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LICF Spectrum as a Fast Detector of Chlorophyll Damage in Safflower Growing under Mutagenic Stress

[1]*Preeti Srivastava and* [2]*Jitendra Pandey*

[1]Division of Genetics, Indian Agricultural Research Institute, New Delhi, India
[2]M.N. Saha Center of Space Studies, I.I.D.S., Nehru Science Center,
University of Allahabad, Allahabad- 211002, U.P., India
[2]Laser Spectroscopy and Nanomaterial Lab, Department of Physics (UGC-CAS),
University of Allahabad, Allahabad-211002 U.P., India

Abstract: The present investigation envisages the *in vivo* measured laser induced chlorophyll florescence spectra of EMS (Ethyl methane sulphonate) treated safflower leaves. Seeds of safflower were treated with 0.5% EMS for three treatment durations (3h, 5h and 7h) to raise the populations. The effect of EMS treatment on chlorophyll content in the leaves was assessed using laser induced chlorophyll fluorescence (LICF) spectra in the early stage of growth. The LICF spectra of leaves were recorded in the region of 650-780 nm using 405 nm violet laser as excitation source and PMT as detector. The chlorophyll fluorescence spectra with peaks at 685 and 735 nm were used for monitoring the growth of plants after the treatment and were analyzed using Gaussian spectral functions with curve fitted parameters to determine the peak positions, area under the spectral curve and the florescence intensity ratio (FIR) F685/F735. FIR is an indicator of chlorophyll content in mesophyll layer and has inverse relation with chlorophyll content in the plant leaves. As the duration of treatment increased FIR ratio increased with corresponding decrease in the chlorophyll content. Total carotenoids content has also been found to be decreased along with the increment of treatment duration. This change in intensity can be discussed in the light of variation in photosynthetic activity. Thus we demonstrate the use of LICF spectra for early, fast and nondestructive detection of chemical induced chlorophyll reduction in safflower.

Key words: Chlorophyll florescence · EMS · FIR · LICF · Safflower

INTRODUCTION

Safflower is one of world's oldest oilseed crops, has been grown commercially for edible oil and natural dye sources around the world [1]. Safflower petals besides being a source of dye, is medicinally important also in curing several chronic diseases like hypertension, coronary heart ailments, rheumatism and male and female fertility related problems [2, 3]. It is an important alternative plant that can be used to increase edible oil sources. It is a highly drought tolerant crop that can be safely grown under arid and saline sodic conditions [4].

Breeders use experimental mutagenesis for creation of new varieties of agricultural crops and for procurement of bigger genetic diversity. Variation in chlorophyll status can be used as test systems for evaluation of genetic action of mutagenic factors. They are also used as markers in genetics, physiological and biochemical investigations [5]. Regarding the LICF study, the LICF is the optical emission from chlorophyll molecules in a plant after absorption of electromagnetic radiation from an active source.

LICF spectra of green leaves exhibited two fluorescence maxima: one near 685nm (red region) and second one in the spectral region around 735nm (far-red region) [6]. The shapes of the chlorophyll florescence spectra and the values of the fluorescence intensity ratio (FIR) at the two maxima (F685/F735) primarily depend upon the total chlorophyll content (a + b) and to a lower degree also on the photosynthetic activity of the leaf [7].

Corresponding Author: Preeti Srivastava, Division of Genetics, Indian Agricultural Research Institute, New Delhi, India.

The LICF spectra technique has been found to be a very important monitoring technique about the responses that the plant shows under stressed condition even before any visual symptoms have appeared [6]. LIF signal can be used to make an inference regarding health and identity of plants and it has a very good correlation with chlorophyll content of the leaves [8]. The fluorescence intensity ratio (FIR) of two maxima F685/F735 is strongly influenced by variation in chlorophyll content and photosynthetic activity of plant leaves [2]. With the increase in duration of treatment with EMS, plants manifested an increase in the value of FIR that may be regarded as the indicator of decrease in the chlorophyll content and photosynthetic activity, which is the response of effective mutagenic treatment. The aim of the present work is to investigate the mutagenic efficiency of different doses of EMS (Ethyl methane sulphonate) in reducing chlorophyll content of safflower leaves through chlorophyll florescence measurements.

