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The Effect of Electrochemical Processing on Biological Behavior of Wheat Seeds Germination

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Using a new electrochemical method to deal with wheat seeds germination is presented in the present paper. The biological effect of the seeds germination treated by electrochemical method is preliminarily studied. The germination potential, germination rate, the length of the seedling bud and root, germination index, vigor index, amylase and peroxidase (POD) activity are determined using biostatistical methods and spectrophotometry. The influence to processing time and voltage are investigated. The results show that the germination potential, germination rate and vigor index increase by about 1%~6%, 0.6%~4.2% and 0.2~2.0, respectively. At the same time, amylase and peroxidase activity were improved by about 0.23~10.34 U and 5.66~28.58 U, separately. The best treatment conditions are the voltage of 8 ~10 V and the processing time of 4 h. The results provide a new method for seeds treatment.

Keywords: Electrochemical method; Wheat seeds; Biological effect; Amylase activity; Peroxidase activity

1. INTRODUCTION

Seeds are the basic and the most important material in crop production. And the vitality of plant seeds will influence the whole life of the growing process. So different ways are applied to keep and improve the vitality of the seeds. It not only is a biology problem worthy discussing but also has great practical significance to the agricultural production [1].

In recent years, scientists often use physical and chemical methods to improve seeds quality, energy and the resistance in order to increase the production and ensure the high and stable yield. The chemical processing methods mainly include seeds soaking and dressing by chemical beforehand sowing[2,3]; Physical methods mainly include ultrasound treatment[4], plasma processing[5], electromagnetic field and static processing[6-10], magnetized water or temperature treatment[11, 12],

microwave processing[13], etc.. Enzyme activity, which is beneficial to transfer starch, protein or other substances into soluble matters that could be adsorbed by embryos, can be improved by different methods of treating seeds. And then it can enhance the seeds germination rate, promote seeds growth and increase the crop yields.

However, the current methods have some shortcomings. Chemical agents may damage the seeds and thus have negative effects on the growth and development of crops due to the high residual medicine in seed. Moreover, the physical treatments, e.g. high pressure electrostatic processing, consume too much energy and have low safety coefficient. Thus, it is necessary to take a safe and low consumption method, electrochemical method, to treat the seeds. Seeds treatment using electrochemical method has great advantages, such as low energy consumption, normal temperature, atmospheric pressure, the system has strong electromagnetic field effect. To our knowledge, there are few literatures about the aspect of seeds germination using electrochemical processing all over the world. So it is worth to further studying. In the present work, treat wheat seeds are treated using electrochemical method and the influence of the process on seeds properties is detailed studied, including germination potential, germination rate and index, and the vigor index and amylase activity of wheat seeds etc..

2. EXPERIMENTAL

2.1 Materials

The tested wheat seeds (Dongfeng No. 1) are provided by Shanxi Academy of Agricultural Sciences.

The reagents used for electrolyte solution are anhydrous Na2SO4 (pure analytical level), purchased from Jiaozuo Chemical III Plant;

The reagents used for seeds disinfection are NaClO solution of pure analytical level, purchased from Tianjin Bodi chemical Co., LTD.

2.2 Methods

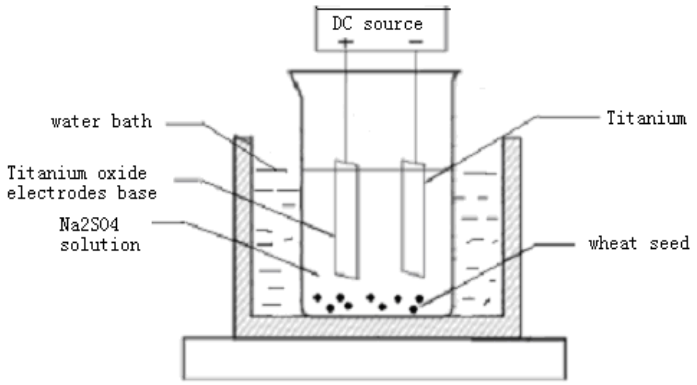

Figure 1. Electrochemical system installation plan on wheat seeds

The used HB 17301 SL5A DC power supply is purchased from Zhejiang Hongbao Electric Group Co., LTD. Details of experiment device is presented as follows (Fig. 1):

