EFFECTS OF LOW-FREQUENCY HIGH-VOLTAGE PULSED ELECTRIC FIELDS ON GERMINATION CHARACTERISTICS OF AGED RICE SEEDS
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低频高压脉冲电场对陈年水稻种子萌发特性的影响

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ABSTRACT
In this study, the effects of different electric field parameters on the germination characteristics of aged rice seeds were determined via low-frequency high-voltage pulsed electric field (LH-PEF) treatment. The Design-Expert software was used to design a rotating combination test, and response surface analysis was used to optimize five germination characteristics of rice seeds. The optimal treatment voltage was 13 kV and the optimal treatment time was 32.72 min. The results show that, under the optimal treatment conditions (P<0.01), the germination potential (GE), germination rate (GP), germination index (GI), vitality index (VI) and high vitality ratio (HVR) of the aged rice seeds increased by 23.7%, 17.7%, 43.2%, 59.7%, and 250%, respectively, compared to the control. The pulsed electric field's biological effect on the aged rice seeds was the most significant when the optimal treatment parameters were used. The results of this study provide a reliable reference for the parameter optimization of high-voltage electric field treatment of crop seeds.

摘要
本研究通过低频高压脉冲电场(LH-PEF)处理陈年水稻种子，研究不同电场参数对其萌发特性的影响，为提高陈年水稻种子活力提供了一种物理方法。本文利用 Design-expert 软件设计旋转组合试验，并利用响应面分析法，对水稻种子的 5 个萌发特性指标进行参数优化，得出最优处理电压为 13kV，最优处理时间为 32.72min。结果表明：陈年水稻种子在最优处理条件下，与对照相比，发芽势(GE)提高了 23.7%，发芽率(GP)提高了 17.7%，发芽指数(GI)提高了 43.2%，活力指数(VI)提高了 59.7%，高活力比(HVR)提高了 250%，各指标都达到极显著差异（P<0.01）。在最优参数的脉冲电场作用下，陈年水稻种子的电场生物学效应最明显，研究结果为后续作物种子高压电场处理的参数优化提供参考。

INTRODUCTION
Nearly half of the world's population consumes staple foods, such as rice (*Cheng et al, 2022*). Rice is a cereal crop that originated in China and is widely planted in the Yangtze and Pearl River Basins, Northeast China, India, Vietnam, Brazil, Japan, and other countries (*Fukushima et al, 2021; Wei et al, 2021*). Nearly half of the world's population consumes rice as staple foods. (*Cheng et al., 2022*). Rice seed quality significantly affects the yield (*Pang et al, 2018; Xu et al, 2018*). Seed vitality and vitality levels are key factors that determine seed quality and directly affect seed germination, seedling growth rate, and plant vitality (*Reed et al, 2022*). The vitality of rice seeds is the highest at harvest and gradually decreases with increasing seed age. Sowing low-vitality rice seeds causes significant economic losses to agricultural production (*Yan et al., 2018*). Therefore, enhancing the vitality of rice seeds is crucial for maximizing the yield of rice crops (*Kudratillaev, 2020*).

To obtain high-quality seeds, different physical, chemical, and biological methods have been recently used to process and pretreat crop seeds. Chemical treatment methods include seed coating, dressing, and soaking, as well as pretreatment with organic and inorganic solutions. Some studies have found that H_2O_2, as an important messenger molecule, can interact with ethylene and salicylic acid (SA) signaling molecules by inducing the expression of Arabidopsis thaliana (*AtNDPK2*) in cells, thereby activating antioxidant enzyme activity to regulate the physiological response of plants (*Barba-Espin et al, 2012*). Biological treatment methods include treating seeds with fungi, bacteria, plant products, or exogenous hormones.

