Australian Journal of Crop Science AJCS
AJCS 8(11):1495-1502 (2014) ISSN:1835-2707

Physiological mechanisms of improving rice (*Oryza sativa L.*) seed vigor through arc-tooth-shaped corona discharge field treatment

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Abstract

Corona discharge field treatment is capable of improving seed vigor, but its physiological mechanisms remain unclear. In this study, *Tianyou* 1, *Tianyou* 3 and *Tianyou* 5 indica hybrid rice seeds were treated with arc-tooth-shaped corona discharge field under 360Kv/m for 4.3min. Then, seven physiological and biochemical indexes, which reflect membrane repair, metabolic enzymes and anti-oxidation enzymes, were analyzed at five germination periods (0, 24, 48, 60,72h). The results showed that the respective levels of respiration rate (26.8%, 30%, 31.5%), dehydrogenase (41.6%, 24.6%,22.3%), α-amylase (23.3%, 32.7%, 22.3%), superoxide dismutase (31.7%, 44.9%, 23.4%), peroxidase (35.2%, 40.1%, 19%) and catalase (22%, 34%, 27.8%) increased compared with the control in three hybrid rice seeds, whereas the electrical conductivity reduced (11.3%, 15.2%, 9.9%). Therefore, the order of the increase on physiology indexes on three kinds of rice seeds was Medium vigor seed > low vigor seed > high vigor seed. Meanwhile, the time of the peak value that appeared various on three kinds of rice seeds, and increase extent of peak value had differences on physiology indexes at different germination periods. Then, a PCA analysis was carried out to explain the contribution rate of the membrane repair, metabolic enzymes and anti-oxidation enzymes for improving rice seeds vigor. The contribution of each part all was 0.34; an intact membrane system was the basis of maintaining and improving of enzyme activity in theory. Therefore, we could deduce that the arc-tooth-shaped corona discharge field treatment improved seed vigor via promoting membrane repair and the activities of metabolic enzymes and anti-oxidation enzymes. This suggests that corona discharge field could be used as a valuable method to promote seed vigor, we recommend that it used to other crop or vegetable seeds for improving the production and quality in practical.

Keywords: Corona discharge field, rice seed, vigor, physiology, principal component analysis.
Abbreviations: CDF_Corona Discharge Field; EF_Electric Field; MF_Magnetic Field; PEF_Pulsed Electric Field; HVEF_ High Voltage Electrostatic Field ; DH_dehydrogenase; SOD_Superoxide Dismutase; POD_Peroxidase; CAT_Catalase; PCA_ Principal Component Analysis.

Introduction

Rice is one of the most important agriculture plants in the world, as it maintains high yield and good quality, which are highly important in agriculture production and development. However, adverse environmental conditions, such as high temperature and high humidity during harvest and storing, may lead to decrease in rice seed vigor, seedling emergence, and uniformity of seedling growth (Tan et al., 2014). Thus, it is important to find an effective treatment strategy for improving seed vigor in rice. The physical methods of seed treatment, characterized by being effective, energy efficient, and environmentally friendly, have attracted the attention of many researchers. The results showed that appropriate magnetic field (MF), electric field (EF) and radiation treatment could improve seed vigor, as well as caused internal physiological and biochemical changes in seeds (Turgay et al., 2012; Uirichs et al., 2006; Gutiérrez et al., 1993; Cramariuc et al., 2005; Chiu et al., 2010; Gaurilčikienė et al., 2013; Radzevičius et al., 2013; Toth et al., 2012). On the aspect of the cell and metabolic enzymes, after treating sunflower seeds with electromagnetic field treatment (EMF) for 2 h, Vashisth et al (2010) found that the electric conductivity was lower than that of the control, whereas the soluble protein content, activities of a-amylase and dehydrogenase were significantly higher than the control. Mung bean seeds have been treated with electromagnetic field treatment (EMF), Reddy et al (2012) found that both the seed vigor and α- amylase activity were significantly increased. After treating chamomile seeds with electromagnetic field treatment (EMF), Pourakbar et al (2013) found that the soluble protein content and activities of α-amylase and dehydrogenase were significantly higher than the control. Similar results have been obtained by Radhakrishnan et al (2012) after treating soybean seeds with pulsed electric field treatment (PEF). In addition to the effects on cells and metabolic enzymes, said method also impacts anti-oxidation enzymes. For instance, both healthy and infected lime trees were treated with a low-frequency current field by Abdollahi et al (2013), who found that the activities of SOD, POD and CAT were significantly increased, and the membranous peroxide was effectively reduced. Lettuce seeds have been treated with electromagnetic field treatment (EMF), the results showed that both the seed vigor and peroxide content were significantly increased in comparison to the control (Mousavizadeh et al. 2013). Xi et al (2013) treated mung bean seeds with high voltage electrostatic field (HVEF), and found that the soluble proteins content, as well as the activities of SOD, POD and CAT, was all significantly improved compared with the control. Therefore, when different kinds of electrical fields such as electrostatic field (EF), electromagnetic field (EMF), and pulsed electric field (PEF), were used to treat crop seeds, inconsistent results were obtained at appropriate treatment conditions. However, these physical methods produce certain beneficial biological effects on both the cell level and enzyme level. In recent years, corona discharge field (CDF) treatment as a new method of electric field (EF), was widely used to treat seeds of various crops, vegetables and cash crops by many researches (Pozeliene et al., 2009; Qi et al., 2013; Li et al., 2010; Xu et al., 2013). Corona discharge field (CDF) treatment can effectively improve the vigor and yield of seeds, but its physiological mechanisms have yet to be reported. In the present paper, an arc-tooth-shaped corona discharge field (CDF) was used to treat three different rice seeds, respectively with low, medium, and high vigor, and a total of seven physiological and biochemical indexes were analyzed at five different germination periods. The results indicated that the increase extent of the integral mean value and peak value were varied on seeds with different vigors, and the peak value of the different physiological and biochemical indexes appeared at different germination periods, as did the peak value of seeds with different vigors. In addition, principal component analysis (PCA) was used to analyze the average of integrals of the seven physiological and biochemical indexes. The aim of this study was to systematically explore the physiological mechanisms of improving rice seed vigor after arc-tooth-shaped corona discharge field treatment. We could deduce that it would be provided a valuable theoretical for explaining the gene expresses in the future studies.

