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Effect of Pulsed Electric Field Treatment on Seed Germination and Seedling Growth of *Scutellaria baicalensis*

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Abstract: To explore the effects of pulsed electric field treatment on the germination of *Scutellaria baicalensis* seeds and the growth of seedlings, this study used the response surface methodology to design the working parameters of the pulsed electric field and treated and cultured *Scutellaria baicalensis* seeds. The results showed that the pulsed electric field treatment was beneficial for the germination of *Scutellaria baicalensis* seeds, improving the metabolic activity and stress resistance of seedlings. When the pulsed electric field treatment's parameters were 0.5 kV·cm^{-1}, 120 µs, and 99 pulses, the germination potential of seeds was significantly increased by 29.25% and the germination index significantly increased by 20.65%, compared to the control. From 5th to 15th day, the activities of SOD, POD, and α-amylase in the seedlings, and the contents of Pro, soluble sugars, and soluble proteins were significantly increased, compared to the control. This study provides a theoretical basis for improving the germination and seedling growth of medicinal herbs such as *Scutellaria baicalensis* and their practical application in production.

Keywords: PEF; *Scutellaria baicalensis* seeds; germination potential; enzymes

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1. Introduction

Scutellaria baicalensis is a plant of the Lamiaceae family. Its dried roots are a frequently used medicinal material in China, with anti-inflammatory, antibacterial, antiviral, anticancer, antioxidant, and hepatoprotective functions [1,2]. With the increasing demand for wild resources in domestic and international markets, wild resources can no longer meet the market demand, and cultivation has become an important source [3]. However, the ability of seeds to emerge from the soil after germination is weak, making them susceptible to drought, and the difficulty in emergence, low emergence rate, and relatively slow growth after emergence [4] hinder the development of production. Improving the emergence rate of *Scutellaria baicalensis* seeds and their resistance during the seedling stage is an important aspect of the *Scutellaria baicalensis* industry development.

Physical, chemical, and biological methods are commonly used to promote seed germination [5]: friction, immersion, change in temperature, light, etc. are examples of physical methods [6]; the use of plant growth hormone, strong acids and alkalis, and other reagents are examples of chemical methods [7]; while biological methods involve the use of microbial metabolites and plant extracts [8,9]. Among them, the physical method and biological method processes are complex and time-consuming, and, although the chemical method is easy to operate, it will result in reagent residues, environmental pollution, and even ecological damage. Therefore, the search for a simple, efficient, green, and environmentally friendly new method of seed treatment has become an important topic in agricultural production [10,11].

In production, hot water immersion and chemical reagent treatment were commonly used to improve the germination rate of *Scutellaria baicalensis* seeds, but they had limited effects on the seedling emergence rate and seedling growth. The pulsed electric field (PEF), as a new physical technology, has been extensively applied in food, medical treatment, and agriculture, for example, as a pre-processing technology for food processing to improve the drying rate of fruits and vegetables [12], fruit juice yield, and plant active substance yield, kill harmful bacteria, and extend food shelf life. In recent years, it has been found that the treatment of crop seeds with a PEF can produce significant biological effects, promote wheat seed germination, and improve seed viability and stress tolerance [13]; promote *Arabidopsis* seed germination and leaf expansion [14]; and promote the germination of kale seeds, root growth, and the quality of kale [15]. However, there is limited research on the application of PEF treatment technology in the production of Chinese medicinal materials, and the germination and seedling growth of medicinal materials directly affect the growth of plants in the later stage and the final yield and quality of medicinal materials [16]. Based on this, this paper combines PEF treatment technology with *Scutellaria baicalensis* seeds, after applying an electric field to *Scutellaria baicalensis* seeds, to measure their germination indices and examine the biological impacts of the therapy on the seeds. Subsequently, the mechanism of the biological impact of an electric field on *Scutellaria baicalensis* seeds was then investigated by the measurement of the seeds' electrical conductivity (EC) during imbibition and the corresponding antioxidant and metabolic indices during the germination and growth stages, in order to provide a theoretical basis for the standardized artificial cultivation technology to improve the seedling emergence rate of *Scutellaria baicalensis*, promote the growth of seedlings, improve the stress resistance of plants, and solve the problems of the difficult emergence of *Scutellaria baicalensis* seedlings and weak stress resistance during the seedling stage.

2. Materials and Methods

2.1. Experimental Materials

The *Scutellaria baicalensis* seeds (with a moisture content of 4.90% $\pm$ 0.013) were generously provided by Industrial Crop Research Institute, Shanxi Agricultural University.

The main research instruments include: ECM830 pulse generator (BTX Corporation, Holliston, MA, USA); Multiskan Go-1510 full-wavelength microplate reader (Thermo Fisher Scientific Oy Ratastie 2, Fl-01620, Vantaa, Finland); DDSJ-308A Conductivity Meter (Shanghai Precision Scientific Instrument Co., Ltd., Shanghai, China); and JS-2000W-A AC power voltage converter (Shanghai Jingsai Electronic Technology Co., Ltd., Shanghai, China).

