

Industrial byproduct corn steep liquor enhanced drought tolerance by preserve membrane lipids and reestablishing redox homeostasis in tea plants (*Camellia sinensis*)

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Abstract

Background

Resource utilization of organic waste to promote sustainable farming is widely recognized in global agriculture systems. As a by-product of corn industry, corn steep liquor (CSL) shows potential in regulating plant stress resistance. Tea plant (*Camellia sinensis*), an important global economic crop, is highly sensitive to drought stress. However, the mechanism by which CSL regulates drought tolerance in tea plants remains unclear. In this study, the effects of CSL on tea growth under drought stress were investigated and the underlying mechanisms were explored.

Results

Under drought stress, tea plants supplied with 0.5 mL/L CSL maintained stronger physiological and photosynthetic performance. CSL-treated plants showed lower levels of reactive oxygen species (ROS) and oxidative damage to the cell membrane. Furthermore, CSL-induced rise in unsaturated fatty acids, which helped maintain cell membrane integrity. It also elevated the reduced forms of ascorbic acid (AsA) and glutathione (GSH), enhancing the efficiency of the AsA-GSH cycle by boosting key enzyme activities, thereby restoring cellular redox homeostasis.

Conclusions

Collectively, our results demonstrate that CSL enhances drought tolerance in tea plants by promoting fatty acid synthesis and increasing unsaturated fatty acid levels, as well as protecting membrane lipid from ROS-induced oxidative damage through the regulation of antioxidant systems, including the AsA-GSH cycle.

1. Introduction

As an important global economic crop, tea (*Camellia sinensis*) is typically cultivated in hilly areas of tropical and subtropical regions, which often lack adequate irrigation facilities, making it highly vulnerable to drought stress. Drought is a critical environmental stress factor that severely constrains plant growth, development, and productivity (Razi and Muneer, 2021). Against the backdrop of current climate change, rising global temperatures and altered precipitation patterns are leading to more frequent, intense, and prolonged drought events. This poses a particular challenge for tea cultivation (Bania et al., 2025; Tian et al., 2025). Therefore, exploring effective cultivation strategies to enhance drought tolerance in tea plants is of great significance for ensuring the sustainability of the industry.

The use of organic waste materials to promote sustainable agricultural practices has gained global recognition, as they provide a cost-effective and environmentally friendly alternative to synthetic fertilizers. Organic waste materials, such as manure, compost, and industrial by-products, not only recycle nutrients but also enhance plant growth by improving soil structure, water retention, and biodiversity, ultimately boosting plant vitality, crop yields, and long-term agricultural sustainability (Le et

al., 2022; Puglia et al., 2021; Raguraj et al., 2023; Zhou et al., 2022b). Corn steep liquor (CSL), a by-product of corn starch production, is derived from the steeping of corn kernels in water (Zhou et al., 2022a; Garcia-Sanchez et al., 2024). With global corn production totaling approximately 1 billion tons annually, CSL represents a significant by-product resource (Garcia-Sanchez et al., 2024). CSL is a yellow-brown organic liquid residue that is highly water-soluble, and contains bioactive compounds such as amino acids, sugars, organic acid, and minerals (Zhou et al., 2022a). Due to its abundance and high organic-matter content, the direct discharge of CSL as wastewater poses significant environmental risks and wastes valuable resources. Consequently, the effective development and utilization of CSL have become key challenges for both industry and society. Due to its low cost and widespread availability, CSL has been increasingly used in microbial fermentation, clean energy production, and biosurfactant manufacturing (Byanju et al., 2023; da Rocha Junior et al., 2019; Joshi et al., 2018). Recognized by the Organic Materials Review Institute (OMRI) as an organic substance, CSL is permitted for use in organic agriculture (Fernandez-Salvador et al., 2015). CSL has been found to exhibit varying levels of growth-promoting effects in several plant species. For instance, a 0.5% CSL solution improved nitrogen utilization efficiency in pepper by enhancing nitrogen assimilation and increasing amino acid and protein levels (Navarro-Morillo et al., 2024). Additionally, low concentrations of CSL have been shown to enhance seed germination, plant growth, biomass production, and flowering in soybeans (Zhu et al., 2018). These studies suggest that CSL exhibits potential growth-promoting effects similar to those of plant biostimulants. However, the effects of corn steep liquor (CSL) on tea plants, and its underlying mechanisms as a biostimulant remain poorly understood.

Excessive accumulation of reactive oxygen species (ROS) is recognized as a key factor inhibiting plant growth under drought stress (Razi and Muneer, 2021; Wang et al., 2022). During drought, the overproduced ROS readily attack fatty acids within the cell membrane, triggering a chain reaction of lipid peroxidation. This disrupts the normal physiological functions of the membrane and leads to physiological impairments such as chloroplast damage and inhibition of photosynthesis (Jiao et al., 2026; Shao et al., 2023). Research indicates that plant tolerance to abiotic stress is closely associated with the degree of fatty acid unsaturation and the composition of unsaturated fatty acids in membrane lipids. For instance, under high-temperature stress, plants reduce the content of unsaturated fatty acids to maintain proper membrane fluidity, thereby alleviating heat-induced membrane damage (Henschel et al., 2024; Liu et al., 2019). Under low-temperature stress, many plants elevate levels of polyunsaturated fatty acids such as linolenic, oleic, and linoleic acid to protect membranes from chilling injury (Tian et al., 2022). Under aluminum stress, increasing unsaturated fatty acid content helps improve membrane fluidity and integrity, mitigating the inhibition of root elongation (Ou et al., 2025).

To counteract oxidative damage caused by excess ROS, plants have established a defense system consisting of enzymatic and non-enzymatic antioxidant mechanisms to maintain ROS homeostasis (Hasanuzzaman et al., 2019; Sun et al., 2018). Among these, ascorbate (AsA), glutathione (GSH), and the AsA-GSH cycle play crucial roles in alleviating drought-induced oxidative damage (Chen et al., 2024). For example, elevating AsA levels in plants significantly alleviates drought-induced suppression of photosynthesis (Jiao et al., 2026). Overexpression of key enzyme genes in the AsA-GSH cycle enhances

drought tolerance (Hasanuzzaman et al., 2019). In addition, in tea plants, secondary metabolites represented by polyphenols as well as the antioxidant enzyme system comprising superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) also contribute importantly to stress resistance (Li et al., 2025; Li, 2023). In recent years, several biostimulants derived from resource recycling, such as mixed liquid extracts from marine sources, endophytic fungi, and animal-derived protein hydrolysates, have been found to promote plant growth and enhance stress tolerance by regulating the antioxidant system under diverse adverse environmental conditions, including drought and salinity. These biostimulants are increasingly recognized as viable alternatives to chemical fertilizers, offering a promising solution for organic cultivation practices (Devi et al., 2022; Rong et al., 2023). However, the feasibility and underlying mechanisms of using CSL to enhance the overall drought resistance in tea plants under drought conditions remain unclear.

In the present study, the primary objective was to assess whether CSL application could stimulate growth, and improve drought stress of tea plants. The hypothesis was based on the presence of key substances in CSL, such as mineral nutrients, amino acids, and reducing sugars, which promote plant growth through various physiological and metabolic processes (Garcia-Sanchez et al., 2024). To test this hypothesis, this study evaluated the effects of CSL on the growth traits and physiological characteristics of tea plants under drought stress, and elucidated the underlying physiological mechanisms of its role in enhancing drought tolerance.

2. Materials and methods

2.1 Plant material, growth condition and treatments

The experiment was conducted in a glass greenhouse at Zhejiang academy of agricultural sciences from March to April 2024. Environmental conditions included a 16 h photoperiod, light intensity of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$, day/night temperatures of 16/10°C, and 70% relative humidity. Two-year-old uniform cuttings of tea cultivar 'Longjing 43' were grown in pots containing a peat : vermiculite : perlite (3 : 1 : 1) mixture and irrigated weekly with 2 L of tea-specific nutrient solution (pH 5.0).

