EFFECTS OF PEF ON DROUGHT STRESS RESPONSE OF SCUTELLARIA BAICALENSIS SEEDS AND ITS PHYSIOLOGICAL MECHANISM
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PEF处理提高黄芩种子干旱胁迫响应及其生理机制研究

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ABSTRACT
PEF pretreatment enhances Scutellaria seed germination and growth, improving drought adaptability. Using PEG-6000 to simulate drought stress, germination potential (42.86%), germination rate (44.76%), and germination index (27.73%) were significantly improved under 12.5% PEG. PEF treatment also increased vigor index, root length, and dry weight, shortened germination time, enhanced SOD and POD activities, reduced MDA content, and elevated levels of soluble sugars, soluble proteins, proline, and α-amylase. Hormonal analysis revealed increased gibberellin and auxin contents accompanied by reduced ABA levels. Overall, PEF pretreatment effectively promoted seed germination and growth under drought stress conditions.

摘要
PEF预处理黄芩种子，可促进发芽和幼苗生长，有利于适应和抵抗不利生长环境。本文以PEG-6000模拟干旱胁迫，观测有、无PEF处理的黄芩种子在不同干旱胁迫下的萌发和生长状况，探讨PEF处理提升黄芩种子抗旱性的生理机制。结果显示：12.5% PEG胁迫下，PEF处理可以使黄芩种子的发芽势、发芽率、发芽指数分别显著提高42.86%、44.76%和27.73%；活力指数、胚根长、干重增加，平均发芽时间缩短。PEF处理使胁迫下生长的幼苗SOD和POD酶活性提高20.13%-40.13%和40.00%-52.69%，丙二醛含量下降35.44%-43.74%，可溶性糖、可溶性蛋白和脯氨酸含量增加28.57%-39.13%、28.41%-79.07%和29.32%-80.07%，α-淀粉酶活性增加15.91%-53.74%；赤霉素和生长素含量在萌发和幼苗期升高4.06%-23.06%和16.62%-26.89%，脱落酸含量下降20.50%-25.45%。因此，PEF预处理黄芩种子能保持干旱胁迫下种子萌发和生长的顺利进行。

INTRODUCTION
Scutellaria baicalensis, a member of the Lamiaceae family, is a commonly used medicinal herb in China. Its dried roots, known as *Radix Scutellariae*, contain bioactive compounds whose extracts have been shown to prevent and treat various inflammatory responses triggered by COVID-19 *(Wang W. et al., 2021)*. *S. baicalensis* seeds are inherently small, therefore encountering drought during germination can result in delayed germination and reduced seedling vitality, or in severe cases, difficulties in seedling emergence *(Wang R. et al., 2024)*. Even if germination occurs, the weak ability to emerge through the soil surface, coupled with drought conditions, leads to low emergence rates and slow seedling growth and severely impacting yield. Seed germination and emergence are the starting points for the growth of medicinal plants and the foundation of herbal production. Improving drought resistance during the germination phase to ensure successful and uniform germination, proper emergence, and strong seedling growth is critical for the healthy development of medicinal plants and the foundation for high-quality, high-yield herbal production. Consequently, enhancing the vitality of *S. baicalensis* seeds, promoting germination, and improving drought resistance during the seedling stage are critical challenges in artificial cultivation and key steps in advancing the development of the *S. baicalensis* industry.

Numerous studies have shown that electric field pretreatment is an effective technique for enhancing seed vitality and seedling emergence rate *(Akdemir et al, 2021; Tan et al., 2014)*. On one hand, electric fields can break seed dormancy and promote germination; on the other hand, they activate enzyme synthesis, facilitate the consumption of stored nutrients, and accelerate the growth of the seed's embryo, cotyledons, and radicle, which aids in rapid seedling emergence *(Ma et al., 2024)*.

PEF treatment of wheat seed grains notably reduced the populations of endogenous microbiota comprising total heterotrophic mesophilic bacteria, total fungi, and yeast under the influence of added energy, while significantly improving germination rate and emergence rate, as well as tolerance to cold and salinity stress *(Akdemir et al., 2021).* Preliminary research of the subject found that PEF treatment with appropriate parameters significantly enhanced the germination potential of *S. baicalensis* seeds, reduced germination time; concurrently, the radicle length, dry weight, and electrical conductivity of seedlings increased. Additionally, physiological and biochemical indicators such as Superoxide Dismutase (SOD), Peroxidase (POD), α-amylase activities, and the contents of soluble sugar, soluble protein, proline, and malondialdehyde (MDA) in seedlings were elevated *(Song et al., 2024)*, indicating that PEF-treated seeds could enhance the ability to resist adverse conditions. To evaluate the potential for drought resistance, this study built on previous work by inducing drought stress on seed germination and early seedling growth using polyethylene glycol-6000 (PEG-6000) *(Jaybhaye et al., 2024; Qi et al., 2023).* The response of PEF-treated and untreated seeds to drought stress was assessed by observing their germination and seedling growth under varying drought conditions. Additionally, the study measured related physiological indicators of seedlings and the differences in microstructural changes during germination.

