



# Article Physiological and Biochemical Effects of Exogenous Calcium on *Camellia oleifera* Abel under Drought Stress

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**Abstract:** High temperature and drought are the main factors restricting the increase in *Camellia oleifera* Abel production that can seriously harm its physiological and biochemical functions. This study aimed to explore the effect by which  $Ca^{2+}$  enhances the drought resistance of *C. oleifera*. Fouryear-old potted plants of the 'Xianglin 210' variety were treated with four levels of CaCl<sub>2</sub> solution (0, 10, 20, and 40 mmol/L) and subjected to natural drought stress. The drought levels were categorized as CS (non-drought), LD (light drought), MD (moderate drought), and SD (severe drought). The effects of drought stress on the growth and physiological-biochemical characteristics of *C. oleifera* were measured. Under drought stress, spraying CaCl<sub>2</sub> solution on leaves could significantly increase the net photosynthetic rate (*Pn*), transpiration rate (*Tr*), stomatal conductance (*Gs*), endogenous hormone indoleacetic acid (IAA) content, abscisic acid (ABA) content, and gibberellic acid (GA) content in organs and tissues; enhance the superoxide dismutase (SOD) activity; and decrease the concentration of intercellular carbon dioxide (*Ci*) and number of dehiscence fruits. Spraying 10 mmol/L CaCl<sub>2</sub> effectively alleviates the damage of drought stress to tung oil trees. The study provides references for alleviating drought stress and increasing economic benefits in *C. oleifera* production.

Keywords: Camellia oleifera; drought stress; exogenous calcium

## 1. Introduction

Camellia oleifera of the genus Camellia (Theaceae family) are small evergreen trees that, alongside Olea europaea Linni., Elaeis guineensis Jacq., and Cocos nucifera Linn., are known as the world's four major woody oilseed species. C. oleifera is widely planted in the low hills of southern China. Precipitation in this region is concentrated from March to June, generally accounting for 60% to 70% of the annual precipitation; July-to-September precipitation is relatively low, accounting for about 20% of the annual precipitation [1,2]. This region is, therefore, also prone to high temperatures and drought weather. High temperature and drought are constraints to cash crop production, and plants experiencing heat stress tend to experience severe physiological and biochemical dysfunctions, which include slowed growth and development, a decreased photosynthetic rate and stomatal conductance (Gs), and damage to various cellular components [3]. Drought affects the growth and fruiting of C. oleifera, causing flower drop, fruit drop, and fruit cracking, which, in turn, reduces the oil tea yield [4–6], and the lower the age of oil tea plants is in the early stage of growth, the weaker the drought resistance is, and the more drastic the response caused by coping with high temperature and drought stress will be [7]. According to previous studies, CaCl<sub>2</sub> can alleviate or even increase the drought tolerance of plants [8].

The calcium ion  $(Ca^{2+})$  is known as "the main regulator of plant metabolism and development". Not only is it an essential nutrient for plant growth and development



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). and a vital structural element that stabilizes and protects the structure and function of the plasma membrane of the cell, but it also supports the internal physiological and metabolic mechanisms of the plant [8,9]. In addition, it is also a second messenger, coupling extracellular stimuli and intracellular responses. Under environmental stress, Ca<sup>2+</sup> can bind to intracellular Ca<sup>2+</sup> receptor proteins and participate in sensing, transmitting, responding, and expressing stress signals, ultimately improving plant resilience to environmental stress directly or indirectly [10].

Research has shown that spraying *Hippophae rhamnoides* Linn. with exogenous calcium effectively promotes photosynthetic properties, including the photosynthetic rate, and increases the activity of antioxidant enzymes under drought stress [11]. In addition, calcium treatment also promotes the accumulation of a variety of osmoregulatory substances, such as soluble sugar, free proline, and betaine, in the leaves of *Handeliodendron bodinieri* (Levl.)Rehd. seedlings to effectively improve the water status of the plant under drought stress [12]. It can be seen that exogenous calcium supplementation is one of the effective measures to improve plant resistance under drought stress. Studies on the effect of exogenous calcium on drought resistance in *C. oleifera* plants have not been reported. In this light, the present study was conducted to investigate the effects of exogenous calcium on moisture content, photosynthesis, endogenous hormones, reactive oxygen species (ROS) accumulation, antioxidant enzymes, and fruits in *C. oleifera* plants under drought stress using the *C. oleifera* cultivar 'Xianglin 210' as the material. This will provide a reference for the development of efficient drought prevention and drought mitigation technologies for *C. oleifera*.

