Effect of drought on photosynthesis, total antioxidant capacity, bioactive component accumulation, and the transcriptome of *Atractylodes lancea*

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Abstract

**Background:** *Atractylodes lancea* (Thunb.) DC, a medicinal herb belonging to the Asteraceae family, often faces severe drought stress during its growth. Until now, there has been no research on the effect of drought stress on the quality formation of *A. lancea*. Therefore, the present study aimed to study the effects of drought stress on *A. lancea* through physical and chemical analysis, and to reveal the related molecular mechanisms via transcriptome analysis.

**Results:** The photosynthesis was markedly inhibited under drought stress. There were alterations to photosynthetic parameters (Pn, Gs, Ci) and chlorophyll fluorescence (Fv/Fm, NPQ), and the chlorophyll content decreased. Twenty genes encoding important regulatory enzymes in light and dark reactions, including the Rubisco gene of the Calvin cycle, were significantly downregulated. After exposure to drought stress for more than 4 days, the activities of four antioxidative enzymes (SOD, POD CAT and APX) began to decrease and continued to decrease with longer stress exposure. Meanwhile, most of the genes encoding antioxidative enzymes were downregulated significantly. The downregulation of 21 genes related to the respiratory electron transport chain indicated that the blocked electron transfer accelerated excessive ROS. The MDA content was significantly elevated. The above data showed that 15 days of drought stress caused serious oxidative damage to *A. lancea*. Drought stress not only reduced the size and dry weight of *A. lancea*, but also lowered the amount of total volatile oil and the content of the main bioactive components. The total volatile oil and atractyloid content decreased slightly, whereas the content of atractylon and β-eudesmol decreased significantly. Moreover, ten significantly downregulated genes encoding sesquiterpene synthase were mainly expressed in rhizomes.

**Conclusions:** After exposed to drought stress, the process of assimilation was affected by the destruction of photosynthesis; stress tolerance was impaired because of the inhibition of the antioxidative enzyme system; and bioactive component biosynthesis was hindered by the downregulation of sesquiterpene synthase-related gene expression. All these had negative impacts on the quality formation of *A. lancea* under drought stress.

**Keywords:** *Atractylodes lancea* (Thunb.) DC, RNAseq, Photosynthetic rate, Antioxidative enzyme, Sesquiterpene, Gene expression, Quality formation

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been traditionally used as an important crude drug for
the treatment of digestive disorders, rheumatic diseases,
night blindness, and influenza for a long time [2–5].
Besides that, it has been well proved that the active
components from *A. lancea* exert great anti-cancer effect,
especially in treatment of cholangiocarcinoma and gas-
tric cancer [6–9]. Recently, it is interesting to find that *A.
lancea* is frequently used in the treatment of Coronavirus
Disease 2019 (COVID-19) and ranks first among the key
Chinese herbal medicines for national epidemic preven-
tion and control in China [10, 11]. So, there is an increas-
ing demand of *A. lancea* with high quality. The main
production areas in China (Jiangsu, Hubei, and Henan)
have a temperate or subtropical climate, with aridity and
little rain in spring and high temperature in summer;
therefore, *A. lancea* often faces severe drought stress
during its growth [12]. In addition, because of the scarcity
of agricultural irrigation resources [13], artificial irriga-
tion cannot be widely implemented to alleviate drought
stress of *A. lancea*. Therefore, drought has become the
main limiting factor affecting the quality formation of *A.
lancea*.

The quality formation of medicinal plants is directly
or indirectly influenced by the physiological state of
plants [14]. Generally, plants with better physiological
characteristics, such as more efficient photosynthesis
and higher stress tolerance, can form better quality than
plants with poor physiological traits [15, 16].

As the most basic and complex physiological process,
photosynthesis is critical to all green plants. It is well
established that drought stress can considerably influence
plants’ photosynthetic physiology by yellowing leaves,
closing stomata, and weakening photosynthesis [17, 18].
The decreased photosynthetic capacity due to drought
stress not only hampers the plant growth but also is
directly/indirectly associated with the reduction of yield
and quality in medicinal plants. Therefore, photosynthe-
sis is a crucial and widely used physiological indicator to
assess the influence of drought stress on the quality for-
mation of medicinal plants [19]. However, there are still
no reports about the photosynthetic response of *A. lan-
cea* to drought stress. This hinder the understanding of
the influence of drought stress on the quality formation
of *A. lancea*.

It is generally believed that drought stress can induce
excessive production of reactive oxygen species (ROS)
which often disrupt the growth and quality formation
of plants [20]. Antioxidative enzyme in plants is the
major defense system to scavenge ROS for maintain-
ing the normal physiological state of plants [21]. Thus,
the high level of antioxidative enzyme activity is impor-
tant for plants to tolerate stress for keeping good growth
state and forming good quality. Generally, when plants
were subjected to drought stress, antioxidative enzymes’
activities increased initially, but decreased with elevated
drought stress level [22]. Zhou et al. [23] and Dai et al.
[24] reported that short-term drought stress could induce
the enhanced activities of antioxidant enzymes in *A. lan-
cea* seedlings to reduce oxidative damage. This indicated
that drought stress could affect the antioxidative enzyme
activities of *A. lancea*. But, the plant material used in
Zhou’s and Dai’s researches were *A. lancea* seedlings.
So, it is essential to analysis the alteration of antioxidative
enzyme activity in the adulted *A. lancea* exposed
drought stress for understanding of the influence of
drought stress on the quality formation of *A. lancea*.