MATERIALS AND METHODS

Seed Treatment and Cultivation

Selected full and intact S. baicalensis seeds were first surface-sterilized to ensure aseptic conditions. The seeds were immersed in 75% ethanol for 1 minute, followed by a 10-minute treatment in 2% sodium hypochlorite solution, and then rinsed thoroughly with sterile distilled water three times. After sterilization, the seeds were soaked in 40 °C warm water for 12 hours. They were then placed on germination beds (comprising a 15 cm diameter dish with a layer of filter paper at the bottom) supplemented with 5 mL of PEG solution. Each bed contained 100 seeds. Germination beds with distilled water served as the blank control. The experiment was conducted with three biological replicates, and seed germination was observed daily to establish the maximum PEG stress concentration.

The maximum PEG-6000 stress concentration was set at 15%, with six concentration gradients: 2.5%, 5%, 7.5%, 10%, 12.5%, and 15%. Randomly selected, soaked seeds (100 per group) were placed in electrode cups with a 4 mm spacing. Distilled water was added to align the liquid level with the electrode height. Based on the parameters in Table 1, which were derived from previous studies demonstrating the effective promotion of *S. baicalensis* seed germination *(Song et al., 2024)*, seeds were treated using an ECM830 square wave electroporation system (BTX, USA). The PEF-treated *S. baicalensis* seeds were then evenly distributed on germination beds, supplemented with 5 mL of PEG solution, covered, and placed in a dark incubator at 22 ℃ Germination progress was recorded and observed daily, with an appropriate amount of gradient solution added as needed. Two controls were established: germination bed culture without PEF treatment with distilled water as blank control CK1 and germination bed culture with PEF treatment with distilled water as control CK2. Each treatment was replicated three times, with daily recording and observation of germination, and supplementation of distilled water and PEG solution to maintain stress concentrations.

Table 1

PEF Treatment Parameter Types and Settings

Pulse strength (kV·cm-1)	Pulse width (μs)	Pulse number (n)
0.5	40	20
1.25	120	60
2	200	99

Germination and seedling growth indexes

Germination was defined as the radicle emergence through the seed coat by 1 mm. The germination potential (GP), germination rate (GR), germination index (GI), and mean germination time (MGT) were determined and calculated according to previously published literature *(Song et al., 2024*). The vigor index (VI) was computed using the method proposed by Ahmed Z.

The germination test was concluded on the 5th day post-initiation. From each group, 10 seedlings were randomly selected to measure radicle length, fresh weight, and dry weight. Radicle length was measured with a ruler. The fresh weight of the seedlings was determined by wiping the surface moisture with filter paper and then weighing them on a one-thousandth precision balance. Subsequently, the seedlings were wrapped in dried tin foil and oven-dried at 80 ℃ until a constant weight was achieved, which was recorded as the dry weight of the seedlings.

Determination of Seedling Physiological Indices

Drought stress cultivation was conducted on PEF-treated and untreated seeds. On the 5th, 10th, and 15th days of cultivation, 0.2 g of seedlings were randomly selected from each group for the determination of physiological indices. The methods for measuring SOD and POD activities, Pro, SS, and SP contents, and α-amylase activity were as described in a previous publication *(Song et al., 2024).*

The determination of MDA content was based on the thiobarbituric acid method by Ying Qi et al. *(Qi et al., 2023),* with slight modifications. Specifically, 0.2 g of seedlings from each treatment were ground in 1.5 mL of 10% trichloroacetic acid in an ice bath, followed by centrifugation at 4000 rpm for 10 min at low temperature. Then, 1 mL of the supernatant was mixed with 1 mL of 0.6% thiobarbituric acid, shaken well, and subjected to a boiling water bath for 10 min. After rapid cooling, the mixture was centrifuged again at 4000 rpm for 10 min at low temperature. The absorbance was measured at 450 nm, 532 nm, and 600 nm.

Determination of hormone content

Four groups of seeds in distilled water culture group (CK), PEF treatment group (PEF), 12.5% PEG treatment group (PEG) and PEF treatment + 12.5% PEG treatment group (PEF+PEG) were used as samples. Samples were collected on the 0th, 2nd, 5th, 10th and 15th days of culture, with 2 g of material per treatment and three biological replicates. Immediately after sampling, the materials were frozen in liquid nitrogen and subsequently stored at -80 ℃ for hormone determination.