### 2. Materials and Methods

### 2.1. Overview of the Experimental Field

The experimental site is located in Tianjiling Experimental Forestry Farm of Hunan Academy of Forestry ( $113^{\circ}01'$  E,  $28^{\circ}06'$  N), which is at an altitude of 80–100 m. It has a subtropical monsoon climate with four distinct seasons. The climate pattern in the study area includes being rainy in late spring and early summer, having a drought in summer and autumn, and having a long, hot summer, which easily causes drought stress in *C. oleifera* plants.

### 2.2. Experimental Materials

The *C. oleifera* varieties for the test were potted plants of 4-year-old 'Xianglin 210' with normal and uniform growth conditions, and there were 15–20 fruits per potted plant (Figure 1A,B). The *C. oleifera* plants were planted in April 2022, and the seedlings were allowed to mature in the greenhouse for 30 days with normal water and fertilizer management. During the management period, the trees were stable and free from pests and diseases, and the number of fruits was standardized (Figure 1C). The pot dimensions were 400 mm height and 500 mm diameter, and the potting soil was red loam:perlite:phosphate fertilizer (19:10:1).



**Figure 1.** Experimental site conditions. (**A**,**B**) The 4-year-old *C. oleifera* potted plants were under identical water and fertilizer management conditions. (**C**) *C. oleifera* potted plants undergoing drought stress.

### 2.3. Experimental Design

There were four different calcium addition treatments in this experiment: CK (distilled water), N1 (10 mmol/L CaCl<sub>2</sub>), N2 (20 mmol/L CaCl<sub>2</sub>), and N3 (40 mmol/L CaCl<sub>2</sub>). Three replicates were set up for each treatment, and 10 C. oleifera seedlings were planted for each replicate. Drought levels were classified according to the volumetric soil water content within the pots, and levels were CS (no drought), LD (mild drought), MD (moderate drought), and SD (severe drought) when the volumetric soil water content reached 0.3, 0.2, 0.15, and 0.1. For consistency, the trees were pruned so that the trees' shapes were roughly similar and the number of fruits was the same on each tree. The soil volumetric water content of *C. oleifera* plants was determined using a soil moisture meter (STEPS, Ludwigshafen, German). When the soil volumetric water content reached 0.3, 0.2, 0.15, and 0.1, photosynthesis parameters and petiole tensile force were determined. Leaf samples were collected and temporarily stored in a -80 °C ultra-low-temperature refrigerator for biochemical indexes, and leaf, fruit, and petiole samples were collected for determination of relative water content. After the fourth sample collection was completed, the C. oleifera plants were immediately rehydrated and managed in a unified manner, and when the fruits were ripe, the fruit samples were taken to determine the oil content.

### 2.4. Experimental Methods

The relative water content values of leaf, pericarp, seed kernel, and petiole were determined using the saturated water content method [13]. Photosynthesis parameters were determined using the 6400XT Portable Photosynthesis System (LI-COR, Lincoln, NE, USA). The endogenous hormones indoleacetic acid (IAA), abscisic acid (ABA), and gibberellic acid (GA) were determined using competitive enzyme-linked immunosorbent assay (ELISA) kits. Malondialdehyde (MDA) (RUIXIN BIOTECH, Quanzhou, China) was measured using the Solepol MDA (Solarbio, Beijing, China) content assay kit. Fruit stalk separation force was determined using the Zhitai DS2-50N Push-Pull tester, and seed kernel oil content was determined using the Soxhlet oil extraction method [14].

### 2.5. Statistical Analysis

The trial used a completely randomized experimental design. All raw data were processed using Excel 2019 (Microsoft, Redmond, DC, USA) for data processing and standard deviation calculation. SPSS 17.0 software (IBM, New York, NY, USA) was used for the significance of difference analysis, Origin 2019 (OriginLab, Northampton, MA, USA) for graphing, and Adobe Photoshop 2019 (Adobe, San Jose, CA, USA) for plate making.

