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REGULAR ARTICLE

INFLUENCE OF HIGH VOLTAGE ELECTROSTATIC FIELD (HVEF) ON VIGOUR OF AGED RICE (*ORYZA SATIVA* L.) SEEDS

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SUMMARY

The vigour restoration of aged rice seeds is of great significance in agriculture. This paper studied the biological effects of high voltage electrostatic field (HVEF) on aged rice seeds, including dry seeds and wet seeds soaked in sterile deionized water for 24 hours. The results showed that HVEF slightly affected the vigour of the aged dry rice seeds while the seed vigour and seedling growth of the aged wet rice seeds were significantly improved. The germination rate and germination potentiality also showed moderate improvement after exposure to HVEF with electric intensity less than t 450 kV•m^{-1}. Compared to control, the vigour index of aged wet rice seeds was increased 31.96%. No significant effects of HVEF on dry aged rice seeds were found.

Keywords: Aged seeds , High voltage electrostatic field (HVEF), Rice, Vigour.

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1. Introduction

During storage all seeds undergo aging and deterioration, with the rate dependent on storage temperature, seed moisture, and crop species, and some measures have been taken to try to alleviate the susceptibility to deterioration and increase storage life or germination [5,13]. However, present restoration techniques require lower temperatures and accurate moisture content and sophisticated technology and it is very difficult to apply on large scale. It is, therefore, urgent to explore new avenues for restore seed viability.

Recent years have witnessed a growing interest in high voltage electrostatic field (HVEF) treatment applications for food processing, inhibition of microorganisms and fruit preservations [7,9,10,16] and other aspects. For example, deterred photosynthesis, slow metabolism and growth, and decreased resistance to stresses happened in growing plants when they were removed from the electrostatic field by the metal net [12]. Some studies also showed that HVEF could increase seeds viability including earlier germination, higher germination rate, faster growth and stronger resistance of seedlings to stress [1,2,18,20,21]. There are also reports on the effects of HVEF on dormant seeds such as corn, carrot, lettuce, brasica, radish, purple clover [19], evening primrose [17] and tomato [14] and the vigour indexes of aged cucumber seeds were increased by 29.50% -59.70% when the seeds were treated by HVEF of 167 kV•m-1 [22].However, up to now there are fewer reports on the application of HVEF on aged rice seeds [11].

In our study, the biological effects of HVEF on aged dry rice seeds and aged wet rice seeds were investigated, respectively. Results from this study can provide information on the application of HVEF in aged plant seeds, allow deeper insights into the healing mechanisms of cell membranes, and provide some theoretic base for application of HVEF in agriculture in the near future.

2. Materials and Methods

Materials and sample preparation

Rice seeds Super-II 725 were obtained from Chongqing Seeds Co., Ltd. They were surface sterilized with 10% sodium hypochlorite solution on a magnetic stirrer for 10 min, washed with deionized water thoroughly, and then air-dried at 30°C. Sterilized rice seeds were subjected to the condition of 40°C and 100% relative humidity for 8 d to accelerate seeds aging process by the procedure proposed by Delouche [3].

HVEF experimental apparatus

The experimental setup for HVEF is shown Fig.1. It mainly consists of two round aluminum boards each with a diameter of 40 cm. The voltage is adjustable and the electric intensity is equal to the ratio of electric voltage between the two boards to the distance between them. For treatment with HVEF, seeds were placed on the down board (Fig. 1).

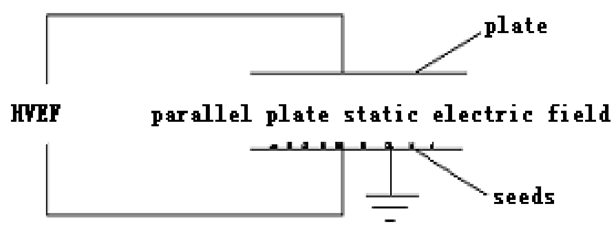

Fig. 1. The equipment implementing the HVEF treatment on rice seeds

Determination of growth parameters

The effect of HVEF on aged seeds was related to the humidity of the seeds [8]. In our study, two groups, wet aged rice seeds and dry aged rice seeds, were used to study the effect of HVEF and the relationship between seed humidity and the effect of HVEF was also study. For wet aged seeds, the aged rice seeds were soaked in sterile deionized water at 30°C for 24 h and then exposed to HVEF with different electric intensity (Table 1). For dry aged seeds, non-soaked in sterile deionized water, the aged rice seeds were directly subjected to HVEF under the same conditions (Table 1). After exposure to HVEF, both the aged dry seeds and aged wet seeds were soaked in water for 24 h at 30°C to accelerate germination, then 100 seeds were germinated at 30°C for 10 days. From day 1 to day 10, the number of germinated seeds was recorded and 15 10-day-old seedlings were randomly selected to measure the length of root and seedling in each treatment. They were then placed in an aerated oven at 80°C for 72 h, and the dry weights were determined. Germination potentiality, germination rate, germination index (GI), and vigour index (VI) of seeds were calculated based on the following formula:

Germination potentiality= number of the germination seeds (0-4d)/number of the total seeds

Germination rate = number of the germination seeds (0-10 d)/ number of the total seeds

GI=∑Gt / Dt (Dt: days for germination, Gt : number of germinated seeds corresponding to Dt)

VI=GI×L (GI: germination index ,L: length of the seedling).

Table 1. Factors and levels for testing the effects of HVEF on aged rice seeds

Levels		1	2	3	4	5	6	7	8	9	10	11
Factors	HVEF intensity ($kV{\cdot}m^{-1}$)	100	150	200	250	300	350	400	450	500	550	600
	Time (min)	5	10	15	20	25	30	35	40	45	50	55

Table 2. Test project for uniform design

Treatment number	Control	1	2	3	4	5	6	7	8	9	10	11
Levels of HVEF ($kV{\cdot}m^{-1}$)	0	100	150	200	250	300	350	400	450	500	550	600
Levels of time (min)	0	25	50	15	40	5	30	55	20	45	10	35

Experimental design

Uniform design is an experimental method provided by Fang [6]. The design provides information on how to scatter the design spots within the experiment range uniformly, which allows one to gain the most information with less design spots. Since 1978 uniform design has been widely applied in such fields as medicine, chemistry, biotechnology, and environmental protection with plentiful achievements and great benefits having been obtained.In our study two factors (electric field intensity and exposure time) were involved, each of which contained 11 levels. Different factors and levels are showed in Table 1 and experiment scheme in Table 2.

Table 1 Factors and levels for testing the effects of HVEF on aged rice seeds

Table 2 Test project for uniform design

Statistical analysis

Each data point was the mean of three replicates. All data obtained were subjected to a one-way analysis of variance (ANOVA), and the mean differences were compared by lowest standard deviations (LSD) test. Comparisons with $P < 0.05$ were considered significantly different.

3. Results and Discussion

Biological effects of HVEF on wet aged seeds

Biological effects of HVEF on aged wet rice seeds were investigated (Table 3). Compared to control, the germination potentiality of wet aged rice seeds was increased, however the negative effect appeared when the electric intensity is more than 400 kV•m-1. Compared to control, the germination rate was higher, except in treatment of 600 kV·m-1/35 min. Length of seedlings, length of root, and dry weight of each seedling were remarkably higher than that of control. Length of seedlings was increased by 5.13%-22%, length of roots by 1.0%-14.78%, and dry weight per plant by 7.07%-18.3% (Table 3). Both germination index and seed vigour index of wet aged rice seeds were significantly increased after exposure to HVEF. Germination index was increased by 1.0%- 12.48% and vigour index by 7.88%-31.96%. Most treatments reached remarkable or highly remarkable levels in vigour index.

Table 3. The effect of HVEF on the wet aged rice seeds

HVEF Treatments	Germination potentiality (%)	Germination rate (%)	Length of seedling (cm)	Length of root (cm)
100 kV·m⁻¹/25 min	85.51±2.28	89.35±3.19	8.41±0.80	9.46±1.40
150 kV·m⁻¹/50 min	79.67±9.50	87.33±4.73	9.33±0.29	10.17±0.67
200 kV·m⁻¹/15 min	83.13±7.40	85.78±5.12	9.68±0.95*	9.83±0.73
250 kV·m⁻¹/40 min	81.81±1.11	86.54±1.06	8.64±1.07	9.04±0.98
300 kV·m⁻¹/5 min	81.00±10.54	89.33±4.16	8.87±0.95	9.31±0.77
350 kV·m⁻¹/30 min	85.61±5.40	88.42±3.80	8.96±0.60	9.16±0.61
400 kV·m⁻¹/55 min	83.80±4.41	90.92±3.62	9.05±0.46	9.94±0.43
450 kV·m⁻¹/20 min	77.95±3.71	88.42±2.44	8.90±0.83	10.33±0.40
500 kV·m⁻¹/45 min	74.83±4.20	86.21±2.04	9.40±0.67*	9.63±0.64
550 kV·m⁻¹/10 min	74.77±5.23	83.83±3.44	9.26±0.53	9.99±0.68
600 kV·m⁻¹/35 min	47.31±4.91	56.65±1.89	9.76±0.17*	9.98±0.44
Control	81.29±8.04	84.96±6.90	8.08±0.13	9.00±0.64

