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EFFECTS OF HE-NE LASER ON GERMINATION RATE OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) SEEDS

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Abstract

Effects of He-Ne laser on growth of sunflower seeds to the changes of germination rate under different irradiation time were investigated. Changes in the contents of protein, nucleic acid, vitamin C, vitamin B1 and vitamin E in seeds and the chlorophyll content in the leaf of sunflower seedlings were observed. With prolonged irradiation time in seedlings, all the above-mentioned parameters showed an increasing trend in the beginning followed by a decreasing pattern later on. However, application of radiation to the seedlings for 0 - 180s promoted the growth of plants.

Introduction

In recent years, He-Ne laser has been widely used in the field of biology as a new technology and research method (Chen *et al.* 2008). After laser irradiation of seeds and seedlings, the internal physiological structure changes, resulting in a variety of non-genetic phenotypic effects. He-Ne laser is widely used due to its good directivity, good monochromaticity and stable control of output power and wavelength (Yang *et al.* 2018). According to research reports, the effect of low-power He-Ne laser on organisms is mainly reflected by the effect of light and the effect of electromagnetic field (Chen *et al.* 2004). The fact is that after the biomolecule absorbs the laser energy, the intramolecular energy level jumps and generates free radicals. Later on, following the direct or indirect action of the free radical causes various changes of the biomolecule, thereby changing the molecular configuration and causing various changes of the biomolecular products. The mechanism shows corresponding physiological responses in seed cells (Yang *et al.* 2018). When the He-Ne laser illuminates the seed, the laser can activate the expression of related genes in the seed. The influence of He-Ne laser on plant growth has been reported on onion, *Arabidopsis* sp., wheat and tartary buckwheat (Duan *et al.* 2011, Jiang *et al.* 2011, Zhang *et al.* 2011, Gao *et al.* 2014,). It is observed that the effect of laser mainly depends on the irradiation dose and irradiation time, either in order to promote plant growth and/or inhibiting plant growth (Chen *et al.* 2011). The results showed that low-dose laser irradiation of seeds can break dormancy (Wang *et al.* 2017), increase germination rate, accelerate metabolism and change the permeability of biofilm. However, excessive radiation time can damage the internal structure of seeds, and even affect the expression of genes.

Comparing different experimental materials, it was found that the optimal irradiation time of different plants was different (Zhang *et al.* 2010). Sunflower has good edible and medicinal value, which is of great significance to solve the food problem. In this experiment, the He-Ne laser was used to treat the sunflower seeds with different irradiation time in order to its physiological effects on the sunflower seed germination process that might be helpful for sunflower planting.

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Materials and Methods

Seeds of *Helianthus annuus* L. were purchased from Harbin Jinlong Agricultural Co., Ltd. The size of the seeds was uniform. The laboratory materials used in the experiment were absolute ethanol (analytical grade, Sinopharm Chemical Reagent Co., Ltd.) and quartz sand (analytically pure, Tianjin Quartz Clock Factory Bazhou Chemical Branch).

The properties of He-Ne laser were: laser wavelength 632.8 nm, power 31.9 mW, power density 5.32 mW/cm^2 beam expander, plane mirror. Other equipments used were one ten thousandth balance: FA2204 balance (Shanghai Hengping Instrument and Meter Factory), UV-visible photometer (Shimadzu UV-2450) and a constant temperature incubator.

The sunflower seeds were soaked in water for 6 hrs at room temperature to release dormancy (Zhang *et al.* 2018). After 6 hrs, the seeds were removed and dehulled. After dehulling, the seeds were randomly grouped and irradiated under the illumination conditions with an irradiation density of 5.32 mW/cm^2. The processing conditions were followed via setting a total of six sets of irradiation time, namely 0, 60, 120, 180, 240 and 300 sec. Each group contained 30 seeds and the experiments were repeated three times under each time condition.

Twenty one dehulled seeds treated with different laser irradiation were selected and sown in a soil culture dish containing nutrient soil, 7 seeds in each dish. The culture environment was 32 ± 1°C, the light conditions were 12 hrs illumination, 12 hrs dark and the humidity was 65% RH. After 7 days of sowing, the germination rate under different irradiation time was counted. The rate of germination was calculated through: GR = The number of seeds that were normally germinated during the germination termination period/the number of seeds tested × 100% (Yang *et al.* 2015).