MATERIALS AND METHODS

Procurement of Seeds and Chemical: Seeds of safflower *(Carthamus tinctorius* L.) were obtained from Division of Genetics, Indian Agricultural Research Institute, New Delhi (India) and EMS was obtained from Merck, India.

Treatment Procedure: Healthy seeds of safflower of uniform size were selected and soaked in distilled water for 2h and then transferred in aqueous solution of 0.5 % EMS for three treatment durations i.e. 3h, 5h, 7h respectively. The treated seeds were then thoroughly washed under tap water to remove residual chemical and then planted in their respective pots along with the untreated control plant.

The germination of seeds was carefully observed and after 7 days of germination uniform size seedlings were selected for the LIF study. LICF spectra are recorded using computer control Acton 0.5 M triple grating monochromator using 1800 grooves/mm grating blazed at 500 nm, Hamamastu R928 PMT as a detector, exited with a violet diode laser (Oxxus CE, made in France, model PS-001, Power 50 mW) light as an excitation source. The beam expander is aligned to obtain 2.0 cm^2 expanded laser light on leaves. The fluorescence radiation is collected on the entrance slit of monochromator. Leaf fluorescence excited and sensed in an angle of 45° to the leaf plain. The monochromator has been calibrated with 546.07 nm Hg lamp. The LICF spectrum has been recorded in the region of 655-780 nm with the help of spectra-sense software.

For chlorophyll content analysis leaf discs from control as well as EMS 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 by using UV-VIS spectrophotometer (Perkin Elmer Lambda 35). The pigment content was calculated by equation allowing a simultaneous determination of the chlorophyll a, chlorophyll b and carotenoids in the same solution, according to the method of Lichtenthaler and Welburn (1983) [9].

The curve fitting has been done using the Levenberg- Marquardt algorithm method for iterative non-linear curve fitting. After choosing the Gaussian spectral function, the individual component peaks were selected. Peak widths were adjusted so as match approximately shapes of the spectrum. It provided a reasonable matching fit for spectral data with good F- statistics, standard error for peak amplitude, peak center and bandwidth (Full width at half intensity maximum (FWHM).

RESULTS AND DISCUSSION

Findings of our experiment revealed that safflower plants treated with EMS at various treatment durations showed variation in growth performance. At lowest time duration the plants showed better growth than 5h and 7h treated plants, as the photosynthetic pigments chlorophyll (a+b) and carotenoids contents of plants were greater. After the treatment the total chlorophyll content was decreased from 11.83µg/ml in control to 6.36µg/ml at 7h duration treatment.

Carotenoids content was also found to be decreased from 3.68 mg g^{-1} fresh weight leaf to 1.85 µg/ml as illustrated in Table 1. EMS induced degradation in chlorophyll content at higher time durations may be due to its inhibitory role in the chlorophyll biosynthesis process. Decrease in chlorophyll content with increase in mutagenic treatment was found in agreement with the studies conducted on mungbean by many authors [9, 10, 5, 11].

The Gaussian spectra resulting from the curve-fitting analysis of LICF for the control and EMS treated safflower plant is presented in Figure 1. The curve fitting parameters such as peak height, band area and bandwidth (FWHM) both in control and treated plants are presented in

Table 1: Photosynthetic pigments content of control as well as of EMS treated Safflower plants

Treatment (h)	Photosynthetic Pigments			
	Chlorophyll a (µg/ml) fresh weight of leaf	Chlorophyll b (µg/ml)) fresh weight of leaf	Total chlorophyll (µg/ml)) fresh weight of leaf	Carotenoids (µg/ml) fresh weight of leaf
Control	9.49	2.34	11.83	3.68
3h	9.32	2.06	11.38	3.48
5h	7.15	1.53	8.68	2.45
7h	5.32	1.06	6.38	1.85