The determination of IAA, GA, and ABA contents was conducted according to the instructions provided with the hormone assay kit (Shanghai Enzyme-linked Biotechnology Co., Ltd.). The sample extraction procedure was as follows: Samples were ground into a powder using liquid nitrogen and then mixed with phosphate buffer (0.01 mol/L, pH 7.2-7.4) at a ratio of 1:9. The mixture was centrifuged at 4000 rpm for 10 minutes at low temperature, and the supernatant was collected *(Kępczyńska & Orłowska, 2021).* The reaction system was prepared as follows: 50 μL of each test sample and standard were added to an enzyme-linked immunosorbent assay (ELISA) plate and incubated at 37 ℃ for 30 min. The plate was then washed five times, after which 50 μL of enzyme-conjugated reagent was added to each well and incubated at 37 ℃ for another 30 min. Following an additional five washes, the color-developing solution was added, and the reaction was allowed to proceed at 37 ℃ for 10 min before the stop solution was applied. Absorbance was subsequently measured at 450 nm.

Microscopic Observation

Seeds from four groups—distilled water culture (CK), PEF treatment (PEF), 12.5% PEG treatment (PEG), and PEF combined with 12.5% PEG treatment (PEF+PEG)—served as samples. On the 2nd day of culture, six seeds from each group were randomly selected. A small portion of the seed coat was removed from the middle of both sides parallel to the embryo bud to facilitate fixative penetration. The seeds were then fixed in FAA solution (70% anhydrous ethanol: glacial acetic acid: formaldehyde = 18:1:1) for over 48 hours. Subsequently, the seeds were processed for paraffin sectioning and double-stained with safranin-fast green. Microstructural characteristics of the seed sections were observed under a Leica fluorescence microscope using a 4× objective.

Statistical Analysis

Microsoft Excel 2016 was used to perform preliminary arrangement of the data, IBMSPSS 20.0 was used to analyze the data, and GraphPad Prism 9.3 was used to draw figures. Differences among multiple groups were examined by one-way analysis of variance (ANOVA) followed by Duncan's new multiple-range test ($p \leq 0.05$); results of these multiple comparisons are indicated by lowercase letters (a, b, c). Additionally, comparisons between each treatment group and the control (CK) were performed using Student's t-test; significant differences are denoted by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). All values are expressed as mean ± SD.

RESULTS

Establishment of PEG concentration range

As shown in Table 2, under 5% PEG stress, the GP and GR were higher than those of the CK. When the PEG concentration increased to 10%, both GP and GR dramatic decreased. At a PEG concentration of 15%, GP and GR dropped dramatically, by 90.44% and 77.59% respectively, compared with the control. When the PEG concentration was ≥20%, *S. baicalensis* seeds did not germinate at all. Therefore, a 15% PEG concentration was used as the maximum stress concentration to simulate drought stress in *S. baicalensis* seeds.

Table 2

Effects of different concentrations of PEG on the germination of *S. baicalensis* seeds

PEG	GP (%)	GR (%)
0/CK1	68.00±3.32	87.00±2.69
5	77.00±3.2	88.50±1.30
10	50.67±2.87***	74.00±1.70**
15	6.50±2.38***	19.50±3.83***
20	0.00±0	0.00±0
25	0.00±0	0.00±0

Note: Asterisks indicate significant differences compared with the CK group according to Student's t-test (** $p < 0.01$, *** $p < 0.001$).

Germination of PEF-treated S. baicalensis seeds under drought stress

Table 3 reveals that seeds without PEF treatment under low PEG concentrations of 2.5%-5% exhibited increased GP, GR, GI, and VI, with a shortened MGT. This promotion of germination might be associated with the reduction of extracellular water potential by low PEG concentrations, leading to slow water absorption and preventing imbibition damage due to rapid water uptake. At a concentration of 7.5%, the GP and VI began to decline, and the germination time extended. As the stress concentration increased further, there were significant decreases in GP, GR, GI, and VI, with a marked prolongation of MGT. PEF treatment can enhance seed GR and VI under normal growth conditions, significantly enhancing GP and GI, and reducing the MGT. Similarly, under drought stress, as illustrated in figures 1A and C, seeds treated with PEF exhibited higher GP and GI compared with untreated seeds at the same PEG concentration. The difference was significant at higher stress concentrations of 10-12.5%, with increases of 27.77%-42.86% and 16.30%-27.73%, respectively. The VI also increased, and the MGT was shortened (Table 3).

Table 3

Effect of PEF treatment on seed germination of *S. baicalensis* under different concentrations of drought stress