Some studies have found that the mechanism of growth-promoting microorganisms primarily involves improving the ability of rhizosphere colonization to produce antibiotics and phytohormones; this promotes signal transduction, gene expression, chlorophyll synthesis, and rhizosphere growth, ultimately inducing plant resistance and promoting growth and yield (*Gutiérrez et al., 2015*). Compared to the possible harm that chemical treatment can inflict on the environment and the longer time periods required for biological treatment, physical treatment is more advantageous. Physical treatment methods can be classified into two types (*Wang et al., 2016; Shin et al, 2010*). Traditional physical treatment methods include mechanically damaging the seed husk, improving its permeability, removing the hard and dormant characteristics of the seeds, and ensuring suitable sand burial. By changing biological and non-biological conditions (e.g. microbial activity, effective radiation of ground photosynthesis, and temperature), the physiological process of seed germination and the survival of seedlings can be effectively promoted (*Yudaev et al, 2019*). Biophysical technologies such as electromagnetic fields, ultrasonic waves, and lasers have been widely used owing to their high efficiency. However, the use of emerging high-voltage electric field technology is becoming more widespread (*Patwardhan and Gandhare, 2016*). Studies have shown that a suitable high-voltage electrostatic field can improve the hydrophilicity of the surfaces of soybean seeds, thereby increasing the germination rate. Other studies have found that applying an appropriate pulsed electric field to barley seeds can improve their metabolic efficiency, thereby improving their emergence rate. Afrasiyab et al. found that chickpea seeds treated with a high-voltage electrostatic field exhibited improved germination activity. In addition, Jianzhong et al. found that appropriate high-voltage electric field treatment can promote the development of foxtail millet. Furthermore, Wang et al. conducted an experiment on the effective time over which electric fields influence seed germination and found that within the effective time, the electric field treatment of Caragana korshinskii seeds significantly improved the germination indices of the seeds and the nutrient content in the seedlings. The electric field treatment also improved the drought resistance of the plants. However, only a few studies have reported the use of low-frequency high-voltage pulsed electric fields (LH-PEFs) for the pretreatment of rice seeds (*Evrendilek et al., 2019; Tantamacharik et al, 2019*).

In this study, aged rice seeds were pretreated with LH-PEF. The Design-Expert software was used to design a rotation combination test, and response surface analysis was used to determine the optimal electric field parameters and to model and optimize five germination characteristics of rice seeds, including germination potential, germination rate, germination index, vitality index, and high vitality ratio. The biological effect of the pulsed electric field on the aged rice seeds was the most significant when the optimal parameters were employed. The results of this study provide a reliable reference for the parameter optimization of the high-voltage electric field treatment of crop seeds.

The rest of this paper is organized as follows. In Section 2, the materials and methods used in the experiment are described. Section 3 presents and analyzes the results, and Section 4 discusses their significance. Finally, Section 5 summarizes the findings of this study and draws the conclusions.

MATERIALS AND METHODS

Samples

Table 1

Physical characteristics of rice seeds.

Name	Hundred-grain mass (g)	Size (mm)		
		Length	Width	Thickness
DaoHuaxiang No. 2	29.95±0.35	9.87±0.36	5.65±0.31	4.12±0.33

The aged rice seeds used in the experiment were DaoHuaxiang No. 2, which were provided by the College of Engineering at Shenyang Agricultural University. The seed was harvested in October 2019 in Fushun City, Liaoning Province, with an initial moisture content of 12.3±0.3%, and was stored at 15 ℃ and a relative humidity of 60-65%. Aged rice seeds of the same size and appearance were selected for germination tests. The initial germination rate without electric field treatment was approximately 40%. The physical characteristics of the aged rice seeds are listed in Table 1.

Experimental equipment

A high-voltage electric field workbench was designed and constructed using a high-voltage power supply, copper mesh, copper plate, plastic insulating board, plastic insulated hollow tubes, and other materials, as shown in Figure 1.

The high-voltage power supply can be disassembled at any time to swap the DC, AC, and pulse power supplies. The output and grounding ends of the electric field generator were connected to the copper mesh and copper plate, which were separated by plastic insulated hollow tubes. The latter were available in a variety of lengths so that the distance between the upper and lower plates could be adjusted, which facilitated the execution of subsequent tests. The positive and negative plates were positioned at a distance of 0.05 m away from each other. After the high-voltage power supply was connected, a continuous and adjustable uniform high-voltage electric field was generated between the copper mesh and copper plate. A slide plate was placed halfway between the upper and lower copper electrode plates, and the test seed was placed at the center of the slide plate, as shown in Figure 1.