Results

Changes in seed electrical conductivity after treatment

Arc-tooth-shaped corona discharge field treatment significantly decreased the electrical conductivity on rice seeds with different vigors (Fig. 3). The results showed the following: 1) the electrical conductivity of Tianyou 1 decreased significantly at the five different soak periods, and the average of the integral was 11.3% lower than the control; 2) the electrical conductivity of Tianyou 3 decreased significantly at the five different soak periods, and the average of the integral was 15.2% lower than the control; and 3) the electrical conductivity of Tianyou 5 decreased at the five different soak periods, and the average of the integral was 9.9% lower than the control. Therefore, the decreasing extent of the electrical conductivity of the three rice seeds with different vigors were in the order of medium > low > high. All of the results indicated that the arc-tooth-shaped corona discharge field treatment can promote membrane repair, maintain membrane function, and improve seed vigor

Changes in respiration rate after treatment

Arc-tooth-shaped corona discharge field treatment significantly increased the respiration rate on rice seeds with different vigors (Fig. 4). The results showed the following: 1) the respiration rate of Tianyou 1 increased significantly at the different germination periods, the average of the integral was 26.8% higher than the control, and the respiration rate increased until 60 h, reaching a peak value which was 24.5% higher than the control ($P \leqslant 0.01$); 2) the respiration rate of Tianyou 3 increased significantly at the different germination periods, the average of integral was 30% higher than the control, and the respiration rate increased until 72 h, reaching a peak value which was 33.9% higher than the control ($P \leqslant 0.01$); and 3) the respiration rate of Tianyou 5 increased significantly at the different germination periods, the average of the integral was 31.5% higher than the control, and the respiration rate increased until 60 h, reaching a peak value that was 32.3% higher than the control ($P \leqslant 0.01$). Therefore, the increased extent of the respiration rate of the three rice seeds with different vigors were in the order of high > medium > low. All the results indicated that the arc-tooth-shaped corona discharge field treatment can improve respiration rate and metabolism.

Changes of dehydrogenase (DH) activity after treatment

Arc-tooth-shaped corona discharge field treatment significantly increased the contents of TTCH, so was the activity of dehydrogenase (DH) on rice seeds with different vigors (Fig. 5). The results showed the following: 1) the dehydrogenase activity of Tianyou 1 increased significantly at the different germination periods, the average of the integral was 41.6% higher than the control, and the dehydrogenase activity increased until 60 h, reaching a peak value that was 44.1% higher than the control ($P \leqslant 0.01$); 2) the dehydrogenase activity of Tianyou 3 increased significantly at the different germination periods, the average of the integral was 24.6% higher than the control , and the dehydrogenase activity increased until 48 h, reaching a peak value that was 23.3% higher than the control ($P \leqslant 0.01$); and 3) the dehydrogenase activity of Tianyou 5 increased significantly at the different germination periods, the average of the integral was 22.3% higher than the control, and the dehydrogenase activity increased until 24 h, reaching a peak value that was 23.3% higher than the control ($P \leqslant 0.01$). Therefore, the increased extent of the dehydrogenase activity of the three rice seeds with different vigors were in the order of low > medium > high. The peak value of the dehydrogenase activity appeared earlier with higher vigor, resulting in the dehydrogenase activity is being activated ahead of time and the metabolism being accelerated.

