

## **Growth and Photosynthetic Performance of *Calendula Officinalis* under Monochromatic Red Light**

**Sasan Aliniaiefard<sup>1\*</sup>, Mehdi Seif<sup>1</sup>, Mostafa Arab<sup>1</sup>, Mahboobeh Zare Mehrjerdi<sup>1</sup>, Tao Li<sup>2</sup> and Oksana Lastochkina<sup>3</sup>**

1. Department of Horticulture, Aburaihan Campus, University of Tehran, Iran.

2. Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Science, Beijing, China.

3. Bashkir Scientific Research Institute of Agriculture, Russian Academy of Sciences, R. Zorge St., 19, 450059 Ufa, Russia.

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### **Abstract**

Light is the driving force for plant photosynthesis. Different attributes of light (e.g. intensity, spectrum and duration) can influence plant growth and development. We studied growth and photosystem II performance of English marigold cut flowers under red (635-665 nm) and white (420-700 nm) LEDs. Although growing plants under red light resulted in morphological deformation such as leaf epinasty, it led to an early flowering and improved growth compared with white light-grown plants. In plants that were grown under red light, flowers were emerged 45 days after germination. In the time of flowering, there were 30 leaves (sum of rosette and lateral leaves) on the red light-grown plants, while 20 leaves were observed on white light-grown plants without flowering on day 45. Fast induction of chlorophyll fluorescence showed that fluorescence intensities of O-J-I-P phases in a typical fluorescence transient exhibited after a 20 min dark-adapted leaves were increased in red light-grown plants. Maximum efficiency of photosystem II (Fv/Fm) and performance index per absorbed light were decreased by red light, while quantum yield of energy dissipation was increased by red light. Most of the energy absorbed by the photosystems in red light-grown plants was dissipated as heat. In conclusion, although red light improved growth and induced early flowering in *Calendula officinalis*, full light spectrum is required to prevent leaf deformation and electron transport disruption under monochromatic red light.

**Keywords:** Early flowering, English marigold, light spectrum, LED, photomorphogenesis, photosynthesis.

### **Introduction**

Light is the driving force for plant photosynthesis. Many of light sources which are used to enhance photosynthetic photon flux density (PPFD) levels have very low energy use efficiency for growing plants. In some types of light sources, parts of their spectrum are not well fitted in that

part of photosynthetically active radiation (PAR) spectrum. This reduces their efficiencies for promoting plant growth and photosynthesis (Kim et al., 2004). In contrast to other light sources, light-emitting diode (LED) lighting system have various advantages, including the ability to set the desired spectral combination, permanence, specific wavelength, low heating and the electrical input (Lin et al.,

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\*Corresponding Author, Email: [aliniaiefard@ut.ac.ir](mailto:aliniaiefard@ut.ac.ir)

2013). These advantages make them suitable light source for growing plants. The LEDs give wavelengths that can be matched with plant photoreceptors to provide optimal production and influence morphology and metabolism of plant (Bourget, 2008; Massa et al., 2008; Morrow, 2008). Experimentations on impact of light variables on plant growth and development is attracting attention of plant scientists. Possibility of using light spectra purposefully enables us to influence plant growth, development and its product quality (Lin et al., 2013). Changes in different light attributes (e.g. intensity, spectra and duration) can influence all aspect of plant growth and physiology especially morphology and photosynthetic responses of the plants. Plant responses to light qualities have an applied importance in the newly-developed plant growth technologies.

Quality of light which refers to the wavelength or color can strongly affect plant development, (Johkan et al., 2010). Red and blue lights have great effects on plant growth because they contain the two main light spectra for photosynthetic CO<sub>2</sub> fixation in plants (Kasajima et al., 2008).

It has been reported that red light is the most important light spectrum for growth and phytochrome responses in plants (Wang et al., 2016). Furthermore, development of photosynthetic apparatus and starch accumulation can be stimulated by light spectrum in plants (Britz and Sager, 1990; Maas, 1992).