The oil content of *A. lancea* reaches about 2.02% to
4.06% [25]. The volatile oil, mainly composed of sesqui-
erpenes, is the main active substance of *A. lancea* and has
liver-protective, analgesic, anti-viral, and gastrointestinal
motility activities [2, 26, 27]. High levels of atractylon,
β-eudesmol, and atractyldolin are present in the volta-
tile oil, and their levels are commonly used as indices to
evaluate the quality of *A. lancea* [28]. Sesquiterpenes
are mainly synthesized by the mevalonate (MVA) pathway in
plants [29]. The universal precursor, farnesyl diphosphate
(FPP), can be converted into different sesquiterpene skel-
etons by different sesquiterpene synthases (SSs) [30].
Previous studies showed that drought stress changed
the content of sesquiterpenes in *Ocimum basilicum* L.
by regulating the expression of key genes encoding enzymes
of terpene biosynthesis [31]. Until now, some researches
on the biosynthetic pathway of sesquiterpenes in *A. lan-
cea* have been reported [32, 33], but, the effect of drought
on accumulation and biosynthesis of sesquiterpenes in
*A. lancea* is still unclear. Thus, it is essential to determine
the accumulation of main bioactive component and to
analysis expression level of genes related to sesquiterpene
biosynthesis in *A. lancea* under drought stress. This will
help us to understand the influence of drought stress on
the quality formation of *A. lancea*.

The main research content of the present study was:
1. To establish a transcriptome database of *A. lan-
cea* leaves and rhizomes under normal water manage-
ment and under drought stress using DNA sequencing,
and to verify the accuracy of the data using quantitative
real-time reverse transcription PCR (qRT-PCR); 2. To
study the effects of drought stress on the photosynthetic
physiology of *A. lancea* by measuring chlorophyll con-
tent, photosynthesis parameters, and chlorophyll fluo-
rescence (ChlF), and to analyze the expression levels of
genes encoding important regulatory enzymes in photo-
synthesis; 3. To study the antioxidant ability of *A. lancea*
derunder drought by measuring the activity of antioxidative
enzymes and analyzing the expression levels of genes
associated with antioxidative enzymes and 4. To study
the effect of drought stress on the yield and quality of *A. lancea* by determining the dry weight and the content of bioactive components and analyzing the expression levels of genes related to bioactive components’ biosynthesis. Through the achievement of the above research content, we hope to provide a detailed understanding of the influence of drought stress on the quality formation of *A. lancea*. This will not only benefit for developing management strategy of *A. lancea* to minimize the quality reduction induced by drought, but also contributes to future drought tolerance breeding in *A. lancea*.

**Results**

**Transcriptomic profiles, differentially expressed genes (DEGs) clustering analysis, and qRT-PCR validation**

A total of 351,123,566 high-quality clean reads with a Q20 ≥ 97.86% and a GC base ratio between 44.84% and 46.01% (Table S1) were generated. After assembling the clean reads, 82,677 unigenes and 14,0145 transcripts were obtained (Table S2). The detailed annotation results of the 82,677 unigenes were shown in Fig. 1A, and the top four similar species were *Cynara cardunculus* (51.96%), *Lactuca sativa* (12.31%), *Helianthus annuus* (9.03%), and *Artemisia annua* (8.67%) (Fig. 1B). These four species all belong to the Asteraceae family, as does *A. lancea*.

Using p-adjust < 0.001 and |log2FC| ≥ 1, 30,510 DEGs were obtained between drought treatment (DT) group and control (CK) group. Using cluster analysis, these 30,510 DEGs could be divided into six clusters according to their pattern of gene expression. Cluster 1 contained 4160 DEGs that were downregulated in both leaves and rhizomes. Cluster 2 comprised 2714 genes whose expression was downregulated in leaves, but was not significantly different in rhizomes. Cluster 3 contained 3377 genes that were downregulated in leaves and upregulated in rhizomes. Cluster 4 contained 6452 DEGs whose expression was downregulated in rhizomes but was not significantly different in leaves. Cluster 5 contained 6452 DEGs that were upregulated in leaves and downregulated in rhizomes. Cluster 6 contained 7480 genes that were upregulated genes in both leaves and rhizomes (Fig. 2A).

GO functional enrichment analysis were performed on the DEGs in the six clusters (Fig. 2B). It is interesting that many DEGs in cluster 1 and cluster 5 were enriched in GO terms associated with redox processes, such as "oxidoreductase activity" (GO:0,016,901), "hydrogen peroxide metabolic process" (GO:0,042,743), and "monooxygenase activity" (GO:0,004,497). In addition, DEGs in cluster 3 were mainly enriched in GO terms related to photosynthesis, such as "chloroplast thylakoid membrane" (GO:0,009,535) and "photosynthetic membrane" (GO:0,034,357), and these genes are all downregulated in leaves, suggesting that the photosynthesis of *A. lancea* was inhibited by drought stress.

These 30,510 DEGs were also associated with 133 KEGG pathways (Figure S1), among which 100 pathways belonged to metabolism, accounting for the majority of DEGs. It is worth noting that three pathways were related to photosynthesis and five pathways were associated with terpenoid biosynthesis (Table 1).

The expression levels of five selected genes (*DN15090_c0_g1*, *DN474_c1_g1*, *DN18330_c1_g1*, *DN47389_c0_g1*, and *DN54795_c0_g2*) calculated using qRT-PCR were listed in Table S3. The gene expression trends were
consistent with those in the transcriptome sequencing data, confirming that the transcriptome-based DEG results were reliable to identify drought-responsive genes in the present study.

Changes in the photosynthesis system and associated gene expression analysis

Under drought stress, all of the measured photosynthesis parameters of *A. lancea* showed obvious alterations. As the duration of drought stress treatment increased, the chlorophyll (CHL) content, net photosynthesis (Pn) and stomatal conductance (Gs) of *A. lancea* all showed continuous downward trends (Fig. 3A-3C). On the 15th of treatment, the values of CHL, Pn and Gs in the DT group were significant smaller than those in the CK group. The intercellular carbon dioxide concentration (Ci) of *A. lancea* started to decrease within 4 days of drought treatment and then began to increase from the 5th day of drought stress treatment, leading to a higher final Ci in the DT group (Fig. 3D). As for chlorophyll fluorescence, drought stress caused a significant reduction in the potential yield of the photochemical reaction of PsII (Fv/Fm) and the longer the drought stress, the greater the reduction (Fig. 3E). The value of non-photochemical quenching (NPQ) also altered significantly as the prolonged drought treatment, increased on the first 4 days, and then decreased from the 5th day (Fig. 3F).