Changes of α-amylase activity after treatment

Arc-tooth-shaped corona discharge field treatment significantly increased the activities of α-amylase on rice seeds with different vigors (Fig. 6). The results showed the following: 1) the α-amylase activity of Tianyou 1 increased significantly at the different germination periods, the average of the integral was 23.3% higher than the control, and the α-amylase activity increased until 60 h, reaching a peak value that was 32.5% higher than the control ($P \leqslant 0.01$); 2) the α-amylase activity of Tianyou 3 increased significantly at the different germination periods, the average of the integral was 32.7% higher than the control, and the α-amylase activity increased until 48 h, reaching a peak value that was 59.4% higher than the control ($P \leqslant 0.01$); and 3) the α-amylase activity of Tianyou 5 increased significantly at the different germination periods, the average of the integral was 22.3% higher than the control, and the α-amylase activity increased until 48 h, reaching a peak value that was 37% higher than the control ($P \leqslant 0.01$). Therefore, the increased extent of the α-amylase activity of the three seed with different vigors were in the order of medium > low > high, the peak value of α-amylase activity appeared earlier with higher vigor. Meanwhile, the arc-tooth-shaped corona discharge field

Table 1. Results of principal component analysis.

Principal composition	Initial Eigen values			Extraction of sum of squares loaded		
	Total given value	Contribution ratio	Cumulative contribution ratio	Total given value	Contribution ratio	Cumulative contribution ratio
1	6.320	90.285	90.285	6.320	90.285	90.285
2	0.498	7.117	97.402	0.498	7.117	97.402
3	0.175	2.504	99.906	0.175	2.504	99.906
4	0.005	0.072	99.978			
5	0.002	0.022	100.00			
6	0.000	0.000	100.00			
7	0.000	0.000	100.00			

Table 2. Component matrix of physiological indexes.

Category	Physiology index	Component 1	Component 2
Repair of membrane system	Electrical conductivity	0.3	0.92
Metabolic enzymes	Respiratory rate	0.39	-0.21
	Dehydrogenase(content of TTCH)	0.38	0.1
	α-amylase	0.39	-0.15
Anti-oxidation enzymes	Superoxide Dismutase	0.4	-0.09
	Peroxidase enzyme	0.39	-0.22
	Catalase	0.39	-0.14

treatment promotes decomposition of stored substance.

Changes of SOD activity after treatment

Arc-tooth-shaped corona discharge field treatment significantly increased the activity of SOD on rice seeds with different vigors (Fig. 7). The results showed the following: 1) the SOD activity of Tianyou 1 increased significantly at the different germination periods, the average of the integral was 31.7% higher than the control, and the SOD activity increased until 60 h, reaching a peak value that was 48.1% higher than the control ($P \leqslant 0.01$); 2) the SOD activity of Tianyou 3 increased significantly at the different germination periods, the average of the integral was 44.9% higher than the control, and the SOD activity increased until 60 h, reaching a peak value that was 73.5% higher than the control ($P \leqslant 0.01$); and 3) the SOD activity of Tianyou 5 increased significantly at the different germination periods, the average of the integral was 23.49% higher than the control, and the SOD activity increased until 60 h, reaching a peak value that was 29.6% higher than the control ($P \leqslant 0.01$). Therefore, the increased extent of SOD activity of the three seed with different vigors were in the order of medium > low > high, and the times of the peak values for SOD activity were similar. All of the results indicated that the arc-tooth-shaped corona discharge field treatment may reduce the membrance lipid peroxidization and maintaining membrane function.

Changes of POD activity after treatment

Arc-tooth-shaped corona discharge field treatment significantly increased the activity of POD on rice seeds with different vigors (Fig. 8). The results showed the following: 1) the POD activity of Tianyou 1 increased significantly at the different germination periods, the average of the integral was 35.2% higher than the control, and the POD activity increased until 60 h, reaching a peak value that was 35.1% higher than the control ($P \leqslant 0.01$); 2) the POD activity of Tianyou 3 increased significantly at the different germination periods, the average of the integral was 40.1% higher than the control, and the POD activity increased until 60 h, reaching a peak value that was 20.1% higher than the control ($P \leqslant 0.01$); and 3) the POD activity of Tianyou 5 increased significantly at the different germination periods, the average of the integral was 19% higher than the control, and the POD activity increased until 60 h, reaching a peak value that was 14.8% higher than the control ($P \leqslant 0.01$). Therefore, the increased extent of the POD activity of the three rice seeds with different vigors were in the order of medium > low > high, and the times of the peak values for POD activity were similar. All of the results indicated that the arc-tooth-shaped corona discharge field treatment may reduce the membrance lipid peroxidization and maintain membrane function.