HVEF Treatments	Dry weight (mg)	Germination index (mg/d)	Seed vigor index (mg/d)
100 kV·m⁻¹/25 min	9.20±0.23**	40.32±0.81	370.89±10.51**
150 kV·m⁻¹/50 min	9.37±0.48**	39.00±3.01	364.79±25.60**
200 kV·m⁻¹/15 min	9.17±0.40**	38.30±3.42	350.23±16.72*
250 kV·m⁻¹/40 min	8.48±0.53	40.24±0.73	341.44±23.32*
300 kV·m⁻¹/5 min	8.93±0.88*	40.92±4.16	366.73±61.29**
350 kV·m⁻¹/30 min	9.23±0.53**	40.41±1.42	372.61±8.80**
400 kV·m⁻¹/55 min	9.30±0.25**	41.72±1.83*	387.73±9.28**
450 kV·m⁻¹/20 min	9.10±0.54**	39.99±1.95	363.24±7.01**
500 kV·m⁻¹/45 min	8.70±0.41	36.41±1.19	316.99±23.19
550 kV·m⁻¹/10 min	9.30±0.33**	37.14±1.52	345.70±25.21*
600 kV·m⁻¹/35 min	8.67±0.26	23.15±2.20	201.00±25.20
Control	7.92±0.18	37.09±2.37	293.82±23.05

*and ** indicate that difference was significant at 95% and 99% levels of probability, respectively.

Regression analysis of the results from wet aged seeds

Regression equation for germination potentiality: Y=56.9656+0.0863X1-0.0002X12-0.0001X1*X2, here X1 refers to intensity of electric field, and X2 refers to the action time (the same as below). When Y=83.67334 (the maximum value of the regression equation, the same as below), X1=215, X2=5, and the maximum germination potentiality of rice seeds was increased 3%, compared to control.

Regression equation for germination rate: Y= 59.1909 + 0.0936X1 - 0.0002 X 12. When Y= 88.4611, X1 =233.5, and the maximum germination rate of rice seeds was increased 4%, compared to control.

Regression equation for germination index : Y=28.4287+0.0903X1-0.0001X12+0.0023X22-0.0005X1×X2.

When Y =47.7582, X1=440, X2=5, and the maximum germination index of rice seeds was increased 28.76% compared to control.

Regression equation for seed vigour index: Y=288.0796+0.6641X1-0.0012X12. When Y =379.9606, X1=276.7, and the maximum seed vigour index of rice seeds was increased 29.3%, compared to control.

The results above indicated that both the germination rate and the germination speed of wet aged seeds were improved when the seeds were exposed to electric intensity less than 400kV•m-1. Appropriate electrostatic field intensity significantly promoted seedling growth and seed vigour, and only slightly increased the germination rate and potentiality. The result above indicated that HVEF with appropriate electric intensity had significant effects on the the vigour of wet aged rice seed.

Biological effect of HVEF on dry aged seeds

No significant effects were found after the dried seeds were exposed to HVEF except the germination of dry aged seeds was increased after the seeds were exposed to 100kV•m-1 for 25 min (Table 4).The vigour of dry aged seeds was decreased when the intensity of electrostatic field was greater than 200 kV•m-1. One possible reason is that cell water flow in dry seeds was restricted by the influence of the HVEF. In agreement with our study, Observation by laser confocal microscope showed that process of starch disassembling was restrained after treatment of HVEF, and further analysis by 1H-NMR showed that

HVEF restricted cell water flow which is related to metabolism and nutrition supply [8].

Table 4. The effect of HVEF on the dry aged rice seeds

HVEF treatments	Germination potentiality (%)	Germination rate (%)	Length of seedling (cm)	Length of root (cm)
100 kV·m⁻¹/25 min	76.08±2.00	79.40±0.53	8.93±0.43	9.95±0.78
150 kV·m⁻¹/50 min	73.09±6.91	77.61±4.85	8.53±0.51	10.98±0.46
200 kV·m⁻¹/15 min	71.58±2.91	75.63±2.02	9.46±0.83	10.70±0.72
250 kV·m⁻¹/40 min	71.63±2.16	78.03±3.26	9.22±0.64	12.10±1.13
300 kV·m⁻¹/5 min	70.15±3.97	77.30±3.81	9.23±1.01	10.51±0.51
350 kV·m⁻¹/30 min	67.72±2.83	71.83±1.67	8.85±0.37	11.14±0.57
400 kV·m⁻¹/55 min	67.92±4.81	75.21±3.23	8.75±0.16	10.04±0.47
450 kV·m⁻¹/20 min	71.48±1.54	76.55±3.21	8.58±0.82	10.32±0.59
500 kV·m⁻¹/45 min	67.88±3.78	75.68±3.04	8.81±0.36	10.04±0.08