#### 3. Results

#### 3.1. Effect of Exogenous Calcium on Water Content in C. oleifera Plants under Drought Stress

The water content of the leaf, pericarp, seed kernel, and petiole in the *C. oleifera* plants decreased by different degrees with the intensification of drought stress (Table 1). The differences between the treatment groups were not significant except for leaves in the CS and LD stages, and the *C. oleifera* plants did not suffer from water deficiency. After entering the MD stage, the water content of organs or tissues decreased significantly, and the differences between the N1 and N2 treatment groups and the control group were significant. In the SD stage, the N1 treatment group had higher water content values in the leaf, pericarp, seed kernel, petiole, and fruit stalk compared to the other three treatment groups. This suggests that exogenous calcium sprayed on leaves can effectively alleviate the water loss in *C. oleifera* plants and thus reduce the damage caused by drought stress on *C. oleifera* plants.

Figure 2 showed the morphological changes of leaves in four groups under drought stress. At the CS and LD stages, no obvious stress damage was observed in the leaves of *C.oleifera* plants. In the MD stage, the leaves in the CK, N2 and N3 treatment groups showed yellowing and slight curling. In the SD stage, the leaves in all four treatment

groups were obviously yellowed, and the leaves of CK and N3 treatment groups appeared curled and browned.

Specimen	Treatment	СК	N1	N2	N3
Leaf	CS	$62.32\%\pm0.62~\mathrm{Aa}$	$62.17\%\pm0.34~\mathrm{Aa}$	$62.21\% \pm 0.76$ Aa	$62.35\% \pm 0.62$ Aa
	LD	$61.99\% \pm 0.35~{ m Aa}$	$62.10\% \pm 0.96$ Aa	$61.43\%\pm0.29~\mathrm{Ba}$	$62.00\% \pm 0.51$ Aa
	MD	$60.12\%\pm0.39~\text{Bb}$	$61.36\%\pm0.12~Ab$	$60.76\% \pm 0.63~{ m ABb}$	$60.54\%\pm0.45~\text{ABb}$
	SD	$58.15\%\pm0.45~\mathrm{Bc}$	$59.88\%\pm0.14~Ab$	$58.90\%\pm0.56~\mathrm{Bc}$	$57.76\% \pm 0.61  \mathrm{Cc}$
Pericarp	CS	$77.29\% \pm 0.25$ Aa	$77.24\%\pm0.19~\mathrm{Aa}$	$76.92\%\pm0.32~\mathrm{Aa}$	$77.18\% \pm 0.10$ Aa
	LD	$77.03\% \pm 0.13$ Aa	$77.43\% \pm 0.17~{ m Aa}$	$77.04\%\pm0.12~\mathrm{Aa}$	$77.05\% \pm 0.08$ Aa
	MD	$70.33\%\pm0.46~\text{Bb}$	$71.33\%\pm0.34~Ab$	$70.84\%\pm0.29~\text{ABb}$	$70.34\%\pm0.30~\text{Bb}$
	SD	$68.10\%\pm0.77~\mathrm{Bc}$	$68.81\%\pm0.24~\mathrm{Ac}$	$68.34\%\pm0.22~Ac$	$67.32\% \pm 0.48~{ m Cc}$
Kernel	CS	$72.35\% \pm 0.50~{ m Aa}$	$71.58\% \pm 0.46$ Aa	$71.79\% \pm 0.27$ Aa	$72.09\% \pm 0.54$ Aa
	LD	$72.05\% \pm 0.38$ Aa	$71.79\% \pm 0.54$ Aa	$71.95\% \pm 0.59$ Aa	$71.74\% \pm 0.46$ Aa
	MD	$68.11\%\pm0.37~\text{ABb}$	$68.49\%\pm0.42~Ab$	$67.85\%\pm0.79~\text{Bb}$	$67.49\%\pm0.57~\mathrm{Bb}$
	SD	$64.66\% \pm 1.11~{ m Bc}$	$65.64\% \pm 0.56~{ m Ac}$	$65.25\%\pm0.36~\text{ABc}$	$63.94\% \pm 0.79~{ m Cc}$
Fruiting stem	CS	$77.29\% \pm 0.30$ Aa	$77.24\% \pm 0.68$ Aa	$76.92\%\pm0.13~\mathrm{Aa}$	77.18% $\pm$ 0.57 Aa
	LD	$77.03\% \pm 0.16$ Aa	$77.43\% \pm 0.32$ Aa	$77.04\% \pm 0.40~{ m Aa}$	77.05% $\pm$ 0.19 Aa
	MD	$70.33\%\pm0.19~\text{Bb}$	$71.33\%\pm1.00~Ab$	$70.84\%\pm0.41~\text{ABb}$	$70.34\%\pm0.49~\text{Bb}$
	SD	$67.43\% \pm 0.21~{ m Bc}$	$68.81\%\pm1.07~{\rm Ac}$	$68.34\% \pm 0.58~{ m Ac}$	$67.32\% \pm 0.75~{ m Bc}$

