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Full Length Article

Biological Effects of Semiconductor Laser Irradiation on Seedling Vigor and Biochemical Attributes in Sunflower

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Abstract

Sunflower is an important economic plant which has been extensively studied in agricultural research. In this work, sunflower seeds were irradiated with semiconductor laser with an irradiation intensity of 12 mW/cm^2. The nucleic acid, protein, vitamin B1, C and E contents in the sunflower seeds were analyzed after irradiation for different times (0, 50, 100, 150, 200, 250, and 300 s). The germinability, germination percentage and vigor index of the seeds were measured. Besides, the chlorophyll, nucleic acid and proteins contents in the sunflower seedlings were also determined. The results showed that with the increase of irradiation time, the nucleic acid, protein, and vitamin B1 contents in the seeds and seedlings exhibited an increasing trend first and then decreased. The germination percentage, germinability and other physico-chemical properties were maximal after irradiation for 150–200 s. The semiconductor laser irradiation intensity at 12 mW/cm^2 for 150 s can effectively affect the seeds and seedlings nucleic acid, protein and vitamin contents. © 2019 Friends Science Publishers

Keywords: Sunflower seed; Semiconductor laser; Germination percentage; Biological Effect

Introduction

Laser irradiation of biological materials has always been an important research direction in life science (Chen and Wu, 2008), which provides a reference for the study of laser breeding and biological effects of laser on organisms. At present, laser processing biomaterials research mainly focuses on the study of laser processing plant seeds, most of the studies are focused on the study of plant material to produce biological effect, such as laser impact on the Chinese cabbage seeds have shown that after a certain time of exposure, its seed germination rate (Jin *et al.*, 2010; Zhang; Zhang, 2010; Chen *et al.*, 2011) and chlorophyll content of seedlings (Jin *et al.*, 2010) showed significant changes. At the same time, relevant studies have been conducted on tomato (Qian *et al.*, 2012; Zhang and Zhang, 2011), maize (Wu and the An, 2002), rape (Wang *et al.*, 1987; Hao *et al.*, 2000), clover (Ćwintal, 2010), soybean (Tehseen *et al.*, 2016), wheat (Chen *et al.*, 2002; Huang, 2016), rice (Liu, 2016) and other crops have shown after laser irradiation of individual biological effect and the control group had a significant change in nucleic acids, proteins and chlorophyll contents and growth status. At the same time, some studies focused on the changes of specific plant systems after laser irradiation. For example, it was found that the activity of enzymes such as superoxide dismutase in breviscapus breviscapus after laser irradiation increased, leading to the improvement of antioxidant system capacity (Zhang *et al.*, 2012; Lin *et al.*, 2017) in calli of breviscapus to a certain extent (Liu *et al.*, 2010). At present, the studies on sunflower, have mainly focused on the cytological level effects caused by laser irradiation sunflower (Wang *et al.*, 2006), and influence on oil quality (Yaqoob *et al.*, 2010). But studies after laser irradiation effects on the changes in the metabolites such as vitamins that have antioxidant properties and proteins content in sunflower seed and seedlings are very limited and are focus of present study.

Materials and Methods

Materials

Helianthus annuus L. seeds were used in this experiment. The seeds were 2.5–3 cm in length and 1 cm in width. These seeds were purchased from Jinlong Agricultural Co., Ltd., Harbin City, China, and the germination percentage was higher than 75%. Anhydrous ethanol (A.R.) and calcium carbonate (A.R.) were purchased from Chemical Reagent Co., Ltd., China. Quartz sand (A.R.) was purchased from Chemical Branch of Tianjin Quartz Clock Factory, Bazhou City, China.

Irradiation Equipment

A VDⅡA DPSS LASER DRIVER laser irradiation system, equipped with a semiconductor laser generator, beam expander, and plane mirror, was employed. The laser wavelength λ was 671 nm, and power was 200 mW. An FA2204-type balance (Hengping Instrument and Instrument Factory, Shanghai City) was used. A 3J2-0023-U-5100-type ultraviolet-visible spectrophotometer was used. An HP450-3LED-type thermostat incubator (Ruihua Instrument Equipment Co., Ltd., Wuhan City) was purchased with an irradiation power meter.