From each group of laser irradiated seeds, 0.4 - 0.5 g were ground with absolute ethanol (volume < 25 ml) and filtered. The filtrate was transferred to a 25 ml volumetric flask by adding extra anhydrous ethanol up to the mark, then the absorbance was measured at a wavelength of 260 nm (A_{260}) with the help of a spectrophotometer (Shimadzu UV-2450 UV-Vis spectrophotometer). The data were used to calculate the content of the nucleic acid. Parallel to this, the absorbance at wavelengths 280 and 260 nm (A_{280} and A_{260}) were also measured, and the protein content was calculated. For calculating vitamin C, absorbance at wavelengths 273.1 and 291.9 nm ($A_{273.1}$ and $A_{291.9}$) were measured. A standard curve for vitamin E was prepared and used to calculate the content of vitamin C and E. The content of vitamin B1 was calculated by measuring the absorbance of the extracted liquid at 246 nm wavelength (A_{246}). After 14 days, 0.2 g of seedling leaves were taken from each irradiation group, ground in absolute ethanol (< 25 ml) and filtered. The aliquot was transferred to a 25 ml volumetric flask and the volume was adjusted exactly by adding extra absolute ethanol up to the mark. The absorbance of the liquid was measured at 645 and 663 nm using absolute ethanol as blank (Connan *et al.* 2015, Anisimovich *et al.* 2017).

In this experiment, the absorbance of seed nucleic acids, proteins, vitamins and seedling chlorophyll was measured by UV-spectrophotometer, and then the absorbance was processed by Graph Pad Prism5 data processing software. The concentration was further calculated and the data was processed by Graph Pad Prism5 data processing software.

Results and Discussion

The seeds of sunflower treated with He-Ne laser irradiation showed different effects on the seed germination rate at different times (Fig. 1). With the extension of irradiation time of laser irradiation to the seed, the germination rate of the seed increased first and then decreased. The germination rate of the seeds was higher compared to the control at irradiation times 60, 120 and 180 sec, the maximum germination rate was 77% at 180 sec. This indicates that the He-Ne laser can promote the germination rate of seeds when the irradiation time ranged from 60 - 180 sec. On the other hand, when the irradiation time exceeds 180 sec, the He-Ne laser inhibits the rate of germination of sunflower seeds.

Fig. 2 shows the effects of He-Ne laser irradiation on the protein content of sunflower seeds at different irradiation times. With the prolongation of laser irradiation time, the protein content first increases and then decreases at irradiation time 240 sec. The germination rate of seeds at 120 and 180 sec was higher than that of the control. The protein content in the seedlings reached a maximum of 1.3 mg/ml at 180 sec, which was 1.9 times that of the CK (control check) group. When the irradiation time of the laser exceeds 180 s, the protein content drops sharply. The results of t-test were highly significant ($p < 0.01$) and therefore, proved to be highly reliable.

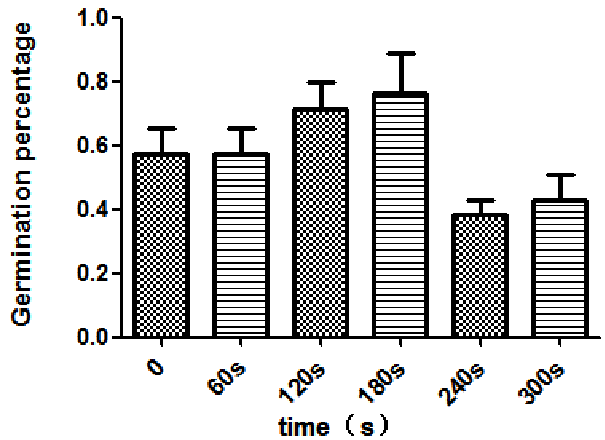

Fig. 1. Effects of He-Ne laser on germination rate of sunflower seeds.