Six concentrations of CSL (0, 0.1, 0.5, 1, 2, and 4 mL L^{-1}) were applied via foliar spraying before bud break (see Fig. S1B for setup). Each treatment included three replicates with nine plants per pot (27 plants total). Spraying was performed after 4 pm every three days for six applications, using 10 mL per plant to cover both leaf surfaces, while protecting the potting medium from runoff. For drought stress, plants were irrigated with nutrient solution containing 20% (w/v) PEG (Fig. S1C). The preparation, basic properties, and main active components of CSL are provided in Fig. S1A and Table S1.

2.2 Measurement of leaf growth traits of tea plants

Following treatment, young leaves (apical bud plus the two youngest leaves) and mature leaves were harvested. The total number of buds and leaf fresh weight were recorded. Samples were then snap-frozen in liquid nitrogen, freeze-dried to constant weight, and the dry weight was determined. For each

plant, the length and width of the penultimate true leaf on the new shoot were measured, and leaf area was calculated as $0.7 \times \text{length} \times \text{width}$ (Wang et al., 2024). Internode length between the first and second true leaves was measured with a vernier caliper (Wang et al., 2024).

2.3 Determination of leaf photosynthetic characteristics

For analysis, the penultimate true leaf of each new shoot was selected. The relative chlorophyll content was measured in situ using a handheld DUALEX chlorophyll-flavonoid meter (Zealquest Scientific Technology Co., Ltd., Shanghai, China) (Wang et al., 2021). After cleaning the surfaces of young (penultimate true leaf) and mature leaves with deionized water, chlorophyll fluorescence characteristics were observed and imaged with a chlorophyll fluorescence phenotype instrument (Zhou et al., 2022b). Fluorescence parameters, such as Fv/Fm (PSII maximum photochemical quantum yield), ETR (PSII reaction center photosynthetic electron transfer efficiency), and NPQ (non-photochemical quenching coefficient) were quantified using PlantExplorer™ software. The net photosynthetic rate (Pr), stomatal conductivity (Gs), water use efficiency, (WUE), transpiration rate (Tr) were measured with a photosynthetic rate analyzer according to the manufacturer's instructions (FS-3080Dpro, Shijiazhuang Fansheng Technology Co., Ltd, Shijiazhuang, China).

2.4 Analysis of antioxidants, antioxidant enzymes and antioxidant activity of tea leaves

Antioxidant metabolites. Ascorbic acid (AsA) and dehydroascorbic acid (DHA) were extracted from fresh leaves (0.1 g) with 1 mL of ice-cold 5% (w/v) metaphosphoric acid (containing 2 mM EDTA). After centrifugation (22000 g, 15 min, 4°C), the supernatant was analyzed following Ou et al. (2025). Reduced and oxidized glutathione (GSH and GSSG) were extracted from fresh leaf tissue (0.1 g) in 1.5 mL sodium phosphate-EDTA buffer and 200 µL of 25% H₃PO₄. Following centrifugation, the supernatant was assayed fluorometrically (excitation/emission: 350/420 nm) according to Ou et al. (2025). Total phenolic content was determined using the Folin-Ciocalteu method (Wang et al., 2023). Freeze-dried leaves (0.05 g) were extracted with 1 mL of 80% (v/v) methanol, centrifuged, and the supernatant reacted with the reagent. Absorbance was measured at 760 nm and quantified against a gallic acid standard curve (results in mg GAE g⁻¹ DW). Flavonoid content was measured in situ using a DUALEX chlorophyll-flavonoid meter, and the nitrogen balance index was calculated as the chlorophyll-to-flavonoid ratio (Wang et al., 2021).

2.5 Antioxidant enzyme activities. The activities of glutathione reductase (GR), ascorbate peroxidase (APX), and catalase (CAT) were assayed as described by Sun et al. (2018), monitoring absorbance changes at 340, 290, and 240 nm, respectively. Activities of monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) were quantified according to Tong et al. (2024) by measuring absorbance at 340 and 265 nm, respectively. Superoxide dismutase (SOD) and peroxidase (POD) activities were determined following Zhou et al. (2022).

2.6 Determination of antioxidant activity of tea plants

Ground freeze-dried leaves (0.06 g) were ultrasonic extracted in 1.5 mL anhydrous ethanol for 1 hour, then centrifuged at 10000 g for 10 min. The supernatant was used for further analysis.

The total antioxidant capacity was quantified using the FRAP (Ferric Reducing Ability of Plasma) assay. The reaction mixture consists 0.1 mL of the supernatant and 2.9 mL of TPTZ working solution. Ethanol was used as a blank control in place of the sample. Incubate the mixture at 37°C in the dark for 10 min. the absorbance at 593 nm was measured. The antioxidant capacity of the sample is expressed as FRAP units, where 1 FRAP unit corresponds to 1 mmol/L FeSO₄ (Atero-Calvo et al., 2024).

The free radical scavenging rate was determined by the DPPH (1,1-Diphenyl-2-picrylhydrazine) method. Transfer a 0.2 mL aliquot of the supernatant to 1.3 mL of a 65 µM DPPH solution dissolved in anhydrous ethanol. Incubate the mixture in a water bath at 30°C for 30 min. The absorbance value A₁ was measured at 517 nm. Transfer a 0.2 mL aliquot of the supernatant to 1.3 mL of anhydrous ethanol, and the water bath steps were the same as above. The absorbance value at 517 nm was A₂. Take another 0.2 mL of ethanol and add 1.3 mL of DPPH, and the water bath steps were the same as above. The absorbance value at 517 nm was A₃. DPPH free radical scavenging rate = $[1 - (A_1 - A_2)/A_3] \times 100\%$ (Zhang et al., 2013).

2.7 Histochemical detection of reactive oxygen and membrane lipid peroxidation of tea leaf

For histochemical detection of H₂O₂ and O₂^{•-} and membrane lipid peroxidation, leaves were stained with 3,3'-diaminobenzidine (DAB, 0.1%, w/v), nitrotetrazolium blue chloride (NBT, 0.5 mM, pH=6), Evans blue solution (0.25%, w/v) and Schiff reagent for 4 h, 8 h, 20 min, and 12 h, respectively. The stained leaves were decolorized in 90% (v/v) ethanol at 90°C and analyzed by taking photos under a microscope (Wang et al., 2023). The content of hydrogen peroxide (H₂O₂), superoxide anion (O₂^{•-}), electrolyte leakage were determined according to (Liang et al., 2021) and lipid hydroperoxide (LPO) were determined according to (Ou et al., 2025)

2.8 Transmission electron microscopy observation of ultrastructure of tea leaf

The observation of the leaf ultrastructure was based on the method reported by Huang et al. (2021). Briefly, leaf segments (1 × 3 × 1 mm³) were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 4 h, washed with the same buffer, and post-fixed in 1% OsO₄ for 2 h. After washing, samples were dehydrated in a graded ethanol series (30%, 50%, 70%, 80%, 90%, 95%) and absolute acetone, each step for 15–20 min. The specimens were infiltrated with Spurr's resin through an acetone–resin gradient and finally with pure resin overnight. After embedding and polymerization at 70 °C for 9 h, ultrathin sections were cut using a Leica EM UC7 ultramicrotome, stained with uranyl acetate and lead citrate, and examined under a Hitachi H-7650 transmission electron microscope.

