environmental conditions (Zhang et al., 2015; Edel et al., 2017; Singh, Parihar & Prasad, 2018; Thor, 2019; Hosseini et al., 2019; Ramírez-Builes et al., 2020). Calcium takes part in most of cellular signaling processes, and its important role in early stress detection and triggering a response has been demonstrated in many crops (Kim, 2009; Upadhyaya et al., 2012; Singh, Parihar & Prasad, 2018; Thor, 2019; Hosseini et al., 2019). Calcium interacts strongly with reactive oxygen species and participates in H$_2$O$_2$ sensing and in the induction of antioxidant defense in plants (Rentel & Knight, 2004; Evans et al., 2005; Noctor, 2006). It is believed that calcium influx and cytoplasmic calcium ([Ca$^{2+}$]c) are important for ABA transduction in guard cells, whereas ABA can regulate stomatal...
aperture in guard cells. In *Arabidopsis thaliana*, amplitudes of extracellular Ca\(^{2+}\) concentration oscillation and of cytosolic Ca\(^{2+}\) concentration oscillation are controlled by soil Ca\(^{2+}\) levels and transpiration rates (*Hetherington & Brownlee, 2004; Kim, 2009*).

Although external calcium application has been useful at increasing abiotic-stress tolerance in several crops, it is not usually considered a tool for improving drought tolerance of tea plantations because tea is an acidophilic crop. Nevertheless, a deficiency of soil calcium was revealed during our long-term observations in tea plantations on the Black Sea Coast of the Caucasus. Soil acidification usually develops during long-term cultivation of tea on acidic soils, thereby significantly diminishing the amounts of available forms of calcium (*Malyukova et al., 2021*). Thus, the aim of the present study was to assess a possible effect of external calcium application on the drought response of the tea plant in terms of physiological parameters and gene expression levels.

**MATERIALS AND METHODS**

**Plant materials, growth conditions, and stress induction**

Three-year-old vegetatively propagated potted plants of the best local tea cultivar Kolkhida were used in this study (Fig. S1). Plants were 50 cm tall and grown in 2-liter polyethylene pots filled with brown forest acidic soil. Before the drought treatment, the experimental plants were precultivated under controlled conditions for 15 days at 20 °C ± 2 °C, humidity of 60% ± 5%, light intensity of 5,000 lux, on a 16/8 h light/dark cycle. At the beginning of this period, one of two fertilizer treatments was started.

- Control treatment: four-time application of an ammonium nitrate solution, 50 ml (150 mg/l, which is equivalent to 100 mg of nitrogen per plant) per pot of soil, during a month: days 0, 10, 20, and 30. Soil pH was 3.9.
- Ca treatment: four-time application of a calcium nitrate solution, 50 ml (400 mg/l, which is equivalent to 100 mg of nitrogen and 150 mg of calcium per plant) per pot of soil during the month: days 0, 10, 20, and 30. Soil pH was 4.3.

On day 30, leaves were sampled for physiological, biochemical, and gene expression analyses and were designated as “no drought” treatment groups. After that, these plants were subjected to soil drought via a gradual decrease in watering: during 7 days, soil humidity was reduced from 70% ± 2% to 16% ± 2%, until cell sap concentration reached a critical level of 10–12%. Next, leaves were sampled for physiological, biochemical, and gene expression analyses and were designated as “drought” treatment groups.

For each analysis, 2\(^{nd}\), 3\(^{rd}\), and 4\(^{th}\) mature leaves from the top of a plant were sampled in the morning between 8 and 9 am. All experiments were performed on three biological replicates (three plants); all experiments were conducted twice in the 2019–2020 period.

**Assays of physiological and biochemical parameters**

The leaf water content was calculated as \(C = \left(\frac{FW - DW}{FW}\right) \times 100\%\), where FW is fresh leaf mass, and DW is dried leaf mass (leaves were dried at 105 °C in an oven for 5 h) (*Yamasaki & Dillenburg, 1999*).
Electrolyte leakage was determined using a ST300C portable conductivity meter (Ohaus, USA) via the following formula:

\[
EL = \frac{L_1}{L_0} \times 100
\]

where \(L_0\) and \(L_1\) are electrical conductivity immediately and 2 h after leaf immersion in deionized water, respectively, and \(L_2\) is conductivity after boiling for 120 min at 100 °C with subsequent cooling to room temperature (Bajji, Kinet & Lutts, 2001).

pH of cell sap was determined by means of a Testo 205 pH-meter (Moscow, Russia) with a hydrogen electrode. For this measurement, 1,000 mg of fresh leaf tissue was homogenized in 20 ml of distilled water (Malyukova et al., 2020).

Amino acids (mg g\(^{-1}\) dried leaf mass), sugars (mg g\(^{-1}\) dried leaf mass) and cations (µg g\(^{-1}\) dried leaf mass) and organic acids (mg g\(^{-1}\) dried leaf mass) were assayed by capillary electrophoresis on a Kapel-105M analyzer (Russia) (Brykalov et al., 2019).

