Explore the designated frequency band (EMF) effect of radiation on biological characteristics of *Ocimum basilicum*

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Abstract. Explore and obtain the effects of irradiated *Ocimum basilicum* seeds on the biological mechanisms of germination and seedling growth in the specified frequency bands. In the experiment, two influencing factors of the specified frequency band and irradiation time were set, and different gradients of irradiated seeds were set. Non-magnetized treatment was used as a blank control group (CK) to determine the changes in the main chemical components in seeds and seedlings, and explore the radiation breeding biology mechanism. The results showed that 90 kHz-100 kHz had a significant inhibitory effect on seed germination, and the 50 kHz-70 kHz band had a significant effect on the germination of *Ocimum basilicum*, with 50 ± 2.0 kHz being the best. In the time gradient exploration, the main chemical content of seeds was the highest when irradiated for 20 min, and the growth effect of seedlings was the best. The specified frequency band (EMF) has the effect of promoting and inhibiting the germination and growth of basil. It stimulates the internal chemical composition to break the dormancy period of the seed, accelerates the anabolism, and increases the molecular assimilation of the building.

1. Introduction

Electromagnetic field (EMF) is a physical field created by a charged object[1]. Electromagnetic irradiation breeding artificially uses physical mutagenic factors, and induces genetic variation of plants by irradiation, and obtains mutants with utilization value in short times[2]. According to the requirements of breeding objectives, it can be directly produced and utilized after manual selection, identification and cultivation[3]. New varieties or new germplasm resources for breeding as a parent. The mutants produced by irradiation mutagenesis show new traits, enrich the germplasm resources, and provide abundant raw materials for breeding. Irradiation mutagenesis can improve a single trait in a short period of time while maintaining excellent characteristics[4]. Treating seeds by EMF has small physiological loss, simple and fast operation, and improve the effect of absorbent fertilizer.[5]. China has done a lot of research on this field since the 1990s, and reported the treatment effects of wheat, corn, rice and soybean, but it is still very shallow for the breeding of *O. basilicum* belonging to aromatic plants. Irradiation of the *O. basilicum* in the frequency band has not been reported yet. *O. basilicum* is a genus of the genus labiatae and basil. In recent years, with the strengthening of people’s green and healthy consumption concept, as a kind of plant with economic value, the market demand is getting bigger and bigger, and it is more and more valued by experts and scholars[6]. In order to
improve the status quo of low natural germination rate, the genetic traits are changed by irradiation treatment. The designated frequency band can affect the change of chemical content in the seed, transport the stored nutrients in the cotyledons to the germ, change the genetic material such as larger molecules in the seedlings to achieve different biological expression mechanisms, increase seed survival rate, and selectively change the expression of stems and leaves and improve economic benefits[7]. The experiment can explore the effects of EMF on the secretion and synthesis of seed enzymes and the changes in the arrangement of various proteins, and to analyze their changes in morphological characteristics, physiological and biochemical characteristics, and to breed new varieties with strong resistance and more economic value[8-9]. Irradiation breeding is an innovation of the O.basilicum breeding technology, which can provide some reference for the subsequent irradiation breeding work, and has important practical significance in enriching the O.basilicum variety. In this study, different doses of irradiated basil seeds were used to induce the latent mutant plants, and the irradiated seeds were subjected to germination test, and the effects of electromagnetic radiation on their morphological, physiological and photosynthetic characteristics were observed. Based on the results of this study, the biological mechanism of basil EMF irradiation was obtained, and the EMF breeding mechanism was improved.

2. Materials and methods

2.1 Experimental Materials

Test materials: O. basilicum seeds, purchased from Shouguang Wentian Seed Industry Co., Ltd.  
Reagents: TTC dye solution, 95% ethanol (or 80% acetone), quartz sand, calcium carbonate powder, 0.1 mol/L hydrogen peroxide, 0.1 mol/L phosphate buffer pH 7.0, 0.1 mol/L phosphate buffer pH 7.8 0.3% guaiacol reaction solution. Instrument: 721-UV-visible spectrophotometer and high freeze speed refrigerated centrifuge.

2.2 Method

2.2.1 Seed irradiation treatment.
Set 4 irradiation gradients 30± 2 kHz, 50± 2 kHz, 70± 2 kHz, 90± 2 kHz to irradiate the O. basilicum seeds, and treat 100 seeds of similar size and fullness at each frequency. With no radiation effect as the control group (CK), the experiment was repeated 3 times.