2.9 Fatty acid analysis

Fatty acid analysis was performed according to (Gowda et al., 2021). Briefly, 50 mg of freeze-dried tea leaves was weighed into a 2 mL microcentrifuge tube. After adding 200 μ L of phosphoric acid solution (0.5%, v/v), the suspension was vortexed for 10 min and ultrasonicated for 5 min, followed by centrifugation at 12,000 r/min for 10 min at 4°C. Subsequently, 100 μ L of the supernatant was transferred to a 1.5 mL tube and mixed with 250 μ L of methyl tert-butyl ether (MTBE) containing an internal standard. The mixture was vortexed for 3 min, ultrasonicated for 5 min, and centrifuged again under the same conditions. The final supernatant was collected for GC-MS/MS analysis using an Agilent 7890B gas chromatograph coupled with a 7000D triple quadrupole mass spectrometer. Separation was achieved on a DB-FFAP capillary column (30 m $\times$ 0.25 mm i.d., 0.25 μ m film thickness). Helium was used as the carrier gas at a constant flow rate of 1.2 mL/min. A liquor of 1 μ L sample was injected in split mode (split ratio 5:1). The oven temperature program was as follows: held at 50°C for 2 min, increased to 220°C at 40°C/min, and then held for 3 min. The temperatures of the injector and transfer line were both set at 230°C. Detection was carried out in multiple reaction monitoring (MRM) mode.

2.10 Statistical analysis

Experimental data were analyzed using one-way ANOVA with Excel 2019 and IBM SPSS Statistics 26. Graphical representations were generated using OriginPro 2024 SR1 (Version 10.1.0.178; OriginLab Corporation, Northampton, USA). Using R language for normalized heatmap analysis of fatty acids.

3. Results

3.1 Effects of CSL on phenotype parameters, chlorophyll content and photosynthetic capacity in tea plants

Under normal conditions, treatment with 0.5 mL/L CSL increased leaf area by 20.20% (Fig. S1 C) and internode length by 31.33%, while 1 mL/L CSL increased internode length by 27.79% (Fig. S1 D). CSL spraying at 0.1, 0.5, and 1 mL/L significantly enhanced young leaf biomass: fresh weight rose by 45.28%, 79.93%, and 48.96%, and dry weight by 45.61%, 72.77%, and 43.07%, respectively (Fig. S1 E). For mature leaves, 0.1 and 0.5 mL/L CSL increased fresh weight by 26.57% and 49.60% and dry weight by 19.65% and 37.39% (Fig. S1 F). Total leaf biomass also increased, with 0.1 and 0.5 mL/L raising fresh weight by 33.84% and 61.38% and dry weight by 25.49% and 45.35% (Fig. S3 C). The nitrogen balance index (NBI), reflecting leaf growth status, increased by 13.46% in young leaves (Fig. S1 M).

Application of 0.5 mL/L CSL increased chlorophyll content by 16.77% in young leaves and 18.24% in mature leaves relative to the control under normal conditions, (Fig. S1 I&J). In young leaves, CSL at 0.5, 1, and 2 mL/L significantly elevated Fv/Fm by 13.50%, 10.02%, and 11.33%, and enhanced ETR by 21.39%, 18.17%, and 17.55%, respectively. NPQ in young leaves increased significantly by 79.35%, 72.30%, 66.76%, and 33.32% after treatment with 0.1, 0.5, 1, 2, and 4 mL/L CSL, respectively. In contrast, CSL did not significantly improve Fv/Fm or ETR in mature leaves, whereas NPQ increased markedly by 1.32-, 1.71-, 1.89-, 2.11-, and 1.84-fold in the 0.1, 0.5, 1, 2, and 4 mL/L CSL groups, respectively. These results

indicate that suitable CSL concentrations can improve photosynthetic efficiency, light utilization, and photoprotection in tea leaves. Among the concentrations tested, 0.5 mL/L CSL showed the most pronounced effect on promoting leaf growth and enhancing chlorophyll fluorescence parameters.

Together, these results show that low-concentration CSL, particularly at 0.5 mL/L, exerts the most pronounced biostimulatory effects on tea plants. Therefore, 0.5 mL/L CSL was selected as the exogenous spraying concentration for subsequent drought experiments

3.2 Effects of CSL on phenotypic attributes, and photosynthesis characteristics under drought stress

As shown in Fig. 1A&B, tea plants subjected to PEG treatment exhibited visible wilting symptoms and leaf chlorosis. Compared with CK, PEG treatment decreased photosynthetic efficiency, significantly reduced Fv/Fm, ETR, and NPQ by 13.8%, 29.46%, and 39.83%, respectively (Fig. 1C). Interestingly, application of CSL markedly alleviated PEG-induced drought stress, as reflected by healthier green foliage and less wilting relative to PEG treatment alone. Accordingly, CSL increased Fv/Fm, ETR, and NPQ by 16.86%, 85.54%, and 20.12%, respectively, compared with the PEG-treated plants. Furthermore, CSL application enhanced chlorophyll content and NBI by 9.73% and 26.32%, respectively, relative to the CK. Notably, under drought stress conditions, these parameters remained elevated by 10.97% and 23.27% compared with the CK. In contrast, CSL did not exhibit a stimulatory effect on flavonoid content.

Compared with the CK treatment, CSL application alone significantly affected the photosynthetic parameters, with Pn, Gs, and WUE increasing by 28.32%, 9.10%, and 52.01%, respectively, while Tr decreased by 15.42%. PEG markedly suppressed Pn, Gs, WUE, and TR, while CSL application alleviated the stress and increased Pn, Gs, and WUE by 96.46%, 17.69%, and 88.21%, respectively (Fig. 1D).

3.3 Effects of CSL on ROS accumulation, oxidative injury, and subcellular structure under drought stress

PEG-induced drought stress significantly induced oxidative stress of tea leaves, as indicated by increased accumulation of H₂O₂ and O₂⁻, along with elevated membrane lipid peroxidation. However, foliar application of CSL effectively alleviated this stress, resulting in significantly lower levels of these oxidative damage markers (Fig. 2A&B).

TEM observation showed intact subcellular structures in the control and CSL-only groups, with chloroplasts having clear membranes, orderly grana lamellae, and abundant, evenly distributed starch grains. PEG-induced drought stress caused blurred chloroplast membranes, disrupted thylakoid stacking, and reduced starch grains. CSL application under drought effectively preserved chloroplast structure and enhanced starch grain synthesis (Fig. 2C).

3.4 The effect of CSL on fatty acid metabolism and related enzyme activity under drought stress

To investigate whether the alleviation of oxidative stress by CSL in tea leaves under drought stress is related to the regulation of membrane lipid metabolism, we employed GC-MS/MS to analyze the extracted fatty acids. As shown in the clustering heatmap, fatty acids in tea leaves of CK and CSL groups clustered together, while the PEG and PEG + CSL groups formed a separate cluster. This pattern indicates that the fatty acid metabolic profile of PEG-treated tea plants remained largely similar to that of the PEG + CSL group, whereas the profile of CSL-treated plants closely resembled that of CK (Fig. 3A). Combined PEG and CSL application significantly elevated the fatty acid content in tea leaves, with a notable increase in unsaturated fatty acids but not in saturated fatty acids (Fig. 3B).

Among long-chain fatty acids (C14:0 to C24:0), C18:3 n-3c was the most abundant components. Content determination of long-, medium-, and short-chain fatty acids combined with principal component analysis (PCA) (PC1: 36.3%, PC2: 20.7%) revealed distinct metabolic changes among treatments (Fig. 3C&D). Drought stress (PEG treatment) significantly affected several fatty acids, with the content of long-chain fatty acids (C24:0, C17:0, C16:1n-7c, C15:0, and C14:0) notably elevated in PEG-treated plants, and a negative correlation with the content of long-chain fatty acid (C18:0). Foliar application with CSL under drought stress is mainly positively correlated with significantly increased long-chain unsaturated fatty acids (C18:2 n-6c, C18:2 n-6t, C18:1 n-9c, and C16:1 n-7c). The treatment of CK and CSL showed a greater positive correlation with many kinds of saturated short-chain and medium-chain fatty acids, like C4:0, C5:0 and i-C10:0.

The activities of three key enzymes ACCase, FAS, FAD involved in fatty acid metabolism in tea plants were determined. Compared with the control, the application of CSL alone increased ACCase activity by 41.27%, while PEG-induced drought stress significantly inhibited it. However, under drought stress, the addition of CSL still maintained ACCase activity at a 20.61% higher level than that of the control. Drought stress also significantly suppressed FAS activity by -12.11%. No significant difference was observed in FAD activity between the four groups (Fig. 3E).