Fabrication of the Laser Irradiation System

The emitter of the semiconductor laser generator was fixed on the right side, and a plane mirror was set on the other side, 34.5 cm away from the emitter, to alter the path of the laser for the irradiation of sunflower seeds (Fig. 1). At about 6 cm under the plane mirror, a beam expander was set. At 12 cm under the beam expander, the seeds were irradiated. The laser irradiation intensity was measured with the irradiation power meter, of which the detector area was about 1 cm^2.

Seeds Treatment

The seeds were soaked at 28ºC for 6 h and randomly divided into 21 groups with each group containing 25 seeds. After shelling, the seeds were irradiated in 12 mW/cm^2 laser. The irradiation time was separately set to be 0, 50, 100, 150, 200, 250 and 300 s. Each irradiation time was tested three times by using three groups of seeds.

Germination Experiment

Twenty shelled laser-treated seeds in each group were selected and evenly placed in the 9-mm-thick soil-based medium in two petri dishes. The experiment was grown at 32 ± 1ºC and illuminated for 12 h in a day. The relative humidity was 65%. The germinability was measured at 3^{rd} day and the germination percentage at 7^{th} day. The number of seeds germinated was recorded every day. Additionally, the vigor index of seeds germinated was calculated to analyze the effects of irradiation conditions on the germination of sunflower seeds.

Germination percentage (%) = number of seeds germinated divided by total number of seeds;

Germination index GI = $\Sigma Dt/Gt$, wherein Gt represents the number of seeds germinated in day Dt.

Germinability (%) = number of seeds germinated in the first three days divided by total number of seeds.

Determination of Substances

The concentrations in the samples were determined by UV-Vis spectrophotometry. The 0.5 g of seeds were ground, and the absorbance values of the leachate at 280 and 260 nm (A_{280} and A_{260}) were measured by using a 3J2-0023 UV-Vis spectrophotometer, with anhydrous ethanol as the control group. The content of nucleic acid was calculated at A_{260} value (Zhang and Zhang, 2010). The proteins were calculated according to the A_{280} and A_{260} values (Zhang and Zhang, 2010). The vitamin C and E were measured through an external standard method at the absorbance 273.1 and 291.9 nm ($A_{273.1}$ and $A_{291.9}$) (Li and Wang, 2002; Yu, 2009). The vitamin B1 was calculated according to the absorbance value at 246 nm (A_{246}) (Gao and Zhu, 2009). On the other hand, chlorophyll contents in seedling leaves (0.2 g) was determined at 645 and 663 nm (A_{645} and A_{663}) (Zhang and Zhang, 2010). These contents were determined three times, and the mean values were taken as the real results.

Results

Effect of Laser Irradiation on Nucleic Acid Contents

The nucleic acid content in the seeds exhibited an increasing trend and then decline over irradiation time (Fig. 2) and positively correlated with laser irradiation time in the range of 0-250 s. The nucleic acid content reached a highest value of 2.87 mg/g (about 1.3 times than in the control group) after laser irradiation continuing for 200 s. With the irradiation time exceeding 250 s, the nucleic acid contents were negatively correlated with laser irradiation time. For irradiation continuing for 300 s, the lowest nucleic acid contents was determined to be 2.00 mg/g, which was about 0.9 times less than control. Under irradiation continuing for ≤ 250 s, the laser promoted the growth of seeds. At the time of 200-250 s, the ability of laser irradiation, promote the generation of nucleic acid decreased, and after 300 s, the generation of nucleic acid was inhibited by laser irradiation ($P<0.01$).

Effect of Laser Irradiation on Protein Contents

The protein contents positively correlated with laser irradiation time in the range of 0-200s (Fig. 3). The protein contents reached a highest value of 56.90 mg/g (about 1.3 times to control group) after laser irradiation continuing for 200 s. With the irradiation time exceeding 250 s, the proteins contents were negatively correlated with laser irradiation time. Under the condition of irradiation continuing for 300 s, the lowest proteins content (38.06 mg/g), about 0.9 times to the control were found. To sum up, under the condition of irradiation continuing for ≤ 250 s, the irradiation facilitated the growth of seeds. At the irradiation time of 200-250 s, the ability of laser irradiation to promote protein production decreased. At the irradiation time of 250s-300 s, the protein contents decreased.