Table 1. Effects of exogenous calcium on water content in Camellia oleifera under drought stress.

Different uppercase letters indicate significant differences between different calcium treatments in the same drought period (p < 0.05); different lowercase letters indicate significant differences between different drought periods for the same calcium treatment (p < 0.05).



**Figure 2.** Development of *C. oleifera* leaves under different drought stresses. CS (no drought), LD (mild drought), MD (moderate drought), and SD (severe drought); CK (distilled water), N1 (10 mmol/L CaCl<sub>2</sub>), N2 (20 mmol/L CaCl<sub>2</sub>), and N3 (40 mmol/L CaCl<sub>2</sub>).

# 3.2. Effect of Exogenous Calcium on Photosynthetic Parameters of C. oleifera Plants under Drought Stress

As shown in Figure 3, the net photosynthetic rate (Pn), Stomatal conductance (GS), and transpiration rate (Tr) of *C. oleifera* plants continued to decrease with the extension of the drought time, and the concentration of intercellular carbon dioxide (Ci) first decreased and then increased. In the LD stage, the Pn decreased significantly, but in the calcium-treated groups, the Pn increased by 25.97%, 23.37%, and 9.70% compared with the control group. In the MD stage, the Pn values of the N2 and N3 treatment groups were lower than that of the control group, and in the N1 treatment group, it increased by 13.64% compared with the control group, it decreased by 21.13% and 25.40% compared with the control group, respectively, while in the N1 treatment group, it increased by 63.10% compared with the control group, indicating that exogenous calcium could significantly increase the Pn in *C. oleifera* plants.



**Figure 3.** Effect of exogenous calcium on photosynthesis parameters of *C. oleifera* plants under drought stress. Different uppercase letters indicate significant differences between different calcium treatments in the same drought period (p < 0.05); different lowercase letters indicate significant differences between different drought periods for the same calcium treatment (p < 0.05).

In the LD stage, there was no obvious decreasing trend of *GS* in the N1 and N3 treatment groups; however, in the MD stage, *GS* of all four treatment groups decreased significantly, among which it was higher in the N2 and N3 treatment groups than in the control group. In the SD stage, only the N1 treatment group had higher *GS* compared to the control group, but the difference was not significant.

In the LD stage, the decreasing trend of *Tr* in the control group was significant, and the calcium-treated groups were all higher than the control group by 63.91%, 44.33%, and 78.57% (p < 0.05). At the MD stage, the difference between the N1 and N2 treatment groups and the control group was significant. At the SD stage, the N1 treatment group increased by 24.67% compared with the control group.

At the CS and LD stages, the decreasing tendency of *Ci* was significant only in the control group. At the LD and MD stages, *Ci* was higher in the N1 and N2 treatment groups than in the control group.

# 3.3. Effects of Exogenous Calcium on Biochemical Indicators in C. oleifera Plants under Drought Stress

Figure 4 showed that the endogenous hormone IAA had the decreasing trend with increasing drought stress. In the CS and LD stages, the decreasing trend of IAA in *C. oleifera* plants was not significant. When the drought entered the MD stage, the IAA in the calcium-sprayed treatment groups was increased by 10.94%, 10.11%, and 6.365% compared with the control group. In the SD stage, the IAA content of *C. oleifera* plants in the N1 treatment group was the highest, and compared with the control treatment group, it was increased by 20.81%. ABA content increased with drought duration. At the MD and SD stages, ABA in the calcium-treated groups was significantly higher than in the control group, with the highest ABA content being in the N1 treatment group (116.34 ng/g). GA content showed a tendency to increase and then decrease with the intensification of drought stress. At the MD stage, the GA levels in the N1 and N2 treatment groups were significantly higher than in the control group by 24.01% and 23.66%, respectively.