The reagents used in the experiment include: coomassie brilliant blue G-250, nitroblue tetrazolium chloride (NBT), ethylenediaminetetraacetic acid disodium salt (EDTA-Na_2), 5-sulfosalicylic acid dihydrate, L-Proline, and bovine serum albumin (BSA) (Beijing Solaibao Technology Co., Ltd., Beijing, China); DL-methionine and riboflavine (Shanghai Yi En Chemical Technology Co., Ltd., Shanghai, China); 2-methoxyphenol (Shanghai Aladdin Biochemical Technology Co., Ltd., Shanghai, China); hydrogen peroxide (Tianjin Damao chemical reagent factory, Tianjin, China); ninhydrin hydrate and sodium citrate (Tianjin Beichen Founder reagent factory, Tianjin, China); citric acid, phenol, and sodium phosphate monobasic dihydrate (Tianjin Kaitong Chemical reagent Co., Ltd., Tianjin, China); 3,5-Dinitro-2-hydroxybenzoic acid (Shanghai Zhanyun Chemical Co., Ltd., Shanghai, China); sodium potassium tartrate, starch, and sucrose (Tianjin Fengboat Chemical reagent Technology Co., Ltd., Tianjin, China); concentrated sulfuric acid (Anhui Jin Yue Guan new material Technology Co., Ltd., Huaibei, China); acetic acid glacial (Tianjin Dengfeng Chemical Co., Ltd., Tianjin, China); disodium hydrogen phosphate dodecahydrate (Tianjin Tianda chemical experiment factory, Tianjin, China); and xylene (Chengdu Jinshan Chemical Reagent Co., Ltd., Chengdu, China); all of which are analytical grade.

2.2. Seed Treatment and Cultivation

Select plump and complete *Scutellaria baicalensis* seeds and soak them in water at 40 °C for 12 h. Randomly select 50 seeds after soaking, place them in an electrode cup with a 4 mm spacing, add distilled water, and ensure the liquid level and the electrode sheet are highly consistent. According to the parameters in Table 1, use a BTX ECM830 Square Wave Electroporation System (BTX, Holliston, MA, USA) based on the protocol described previously [12,17]; apply PEF treatment to the seeds, then place them on the germination bed (the germination bed is composed of a 15 cm diameter dish and a layer of filter paper at the bottom of the dish), add 5 mL distilled water, cover with lid, and place it in 22 °C incubator for dark cultivation, using seeds without treatment as the control. Repeat each treatment 3 times, and record every day.

Table 1. PEF treatment parameter types and parameter settings of single-factor experiment.

	Fixed Value	Single Variable						
1	Pulse width: 50 Number of pulses: 50	0	0.5	1	1.5	2	2.5	Pulse intensity ($kV\cdot cm^{-1}$)
2	Pulse intensity: 0.5 Number of pulses: 50	0	40	80	120	160	200	Pulse width (μs)
3	Pulse intensity: 0.5 Pulse width: 40	0	20	40	60	80	99	Number of pulses (*n*)

2.3. Experimental Design

The experiment of PEF treatment is designed based on the instrument performance, seed tolerance, and preliminary experiment results. Based on the results of the preliminary tests, the Box–Behnken design (BBD) with the software package Design expert 10 was used to conduct a three-factor, three-level response surface design experiment on the pulse parameters (Table 2). The germination potential was used as the response value, with each treatment repeated 3 times.

Table 2. PEF treatment parameter types and parameter settings.

Pulse Intensity ($kV\cdot cm^{-1}$)	Pulse Width (μs)	Number of Pulses (*n*)
0.5	40	20
1.25	120	60
2	200	99

2.4. Statistics of Seed Germination Indices

The radicle breakthrough seed coat of 1 mm was used as the seed germination standard. The germination potential (*GP*) [18] is counted on the 2nd day of the germination test, the germination rate (*GR*) [19] is counted on the 5th day, and the germination test is completed; then, the germination index (*GI*) and the average germination time (*MGT*) [20] are calculated. The specific calculation formulae are as follows:

$$GP = \frac{m_1}{50} \times 100\% \quad (1)$$

$$GR = \frac{m_2}{50} \times 100\% \quad (2)$$

$$GI = \sum G_t \div D_t \quad (3)$$

$$MGT = \sum \frac{n \times d}{N} \quad (4)$$

where m_1 is the number of germinated seeds on 2nd day, m_2 is the number of germinated seeds on 5th day, t and d are the number of days that have elapsed since the germination test, G is the number of seeds germinated on the t day, D is the corresponding germination days, n is the number of seeds germinated per day, and N is the total number of seeds germinated at the end of the test.

2.5. Determination of Seed Conductivity

Seeds treated with PEF and the control were put into a dry beaker; 20 mL distilled water was immediately added, sealed with plastic wrap, placed in an incubator at 25 ± 0.5 °C, and the conductivity was determined by a conductivity meter at 0.1, 4, 12, 24, and 48 h [20]. Each treatment included 100 seeds and is repeated 3 times.

$$EC = \frac{E_1 - E_2}{s} \quad (5)$$

where E_1 is the EC of samples, E_2 is the EC of water, and s is the weight of samples.