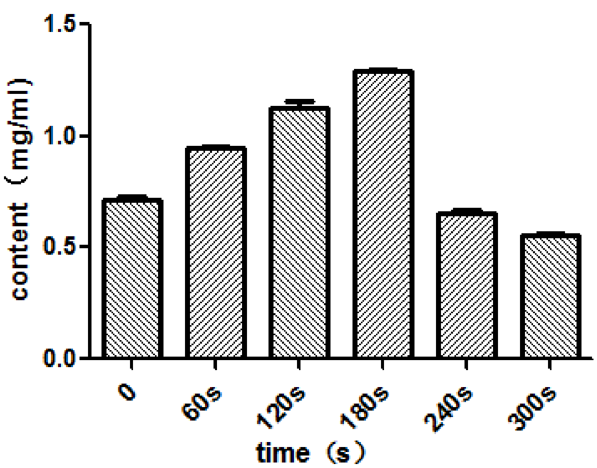

Fig. 2. Effects of He-Ne laser on protein content of sunflower seeds.

As can be seen from Fig. 3, He-Ne laser irradiation of seeds has different effects on nucleic acid content at different times. The content of nucleic acid increases first and then decreases with the increase of exposure time. The nucleic acid content in the seed reached the maximum value of 2.87 Mg/g at 180 sec, this value is 1.5 times higher than that of the control group, but later on the values dropped sharply when the irradiation time of the laser exceeded 180 sec. This indicates that He-Ne laser can promote the accumulation of seed nucleic acid when the irradiation time is 60 - 180 sec, but at higher irradiation time i.e., >180 sec, the He-Ne laser can inhibit the accumulation of sunflower seed nucleic acid ($p < 0.01$)

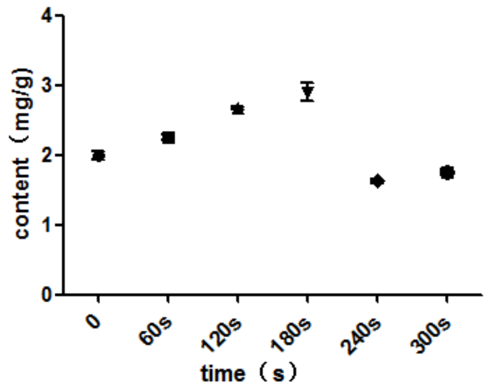

Fig. 3. Effects of He-Ne laser on Nucleic acid content of sunflower seeds.

Fig. 4 shows that the vitamin C content of irradiated sunflower seeds is variable at different doses of He-Ne laser irradiation. The increment of vitamin C was a function of He-Ne irradiation at 60, 120 and 180 sec, but the vitamin C content dropped at radiation time 240 and 300 sec. At irradiation time 180 sec, the vitamin C content in the seed reached its maximum value 0.00042 mol/l, which is 1.14 times more than the control. A sharp decrease in the vitamin C content of the irradiated seeds was observed when the laser irradiation time exceeded 180 sec. This indicates that He-Ne laser can promote the accumulation of vitamin C in seeds when the irradiation time ranged within 60 - 180 sec. The accumulation of vitamin C in sunflower seeds was inhibited when the irradiation time exceeded 180 sec ($p < 0.01$).

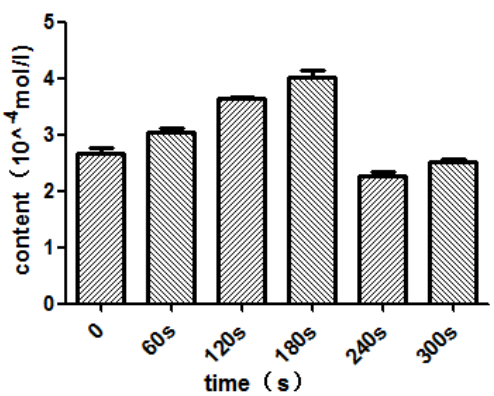

Fig. 4. Effect of He-Ne laser on vitamin c content in sunflower seeds.

Fig. 5 reveals positive effect on the vitamin E content of sunflower seeds at 60, 120 and 180 sec He-Ne laser irradiation time. The content of vitamin E in the seed reached the maximum value of 0.00996 mol/l at 180 sec irradiation time. This value is 1.6 times more than that of the control. As it was evident in case of vitamin C and E content of the irradiated seeds also dropped sharply when the laser irradiation time exceeded 180 sec. This indicates that He-Ne laser can promote the accumulation of vitamin E in seeds when the irradiation time ranged between 60 and 180 sec. However, vitamin E content dropped significantly when the irradiation time exceeded 180 sec ($p < 0.01$).