2.2.2 Germination and determination.
100 seeds were taken from the treatment group and the control group, and the seeds were placed neatly in a petri-dish which was sterilized by alcohol and covered with a layer of moist filter paper. 5.0 mL of distilled water was added and placed in a 25°C light incubator. Replace the filter paper once in 2d, add distilled water regularly to keep the dish moist, observe and record the germination. The seed germination rate was counted. The radicle length was equal to the seed length, which was the seed germination. The 4d germination number was recorded as the germination potential. When the germination of the seeds in the dish was basically unchanged, the germination rate was recorded and calculated. The experiment was repeated 3 times and the average value was calculated.

\[ D = \frac{T_4}{G} \times 100\% \quad (1) \]
\[ T\% = \frac{T}{G} \times 100\% \quad (2) \]
\[ GI = \frac{\sum G_t}{D_t} \quad (3) \]

Note: \( D \) was the germination potential, \( T \) was the number of germination of the tested seeds, \( T_4 \) means the number of germination of the tested seeds within 4 days, \( G \) means the number of seeds tested, \( T\% \) is the germination rate, \( GI \) is the germination index, and \( G_t \) is the end of the germination test number of daily germination period, \( D_t \) is the number of days germination, \( \sum \) means the sum.

2.2.3 Seed irradiation treatment and sowing.
The optimal frequency of germination effect was selected, and three time gradients were set up for 10 min, 20 min, and 30 min for irradiation treatment. Each group was set with 100 seeds of similar size and fullness, and three repeated experiments were performed. The effect of no magnetic field was used as the control group (CK). Irradiated O.
basilicum seeds were sown in a tray according to a time gradient, and 100 seeds were seeded per tray, repeated 3 times. The plugs were placed in a 25°C environment, watered once for 1d, and the emergence rate was observed at 3d. When the seed emerges to four true leaves, it is transplanted into the pot. Normal water and fertilizer management, observe the changes in morphological, physiological and photosynthetic characteristics of seedlings. It is convenient for the determination of biological indicators related to late seedlings.

2.2.4 Measuring index and method. O. basilicum seeds before and after irradiation were placed in distilled water for soaking to ensure full contact with distilled water. Distilled water was discarded after 2, 4, 8 h, respectively, and the water on the outer skin of the seeds was wiped dry with filter paper. Test the quality of the seeds before and after and record the data. Repeat the test 3 times.

\[
Q(\%) = \frac{m_1 - m_0}{m_0} \times 100\%
\]

(4)

Note: \( Q \) means the water absorption rate of the seed, \( m_0 \) means the weight before soaking, and \( m_1 \) means the weight after the inflation.

1) Peroxidase (POD) activity was measured by using the guaiacol method: 0.2 g of the seed was weighed, and the supernatant of the extracted enzyme was obtained by grinding and centrifugation to be measured by a spectrophotometer.
2) Determination of catalase (CAT) activity by ultraviolet absorption spectrophotometry: 0.3 g of the leaves and a phosphate buffer of pH 7.8 were ground and centrifuged to obtain a crude enzyme solution. The supernatant enzyme solution was added to 0.1 mol/L of H2O2 and measured by an ultraviolet spectrophotometer at 240 nm.
3) Determination of amylase activity using 3, 5-dinitrosalicylic acid reduction method: A maltose standard solution was prepared and a regression equation for the mass concentration of maltose was established. Complete the preparation of the maltose standard curve to facilitate the calculation of enzyme concentration. 1.0 g of seeds germinated for 4 days were weighed in a mortar, ground and centrifuged, and the supernatant was diluted with distilled water to a volume of 100 mL as an amylase stock solution for measuring \( \alpha \)-amylase activity. A 10.0 mL amylase stock solution was taken and diluted to 50 mL with distilled water to prepare an amylase dilution for determining amylase activity.
4) Determination of chlorophyll content in seedlings: Wash the fresh leaves into 1mm filaments, accurately weigh 0.25g into the test tube, add 8mL 95% ethanol extract, and leave it in the dark for 24 hours until the leaves are completely white, filter and bring to 25.0 mL. Shake well. The absorbance values of the extracts at wavelengths of 665 nm, 649 nm, and 470 nm were measured by using a 721 spectrophotometer, and the chlorophyll concentration was calculated by referring to the correlation formula, and three repeated experiments were performed.