	GP (%)	GR (%)	GI (%)	VI (%)	MGT (d)
Untreated					
CK1	66.00±0.27bcd	87.50±0.02a	28.49±0.07bc	0.80±0.09abc	1.84±0.13bc
2.5% PEG	79.33±0.29a	91.33±0.04a	34.38±0.30a	0.88±0.32abc	1.72±0.36c
5% PEG	76.33±0.28abc	89.00±0.03a	33.61±0.22a	0.89±0.24abc	1.78±0.28bc
7.5% PEG	65.00±0.28cd	88.67±0.03a	29.74±0.15b	0.65±0.17abc	2.13±0.21bc
10% PEG	48.00±0.27e	73.00±0.02c	22.52±0.17d	0.45±0.19bc	2.29±0.23bc
12.5% PEG	31.50±0.25f	52.50±0.03d	16.19±0.09e	0.21±0.11c	2.28±0.15bc
15% PEG	5.50±0.5g	18.67±0.08e	3.42±0.22f	0.03±0.24d	3.48±0.28a
PEF Treated					
CK2	83.00±0.31a	88.00±0.08a	33.12±0.11a	1.02±0.11ab	1.57±0.09c
2.5% PEG	82.00±0.34a	87.50±0.03a	34.28±0.34a	1.10±0.09a	1.55±0.32c
5% PEG	77.33±0.33ab	86.00±0.01ab	33.18±0.26a	1.00±0.05ab	1.69±0.24c
7.5% PEG	73.00±0.23abcd	84.67±0.08ab	30.06±0.19b	0.72±0.05abc	1.82±0.17bc
10% PEG	61.33±0.32d	81.00±0.03abc	26.19±0.21c	0.45±0.08bc	2.00±0.19bc
12.5% PEG	45.00±0.3e	76.00±0.31bc	20.68±0.13d	0.29±0.08c	2.15±0.11bc
15% PEG	6.50±0.25g	18.50±0.03e	3.98±0.26f	0.03±0.01d	2.92±0.24ab

Note: Different lowercase letters in the same column indicate significant differences at $P<0.05$.

The GR was lower in the PEF-treated group than in the untreated group at low stress concentrations of 2.5-7.5%, but it was higher at higher PEG concentrations of 10%-15%, reaching a significant level at a 12.5% stress concentration (Figure 1B). In summary, under PEG stress ranging from 2.5% to 15%, PEF treatment enhanced seed GP, VI, and GI, reduced MGT, and increased the GR at high stress concentrations.

Consequently, PEF treatment can improve the response ability of *S. baicalensis* seeds to drought during germination. To further investigate the physiological mechanisms by which PEF pretreatment enhances the drought tolerance of *S. baicalensis* seeds, a 12.5% PEG-6000 solution was used as the optimal stress induction concentration, as it reduced the germination rate (GR) and germination potential (GP) by 40% and 52%, respectively. In studies on drought stress during early germination and seedling growth in soybean seeds, the concentration causing a 50% inhibition of germination rate was also used for subsequent mechanistic analysis. For soybean, this concentration was 15%, indicating that different plant species exhibit varying levels of drought tolerance *(Jaybhaye et al., 2024).*

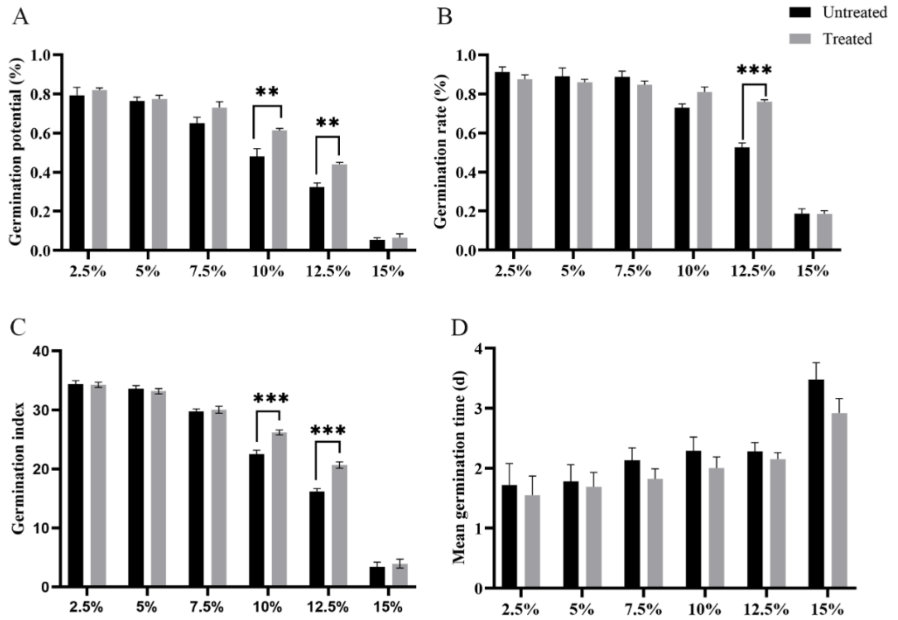

Fig. 1 - Effect of PEF treatment on seed germination of *S. baicalensis* under different concentrations of drought stress. (**A**) GP, (**B**) GR, (**C**) GI, and (**D**) MGT.

Note: Asterisks indicate significant differences compared with the CK group according to Student's t-test (** $p < 0.01$, *** $p < 0.001$).