**Analyses of gene expression profiles by quantitative reverse-transcription PCR (qRT-PCR)**

Total RNA was extracted from the third mature leaf in three biological replicates by the guanidine method according to the manufacturer’s protocol (Biolabmix, Novosibirsk, Russia; https://biolabmix.ru/). The concentration and quality of RNA were determined on a Bio-drop µLite spectrophotometer (Biochrom, Cambridge, UK) and RNA integrity was assessed by electrophoresis in a 1% agarose gel. Then, 1,000 ng of RNA was treated with 1 µl of DNaseI Buffer and 1 µl DNaseI (Biolabmix, Novosibirsk, Russia; https://biolabmix.ru/) for 30 min at 42 °C with subsequent DNase inhibition by heating. After that, 1,000 ng of RNA was employed to prepare 20 µl of cDNA using the M-MuLV–RH-kit (Biolabmix, Novosibirsk, Russia; https://biolabmix.ru/) with subsequent quality evaluation by gel electrophoresis and qRT-PCR on LightCycler96 (Roche Life Sciences, Penzberg, Germany; https://lifescience.roche.com/global_en.html). After cDNA preparation, all samples were diluted to the same concentration of 700 ng µl\(^{-1}\) according to standardization by means of the expression of a reference gene, actin (NCBI Gene ID: 114316878). To measure gene expression, qPCR was carried out in a 15 µl reaction mixture consisting of 7.5 µl of 2× SybrBlue qRT-PCR buffer with hot-start polymerase (Biolabmix, Novosibirsk, Russia; https://biolabmix.ru/), 0.2 µl of each primer (forward and reverse), 1 µl of cDNA, and the rest of the volume was PCR grade water. A two-step amplification program was as follows: preheating for 5 min at 95 °C, 40 cycles of amplification (10 s at 95 °C and 30 s at 56–62 °C), final extension for 5 min at 72 °C, and melting for 3 min at 95 °C. In total, more than 40 genes were analyzed in this study (Table S1).

The relative gene expression level was calculated by the \(2^{-\Delta\Delta Cq}\) method of Livak & Schmittgen (2001), where
\[ \Delta \Delta \text{Cq} = (C_{\text{q, gene of interest}} - C_{\text{q, internal control}})^{\text{treatment}} - (C_{\text{q, gene of interest}} - C_{\text{q, internal control}})^{\text{control}}. \]

**Data analysis and visualization**

The experimental design was completely randomized. One-way ANOVA and Student’s *t* test were performed to find significant differences in effects among the treatments. The significance of the differences was evaluated by the Fisher test, LSD$_{0.05}$, and standard deviations from the mean. In addition, principal component analysis (PCA) and hierarchical clustering were conducted to examine the relations and visualize genetic and biochemical results. Dissimilarities were calculated using the DICE coefficient, with agglomeration by Ward’s method. Two separate matrices of biochemical and the genetic data were subjected to PCA. Before this procedure, the data were normalized: all data were converted to the ratio of a drought treatment group to a no-drought treatment group. After that, the normalized matrices were analyzed separately by Pearson (n)-type PCA, and two plots (biochemical and genetic) were superimposed. Statistical analyses of the data were carried out in the XLSTAT software (free trial version) ([https://www.xlstat.com/](https://www.xlstat.com/)).

**RESULTS**

The effect of Ca application on physiological and biochemical parameters of the tea plant under drought

Under drought stress, three-time elevation of electrolyte leakage was noted in control plants but not in Ca-treated plants (Fig. 1A). A significant decrease in the water content...
(from 76–77% to 71–72%) was detected in both control and Ca-treated plants (Fig. 1B). Furthermore, pH of cell sap increased significantly under drought stress in both groups of plants, and Ca-treated plants experienced higher elevation of pH as compared to control plants (Fig. 1C).

As for biochemical parameters, Ca-treated plants manifested significantly higher glucose (23 mg g$^{-1}$) and fructose (17.6 mg g$^{-1}$) accumulation as compared to control plants (18.6 and 11.9 mg g$^{-1}$, respectively) under drought (Fig. 2A). Additionally, in both groups of plants, cation contents changed significantly in tea leaves under drought (Fig. 2B). That is, control plants showed a significant decrease in the Na$^+$ content from 506 to 193 µg g$^{-1}$ dry leaf mass, which was not observed in Ca-treated plants, but significant elevation of the Ca$^{2+}$ content from 253 to 398 µg g$^{-1}$ dry leaf mass and increased Mg$^{2+}$ accumulation were registered in Ca-treated plants under drought.

Drought stress diminished the content of several amino acids, namely Met, Val, Phen, Arg, and Leu, in mature tea leaves. In Ca-treated tea plants, Met, Val, and Tyr accumulated more strongly under drought stress (Fig. 2C). Among these compounds, the highest

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**Figure 2** Biochemical parameters in the tea tree under drought. Accumulation of biochemical compounds in mature tea leaves under drought stress: sugars (A), cations (B), amino acids (C), and organic acids (D). Different lowercase letters indicate significant differences at $P < 0.05$. DOI: 10.7717/peerj.13997/fig-2
accumulation was detected for two amino acids (Met (3-fold) and Val (2-fold)) in comparison with no-drought conditions. The levels of Pro, Ser, Thr, and Ala were not influenced either by Ca application or by drought induction.

Different levels of organic acids were present before drought induction: control plants had 1.5–2.0-fold higher levels of malic, citric, succinic, and acetic acids than the Ca-treated plants did (Fig. 2D). Under drought stress, contents of malic, succinic, citric, and acetic acids declined 2–3-fold in control plants. By contrast, only the citric acid content decreased in Ca-treated plants. To summarize, drought treatment significantly affected physiological integrity of tea plants, but Ca-treated and control plants showed different responses to drought, indicating better physiological status under stress in Ca-treated plants.

The impact of Ca application on gene expression profiles of the tea plant under drought and relations with biochemical data

Hierarchical clustering based on the gene expression profiles gave five distant clusters (Fig. 3). The upper cluster contained 13 genes including nine genes upregulated by drought (CRK45, NAC26, TPS11, LOX1, DREB26, LOX6, GS, ADC, and BAM).

The second cluster was the biggest one and comprised 26 genes. Among them, 13 genes (WRKY2, FLS2, FLSb, LDOX, F3′H, F3H, WRKY42, FLS, bHLH102, TCS, TS, CAU1, and CHI) were downregulated by drought stress, and the other 13 genes (SnRK1.1, SnRK1.2, SnRK1.3, IMPDH, DHNI, RS1, Anase, DHN3, ANR, SUS, RS2, GR-RBP3, and CHS) were not affected by drought.