Photosystem I and II (PSI and PSII) in the electron transport chain of photosynthetic apparatus are involved in converting solar energy to chemical compounds in plants (Jordan et al., 2001). It has been found that the PSII is sensitive to light quality (Miao et al., 2016). Patterns of electron transport under monochromatic light source can help to better understand the physiological response of photosynthesis to light spectra. Previous studies have investigated the plant

photomorphogenesis under red light. However, little is known regarding the structure and function of photosynthetic apparatus under monochromatic red light spectrum. Therefore, in the current study, growth, development and photosynthetic performance of *Calendula officinalis* plants were investigated after their growth under monochromatic red and white LEDs.

English Marigold (*Calendula officinalis*) is an annual or biennial plant species with hairy leaf that belongs to the Asteraceae family. It is native to North Africa and Southern Europe and represents as one of the most versatile ornamental plants in the gardens and green spaces. English Marigold has different applications (e.g. as a pot plant, garden plant, cut flower, food and medicinal plant) in horticulture industry. Nowadays, using LEDs in green space designing and also in greenhouse production of plants is widely developing. However, little is known regarding the morphological and photosynthetic aspects of plant under monochromatic lights. Therefore, objective of the current study was to determine the effects of monochromatic red light spectrum on morphology and photosynthetic performance of calendula flowers by investigating morphology and photosynthetic biophysical properties related to PSII performance.

Fate of absorbed light energy and other information about structure and function of photosynthetic apparatus can be analyzed through chlorophyll fluorescence data by the so-called OJIP test. This test relays on energy flow in thylakoid membranes, which provides detailed information about biophysics of the photosynthetic system through measurement of fluorescence signals (Kalaji et al., 2017). Therefore, this test was mainly used in current study to investigate the effects of light spectrum on photosynthetic apparatus of calendula plants.

## Materials and Methods

### *Plant materials and treatments*

*Calendula* (*Calendula officinalis* cv.

Orange star) seeds were germinated in trays filled with a mixture of cocopeat and perlite (2:1, V:V) in research greenhouse, Aburaihan campus, University of Tehran, Iran in 2017. When two true leaves were emerged (10 days after germination), seedlings were transplanted into plastic pots (15 cm diameter and 20 cm depth, one seedling per pot) containing a mixture of perlite and sand (2:1, V:V). The seedlings were irrigated with half strength Datis Agrochemicals fertilizer (N:P:K+microelements; 20:20:20+ME) and grown under the natural light in the greenhouse (28/18 °C day/night temperature and 40% relative humidity) until the four leaf stage. Then the seedlings were transferred to two growth chambers

with same temperature and relative humidity but with different light spectra. Four plants were kept under white LEDs in the range of 380-750 nm in closed growth chamber (Figure 1). Four plants were also transferred to another growth chamber with red LED with peaks at 635 and 665 nm (Figure 1). Light-emitting diode (24W, Iran Grow Light Co, Iran) was used as the source for red and white spectra providing a photosynthetic photon flux density (PPFD) of  $250 \mu\text{mol m}^{-2} \text{s}^{-1}$  with 12 hours light/dark photoperiod. PPFD intensities and light spectra were monitored using a Sekonic light meter (Sekonic C-7000, Japan). The day/night temperature was 28/18 °C and relative humidity was 40%.