As shown in Figure 5, drought stress significantly increased the MDA content in the *C. oleifera* plants. At the MD stage, the MDA content in the *C. oleifera* plants in each treatment group increased rapidly; however, its values were 31.46%, 21.93%, and 11.33% lower in the N1, N2, and N3 treatment groups, respectively when compared with the control group. At the SD stage, the differences between the N1, N2, and the control groups were significant, with the N1 treatment group having the lowest MDA content in *C. oleifera* plants, which was 11.32 nmol/g. From the CS stage to the MA stage, drought stress significantly increased the superoxide dismutase (SOD) activity in the *C. oleifera* plants. In the MD stage, the SOD activities of the N1, N2, and N3 treatment groups were increased by 31.91%, 31.53%, and 10.39%, respectively compared with the control group. In the SD stage, the SOD activity decreased significantly; still, the SOD activity levels of the N1 and N2 treatment groups (116.37 and 93.98  $\mu/g$ , respectively) were significantly higher than that of the control group. The above indicated that spraying exogenous calcium had had a positive effect on the *C. oleifera* plants to alleviate drought stress.

### 3.4. Effect of Exogenous Calcium on Fruits of C. oleifera Plants under Drought Stress

Drought stress in *C. oleifera* plants manifests as fruit dehiscence. The control and N3 treatment groups had the highest numbers of dehiscent fruits and the N1 treatment group had the least (Figure 6). At the end of the drought stress treatment, the *C. oleifera* plants were rehydrated and managed uniformly. The fruits of the various treatment groups were collected in the middle of October, and the oil contents of the seed kernels were determined. According to the results, the seed kernel oil content was the highest in the N1 treatment group, at 44.72%. By contrast, the control treatment group had been affected by drought stress; the oil content was only 34.40%, proving that spraying exogenous calcium can effectively improve the oil content of the seed kernel of *C. oleifera* under drought stress. The push–pull tester was used to determine the stalk pulling force in the SD period of the *C. oleifera* plants in the various treatment groups. From the results, it was noted that the fruit stalk pull force was highest in the N1 treatment group and lowest in the CK treatment group.



**Figure 5.** Effect of exogenous calcium on MDA and SOD content in *C. oleifera* plants under drought stress. Different uppercase letters indicate significant differences between different calcium treatments in the same drought period (p < 0.05); different lowercase letters indicate significant differences between different drought periods for the same calcium treatment (p < 0.05).



**Figure 6.** Effect of exogenous calcium on fruits of *C. oleifera* plants under drought stress. Different capital letters indicate significant differences between calcium treatments (p < 0.05).

### 4. Discussion

### 4.1. Effect of Exogenous Calcium on Photosynthesis in C. oleifera under Drought Stress

When plants are subjected to heat and drought stress, stomata are usually closed, Pn decreases, and growth is inhibited. Research has shown that the photosynthetic rate in plants is affected by both stomatal and non-stomatal factors [15]. The cause of the decrease in the photosynthetic rate under mild drought stress in plants is the decrease in *GS*, which leads to a decrease in the concentration of *Ci* and an increase in the stomatal limiting value,