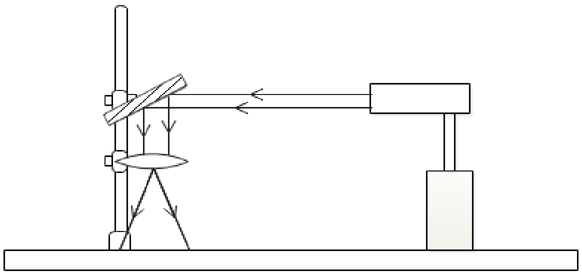

Fig. 1: Schematic illustration of the laser irradiation system
(1) Bracket, (2) plane mirror, (3) beam expander, (4) semiconductor laser generator, (5) Working table, (6) slide

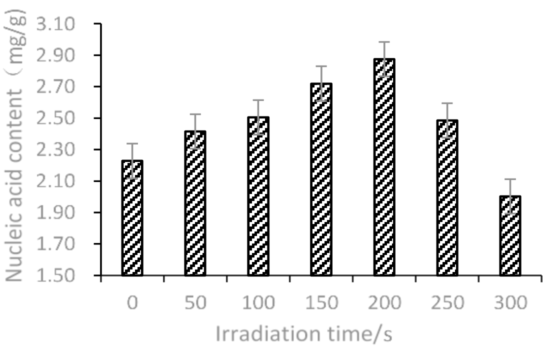

Fig. 2: The laser irradiation time effects on nucleic acid contents in sunflower seed

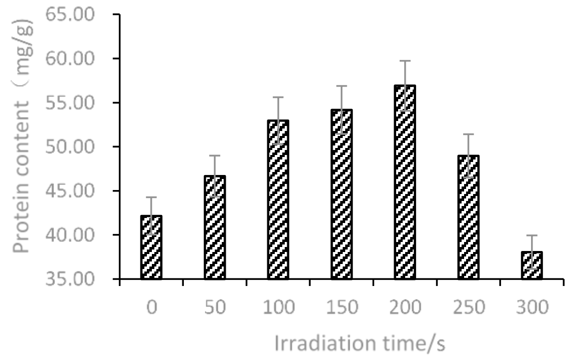

Fig. 3: The laser irradiation time effects on protein contents in sunflower seed

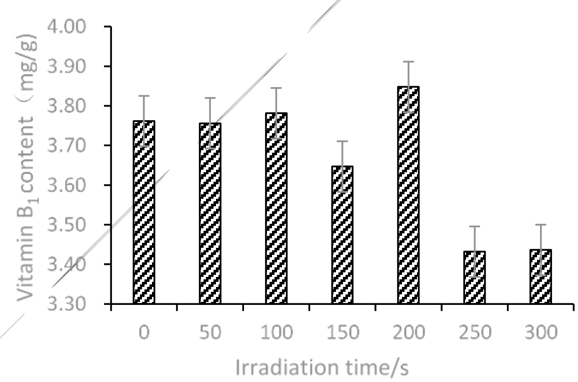

Fig. 4: The laser irradiation time effects on vitamin B1 contents in sunflower seeds

Effect on Vitamin B1 Contents in Seeds

The vitamin B1contents reached highest 3.85 mg/g after laser irradiation continuing for 200 s. With the irradiation time exceeding 250 s, the vitamin B1 contents decreased drastically and then leveled off. The lowest value of vitamin B1 contents was 3.43 mg/g (about 0.9 times to the control) when the irradiation continued for 250 s. These results imply that when the irradiation time was more than 250 s, vitamin B1 contents showed a downward trend. When the irradiation time was more than 300 s, the generation and accumulation of vitamin B1 were inhibited (Fig. 4).

Effects on Vitamin C. Contents in Seeds

The vitamin C content positively correlated with laser irradiation time in the range of 0-250 s. The content reached a highest value of 0.004 mg/g (about 1.29 times to control) after laser irradiation continuing for 200 s (Fig. 5). With the irradiation time exceeded 200 s, the content decreased gradually over laser irradiation time. Under the condition of irradiation continuing for 300 s, the lowest vitamin C content (0.0028 mg/g), about 0.9 times to control were found. When the irradiation time was between 200 -250 s, the accumulation of vitamin C decreased, but still higher than the control. When the irradiation time was greater than 250 s, the laser had an inhibitory effect on the accumulation of vitamin C in seeds ($P<0.01$).