Seedling growth of PEF-treated S. baicalensis seeds under drought stress levels

As shown in Table 4, without electric field treatment, drought stress inhibited the radicle length, fresh weight, and dry weight of seedlings. Under normal cultivation conditions, PEF treatment increased radicle length, fresh weight, and dry weight of seedlings. At the 12.5% PEG stress level, PEF treatment resulted in higher radicle length and dry weight compared with the untreated group, while fresh weight was lower, though the differences were not significant. This PEF treatment led to an increase in dry weight without a corresponding increase in fresh weight, which may be related to the effect of PEF treatment on the rearrangement of intramolecular dipoles, increased bound water content, and relatively lower free water content *(Ma et al., 2024),* thus favoring drought resistance.

Table 4

Effect of pulsed electric field treatment on seedling growth of *S. baicalensis* seeds under 12.5% PEG stress

Growth index	Radicle length (cm)	Fresh weight (g)	Dry weight (g)
CK	3.02±0.18a	0.28±0.03a	0.14±0.02a
PEG	2.29±0.31b	0.14±0.04b	0.11±0.02a
PEF	3.29±0.31a	0.31±0.02a	0.15±0.01a
PEF+ PEG	2.61±0.13a	0.13±0.02b	0.13±0.014a

Note: Different lowercase letters in the same column indicate significant differences at P<0.05.

Effect of PEF treatment on MDA content and SOD and POD activities in seedlings under drought stress

O_2 is essential for plant growth, and when O_2 is not fully reduced during its utilization, certain oxygen metabolites and their derivatives are produced. These are more chemically reactive than O_2 and are termed reactive oxygen species (ROS). An appropriate amount of ROS can act as signaling molecules to regulate plant growth favorably *(Farooq et al., 2022)*.

Under normal conditions, cells eliminate excess ROS through enzymatic and non-enzymatic antioxidant systems, maintaining a dynamic balance of ROS that is beneficial for growth. However, under stress, the production rate of ROS increases, disrupting the ROS balance, and triggering lipid peroxidation of unsaturated fatty acids, producing MDA, which further aggravates membrane damage and leads to the loss of membrane function. Consequently, MDA content can reflect the degree of membrane damage and is a common indicator for assessing the level of stress damage. ROS also cause the fragmentation of nucleic acids and the degradation of proteins and polysaccharides *(Dat et al., 2000)*. SOD catalyzes the reaction between superoxide anions (O^{2-}) and H^+, forming H_2O_2 and O_2, serving as the first line of defense against oxidative damage induced by drought stress *(Altaf et al., 2022)*, and acting as the primary defense against ROS under drought conditions. Subsequently, POD converts H_2O_2 into H_2O, reducing free radical-induced damage to cells.

Figure 2 shows that under normal cultivation conditions without PEF treatment, the SOD activity of seedlings reached its peak on day 10 of cultivation and slightly declined on day 15. POD activity and MDA content remained relatively stable. Under 12.5% PEG stress, MDA content increased on day 5 and day 10, with a significant rise in SOD activity, while POD activity increased, though not significantly. After PEF treatment under 12.5% PEG stress, the activities of both enzymes markedly increased compared with the untreated group. SOD activity increased by 20.12%-40.15%, and POD activity increased by 40.00%-52.69%, enhancing the ability to scavenge ROS. Consequently, MDA content notably decreased by 35.44%-45.78% from day 5 to day 15 of cultivation. It is believed that the upregulation of SOD activity under PEG-induced drought stress is crucial for alleviating oxidative stress and ensuring plant survival under drought conditions *(Liu et al., 2022)*. In this study, although antioxidant enzyme activity increased under PEG stress, MDA content also rose, indicating ROS-induced damage to cells. This finding is consistent with reports in the literature showing that drought stress caused greater oxidative damage to tomato and cucumber plants compared with controls. However, after PEF treatment, MDA content decreased, suggesting that PEF treatment may have prevented membrane damage under stress, thereby maintaining optimal plant growth under adverse conditions.

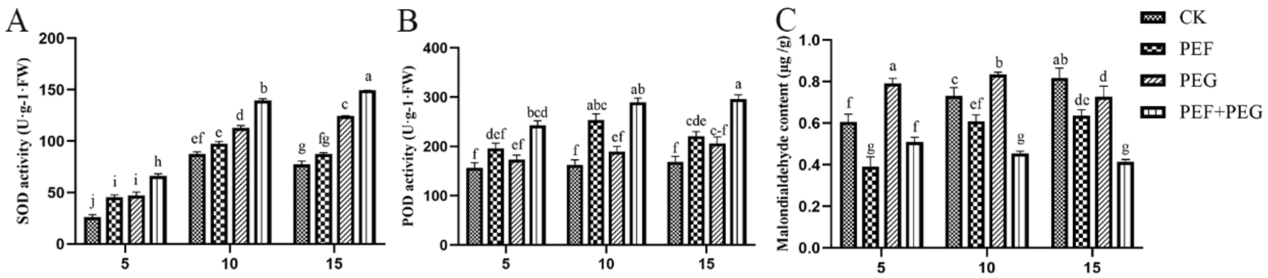

Fig. 2 - Effect of PEF electric field treatment on the activity of oxidative stress indicators in *S. baicalensis* seedlings under 12.5% PEG stress. (A) SOD activity, (B) POD activity and (C) MDA content. Different small letters on vertical bars indicate significant differences between means at the p < 0.05 level