Changes of CAT activity after treatment

Arc-tooth-shaped corona discharge field treatment significantly increased the activity of CAT on rice seeds with different vigors (Fig. 9). The results showed the following: 1) the CAT activity of Tianyou 1 increased significantly at the different germination periods, the average of the integral was 22% higher than the control, and the CAT activity increased until 60 h, reaching a peak value that was 29.1% higher than the control ($P \leqslant 0.01$); 2) the CAT activity of Tianyou 3 increased significantly at the different germination periods, the average of the integral was 34% higher than the control, and the CAT activity increased until 60h, reaching a peak value that was 51.8% higher than the control ($P \leqslant 0.01$); and 3) the CAT activity of Tianyou 5 increased significantly at the different germination periods, the average of the integral was 27.8% higher than the control, and the CAT activity increased until 60 h, reaching a peak value that was 25.2% higher than the control ($P \leqslant 0.01$). Therefore, the increased extent of the CAT activity of the three rice seeds with different vigors were in the order of medium > low > high, and the times of the CAT activity peak values were similar. All of the results indicated that the arc-tooth-shaped corona field treatment may reduce the membrance lipid peroxidization and maintain membrane function.

Principal component analysis of factors affecting seed vigor

After the arc-tooth-shaped corona discharge field treatment, the physiological and biochemical indexes were shown to have changed significantly compared with the control. Principal component analysis was used to further determine

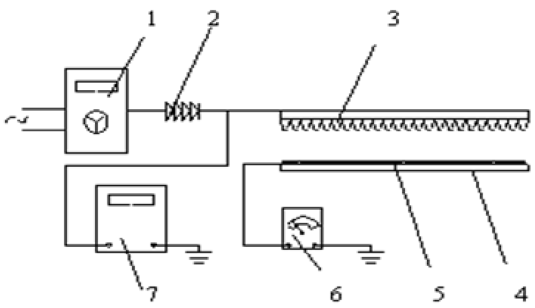

Fig 1. Schematic of high voltage electric field treatment equipment.

AC test transformer 2. High voltage silicon stacks 3. Arc-shaped electrode treated head 4. Stainless steel bottom plate 5. Rice seeds 6 digital ammeter 7 .high pressure gauge 1.

Fig 2. Photo of high voltage electric field treatment equipment.

the effects of each of the physiological and biochemical indexes (membrane repair, metabolic enzymes, and anti-oxidation enzyme) for rice seed vigor. The contribution rate and eigenvector are important indicators for choosing the principal component. In the present paper, Principal component analysis was used to analyze the average of the integrals on seven physiological and biochemical indexes at five germination periods. As shown in Table 1, the cumulative contribution rate of the principal components from the three components were as high as 99.906%, thus this three principal components were used to further analyze these physiological and biochemical indexes affecting seed vigor. As shown in Table 2, the contribution rate and load coefficient of the first and second principal components were analyzed, and results showed that the physiological and biochemical indexes, which reflect the membrane repair, metabolic enzymes and anti-oxidation enzymes, both had an equal contribution rate of 0.34. Therefore, the membrane repair, metabolic enzymes and anti-oxidation enzymes contributed equally to promoting rice seed vigor. However, given that the intact membrane is the basis of maintaining and improving of enzyme activity, despite the fact that the contribution rates of the three were equal, we concluded that membrane repair plays a more important role, followed by metabolic enzymes and anti-oxidation enzymes.

Discussion

The results obtained in this experiment showed a positive impact of corona discharge field treatment on rice seed physiology (Tan et al., 2014). Electric field (EF) treatment as an effective physical technology have been widely studied in the fields of agriculture, biology and medicine, most of results showed that electric field treatment increased seed vigor and emergence (Carbonell et al., 2011; Rochalska et al., 2005; De Souza et al., 2010; Soltani et al., 2006), so was the magnetic field (MF) treatment (Bilalis et al., 2013). Meanwhile, the biological effects of electric field treatment (EF) have been thoroughly investigated by researchers, but the results have been varied (Guderjan et al., 2007; Tkaleca et al., 2009; López et al., 2008). Diversities among biological effects depend on many factors. One is the states of the seeds, including size, moisture content and vigor, and the other is the electric field type. Thus, biological effects were shown to be different due to the physiological and biochemical indexes were measured with different types of electric fields and seeds by researchers previously, as a result the explanation of the physiological mechanisms were not the same. Corona discharge field treatment as a new method of electric field (EF) has little research on rice seeds. In this paper, for the purpose of understanding the physiological mechanisms of arc-tooth-shaped corona discharge field treatment (CDF), three hybrid rice seeds with different vigors were used to treat and a total of seven physiological and biochemical indexes were analyzed at five different germination periods.