2.3 The design of experiments

Wheat seeds are chosen as the research objects. Seeds are disinfected in 5% NaClO solution for 10 min and then are washed with deionized water for 2~3 times , putting them into a small beaker followed by adding Na_2SO_4 electrolyte solution with concentration of6×10-2 mol/L. The inert materials titanium oxide electrodes and titanium electrode are used as anode and cathode, respectively in the electrochemistry system. The wheat seeds are treated by DC voltage power ranging from 2 V to10 V at different time intervals (1.0, 2.0, and 4.0 h). The treated seeds are put on the filter paper in petri dishes followed by adding 10ml deionized water to keep the dishes moist. Finally, sample seeds are placed into the incubator under constant light and temperature to observe the germination. It should be noticed that there are 50 seeds in each dish, and each treatment is repeated three times. Moreover, two groups seeds (CK 1 and CK 2) untreated using electrochemical devices are carried out in order to well observe the differences caused by electrochemical treatment. For groups CK 1 and CK 2, seeds are soaked in distilled water and Na_2SO_4 electrolyte solution, respectively.

2.4 The apparent characteristics of wheat seeds

A. Germination characteristics

The germination time and status are recorded to calculate germination characteristics including germination potential, germination rate, germination index and vigor index.

Gp (Germination potential) = (the number of all the normal germinating seeds in specified dates/ the seeds number for the test)×100%; Gp should be confirmed by the germination rate at the sprouted peak (3 d).

Gr (Germination rate) = (the number of all the normal germinating seed/the seed number for the test) ×100%; Gr should be confirmed by the germination rate at the seventh day.

Gi (Germination index) = Σ (Gt/Dt);

Where, Gt is the number of germinating seeds in the t days, and Dt is the day number of germination;

Vi (Vigor index) = S×Gi; S is the average fresh weight of seedlings

B. Seedling morphological characters

The budand root length of sample seeds, the fresh weight and dry weight of seedlings are respectively measured at the seventh day. The dry weight is determined using re-drying method.

2.5 Amylase activity of the wheat seeds

Amylase activity is determined by 3, 5-dinitrosalicylic acid colorimetry[14]. The unit of amylase activity is U. A unit of enzyme activity is confirmed by the enzyme quantity needed when 1mg maltose is released during starch hydrolysis process within 3 min under 25℃.

2.6 POD activity of sample seeds

POD activity is determined by the method of Guaiacol[15]. When the wavelength is 470 nm, the absorbance changed by 0.01 per min is as 1 peroxidase activity unit (U).

3. RESULTS AND DISCUSSION

3.1 The influence of apparent characteristics on wheat seeds

Tables 1-3 show wheat seeds growth vitality treated with various voltages after different time Table 1 shows the germination characteristics of CK 2 is better than that of CK 1 treated after 1.0 h, but the differences are not obvious. In the case of different voltages treatment, seeds present best characteristics after being treated using 10 V. Compared with CK 1 (CK 2), the seedling bud length, root length, the germination rate, germination potential, germination index and vigor index of seeds treated using 10 V increase by 0.75 (0.65) cm, 0.60 (0.14) cm, 3.2% (2.7%), 1.8% (1.8%), 1.8 (2.8) and 1.6 (1.27) respectively.

Table 1. The apparent characteristics of wheat seeds germination treated with different voltages (processing time 1.0 h)

Treatment Group	The Bud Length/cm	The Root Length/cm	Germination Potential/%	Germination Rate/%	Germination Index	Vigor Index
2V	9.98	10.91	91.0	95.7	73.9	14.20
4V	9.58	10.32	92.6	95.3	72.3	13.85
6V	9.89	11.27	93.0	97.0	74.5	13.36
8V	10.55	12.03	88.7	94.7	70.1	13.78
10V	10.38	11.18	92.3	97.7	74.2	14.41
CK 1	9.63	10.58	90.5	94.5	72.4	12.81
CK 2	9.73	11.04	90.5	95.0	71.4	13.14