To gain a deeper understanding of the mechanisms underlying the reduced photosynthesis of *A. lancea* under drought stress, 31 DEGs encoding key enzymes participating in the light and dark reactions of photosynthesis were identified in leaves. Among these 31 DEGs, 11 were upregulated and 20 were downregulated. In total, among seven DEGs involved in the light reaction (Fig. 4A), *DN31152_c0_g2* (F-type H+/Na+ ATP synthase gene) and *DN1739_c0_g1* (photosystem I P700 apoprotein A1gene) were upregulated. The other five downregulated DEGs associated with the light reaction were *DN21336_c0_g1* (photosystem I P700 apoprotein A1 gene), *DN19968_c0_g1* and *DN32471_c0_g1* (F-type H+/Na+ ATP synthase gene), *DN18889_c0_g1*

![Fig. 2](image_url)
and DN60086_c0_g1 (photosystem II reaction center W protein gene). Twenty-four DEGs were involved in dark reaction, among which 9 DEGs were upregulated and 15 were downregulated (Fig. 4B). The upregulated 9 DEGs included five malate dehydrogenase genes (DN7011_c0_g1, DN18398_c0_g1, DN48988_c0_g1, DN69445_c0_g1 and DN39038_c0_g1), a glyceraldehyde-3-phosphate dehydrogenase gene (DN1992_c4_g1), a ribose-5-phosphate isomerase gene (DN1163_c0_g2), a phosphoglycerate kinase gene (DN34957_c0_g1) and a pyruvate, phosphate dikinase gene (DN23841_c0_g1). The 15 downregulated DEGs included six malate dehydrogenase genes (DN1618_c0_g2, DN45011_c0_g1, DN3381_c1_g1, DN16280_c0_g1, DN61455_c1_g1, and DN17392_c0_g1), two triosephosphate isomerase genes (DN7162_c3_g1 and DN24632_c0_g2), a ribulose bisphosphate carboxylase gene (DN1343_c0_g2), a glyceraldehyde-3-phosphate dehydrogenase gene (DN4492_c0_g3), an alanine aminotransferase gene (DN14431_c0_g1), a pyruvate, phosphate dikinase gene (DN4030_c0_g2), a phosphoglycerate kinase gene (DN9649_c0_g1), a phosphoenolpyruvate carboxykinase gene (DN3731_c0_g1), and a fructose-1,6-bisphosphatase gene (DN39571_c0_g2).

Changes in the protective enzymes system and associated gene expression analysis

The activities of SOD, POD, CAT and APX were higher in the DT group than those in the CK group during the early stage of drought stress. For SOD and POD, the early stage of drought stress was 0 to 8 days. For CAT and APX, it was 0 to 4 days. After the early stage, the activities of these enzymes began to decrease. On the 15th day of drought stress, the activity of the four antioxidative enzymes were all different with those in the CK group. The activities of SOD and APX in the DT group were significantly lower than those in the CK group. The POD activity was significantly higher than that in the CK group and CAT activity is slightly (non-significantly) lower than that in the CK group (Fig. 5A-5D). In addition, the amount of MDA increased rapidly in A. lancea under drought stress, while there was no change in MDA levels in the CK group (Fig. 5E). The relative conductivity showed a similar result to the MDA content (Fig. 5F).

The detailed analysis of transcriptome of A. lancea under normal water management and under drought stress provided a large number of DEGs encoding antioxidative enzymes. There were five SOD genes (DN3525_c0_g1, DN19179_c0_g3, DN19917_c0_g1, DN474_c1_g1, and DN53562_c0_g1) and ten CAT genes (DN5539_c0_g4, DN42828_c0_g1, DN3870_c0_g1, DN49828_c0_g1, DN9135_c1_g1, DN5359_c0_g1, DN3848_c0_g1, DN7441_c0_g1, DN25688_c0_g1, and DN3848_c0_g2). These 15 DEGs were all downregulated under drought stress. In addition, 18 POD genes were obtained, including six upregulated DEGs (DN21191_c0_g1, DN4672_c0_g2, DN51705_c0_g1, DN35661_c0_g1, DN2622_c1_g1, and DN51705_c0_g1) and 12 downregulated DEGs (DN38_c0_g1, DN68449_c0_g1, DN7681_c1_g1, DN75393_c0_g2, DN9309_c0_g2, DN9309_c0_g2).
Fig. 4 DEGs involved in light reaction (A) and dark reaction (B) of photosynthesis
DN40830_c0_g1, DN52474_c0_g4, DN42408_c0_g1, DN61971_c0_g1, DN45395_c0_g1, DN19722_c0_g2, and DN15530_c0_g3).

It has been reported that ROS can act as second messengers to transduce the signal to transcription factors, thus in turn activate gene expression of antioxidative enzymes. So, we also paid attention to DEGs associated with ROS production and found that 21 downregulated DEGs were related to the respiratory electron transport chain under drought stress (Fig. 6). The 21 DEGs comprised four NADH:ubiquinone oxidoreductase genes (DN215_c1_g1, DN13447_c0_g2, DN5922_c0_g2, and DN30220_c0_g1), two cytochrome-c oxidase genes (DN7184_c0_g1 and DN26312_c0_g1), and 15

Fig. 5  SOD (A), POD (B), CAT (C), APX (D) activities, MDA content (E) and relative conductivity (F) of A. lancea under drought stress. The values are presented as the means±SD. One asterisk and two asterisks indicate significantly different values in DT compared to CK at p≤0.05 and p≤0.01, respectively.