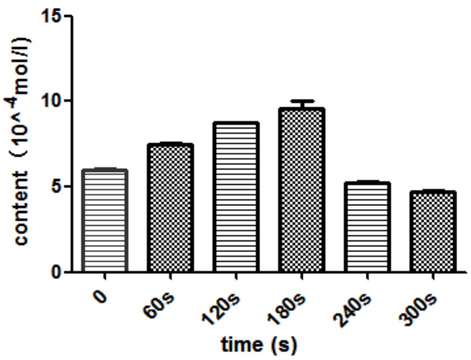

Fig. 5. Effect of He-Ne laser on vitamin e content in sunflower seeds.

Fig. 6 shows the effect of He-Ne laser irradiation on the vitamin B1 content of the sunflower seeds. Similar to the other measurements as mentioned above, vitamin B1 increases first and then decreases with the increase of laser irradiation time. The content of vitamin B1 at 60, 120 and 180 sec irradiation time was higher compared to control. At 180 sec irradiation time, the content of vitamin B1 in the seed reached the maximum value 47.29 µg/ml, which was 1.75 times more than that of the control. Vitamin B1 content fell sharply when the irradiation time exceeded 180 sec. So, it indicates that He-Ne laser can promote the accumulation of vitamin B1 in the seed when the irradiation time ranged 60-180 sec, but at higher irradiation time above 180 sec, He-Ne laser inhibits the accumulation of vitamin B1 in sunflower seeds. The reliability of the effect was proved via t test ($p < 0.01$).

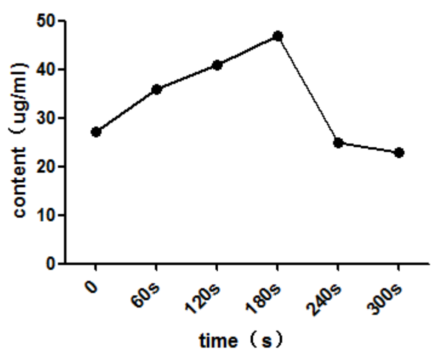

Fig. 6. Effects of He-Ne laser on vitamin B1 content in sunflower seeds.

It can be seen from Fig. 7 that He-Ne laser irradiation seed treatment has different effects on the chlorophyll content of sunflower seedling leaves at different times. The content of chlorophyll did not increase significantly with the increase of laser irradiation time. At 180 s irradiation time, the content of chlorophyll of the tested sunflower seedlings was found higher than other treatments, the value of chlorophyll content in the leaves of seedlings reached the maximum 1.02 mol/l, which was 5.6 times higher than that of the control group.

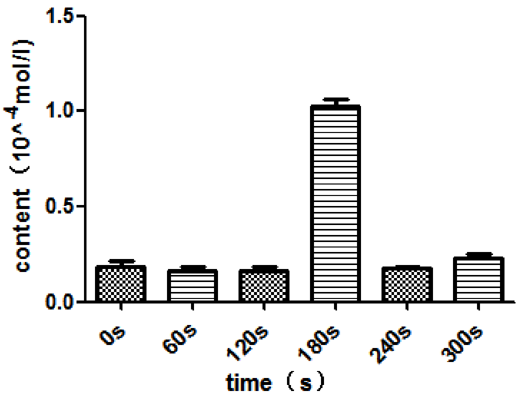

Fig. 7. Effects of He-Ne laser on chlorophyll content in sunflower seedling leaves.

In this experiment, the trends of vitamin, protein and nucleic acid content in seeds were basically the same. After laser irradiation, the stimulation of the laser causes changes in the expression of the nucleic acid, the transcription and translation of the gene also changes, and vitamins, proteins and nucleic acids are also involved in metabolic pathways. We studied the changes in sunflower seed content after laser irradiation of seeds. This is the first time that the seed has been shown to react immediately after laser irradiation, resulting in gene expression. The changes in metabolic activity have paved the way for us to study specific metabolic changes, and have also proved that the results of seedling changes are correct.

He-Ne laser irradiation with an appropriate duration has been shown to have a significant effect on the germination of sunflower seeds. In a certain period of time, it inhibits seed germination, indicating that the effect of He-Ne laser on the organism is related to the length of the radiation. In the results of this experiment, the optimum irradiation time was found to be 180 seconds.

Acknowledgements

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