3.5 Effects of CSL on antioxidant substances, antioxidant enzymes, and antioxidant capacity

Ascorbic acid (AsA) and glutathione (GSH) are widespread low molecular-weight antioxidants that play key roles in maintaining redox homeostasis by regulating ROS levels. PEG treatment increased the content of reduced ascorbate (AsA), glutathione (GSH), and glutathione disulfide (GSSG) in tea leaves. Application of CSL increased the concentration of AsA and GSH by 27.33% and 7.66%, compared to PEG treatment (Fig. 4). Analysis of the activities of enzymes involved in the AsA-GSH cycle demonstrated that the activity of dehydroascorbate reductase (DHAR) significantly increased under PEG treatment. Compared to PEG treatment, the application of CSL lead to further enhancement in the activities of APX, MDHAR, and GR increasing by 38.56%, 64.11%, and 69.47%, respectively. These results indicate that CSL could regenerate AsA and GSH and increased their total levels by regulating the activity of key enzymes in the AsA-GSH cycle. Under PEG-induced drought stress, CAT activity increased by 60.82%. Foliar application of CSL further enhanced POD activity by 38.15% compared to the PEG-treated group.

However, CSL application did not exert a positive effect on polyphenol (TP) content under drought conditions. The AsA/DHA and GSH/GSSG ratios represent the redox status of the AsA-GSH cycle systems in plants, with a higher ratio indicating greater antioxidant capacity. CSL supplementation restored the redox pair ratio of AsA/DHA and GSH/ GSSG under drought stress (Fig. 4N&O). Consistent with elevated DPPH and FRAP values confirmed an enhanced total antioxidant capacity (Fig. 4P&Q). Collectively, these results demonstrate that CSL mitigates drought-induced oxidative damage by reinforcing the cellular antioxidant system, thereby protecting plasma membrane integrity.

3.5 Correlation analysis and principal component analysis

Correlation analysis revealed significant relationships among photosynthetic parameters, ROS metabolism, fatty acids, AsA-GSH cycle, and antioxidant system in tea plants. Photosynthetic parameters (Fv/Fm, ETR, Pr, and Gs) were positively correlated with GSH, while showed negative correlations with ROS content (H_2O_2 , $O_2^{\cdot -}$), and ROS-induced LPO. Meanwhile, UFA was positively correlated with antioxidant indicators (DPPH, FRAP, AsA, GSH, APX and AsA/DHA) and photosynthetic parameters (Chl, Fv/Fm, ETR) (Fig. 5A). Principal component analysis (PCA) clearly differentiated the treatments, with the first two principal components (PC1 and PC2) accounting for 42.7% and 29% of the total variance, respectively. In the two-dimensional score plot, the four treatments formed distinct clusters. Foliar application with CSL whether under control conditions or drought conditions significantly increased plant antioxidant (GSH) reduced antioxidant ratios (GSH/GSSG, AsA/DHA), and photosynthetic efficiency (Fv/Fm, ETR). In contrast, PEG-simulated drought stress led to the accumulation of reactive oxygen species (H_2O_2 , $O_2^{\cdot -}$), increased LPO and EL. The combined treatment (PEG + CSL) effectively alleviated PEG-induced drought stress damage by elevated UFA and antioxidant capacity (DPPH and FRAP), making the plant physiological status more similar to CSL and CK treatment rather than PEG treatment (Fig. 5B).

4. Discussion

Many waste materials from industrial and agricultural production, when properly utilized, can promote plant biological growth, making them valuable raw materials for the development of plant biostimulants (Devi et al., 2022; Raguraj et al., 2023; Rong et al., 2023). The present study demonstrated that CSL, a by-product of the corn industry, effectively promoted tea growth, improved tea quality, and enhanced the tea plant's resistance to drought stress at low concentrations, indicating its potential as an effective biostimulant. These beneficial effects may be attributed to the presence of essential nutrients in CSL that support plant growth and development, as well as its potential regulatory effects on fatty acid metabolic processes and redox homeostasis in tea plants.

The present study found positive effects of CSL on biomass accumulation and apparent features in tea plants. In crops such as corn, grapes, and spinach, exogenous biostimulants have significantly promoted growth parameters including root length, plant height, leaf area, and biomass (Boselli et al., 2019; Carillo et al., 2019; D'Amato and Del Buono, 2021). In tea breeding programs, fresh leaf characteristics, such as

leaf size, area, bud density, and fresh weight, are commonly used to evaluate apparent quality and potential yield (Zaman et al., 2023). Our results demonstrated that the effects of CSL on the apparent leaf characteristics of tea plants, including leaf area, internode length, and leaf biomass, exhibited a dose-dependent response (Fig. S2). Lower concentrations significantly promoted leaf area growth and biomass increase in tea leaves, with 0.5 mL/L being the optimal concentration. Particularly, the promoting effect of CSL on internode length holds significant implications for the future mechanized harvesting of tea. However, higher concentrations of CSL did not result in further promotion of tea plant growth. These findings are consistent with the growth-promoting effects of biostimulants observed in Chinese cabbage (Li et al., 2024) and pepper (Navarro-Morillo et al., 2023). The promotive effect of CSL on tea plant growth may be attributed to its content of a wide variety of active ingredients with synergistic effects, such as high content of free amino acids and small molecule sugars (Table S1), which are easily absorbed by plants and can induce the biosynthesis of endogenous plant hormones, further enhancing CSL's action (Navarro-Morillo et al., 2023).

Prolonged drought stress accelerates leaf senescence and reduces photosynthesis, thereby impairing the plant's capacity to capture and utilize light energy, which in turn limits biomass accumulation and substance production (Tian et al., 2025). Previous studies have shown that when plants are exposed to drought, their chlorophyll content decreases and stomata close thereby reducing photosynthetic efficiency (Jiao et al., 2026). In the present study, this protective effect of CSL application on robust photosynthetic performance, such as chlorophyll content increases, stomata open, and higher photosynthetic rate was observed under PEG-induced drought stress (Fig. 1). This result is consistent with previous findings (Huang et al., 2021), suggesting that CSL is beneficial in help preserve chloroplast structural integrity and stomatal function under stress conditions. Notably, in situ chemical staining showed that CSL effectively reduced the accumulation of reactive oxygen species (ROS) and mitigated membrane lipid peroxidation damage (Fig. 2). This is likely a direct result of the CSL-induced enhancement of the tea plant's antioxidant capacity, which helped protect against the poor photosynthetic performance under drought stress. Similarly, in *Brassica rapa*, CSL enhanced adaptability to salt stress (Zhang et al., 2023), and in Chinese cabbage, it improved tolerance to cadmium stress (Li et al., 2024), both by alleviating ROS accumulation and oxidative damage. In conclusion, CSL functions as a stress protectant, conferring resilience to tea plants under drought conditions.

Under stress conditions, lipids in the plant cell membrane system, particularly polyunsaturated fatty acids, are vulnerable to attack by ROS, leading to membrane lipid peroxidation and subsequent oxidative cell damage (Mano, 2012; Takagi et al., 2016). Excessively accumulated ROS readily targets fatty acids in the plasma membrane, initiating a chain reaction of lipid peroxidation. This disrupts the normal physiological functions of the plasma membrane, thereby impairing plant physiological and metabolic processes (Sakihama & Yamasaki, 2002; Yin et al., 2010; Liang et al., 2021). Fatty acids, as essential components of plant membrane lipids, not only function in energy storage and membrane structure but are also broadly involved in plant responses to various environmental stresses (Gogna et al., 2020; Li et al., 2022; Zhang et al., 2025). Under abiotic stress, plants undergo lipid remodeling, altering lipid composition and content to effectively maintain membrane stability and enhance stress tolerance

(Henschel et al., 2024; Ou et al., 2025). Alterations in fatty acid composition and content are critical for plant adaptation to stresses such as temperature extremes, salinity, and drought. For instance, under cold stress, plants accumulate higher levels of unsaturated fatty acids to enhance cold resistance (Hoh et al., 2022; Kang et al., 2025). Under salt stress, rice increases the accumulation of linoleic acid and α -linolenic acid to maintain membrane integrity (Srivastava and Yadav, 2024). Similarly, during drought stress, plants increase the proportion of specific unsaturated fatty acids in membranes to preserve stability and improve drought tolerance (Wang et al., 2022; Yin et al., 2024). In the present study, foliar application of CSL under drought stress increased the content of unsaturated fatty acids in tea leaves, with a notable rise in C18:3 n-3c. Additionally, CSL significantly reversed the drought-induced changes in medium-chain fatty acids such as C6:0. Furthermore, foliar application of corn steep liquor enhanced the activities of critical fatty acid biosynthetic enzymes acetyl-CoA carboxylase (ACCase) and fatty acid synthase (FAS) under drought conditions (Fig. 3). These results suggest that CSL may play an important role in modulating fatty acid composition and content during the drought response in tea plants.