The test equipment included a pulsed electric field generator (*Dalian Zhongxing Electronic Technology Co., LTD.*), a Vp-5512a oscilloscope (*Japan Panasonic Corporation*), a Bsa124s-CW electronic balance (Germany Sartorius company, accuracy of 0.0001 g), an Rtop-268d intelligent artificial climate incubator (*Zhejiang Top Instrument Co., LTD*.; temperature control range: 0-50 ℃, temperature fluctuation: ±0.5 ℃, temperature uniformity: ±1 ℃, humidity control range: 50-95% RH, humidity fluctuation: ±5% RH), a copper plate, an acrylic insulation board and acrylic hollow tube (*Zhejiang Wenzhou Jumeng Technology Co., LTD*.), and vernier calipers with an electronic digital display and a precision of 0.01 mm. Additional items used in the experiment included a hygrometer, absorptive paper, distilled water, germinating box, germinating paper, stainless-steel tweezers, plastic partition, beaker, and burette.

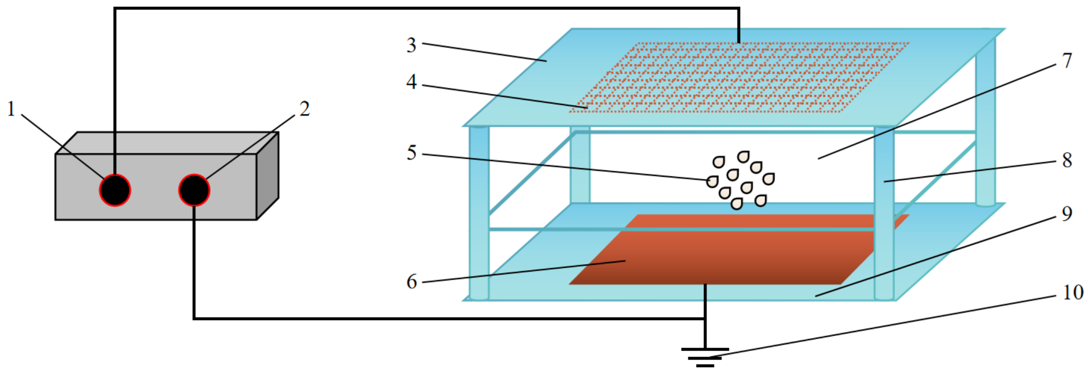


Fig. 1 - Schematic diagram of the workbench used for high-voltage electric field treatment
1) High-voltage power supply positive pole; 2) High-voltage power supply negative pole;
3) Positive plastic insulation board; 4) Copper mesh; 5) Rice seeds; 6) Copper plate; 7) Slide plate;
8) Plastic insulated hollow tubes; 9) Negative plastic insulation board; 10) Ground.

Methods and procedures

Experimental design of electric field treatment of rice seeds

Table 2

Experimental factors and coding values

Coding value	Electric field voltage (kV)	Treatment time (min)
-1.414	6	5
-1	8.05	13.05
0	13	32.5
1	17.95	51.95
1.414	20	60

Based on the results of the preliminary tests, the values of the low-frequency pulsed electric field parameters were as follows: the electric field voltage was 6-20 kV, the treatment time was 5-60 min, the direction of the field intensity was vertically downward, the electric field frequency was 10 Hz, and the electric field pulse width was 10 ms.

Using 13 kV and 32.5 min as the median values for the electric field voltage and treatment time, respectively, a secondary general rotation combination test was designed and analyzed using the Design-Expert software. Thirteen treatment groups and one control group were designed. The experimental factors and coding values are listed in Table 2.

Germination experiment of seeds

The experiment was conducted on June 14, 2021, at Laboratory 513, College of Information and Electrical Engineering, Shenyang Agricultural University, China. DaoHuaxiang No. 2 rice seeds with full grains of equal size were used. Fifty seeds were divided into several portions and numbered.

The seeds were treated according to the values listed in Table 2. Then they were soaked in 0.01% potassium chloride solution for 10 min, sterilized, and washed with distilled water three times before being placed in an incubator at a constant temperature for the germination test (*Wang et al., 2020*). The environment was established according to the following national standards: a constant temperature of 25℃, continuous light, and an equivalent humidity of 100%. For each electric field setting, four box tests were performed, and the average value of each quantity across the four tests was calculated.