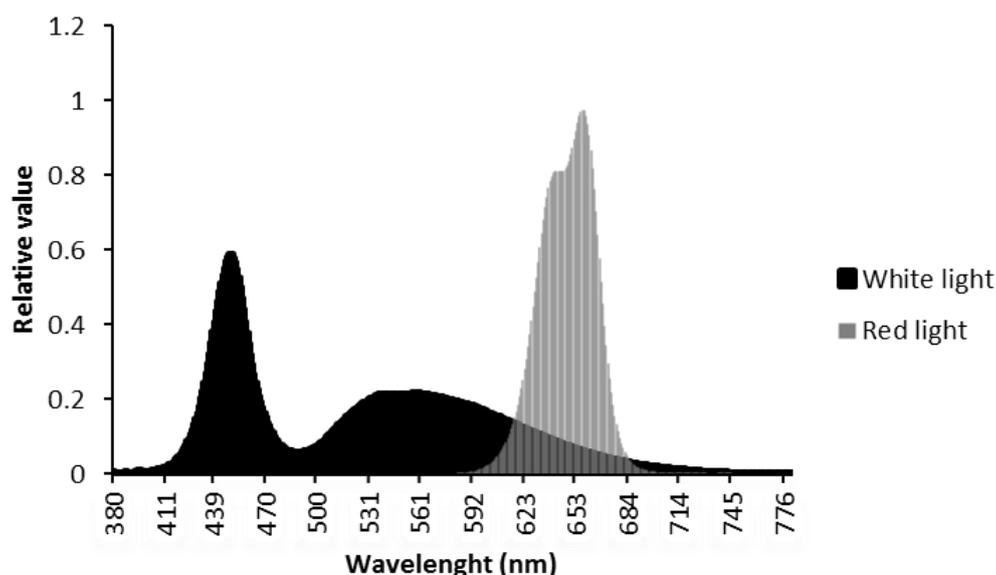


Fig. 1. Relative distribution spectrum of white and red lights that were used for growing English marigold plants

#### *Chlorophyll a fluorescence parameters*

Youngest fully developed leaves were used for measuring maximum quantum efficiency of PSII ( $F_v/F_m$ ) with a PAR-fluorPen FP 100-MAX (Photon Systems Instruments, PSI, Czech Republic). Intact leaves still attached to the plants were dark-adapted for 20 min. After dark adaptation intact plants were immediately used to measure  $F_v/F_m$ . The Fluorcam

consisting of a CCD camera and four fixed LED panels, one pair supplying the measuring pulses and the second pair providing actinic illumination and saturating flash was used. The  $F_v/F_m$  was calculated using a custom-made protocol (Genty et al., 1989; Aliniaiefard et al., 2014; Aliniaiefard and van Meeteren, 2014). Images were recorded during short measuring flashes in darkness. At the end

of the short flashes the samples were exposed to a saturating light pulse ( $3900 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) that resulted in a transitory saturation of photochemistry and reduction of primary quinone acceptor of PSII (Genty et al. 1989). After reaching steady state fluorescence, two successive series of fluorescence data were digitized and averaged, one during short measuring flashes in darkness ( $F_0$ ), and the other during the saturating light flash ( $F_m$ ). From these two images,  $F_v$  was calculated by the expression  $F_v = F_m - F_0$ . The  $F_v/F_m$  was calculated using the ratio  $(F_m - F_0)/F_m$ . The average values, and standard deviation of  $F_v/F_m$  per image were calculated using version 7 of FluorCam software 7.0.

Youngest fully developed leaves were used for measuring transient chlorophyll a (Chl a) fluorescence with a PAR-fluorPen FP 100-MAX (Photon Systems Instruments, PSI, Czech Republic). After applying the protocol, standard deviation per plants was calculated using version 1.0 fluorpen software.

The OJIP test were measured with PAR-fluorPen FP 100-MAX on young fully expanded English-marigold leaves with at least 20 minutes dark adaptation on intact leaves still attached to the plants. After dark adaptation,  $F_0$  was measured at 50  $\mu\text{s}$ , fluorescence intensity at J-step was measured at 2 ms, and fluorescence intensity at I-step was measured at 60 ms. Image were generated using PAR-Fluorpen software version 1.0. Performance index was calculated on the absorption basis ( $\text{PI}_{\text{ABS}}$ ) and densities of  $\text{QA}^-$  reducing PSII reaction centers at time 0 and time to reach maximum fluorescence. Furthermore, the yield ratios including: The probability that a trapped exciton moves an electron in electron transport chain beyond  $\text{QA}^-$  ( $\psi_0$ ), quantum yield of electron transport ( $\phi_{\text{E0}}$ ), quantum yield of energy dissipation ( $\phi_{\text{D0}}$ ) and maximum quantum yield of primary photochemistry ( $\phi_{\text{P0}}$ ) were also calculated based on the following equations (Strasser et al., 2000; Kalhor et al., 2018):

$$V_j = (F_j - F_0) / (F_m - F_0)$$

$$M_0 = \frac{\text{TR}_0}{\text{RC}} - \frac{\text{ET}_0}{\text{RC}} = \frac{4(F_{300} - F_0)}{F_m - F_0}$$

$$\phi_{\text{P0}} = 1 - \left( \frac{F_0}{F_m} \right) \left( \text{or } \frac{F_v}{F_m} \right)$$

$$\phi_{\text{E0}} = \left( 1 - \left( \frac{F_0}{F_m} \right) \right) \cdot \text{Psi}_{-0}$$

$$\phi_{\text{D0}} = 1 - \text{Phi}_{\text{P0}} - \left( \frac{F_0}{F_m} \right)$$