Currently, research regarding the physiological mechanisms of electric field treatment has gradually become more common, but most previously researchers measured the physiological and biochemical indexes at the single germination time, resulting in imprecise explanations for the physiological mechanisms. Deng et al(2006), who treated dry and wet cucumber seeds with high voltage electrostatic field (HVEF), found that the conductivity of wet seeds was significantly lower than that of dry seeds at 4 h, and the respiration rate and α-amylase activity of wet seeds were higher significantly than those of dry seeds at 6 h. Wang et al (2009), who treated aged rice seeds with a high voltage electric field (HVEF), found that the leachate conductivity and malondialdehyde were significantly decreased, whereas the activities of SOD, POD and CAT were significantly improved compared with control at 4 and 9 days. In the present paper, the physiological and biochemical indexes of three hybrid rice seeds were analyzed at five germination periods. On the cell level, the electrical conductivity was reduced by varying degrees, so were the averages of the integral. On the metabolism level, the activities of dehydrogenase and α--amylase were increased by varying degrees, so were the averages of the integrals and peak values. On the anti-oxidation enzyme level, the activities of SOD, POD and CAT were increased by varying degrees, so were the averages of the integrals and peak values. Therefore, it is more accurate to make a conclusion for the changes of enzymes in seeds by determining the averages of the integrals and peak values at different germination periods. In addition, the time of the peak values that appeared various at 5 germination periods for different physiological and biochemical indexes, and the peak values of the metabolic enzymes occurred earlier than those of the anti-oxidation enzymes, which may be due to the enzyme activation time reflecting the metabolic and anti-oxidation functions were different. What's more, the time of the peak values for the same physiological and biochemical indexes, such as dehydrogenase and α- amylase, were different for the three rice seeds. The time occurred earlier for the seeds with high vigor, which may be due to different dynamic abilities for enzymes and metabolism.

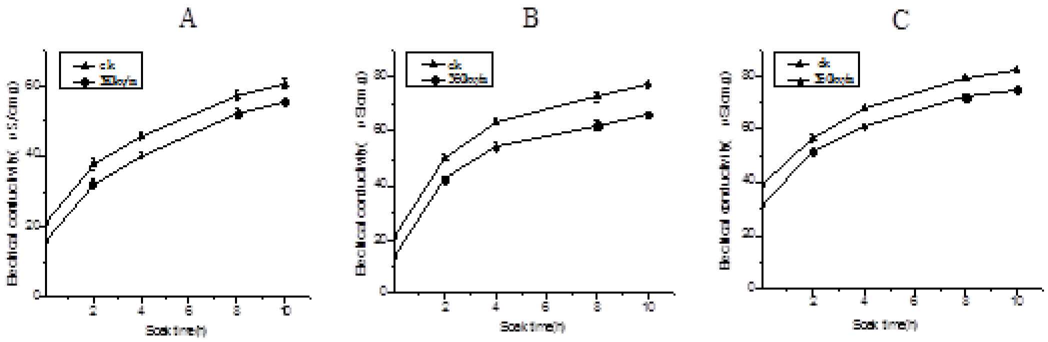

Fig 3. Comparisons of Electrical conductivity on three kinds of rice seed with different vigors. A: "Tian You 1" B: "Tian You 3" C: "TianYou 5". CK: Control group, 360Kv/m: Treated group . Note: P≤0.01that significant differences at the 1% level.