Drought stress induces the overaccumulation of reactive oxygen species (ROS) in plants, leading to cellular oxidative damage, which is a major cause of plant injury (Na et al., 2022; Razi and Muneer, 2021). To maintain ROS homeostasis and combat drought-induced oxidative stress, plants have evolved a defense system comprising antioxidant enzymes and non-enzymatic antioxidants (Na et al., 2022; Razi and Muneer, 2021). Among these, ascorbate (AsA) and glutathione (GSH) serve as crucial small-molecule primary antioxidants. They effectively scavenge ROS and regulate ROS levels via the AsA-GSH cycle, playing a vital role in mitigating oxidative damage under drought stress (Chen et al., 2024). In this cycle, ascorbate peroxidase (APX) catalyzes the reaction between AsA and H_2O_2 , ultimately reducing H_2O_2 to water while oxidizing AsA to dehydroascorbate (DHA). Concurrently, key enzymes such as glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR) work coordinately to regenerate AsA and GSH (Li, 2023). Studies have shown that AsA and GSH are critical in alleviating oxidative damage in plants under drought, and overexpression of key AsA-GSH cycle enzymes significantly enhances plant drought tolerance (Hasanuzzaman et al., 2019). The present study demonstrates that under drought stress, exogenous application of CSL significantly enhanced the activities of AsA-GSH cycle enzymes in tea leaves (Fig. 4) and maintained higher levels of reduced AsA and GSH (Fig. 4). Furthermore, CSL application notably increased the activity of other antioxidant enzymes, such as peroxidase (POD). While tea polyphenols and flavonoids represent important secondary antioxidants in tea leaves, CSL did not positively influence their content under drought stress, indicating a limited effect on these secondary metabolic pathways. Additionally, CSL significantly improved the total antioxidant capacity of tea leaves (Fig. 4P & Q), markedly reduced lipid peroxidation in root tips (Fig. 2A & C). Correlation analysis and principal component analysis revealed a significant positive correlation between antioxidant capacity and the accumulation of AsA and GSH with the application of CSL under drought stress (Fig. 5A&B). These results collectively suggest that CSL enhances drought tolerance in tea plants by activating the AsA-GSH cycle, maintaining elevated AsA and GSH pools, thereby promoting ROS scavenging and alleviating lipid peroxidation damage (Fig. 6).

Conclusions

In summary, the application of the industrial byproduct corn steep liquor (CSL) has demonstrated positive effects on improving both the growth and stress resistance of tea plants. Foliar application of CSL at an appropriate concentration (0.5 mL/L) significantly enhanced photosynthesis and biomass accumulation under normal conditions. Moreover, under drought stress, CSL application effectively mitigated lipid peroxidation damage by promoting the synthesis of unsaturated fatty acids and enhancing the AsA-GSH cycle.

Declarations

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the research reported in this paper.

Credit authorship contribution statement

Xin Liang: Writing, review & editing, Funding acquisition, Formal analysis. Yanhua Jiang: Writing, review & editing, Formal analysis. Conceptualization. Peibin Dai: Investigation, Formal analysis. Yike Shao: Investigation, Formal analysis. Yang Liu: Investigation. Yu Duan: Review. Shanshan Wang: Methodology. Yufeng Lu: Investigation. Haiqiang Feng: Investigation. Li Ruan: Writing, review & editing, Funding acquisition. All authors reviewed and approved the final version of the manuscript.

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Author Contribution

X. L.: Writing, review & editing, Funding acquisition, Formal analysis. Y. J.: Writing, review & editing, Formal analysis. Conceptualization. P. D.: Investigation, Formal analysis. Y. S.: Investigation, Formal analysis. Y. L.: Investigation. Y. D.: Review. S. W.: Methodology. Y. L.: Investigation. H. F.: Investigation. L. R.: Writing, review & editing, Funding acquisition. All authors reviewed and approved the final version of the manuscript.

