

Effects of drought stress on seed germination and early seedling growth in *Ferula ferulaeoides* (Steud.) Korov.

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Abstract

Ferula ferulaeoides (Steud.) Korov. is a well-known perennial herb whose growth and distribution are negatively affected by drought. However, the seed germination stage and early seedling growth stage, which may be more sensitive to drought, have not been studied in detail. To reveal the effects of drought on seeds and seedlings of *F. ferulaeoides*, five water stress conditions induced by PEG-6000 treatment at five different concentrations (0, 5%, 10%, 15%, and 20%) were developed, in a completely randomized order for seeds and seedlings. The results revealed that drought stress significantly reduced seed germination percentage, and that no seeds germinated at a PEG-6000 concentration of 20%. In histomorphology, it was noted that the chloroplast of seedling leaf cells was deformed and vacuolized. The mitochondria structure was damaged in the leaf and root cells. By further analyzing part of the physiological and biochemical parameters of seedlings under drought stress, we showed that antioxidant enzymes such as catalase, peroxidase, and superoxide dismutase responded rapidly under drought stress. The seedlings accumulated a large amount of malondialdehyde under high drought stress. Additionally, drought stress affected the accumulation of proline and photosynthetic pigments. To summarize, drought stress affects seed germination and seedling growth in *F. ferulaeoides*; heavy drought should be avoided during the germination stage; and seedlings should not be exposed to long-term drought or severe drought. Our results provide information that may be useful for future artificial cultivation.

Keywords

Germination; Physiological and biochemical parameters; Ultrastructure; Water stress;

Introduction

Ferula ferulaeoides (Steud.) Korov. is a perennial herb belonging to Umbelliferae (Apiaceae) (Liu et al. 2021). As a classical medicinal plant, *F. ferulaeoides* is commonly used in treating rheumatic pain, swelling, and blood stasis in the Chinese province of Xinjiang (Sun et al. 2019). Recent studies have also shown that its roots and resin have many pharmacological activities, such as antibiosis and anti-inflammation (Liu et al. 2020). Therefore, wild *F. ferulaeoides* is widely used in local medicinal substances, but its availability is under threat due to its yield decrease (Guo et al. 2021).

Wild *F. ferulaeoides* is primarily distributed along the extremely arid edge of the Junggar Basin, Xinjiang Province, where dunes and gravel predominate (Fu et al. 2012). This harsh geographic environment, which is characteristic of serious water deficiency, limits the growth and distribution of wild *F. ferulaeoides* (Li et al. 2011; Tang et al. 2016). Prior resource investigation studies showed that an arid environment is a significantly negative factor for the seed germination stage and early seedling growth stage of *F. ferulaeoides* (Li et al. 2011; Li et al. 2011). Research on other Apiaceae plants has shown that water deficiency affects plant growth in different ways: 1) by reducing the germination percentage (GP) of plant seeds, and even causing a nonbudding condition when drought is severe (Yang et al. 2011; Yan et al. 2020; Liu et al. 2021; Gammoudi et al. 2021); 2) by causing morphology changes, such as irreversible damage in chloroplasts, as well as in leaf and root cell mitochondria (Chaves et al. 2009; Krichen et al. 2014); and 3) by causing level changes in partial physiological and biochemical indexes, such as the activation of antioxidant systems ((Asada 2006; Gill and Tuteja 2010; Hosseini et al. 2018; Corpas et al. 2020; Gechev and Petrov 2020; Mukarram et al. 2021). However, no quantitative studies have yet reported on *F. ferulaeoides*.

Currently, the cultivation of *F. ferulaeoides* is a popular research subject, and this hypothetically either provides an alternative resource in the market or protects wild resources, so understanding the effects of drought on seed germination and the seedling stage in *F. ferulaeoides* is crucial. In this study, we observed fluctuations in the GP of *F. ferulaeoides* seeds depending on the degree of drought stress, and we continued to observe changes in the ultrastructure and partial physiological and biochemical indexes of *F. ferulaeoides* seedlings at different soil water levels. Our data explain the characteristics of *F. ferulaeoides* seed germination and water demand at the seedling stage. This study is a preliminary exploration of the effects of different drought stress on the seeds and seedlings of *F. ferulaeoides*, and it will provide helpful information for future cultivation.