The third cluster consisted of three genes with increased expression under drought: GOLS3, SWEET2, and DHN2. The greatest fold change was registered for two genes—RHL41 and GOLS1—which were outliers.

Finally, among the drought-induced genes, four genes (RHL41, GOLS1, LOX1, and DREB26) were more strongly expressed in Ca-treated plants than in control plants.

Pairwise comparisons of the four treatment groups by PCA uncovered high Pearson’s correlation between two principal components and factors at a significance level of α = 0.05. Several genes and biochemical parameters clearly separated two groups (Drought-Control and Drought-Ca) with high positive loadings (Fig. 4). The largest square cosines (0.78) were observed in principal component 1 (PC1). Examination of the biplot (superimposed PCA plots) revealed that the Drought-Control and Drought-Ca vectors are positioned on the positive and negative sides of PC1, respectively, with high loadings. Several data points related to each vector were found to be grouped closely with them. For example, the highest positive loading belongs to genes RHL41, GOLS1 and LOX1, and amino acids Met and Val are distributed with high loading along the Drought-Ca vector. In addition, many genes and biochemical parameters that were more pronounced in the control (no drought) treatment group are densely clustered and located on the PC2 side, orthogonally to the drought-related vectors. Nonetheless, most of PC2 scores are low, meaning weak correlations between the data points.
Figure 3  Gene expression in the tea tree under drought. A heat map of relative gene expression and hierarchical clustering of genes in the tea plant under drought stress. Genes highlighted in green: upregulated under drought in both groups of plants vs. no drought; blue: genes with greater upregulation in Ca-treated plants; orange: genes with greater upregulation under drought in control plants; gray: genes with decreased expression in both groups of plants vs. no drought. Different lowercase letters indicate significant differences at $P < 0.05$. DOI: 10.7717/peerj.13997/fig-3
DISCUSSION

The effect of Ca application on physiological and biochemical parameters of the tea plant under drought

In this study, we assessed the effect of calcium application to soil on the tea plant drought response. Under drought stress, typical physiological changes were observed in plants such as alterations in the leaf water content, electrolyte leakage, and pH of cell sap, which are prominent phenotypical indicators of the drought response in plants. Nonetheless, Ca-treated plants manifested less electrolyte leakage and elevated pH, pointing to less damage to cell membranes under drought stress. Cell sap pH is the one of the first signals leading to ABA synthesis and stomata closure; therefore, it is a sensitive physiological indicator of the drought response (Song et al., 2008), consistently with our results.

The higher concentration of sugars observed in Ca-treated tea plants before stress induction is probably due to predominance of the sucrose biosynthetic pathway over the starch biosynthetic pathway; this arrangement can ensure higher viscosity of the cytoplasm to prevent water evaporation under drought, in agreement with previous data (Kaelke & Dawson, 2005; Kasuga, Arakawa & Fujikawa, 2007; Regier et al., 2010).

Among the other physiological parameters, the cation content is an important diagnostic parameter for site-specific and efficient nutrient management (Hauer-Jákli &

Figure 4  PCA of biochemical and genetic parameters. A PCA biplot representing superimposed data from biochemical and genetic principal component analyses in Ca-treated (Ca) and Ca-untreated (Control) tea plants under drought. Green: genetic data, blue: biochemical data.

Full-size DOI: 10.7717/peerj.13997/fig-4
Our results uncovered drought-induced elevation of the Mg$^{2+}$ content in Ca-treated plants; this alteration can provide better antioxidant defense under drought stress, as reported by some researchers (Hauer-Jákli & Tränkner, 2019). Magnesium, the most abundant free divalent cation in the cell, participates in carbon fixation and photosynthesis. It acts as an activator or cofactor of enzymes in carbohydrate metabolism and plays an important part in photo-oxidative defense (Guo et al., 2015; Hauer-Jákli & Tränkner, 2019; Grzebisz, 2013; Chen et al., 2018). On the other hand, some studies indicate antagonistic relations between Mg$^{2+}$ and Ca$^{2+}$ in plants (Gransee & Führs, 2013), a synergistic effect of Ca$^{2+}$ and K$^{+}$ uptake, and no influence on Mg$^{2+}$ uptake in the coffee plant (Ramírez-Builes et al., 2020). Nonetheless, these studies mostly describe competitive absorption of Mg$^{2+}$ and Ca$^{2+}$ from the soil fertilized with these nutrients. In our work, the Mg$^{2+}$ accumulation can be explained by relocation of the magnesium ions transported from other organs into leaves; this process was positively affected by Ca$^{2+}$ application.

Accumulation of amino acids is a well-known mechanism of osmotic adjustment, of detoxification of reactive oxygen species, and of intracellular pH regulation under various osmotic stresses (Silvente, Sobolev & Lara, 2012). Among different amino acids, Met and Val accumulated to a greater extent under drought stress in our Ca-treated tea plants than in control tea plants. Both are protein-bound amino acids and play an important role in plant metabolism (Jander & Joshi, 2010; Binder, 2010; Hildebrandt, 2018). Aspartate-derived amino acid Met is tightly connected with the metabolism of branched-chain amino acids Val, Leu, and Ile, which activate jasmonic acid (JA) signaling; the latter is crucial for plants’ resistance to biotic and abiotic stressors (Jander & Joshi, 2010; Binder, 2010). Thus, higher accumulation of Met and Val in the tea plant may be one more piece of evidence for activation of JA signaling by Ca$^{2+}$ under drought. Of note, under drought stress, Gly accumulation was higher in control plants than in Ca-treated plants. Gly is a major component of glycine-rich proteins and is involved in RNA post-transcriptional processing, including splicing and polyadenylation, which are believed to perform a crucial function in plants’ responses to abiotic stressors (Khan et al., 2017; Czolpinska & Rurek, 2018). Further studies are necessary to assess the role of Gly and of the Gly metabolic pathway in the tea plant under drought conditions.