During germination, rice seeds should be replenished regularly to ensure that a sufficient amount of water is available for seedling development. During this period, if mildew existed inside the sprouting box, it was treated immediately to prevent it from affecting the test results. To ensure the ventilation of the seed-growing environment, the box cover was removed in time to prevent the growth of seedlings. The number of germinations was recorded daily from day three until the seedlings were washed on day seven, and the relevant data were measured. Statistical criteria were defined according to the "Seed Examination," and all rice seedlings in each box were measured.

While the rice seeds were germinating, the germination potential, germination rate, and vitality index were used as germination indices. The germination potential (GE) is defined as:

$$GE = \frac{m_1}{M} \times 100\% \tag{1}$$

where m_1 is the number of germinated seeds on the third day, and M is the number of seeds used in the experiment. The germination rate (GP) is defined as:

$$GP = \frac{m_2}{M} \times 100\% \tag{2}$$

where m_2 is the number of seeds that grew into seedlings at the end of the experiment. The germination index (GI) is defined as:

$$GI = \sum (Gt / Dt) \tag{3}$$

where Dt is the number of germinating days and Gt is the number of germinating seeds per day corresponding to Dt. The vitality index (VI) is defined as:

$$VI = GI \times S \tag{4}$$

where S is the length (cm) or fresh weight (g) of a single normal seedling over a certain period.

The high vitality ratio (*HVR*) is defined as:

$$HVR = \frac{m_3}{M} \tag{5}$$

where m_3 is the number of seeds with a seedling height of at least 4 cm at the end of the experiment.

After the germination test, the seedlings were washed with clean water, and the surface was dried with absorbent paper. The lengths of the rice seedlings were measured using digital vernier calipers, and 100 plants were placed in each box. For each of the four tests using a given electric field, the average length of the seedlings in each box was calculated, and the average value for all four tests was taken as the seedling length for the given electric field and compared to the CK control group.

Data processing

The SPSS 23 and Design-Expert 8.0.5 data processing software programs were used to statistically analyze the data, and Origin and MATLAB were used to generate plots.

RESULTS

Effect of pulsed electric field on seed vitality of aged rice

Thirteen treatments for the rotary combination test and four repetitions at the median values were conducted. The test results are displayed in Table 3. As shown in the table, the germination potential, germination rate, germination index, vitality index, and high vitality ratio of the aged rice seeds after the LH-PEF treatment were significantly higher than those of the CK control group. When the electric field voltage was 13 kV and the treatment time was 32.5 min, all indices exhibited significant differences compared to the CK ($P < 0.01$) control group.

The Design Expert software was used to perform multiple regression fitting on the test data shown in Table 3, and a quadratic multinomial regression model was established for the germination potential, germination rate, germination index, vitality index, high vitality ratio, voltage, and treatment time of the rice seeds. A variance analysis for each item was conducted, and the results of the analysis are presented in Table 4.

Table 3

Design and results of rotary combination test

Test No.	Electric field voltage (kV)	Treatment time (min)	Germination potential (%)	Germination rate (%)	Germination index	Vitality index	High vitality ratio
CK	0	0	61.1	82.3	22.2	1.34	0.04
1	13	32.5	74.4**	97.1**	29.8**	2.2**	0.13**
2	8.05	51.95	68.1*	89.8**	26.1*	1.59*	0.05*
3	8.05	13.05	68.9*	90.2**	26.5**	1.71*	0.05*
4	13	5	70.3**	92.7**	27.4**	1.82*	0.08*
5	13	32.5	74.9**	97.7**	31**	2.27**	0.14**
6	13	32.5	74.7**	96.5**	30.8**	2.16**	0.13**
7	13	60	71.1*	92.1*	28.2**	1.84*	0.08*
8	6	32.5	65.5*	85.9*	24.1*	1.55*	0.07*
9	20	32.5	66.6*	85.7*	23.8*	1.48*	0.05*
10	17.95	13.05	69.1*	89.7**	27.1**	1.59**	0.06*
11	13	32.5	74.9**	96**	30.5**	2.22**	0.11**
12	13	32.5	75.1**	96.6**	31.6**	2.15**	0.12**
13	17.95	51.95	68.7*	88.7*	26.8*	1.69*	0.07*

Note: '*' represents significance ($P < 0.05$); '**' represents extreme significance ($P < 0.01$).