Fig. 6  The hypothetical regulatory network about the effect of drought stress on the oxidative enzyme activities of A. lancea.
ATP synthase genes (DN7129_c0_g1, DN1539_c0_g1, DN1467_c0_g1, DN19968_c0_g1, DN5265_c0_g1, DN14586_c0_g1, DN4776_c0_g1, DN32471_c0_g1, DN5303_c1_g2, DN2129_c1_g1, DN2238_c0_g2, DN1584_c1_g3, DN17811_c0_g1, DN27345_c0_g1 and DN77850_c0_g1).

Changes in bioactive component contents and associated gene expression analysis

The rhizomes of *A. lancea*, sampled after 15 days of drought stress treatment, were used as the experimental materials for quality assessment. Morphologically, the rhizomes of *A. lancea* from the DT group were smaller than those from the CK group (Fig. 7A). Moreover, drought stress treatment led to a reduction in the dry weight of *A. lancea* rhizomes. The average dry weight of the rhizomes from the DT group was 7.85 ± 2.01 g, while the average dry weight of the rhizomes from the CK group was 14.23 ± 4.16 g. The proportion of essential oil also showed difference between the two groups. The essential oil content of *A. lancea* in the CK and DT group was 0.111 ml/g and 0.096 ml/g, respectively. In addition, the content of three bioactive components (attractylon, β-eudesmol, and atractylodin) all showed decreasing trends in *A. lancea* in the DT group compared with that in the CK group (Fig. 7B). Importantly, the amount of attractylon decreased significantly (P < 0.05). Notably, a sharp reduction (P < 0.01) was observed in the content of β-eudesmol.

We next turned our attention to DEGs associated with “terpenoid backbone biosynthesis” (map00900) and “sesquiterpenoid and triterpenoid biosynthesis” (map00909) and identified 18 DEGs associated with sesquiterpenoid biosynthesis. Among them, eight DEGs were related to the MVA pathway, including two hydroxymethylglutaryl-CoA synthase genes (DN8392_c0_g1 and DN2865_c0_g3), four hydroxymethylglutaryl-CoA reductase genes (DN48911_c0_g2, DN1889_c2_g2, DN86031_c0_g1, and DN16497_c0_g1), and two diphosphomevalonate decarboxylase genes (DN79187_c0_g1 and DN23531_c0_g1). Under drought stress, some of these 8 genes were upregulated and some were downregulated, i.e., they did not show a consistent pattern. In addition, 10 DEGs associated with sesquiterpene synthase were obtained. Among them, seven (DN56449_c0_g2, DN2689_c0_g2, DN1597_c0_g1, DN54795_c0_g2, DN18787_c0_g2, DN1159_c0_g1, and DN3448_c0_g2) were significantly downregulated (Fig. 8) and three (DN18629_c0_g2, DN18787_c0_g1, and

**Fig. 7** Morphology, dry weight (A) and bioactive component content (B) of *A. lancea* under drought stress. The determination of each component was performed for three times. One asterisk represents a significant difference of p < 0.05 and two asterisks represent a significant difference of p ≤ 0.01.

**Fig. 8** The effect of drought stress on the biosynthesis pathway of sesquiterpene in *A. lancea*
DN85030_c0_g1) were upregulated. These sesquiterpene synthase genes were mainly differentially expressed in rhizomes (n = 8) with only two expressed in leaves.

Discussion
The quality formation of medicinal plant is an extreme complex process which was mainly determined by two aspects: heredity and environment [34]. Certainly, suitable environment is quite important for the quality formation of medicinal plants [35]. However, with the deterioration of the global climate, encountering drought stresses has been more common and frequent during medicinal plants’ growth. Thus, drought has become a crucial limiting factor that influence the quality formation of medicinal plants [36, 37]. In present study, the effect of drought stress on the quality formation of A. lancea was studied by analyzing photosynthesis, antioxidative enzyme activity and bioactive component accumulation.

Photosynthesis is particularly sensitive to drought stress and often is inhibited because of stomata closure induced reduced CO₂ supply and further metabolic impairment by drought stress [38, 39]. Generally, the total dry matter production of a plant is equal to its net result was consistent with previous studies [41, 42]. The obvious photosynthesis inhibition in A. lancea was studied by analyzing photosynthesis, antioxidative enzyme activity and bioactive component accumulation.

In present study, the significant decrease of Pn in A. lancea from the DT group confirmed that drought stress lasted for 15 days caused obvious photosynthesis inhibition in A. lancea. This result was consistent with previous studies [41, 42]. The continuous declined Gs indicated that limitations of CO₂ uptake caused by stomata closure was responsible for photosynthesis inhibition [43]. With prolonged drought stress, Ci began to increase, suggesting that metabolic impairment induced by drought became another main limiting factor. Rubisco (ribulose bisphosphate carboxylase) is the key enzyme participated in the first stage of the Calvin cycle by catalyzing ribulose-1,5-bisphosphate and CO₂ to form two molecules of 3 phosphoglyceric acid (3-PGA) [44]. In the present study, a Rubisco gene (DN1343_c0_g2) was significantly downregulated under drought stress, indicating that drought stress caused metabolic impairment in A. lancea by inhibiting the carboxylation reaction of CO₂. The rate of photosynthesis also depends on the regeneration of RuBP [45]. Fructose-1,6-bisphosphatase (FBP) and triosephosphate isomerase (TPI) can catalyze glyceraldehyde-3-phosphate (GAP) through a series of transformations to reform RuBP [46, 47]. A FBP gene (DN39571_c0_g2) and two TPI genes (DN7162_c3_g1 and DN24632_c0_g2) were significantly downregulated, indicated that drought stress could also cause metabolic impairment in A. lancea by hindering the regeneration of RuBP.