Organic-acid metabolism not only equilibrates redox potential in plant cells but also transfers redox equivalents between cell compartments thereby supporting various metabolic processes (Igamberdiev & Eprintsev, 2016; Igamberdiev & Bykova, 2018). Here, before drought induction, several organic acids (malic, citric, succinic, and acetic) steadily accumulated in control plants in comparison with Ca-treated plants (Fig. 2D). By contrast, under subsequent drought stress, the contents of malic, succinic, citric, and acetic acids diminished in control plants but not in Ca-treated plants. Other authors reported that the total amount of organic acids decreases under osmotic stress in bean leaves (Sassi et al., 2010). On the other hand, drought does not trigger the accumulation of organic acids except for succinate in soybean (Silvente, Sobolev & Lara, 2012). We observed a lower level of citric acid in our plants treated with calcium. Some researchers report that citric acid can improve photosynthetic rates, reduce reactive oxygen species, and provide better osmoregulation under drought stress (Tahjib-Ul-Arif et al., 2021). Therefore, additional
studies are necessary to evaluate the effect of drought stress on the organic acid fluctuations in tree crops.

The influence of Ca application on gene expression profiles of the tea plant under drought and relations with biochemical data

Most of crop quality–related genes proved to be downregulated under drought stress in the tea plant, in line with other studies (Li et al., 2015; Li et al., 2019; Wang et al., 2016a). On the contrary, several stress-related genes were upregulated by drought in both control and Ca-treated plants, in agreement with other research pointing to their involvement in stress responses (Wang et al., 2016b; Liu et al., 2016b; Samarina et al., 2020; Wrzaczek et al., 2010; Tanaka et al., 2012; Paul & Kumar, 2013; Li et al., 2016; Ban et al., 2017; Cheng et al., 2016; Yin et al., 2022).

Among the upregulated genes, three (LOX1, RHL41, and GOLS1) showed 2–3 times greater relative expression in Ca-treated plants as compared to control plants. GolS is the key enzyme for the synthesis of raffinose family oligosaccharides, which serve as osmoprotectants in plant cells and protect salicylate from an attack by hydroxyl radicals (e.g., galactinol and raffinose perform this function) (Panikulangara et al., 2004; Nishizawa, Yabuta & Shigeoka, 2008; Falavigna et al., 2018; Li et al., 2019). The plants overexpressing GOLS1 accumulate galactosyl inositol, which acts as a sugar signal in the ethylene signaling cascade (Li et al., 2019). Gols1- or Gols2-overexpressing Arabidopsis thaliana has high intracellular levels of galactinol and raffinose, which correlate with higher tolerance of drought stress (Panikulangara et al., 2004). Based on our results, it can be hypothesized that calcium affects GOLS1 expression and participates in the galactinol biosynthesis pathway leading to better acclimation of the tea plant to drought.

RHL41 (responsive to high light) is a member of the C2H2 family and is related to zinc-finger protein Zat12. Transgenic Arabidopsis plants overexpressing RHL41 possess thick dark green leaves and higher anthocyanin and chlorophyll contents (Iida et al., 2000). RHL41 plays a critical part in salt and drought responses by participating in the ABA-dependent pathway (Miller, Shulaev & Mittler, 2008; Ghorbani, Alemzadeh & Razi, 2019; Samarina et al., 2020). In our previous study, increased accumulation of RHL41 transcripts was observed, indicating specific involvement of this gene in drought defense (Samarina et al., 2020). In the present study, Ca treatment enhanced the upregulation of RHL41 in the tea plant, pointing to a stronger ABA-mediated response to drought; this phenomenon can explain better protection of membranes from oxidative stress.

The LOX gene family is known to be involved in lipid catabolism for oxylipin synthesis playing an important role in the JA-dependent pathway in various stress responses (Liavonchanka & Feussner, 2006). CsLOX1 is induced by a pathogen infection and brief cold treatment in the tea plant and partakes in the JA-responsive pathway (Zhu et al., 2018). In our work, calcium treatment affected the upregulation of LOX1, meaning the activation of the ABA-independent stress response. This finding is consistent with data from Montillet et al. (2013), who demonstrated that LOX1 performs a major function in the control of stomatal defense and plant innate immunity. They reported that the activities of an oxylipin- and an ABA-dependent pathway converge on anion channel
SLAC1 thereby regulating stomatal closure. Thus, we can speculate that calcium participates in the regulation of stomatal aperture in guard cells not only through activation of ABA signaling but also through oxylipin signaling activation by inducing the LOX1 expression. This theory is supported by PCA: the positive association of Met and Val contents with the LOX1 gene and high positive loading along the Ca vector observed in PCA (Fig. 4) confirmed the important role of calcium in the JA-mediated drought response in the tea plant. As mentioned above, under drought stress, Met and Val accumulated more in Ca-treated tea plants, and these amino acids participate in JA pathway activation.

Notably, in our study, three stress-related transcription factors (bHLH102, WRKY2, and WRKY42) were downregulated by drought stress in both control and Ca-treated plants. These genes are involved in stress and hormone signaling, particularly in the ABA-mediated abiotic-stress response (Wang et al., 2016a; Phukan, Jeena & Shukla, 2016; Jiang et al., 2017; Cui et al., 2018). This contradictory result may be due to genotype-specific responses or dissimilar stress conditions used in various studies. A limitation of our study is that we did not evaluate a short-term drought response. Further investigation will help to assess temporal and spatial expression alterations of the aforementioned genes.