Table 4

Results of variance analysis for each index

Source of variation	Germination potential		Germination rate		Germination index		Vitality index		High vitality ratio	
	F value	*P* value	*F* value	*P* value	*F* value	*P* value	*F* value	*P* value	*F* value	*P* value
Model	161.22	0.0011**	123.99	0.0015**	29.38	0.0001**	118.52	0.0064**	13.63	0.0017**
V	3.91	0.0885	1.28	0.2951	0.183	0.6817	1.03	0.3431	0.002	0.9653
f	0.0033	0.9557	1.83	0.2187	0.0444	0.8391	0.005	0.9455	0.0689	0.8005
Vf	0.2254	0.6494	0.26	0.6258	0.0048	0.9469	7.07	0.0325*	0.1378	0.7214
V^2	722.82	0.0021**	578.49	0.0007**	136.59	0.0008**	495.99	0.0009**	50.05	0.0002**
f^2	151.96	0.0017**	85.7	0.001**	21.84	0.0023**	149.6	0.0003**	26.18	0.0014**
R^2	0.9914		0.9888		0.9545		0.9883		0.9069	
SNR	31.4637		27.6787		13.3595		25.6064		7.9298	

Note: '*' represents significance ($P < 0.05$); '**' represents extreme significance ($P < 0.01$); V is pulsed voltage in kV; *f* is treatment time in min.

Response surface analysis of the effect of pulsed electric field on germination potential of aged rice seeds

As shown in Table 3, the germination potential of the aged rice seeds after the LH-PEF treatment was significantly higher than that of the CK group ($P < 0.01$ or $P < 0.05$). Figure 2(a) shows a plot of the response surface analysis of the effect of pulsed electric field on the germination potential of the rice seeds. As shown in the figure, the germination potential of the rice seeds reached an optimal value with the increase in a certain combination of electric field voltage and treatment time.

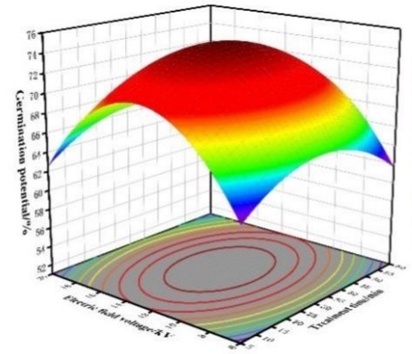
A quadratic multinomial regression model for the relationship between the germination potential of the aged rice seeds and the electric field voltage and treatment time was established, and it was expressed as:

$$GE = 39.36 + 4.58V + 0.32f + 0.0001Vf - 0.18V^2 - 0.005f^2 \left(R^2 = 0.9914\right) \tag{6}$$

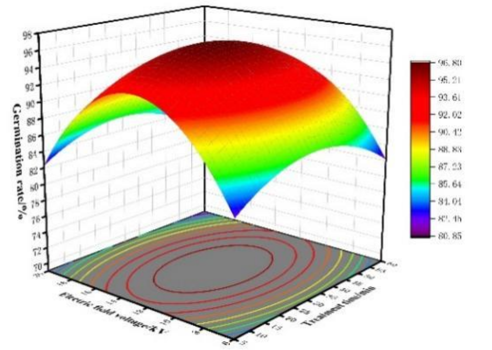
where:

GE is the germination potential of the rice seeds treated with a pulsed electric field (in %), V is the pulse voltage (in kV), and f represents the treatment time (in min).

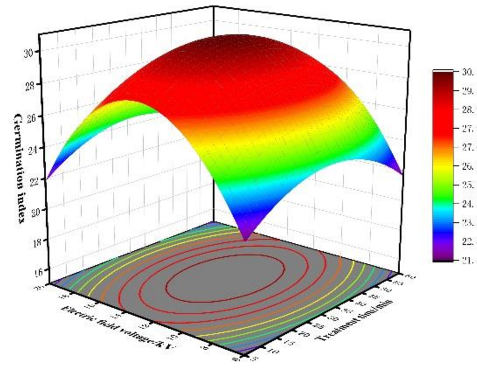
Table 4 shows that the F value of the germination potential was 161.22 and the P value was 0.0011, indicating that the established expression between the germination potential, electric field voltage, and treatment time was extremely significant (P < 0.01). The signal-to-noise ratio of the relational model was 31.4637, indicating that the model was superior and the accuracy and reliability of the test were high. The data in Table 4 show that the squares of the electric field voltage and treatment time had a significant influence on the germination potential, but the electric field voltage, treatment time, and their interaction had no significant influence on the germination potential.