The Fv/Fm ration represent the potential yield of the photochemical center of photosystem II (PSII) [48]. The value of Fv/Fm ration has been reported to stay around 0.83 among many different plant species under normal growth conditions [49]. When the plant was exposed to drought stress, Fv/Fm is particularly sensitive and its value decreased greatly [50]. Thus, Fv/Fm has already become an important parameter to judge the physiologi cal state of plants under drought stress. In present study, the Fv/Fm value of A. lancea in the CK group was kept at a relative stable level, while the Fv/Fm in the DT group showed a significantly reduction. This demonstrated that the efficiency of light energy conversion and electron transfer activities were all markedly inhibited under drought stress. The characteristic decrease of Fv/Fm induced by drought stress was consistent with previous studies [51, 52]. The reduction in Fv/Fm is often associated with an increase in the efficiency of non-photochemical quenching (NPQ) [53]. Under drought stress, the significantly increased NPQ showed that excessive irradiation could be dissipated into heat to protect A. lancea from drought-induced damage. The observed reduction in efficiency of PSII was concomitant with the down-regulation of genes belonging to the PSII category induced by drought stress, evidencing that the reduced efficiency of PSII was the other main limiting factor of photosynthesis inhibition in A. lancea under drought stress.

Combined with the lower dry weight of A. lancea under drought stress, we inferred that drought inhibited photosynthesis of A. lancea by closing stomata, reducing the CHL content, limiting the efficiency of PSII and inducing metabolic impairment, which led to a yield decline, thereby affecting the quality formation of A. lancea.

Drought stress inevitably causes excessive production of ROS, which can oxidize biofilms, form lipid peroxidation products, and damage cells [54]. Antioxidative enzymes form the first line of defense against ROS and have been reported to contribute directly or indirectly in drought tolerance of many plants, such as rice [55], alfalfa [56] and canola [57]. Adebayo (2015) stated that sustained yields in maize under drought stress were directly related to better antioxidant activities [58]. In present study, the activities of SOD, POD, CAT and APX were higher in the DT group than those in the CK group during the early stage of drought stress. This was consistent with Zhou’s and Dai’s researches. This implied that during the early stage of drought stress, ROS acted as second messengers involved in the stress signal transduction pathway to activate antioxidative enzyme activity. Like other plants, the induction of antioxidant enzyme activity
is an adaptation strategy which A. lancea use to overcome oxidative stresses. The balance between ROS production and activities of antioxidative enzymes determined whether oxidative signaling and/or damage would occur [59]. As the duration of drought treatment increased, the activities of all four antioxidative enzymes decreased. On the 15th day, except for POD, the activities of SOD, CAT and APX were lower compared with those in the CK group. And gene expression analysis identified 35 antioxidative enzyme genes and 26 of them were significantly downregulated under 15 days’ drought stress. This may reflect the low ROS scavenging capacity and increased damage in A. lancea under 15 days’ drought stress. The decrease of antioxidative enzyme activity under progressive drought stress also has been reported by many previous studies. In addition, 21 significantly downregulated genes related to the respiratory electron transport chain in A. lancea under drought stress, suggested that the blocked electron transport would accelerate the accumulation of ROS [60]. So, it could be concluded that the level of ROS induced by 15 days of drought stress in A. lancea was beyond the adjustable range of the plant itself, and the antioxidative enzyme system could no longer remove the excessive ROS. The markedly elevated level of MDA and MDA relative conductivity under drought stress also evidenced the severe oxidative damage to A. lancea caused by drought stress. Taken together, our data suggested that the significant decline of antioxidative enzyme activity was an important factor affecting A. lancea’s stress tolerance, thereby affecting the quality formation of A. lancea.

The amount of volatile oil and the content of atractylon, β-eudesmol, and atractylopin decreased in A. lancea under 15 days’ drought stress. This may reflect the low ROS scavenging capacity and increased damage in A. lancea under15 days’ drought stress. The decrease of antioxidative enzyme activity under progressive drought stress also has been reported by many previous studies. In addition, 21 significantly downregulated genes related to the respiratory electron transport chain in A. lancea under drought stress, suggested that the blocked electron transport would accelerate the accumulation of ROS [60]. So, it could be concluded that the level of ROS induced by 15 days of drought stress in A. lancea was beyond the adjustable range of the plant itself, and the antioxidative enzyme system could no longer remove the excessive ROS. The markedly elevated level of MDA and MDA relative conductivity under drought stress also evidenced the severe oxidative damage to A. lancea caused by drought stress. Taken together, our data suggested that the significant decline of antioxidative enzyme activity was an important factor affecting A. lancea’s stress tolerance, thereby affecting the quality formation of A. lancea.

Conclusions
Drought stress affected the quality formation of A. lancea from the following three aspects. First, genes encoding important regulatory enzymes in dark reaction were downregulated significantly, the efficiency of PSII was limited, thus, photosynthesis was significantly inhibited. Second, gene expression and activities of antioxidative enzymes were mostly downregulated, thus, the stress tolerance of A. lancea was impaired. Third, gene expression of key enzymes involved in sesquiterpene biosynthesis were down-regulated, thus, the accumulation of bioactive components was slowed down in A. lancea.