CONCLUSIONS
Effects of external Ca application on drought responses of the tea tree were evaluated. Under drought, a greater increase in cell sap pH; higher accumulation of Tyr, Met, and Val; greater contents of the malic and citric acids; and higher Mg\(^{2+}\) concentration were observed in Ca-treated tea plants. Among the upregulated genes, three genes (LOX1, RHL41, and GOLS1) showed 2–3 times greater relative expression in Ca-treated plants than in control plants. PCA results indicate a positive correlation of Met and Val contents with LOX1 mRNA expression, confirming the important function of calcium in the activation of JA signaling in the tea plant under drought stress. On the basis of these results, it can be theorized that calcium affects the galactinol biosynthesis pathway and participates in the regulation of stomatal aperture in guard cells not only through ABA signaling activation but also through oxylipin pathway activation. Thus, calcium improves the drought response in the tea tree. These findings improve our understanding of calcium-mediated drought defense in tree crops. Further studies will reveal temporal and spatial changes of expression of the above-mentioned genes.

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**Competing Interests**
Yuriy Orlov is an Academic Editor for PeerJ.

**Author Contributions**
- Lyudmila S. Malyukova conceived and designed the experiments, performed the experiments, prepared figures and/or tables, and approved the final draft.
- Natalia G. Koninskaya conceived and designed the experiments, performed the experiments, prepared figures and/or tables, and approved the final draft.
- Yuriy L. Orlov analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Lidiia S. Samarina conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

**Data Availability**
The following information was supplied regarding data availability:
The raw data is available in the Supplemental Table.

**Supplemental Information**
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.13997#supplemental-information.

**REFERENCES**
Bajji M, Kinet J-M, Lutts S. 2001. The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. *Plant Growth Regulation* 36:61–70 DOI 10.1023/A:1014732714549.

Ban Q, Wang X, Pan C, Wang Y, Kong L, Jiang H, Xu Y, Wang W, Pan Y, Li Y, Jiang Ch. 2017. Comparative analysis of the response and gene regulation in cold resistant and sensitive tea plants. *PLOS ONE* 12(12):e0188514 DOI 10.1371/journal.pone.0188514.

Baruah RD, Bhagat RM. 2012. Climate trends of Northeastern India: a long-term pragmatic analysis for tea production. *Two and a Bud* 59(2):46–49.

Bhagat RM, Baruah RD, Cacigue S. 2010. Climate and tea (*Camellia sinensis* (L.) O. Kuntze) production with special reference to north eastern India: a review. *Journal of Environmental Research and Development* 4(4):1017–1028.
Binder S. 2010. Branched-chain amino acid metabolism in arabidopsis thaliana. The Arabidopsis Book 1–14:e0137 DOI 10.1199/tab.0137.

Brykalov AV, Yakub YuF, Shanaeva EA, Belik EV, Gryadskikh DA. 2019. The use of capillary electrophoresis and gas chromatography for the study of biologically active compounds. Krasnodar: KubSAU, 115.

Chen J, Gao T, Wan S, Zhang Y, Yang J, Yu Y, Wang W. 2018. Genome-wide identification, classification and expression analysis of the hsp gene superfamily in tea plant (Camellia sinensis). International Journal of Molecular Sciences 19(9):2633 DOI 10.3390/ijms19092633.

Cheng L, Wang Y, He Q, Li H, Zhang X, Zhang F. 2016. Comparative proteomics illustrates the complexity of drought resistance mechanisms in two wheat (Triticum aestivum L.) cultivars under dehydration and rehydration. BMC Plant Biology 16(1):188 DOI 10.1186/s12870-016-0871-8.

Cui X, Wang Y-X, Liu Z-W, Wang W-L, Li H, Zhuang J. 2018. Transcriptome-wide identification and expression profile analysis of the bHLH family genes in Camellia sinensis. Functional & Integrative Genomics 18(5):489–503 DOI 10.1007/s10142-018-0608-x.

Czolpinska M, Rurek M. 2018. Plant glycine-rich proteins in stress response: an emerging, still prospective story. Frontiers in Plant Science 9(302):276 DOI 10.3389/fpls.2018.00302.

Damayanthi MMN, Mohotti1 AJ, Nissanka SP. 2010. Comparison of tolerant ability of nature field grown tea (Camellia sinensis L.) Cultivars exposed to a drought stress in passara area. Tropical Agricultural Research 22(1):66–75 DOI 10.1038/hortres.2014.29.

Edel KH, Marchadier E, Brownlee C, Kudla J, Hetherington AM. 2017. The evolution of calcium-based signalling in plants. Current Biology 27(13):R667–R679 DOI 10.1010/j.cub.2017.05.02.

Evans NH, McAinsh MR, Hetherington AM, Knight MR. 2005. ROS perception in arabidopsis thaliana: the ozoneinduced calcium response. Plant Journal 41(4):615–626 DOI 10.1111/j.1365-313X.2004.02325.x.

Falavigna VS, Denardi PD, Evelyn MY, Dos SHP, De OPRD, Márcia MP, Giancarlo P, Fernando RL. 2018. Evolutionary diversification of galactinol synthases in Rosaceae: adaptive roles of galactinol and rafinose during apple bud dormancy. Journal of Experimental Botany 69(5):1247–1259 DOI 10.1093/jxb/erx451.

Fleta-Soriano E, Munné-Bosch S. 2016. Stress memory and the inevitable effects of drought: a physiological perspective. Frontiers in Plant Science 7(740):143 DOI 10.3389/fpls.2016.00143.

Ghorbani R, Alemzadeh A, Razi H. 2019. Microarray analysis of transcriptional responses to salt and drought stress in Arabidopsis thaliana. Heliyon 5(2019):e02614 DOI 10.1016/j.heliyon.2019.e02614.

Gransee A, Führs H. 2013. Magnesium mobility in soils as a challenge for soil and plant analysis, magnesium fertilization and root uptake under adverse growth conditions. Plant and Soil 368(1–2):5–21 DOI 10.1007/s11104-012-1567-y.

Grzebisz W. 2013. Crop response to magnesium fertilization as affected by nitrogen supply. Plant and Soil 368(1–2):23–39 DOI 10.1007/s11104-012-1574-z.