leading to a subsequent decrease in the photosynthetic rate. In this study, the Pn in C. oleifera plants decreased slowly from the CS stage to the LD stage, and GS, Tr, and concentration of Ci also decreased, which indicated that when C. oleifera plants were in the LD stage, the main factor for the decrease in the photosynthetic rate was the stomatal index; this was consistent with the findings of a previous study [15]. When C. oleifera plants were in the MD-to-SD stage, the *Pn*, *GS*, and *Tr* decreased rapidly, but the *Ci* concentration increased, which indicated that the decrease in the Pn at this stage was affected by non-stomatal factors. Studies have shown that the main reasons for the decrease in the photosynthetic rate when plants are subjected to high temperature and moderate drought are chlorophyll disintegration, a decrease in photosystem II activity, and the inhibition of RuBP carboxylase activity, which makes the Ci concentration increase stomatal limitation [16,17]. In the present study, the *Ci* concentration did not increase in the N1 and N2 treatment groups at the MD and LD stages, and the Pn of the N1 treatment group increased by 63.10% at the SD stage compared to the control treatment group. It has been suggested that Ca<sup>2+</sup> can both enhance the activity of enzymes on plant cell membranes and improve the efficiency of carbon dioxide carboxylation in chloroplasts as well as enhance the agility of the stomatal response and self-regulation, which makes the leaves maintain a relatively higher water content and be conducive to the enhancement of photosynthesis [18,19]. This suggests that spraying the appropriate concentration of exogenous calcium can delay the transition of photosynthesis from stomatal limitation to non-stomatal limitation and thus enhance drought resistance in plants.

# 4.2. Effect of Exogenous Calcium on Endogenous Hormone Content in C. oleifera under Drought Stress

The plant growth hormone (IAA) is mainly synthesized in the apical meristematic tissue in plants, possessing the ability to regulate plant resistance to abiotic stress. Changes in endogenous plant hormone IAA content under drought stress are complex and differ among plants under drought conditions. Studies have shown that the IAA content in the leaves of tall fescue seedlings increases with drought stress enhancement [20]. In the present study, the IAA content in the leaves of *C. oleifera* 'Xianglin 210' decreased with the extension of drought stress time, which is consistent with the changes in the IAA content in Pinus massoniana seedlings [21] and *Picea asperata* seedlings [22] under drought stress.

ABA, also known as the "stress hormone", can act as a signaling molecule to induce stomatal closure, thereby enhancing drought tolerance in plants [23,24]. In the present study, the ABA content in *C. oleifera* plants increased with the increase in drought stress, which was conducive to the stomatal closure of *C. oleifera* leaves, thus reducing water transpiration and the degree of cell membrane damage and ultimately improving drought resistance and water retention in *C. oleifera* plants.

Gibberellin (GA) can change the use of soil moisture by plants, promote the closure of plant stomata, reduce plant transpiration, and promote elongation and growth in plant cells [25]. In this study, the leaf GA content in *C. oleifera* plants was significantly higher in the MD-to-SD stage compared with the control treatment group, which was consistent with the changes in GA content in *Festuca elata* and *Ulmus pumila* under drought stress [20,25].

After spraying exogenous calcium, compared with the control group, IAA decreased with the prolongation of drought time, and the contents of ABA and GA were significantly increased. Such findings might be related to the fact that Ca<sup>2+</sup> ions are the secondary messengers of cellular signal transduction, and calcium binds to calmodulin (CaM) to transmit external drought stress signals. It is also known that calcium is involved in the response and expression of signals. *C. oleifera* plants reduce water transpiration to improve their drought resistance through increases in the contents of ABA and GA in order to induce stomatal closure and reduce water transpiration [26,27], which suggests that foliar calcium spraying can reduce the damage caused by drought stress on *C. oleifera*.

### 4.3. Effect of Exogenous Calcium on Biochemical Indicators in C. oleifera under Drought Stress

MDA is one of the products of membrane lipid peroxidation in plants under high temperatures and drought [28]. In the present study, the MDA content increased with the drought stress. Especially in the MD stage, the MDA content in the control treatment group increased sharply, which indicated an imbalance between the production and scavenging of reactive oxygen radicals, causing the oxidation of the cell membrane. However, the MDA contents in the calcium-sprayed treatment groups were significantly reduced compared with the control group. Because calcium is an important component of plant cell membranes and a stabilizer of biological membranes, calcium cross-links with the polar heads of membrane phospholipids and enhances the binding of membrane proteins to phospholipids, which reduces the permeability of the cell membrane, thus reducing the MDA content in *C. oleifera* plants under drought conditions [29]. This is consistent with our results, wherein exogenous calcium effectively slowed the membrane lipid peroxidation process.

