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SHORT COMMUNICATION

EFFECT OF LASER-IRRADIATION ON PHOTOSYNTHETIC EFFICIENCY OF SAFFLOWER LEAVES

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SUMMARY

The present investigation has been undertaken to determine the influence of laser light at a wavelength of 632.8 nm on safflower leaves. Our study illustrates that non-irradiated plants possessed higher amount of chlorophyll (a+b) and carotenoid content as compared to irradiated plants. However a significant increase in total chlorophyll (Chl) content has been noticed on increasing the treatment duration from ½ h to 1½ h. This increase in photosynthetic efficiency can also be measured by calculating the peak ratio (F_{685}/F_{735} ratio) through LICF (Laser induced chlorophyll fluorescence) study. This ratio is dependent on the chlorophyll content of the leaves. It decreases with increase in chlorophyll content. Thus this method could be successfully deployed to measure change in photosynthetic activity of leaves and that too in a non-destructive manner. From our study it can be concluded that the mutagenic effectiveness of laser rays decreased with the increase in treatment dose.

Key words: Laser rays, chlorophyll and carotenoid content, LICF, FIR, safflower
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1. Introduction

Laser irradiation is emerging as a new means for creating phenotypic and genotypic alterations in agriculture, especially in medicinal plants. Experiments conducted by various workers supports that laser light could be successfully utilized in improving sowing qualities of seed, shortening the phases of plant development, produced more vigorous plants, increased yield of both stems and seeds and increased germination by 10-15% (1, 2). Some researchers often propounded that laser beam could be successfully used in the field of genetics and plant breeding where they induce biostimulation effect, and higher doses of laser beams for damaging of cell genetic material leading to mutation process (3).

Safflower (an annual herb) belonging to family Asteraceae is an ethnopharmacologically important plant as it can reduce coronary heart ailments due to the presence of polyunsaturated fatty acids. Its flowers are used as sedative, in bronchitis and as liver tonic besides being a good source of dyestuff. Seeds of safflower are used in treating arthritis and foul ulcers (4).

Regarding the LICF spectra it is a means for early detection of any stress induced impact on plants where chlorophyll fluorescence intensity ratio (FIR) F_{685}/F_{735} were recorded. The inverse relationship between in vivo chlorophyll fluorescence and photosynthetic activity first proposed by Kautsky and Frank (5) can be applied to study the potential photosynthetic capacity of plants as well as to detect damage to the photosynthetic apparatus. An increase in FIR ratio depicts a decrease in chlorophyll content whereas decrease an increase in chlorophyll content. Therefore it can be used to monitor changes in the chlorophyll content during leaf development (6, 7, 8) autumnal chlorophyll breakdown (9, 10), course of a year (11), and also as a result of

natural and anthropogenic stress or damage events (12, 13).

2. Material and methods

Seeds of safflower (*Carthamus tinctorius* L.) were procured from NBPGR (IARI), New Delhi, India. Healthy seeds of safflower of uniform size were selected and exposed to laser light at 632.8 nm for three treatment durations of ½ h, 1 h and 1½ h (source He-Ne laser). The seeds were soaked overnight and planted in their respective pots alongwith the untreated control plants inorder to record variation in photosynthetic activity. The germination of seeds were carefully observed and after 7 days of germination uniform size seedlings were selected for the LICF study and the rest were grown for observation. The experiment was repeated six times to determine each parameter. For chlorophyll content analysis leaf discs from control as well as laser treated plants, were extracted in 80% acetone (v/v in double distilled water), and were used for the measurement of pigment content. The pigment content was determined from the transparent, centrifuged acetone extract solution by measuring the absorbance in the region of 380-700 nm using an UV/VIS spectrophotometer (Perkin Elmer Lambda 35). The pigment content were calculated by equation allowing a simultaneous determination of the chl.a, chl.b and carotenoids in the same solution, as in the work of Lichtenthaler, 1987 (9).

For studying Laser induced Fluorescence spectra by violet laser (405 nm), the leaves of safflower have been exposed to 405 nm light of violet laser **(**OXXUS C.E., made in France model PS-001), power, 50mW. The laser induced fluorescence radiation was collected on the entrance slit of the computer controlled Action 0.5M triple grating monochromator using 1800 grooves/mm grating blazed at 500nm hamamastu R928 PMT as a detector and spectra sense software. Leaf fluorescence excited and sensed in an angle of 45° to the leaf plain. The monochromator has been calibrated with 546.07nm Hg lamp. The LIF spectrum has been recorded in the region of 650-780 nm.

3. Results and Discussion

Findings of our experiment revealed that safflower plants treated with laser rays at three different treatmnt durations showed variation in photosynthetic activity .At the highest time duration the plants showed better photosynthetic rate than ½ h and 1 h as the photosynthetic pigments chlorophyll a, b and carotenoid content of plants were found to be elevated. The total chlorophyll content was hyped from 4.05µg/ml fresh weight leaf in ½ h treated set to 5.72µg/ml fresh weight leaf at 1½ h duration treatment as compared to total chlorophyll content of 6.36µg/ml fresh weight leaf recorded in control. Carotenoid content was also increased from 0.78µg/ml fresh weight leaf to 0.88µg/ml fresh weight leaf as illustrated in table 1. Laser induced upgradation in chl content may be due to its stimulatory role in the chl biosynthesis process.

Table 1-Effect of laser rays on photosynthetic pigments of safflower leaves

Treatment (h)	Photosynthetic pigments			
	Chlorophyll a µg/ml fresh weight leaf	Chlorophyll b µg/ml fresh weight leaf	Total Chlorophyll µg/ml fresh weight leaf	Carotenoids µg/ml fresh weight leaf
Control	4.87	1.49	6.36	0.86
½ h	3.17	0.88	4.05	0.78
1 h	3.96	1.28	5.24	0.84
1½ h	4.36	1.36	5.72	0.88

The fluorescence intensity ratio (FIR) of two maxima (F_{685}/F_{735}) for peak height, band area and bandwidth i.e. Full width at half maxima (FWHM) both in control and treated plants has been presented in Table 2.

Table 2- The F_{685}/F_{735} ratio for peak height, band area and bandwidth for the control and laser treated plants

Treatment (h)	(F_{685}/F_{735}) ratio		
	Peak height	Band width	Band area
Control	2.37	0.41	1.07
½ h	3.15	0.45	1.13
1 h	3.03	0.44	1.12
1½ h	2.75	0.42	1.10

The FIR has been established as an indicator of the increase or decrease in the Chl content and photosynthetic activity as compared to the control plant. A decrease in the 685nm region is much higher than decrease the 735nm region, thus a higher Chl content increase the value of F_{685}/F_{735} at the higher doses. The result exhibit that the fluorescence come from the mesophyll layers largely depends on the content of photosynthetic pigment in leaves and partial reabsorption of the emitted fluorescence and established FIR as a very sensitive indicator of change in photosynthetic activity and Chl content of leaves. Thus, by assessing the increase or decrease in FIR ratio we can predict that whether the treatment given to the plant has positive or negative impact on the growth and development of crop plants as illustrated by Gopal et al., 2002 (14).

From the study conducted it can be summarized that laser rays possessed a positive effect on safflower seedlings and has marked influence on the photosynthetic activity. It has brought about an increase in total chl content and carotenoid content with the increase in duration of mutagenic treatment. Thus through LIF spectra damage thresholds and chl regeneration studies can be successfully carried out in crop plants.

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