Effect on Vitamin E Contents in Seeds

The vitamin E content reached a highest value of 0.0172 mg/g (about 1.3 times to control) after laser irradiation continuing for 200 s. With the irradiation time exceeded 200s (Fig. 6), the content decreased gradually over laser irradiation time. The lowest vitamin E content (0.0149 mg/g) about 1.1 times that in the control group were found. However, the content slightly improved under the condition of irradiation continuing for 300 s. These results indicate that the irradiation promoted the production of vitamin E for ≤ 200 s, and suppressed the production of vitamin E irradiation exceeded for > 200 s ($P<0.05$).

Effects on Germinability, Germination Percentage and Vigor of Seeds

Over the irradiation time, the germinability of seeds at 3rd day exhibited an increasing trend first and then decreased (Table 1). Under irradiation continuing for 150 s, the germinability reached a maximum of 0.6 and the highest vigor also observed. Under irradiation continuing for > 150 s, the germinability decreased gradually to a minimum of 0.42. On the other hand, the germination percentage, index, and vigor

Table 1: The laser irradiation effects on germination potential, rate and index of sunflower

Irradiation time	0s	50s	100s	150s	200s	250s	300s
Germinability	0.48	0.40	0.53	0.60	0.42	0.45	0.42
Germination percentage	0.55	0.57	0.68	0.77	0.58	0.65	0.63
Germination index	15.73	15.21	21.50	23.43	17.46	19.05	20.88

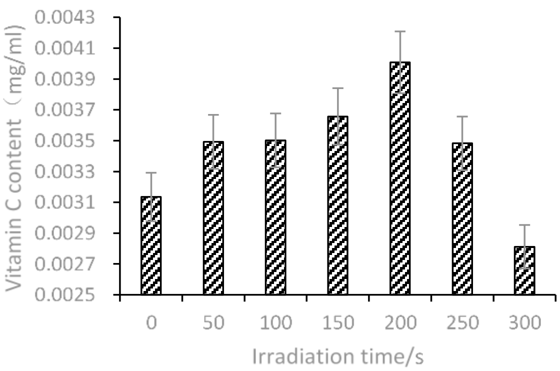

Fig. 5: The laser irradiation time effects on vitamin B1 contents in sunflower seed

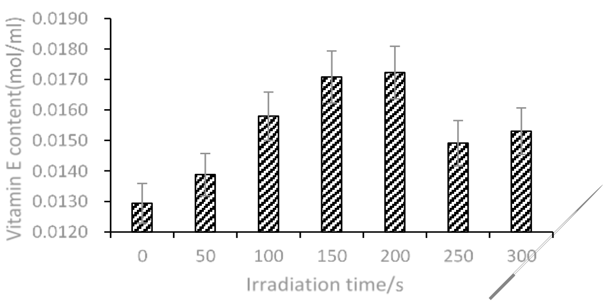

Fig. 6: The laser irradiation time effects on vitamin E contents in sunflower seed

also showed similar varying trends against the irradiation time. The maximum values of germination percentage and index were 0.77 and 3.43, respectively, irradiation continuing for 150 s, the highest germination vigor was observed.

Effects on Nucleic Acid Contents in Seedlings

The total contents of nucleic acid in corresponding seedlings changed greatly after the irradiation of seeds (Fig. 7). The nucleic acid contents in all experimental groups were higher than those in the control. The highest content after laser irradiation was recorded continued for 150 s. With the irradiation time exceeded 150 seconds, the content decreased dramatically over laser irradiation time. The lowest content of nucleic acid were found under the condition of irradiation continuing for 250 s (*p=0.05*).