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Figures

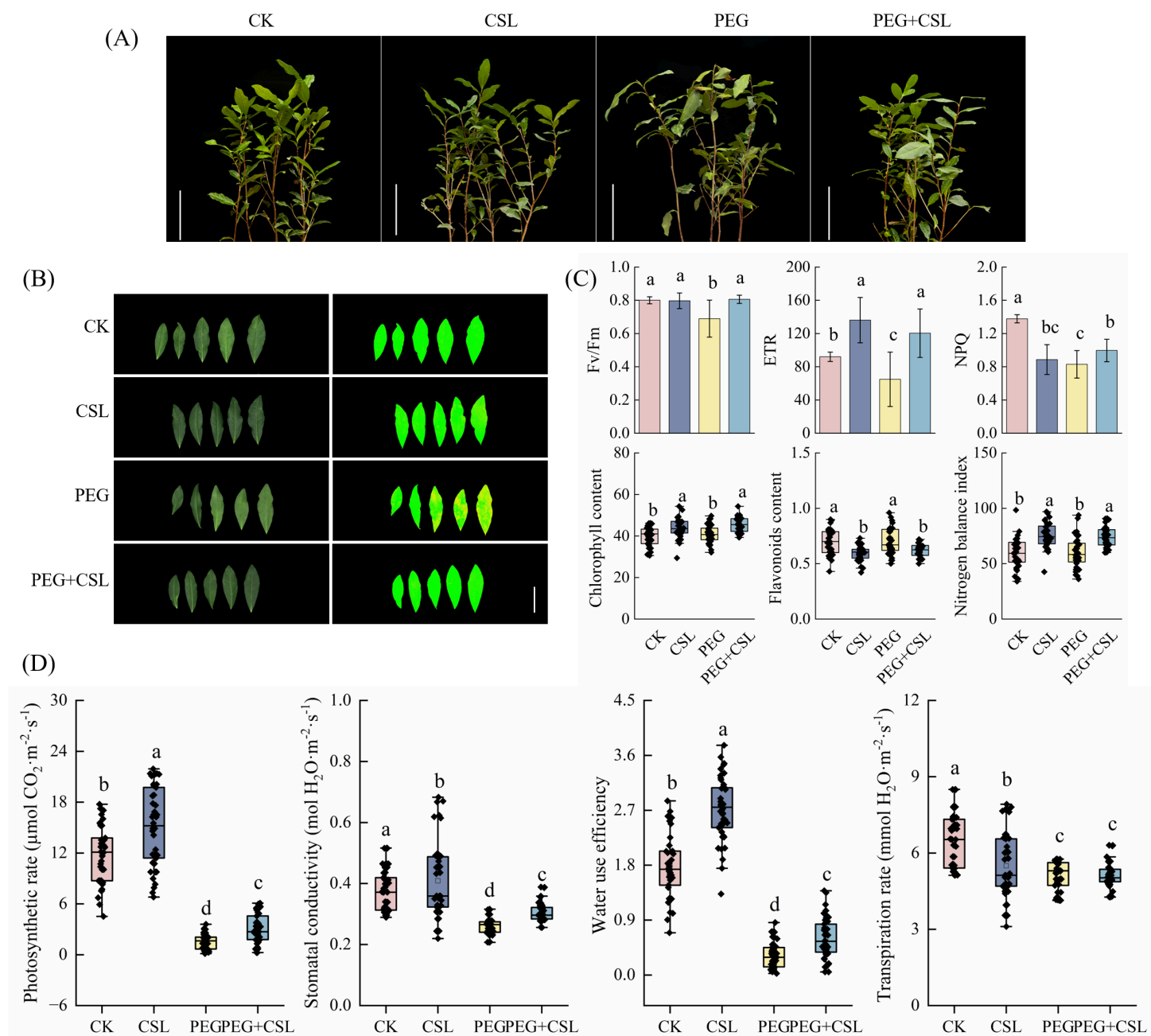


Figure 1

Effects of CSL on morphological attributes(A&B), photosynthetic parameters (C&D). Tea plants were treated with the following treatments: CK, normal nutrient solution treatment. CSL, normal nutrient solution + foliar application with CSL on leaves. PEG, simulating drought stress with polyethylene glycol added in nutrient solution for root irrigation. PEG+CSL, root irrigation with PEG added nutrient solution, while leaf spraying with CSL. The results are mean $\pm$ SD. Different letters mean significant differences at $p < 0.05$. Scale bar in fig. A, B&E was 15 cm, 2 cm, and 500 μm , respectively. CW, cell wall; PM, plasma membrane; Ch, chloroplast; GL, grana lamellae; SG, starch grains.

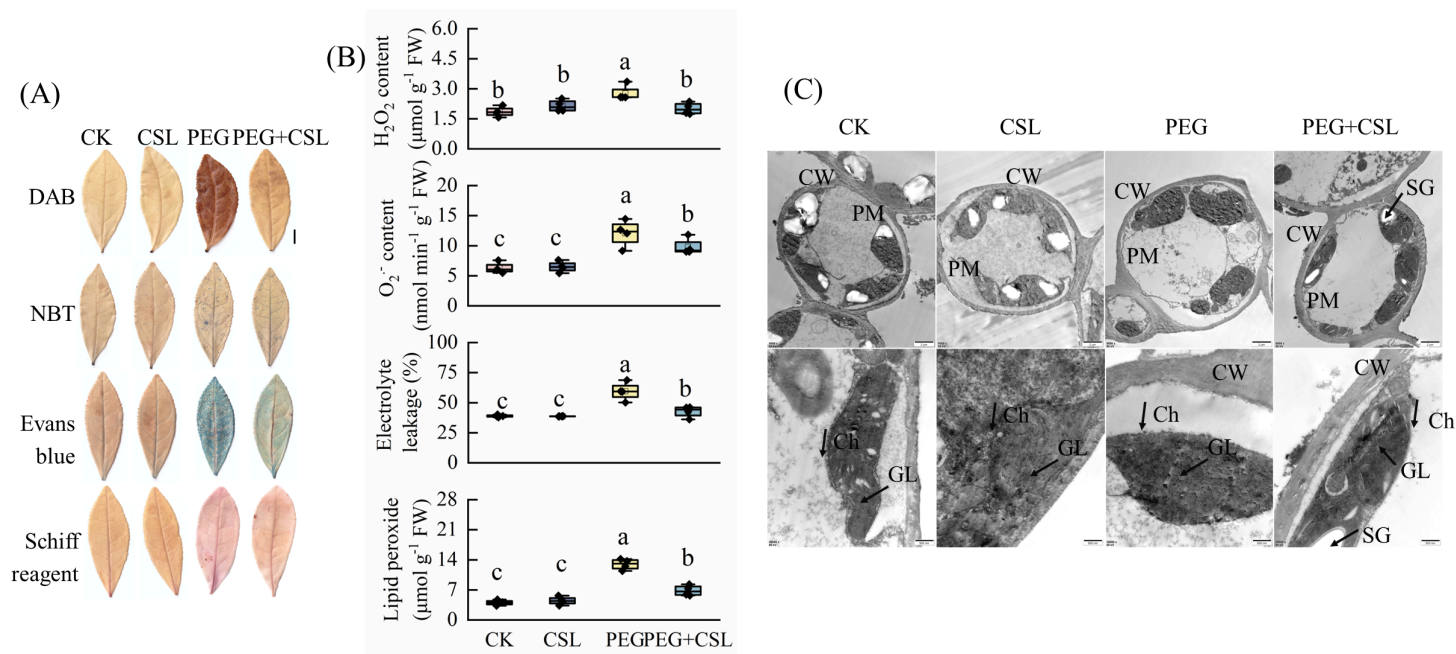


Figure 2

Effects of CSL on redox indicators (A&B), and cell ultrastructure microstructure (C). Tea plants were treated with the following treatments: CK, normal nutrient solution treatment. CSL, normal nutrient solution + foliar application with CSL on leaves. PEG, simulating drought stress with polyethylene glycol added in nutrient solution for root irrigation. PEG+CSL, root irrigation with PEG added nutrient solution, while leaf spraying with CSL. The results are mean±SD. Different letters mean significant differences at $p<0.05$. Scale bar in fig. A was 500 μm, respectively. The abbreviation in image C is: CW, cell wall; PM, plasma membrane; Ch, chloroplast; GL, grana lamellae; SG, starch grains.