2.2.5 Data processing. Excel 2003 was used for data statistics, and software SPSS was used to analyze mathematical relationships such as correlation.

3. Results and analysis

3.1 Effects of Different Magnetic Field Frequencies on Seed Germination Index

When plant seeds are exposed to different frequency bands, the internal matter will be affected to varying degrees, and the germination rate will change. O. basilicum seeds have greater irradiance sensitivity and the germination index after irradiation treatment increases first and then decreases with the increase of EMF. When the specified frequency band was 30±2 kHz, the germination rate and germination index of the seeds were not significantly different from the control; the germination potential of O. basilicum was significantly different from that of the control under 50±2.0 kHz irradiation, and the germination rate was 1.5 times that of the CK group; The germination rate of 2.0 kHz EMF seeds was significantly different from that of the control. When the irradiation frequency
was 90±2.0 kHz, the germination rate, germination potential and germination index were lower than the control, indicating that the interval was inhibited. In summary, within the experimentally set frequency range, the 50±2.0 kHz interval promotes the best results. (in Table. 1).

Table 1. Effect of different magnetic field frequencies on the germination of *O. basilicum* seeds

| Frequency / kHz | 30±2 | 50±2 | 70±2 | 90±2 | CK    |
|----------------|------|------|------|------|-------|
| Germination potential /% | 29.0±1.2c | 45.0±0.66c | 31.0±1.2c | 8.0±1.43c | 30.0±0.7c |
| Germination rate /% | 61.2±0.5b | 86.0±0.36b | 73.6±0.5b | 12.8±2.1b | 56.0±1.2b |
| Germination index /% | 26.2±1.2a | 52.6±0.5a | 41.7±0.76a | 5.7±2.5a | 37.3±1.2a |

3.2 Effect of Different Irradiation Time on Water Absorption of Seeds

The difference in water absorption between the groups treated by electromagnetic frequency was not significant, and the difference was significant in the control. Compared with the group of CK, the average water absorption of the experimental group increased by nearly 100%. By comparing the data on the soaking time of the two groups of 2, 4 and 8h, the water absorption of the basil seed is a curve change, which increases first and then decreases with the increase of the soaking time, and shows a negative growth trend after the critical value (in Table. 2).

Table 2. Effect of different magnetization time on seed water absorption

| Water absorption time/h | 10 | 20 | 30 | CK    |
|-------------------------|----|----|----|-------|
| 2                       | 540.81%±0.63b | 563.32%±1.2b | 551.41%±1.2b | 368.53%±1.5b |
| 4                       | 496.12%±1.6a | 525.35%±1.2a | 556.90%±0.77a | 412.75%±1.76a |
| 8                       | 469.04%±0.8a | 471.68%±0.7a | 493.62%±0.66c | 321.09%±1.5a |

3.3 Effect of Different Irradiation Time on Seed Enzyme Activity

Under the irradiation condition of 50 kHz frequency band, the activities of POD, CAT and amylase in different time gradients increased first and then decreased. The difference between 20 min enzyme activity and control was the most significant. The difference between 30 min and control was significant, and the difference between 10 min and control was not obvious. It has an inhibitory effect. It can be seen that the internal enzyme activity in the basil seed is significantly improved when the electromagnetic irradiation is performed at 50 kHz for 20 min (in Fig. 1).

3.4 Effects of Different Irradiation Time on Chlorophyll Content of Seedlings

Time-gradient irradiation of *O. basilicum* seeds was established in the 50 kHz band, and the total amount of chlorophyll showed an overall trend of increasing first and then decreasing with increasing irradiation time. The chlorophyll of the treated seedlings was significantly different from the control.
The optimal order of chlorophyll content growth was 20 min>30 min>10 min, and the 20 min chlorophyll a content was especially significant compared with the control.

4. Discuss

4.1 Effect of time on water absorption of O. basilicum seeds
The water absorption capacity of seeds is of great significance in judging the quality of seeds. Enhanced water absorption enhances the germination and growth of basil. Stimulate the cotyledons of O. basilicum seeds, improve the water storage mechanism, accelerate the late endosperm development and form leaves and stems, further increasing the height of the O. basilicum plant. It can be seen from the experiment that EMF irradiation has an excellent effect on promoting water absorption of seeds, and it is obviously different from the control; in the scope of this study, the water absorption of 2 hours of immersion for 2 hours at 50 kHz irradiation treatment is the best.