Effects on Proteins Contents in Seedlings

The total contents of proteins in seedlings also exhibited

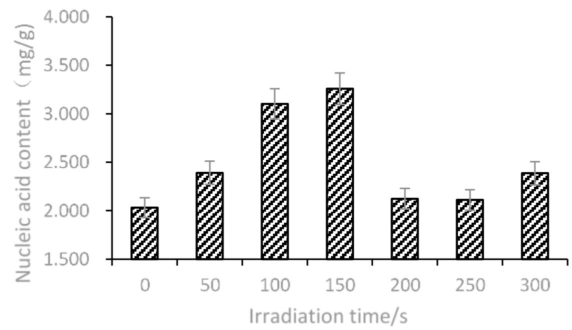

Fig. 7: The laser irradiation time effects on nucleic acid contents in sunflower seedlings

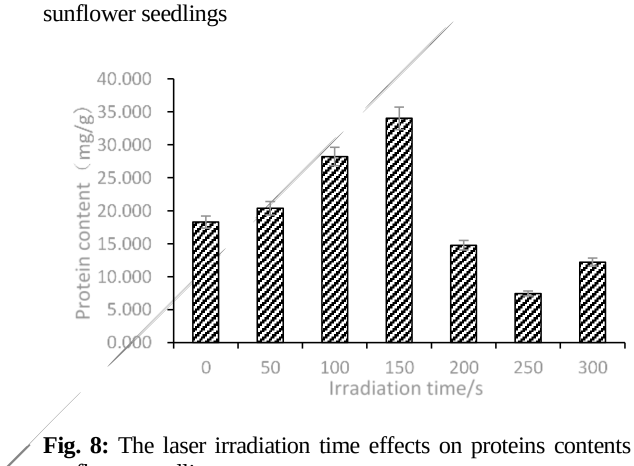

Fig. 8: The laser irradiation time effects on proteins contents in sunflower seedlings

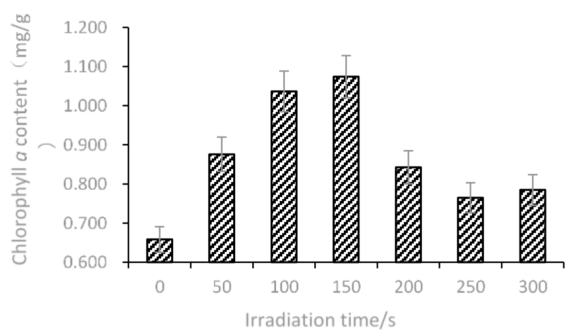

Fig. 9: The laser irradiation time effects on chlorophyll *a* contents in sunflower seedlings

a volcanic trend, similar to the variation trend of nucleic acid (Fig. 8). Under irradiation continued for 150 s, the proteins contents reached a maximum, about 1.9 times than in the control. Under the condition of irradiation continued for > 150 s, the proteins contents decreased drastically (irradiation continuing for 250 s), about 0.4 times than in the control (*p=0.01*).

Effects on Chlorophyll Contents in Seedlings

The chlorophyll contents (*a* and *b*) exhibited increasing trends first and then decreased, and both reached maximum

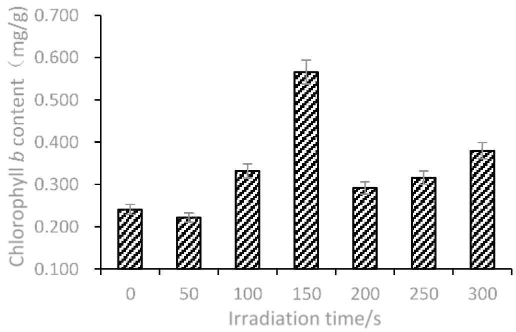

Fig. 10: The laser irradiation time effects on chlorophyll *b* contents in sunflower seedlings

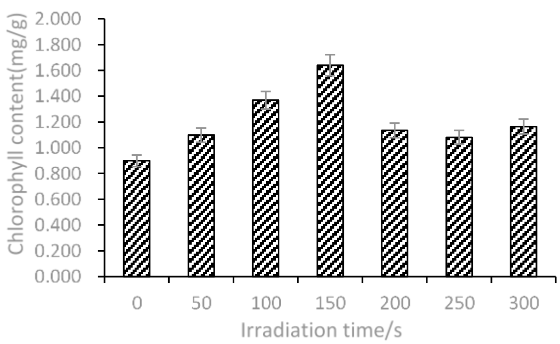

Fig. 11: The laser irradiation time effects on total chlorophyll contents in sunflower seedlings