SOD has the function of scavenging superoxide radicals to protect plants from adversity. Firstly [30], SOD converts superoxide radicals into hydrogen peroxide ( $H_2O_2$ ), and then, catalase (CAT) and peroxidase (POD) decompose  $H_2O_2$  into water [31], which in turn mitigates the damage caused by drought stress to plants. Consistent with the results of previous studies [30], SOD activity showed a rising post-trend from the CS stage to the MD stage in this study. However, in the SD stage, SOD activity decreased, possibly due to the overproduction of ROS in C. oleifera plants under drought stress. Within a certain concentration range of ROS, C. oleifera can effectively alleviate the membrane lipid peroxidation caused by superoxide radicals by increasing SOD activity to scavenge the free radicals; however, when the ROS level exceeds the antioxidative capacity of the plant, the activity of SOD will decrease, leading to the accumulation of ROS, which results in oxidative damage to plant tissues. Calcium is involved in most cell signaling processes and interacts strongly with reactive oxygen species (ROS) [9]. In this study, the application of exogenous calcium to the leaves of C. oleifera plants significantly enhanced SOD activity, which was consistent with previous findings involving *H. rhamnoides* [11]. It is likely that after binding to receptors, such as the ligand-modulated CaM receptor, Ca<sup>2+</sup> ions reduced the accumulation of ROS in the bodies of the *C. oleifera* plants through the amplification and transmission of signals and, at the same time, enhanced the activity of antioxidant enzymes, which indicated that spraying exogenous calcium significantly enhanced drought resistance in the *C. oleifera* plants.

### 4.4. Effect of Exogenous Calcium on C. oleifera Fruits under Drought Stress

Fruit cracking is the phenomenon of the early cracking of fruits before maturity. In *C. oleifera*, fruit growth surges in July–August. There are many reasons for fruit cracking such as high temperature, drought, and other adverse climatic conditions. In this study, it was found that the number of cracked fruits in the control group increased sharply in the SD stage, contrary to the lower number of cracked fruits in the LD stage, which indicated that the cracking of *C. oleifera* fruits was positively correlated with the moisture content of the soil and in the *C. oleifera* plant; the lower the soil moisture content was, the more prone to cracking the *C. oleifera* fruits were. The difference between the number of cracked fruits in the N1 treatment group and the control group was significant. This may have been because the exogenous calcium raised the content of protopectin in the *C. oleifera* fruit, thereby delaying or preventing the degradation of pectin into soluble pectin, which made the fruits less likely to crack [32,33].

According to previous studies, July–September is the critical period for fruit expansion and the growth of oils and fats in *C. oleifera*, and drought stress will limit the growth of *C. oleifera* fruits and oil conversion, resulting in low oil content and poor quality [34,35]. In this experiment, the highest oil content in seed kernels was observed in the N1 treatment group, with a significant difference from what was found in the control group. This observation may be attributed to the fact that calcium ions increased the enzyme related to triglyceride synthesis activity, slowing down the disintegration of liposomes that synthesize and store fats and thus promoting the synthesis and operation of oils [36]. This suggests that exogenous calcium sprayed under drought stress can significantly increase the oil content.

Fruit stalk tensile strength refers to the degree of the difficulty of fruit shedding from the stalk; the lower the stalk tensile strength is, the easier it is for the fruits to fall off. The formation of the stalk off-zone is the main cause of fruit shedding. In this experiment, compared to the control group, the stalk tensile strength in the N1 treatment group was significantly higher, and the stalk tensile strengths of the N2 and N3 treatment groups were slightly higher. The reason might be that spraying calcium solution inhibited the formation of a fruit off-zone and increased the content of calcium pectinate in the fruit stalks, thus increasing the fruit stalk pulling force [37].

### 5. Conclusions

The results of this experiment showed that under drought stress, the leaf-spraying of CaCl<sub>2</sub> solution on *C. oleifera* plants significantly increased organ and tissue water content, enhanced SOD activity, reduced MDA content, increased photosynthetic efficiency, and increased the IAA, ABA, and GA endogenous hormone contents in *C. oleifera* plants, as well as reduced the number of cracked fruits and increased the stipe pulling force and oil content in seed kernels. During *C. oleifera* production, the damage caused by drought stress can be reduced by leaf-spraying with CaCl<sub>2</sub> solution, in which the treatment of leaf-blade-spraying with 10 mmol/L CaCl<sub>2</sub> solution is the most effective, followed by that with 20 mmol/L CaCl<sub>2</sub> solution.

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