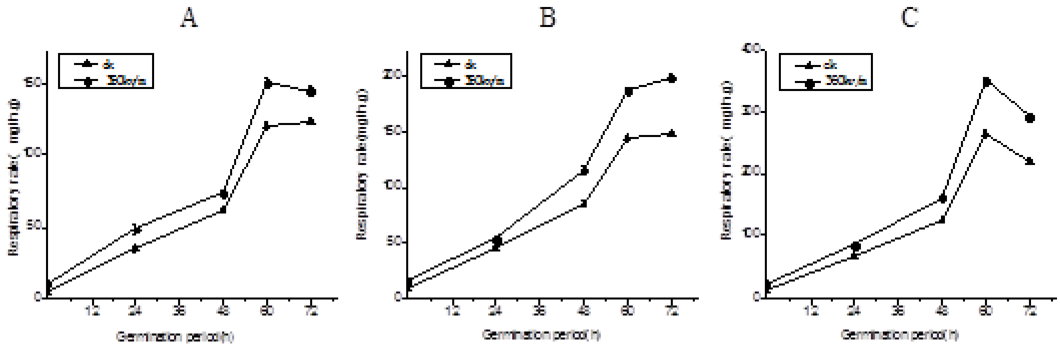

Fig 4. Comparisons of Respiratory rate on three kinds of rice seed with different vigors. A: "Tian You 1" B: "Tian You 3" C: "TianYou 5". CK: Control group, 360Kv/m: Treated group . Note: P≤0.01that significant differences at the 1% level.

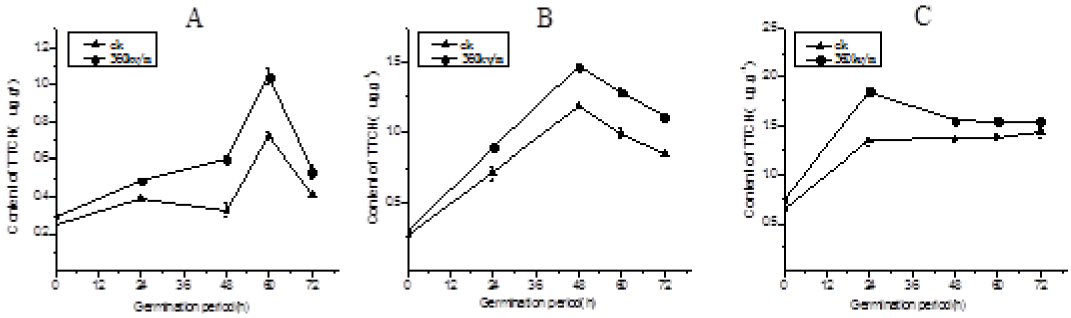

Fig 5. Comparisons of Content of TTCH on three kinds of rice seed with different vigors. A: "Tian You 1" B: "Tian You 3" C: "TianYou 5". CK: Control group, 360Kv/m: Treated group . Note: P≤0.01that significant differences at the 1% level.

Thus, it is imprecise to analyze the physiological mechanisms at a single time point, and more accurate and persuasive to determine the comprehensive mechanisms on different physiological and biochemical indexes at different germination periods. In most studies, the mechanisms were determined simply by change of various physiological and biochemical indexes, and there has yet to be any research performed in which the principal indexes affecting seed vigor were determined by using principal component analysis. In the present paper, the principal component analysis method was used to analyze the contribution rates of the physiological and biochemical indexes, which reflect membrane repair, metabolic enzymes and anti-oxidation enzymes. The results showed that although the three categories of physiological and biochemical indexes had equal contribution rates on rice seed vigor, membrane repair played the most important role on seed vigor in theory. Intact membrane is the basis of maintaining and improving enzyme activity, followed by metabolic enzymes and anti-oxidation enzymes. These results indicate that the arc-tooth-shaped corona discharge treatment can significantly improve seed vigor by promoting membrane repair and the

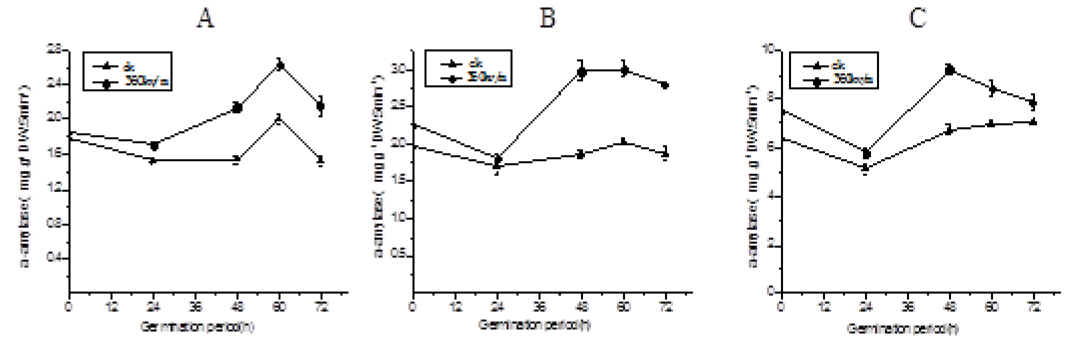

Fig 6. Comparisons of α-amylase activity on three kinds of rice seed with different vigors. A: "Tian You 1" B: "Tian You 3" C: "TianYou 5". CK: Control group, 360Kv/m: Treated group . Note: P≤0.01that significant differences at the 1% level.