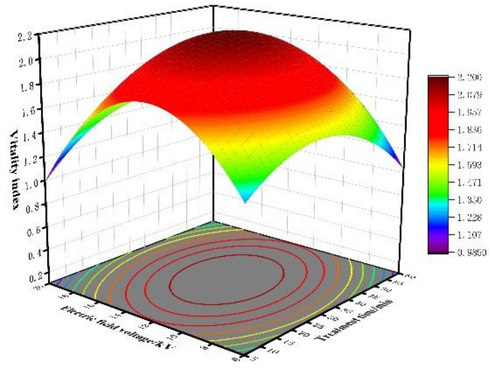
(a) Germination potential



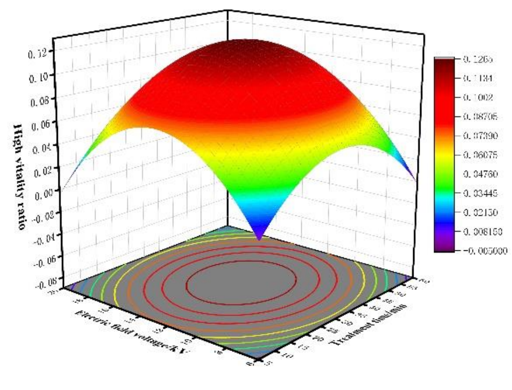
(b) Germination rate



(c) Germination index



(d) Vitality index



(e) High vitality ratio

Fig. 2 - Response surface analysis of germination indices of rice seeds after pulsed electric field treatment

Response surface analysis of effect of pulsed electric field on germination rate of aged rice seeds

As shown in Table 3, the germination rate of aged rice seeds after the LH-PEF treatment was significantly higher than that of the CK group, and most of them showed extremely significant differences (P < 0.01). Significant differences were observed for the individual treatments (P < 0.05). Figure 2(b) shows a plot of the response surface analysis of the effect of the LH-PEF treatment on the germination rate of the rice seeds. As shown in Fig. 2(b), for different treatment times, the influence of the electric field voltage on the germination rate of the aged rice seeds was similar to that on the germination potential.

The effects of an extended treatment time on the germination rate of the aged rice seeds were similar for different electric field voltages. In summary, the germination rate of the rice seeds after the LH-PEF treatment reached an optimal value for a certain combination of electric field voltage and treatment time. A response surface analysis established a quadratic multinomial regression model for the relationship between the germination rate of the aged rice seeds and the electric field voltage and treatment time, and it was expressed as:

$$GP = 96.78 - 0.24V - 0.28f - 0.15Vf - 5.36V^2 - 2.07f^2 \quad \left(R^2 = 0.9888\right) \tag{7}$$

where: GP is the germination rate of the rice seeds treated with an LH-PEF (in %).

Table 4 shows that the F value of the germination rate was 123.99 and the *P* value was 0.0011, indicating that the established expression between the germination rate, electric field voltage, and treatment time was extremely significant ($P< 0.01$). The signal-to-noise ratio of the model was 27.6787, indicating that the model was superior and the accuracy and reliability of the test were high. The data in Table 4 indicate that the squares of the electric field voltage and treatment time had a significant influence on the germination rate, while the electric field voltage, treatment time, and their interaction had no significant influence on the germination rate.