2.6. Determination of Physiological Indices of Seedlings

On the 5th, 10th, and 15th day of culture, seedlings were taken to measure the indices. Among them, the activity of superoxide dismutase (SOD) and peroxidase (POD) were analyzed following Liu Hai et al. [7], with a slight change: 0.1 g fresh tissues were fully homogenized with 1.5 mL (pH = 7.8) phosphate buffer, then centrifugated at 12,000 rpm for 20 min. The whole process is at 4 °C, SOD activity was determined by nitroblue tetrazolium assay, and POD activity was determined by guaiacol method. The content of proline (Pro), soluble sugar, and soluble protein were analyzed following Li Haipeng et al. [18,21,22], with a slight change; details are as follows: soluble protein extraction was performed as described before, the content was determined by Coomassie brilliant blue G-250 staining, and light absorption was measured using a spectrophotometer at 595 nm. Pro content determination is as follows: use 0.1 g of fresh tissues, add 1 mL of 3% 5-sulfosalicylic acid dihydrate (w/v), place in boiling water bath for 10 min; after cooling, perform centrifugation at 10,000 rpm for 15 min, at 4 °C, and then take 0.5 mL of supernatant and add 0.5 mL glacial acetic acid and 0.5 mL 2.5% ninhydrin hydrate (w/v), place in boiling water bath for 30 min, then add 1 mL xylene; after eddy current oscillation for 30 s, the supernatant was taken and xylene was used as blank control, and light absorption was measured using a spectrophotometer at 520 nm. Soluble sugar extraction is as follows: use 0.1 g of fresh tissues, add 1 mL of distilled water, place in boiling water bath for 30 min; after cooling, perform centrifugation at 2000 rpm for 15 min. The content was determined by phenol-sulfuric acid method; the activity of α-amylase was analyzed following Wang Xuekui et al. [23], with a slight change: use 0.1 g fresh tissues, add 1 mL of (pH = 5.6) citrate buffer solution, then perform centrifugation at 12,000 rpm for 10 min, at 4 °C; the activity was determined by DNS method.

2.7. Statistical Analysis

Microsoft Excel 2016 was used to perform preliminary arrangement of the data, IBM-SPSS 20.0 was used to analyze the data, and GraphPad Prism 9.3 was used to draw figure. The differences were examined by one-way analysis of variance (ANOVA), followed by the new multiple-range (Duncan) test ($p \leq 0.05$). Values are given as mean ± SD.

3. Results

3.1. Study on the Effect of Different Pulse Intensities on Seeds

When the pulse width was 50 μs, the number of pulses was 50 pulses, and the effects of PEF treatment with different pulse intensities on the seed germination indices of *Scutellaria baicalensis* are shown in Table 3. The GP of seeds treated with an electric field of 0.5–2.5 kV·cm^{-1} pulse intensity increased by 1.5–9% compared to the untreated seeds, and, except for the 2.0 kV·cm^{-1} treatment, the other treatments reached significant to extremely significant levels. The GR of seeds decreased under electric field treatment; the inhibition reached a significant level with a 2.5 kV·cm^{-1} high-pulse-intensity treatment. The germination index was significantly higher than that of the control group at a low electric field intensity of 0.5 kV·cm^{-1}, and decreased significantly under the high-intensity treatment of 2.5 kV·cm^{-1}. MGT was significantly shortened compared with the control under a 0.5 kV·cm^{-1} electric field strength treatment, while the differences in the other treatments were not significant. Overall, according to Table 3, low-intensity electric field treatment can improve the germination potential of *Scutellaria baicalensis*, enhance the germination index, and shorten the germination.

Table 3. The effects of different pulse intensities on the germination of *Scutellaria baicalensis* seeds.

Pulse Intensity (kV·cm^{-1})	GP (%)	GR (%)	GI	MGT (d)
0	70.50 ± 1.50	87.10 ± 0.58	51.74 ± 0.34	2.04 ± 0.01
0.5	79.50 ± 1.50 ***	87.00 ± 2.50	55.27 ± 0.71 **	1.82 ± 0.09 *
1	75.50 ± 2.50 **	83.00 ± 2.50	51.04 ± 0.79	1.93 ± 0.05
1.5	76.50 ± 1.50 ***	83.50 ± 20	50.76 ± 0.50	1.92 ± 0.05
2	72.00 ± 1	83.42 ± 1.08	49.08 ± 0.26	1.96 ± 0.02
2.5	74.00 ± 1.00 *	80.50 ± 0.50 ***	45.42 ± 1.37 ***	1.98 ± 0.06

The '*' means $p < 0.05$; ** means $p < 0.01$; *** means $p < 0.0005$.

3.2. Study on the Effect of Different Pulse Widths on Seeds

When the pulse intensity was 0.5 kV·cm^{-1}, the number of pulses was 50 pulses, and the effects of PEF treatment with different pulse widths on the seed germination indices of *Scutellaria baicalensis* are shown in Table 4. The GP of seeds increased by 9.10–21.35% compared with the control after treatment with a pulse width of 40–200 µs, reaching a significant level, among which the GP reached a maximum of 77.3% ± 1.25 when the pulse width was 160 µs. Except for the 200 µs treatment, the GR was slightly lower than that of the control; the rest of the treatments were higher than the control. The GI was significantly higher than that of the control with a pulse width of 40 µs, and significantly lower than that of the control when the pulse width reached 160 µs and 200 µs. Except for the 80 µs treatment, the MGT of all other treatments was reduced.

Table 4. The effects of different pulse widths on the germination of *Scutellaria baicalensis* seeds.