2 Materials and methods

2.1 Seed materials

Seeds of *F. ferulaeoides* were collected from Shihezi City, Xinjiang Province, China (43°12'N, 85°57'E) in May 2021. The seed variety was identified by Prof. Yan Ping (College of Life Science, Shihezi University) according to the *Flora of China* and the fruit anatomical characteristics of *F. ferulaeoides*. Healthy seeds identified as *F. ferulaeoides*, as opposed to those eliminated as being wormy or sick ones, were selected and stored at 4°C.

2.2 Seed germination

To break seed dormancy, the seeds were soaked in water at 60°C for 3 min, washed 3 times with distilled water, and then soaked in distilled water for 24 h. A total of 20 resuscitated seeds per group were placed in Petri dishes (9 cm in diameter) and cultured in an incubator at 5°C without light. The drought simulation conditions were set according to Muscolo and Parida et al. (2010; 2013; 2014). In brief, PEG-6000 was configured at gradient concentrations (5%, 10%, 15%, and 20%) by distilled water. The filter paper in Petri dishes was soaked in distilled water or different PEG-6000 solutions. Each group was cultured for 30 days. Papers and solution were changed every three days so that the water potential was constant. This experiment was repeated three times. Seeds were considered germinated when the primary roots were at least 2 mm long, and the number of germinated seeds was counted daily to calculate GP.

GP was calculated according to the following formula:

$$GP = (n_1 + n_2 + n_3 + \dots + n_i) / N,$$

where n is the number of germinated seeds on each counting day at a time and N represents the total number for each group, 20.

2.3 Seedling cultivation

Another batch of resuscitated seeds was used to culture seedlings, which were planted in pots (26 cm in width and 24 cm in height). These pots contained a soil mixture of peat, vermiculite, and perlite in a ratio of 2:1:1. The day–night temperature was maintained at 15/5°C for 30 days. The soil was wet throughout the process. After that, healthy seedlings with the same growth vigor were selected and transferred to a container where seedling roots were immersed in 1/2 Hoagland's nutrient solution. After one day of balance recovery treatment, the nutrient solution was replaced with 1/2 Hoagland's nutrient solution, which contained PEG-6000 at different concentrations (5%, 10%, 15%, and 20%) for nine days, while 1/2 Hoagland's nutrient solution served as the control. The experiment was performed in three replicates, with three plants per replication.

2.4 Transmission electron microscopy (TEM) observation

The control, 10%, and 20% groups were selected to observe the ultrastructure of their roots and leaves on the sixth day. The samples were cut into 1 mm² segments and placed in 2.5% glutaraldehyde (v/v) for 24 h. After being washed three times with phosphate-buffered saline (PBS, pH = 7.0), the segments were immersed in 1% osmium tetroxide and then were rinsed three times with the same PBS. The segments were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, 95%, and 100% v/v) at each stage for 15 min and then left in acetone for 20 min. The dehydrated segments were placed at room temperature for 1 h in a 1:1 mixture of absolute acetone and the Spurr resin mixture. They were then transferred to a 1:3 mixture of absolute acetone and resin and left there for 3 h. The segments were kept overnight in the final Spurr resin mixture. After embedding in ERL-4206, the specimens were heated at 70°C overnight. The blocks were sectioned using an ultramicrotome (LEICA EM UC7 Ultramicrotome), and the sections (70–90 nm) were treated with uranyl acetate and alkaline lead citrate

for 5 min. These stained sections were placed on a copper grid and observed via transmission electron microscopy (JEM-1230, Japan) at 80 kV.

2.5 Physiological and biochemical parameters of seedlings

Physiological and biochemical parameters of seedlings in each group were determined on the third, sixth, and ninth days. The antioxidant enzyme system (including superoxide dismutase [CAT NO: BC0175], peroxidase [CAT NO: BC0095], and catalase [CAT NO: BC0205]), malondialdehyde [CAT NO: BC0025], proline [CAT NO: BC0295], and chlorophyll [CAT NO: BC0995] were assessed using an assay kit (Beijing Solarbio Science & Technology Co., Ltd.). The detection method strictly followed the corresponding kit instructions.