Table 2. The apparent characteristics of wheat seed germination treated with different voltages (processing time 2.0 h)

Treatment Group	The Bud Length/cm	The Root Length/cm	Germination Potential/%	Germination Rate/%	Germination Index	Vigor Index
2V	10.47	10.57	91.3	98.0	72.7	14.31
4V	9.98	10.58	92.7	97.3	73.1	13.93
6V	8.66	8.51	91.3	96.7	71.2	13.24
8V	10.95	11.11	96.0	98.7	75.0	14.30
10V	10.68	11.63	87.3	94.7	71.0	13.97
CK 1	8.50	8.37	88.0	94.7	67.3	12.30
CK 2	8.47	8.40	90.0	94.0	68.4	12.89

The apparent characteristics of the seeds in other treat groups are not better than that treated under 10 V, but better than samples seed without electrochemical treatment, i.e. groups CK 1 and CK 2.

Table 2 shows the germination characteristics of CK 2 is better than that of CK 1 treated after 2.0 h, but the difference is not obvious. For samples with different voltages treatment, seeds present best characteristics after being treated using 8 V. Compared with CK 1 (CK 2), the seedling bud length, root length, the germination rate, germination potential, germination index and vigor index of seeds treated using 8 V increase by 2.45 (2.48) cm, 2.74 (0.11) cm, 4.0% (4.7%), 8.0% (6.0%), 7.70 (6.60) and 2.00 (1.41) respectively

Table 3 shows the germination characteristics of CK 1 is better than that of CK 2 treated after 4.0 h, but the difference is not obvious. For samples with different voltages treatment, seeds present best characteristics after being treated using 4 V. Compared with CK 1 (CK 2), the seedling bud length, root length, the germination rate, germination potential, germination index and vigor index of seeds treated using 8 V increase by 0.99 (2.48) cm, 0.24 (2.86) cm, 0.6% (0.6%), 8.6% (8.6%), 5.60 (4.80) and 1.19 (1.66), respectively.

Table 3. The apparent characteristics of wheat seed germination treated with different voltages (processing time 4.0 h)

Treatment Group	The Bud Length/cm	The Root Length/cm	Germination Potential/%	Germination Rate/%	Germination Index	Vigor Index
2V	10.00	11.42	92.7	97.3	72.8	15.19
4V	10.23	11.45	95.3	97.3	74.4	15.81
6V	9.32	10.08	88.0	96.7	71.1	14.80
8V	9.61	8.44	94.0	97.0	72.2	13.79
10V	10.04	9.13	93.3	96.7	72.2	14.43
CK 1	9.24	11.21	86.7	96.7	68.8	14.62
CK 2	8.69	8.59	86.7	96.7	69.6	14.15

On the basis of the above result obtained from Tables 1-3, it can be found that the appropriate electrochemical system benefits for the growth of the seedling root and bud, leading to the enhance of the seed vigor. And it can accelerate stored materials to decompose, transform and recycle in the process of seeds germination. In addition, matter accumulation accelerates seedling growth and positively stimulates the self-adjusting ability of seeds. thus increasing the nutrient absorption and transformation of energy during seeds growth process. The content of free radicals within the seeds increases with electromagnetic field caused by electrochemical system, which leads to the increase of membrane permeability, enzyme activity the acceleration of seed germination and the improvement of seed vigor [16, 17].

3.2 The wheat seedling growth situation

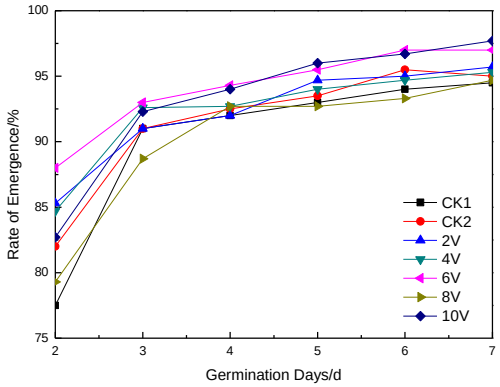

Figure 2. The effect of the emergence rate under different voltages (processing time 1.0h)