Pulse Width (µs)	GP (%)	GR (%)	GI	MGT (d)
0	63.70 ± 1.25	83.30 ± 1.70	41.05 ± 0.43	2.86 ± 0.11
40	74.30 ± 2.05 ***	86.00 ± 1.00	51.26 ± 0.17 ***	2.71 ± 0.63
80	69.50 ± 0.50 **	84.70 ± 1.70	40.79 ± 0.97	2.94 ± 0.23
120	71.50 ± 0.50 ***	86.00 ± 0.82	40.58 ± 0.63	2.69 ± 0.36
160	77.30 ± 1.25 ***	86.30 ± 0.94	36.02 ± 0.62 ***	2.36 ± 0.95
200	74.50 ± 0.50 ***	82.70 ± 1.25	37.61 ± 0.73 *	2.50 ± 0.78

The '*' means $p < 0.05$; ** means $p < 0.01$; *** means $p < 0.0005$.

3.3. Study on the Effect of Different Pulse Numbers on Seeds

When the pulse intensity was 0.5 kV·cm^{-1}, the pulse width was 40 µs, and the effects of the electric field treatment with a different number of pulses on the seed germination indices of *Scutellaria baicalensis* are shown in Table 5. The GP of seeds treated with different pulse numbers was significantly higher, with an increase of 19.18–26.23% compared to the control. The treatment with 20 pulses had the highest germination potential at 77% ± 1. Except for the treatment with 40 pulses, the GR of all other treatments were higher than the control. The GI of each treatment was significantly different from that of the control, with the treatment of 20 pulses showing a 19.57% increase compared to the control, and the treatment of 40 pulses showing a 15.29% decrease compared to the control. The MGT of the treatment with 40 pulses was longer than that of the control, while the rest of the treatments had shorter germination times, but the differences were not significant.

Table 5. The effects of different pulse numbers on the germination of *Scutellaria baicalensis* seeds.

Number of Pulses (*n*)	GP (%)	GR (%)	GI	MGT (d)
0	61.00 ± 0.50	83.67 ± 0.94	58.32 ± 0.34	2.86 ± 0.17
20	77.00 ± 1.00 ***	86.00 ± 0.82	62.02 ± 0.71 ***	2.71 ± 0.54
40	72.50 ± 0.25 ***	83.67 ± 0.47	49.40 ± 0.96 ***	2.94 ± 0.65
60	73.50 ± 0.25 ***	85.00 ± 0.82	52.47 ± 0.52 ***	2.69 ± 0.35
80	72.70 ± 1.64 ***	85.67 ± 1.25	50.48 ± 0.27 ***	2.36 ± 0.68
99	75.70 ± 1.66 ***	84.33 ± 1.25	50.20 ± 0.38 ***	2.50 ± 0.45

The '***' means $p < 0.0005$.

3.4. Using the Response Surface Method to Optimize the Parameters of PEF Treatment

The effect of a preliminary PEF treatment on seeds showed that the PEF had a significant effect on the GP and the GP could indicate the vitality and germination speed of the seeds [24,25]. Therefore, the response surface test was carried out with the GP as the response value Y, with the pulse intensity (A), pulse width (B), and number of pulses (C) as the independent variables; the results are shown in Table 6. The Box–Behnken design (BBD) with the software package Design expert 10 is used to perform multiple regression analysis on the obtained data and the polynomial equation of the relationship between GP and three factors is obtained:

$$Y = 40.30 - 1.69A + 0.063B + 1.50C - 0.63AB + 0.75AC - 1.75BC - 1.21A^2 - 1.71B^2 + 0.16C^2 \quad (6)$$

Table 6. Response surface test and results.

Test No.	Pulse Intensity (kV·cm^{-1})	Pulse Width (µs)	Number of Pulses (*n*)	GP (%)
1	1.25	200	20	78%
2	0.5	120	99	84%
3	1.25	120	60	80%
4	1.25	40	99	84%
5	1.25	40	20	72%
6	1.25	120	60	84%
7	2	40	60	72%
8	1.25	200	99	76%
9	1.25	120	60	78%
10	2	120	99	80%
11	1.25	120	60	79%
12	0.5	120	20	80%
13	1.25	120	60	82%
14	0.5	40	60	76%
15	0.5	200	60	80%
16	2	120	20	70%
17	2	200	60	71%

The results of ANOVA (Table 7) show that the F value of the model is 8.35 and the p value is 0.0053 < 0.05, indicating that the model is significant; the p value of the lack of fit is 0.8238 > 0.05, which is not misfitted, indicating that the model is established and fits well with the actual situation. The coefficient of determination $R^2 = 0.9148$ further proves that the model has a high fit, which can truly reflect the relationship between the three factors and the GP and can reasonably predict the germination effect of the pulse treatment on *Scutellaria baicalensis* seeds. The three factors that influence the GP, in order, are pulse intensity > pulse number > pulse width, with pulse intensity (A) and pulse number (C) having a significant effect.

Table 7. Results of variance analysis.

Source of Variation	F Value	p Value	Sig.
Model	8.35	0.0053	Significant
A	22.42	0.0021	**
B	0.031	0.8658	
C	17.72	0.0041	**
AB	1.54	0.2549	
AC	2.21	0.1803	
BC	12.06	0.0104	*
A2	6.09	0.0429	*
B2	12.15	0.0102	*
C2	0.11	0.7505	
Lack of fit	0.30	0.8283	Not significant

The '*' means $p < 0.05$; ** means $p < 0.01$.