2.6 Data analysis

All of the statistical analyses were performed using GraphPad Prism 8. The data of seed germination were analyzed using one-way analysis of variance (ANOVA). $P < 0.0001$ (****); 0.0002 (***), 0.0021 (**); 0.0332 (*); and 0.1234 (ns).

3. Results

3.1 Measurements of seed germination

In general, GP decreased significantly with the increase in PEG-6000 concentrations. The highest GP was observed in the CK group (71.7%). GP at PEG-6000 concentrations of 5%, 10%, and 15% was 51.7%, 31.7%, and 18.3%, respectively. No seeds germinated at the 20% PEG-6000 concentration (Fig. 1).

3.2 Ultra-microscopic examination by TEM

Under control conditions, the leaf cells had a granular cytoplasm densely filled with numerous organelles (Fig. 2-a-CK-L). The chloroplast had an ellipsoidal shape with well-arranged thylakoid membranes in distinct grana regions, and the stroma contained starch grains (Fig. 2-b-CK-L and 2-c-CK-L). The mitochondria structure was intact in leaf cells with clear cristae (Fig. 2-d-CK-L). The mitochondria of root cells also showed the same ultrastructure (Fig. 2-e-CK-R). Under PEG-6000-treated conditions, considerable ultrastructural differences in organelles were observed. Specifically, the chloroplast of leaf cells deformed and vacuolized, grana-stacking was less frequent, and the thylakoids were loosened and distorted (Fig. 2-b-10%-L, 2-c-10%-L, and 2-b-20%-L). Vague cristae were observed in the mitochondria of leaf cells, revealing a lack of integrity (Fig. 2-a-10%-L, 2-a-20%-L). In root cells, the mitochondria had vague cristae, and the structure was incomplete (Fig. 2-d-10%-R). At 20% PEG-6000 concentrations, the mitochondrial outer membrane of the root cells exhibited loss of integrity (Fig. 2-c-20%-R).

3.3 Measurement of antioxidant enzyme system markers

The antioxidant enzyme system plays a crucial role in protecting cells from damage under drought stress. Overall, except for the 20% PEG-6000 concentration group, the superoxide dismutase (SOD) activity of the other stress groups was not significantly different from that of the control group during the first three days, while after the sixth day, the SOD activity in the 20% PEG-6000 concentration

group significantly increased, and the SOD activities of the other groups were significantly lower than the control group (Fig. 3-A). The peroxidase (POD) activity in the 10% PEG-6000 concentration group increased all of the time. However, it increased and then decreased at 15% and 20% PEG-6000 concentrations (Fig. 3-B). On the third day, the catalase (CAT) activity of the other groups was significantly higher than that of the control group, except in the 5% PEG-6000 concentration group. After the sixth day, the CAT activity of each group was significantly higher than that of the control group. Moreover, the CAT activity in all of the experimental groups exhibited a trend of first increasing and then decreasing (Fig. 3-C).

3.4 Measurement of Malondialdehyde

The extent of oxidative damage can usually be seen by monitoring changes in the level of malondialdehyde (MDA). In the 20% PEG-6000 concentration group, as compared to the control group, the low MDA content may be due to the low MDA content in the sample on the third day. The MDA content in each PEG-6000 concentration group was significantly higher than that in the control group on the sixth day. The MDA content only decreased on the third day in the 20% PEG-6000 concentration group. At other PEG-6000 concentrations, the MDA content increased with time (Fig. 4).

3.5 Measurement of Proline

When it comes to responding biochemically to moisture deficit, plants accumulate proline (Pro) to regulate osmosis. In this study, the Pro content of each experimental group first increased and then decreased with the increase in time (Fig. 5). The Pro content in the 5% PEG-6000 concentration group was not significantly different from that in the control group on the third day. The 5% and 10% PEG-6000 concentrations gradually increased after the third day and gradually declined on the ninth day. At 15% and 20% PEG-6000 concentrations, the Pro content reached the highest level on the third day and then gradually declined.

3.6 Measurement of Chlorophyll

The photosynthetic performance of plants can be evaluated by leaf chlorophyll (Chl) content. The Chl content in the control group accumulated with the increase in time. The Chl content in the different PEG-6000 concentrations groups was lower than in the control group, and there was less fluctuation over time and at different concentrations (Fig. 6).