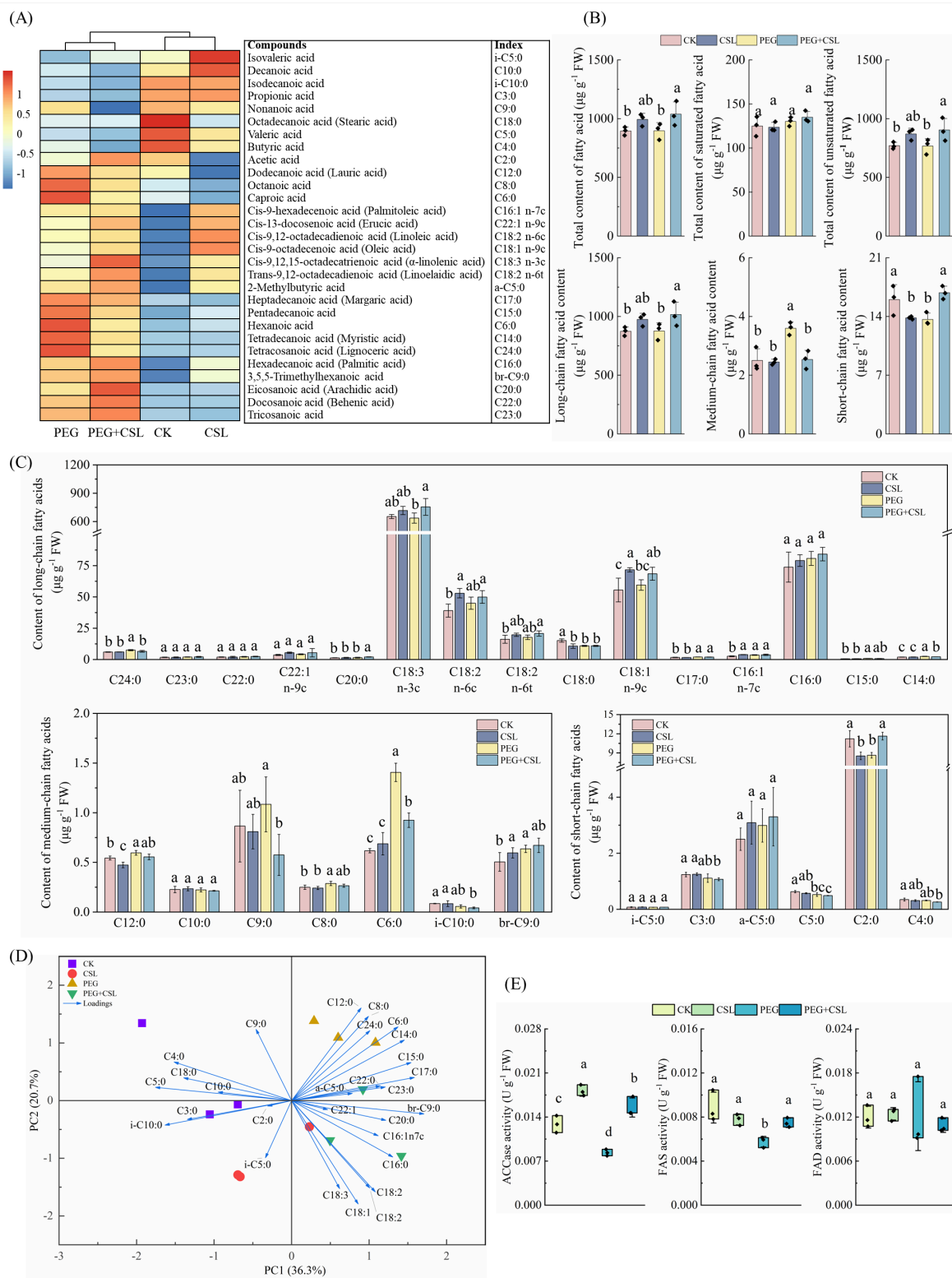


Figure 3

Effects of CSL on the content of fatty acids and activities of key enzymes related to fatty acid synthesis and desaturase in tea plants. CK, CSL, PEG, and PEG+CSL represent the control, foliar application of corn steep liquor under control condition, drought stress, and the combined treatment of corn steep liquor and drought stress, respectively. (A) Normalized heat map analysis of the fatty acid content in tea leaves. (B&C) The content of different fatty acids. (D) Principal co-ordinate analysis of fatty acid

contents in tea leaves. (E) The activities of acetyl-CoA carboxylase (ACCCase), fatty acid synthase (FAS) and fatty acid desaturase (FAD). Different letters above the bars represent statistically significant difference ($p < 0.05$). The abbreviations and detailed names of fatty acids can be found in the Table S2.

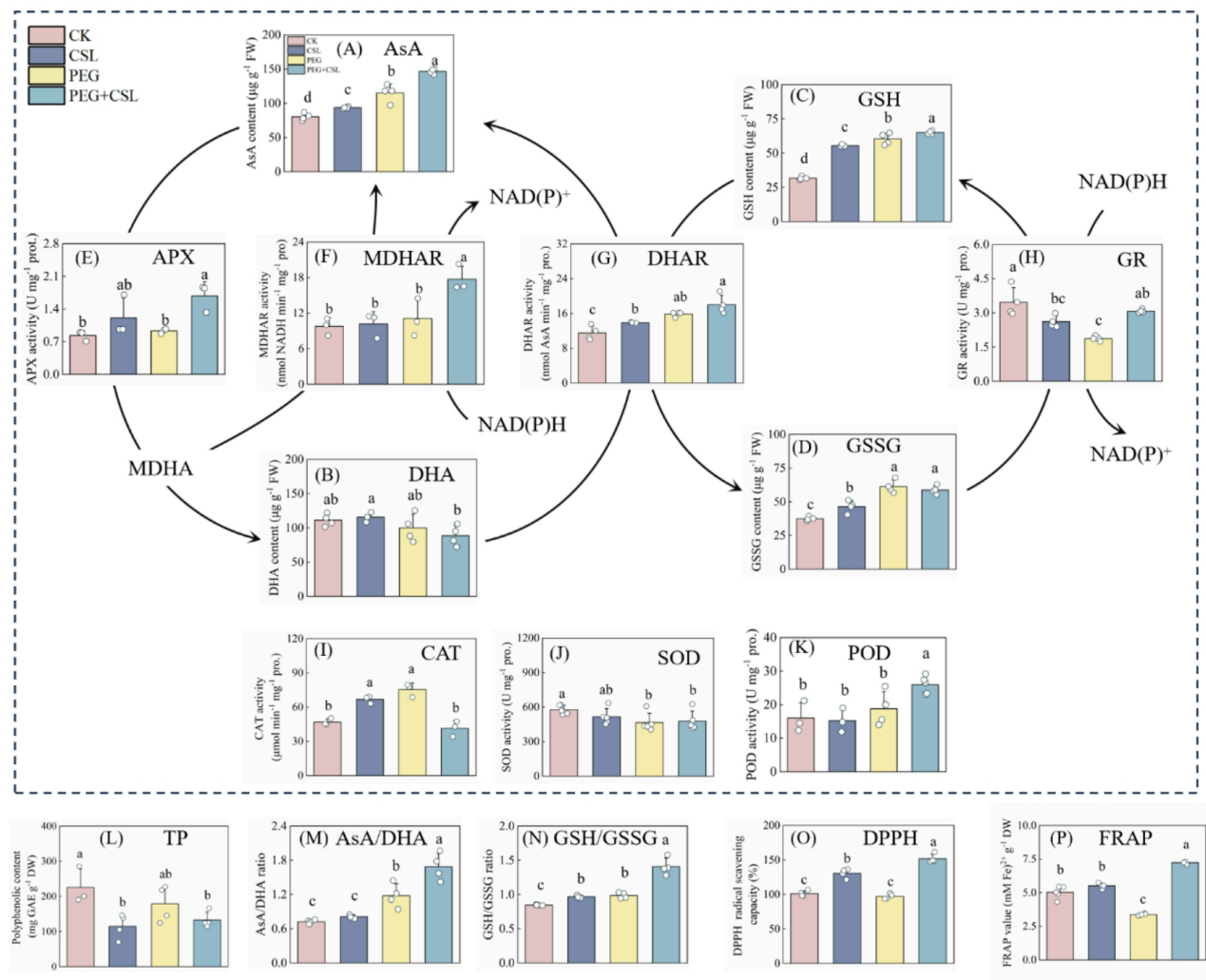


Figure 4

Effects of CSL on AsA-GSH cycle, antioxidant content antioxidant enzyme activity and total antioxidant capacity of tea leaves. (A) Main pathway of ROS formation and the AsA-GSH cycle in the plant cell. Effect of CSL or/and PEG treatments on the content of reduced forms of ascorbic acid (AsA) (B), oxidized forms dehydroascorbic acid (DHA) (C), reduced sglutathione (GSH) (D), oxidized glutathione (GSSG) (E), polyphenolic (TP) (M). Effect of CSL or/and PEG treatments on the activity of scorbate peroxidase (APX) (F), monodehydroascorbate reductase activity (MDHAR) (G), dehydroascorbate dehydrogenase activity (DHAR) (H), gluathione reductase activity (GR) (I), catalase (CAT) (J), superoxide dismutase (SOD) (K), and peroxidase (POD) (L). Effect of CSL or/and PEG treatments on the ratio of AsA to DHA (N), GSH to GSSG (O), free radical scavenging rate (DPPH) (P) total antioxidant activity (FRAP)

(Q). Values are means $\pm$ standard error. The different lowercase letters on the bars indicate significant differences among the treatments ($p < 0.05$).