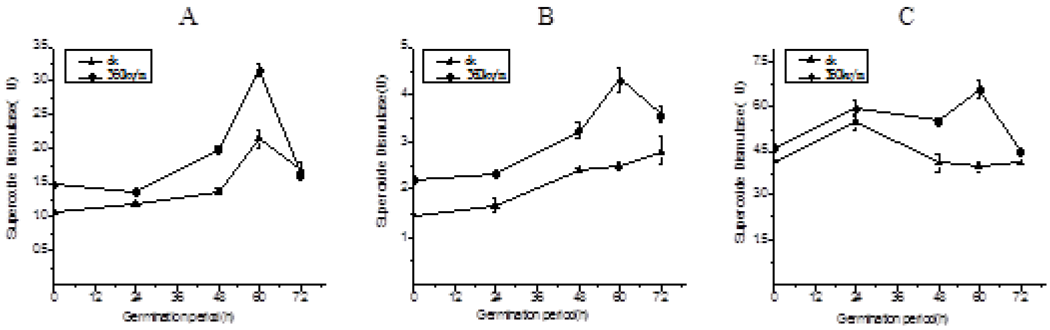

Fig 7. Comparisons of Superoxide dismutase on three kinds of rice seed with different vigors. A: "Tian You 1" B: "Tian You 3" C: "TianYou 5". CK: Control group, 360Kv/m: Treated group . Note: P≤0.01that significant differences at the 1% level.

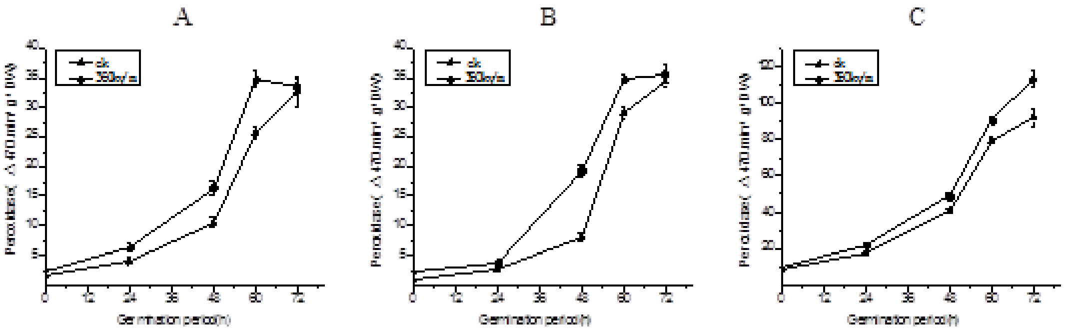

Fig 8. Comparisons of Peroxidase on three kinds of rice seed with different vigors. A: "Tian You 1" B: "Tian You 3" C: "TianYou 5". CK: Control group, 360Kv/m: Treated group. Note: P≤0.01that significant differences at the 1% level.

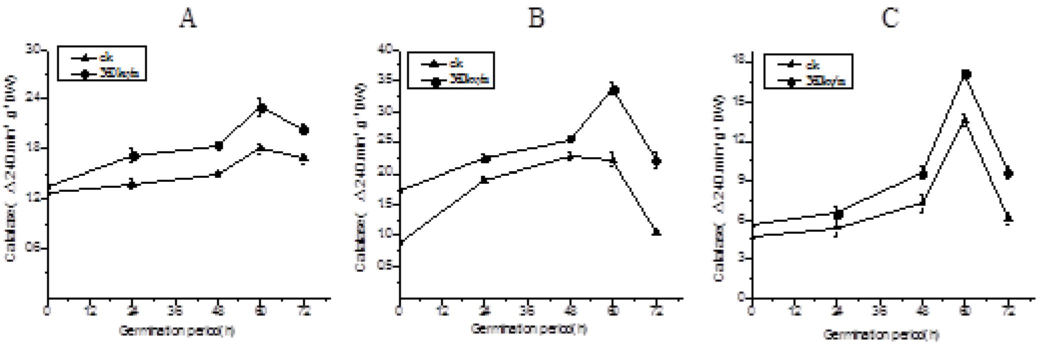

Fig 9. Comparisons of Catalase on three kinds of rice seed with different vigors. A: "Tian You 1" B: "Tian You 3" C: "TianYou 5" CK: Control group, 360Kv/m: Treated group. Note: P≤0.01that significant differences at the 1% level.