Effect of PEF treatment on SS, SP and Pro contents of seedlings under drought stress

SP is a crucial component of many plant metabolic enzymes in plant cells, including unbound proteins on cellular and organelle membranes. Consequently, their content can predict the growth and physiological status of plants *(Liang et al., 2007).* Starch is hydrolyzed into soluble sugars, which serve as the primary energy source for the early development of nearly all seed embryos. SS can also act as osmoprotectants, enhancing the cell's water absorption and retention under stress conditions. Pro, another osmoprotectant, can increase the relative water content (RWC) of cells, as well as enhance membrane and chlorophyll stability, boost seedling vigor, and play a significant role in overcoming drought stress *(Ghani et al., 2022)*.

Figure 3 shows that under normal culture conditions without PEF treatment, the contents of SS and SP in the seedlings increased over the growth period, while Pro content increased rapidly and remained at a high level. Under 12.5% PEG stress, the SS content in the seedlings was higher than that in the control, and the Pro content was higher on days 5 and 15 (notably so on day 15). SP content was higher between day 5 to 10 but lower on day 15 compared with the control, which might be attributed to the inhibition of metabolic activity as stress duration increased.

Following PEF treatment, the contents of SS, Pro, and SP in the seedlings all increased, with SP content significantly enhanced by 28.89%-79.39%, SS content notably increased by 28.57%-39.13%, and Pro content substantially raised by 29.32%-80.07%.

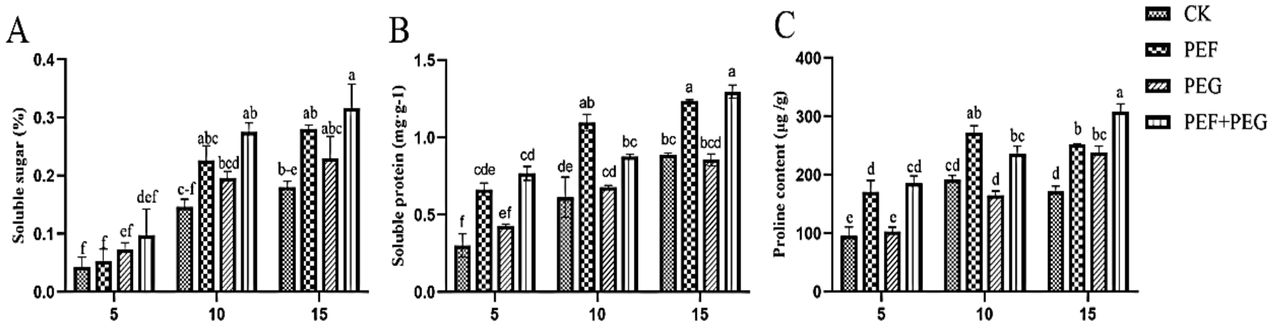

Fig. 3 - Effect of PEF electric field treatment on the content of osmoregulatory substances in stressed *S. baicalensis* seedlings under PEG stress. (A) SS; (B) SP;(C) Pro content. Different small letters on vertical bars indicate significant differences between means at the p< 0.05 level.

Effect of PEF treatment on α-amylase activity of S. baicalensis seedlings under different drought stresses

The energy required for embryo development before seed germination is derived from the hydrolysis of stored nutrients such as starch and lipids within the seeds, a process facilitated by the involvement of hydrolytic enzymes like amylase and lipase. α-Amylase, in particular, can break down starch into SS, which serve as the primary energy source for seed germination and growth. Consequently, an increase in the activity of this enzyme can promote robust early growth and lead to the establishment of healthy crops. Figure 4 shows that under 12.5% PEG stress without PEF treatment, α-amylase activity was higher than in the control, with significant differences observed on days 10 and 15. Following PEF treatment, α-amylase activity in the seedlings under stress was significantly enhanced by 15.92%-53.74% from days 5 to 15. The high activity of α-amylase during the initial germination phase catalyzes the breakdown of starch into polysaccharides, providing the necessary substances and energy for germination. Additionally, the increased content of soluble sugars is beneficial for enhancing the osmotic potential of cells under stress, promoting water uptake and retention capabilities *(Zhang et al., 2022).*