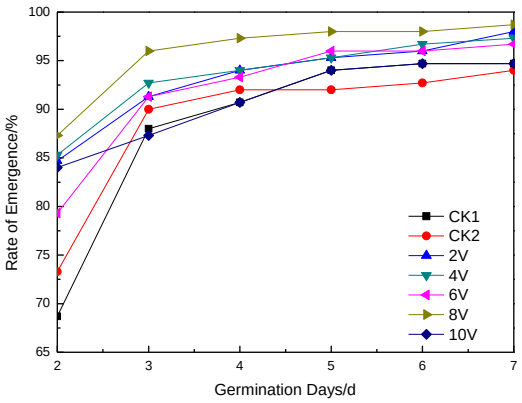

Figure 3. The effect of the emergence rate under different voltages(processing time 2.0h)

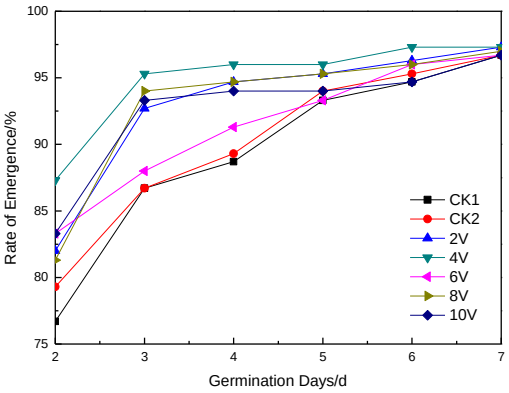

Figure 4. The effect of the emergence rate under different voltages (processing time 4.0)

Figs. 2-4 show the growth of wheat seeds treated by electrochemical method. Compared with seeds without electrochemical treatment (groups CK 1 and CK 2), the germination rate of the treated wheat seeds is significantly improved. The experimental results show that there is driving force for the

seeds germination by the electrochemical treatment with appropriate voltage and time, which could not only increase permeability on seed coat, promote cell activation, break the seed dormancy state but also improve the germination rate and accelerate seedling growth.

As other researchers' report, the above electric and magnetic field effect is responsible for enhancing seed germination capacity, accelerating morphogenetic processes, and increasing seeds water absorption [18, 19]. The absorbed water in seeds, to some extent, take place changes due to the electrochemical system, so that it penetrates fast inside seeds, at the same time affects the rate of enzymatic reactions [19].

3.3 The influence of amylase activity

Amylase，the main hydrolysis enzymes during seeds germination，plays an important role in the quickly starting of the material and energy metabolism. Its activity level is the main reason which leads to fast seeds germination.

The comparison between CK1 and CK2 in Table 4 shows that amylase activity of seedlings does not significant change. It suggests that the effect of seeds germination treated by Na_2SO_4 aqueous solution is not obvious. However, compared with the groups CK 1 and CK 2.amylase activity of seeds treated by the electrochemical method improved to some extent. Amylase activity was improved when the voltage is 8 V or 10 V. Especially for sample seeds treated for 4 h, amylase activity of seedlings was significantly enhanced (about 10 U increases) [20].

The above results indicate that the increase of amylase activity could ensure nutrients rapidly and sufficiently enter into the embryo in the appropriate electrochemical system. At the same time, the cell division accelerates, leading to the increasing synthesis speed of protein and nucleic acid and the seed metabolism [21].

Table 4. The effect of the amylase activity

Factor Time	CK1	CK2	2V	4V	6V	8V	10V
1.0h	26.83U	26.14U	26.76U	27.12U	31.27U	27.41U	27.95U
2.0h	22.85U	20.88U	23.84U	23.38U	24.17U	26.39U	27.50U
4.0h	22.78U	21.77U	14.21U	14.02U	23.01U	32.11U	31.11U

3.4 The influence of peroxidase activity

There is a great quantity of peroxidase, one kind of enzyme with higher activity, in plants. It is a. With respiration, photosynthesis and growth hormone strongly depend on the oxidation. POD, one kind of oxidase in plant organs and tissues ubiquitously, is related with many physiological and

metabolic processes in the plants. It is also closely related with cell growth and differentiation. And then it can affect plant growth and development[22].