Response surface analysis of effect of pulsed electric field on germination index of aged rice seeds

As shown in Table 3, the germination index of the aged rice seeds after the LH-PEF treatment was significantly higher than that of the CK group ($P < 0.01$ or $P < 0.05$). Figure 2(c) shows a plot of the response surface analysis of the effect of the LH-PEF on the rice seed germination index. As shown in Fig. 2(c), the rice seed germination index after the LH-PEF treatment reached an optimal value for a certain combination of electric field voltage and treatment time. A response surface analysis established a quadratic multinomial regression model for the relationship between the germination index of the aged rice seeds and the electric field voltage and treatment time, and it was expressed as:

$$GI = 30.74 + 0.1095V + 0.0539f + 0.025Vf - 3.21V^2 - 1.28f^2 \quad \left(R^2 = 0.9545\right) \tag{8}$$

where GI is the germination index of the rice seeds treated with an LH-PEF (in %).

Table 4 shows that the F value of the germination index was 29.38 and the *P* value was 0.0001, indicating that the established expression between the germination index, electric field voltage, and treatment time was extremely significant ($P < 0.01$). The signal-to-noise ratio of the relational model was 13.3595, indicating that the model was superior and the accuracy and reliability of the test were high. The data in Table 4 indicate that the squares of the electric field voltage and treatment time had a significant influence on the germination index, while the electric field voltage, treatment time, and their interaction had no significant influence on the germination index.

Response surface analysis of effect of pulsed electric field on vitality index of aged rice seeds

As shown in Table 3, the vitality index of the aged rice seeds after the LH-PEF treatment was significantly higher than that of the CK group, and most of the tests generated extremely significant differences ($P < 0.01$). Figure 2(d) shows a plot of the response surface analysis of the effect of the LH-PEF treatment on the vitality index of the rice seeds. As shown in Fig. 2(d), the vitality index after the LH-PEF treatment reached an optimal value for a certain combination of electric field voltage and treatment time. Moreover, the effect of treatment time on the vitality index was significant. A quadratic multinomial regression model was established for the relationship between the vitality index of the aged rice and the electric field voltage and treatment time, and it was expressed as:

$$VI = 2.20 - 0.0149V + 0.0010f + 0.0550Vf - 0.3494V^2 - 0.1919f^2 \quad \left(R^2 = 0.9983\right) \tag{9}$$

where VI is the vitality index of the rice seeds treated with an LH-PEF (in %).

Table 4 shows that *the* F value of the vitality index was 118.52 and the *P* value was 0.0064, indicating that the established relationship between the vitality index, electric field voltage, and treatment time was extremely significant ($P < 0.01$). The signal-to-noise ratio of the relational model was 25.6064, indicating that the model was superior, and the accuracy and reliability of the test were high. The data in Table 4 indicate that the squares of the electric field voltage and treatment time and the interaction between the electric field voltage and treatment time had a significant influence on the vitality index, but the electric field voltage and treatment time had no significant influence on the vitality index.

Response surface analysis of the effect of pulsed electric field on high vitality ratio of aged rice seeds

As shown in Table 3, the high vitality ratio of the aged rice seeds after the LH-PEF treatment was significantly higher than that of the CK group, and most of the tests generated extremely significant differences (P < 0.01). Figure 2(e) shows a plot of the response surface analysis of the effect of the LH-PEF treatment on the high vitality ratio of the rice seeds. As shown in Fig. 2(e), the high vitality ratio after the LH-PEF treatment reached an optimal value for a certain combination of electric field voltage and treatment time.

Moreover, the effect of treatment time on the high vitality ratio of the rice seeds was evident. A quadratic multinomial regression model was established for the relationship between the high vitality ratio of the aged rice and the electric field voltage and treatment time, and it was expressed as:

$$HVR = 0.1260 + 0.0002V + 0.0012f + 0.0025Vf - 0.0361V^2 - 0.0261f^2 \quad \left(R^2 = 0.9069\right) \tag{10}$$

where HVR is the high vitality ratio of the rice seeds treated with an LH-PEF (in %).

Table 4 shows that the F and P values of the high vitality ratio were 13.63 and 0.0017, respectively, indicating that the established relationship between the high vitality ratio, electric field voltage, and treatment time was extremely significant (P < 0.01). The signal-to-noise ratio of the model was 7.9298, indicating that the model was superior and the accuracy and reliability of the test were high. The data in Table 4 indicate that the squares of the electric field voltage and treatment time had a significant influence on the high vitality ratio, but the electric field voltage, treatment time, and their interaction had no significant influence on the high vitality ratio.