3.5. Using the Response Surface Method to Optimize the Parameters of PEF Treatment

Through a regression model analysis, three groups of parameters with the highest (H), medium (M), and lower (L) GP were selected for validation, with each treatment repeated three times. The results are shown in Table 8. The predicted values are basically consistent with the experimental values, indicating that the parameters model obtained from the response surface optimization can be used to predict the pulse parameters of the PEF.

Table 8. Verification results of PEF parameters for promoting seed germination.

Test No.	Pulse Intensity ($kV \cdot cm^{-1}$)	Pulse Width (μs)	Number of Pulses (n)	Predicted Values	Experimental Values
CK	0	0	0	0.66	0.66 ± 0.05
H	0.5	120	99	0.85	0.85 ± 0.02
M	0.5	40	60	0.76	0.73 ± 0.04
L	1.705	59	28	0.71	0.71 ± 0.02

3.6. Effects of PEF Treatment on Seed Germination

As shown in Table 9, compared with the control, the GP and GI were significantly increased in the three groups, and Group H was higher than Group M, and Group M was higher than Group L. The two indicators of Group H increased by 29.25% and 20.65%. The GR only increased slightly in the Group H; both Group M and L were significantly lower than controls. MGT was shorter than the control; the difference was not significant.

Table 9. Effect of different pulse parameters on seed germination of *Scutellaria baicalensis*.

Test No.	GP (%)	GR (%)	GI	MGT (d)
CK	65.50 ± 0.05	87.34 ± 0.47	27.41 ± 0.24	1.94 ± 0.16
H	84.66 ± 0.02 ***	88.66 ± 1.70	33.07 ± 0.23 ***	1.53 ± 0.23
M	72.50 ± 0.04 ***	79.00 ± 2.60 **	29.19 ± 0.42 **	1.43 ± 0.41
L	71.00 ± 0.02 ***	81.00 ± 0.50 *	28.85 ± 0.36 *	1.54 ± 0.33

The '*' means $p < 0.05$; ** means $p < 0.01$; *** means $p < 0.0005$.

3.7. Determination of Electrical Conductivity of Scutellaria baicalensis Seeds

As shown in Table 10, the conductivity of each group increased with the prolongation of the measurement time; the conductivity of each treatment group at different times was significantly lower than that of the control. The conductivity of treatment groups was the lowest at 0.1 h, with reductions of 54.50%, 55.67%, and 64.60% compared to the control and showing minimal changes from 24 h to 48 h, while the control continued to increase rapidly. Some studies [26] suggest that electric field treatment can promote cell membrane repair and improve membrane stability, which can explain the consistently low seed conductivity of *Scutellaria baicalensis* under PEF treatment in this study.

Table 10. Effects of PEF treatment on the electrical conductivity of *Scutellaria scutellaria* seeds.

Measurement Time/h	Electrical Conductivity/μs·cm^{-1}·g^{-1}			
	CK	H	M	L
0.1	31.67 ± 0.94	14.41 ± 0.94 ***	14.04 ± 0.53 ***	11.21 ± 0.30 ***
4	65.06 ± 0.23	50.59 ± 0.21 ***	48.93 ± 0.31 ***	49.21 ± 0.44 ***
12	96.89 ± 1.02	74.65 ± 0.51 ***	76.99 ± 0.83 ***	75.99 ± 0.66 ***
24	137.59 ± 0.77	106.82 ± 0.49 ***	109.21 ± 0.62 ***	110.01 ± 0.45 ***
48	167.08 ± 0.48	118.28 ± 0.35 ***	120.28 ± 0.38 ***	119.98 ± 0.29 ***

The '***' means $p < 0.0005$.

3.8. PEF Treatments Affect the Activities of SOD and POD in Seedlings

As shown in Figure 1a,b, after treatment, the enzyme activity in seedlings were higher than those in the control on the 5th–15th day after germination. In the H group, the SOD activity was significantly increased by 78.21%, 7.38%, and 4.85% on the 5th, 10th, and 15th day; the POD activity significantly increased by 126.45% and 71.88%, on the 10th and 15th days. In the M group, the SOD activity reached a significant level on the 5th and 10th day, while the POD activity significantly increased on the 10th and 15th day. In the L group, the SOD activity was only significantly higher than the control on the 5th day, while the POD activity was significantly higher than the control on the 15th day. After treatment, the SOD activity increased earlier than POD, which is related to their functions. SOD is the first enzyme in the antioxidant system to defense, which catalyzes the superoxide anions into H_2O_2 and O_2, while POD is responsible for converting H_2O_2 into H_2O, thus reducing the damage of free radicals to cells [7].

3.9. PEF Treatments Affect the Contents of Soluble Sugars and Soluble Proteins in Seedlings

As shown in Figure 1c,d, after treatments, the contents of soluble sugars and soluble proteins showed an increasing trend and were higher than those in the control. In the H group, the content of soluble proteins was significantly higher than that in the control, with increases of 77.13%, 69.32%, and 40.66% on the 5th–15th day; the content of soluble sugars was significantly higher than the control on the 10th and 15th day, with increases of 95.32% and 90.63%. In the M group, compared to the control, the content of soluble sugars was significantly higher on the 10th day, and the content of soluble proteins was significantly higher on the 5th and 10th day. In the L group, there was no significant difference in the content of soluble sugars compared to the control; the content of soluble proteins in the seedlings was significantly higher than the control on the 5th day, compared to the control.