There is no significant difference for POD activity of samples seeds between CK 1 and CK 2as shown in Fig. 5. C However, one can see that POD activity is improved significantly when voltage ranging from 6 V to 10V is applied in electrochemical system. Compared with CK 2, POD activity significantly improved in 0.05 level and 0.01 level when seeds are treated with voltage of 6 V and 8 V, respectively. When the voltage was 8V, compared with CK1 and CK2, POD activity was significantly improved in 0.01 level. When the voltage was 10V, compared with CK1, POD activity was significantly improved in 0.05 level, also compared with CK2, POD activity was significantly improved in 0.01 level.

The results show that the appropriate electrochemical treatment for wheat seeds can enhance the POD activity. The enzyme could not only promote the formation of new cells, enhance gene expression activities but also maintain the balance between free radicals, protect the membrane structure and be conducive to seed germination and growth.

It is well known that appropriate amount of free radical is the inevitable product in the process of oxygen metabolism. Free radical plays a very important role in storing energy of metabolism, eliminating waste and clearing away poison matter [23]. But when the free radical was excessive，it would cause the serious injury for cell tissue. Therefore maintaining free radical metabolic balance in organism is very important. In the appropriate electrochemical system, metabolism of seed cell was activated by electromagnetic field effect, which can accumulate the free radical to stimulate the synthesis of peroxidase. So POD activity of the processing seed was improved in appropriate electrochemical system. The present work provides the experiment basis for the electrochemical system applied in practice applications and is helpful for the exploration of electrochemical reaction mechanism.

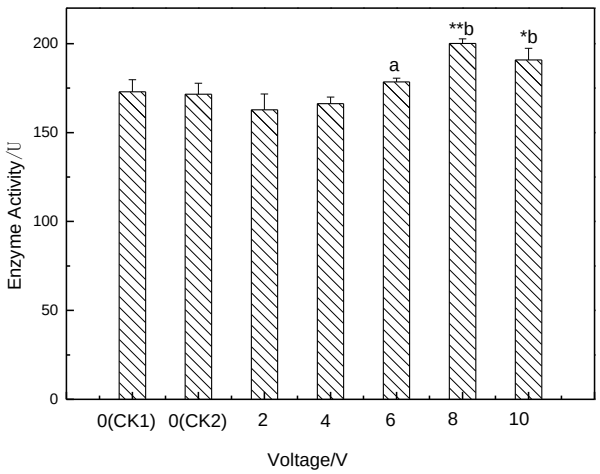

Figure 5. The relationship between peroxidase activity and applied voltage
(* and ** indicate significant difference between CK1 and treatment at 0.05 and 0.01 level respectively **a** and **b** indicate significant difference between CK2 and treatment at 0.05 and 0.01 level respectively)

4. CONCLUSIONS

The change of physiological characteristics, including germination potential, germination rate, germination index, and vigor index etc., and the enzyme activity size were measured during seedling stage. The following conclusions have been obtained:

The wheat seeds treatment using electrochemical methods reported in the present paper, which are latest at home and abroad, provide a new way for seeds germination processing.

The biological effect of wheat seeds after electrochemical treatment is related to the experimental conditions. The used treatment voltage and time have a different effect on germination characteristics, such as germination potential or rate, vigor index and enzyme activity of the seeds. The results show that the germination potential, germination rate and vigor index increase by about 1%~6%, 0.6%~4.2% and 0.2~2.0 respectively, at the same time amylase activity and peroxidase activity improve by about 0.23~10.34 U and 5.66~28.58 U respectively. Electrochemical methods could increase seeds coat permeability, promote cell activation within the seeds, break dormancy status of the seeds, which results in the improvement of germination rate and acceleration of seedlings growth;

The used voltage ranging from 8 V to 10 V and 4 h processing time is the optimization treatment conditions. It indicates that appropriate electrochemical system is helpful to improve wheat seed vigor.

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