4.2 Effect of time on the internal enzyme activity of seeds
Peroxidase (POD) is a highly active enzyme that is ubiquitous in plants and is a protective enzyme associated with plant stress resistance. Peroxidase is a protein encoded by a multigame family of genes that is involved in many important physiological and developmental processes. Obtaining 50 kHz electromagnetic radiation significantly changed the peroxidase in the seeds of O. basilicum, and the activity was highest at 20 min, which proved that the seed had the best stress resistance. Catalase (CAT) is the main enzyme that scavenges hydrogen peroxide in plants. It protects plant cells against oxidative damage and reveals the plant's response to the environment, so that EMF can be used to determine whether the seeds are damaged. The experimental results show that 50 kHz is basically harmless to basil, and the enzyme activity is the largest when magnetized for 20 min. Therefore, in EMF, O. basilicum is basically not damaged, and can enhance the activity of O. basilicum catalase. Amylase is an enzyme commonly found in plants. Amylase activity can be used to assess seed germination rate. The results showed that the amylase activity in the seed increased gradually with the increase of irradiation time in the set time range. The activity of α-amylase and total amylase was the highest under the condition of 50 kHz electromagnetic radiation for 20 min. Seed germination has the best effect.

4.3 Effect of treatment time on chlorophyll content of O. basilicum
Chlorophyll is the main pigment of photosynthesis, and its content can reflect the photosynthetic capacity of the plant. Photosynthesis is the physiological basis for high yield of plants. EMF irradiation changes the content of physiological active substances in seeds, controls the concentration of germ auxin, and rapidly develops into stems and leaves, absorbs light energy for photosynthesis, increases the production of chlorophyll, and provides energy for seedlings. Studies have shown that irradiation has a certain effect on the chlorophyll content of O. basilicum dormant seeds and seedlings, which can indirectly grasp the growth of plants. The results of this investigation showed that the chlorophyll content of O. basilicum increased to different extents under the irradiation of the specified frequency band, and the optimal treatment time was 20 min.

As can be seen from the above, within the scope of this inquiry, the value of each index is the best when 50 kHz affects 20 min. In the later stage, the optimal treatment time of O. basilicum germination in 20-30 min can be continued at 50 kHz, and this mechanism is used to explore the optimum time of other frequencies and improve the electromagnetic irradiation breeding system.

5. Conclusions
The Seed germination and seedling growth are a complex physiological process. The content of each indicator in the seed is highly susceptible to environmental stress. EMF irradiation treatment should be determined by the specific conditions of the seed. This investigation shows that low frequency irradiation promotes the germination of O. basilicum seeds, and high frequency irradiation has an
inhibitory effect on the seed germination of *O. basilicum*. The germination rate increased in the range of 50-70 kHz, and 90±2.0 kHz inhibited the growth of basil. The growth effects of *O. basilicum* seeds and plants under different irradiation time were explored, and the physiological mechanisms of EMF affecting seed water absorption, internal enzyme activity and seedling chlorophyll content were obtained. It was concluded that the biological parameters were the best at 20 min, and the growth mechanism of *O. basilicum* was the best. The main chemical components of seeds are composed of vitality matrix, vital energy, physiologically active substances, mediators active in various substances, and participants. The first three are the most important for seed growth and germination. EMF stimulates the cotyledons of *O. basilicum* seeds, changes the secretion of major chemical substances, increases the internal enzyme activity, provides sufficient nutrition for the germ, rapidly develops to form seedlings, accelerates the production of chlorophyll, and provides energy for plants. At the same time, cotyledons store nutrients for assimilation of seedlings, synthesize amino acids and nucleotides into proteins and nucleic acids, and facilitate a series of biological activities such as anabolism and biosynthesis of *O. basilicum* plants. Under the appropriate frequency band and processing time, EMF irradiation can promote the seed germination of *O. basilicum*, and can break the seed dormancy period and promote the seed cell division. Through the later adjustment of the treatment time, the main chemical composition changes can be affected, and then the growth mechanism of the *O. basilicum* can be changed.

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