3.10. PEF Treatments Affect the Activities of α-Amylase in Seedlings

As shown in Figure 1e, with the growth of the seedlings, the activity of α-amylase in the control and different treatments showed a trend of high to low, and then tended to stabilize. In the H and M groups, the enzyme activities on the 10th and 15th day were significantly higher than that in the control, with increases of 46.74% and 38.85% on the 10th day, and 66.51% and 45.99% on the 15th day; in the L group, it was significantly higher than the control on the 15th day. The high activity of α-amylase in the early stage of germination can catalyze the decomposition of starch into polysaccharides, providing materials and energies for germination. When the cotyledons have unfolded on the 10th day of growth, photosynthesis can take place; at the same time, the starch in the seed is consumed in large quantities, so the enzyme activity decreases. However, after the cotyledons have unfolded, the PEF treatment significantly increases the enzyme activity, indicating that PEF treatment can accelerate carbohydrate metabolism and promote growth. These results also indicate that, during the germination of *Scutellaria baicalensis* seeds, starch and sugar metabolism are the main processes [27].

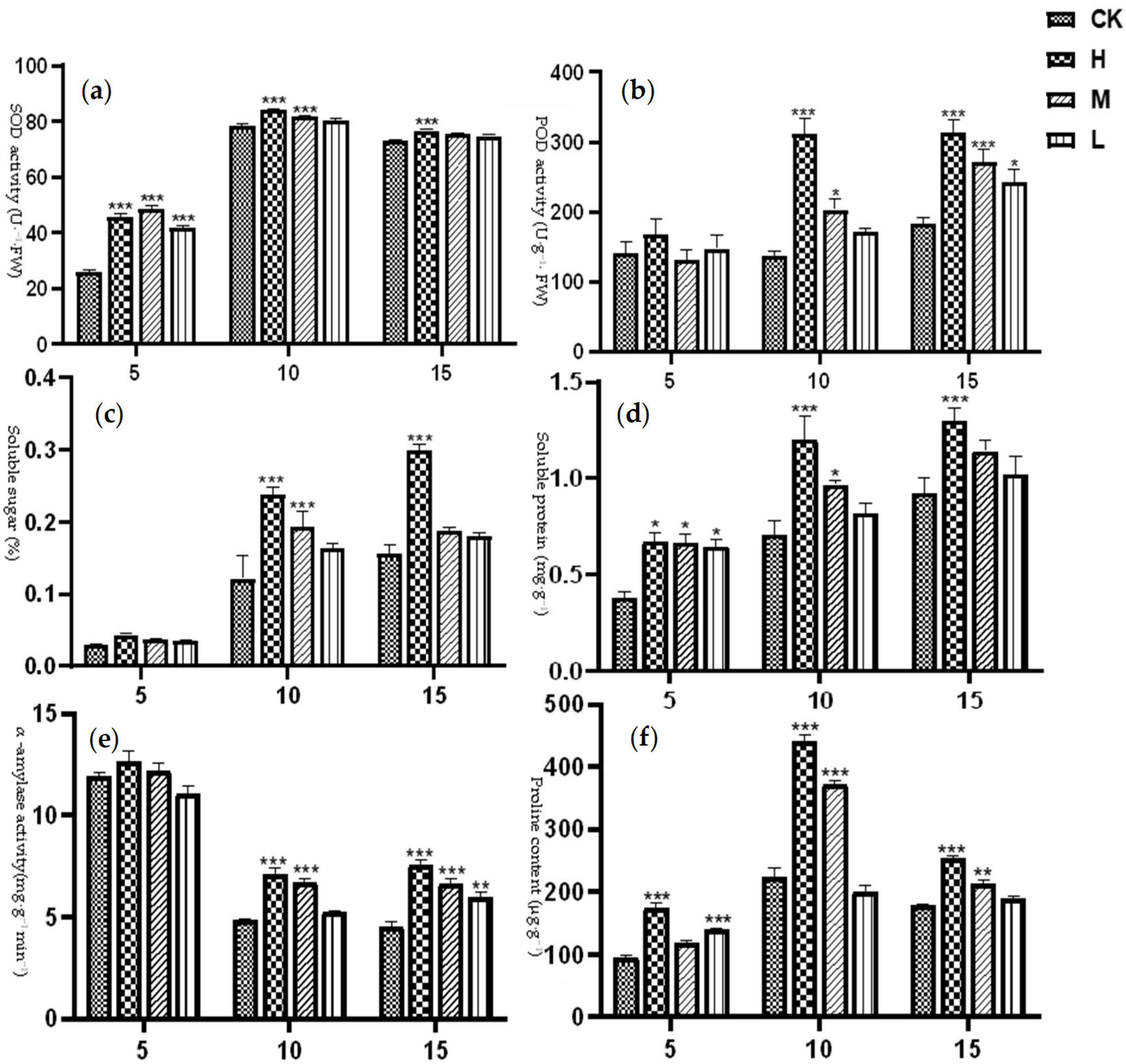

Figure 1. Effects of different PEF treatments on metabolism of seedlings: (**a**) the activities of SOD in seedlings; (**b**) the activities of POD in seedlings; (**c**) the content of soluble sugars in seedlings; (**d**) the content of soluble proteins in seedlings; (**e**) the activities of α-amylase in seedlings; and (**f**) the content of proline in seedlings. The '*' means $p < 0.05$; ** means $p < 0.01$; *** means $p < 0.0005$.