Methods
Plant materials and drought stress treatment
A. lancea was obtained from Maoshan (N31°79’14.98", E119°32’19.56"), Jurong, Jiangsu Province, China and identified as Atractylodes lancea (Thunb.) DC by Professor Wei Gu (School of Pharmacy, Nanjing University of Chinese Medicine, China). The voucher was deposited in Department of Chinese Medicine Resources, Nanjing University of Chinese Medicine with ID MCZ-19–001. The A. lancea used in the present study were artificially cultivated, so, there was no permission needed for the collection of A. lancea. Then the plants were transplanted in the Medicinal Botanical Garden, Nanjing University of Traditional Chinese Medicine (NUTCM). Five A. lancea plants were in a plastic pot (top diameter 38 cm, bottom diameter 32 cm, height 35 cm) with approximately 20 kg dry soil. The potted plants were grown in a greenhouse with 25 °C, 60% relative humidity and 14 h of light and 10 h of darkness. Soil taken from the Medicinal Botanical Garden of NUTCM was air-dried naturally and then passed through a 5 mm sieve. The experimental soil was prepared by mixing the air dried soil and farmyard manure evenly in a ratio of 4:1. The detailed information of the prepared experimental soil was as followed: texture, loam; organic matter, 12.74 g/kg; total nitrogen, 2.12 g/kg; total phosphorus, 3.57 g/kg; total potassium, 12.53 g/kg; alkalihydro nitrogen, 63.38 mg/kg; available phosphorus, 20.53 mg/kg; available potassium, 49.86 mg/kg; pH, 6.52. After adaption for two weeks, sixty A. lancea plants with similar growth rates were randomly divided into two groups: CK group and DT group. Soil water content was measured using a TDS-IIW Soil Moisture Meter (Zhejiang Top Instrument Co., Ltd, Zhejiang, China). The water content was kept at 25% to 35% of saturated soil water content in the CK group and the DT group for 15 days, respectively. At 0, 2, 4, 8, and 15 days of the stress treatment, leaves of the two groups were collected and divided into two parts. One part was used for physiological index measurements and
the other part was immediately frozen in liquid nitrogen for RNA extraction. In addition, the rhizomes of the two groups were collected at 0, 2, 4, 8, and 15 days and were also divided into two parts. One half of each sample was frozen in liquid nitrogen to be used for RNA extraction, and the other half was oven-dried at 40 °C to a constant weight for quality analysis.

**Illumina sequencing and functional annotation**

Leaves and rhizomes of the two groups collected at 15 days were used to isolate total RNA using an RNeasy Plus Mini Kit (#74,134; Qiagen, Hilden, Germany). The cDNA was synthesized using the fragmented mRNA as a template. PCR amplification was then performed, and then high-throughput sequencing was performed on an Illumina HiSeq TM 4000 instrument by Shanghai Megi Biomedical Technology Co., Ltd (Shanghai, China). Fastx_toolkit_0.0.14 (http://hannonlab.cshl.edu/fastx_toolkit/) was used for quality assessment of the raw sequencing data. After obtaining high-quality clean reads, Trinity_v2.8.5 (https://github.com/trinityrnaseq/trinityrnaseq) was used to assemble the clean reads into transcript sequences, and TransRate (http://hiberlab.com/ transrate) was applied to filter the transcripts. The optimized transcript sequences were stored as FASTQ files under the project accession numbers (SRR8699030, SRR8699031, SRR8699032, and SRR8699033) in the Short Read Archive database of the NCBI.

Gene annotation information was obtained by BlastX searching in the NCBI non-redundant protein database (NR, http://www.ncbi.nlm.nih.gov), and by analysis using the following database resources: Cluster of Orthologous Groups (COG), Swissprot (http://www.expasy.ch/sprot), Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg), Pfam (http://pfam.xfam.org/), and Gene Ontology (GO, http://www.geneontology.org). The E-value was set to 1E−5.

**Gene expression analysis and qRT-PCR validation**

Gene expression was calculated using RSEM (http:// deweylab.github.io/RSEM/) and normalized by the FPKM ( Fragments per Kilobases per Million reads) value. EdgeR_3.24.3 (http://bioconductor.org/packages/stats/bioc/edgeR/) were used to obtain DEGs, and Bonferroni one-step correction was used as a multiple test correction method, and p-adjust<0.05 and |log2fold-change (FC)|≥2 were used as criteria. Fisher’s exact test was used for DEG enrichment analysis. When the false discovery rate (FDR) was <0.05, the GO function item or the KEGG pathway was considered to be significantly enriched for the DEG.

Expression levels of five randomly selected genes (DN15090_c0_g1, DN474_c1_g1, DN18330_c1_g1, DN47389_c0_g1, and DN54795_c0_g2) were analyzed to validate the accuracy of transcription sequencing results. The qRT-PCR reaction was performed according to the Platinum® SYBR® Green qPCR Supermix UDG real-time PCR kit instructions (Thermo Fisher Scientific, Waltham, MA, USA). The qPCR reaction comprised: Pre-denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 62 °C for 30 s, and extension at 72 °C for 40 s. EF1A1 (encoding EF-1α) was used as an internal reference gene, and the 2−ΔΔCt method was used to calculate the relative expression level of a gene. The experiment was performed three times, and each sample was assessed three times. The specific qPCR primers were designed using Primer Premier 5.0 software (PREMIER Biosoft International, San Francisco, CA, USA) and were supplied by Generay Biotech Co., Ltd. (Shanghai, Chins; Table S4).

**Photosynthesis measurement**

An LI-6400XT photosynthesis-fluorescence measurement system was applied to measure photosynthesis and chlorophyll fluorescence parameters. The determination of net photosynthesis (Pn), stomatal conductance (Gs), and the intercellular carbon dioxide concentration (Ci) were carried out between 9:00 and 11:00 in the morning. After being subjected to dark adaptation treatment for 2 h from 12 noon, the plants were subjected to photodynamic efficiency measurement to obtain the Fv/Fm (the ratio of variable to maximum fluorescence). Then, after 1 h of light activation, non-photochemical quenching (NPQ) was measured. The content of CHL was determined using Arnon’s method [61].

**Protective enzyme activity**

The content of MDA was measured using the thiobarbituric acid method [62]. A conductivity meter, BDS-6300 (Shanghai Yidian Scientific Instrument Co., Ltd. Shanghai, China), was used to determine the relative conductivity. The riboflavin-trinitrotoluene chloride (NBT) method and guaiacol method were applied to detect SOD and POD activity [63]. The activities of CAT and APX were determined using a UV spectrophotometer UV 5200 (Shanghai Metash Instruments Co., Ltd. Shanghai, China) at 240 nm and 290 nm, respectively [62].