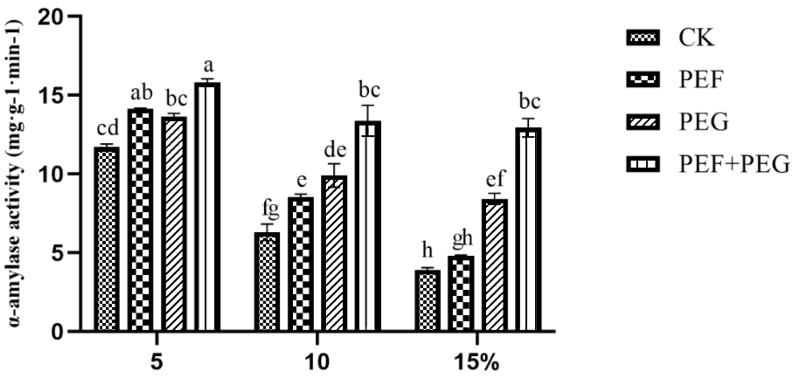

Fig. 4 - Effect of PEF electric field treatment on α-amylase activity in stressed *S. baicalensis* seedlings under 12.5% PEG stress. Different small letters on vertical bars indicate significant differences between means at the p < 0.05 level

Effect of PEF treatment on endogenous hormone contents in S. baicalensis seedlings under 12.5% PEG stress

During seed maturation, endogenous ABA accumulates within the seeds, inducing and maintaining seed dormancy to prevent germination. In contrast, as the germination process initiates, the level of endogenous ABA in the seeds is reduced, while the increase in GA content stimulates seed germination.

Figure 5A and B reveal that, compared with the CK, the PEF-treated seedlings exhibited increased contents of GA and IAA, with GA markedly higher by 15.81%, 32.08%, and 9.14% on days 0, 2, and 10, respectively, and IAA markedly increased by 16.93% and 13.80% on days 2 and 10, respectively. Both GA and IAA reached their peak concentrations on day 2. This is because, during the imbibition process, the embryonic cells promptly resume the metabolic activities necessary for seed growth, synthesizing GA, which induces the production of hydrolases, with α-amylase being the most abundant *(Mumtaz, Javed, Rana, Iqbal, & Choi, 2024)*. Under PEG stress, the contents of gibberellic acid (GA) and auxin (IAA) in seedlings significantly decreased from days 2 to 15 compared with the CK. However, after PEF treatment, the GA and IAA contents in stressed seedlings were lower than the CK group but higher than the non-PEF-treated PEG group from days 2 to 15. Specifically, GA content increased significantly by 23.06%, 13.84%, and 15.77% on days 2, 10, and 15, respectively, compared with the untreated group, while IAA content was notably elevated by 17.88%, 16.62%, and 26.89% on days 2, 5, and 15, respectively, compared with the PEG-treated group. In contrast, the ABA content showed an opposite trend. Compared with CK, the ABA content in PEF-treated seedlings significantly decreased by 15.50%, 10.85%, and 14.78% on days 0, 2, and 15, respectively. Under stress concentrations, the ABA content in the PEG group markedly increased compared with CK, whereas in the PEF+PEG group, the ABA content in seedlings was considerably reduced by 20.50%, 21.03%, 24.99%, and 25.45% on days 2 to 15 compared with the PEG group, although it remained higher than the CK group (Figure 5C).

In a study on barley seeds treated with high-power microwave (HPM), an increase in germination rate was observed, along with increased IAA content and decreased ABA content, which aligns with the findings of this study where PEF pretreatment reduced ABA content and increased IAA and GA levels. In summary, PEF treatment can enhance the contents of GA and IAA in seedlings under normal growth conditions and drought stress, while reducing ABA content, thereby regulating germination and growth.

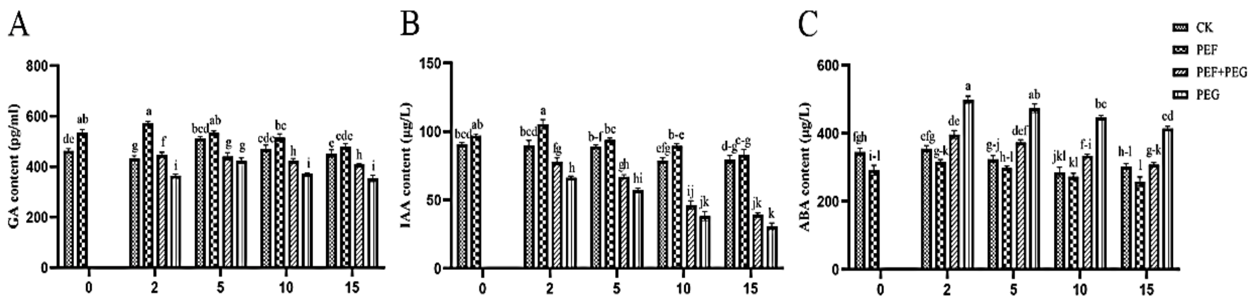

Fig. 5 - Effect of PEF treatment on hormone contents of *S. baicalensis* seedlings under 12.5% PEG stress. (A) GA, (B) IAA,(C) ABA content. Different small letters on vertical bars indicate significant differences between means at the p < 0.05 level.

Microstructural changes during seed germination

Microstructural observations demonstrated that PEF treatment significantly enhanced both germination vigor and drought tolerance in S. baicalensis seeds. After 2 days of culture, PEF-treated seeds (Fig. 6B) displayed accelerated radicle protrusion and more active cell division compared with the control (Fig. 6A).