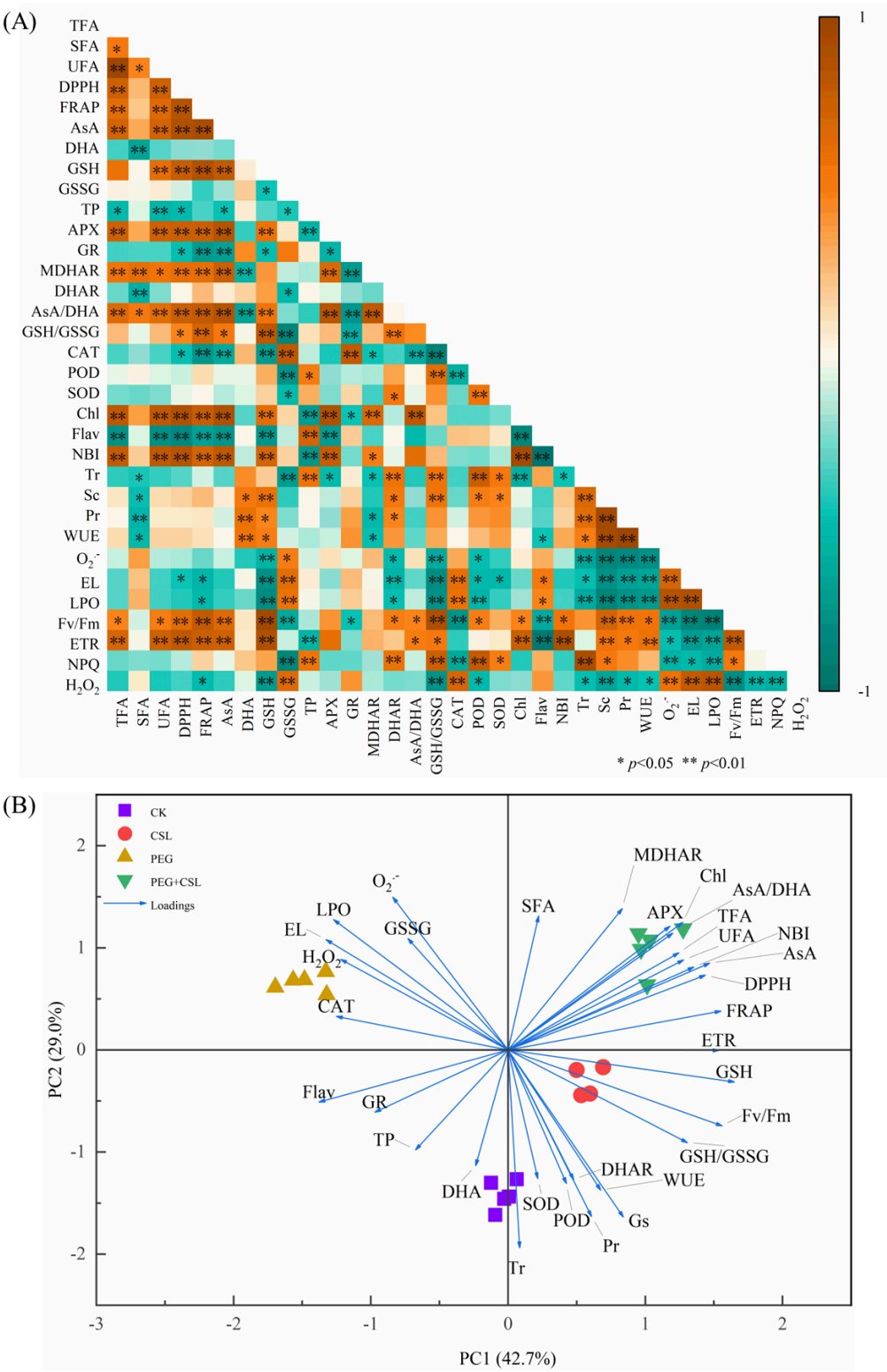


Figure 5

The correlation matrix (A) and principal component analysis (PCA) (B) of photosynthetic parameters, ROS metabolism, fatty acids, and redox indicators in tea plants after the treatments. The symbol *, and

** indicate significance of $p < 0.05$, and 0.01 , respectively. CK, CSL, PEG, and PEG+CSL represent the control, foliar application of corn steep liquor under control condition, drought stress, and the combined treatment of corn steep liquor and drought stress, respectively. TFA, toatal fatty acid. SFA, saturated fatty acid. UFA, unsaturated fatty acid. DPPH, free radical scavenging rate. FRAP, total antioxidant capacity. AsA, reduced ascorbic acid. DHA, oxidized ascorbic acid. GSH, reduced glutathione. GSSG, oxidized glutathione. TP, polyphenol. APX, ascorbate peroxidase. GR, glutathione reductase. MDHAR, monodehydroascorbate reductase. DHAR, dehydroascorbate reductase. CAT, catalase. POD, peroxidase. SOD, superoxide dismutase. Chl, chlorophyll. Flav, flavonoid. NBI, nitrogen balance index. Tr, transpiration rate. Gs, stomatal conductivity. Pr, net photosynthetic rate. WUE, water use efficiency. Fv/Fm, PSII maximum photochemical quantum yield. ETR, PSII reaction center photosynthetic electron transfer efficiency. NPQ, non-photochemical quenching coefficient. H_2O_2 , hydrogen peroxide. $O_2^{\cdot -}$, superoxide anion. EL, electrolyte leakage. LPO, lipid hydroperoxide.

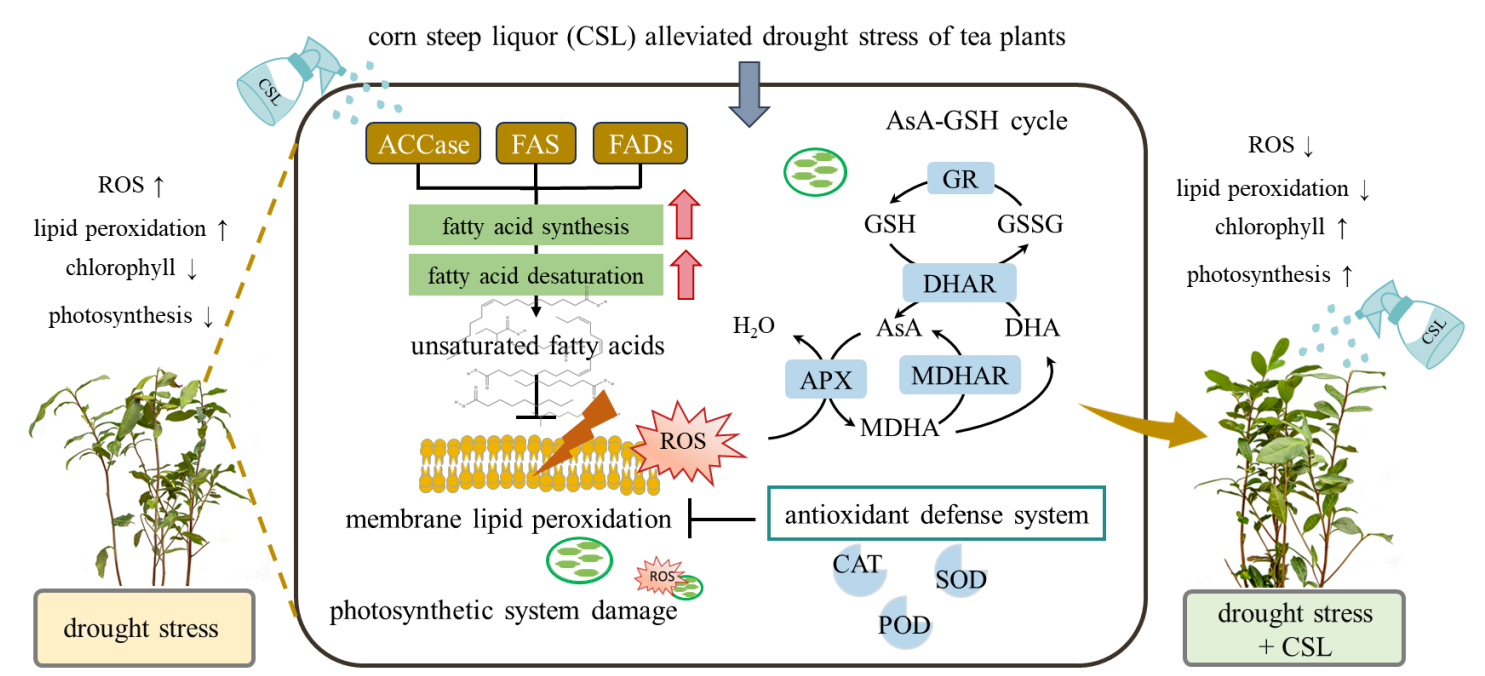


Figure 6

CSL, a by-product of corn processing, is an effective biostimulant for improving drought tolerance of tea plants by regulating fatty acid metabolism and redox homeostasis.

Supplementary Files

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