**Determination of bioactive components**

Essential oil was collected by steam distillation according to the volatile oil extraction method in the 2020 edition of the Chinese Pharmacopoeia [64].

A Waters e2695 series high performance liquid chromatography (HPLC) system (Waters Technology (Shanghai) Co. Ltd., Shanghai, China) was employed to analyze the samples. A Welchrome C18 analytical column
(250 mm × 4.6 mm, 5 mm; Shanghai Welch Technology Co. Ltd., Shanghai, China) was used and the mobile phase was acetonitrile (A) and 0.1% v/v formic acid solution (B) at a flow rate of 1 mL min⁻¹. The gradient was as follows: 0–5 min, 10% A, 5–6 min, 14% A, 6–22 min, 30% A, 22–27 min, 52% A, 27–55 min, 80% A, 55–65 min, 100% A. The column temperature was 30 °C and the injection volume was 10 μL. The detection wavelength was 340 nm for atractyloside and 203 nm for atractyloside and β-eudesmol, respectively. The reference standards of atractyloside, atractylon, and β-eudesmol were purchased from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China).

Four hundred milligrams of rhizome sample powder and 10 mL of methanol were placed together in a brown triangular bottle and weighted precisely. After ultrasonic extraction for 40 min and cooling to room temperature, methanol was added to the mixture to make up for weight loss. Then, the supernatant was passed through a 0.45-μm micro-porous membrane to prepare the sample solution for HPLC analysis.

Statistical analysis
All data were assessed for significant differences (P < 0.05) using one-way analysis of variance (ANOVA) with Bonferroni correction using SPSS 20.0 software (IBM Corp., Armonk, NY, USA). Each experiment was performed three times independently and all data were presented as the mean and standard deviation (SD). The GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) was used to construct graphs.

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Not applicable.

Authors' contributions
AZ and ML conducted the experiments, performed the data analysis, and wrote the manuscript; ZC, YG, LP, and RT assisted in the data analysis; WG supervised the experiment, and led the data analysis and writing of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The data was accessible in NCBI with the direct link and login number: https://www.ncbi.nlm.nih.gov/sra/?term=SRR8699031, SRR8699032. https://www.ncbi.nlm.nih.gov/sra/?term=SRR8699033.

Declarations
Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare that they have no conflicts of interest.

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References
1. Zhou JY, et al. Volatiles released by endophytic Pseudomonas fluorescens promoting the growth and volatile oil accumulation in Atractylodes lancea. Plant Physiol Biochem. 2016;101:132–40.
2. Yu M, et al. Atractylodes lancea volatile oils attenuated helicobacter pylori NCTC11637 growth and biofilm. Microb Pathog. 2019;135:103641.
3. Liu R, et al. The Suppressive Effects of the Petroleum Ether Fraction from Atractylodes lancea (Thunb.) DC. On a Collagen-Induced Arthritis Model. Physiother Res. 2016;30(10):1672–9.
4. Wei Y, et al. Atractylodes lancea rhizome water extract reduces triptolide-induced toxicity and enhances anti-inflammatory effects. Chin J Nat Med. 2017;15(12):905–11.
5. Zhang WJ, et al. Atractylodis Rhizoma: A review of its traditional uses, phytochemistry, pharmacology, toxicology and quality control. J Ethnopharmacol. 2020,266:113415.
6. Mathema VB, Chajaroenkul W, Na-Bangchang K. Cytotoxic activity and molecular targets of atractyloside in cholangiocarcinoma cells. J Pharm Pharmacol. 2019;71(2):185–95.
7. Na-Bangchang K, Plengsuriyakarn T, Karbwang J. Research and Development of Atractylodes lancea (Thunb.) DC as a Promising Candidate for Cholangiocarcinoma Chemotherapeutics. Evid Based Complement Alternat Med. 2017;2017:5929234.

Supplementary Information
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Additional file 1: Table S1. Summary of RNA-Seq database from A.lancea under drought stress. Table S2. The detailed information for assembled unigenes of A. lancea under drought stress. Table S3. qRT-PCR validation of DEGs from A. lancea. Table S4. The primer list of DEGs for qRT-PCR validation. Figure S1. Functional classification for assembled unigenes of A. lancea by KEGG.
8. Zhao M, et al. Selective fraction of Atractylodes lancea (Thunb.) DC. and its growth inhibitory effect on human gastric cancer cells. Cytotecnol. 2014;66(2):201–8.

9. Bai Y, et al. Atractylodin Induces Myosin Light Chain Phosphorylation. 2021;2:186798.

10. Yang LP et al. Network pharmacology study of ephedra herba-atactylodis rhizoma in the treatment of novel coronavirus pneumonia. J Chinese Med Materials. 2020;43(7):1784–90.

11. Ma LJ et al. Prescription rules and mechanisms of herbal prevention prescriptions for COVID-19 by data mining and network pharmacology. Modernization of Traditional Chinese Medicine and Materia Materia-World Science and Technology. 2020;22(3):566–72.

12. Li MY et al. Effects of high temperature stress on chlorophyll fluorescence characteristics of Atractylodes lancea from different habitats. Plant Physiology. Journal. 2015;51(11):1861–6.

13. D’Odorico P et al. The global value of water in agriculture. Proc Natl Acad Sci U S A. 2020;117(36):21985–93.

14. Habermann E et al. How does leaf physiological acclimation impact for age production and quality of a warmed managed pasture of Stylasonthes capitata under different conditions of soil water availability? Sci Total Environment. 2021;759:143505.