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activities of metabolic enzymes and anti-oxidation enzymes.

Materials and Methods

Plant materials

Three types of hybrid rice seeds with respective initial germination rates of 45%, 65% and 85%, namely Tianyou 1, Tianyou 3 and Tianyou 5, were used to treat with arc-tooth-shaped corona discharge field as the test materials (Tan et al., 2014). Furthermore, the rice seeds of same vigors without arc-tooth-shaped corona discharge field as the control. All the rice seeds are F_1 hybrid indica rice seeds from the seed company.

Experimental equipment

The corona discharge field treatment equipment was shown in figure 1 and figure 2. It mainly consisted of an YDJ-50 AC transformer, a 2 CL high voltage silicon stack, an FRC-100 high-pressure gauge, a 2 mA digital ammeter, an electric arc-tooth steel head, and a stainless steel polar plate. A total of 10 arc-tooth-shaped electrode slices were fixed on the test bed through the holes on either side, the distance from the tip of the arc-tooth-shaped stainless steel plate (ground) was 50 mm.

Corona discharge field treatment

Three hybrid rice seeds were treated with the arc-tooth shaped corona discharge field of 360 kV/ m for 4.3 min (Fig.2). During treatment, a single layer of rice seeds (60g) was treated with positive electric field. Meanwhile, the voltage was adjusted by the knob on the AC test transformer. During the processing, the optimum humidity of three hybrid indica rice seeds was 12.5%, we could adjust the humidity of rice seed to ensure 12.5%. It was measured by cereal moisture tester before processing.

Seed germination and sampling

One replicate of 40 seeds was randomly selected from both the treated and control (CK) sample groups. The seeds were then disinfected with 1% sodium hypochlorite solution and thoroughly washed with deionized water, and this procedure was repeated three times. Finally, the paper bed was used for seed germination under the conditions of 25℃ and continuous light for 0h, 24h, 48h, 60h and 72 h. All samples were used to assay respiration rate, dehydrogenase activity (content of TTCH), α-amylase activity, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT).

Electrical conductivity

After arc-tooth-shaped corona discharge treatment, 40 intact seeds were selected and weighed, and this procedure was repeated three times. Then the rice seeds were washed with deionized water three times, dried with filter paper and placed into the 50 ml graduated test tube with distilled water (30 ml). A graduated test tube with distilled water was used as the blank control. All of the graduated tubes were sealed with stoppers and soaked in a 25℃ incubator for different soak times (0, 2, 4, 8 and 10 h). Finally, the electrical conductivity of the rice seeds was assayed by using a DDS-307 conductivity meter at room temperature.

Respiration rate

40 seeds from each germination period were sampled and weighed, and this was repeated three times. The seeds were placed in a clean wide-mouthed bottle with moistened filter paper, and the initial CO_2 emission was measured using an infrared CO_2 analyzer, then the CO_2 emission was measured again after being placed for 1 h. Then the respiration rate was calculated.

Dehydrogenase activity (content of TTCH)

The greater the content of TTCH was, the higher activity of dehydrogenase was. The activity of dehydrogenase (DH) was measured according to the TTC method modified from Hu (1986).

α-amylase assay

The activity of the α-amylase was assayed using the 3, 5 - dinitrosalicylic acid method (DNS) method, according to a previous report by Bernfield et al (1951).

Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) assay

The respective activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were assayed according to a previous report (Cakmak et al 1993; Dhindsa et al 1980). The supernatant was used for measuring the activities of SOD, POD and CAT by the nitro-blue tetrazolium (NBT) method, guaiacol method and UV absorption, respectively.

Statistical analysis

All the data obtained for physiological mechanism analyses were the mean of three readings per replicate per treatment, each physiological and biochemical experiments were repeated thrice with independent extractions to verify the results obtained in Excel 2003. The data of physiological and biochemical indexes at different germination periods were subjected to analysis of variance (ANOVA) and Paired Samples T Test using SPSS 18.0. The integral mean values were calculated using Origin 8.6. The contributions of physiological and biochemical indexes were calculated using the principal component analysis in SPSS 18.0.

Conclusion

Our results indicated that arc-tooth-shaped corona discharge field treatment as a physical treatment can significantly improve rice seed vigor. Moreover, it is accurate to analyze the comprehensive mechanisms with different physiological and biochemical indexes at different germination periods. Thus, arc-tooth-shaped corona discharge field treatment increased rice seed vigor by promoting membrane repair and the activities of metabolic enzymes and anti-oxidation enzymes.

Acknowledgements

Funding support was received from Special Fund for Agro-scientific Research in the Public Interest of china (201203052, 201303005).

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