Discussion

Soil water deficiency is a typical abiotic stress that limits the survival and growth of young plants (Li et al. 2011). Previous studies have shown that drought has an adverse effect on the GP of different Apiaceae plants such as *Angelica dahurica* (Fisch.) Benth. et Hook seeds and *Foeniculum vulgare* Mill (Yang et al. 2011). Similarly, our study confirmed that seeds of *F. ferulaeoides* are water-sensitive and could not germinate under severe drought conditions. This germination trait is an important way of survival for seeds to adapt to the environment.

With respect to organelle morphology, there are noticeable differences in terms of chloroplast and mitochondria between leaf and root cells of *F. ferulaeoides* seedlings cultivated under drought

conditions or normal water conditions. These changes confirm that drought is detrimental to the photosystem and energy supply system of *F. ferulaeoides* (Rodrigues et al. 2014).

To examine the activities of antioxidant enzymes, our results revealed that CAT, POD, and SOD responded rapidly under drought stress, but CAT and POD increased and then decreased as time progressed. These findings partly demonstrate that *F. ferulaeoides* seedlings can rapidly respond to drought stress but cannot endure long-term drought or severe drought, which is consistent with previous drought stress studies on *Ligusticum chuanxiong* Hort. and *Notopterygium inchum* Ting ex H.T. Chang (Zhou et al. 2009; Yang et al. 2018). In addition, previous studies showed that MDA content in seedlings might increase with the deepening of drought stress (Zhou et al. 2009; Yang et al. 2011; Liu et al. 2022). However, we observed that slight drought stress had little impact on the MDA content, whereas higher drought stress increased the MDA content. The above data showed that the *F. ferulaeoides* seedlings suffered only minor damage due to mild drought stress, but under severe drought stress, a strong lipid peroxidation of cell membranes occurred.

One of the osmotic defense mechanisms is the accumulation of Pro to maintain the water potential of plants under drought stress (Sales et al. 2015; Ingram and Bartels 1996). Our study indicated that the *F. ferulaeoides* seedlings can accumulate Pro rapidly to resist drought. This phenomenon may partly explain why *F. ferulaeoides* seedlings can tolerate mild drought stress. However, the Pro content showed a decreasing trend after long-time drought stress, indicating that the ability to balance the osmotic potential by Pro accumulation is restricted at the seedling stage. This result was consistent with the research of Liu and Han (2017; 2022). Chl content is an important indicator of the photosynthetic performance of plants (Yang et al. 2018). Our study showed that drought stress influenced the accumulation of photosynthetic pigments in *F. ferulaeoides*. It is possible that water is the raw material for photosynthesis, and drought destroys the chloroplast structure of the leaves, which influenced the photosynthesis of *F. ferulaeoides* in our study. Therefore, drought can comprehensively affect the changes in the antioxidant enzyme system, osmoregulation, and photosynthesis of *F. ferulaeoides* as a strategy to withstand adverse circumstances. This suggests that the impact of drought on the early development of *F. ferulaeoides* is multidimensional.

In brief, our data demonstrate that drought stress affected the germination and early growth of *F. ferulaeoides*. *F. ferulaeoides* seedlings can rapidly respond to drought stress but cannot endure drought for an extended period, which may be evidence that drought is one of the reasons for the low survival rate of *F. ferulaeoides*. Presently, reports on *Ferula* L. cultivation are limited. In order to be able to cultivate *F. ferulaeoides*, severe drought should be avoided during germination, and seedlings should not be exposed to long-term drought or severe drought at the seedling establishment phase.

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Author contribution YM contributed to the study conception and design. Data collection and experiments were performed by YM, XL and KW. SSL and YYZ analyzed the data. The first draft of the manuscript was written by YM and KL, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability All data generated or analysed during this study are included in this published article.

Declarations

Conflict of interest The authors declare no potential conflict of interest.

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Fig. 1 Germination percentage of *F. ferulaeoides* seeds at different PEG-6000 concentrations

Error bars represent the standard deviation among the three replicates.