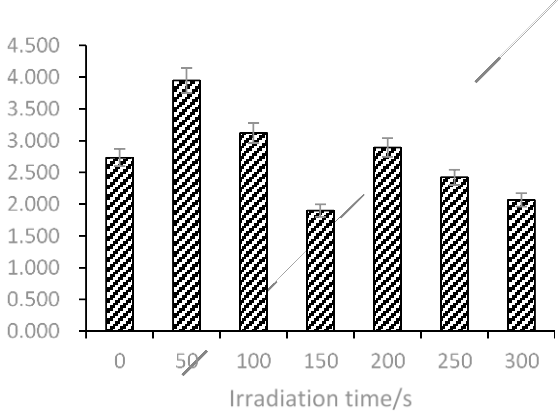

Fig. 12: The laser irradiation time effects on ratios of contents of chlorophyll *a* to contents of chlorophyll *b*

for irradiation continued for 150 s (Fig. 9, 10 and 11). Under irradiation continued for > 150 s, both contents decreased drastically, but chlorophyll *a* was much higher than chlorophyll *b*.

Under irradiation continuing for 150 s, the chlorophyll *a* content were 1.6 times than the control (Fig. 9). Under irradiation continuing for 150 s, the chlorophyll *b* contents reached to 0.566 mg/g and were 2.3 times in the control group. From this point of view, the chlorophyll *a* and *b* in seedlings greatly increased by the laser irradiation.

Besides, the ratio of content of chlorophyll *a* to *b* was also affected by the irradiation time. The ratio decreased at first and then increased, and reached the minimum value of 1.899 (0.7 times than control) under the condition of irradiation continuing for 150 s (Fig. 12) ($P<0.05$).

Effects on the Seedling Phenotype

The plant height, leaf length and width of the seedlings also showed an increasing trend first and then decline trends over laser irradiation time (Fig. 13, 14 and 15). All of these indices reached their maximum values under the condition of irradiation continuing for 150 s.

Discussion

Effects of Laser Irradiation on the Sunflower Seeds

When the moisture content in seeds reaches 20%, the metabolism of amino acids and weak respiration is initiated. At moisture content of 40%–60%, the production of proteins and RNA is strongly initiated (Zheng *et al.*, 2017). Therefore, after soaking, the sunflower seeds swelled, commenced to germinate, and the gene expression was activated.

The laser could activate the expression of some genes in seeds. The different nucleic acid contents in seeds under the conditions of different irradiation times may be ascribed to the different effects of laser (Zhang *et al.*, 2011). Laser continuing for a short time stimulated the relative genes to a small extent, and thus the replication of genetic substances and transcription in cells were thoroughly improved. In contrast, the long-time laser irradiation might damage the genes, resulting in inactive replication and transcription (Yan *et al.*, 2014).

When cellular dormancy was terminated, the nucleic acid contents was changed due to the laser stimulation, and gene expression activity also changed, resulting in changes in cellular metabolism and the contents of vitamin B1, C, and E in cells (Zhang *et al.,* 2011). In the present work, under the condition of irradiation continuing for < 200 s, the effect of laser on seeds was positive. The irradiation treatment promoted the cellular gene expression, cellular metabolism, and life activity of seeds. In contrast, under the condition of irradiation continuing for > 200 s, the positive effect disappeared gradually, and the inhibition effect of laser were observed.

Effects on Sunflower Seedlings

According to germinability and germination percentage, the effect of laser irradiation on the seeds continued to the germination and seedling stages. It is implied that the laser irradiation activated and enhanced the germination genes in