3.11. Effects of Different PEF Treatments on the Content of Proline in Seedlings

As shown in Figure 1f, the content of proline showed a trend of first increasing and then decreasing after treatment. In the H and M groups, the content of proline was significantly higher than that in the control, on the 5th, 10th, and 15th day, and, in the H group, it was higher than that in the other groups. In the H and M groups, it was 84.00% and 24.50% higher than the control on the 5th day, 97.31% and 66.77% higher than the control on the 10th day, and 41.95% and 19.29% higher than the control on the 15th day, while there was no significant difference in the content of proline of the L group compared to the control.

4. Discussion

4.1. Suitable PEF Treatment Can Promote Seed Germination of Scutellaria baicalensis

Seed imbibition and germination involve a series of physiological and biochemical processes, which can be regulated by PEF technology to affect seed germination and subsequent growth [28]; many studies have also shown that using an electric field to treat seeds is an effective technology to improve the seed vitality and emergence rate [29]. The beneficial biological effects can be produced by treating seeds of a certain state (including size, water content, and vitality) with the appropriate electric field type and process parameters. When using low-frequency high-voltage PEF treatment of aged rice seeds with a voltage of 13 kV and time of 32.72 min, the GP, GR, GI, and VI of seeds can be significantly improved [30]. Akdemir et al. found that, when wheat seeds were treated with PEF at 1.07–17.28 J, the GR and emergence rates were significantly increased by 10% and 28% [20]. All of these studies used dried seeds, whereas Sze Ying Leong et al. [28] studied the treatment of wheatgrass (*Triticum aestivum* L.) seeds with different water contents by the PEF, and found that, when the water content of the seeds reached 45% or higher, the PEF had greater metabolic stimulation on the seeds and increased the activities of antioxidant enzymes such as glutathione (GSH) SOD and CAT. This may be related to the fact that the resistance of the seed decreases with the increasing water content, allowing the PEF to be better distributed around or within the seed, resulting in the embryo contained within the seed receiving greater electrical stimulation. In this paper, we treated fully absorbent *scutellaria baicalensis* seeds with a PEF; when the treatment parameters were 0.5 kV·cm^{-1}, 120 μs, and 99 pulses, SOD and POD activities significantly increased by 78.21% and 126.45%, the GP and GI significantly increased by 29.25% and 20.65%, and the MGT reduced by 21.23%. The treatment promoted the antioxidant enzyme activities of the seeds, significantly improved the GP and GI, and shortened the germination time, which was conducive to the rapid and orderly emergence of *Scutellaria baicalensis* seeds and the resistance to various factors that were advised for germination in the field, laying a good foundation for later growth.

4.2. PEF Treatment Can Accelerate Cellular Metabolism in Scutellaria baicalensis Seeds

The energy in the growth process of seeds before germination and cotyledon development comes from the hydrolysis of stored nutrients such as starch and fat in seeds. Amylase and lipase, which hydrolyze these macromolecular substances, play a crucial role in the early growth and development of embryos; the increase in the activity of these enzymes can make plants grow vigorously in the early stage and form good crops [31]. α-amylase can hydrolyze starch to soluble sugars; it is the main source of energy for the germination and growth of almost all seeds. As the seeds absorb water and expand, the embryo synthesizes gibberellin and then induces α-amylase production, thus accelerating seed germination. Seed germination was accelerated when treated with an alternating electric field, which may be related to the induction of gibberellin and α-amylase synthesis by electric field treatment, which is similar to the results of this study [13,32]. This study showed that the activity of α-amylase in seedlings was highest on the 5th day, and it speaks volumes about that. But the soluble sugars content was very low on the 5th day; this indicates that the soluble sugars formed by starch hydrolysis are used for respiration and various metabolic activities, providing materials and energy for cell growth and metabolism. Subsequently, due to the extensive consumption of starch, the activity of α-amylase began to decline. On the 10th day, the cotyledons unfolded to conduct photosynthesis and synthesize carbohydrates, providing continuous energy for seedlings; the activity of α-amylase remained at a stable level. PEF treatment significantly increased α-amylase activity, soluble sugar content after cotyledon development, and soluble protein content during the whole growth process. The soluble protein content of plants can predict the growth status and physiological condition of plants [7]; it is an important component of many plants' metabolic enzymes, including unbound proteins on the membranes of cells and organelles [33]. Zahoor Ahmed et al. also found that the soluble protein content of wheat seedlings increased by 16.76% when treated with 6 kV·cm^{-1} and 50 pulses [20]. In the H group, the soluble proteins content in seedlings on the 5th–15th day was 77.13%, 69.32%, and 40.66% higher than the control group. In this study, the electric field treatment increased the metabolic enzyme activity of *Scutellaria scutellaria* seeds and enhanced the cell metabolism, which laid a material foundation for the early good growth of *Scutellaria scutellaria* seedlings.