Guo W, Chen S, Hussain N, Cong Y, Liang Z, Chen K. 2015. Magnesium stress signaling in plant: just a beginning. Plant Signaling & Behavior 10(3):e992287 DOI 10.4161/15592324.2014.992287.

Hauer-Jákli M, Tránkner M. 2019. Critical leaf magnesium thresholds and the impact of magnesium on plant growth and photo-oxidative defense: a systematic review and meta-analysis from 70 years of research. Frontiers in Plant Science 10:766 DOI 10.3389/fpls.2019.00766.
Hetherington AM, Brownlee C. 2004. The generation of Ca2+ signals in plants. *Annual Review of Plant Biology* 55(1):401–427 DOI 10.1146/annurev.arplant.55.031903.141624.

Hildebrandt TM. 2018. Synthesis versus degradation: directions of amino acid metabolism during Arabidopsis abiotic stress response. *Plant Molecular Biology* 98(1–2):121–135 DOI 10.1007/s11103-018-0767-0.

Hosseini SA, Réthoré E, Pluchon S, Ali N, Billiot B, Yvin JC. 2019. Calcium application enhances drought stress tolerance in sugar beet and promotes plant biomass and beetroot sucrose concentration. *International Journal of Molecular Sciences* 20(15):3777 DOI 10.3390/ijms20153777.

Hu Z, Ban Q, Hao J, Zhu X, Cheng Y, Mao J, Lin M, Xia E, Li Y. 2020. Genome-wide characterization of the C-repeat binding factor (CBF) gene family involved in the response to abiotic stresses in tea plant (*Camellia sinensis*). *Frontiers in Plant Science* 11:921 DOI 10.3389/fpls.2020.00921.

Igamberdiev AU, Bykova NV. 2018. Role of organic acids in the integration of cellular redox metabolism and mediation of redox signalling in photosynthetic tissues of higher plants. *Free Radical Biology and Medicine* 122:74–85 DOI 10.1016/j.freeradbiomed.2018.01.016.

Igamberdiev AU, Eprintsev AT. 2016. Organic acids: the pools of fixed carbon involved in redox regulation and energy balance in higher plants. *Frontiers in Plant Science* 7(e0149850):1042 DOI 10.3389/fpls.2016.01042.

Iida A, Kazuoka T, Torikai S, Kikuchi H, Oeda K. 2000. A zinc finger protein RHL41 mediates the light acclimatization response in Arabidopsis. *The Plant Journal* 24(2):191–203 DOI 10.1046/j.1365-313x.2000.00864.x.

Jander G, Joshi V. 2010. Recent progress in deciphering the biosynthesis of aspartate-derived amino acids in plants. *Molecular Plant* 3(1):54–65 DOI 10.1093/mp/ssp104.

Jiang J, Ma S, Ye N, Jiang M, Cao J, Zhang J. 2017. *WRKY* transcription factors in plant responses to stresses. *Journal of Integrative Plant Biology* 59(2):86–101 DOI 10.1111/jipb.12513.

Kaelke CM, Dawson JO. 2005. The accretion of nonstructural carbohydrates changes seasonally in *Alnus incana* ssp. rugosa in accord with tissue type, growth, N allocation, and root hypoxia. *Symbiosis* 39(2):61–66.

Kasuga J, Arakawa K, Fujikawa S. 2007. High accumulation of soluble sugars in deep supercooling Japanese white birch xylem parenchyma cells. *New Phytologist* 174(3):569–579 DOI 10.1111/j.1469-8137.2007.02025.x.

Khan N, Sh Ali, Shahid MA, Kharabian-Masouleh A. 2017. Advances in detection of stress tolerance in plants through metabolomics approaches. *Plant Omics* 10(3):153–163 DOI 10.21475/poj.10.03.17.pne600.

Kim MC. 2009. Calcium and calmodulin-mediated regulation of gene expression in plant. *Molecular Plant* 2(1):13–21 DOI 10.1093/mp/ssn091.

Li Y, Wang X, Ban Q, Zhu X, Jiang Ch, Wei Ch, Bennetzen JL. 2019. Comparative transcriptomic analysis reveals gene expression associated with cold adaptation in the tea plant *Camellia sinensis*. *BMC Genomics* 20(1):624 DOI 10.1186/s12864-019-5988-3.

Li YY, Zhou YQ, Xie XF, Shu XT, Deng WW, Jiang CJ. 2016. Cloning and expression analysis of dehydrin gene (CsDHN) in tea plant (*Camellia sinensis*). *Journal of Agricultural Biotechnology* 24:332–341.

Li Ch-F, Zhu Y, Yu Y, Zhao Q-Y, Wang Sh-J, Wang X-Ch, Yao M-Zh, Luo D, Li X, Chen L, Yang Y-J. 2015. Global transcriptome and gene regulation network for secondary metabolite biosynthesis of tea plant (*Camellia sinensis*). *BMC Genomics* 16(1):560 DOI 10.1186/s12864-015-1773-0.
Liavonchanka A, Feussner I. 2006. Lipoxygenases: occurrence, functions and catalysis. *Journal of Plant Physiology* 163(3):348–357 DOI 10.1016/j.jplph.2005.11.006.

Liu S-C, Jin J-Q, Ma J-Q, Yao M-Z, Ma C-L, Li C-F, Ding Z-T, Chen L. 2016b. Transcriptomic analysis of tea plant responding to drought stress and recovery. *PLOS ONE* 11(1):e0147306 DOI 10.1371/journal.pone.0147306.

Liu Y, Liang J, Sun L, Yang X, Li D. 2016a. Group 3 LEA protein, *ZmLEA3*, is involved in protection from low temperature stress. *Frontiers in Plant Science* 7:1011 DOI 10.3389/fpls.2016.01011.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using realtime quantitative PCR and the 2^{−ΔΔCT} method. *Methods* 25(4):402–408 DOI 10.1006/meth.2001.1262.