15. He J et al. Changes in the photosynthesis properties and photo-biochemical processes as affected by growth CO2 and phosphorus nutrition in cotton. J Plant Physiol. 2013;170(9):3473–86.

16. Huang Q, et al. Differential Gene Expression between Leaf and Rhizome in Atractylodes lancea: A Comparative Transcriptome Analysis. Front Plant Sci. 2016;7:548.

17. Ahmed S, et al. The Transcript Profile of a Traditional Chinese Medicine. Atractylodes lancea, Revealing Its Sesquiterpenoid Biosynthesis of the Major Active Components. PLoS ONE. 2016;11(3):e0151795.

18. Huang L, et al. Top-geophers of traditional Chinese medicine: common traits, quality characteristics and formation. Front Med. 2017;11(52):185–94.

19. Li Y, et al. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiol Biochem. 2020;148:80–9.

20. Omidi H, et al. Balangu (Ilexema sp.) growth and physiology under field drought conditions affecting plant medicinal content. Plant Physiol Biochem. 2018;130:641–6.

21. Ashrafi M, et al. Effect of drought stress on metabolite adjustments in drought tolerant and sensitive thyme. Plant Physiol Biochem. 2018;132:391–9.

22. Kruse J, et al. Optimization of photosynthesis and stomatal conductance in the date palm Phoenix dactylifera during acclimation to heat and drought. New Phytol. 2019;223(4):1973–88.

23. Hu L, Wang Z, Huang B. SAH/5ahmU concentrations and metabolic factors associated with inhibition and recovery of photosynthesis from drought stress in a C perennial grass species. Physiol Plant. 2010;139(1):93–106.

24. Marco-Silva E, et al. Phenotyping of field-grown wheat in the UK highlights contribution of light response of photosynthesis and flag leaf longevity to grain yield. J Exp Bot. 2017;68(13):3473–86.

25. Chastain DR, et al. Water deficit in field-grown Gossypium hirsutum primarily limits net photosynthesis by decreasing stomatal conductance, increasing photorespiration, and increasing the ratio of dark respiration to gross photosynthesis. J. Physiol 2014;171(17):1576–58.

26. Marček T, et al. Metabolic response to drought in six winter wheat genotypes. PLoS ONE. 2019;14(2):e0212411.

27. Singh SK et al. Carbon dioxide diffusion across stomata and mesophyll and photo-biochemical processes as affected by growth CO2 and phosphorus nutrition in cotton. J Plant Physiol. 2013;170(9):801–13.

28. Huang F, et al. Rubisco accumulation factor 1 (RaFl) plays essential roles in mediating Rubisco assembly and carboxysome biogenesis. Proc Natl Acad Sci U S A. 2020;117(29):17418–28.

29. Sun J, et al. Decrease in leaf sucrose synthesis and photo-respiration, and increasing the ratio of dark respiration to gross photosynthesis in Arabidopsis null mutants of SPSA1. Plant Cell Environ. 2015;51(11):1861–6.

30. Liu Q, et al. Differentiation of original oils in Atractylodes lancea and Atractylodes koreana by gas chromatography with mass spectrometry. J Sep Sci. 2016;39(24):4773–80.

31. Xu K, et al. Six new compounds from Atractylodes lancea and their antioxidant capacity. Indian J Biochem Biophys. 2013;50(2):150–8.

32. Huang Q, et al. Drought stress affects the expression of key genes involved in the biosynthesis of phenylpropanoids and essential oil components in basal (Ocimum basilicum L.). Phytochemistry. 2017;139:1–7.

33. Huang Q, et al. Differential Gene Expression between Leaf and Rhizome in Atractylodes lancea: A Comparative Transcriptome Analysis. Front Plant Sci. 2016;7:348.
54. Jha RK, et al. Introgression of a novel cold and drought regulatory-protein encoding CORA-like gene, SbCDR, induced osmotic tolerance in transgenic tobacco. Physiol Plant. 2021;172:1170–88.
55. Singh DP, et al. Microbial inoculation in rice regulates antioxidative reactions and defense related genes to mitigate drought stress. Sci Rep. 2020;10(1):4818.
56. Zhang, C, et al., Drought tolerance in alfalfa (Medicago sativa L.) varieties is associated with enhanced antioxidative protection and declined lipid peroxidation. J Plant Physiol, 2019. 232: p. 226–240.
57. Rezayian M, Niknam V, Ebrahimzadeh H. Penconazole and calcium ameliorate drought stress in canola by upregulating the antioxidative enzymes. Funct Plant Biol. 2020;47(9):825–39.
58. Adebayo AR, Sebetha ET. Data on influence of different nitrogen fertilizer rates and plant density on grain yield and yield components of Water Efficient Maize (WEMA) variety. Data Brief. 2020;30:105582.
59. Paradiso A, et al. ROS Production and Scavenging under Anoxia and Re-Oxygenation in Arabidopsis Cells: A Balance between Redox Signaling and Impairment. Front Plant Sci. 2016;7:1803.
60. Barros Junior Ud.O., et al. Unraveling the roles of brassinosteroids in alleviating drought stress in young Eucalyptus urphylla plants: Implications on redox homeostasis and photosynthetic apparatus. Physiol Plant. 2021;172:748–661.
61. Esteban R, et al. On the recalcitrant use of Arnon’s method for chlorophyll determination. New Phytol. 2018;217:474–6
62. Hao A, et al. Effects of substrate and water depth of a eutrophic pond on the physiological status of a submerged plant Vallisneria natans. PeerJ. 2020;8:e10273.
63. Yang S, Zhang J, Chen L. Growth and physiological responses of Pennisetum sp. to cadmium stress under three different soils. Environ Sci Pollut Res Int. 2021;28:14867.
64. National Pharmacopoeia Committee. Pharmacopoeia of People’s Republic of China. Part 4. Beijing: Chemical Industry Press; 2020. p. 233.

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