By the 4th day, this promotive effect was further evidenced by advanced morphological differentiation, including distinct bud and root formation as well as more mature cotyledons in the PEF-treated group (Fig. 6F). Under drought stress (12.5% PEG), seed growth was markedly inhibited, exhibiting tightly packed cotyledon cells and arrested radicle development at both day 2 (Fig. 6C) and day 4 (Fig. 6G).

In contrast, PEF pretreatment effectively counteracted this stress-induced inhibition. The PEF+PEG-treated seeds not only showed alleviated radicle growth arrest at day 2 (Fig. 6D), but also developed key adaptive features—such as thickened cell walls in the embryonic axis and enhanced radicle differentiation—by day 4 (Fig. 6H), which collectively contributed to improved stress resistance.

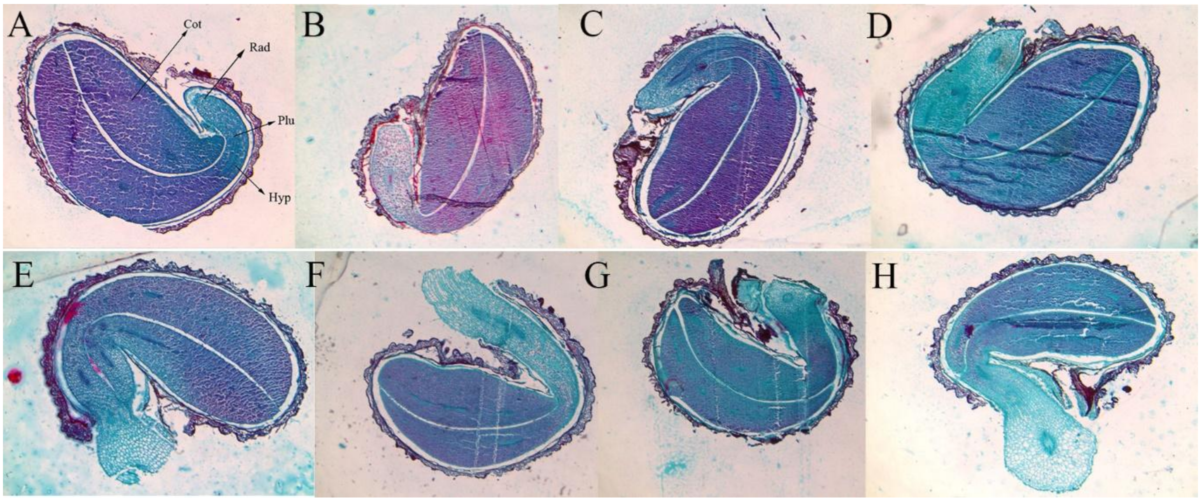

Fig. 6 - PEF *S. baicalensis* seed microstructure. (A) CK 2-day, (B) PEF-treated 2-day, (C) PEG 2-day, (D) PEF+PEG-treated 2-day, (E) CK 4-day, (F) PEF-treated 4-day, (G) PEG 4-day, (H) PEF+PEG-treated 4-day *S. baicalensis* seeds. Cot: cotyledon, Rad: radicle, Plu: plumule, Hyp: hypocotyl

CONCLUSIONS

Drought is one of the crucial ecological factors affecting the growth and final yield of traditional Chinese medicinal herbs. This study simulated drought stress using PEG-6000 and investigated the drought resistance responses of *S. baicalensis* seeds and seedlings to PEF pretreatment. Under 12.5% PEG-6000 stress, compared with the untreated group, PEF treatment notably promoted seed germination and seedling growth, enhancing their metabolic capacity and stress resistance. Among them, the activities of SOD and POD enzymes were significantly increased by 20.13%-40.13% and 40.00%-52.69%, respectively; the content of MDA significantly decreased by 35.44%-45.78%, enhancing antioxidant activity and reducing the damage caused by adversity. Soluble protein content considerably increased by 28.41%-79.07%, soluble sugar content increased by 28.57%-39.13%, proline content considerably rose by 29.32%-80.07%, and α-amylase activity markedly enhanced by 15.91%-53.74%, strengthening membrane stability while promoting metabolism; gibberellic acid content notably increased by 23.06%, 13.84%, and 15.77% on days 2, 10, and 15, respectively, while auxin content notably rose by 17.88%, 16.62%, and 26.89% on days 2, 5, and 15, respectively. Abscisic acid content dramatic decreased by 20.50%-25.45% from days 2 to 15. This broke seed dormancy, promoted seed germination, accelerated cell division, and maintained seedling growth under stress. In summary, PEF pretreatment can enhance the drought stress response capacity of *S. baicalensis* during germination and early seedling growth, serving as an effective treatment technique to improve the resilience of traditional Chinese medicinal herbs and ensure their healthy and sustainable development.

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