Ma Q, Zhou Q, Chen C, Cui Q, Zhao Y, Wang K, Arkorful E, Chen X, Sun K, Li X. 2019. Isolation and expression analysis of *CsCML* genes in response to abiotic stresses in the tea plant (*Camellia sinensis*). *Scientific Reports* 9(1):e8211 DOI 10.1038/s41598-019-44681-7.

Malyukova LS, Nechaeva TL, Zubova MYu, Gvasalia MV, Koninskaya NG, Zagoskina NV. 2020. Physiological and biochemical characterization of tea (*Camellia sinensis* L) microshoots in vitro: The norm, osmotic stress, and effects of calcium. *Sel'skokhozyaistvennaya Biologiya* 55(5):970–980 DOI 10.15389/AGROBIOLOGY.2020.5.970ENG.

Malyukova LS, Pritula ZV, Kozlova NV, Veliky AV, Rogozhina EV, Kerimzade VV, Samarina LS. 2021. Effects of calcium-containing natural fertilizer on *Camellia sinensis* (L.) Kuntze. *Bangladesh Journal of Botany* 50(1):179–187 DOI 10.3329/bjb.v50i1.52686.

Man D, Bao YX, Han LB, Zhang XZ. 2011. Drought tolerance associated with proline and hormone metabolism in two tall fescue cultivars. *Horticultural Science* 46(7):1027–1032 DOI 10.21273/HORTSCI.46.7.1027.

Marciańska I, Czyczyło-Mysza I, Skrzypek E, Filek M, Grzesiak S, Grzesiak MT, Janowiak F, Hura T, Dziurka M, Dziurka K, Nowakowska A, Quarrie SA. 2013. Impact of osmotic stress on physiological and biochemical characteristics in drought-susceptible and drought-resistant wheat genotypes. *Acta Physiologiae Plantarum* 35(2):451–461 DOI 10.1007/s11738-012-1088-6.

Maritim TK, Kamunya SM, Mireji P, Wendia CM, Muoki RC, Cheruiyot EK, Wachira FN. 2015. Physiological and biochemical response of tea (*Camellia sinensis* (L.) O. Kuntze) to water-deficit stress. *The Journal of Horticultural Science and Biotechnology* 90(4):395–400 DOI 10.1080/14620316.2015.11513200.0.4236/ajps.2012.34054.

Miller G, Shulaev V, Mittler R. 2008. Reactive oxygen signaling and abiotic stress. *Physiologia Plantarum* 133(3):481–489 DOI 10.1111/j.1399-3054.2008.01090.x.

Montillet JL, Leonhardt N, Mondy S, Tranchimand S, Rumeau D, Boudsocq M, Garcia AV, Douki T, Bigeard J, Laurière C, Chevalier A, Castresana C, Hirt H. 2013. An abscisic-acid-independent oxylipin pathway controls stomatal closure and immune defense in Arabidopsis. *PLOS Biology* 11:e1001513 DOI 10.1371/journal.pbio.1001513.

Nishizawa A, Yabuta Y, Shigeoka S. 2008. Galactinol and raffinose constitute a novel function to protect plants from oxidative damage. *Plant Physiology* 147(3):1251–1263 DOI 10.1104/pp.108.122465.

Njoloma C. 2012. Application of foliar spray containing copper, zinc and boron to mature clonal tea (*Camellia sinensis*): affect on yield and quality. A thesis for the degree in M.Sc. (Agric) Agronomy in the Faculty of Natural and Agricultural Sciences University of Pretoria. 116.

Noctor G. 2006. Metabolic signalling in defence and stress: the central roles of soluble redox couples. *Plant Cell and Environment* 29(3):409–425 DOI 10.1111/j.1365-3040.2005.01476.x.
Panikulangara TJ, Eggers-Schumacher G, Wunderlich M, Stransky H, Schöffl F. 2004. 
Galactinol synthase1 a novel heat shock factor target gene responsible for heat-induced synthesis of rafinose family oligosaccharides in arabidopsis. *Plant Physiology* **136**(2):3148–3158 
DOI 10.1104/pp.104.042606.

Paul A, Kumar S. 2013. Dehydrin2 is a stress-inducible, whereas Dehydrin1 is constitutively expressed but up-regulated gene under varied cues in tea (*Camellia sinensis* (L.) O. Kuntze). *Molecular Biology Reports* **40**(5):3859–3863 DOI 10.1007/s11033-012-2466-2.

Phukan UJ, Jeena GS, Shukla RK. 2016. WRKY Transcription factors: molecular regulation and stress responses in plants. *Frontiers in Plant Science* **7**(807560):760 
DOI 10.3389/fpls.2016.00760.

Ramirez-Builes VH, Küsters J, deSouza TR, Simmes C. 2020. Calcium nutrition in coffee and its influence on growth, stress tolerance, cations uptake, and productivity. *Frontiers in Agronomy* 2:590892 DOI 10.3389/fagro.2020.590892.

Regier N, Streb S, Zeeman SC, Frey B. 2010. Seasonal changes in starch and sugar content of poplar (*Populus deltoides × nigra* cv. Dorskamp) and the impact of stem girdling on carbohydrate allocation to roots. *Tree Physiology* **30**(8):979–987 DOI 10.1093/treephys/tpq047.

Rentel MC, Knight MR. 2004. Oxidative stress-induced calcium signaling in arabidopsis. *Plant Physiology* **135**(3):1471–1479 DOI 10.1104/pp.104.042663.

Samarina LS, Bobrovskikh AV, Doroshkov AV, Maluykova LS, Matskiv AO, Rakhmangulov RS, Koninskaya NG, Malaryovskaya VI, Tong W, Xia E, Manakhova KM, Ryndin AV, Orlov YL. 2020. Comparative expression analysis of stress-inducible candidate genes in response to cold and drought of tea plant (*Camellia sinensis* (L.) Kuntze). *Frontiers in Genetics* **11**:507 DOI 10.3389/fgene.2020.611283.