4.3. PEF Treatment Improved the Functional Stability of Seedling Cells after Germination

Some enzymatic reactions in plant cells, oxidation of biological substances, and self-respiration can produce free radicals, especially under adversity, forming a large number of free radicals. Free radicals can induce the lipid peroxidation of the cell membrane, cause damage to the plasma membrane, and even attack nucleic acids and proteins in cells. The SOD enzyme is the first enzyme that catalyzes reactive oxygen species to produce non-toxic O_2 and H_2O_2, followed by the decomposition of H_2O_2 into H_2O and O_2 by protective enzymes such as POD, which are important members of the antioxidant defense system in cells. Maize seedlings were applied to an extremely low-frequency pulsed electric field (ELF-PEF) with an electric energy intensity of 200 kV/m, pulse width of 80 ms, and frequency of 1 Hz; SOD activity was increased, membrane peroxidation was decreased, dry weight was increased, and drought resistance was improved. Additionally, it was proposed that the scavenging of oxygen free radicals was connected to the increase in the growth rate of maize seedlings. [33]. In this study, the electric field treatment also improved the activity of SOD and POD enzymes, among which the SOD activity of seedlings treated with 0.5 kV·cm^{-1}, 120 µs, and 99 pulses was significantly higher than the control on the 5th–15th day by 78.21%, 7.38%, and 4.85%, and the POD activity on the 10th and 15th day was significantly higher than the control by 126.45% and 71.88%. The peak of antioxidant enzymes appeared later than that of amylase, which is consistent with the results of Min Tan et al.'s study on treating rice seeds with an arc-tooth-shaped corona discharge field [29]. It is believed that it is related to the different activation times of metabolic enzymes and antioxidant enzymes. At the same time, the proline content was 19.29–97.31% higher than the control from the 5th to 15th days. Proline, as an organic molecule in cells, can regulate cell osmotic pressure and increase the adaptability of cells to different environments. In conclusion, treating *Scutellaria scutellaria* seeds with PEF can reduce lipid peroxidation of the membrane, maintain membrane function, promote osmotic substance accumulation, and improve plant stress resistance.

4.4. PEF Treatment Is Beneficial to the Metabolic Repair of Scutellaria scutellaria Seeds during Imbibition

A large number of studies have shown that EC has a significant negative correlation with seed vitality and the seedling emergence potential [34,35]. The higher the EC value, the slower or worse the germination, or there could even be no germination [35,36]. Among the artificially aged brassica seeds [26,37], the seeds with a high EC value were ungerminated seeds and abnormally emerging seeds; this is because electrical conductivity can reflect the integrity and stability of cell membranes [26,38], and, when seeds deteriorate or are damaged, the seed membrane becomes more permeable, the electrolyte leakage degree of amino acids and organic acids from the membrane is high, and the conductivity is increased, and the nutrients of the high-quality seeds are still preserved in a well-structured membrane. At present, the conductivity measurement is considered to be a simple and rapid method to assess the seed viability, emergence rate [39], and the ability of seeds to emerge in the field [40]. It is well-known that the initial stage of seed germination is imbibition, and some leakage of ions, amino acids, and other electrolytes can also be observed during this process [36]. However, the pre-hydration treatment of *Cyanus segetum* seeds can reduce EC and improve germination [35], and it is believed that pre-hydration is conducive to the metabolic repair, DNA repair, and membrane repair of seeds before germination [35,41]. In this study, PEF treatment significantly reduced the conductivity of *Scutellaria baicalensis* seeds, while the GP increased, indicating that the appropriate electric field treatment can reduce the permeability of the cell membrane [42] and promote membrane repair during imbibition before germination. In the stage of imbibition before germination, there was also a negative correlation between seed conductivity and germination activity. However, in the study of wheat grains treated with PEF with an energy range of 1.07–17.28 J, the conductivity at 4 h and 8 h after treatment is significantly lower than that without treatment. However, when measured at 24 h, with 2.16, 5.35, 5.76, and 17.27 J, the EC values were higher than the control, and the germination rate was also higher [20]; then, it is concluded that electrical conductivity was positive correlated with germination, which might be related to different seed species and different seed water state.

5. Conclusions

The results showed that the suitable PEF treatment of *Scutellaria baicalensis* seeds could improve the seed germination potential and shorten the germination time; when the treatment parameters were 0.5 $kV \cdot cm^{-1}$, 120 µs, and 99 pulses, the GP and GI increased by 29.25% and 20.65%. The significant increase in germination potential can make germination fast and orderly, which is beneficial for resisting various factors that are not conducive to germination in the field, and laying a good foundation for the later plant growth.

Further study revealed that the electric field treatment of *Scutellaria baicalensis* seeds reduced the electrical conductivity and promoted the membrane repair process during the imbibition process before seed germination, enhanced metabolic enzyme activity, promoted respiration, and accelerated starch decomposition and soluble sugar consumption; it can improve the activity of SOD and POD antioxidant enzymes, reduce membrane lipid peroxidation, maintain the stability of the cell membrane, promote the accumulation of proline in cells, and improve the water retention ability of cells. These physiological and biochemical changes are conducive to the adaptation and resistance to the unfavorable environment of the germination process, thus improving the early growth ability of the plant and laying a good foundation for production. It also provides a new research idea for solving the problems of the low germination rate, difficult emergence, and weak stress resistance of *scutellaria scutellaria* in the field.

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