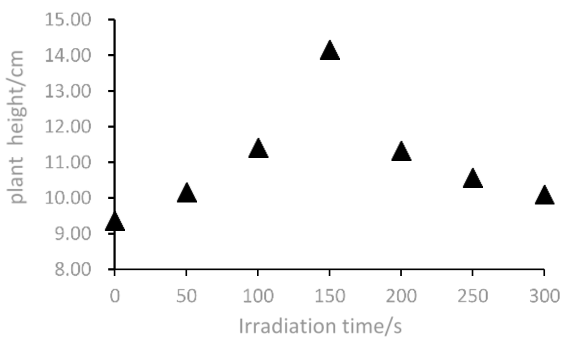

Fig. 13: The laser irradiation time effects on sunflower seedling heights

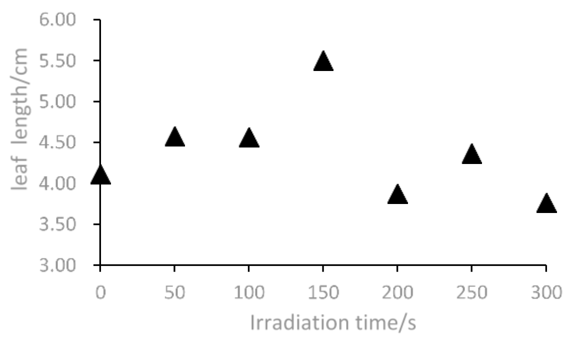

Fig. 14: The laser irradiation time effects on sunflower seedling leaf lengths

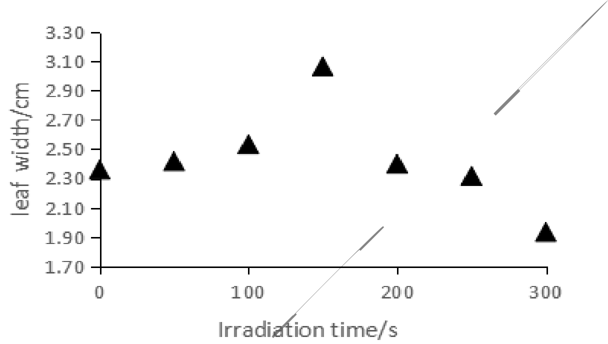

Fig. 15: The laser irradiation time effects on sunflower seedling leaf widths

seeds (Li and Ye, 2005; Zhang *et al.,* 2011). Therefore, during the process of germination, the changing trends of germinability and germination percentage were similar to nucleic acid content in seeds.

Vitamin B1 participates in the respiratory response in mitochondria (Liu, 2016). During the experiment, with the increase of irradiation time, the life activity of plants is enhanced. This process may promote the high-level synthesis of vitamin B1, which leads to the apparent higher content of vitamin B1, and leads to the enhancement of metabolic activity in sunflower, thus promoting the increase of germination rate. The increase of protein and other substances makes sunflower grow better.

Vitamin C is involved in a series of metabolic processes such as free radical scavenging in plants, and vitamin E is involved in antioxidant processes in plants (Chen *et al.*, 2004; Jin *et al.*, 2009; Guo *et al.*, 2011). Therefore, it can be concluded that sunflower seeds were enhanced with vitamin C and other beneficial metabolic processes after 150s-200s of light, which promoted the enhancement of plant life activities and made the plants grow best under this condition.

Chlorophyll *b* is an important component of LHC9 (Guo *et al.*, 2006) and plays an important role in the uptake and delivery of light energy, regulation of photosynthesis, maintaining the stability of LHCII, and adaptability to various environments. In addition the better the growth of plants, Chlorophyll *a* has the ability to absorb light energy, and the ability to convert light energy into electrical energy. The ratio of chlorophyll *a* to *b* can reflect the photosynthetic rate to some extent. Studies have shown that when the chlorophyll *a/b* value is reduced under appropriate conditions, the photosynthetic rate can increase to a certain extent, resulting in increased production of plants. In this study, it was speculated that the decrease of chlorophyll *a/b* ratio also led to the increase of photosynthetic rate and plants with low chlorophyll *a/b* had better growth than other groups.

Because after laser irradiation, activities to strengthen the gene expression of sunflower seeds, lead to the nucleic acid and protein content increased, while vitamin B1 to express a lot of, also promoted the respiration, and, in turn, promote the growth of plants (Liu, 2016). At the same time due to laser irradiation caused some damage to a plant, thus represented by vitamins C and E antioxidant system (Guo *et al.,* 2011) to protect the sunflower seeds and seedlings.

Conclusion

The present study concluded that the laser irradiation for 12 mW/cm^2, adopt laser irradiation 150 s to 200 s can effectively promote the seeds activities, and help the growth of seedlings sprout and later, can consider when laser of sunflower breeding.

Based on results, it can be concluded that when the laser irradiation is less than 150 s, sunflower seeds and seedlings nucleic acid, protein and vitamin content increased, the growth of seedling was better with the increase of irradiation time until the 150 s. When the irradiation time exceeded 150s, the promoting effect of irradiation on seedlings gradually decreased. When the irradiation time exceeds 250s, the nucleic acid, protein, vitamin contents and other substances decreased significantly, resulting in the inhibition of sunflower seedling growth by irradiation.

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