Fig. 2 Ultrastructural changes in the leaf cells and root cells of *F. ferulaeoides* seedlings under different drought stress

(CK control group, 10%, 20% different PEG-6000 concentration groups, L leaf, R root). Va vacuole, Sg starch grain, N nucleus, Chl chloroplast, M mitochondria.

Fig. 3 Antioxidative enzyme activities in *F. ferulaeoides* seedlings at different PEG-6000 concentrations

Activities of superoxide dismutase (A), peroxidase (B), and catalase (C) were measured after the experiment. Values are mean $\pm$ SE (n = 3). Error bars represent the standard deviation among the three replicates.

Fig. 4 MDA content in *F. ferulaeoides* seedlings at different PEG-6000 concentrations

Values are mean $\pm$ SE (n = 3). Error bars represent the standard deviation among the three replicates.

Fig. 5 Pro content in *F. ferulaeoides* seedlings at different PEG-6000 concentrations

Values are mean $\pm$ SE (n = 3). Error bars represent the standard deviation among the three replicates.

Fig. 6 Chlorophyll content in *F. ferulaeoides* seedlings at different PEG-6000 concentrations

Values are mean $\pm$ SE (n = 3). Error bars represent the standard deviation among the three replicates.

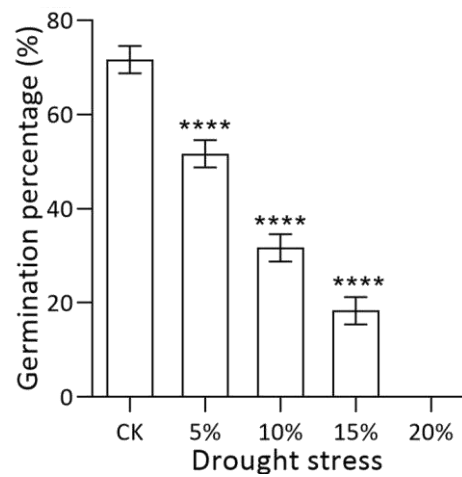


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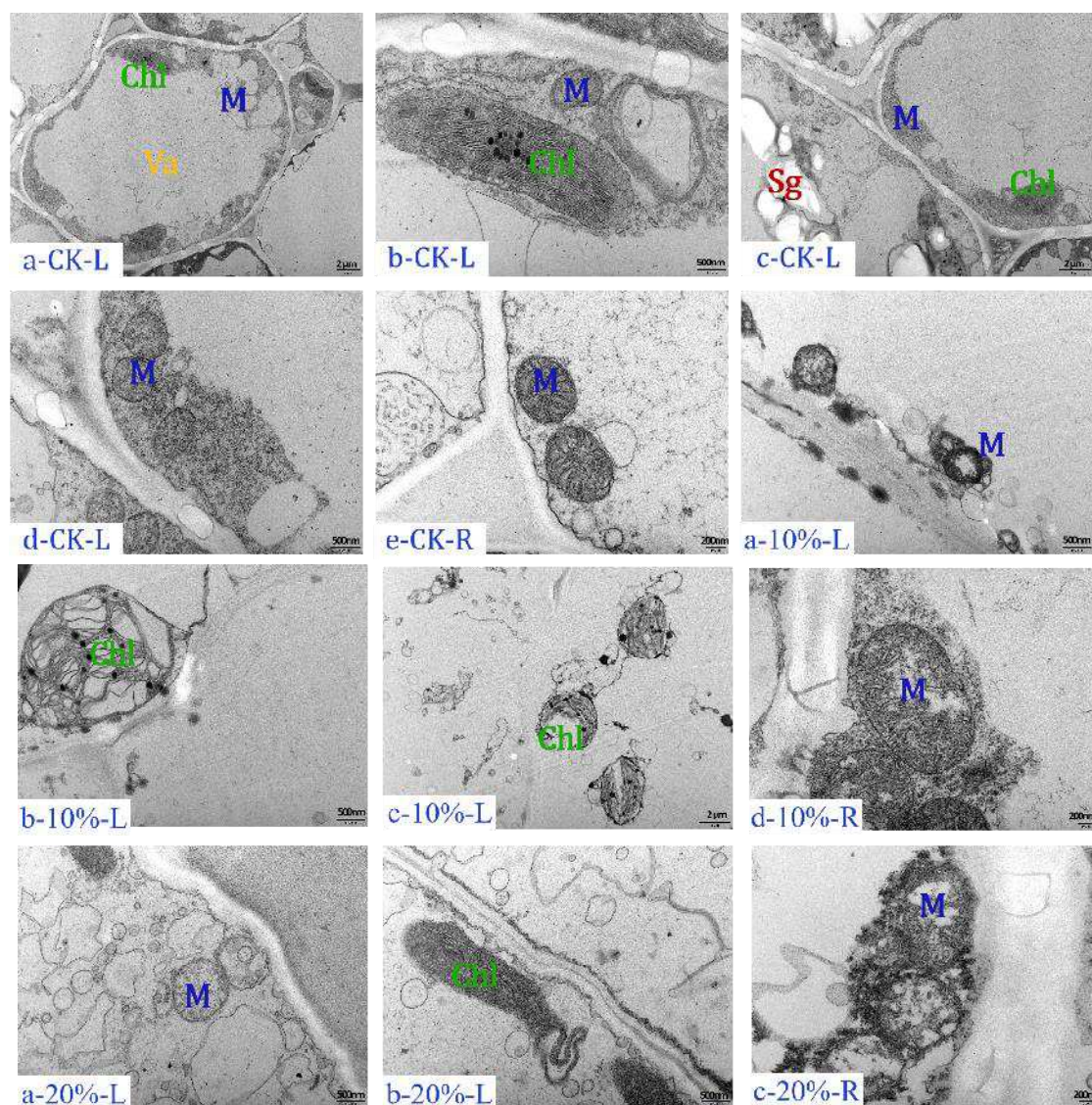