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

Treatment (h)	Peak height	(F685/ F735) ratio Band Width	Band Area
Control	2.08	0.36	0.84
3h	2.30	0.36	0.83
5h	2.36	0.37	0.85
7h	2.46	0.42	0.89

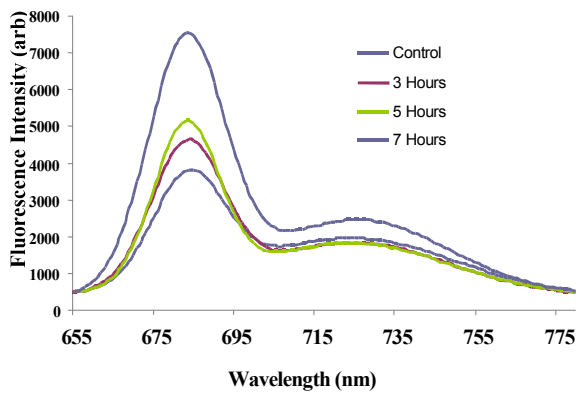

Fig. 1: Gaussian Curve-fitted LICF Spectra of control and EMS treated safflower leaves excited by 405nm violet laser.

Table 2. The chlorophyll fluorescence of leaves consists of two maxima in the red (near 685-690 nm) and far-red region (near 730-740 nm). The intensity and the shape of the chlorophyll fluorescence spectra depend on the concentration of the fluorophore chlorophyll *a*. Although in an organic solvent Chlorophyll b exhibits a red fluorescence, it does not do so *in vivo* because in a leaf the excitation energy is transferred completely to chlorophyll *a* and to a lower degree also on the leaf structure, the photosynthetic activity and the leaf's optical properties. The latter determine the penetration excitation light into the leaf as well as the emission of chlorophyll fluorescence from different depths of the leaf. The increase of chlorophyll fluorescence with increasing chlorophyll concentration is mainly detected in the long wavelength range (far-red fluorescence), whereas short-wavelength red fluorescence levels off and then decreases due to the re-absorption of the emitted red chlorophyll fluorescence by the chlorophyll absorption bands, which reduce the short-wavelength fluorescence with rising chlorophyll content. The effect of the decrease in the Chlorophyll content is mainly detected in short-wavelength range (red chlorophyll fluorescence), where short-wavelength red chlorophyll fluorescence increases with decrease in the Chlorophyll content due to the reduction of the re-absorption of the emitted red Chlorophyll fluorescence by the chlorophyll absorption band. In the green leaves about 90% of the emitted chlorophyll fluorescence at 685 nm, reabsorbed by the chlorophyll molecules of the leaf [5] and the re-absorption are caused by the overlapping of short-wavelength range of the chlorophyll fluorescence emission spectrum with the long-wavelength of the chlorophyll absorption spectrum. Since the red chlorophyll fluorescence maxima near 690 are more strongly affected by the re-abortion than the long-wavelength maximum near 730-740 nm, the ratio F685/F735 increase with decreasing chlorophyll content and *vice-vers*a. Thus FIR is strongly influenced by variation in chlorophyll content and photosynthetic activity of the leaf. In the present study the increase in time duration of EMS treatment caused an increase in FIR ratio thereby depicting a decrease in chlorophyll content and photosynthetic activity of the 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.

CONCLUSIONS

- EMS possessed positive mutagenic effect on safflower seedlings.
- EMS has marked influence on pigments content and photosynthetic activity. Both these parameters were decreased with the increase in the duration of mutagenic treatment.

- Chlorophyll fluorescence ratio represents an ideal tool for detecting differences and changes of chlorophyll content in plant species and leaf tissue.
- We can use LICF spectroscopy as an asset for early detection of EMS induced deduction of chlorophyll fluorescence in response to chemical treatment in safflower leaves.

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