HVEF treatments	Dry weight (mg)	Germination index (mg/d)	Seed vigour index (mg/d)
100 kV·m⁻¹/25 min	8.43±0.88	33.21±2.66	281.52±50.67
150 kV·m⁻¹/50 min	8.53±0.32	32.40±3.40	277.15±38.86
200 kV·m⁻¹/15 min	8.73±0.10	31.76±0.95	277.37±8.86
250 kV·m⁻¹/40 min	8.38±0.63	32.10±0.68	269.13±20.96
300 kV·m⁻¹/5 min	8.80±0.25	31.01±0.88	272.75±2.27
350 kV·m⁻¹/30 min	8.28±0.26	31.04±1.77	256.84±6.52
400 kV·m⁻¹/55 min	8.70±1.00	31.39±3.77	275.59±66.21
450 kV·m⁻¹/20 min	8.08±0.29	30.90±0.36	249.85±11.37
500 kV·m⁻¹/45 min	8.63±0.33	29.30±1.53	253.32±22.67
550 kV·m⁻¹/10 min	8.50±0.35	30.49±1.01	258.92±4.30
600 kV·m⁻¹/35 min	8.68±0.21	30.17±1.04	262.08±14.97
Control	8.97±0.36	32.40±1.72	290.90±26.75

Regression analysis of the results from dry aged seeds

Regression equation for germination potentiality: Y=62.9733-0.0287X1+0.00003X12-0.00004X1× X2, here X1 refers to the intensity of electric field, and X2 refers to the action time. When the maximum value of the regression equation Y=75.5771, X1=100, and X2=5, the maximum germination potentiality of rice seeds was increased 6% compared to the control. When 100<X1<117, 5<X2<15, germination potentiality will be greater than 75.

Regression equation for germination index: Y=33.2514-0.0059X1+0.0017X2 When Y =32.7549, X1=100, X2=55, and the maximum germination index was increased 1.0%. When 100<X1<210, 5<X2<55, germination index is greater than 32.

Regression equation for seed vigour index: Y=314.3904-0.1753X1-1.2541X2+0.0002X12+0.0218X22.

When Y =295.8299, X1=100, X2=55, and the maximum seed vigour index increased 2.0%. When 100<X1<106, X2=55, seeds vigour index is greater than 295.The results above indicated that HVEF has little effect on the dry aged rice seeds.

Comparison of the effects of HVEF on dry aged seeds and wet aged seeds

The effects of HVEF on vigour index of dry seeds and wet seeds were compared (Fig.2). Compared to the dry aged rice seeds, the vigour index of wet aged rice seeds was significantly higher after the seeds was exposed to HVEF with the electric intensity less than 400kV•m-1. Compared to dry aged seeds, when the wet aged rice seeds were exposed to HVEF, the physiological activities resumed and seeds gained energy from HEVF to form active molecules with high energy. This made water electrolyzed, basic nutrition substances dissolved quickly, and activities of enzymes changed causing seed vigour to increase [4]. This maybe explains why there is a remarkable difference between the wet and dry rice seeds exposed to HVEF.

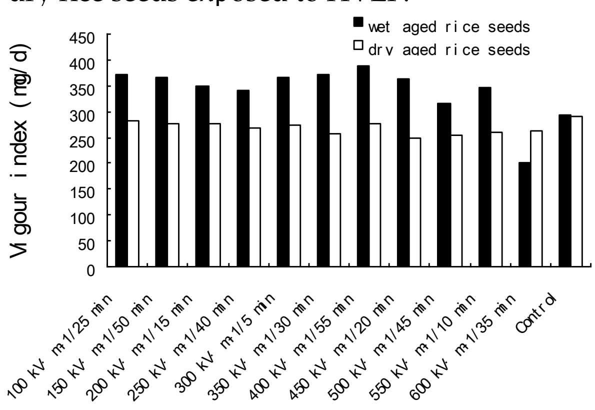

Fig. 2. Vigour index of wet aged rice seeds and dry aged rice seeds

In China, approximately 20 million kilograms of aged rice seeds are used to cope with natural calamities every year. However, germination requirements are frequently not met because of low seed vigour and germination rate, and also affecting subsequent growth Therefore it is very important to find appropriate measures to improve the vigour of aged rice seeds. Our results showed that the vigour of wet aged rice seeds could be improved by HVEF treatment, which is of great importance in agriculture.

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