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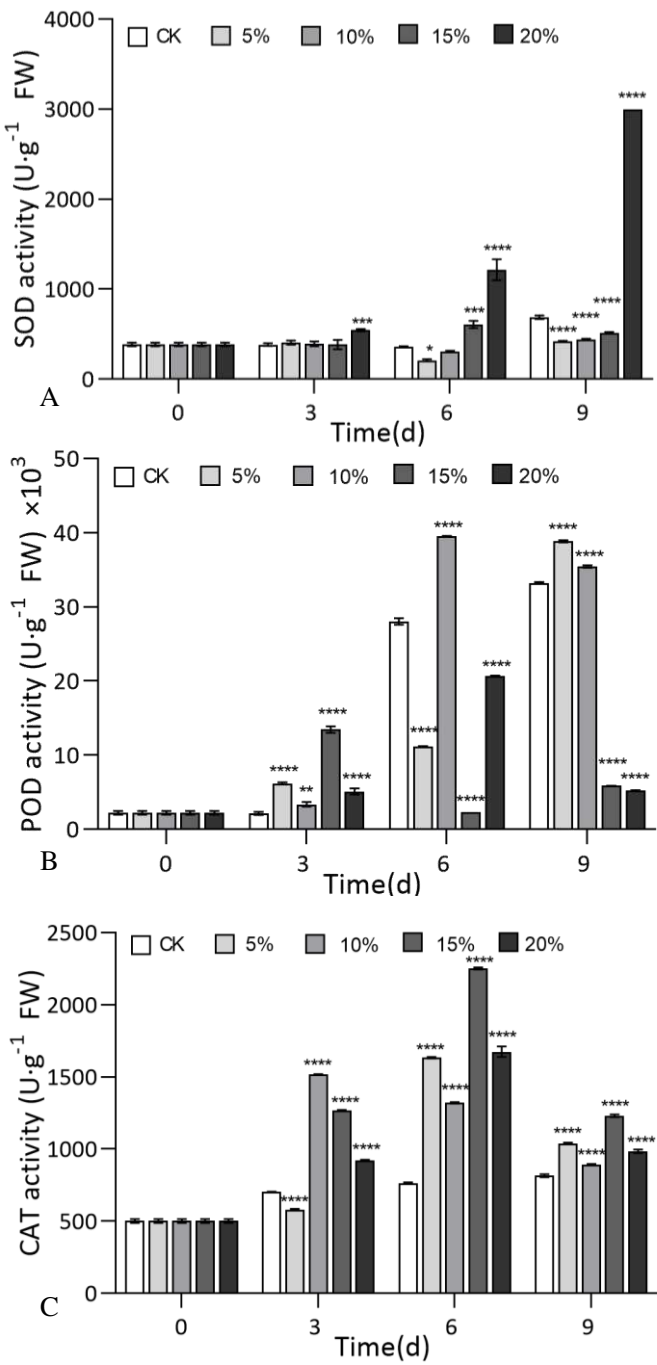