Parameter optimization

The maximum values of germination potential, germination rate, germination index, vitality index, and high vitality ratio of the aged rice seeds were used as the optimization objectives, and the weights of each of these five quantities were all 1. The binomial regression mathematical model, as expressed in Equations (6)–(10), was optimized using the response surface analysis method. The optimal combination of electric field voltage and treatment time for the LH-PEF treatment of the aged rice seeds was an electric field voltage of 13 kV and a treatment time of 32.72 min. Under the optimal conditions, the expected values of germination potential, germination rate, germination index, vitality index, and high vitality ratio of the aged rice seeds were 74.8%, 96.8%, 30.8, 2.20, and 0.13, respectively, which were significantly higher than those of the CK group (P < 0.01).

Validation test

Based on the results of the parameter optimization, the aged rice seeds were treated with a pulsed electric field voltage of 13 kV and a treatment time of 32.72 min. As shown in Figure 3 and Table 5, the comparison of rice seed germination between the treatment group and the control group.

Table 5

Comparison of parameters between treatment group (TG) and control group (CK)

Test No.	Electric field voltage (kV)	Treatment time (min)	Germination potential (%)	Germination rate (%)	Germination index	Vitality index	High vitality ratio
TG	13	32.72	75.6	96.4	31.8	2.14	0.14
CK	0	0	61.1	82.3	22.2	1.34	0.04

The germination potential, germination rate, germination index, vitality index, and high vitality ratio of aged rice seeds were 75.6%, 96.4%, 31.8, 2.14, and 0.14, respectively. Compared to the CK group, the germination potential increased by 23.7%, the germination rate increased by 17.7%, the germination index increased by 43.2%, the vitality index increased by 59.7%, the high vitality ratio increased by 250%, and all indices reached extremely significant differences (P < 0.01), consistent with the results of the parameter optimization.

Fig. 3 - Comparison between rice seed germination of treatment group (a) and control group (b). Scale bar, 1 cm

DISCUSSION

As a natural phenomenon, an electric field can affect the growth of organisms to various degrees (*Kovalyshyn., 2016*). The biological effects of LH-PEFs on organisms are the physical and chemical reactions that occur in them as a result of such an electric field, thus generating a comprehensive aftereffect (*Feng et al., 2020; Zhang et al., 2019*). In this study, electric field treatment with the appropriate parameters promoted rice seed germination. According to the test results, the germination potential, germination rate, germination index, vitality index, and high vitality ratio of the aged rice seeds reached an optimal value for a certain combination of electric field voltage and treatment time. When the electric field voltage was 13 kV, the plate spacing was 50 mm, the treatment time was 32.72 min, and the electric field frequency was 10 Hz, the germination indices of the aged rice seeds reached their maximum values.

According to the hypothesis of energy stimulation for seed germination, complex physical and chemical changes occurred in the interior of aged rice seeds after they were treated with an electric field with a voltage of 13 kV and a treatment time of 32.72 min, which provided appropriate energy stimulation for the germination of the aged rice seeds. As a result, the germination potential, germination rate, germination index, vitality index, and high vitality ratio of the aged rice seeds significantly improved. Since the test varieties used in this experiment are relatively simple, it is hoped that the wide applicability of this pretreatment method can be explored in the future.

CONCLUSIONS

1) Aged rice seeds were treated with LH-PEFs, and a rotating combination test was performed. The results showed that the germination potential, germination rate, germination index, vitality index, and high vitality ratio of the aged rice seeds reached an optimal value for a certain combination of electric field voltage and treatment time. Accordingly, the biological effect of the electric field on aged rice seeds depended on both the electric field voltage and treatment time.

2) When the electrode plate spacing was 50 mm and the electric field frequency was 10 Hz, the optimal LH-PEF treatment conditions were an electric field voltage of 13 kV and a treatment time of 32.72 min. After the aged rice seeds were treated with this electric field dose, all the germination indices were significantly different compared to the control group ($P < 0.01$). Specifically, the germination potential, germination rate, germination index, vitality index, and high vitality ratio were increased by 23.7%, 17.7%, 43.2%, 59.7%, and 250%, respectively, compared to the CK control group.

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