Sassi S, Aydi S, Hessini K, Gonzalez EM, Arrese-Igor C. 2010. Long-term mannitol-induced osmotic stress leads to stomatal closure, carbohydrate accumulation and changes in leaf elasticity in Phaseolus vulgaris leaves. *African Journal of Biotechnology* **9**:6061–6069.

Silvente S, Sobolev AP, Lara M. 2012. Metabolite adjustments in drought tolerant and sensitive soybean genotypes in response to water stress. *PLOS ONE* **7**(6):e38554 DOI 10.1371/journal.pone.0038554.

Singh R, Parihar P, Prasad SM. 2018. Sulfur and calcium simultaneously regulate photosynthetic performance and nitrogen metabolism status in as-challenged *Brassica juncea* L. *Frontiers in Plant Science* **9**:772 DOI 10.3389/fpls.2018.00772.

Song WY, Zhang ZB, Shao HB, Guo XL, Cao HX, Zhao HB, Fu ZY, Hu XJ. 2008. Relationship between calcium decoding elements and plant abiotic-stress resistance. *International Journal of Biological Sciences* **4**(2):116–125 DOI 10.7150/ijbs.4.1.116.

Tahjib-Ul-Arif M, Zahan MI, Karim MM, Imran S, Hunter CT, Islam MS, Mia MA, Hannon MA, Rhaman MS, Hossain MA, Brestic M, Skalicky M, Murata Y. 2021. Citric acid-mediated abiotic stress tolerance in plants. *International Journal of Molecular Sciences* **22**(13):7235 DOI 10.3390/ijms22137235.

Tanaka H, Osakabe Y, Katsura S, Shinji Mizuno S, Maruyama K, Kusakabe K, MizoI J, Shinozaki K, Yamaguchi-Shinozaki K. 2012. Ablastic stress-inducible receptor-like kinases negatively control ABA signaling in Arabidopsis. *The Plant Journal* **70**(4):599–613 DOI 10.1111/j.1365-313X.2012.04901.x.

Thor K. 2019. Calcium—nutrient and messenger. *Frontiers in Plant Science* **10**:440 DOI 10.3389/fpls.2019.00440.
Upadhyaya H, Dutta BK, Sahoo L, Panda SK. 2012. Comparative effect of Ca, K, Mn and B on post-drought stress recovery in tea (Camellia sinensis (L.) O. Kuntze). American Journal of Plant Science 3(4):443–460 DOI 10.4236/ajps.2012.34054.

Upadhyaya H, Panda SK. 2004. Responses of camellia sinensis to drought and rehydration. Biologia Plantarum 48(4):597–600 DOI 10.1023/B:BIOP.0000047158.53482.37.

Wang Y-X, Liu Z-W, Wu Z-J, Li H, Zhuang J. 2016b. Transcriptome-wide identification and expression analysis of the NAC gene family in tea plant (Camellia sinensis (L.) O. Kuntze). PLOS ONE 11(11):e0166727 DOI 10.1371/journal.pone.0166727.

Wang Y, Shu Z, Wang W, Jiang X, Li D, Pan J, Li X. 2016a. CsWRKY2, a novel WRKY gene from Camellia sinensis, is involved in cold and drought stress responses. Biologia Plantarum 60(3):443–445 DOI 10.1007/s10535-016-0618-2.

Wrzaczek M, Brosché M, Salojärvi J, Kangasjärvi S, Idänheimo N, Mersmann S, Robatzek S, Karpiński S, Karpińska B, Kangasjärvi J. 2010. Transcriptional regulation of the CRK/DUF26 group of Receptor-like protein kinases by ozone and plant hormones in Arabidopsis. BMC Plant Biology 10(1):95 DOI 10.1186/1471-2229-10-95.

Yamasaki S, Dillenburg LC. 1999. Measurements of leaf relative water content in araucaria angustifolia. Revista Brasileira De Fisiologia Vegetal 11:69–75.

Yin H, Ma Y, Deng Y, Xu Z, Liu J, Zhao J, Dong J, Yu J, Chang Z. 2016. Genome shuffling of Saccharomyces cerevisiae for enhanced glutathione yield and relative gene expression analysis using fluorescent quantitation reverse transcription polymerase chain reaction. Journal of Microbiological Methods 127:188–192 DOI 10.1016/j.mimet.2016.06.012.

Yin H, Yang F, He X, Du X, Mu P, Ma W. 2022. Advances in the functional study of glutamine synthetase in plant abiotic stress tolerance response. The Crop Journal 10(4):917–923 DOI 10.1016/j.cj.2022.01.003.

Yue C, Cao HL, Wang L, Zhou YH, Huang YT, Hao XY, Wang YC, Wang B, Yang YJ, Wang XC. 2015. Effects of cold acclimation on sugar metabolism and sugar-related gene expression in tea plant during the winter season. Plant Molecular Biology 88(6):591–608 DOI 10.1007/s11103-015-0345-7.

Zhang XC, Gao HJ, Wu HH, Yang TY, Zhang ZZ, Mao JD, Wan XC. 2015. Ca2+ and CaM are involved in Al3+ pretreatment-promoted fluoride accumulation in tea plants (Camellia sinensis L.). Plant Physiology and Biochemistry 96(9):288–295 DOI 10.1016/j.plaphy.2015.08.007.

Zhu JK. 2016. Abiotic stress signaling and responses in plants. Cell 167(2):313–324 DOI 10.1016/j.cell.2016.08.029.

Zhu J, Wang X, Guo L, Xu Q, Zhao S, Li F, Yan X, Liu S, Wei C. 2018. Characterization and alternative splicing profiles of the lipoxygenase gene family in tea plant (Camellia sinensis). Plant and Cell Physiology 59(9):1765–1781 DOI 10.1093/pcp/pcy091.