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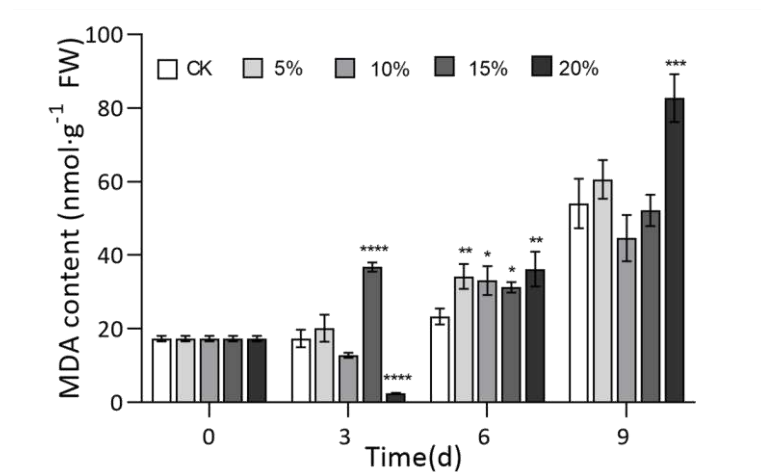


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Values are mean $\pm$ SE (n = 3). Error bars represent the standard deviation among the three replicates.

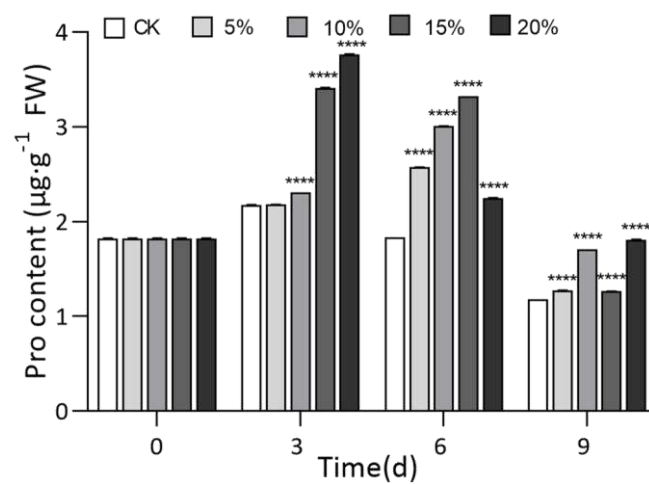


Fig. 5 Pro content in *F. ferulaeoides* seedlings at different PEG-6000 concentrations

Values are mean $\pm$ SE (n = 3). Error bars represent the standard deviation among the three replicates.

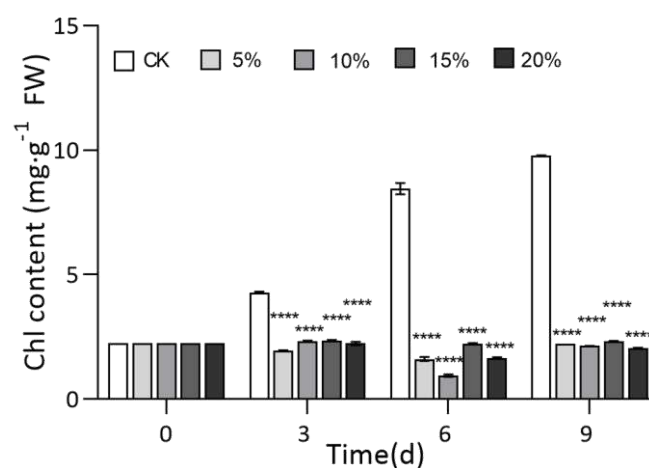


Fig. 6 Chlorophyll content in *F. ferulaeoides* seedlings at different PEG-6000 concentrations

Values are mean $\pm$ SE (n = 3). Error bars represent the standard deviation among the three replicates.