From these data the following parameters were calculated: the specific energy fluxes per reaction center (RC) for energy absorption ( $\text{ABS}/\text{RC} = M_0 \cdot (1/V_j) \cdot (1/\phi_{\text{P0}})$ ). Performance index for energy conservation from excitation to the reduction of intersystem electron acceptors:  $\text{PI}_{\text{ABS}} = (\text{RC}/\text{ABS}) [(\phi_{\text{P0}}/(1 - \phi_{\text{P0}}))] [(1 - V_j)/1 - (1 - V_j)]$ .

### Statistical analysis

Differences in plant growth characteristics and photosynthetic performance were evaluated by t test using GraphPad Prism 7.01 for Windows (GraphPad software, Inc., San Diego, CA). *P*-values smaller than 0.05 were considered as significantly different.

## Results

### Effect of red and white lights on plant growth

Growing calendula plants under red and white LEDs resulted in different growth phenotypes. Figure 3 shows the effect of the red light on the plant growth and plant morphology. Growing plants under monochromatic red light caused leaf epinasty, while the plants that were grown under white light had normal morphology. Although, growing plants under red light caused abnormal morphology, higher vegetative growth were observed in the plants that were grown in the growth chamber with red LED. Forty five days following germination, flowers buds were observed on the plant that were grown under monochromatic red light when they

had 16 rosette and 14 lateral leaves, while, during the same time period, the plants that were grown under white light had 14 rosette and 6 lateral leaves with no flower buds observed (Fig. 3).

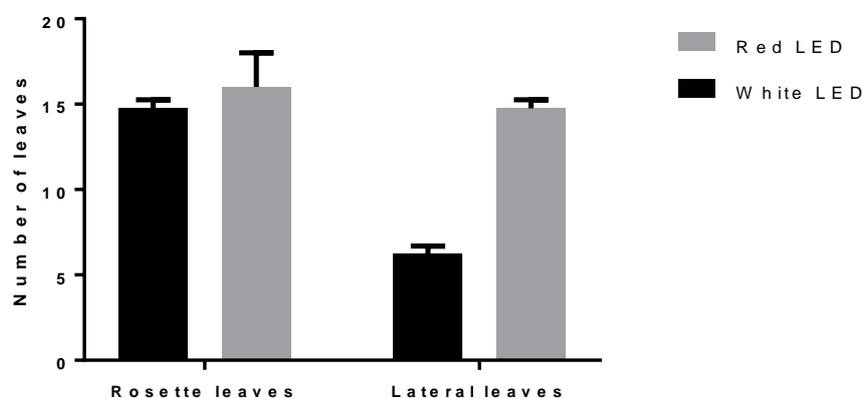
#### **Chlorophyll fluorescence parameters**

Growing plants under red and white LEDs resulted in different patterns of fluorescence emission from the calendula leaves. As can be seen in Figure 4 growing plants under red light led to higher fluorescence intensities in different steps of OJIP curve. Although, higher  $F_0$ ,  $F_j$ ,  $F_i$  and

$F_m$  were observed in the leaves of plants that were grown under red light, the differences among these parameters in red light-grown plants were lower than the differences among them in white light-grown plants (Table 1). This resulted in higher  $V_j$ ,  $V_i$  and  $F_v$  on the leaves of plants that were grown under white LED. Furthermore, higher  $F_v/F_m$ ,  $F_v/F_0$  and  $F_v/F_0$  ratios were observed in the plants that were grown under white light when compared to their value in red light-grown plants (Fig. 5 and Table 1).



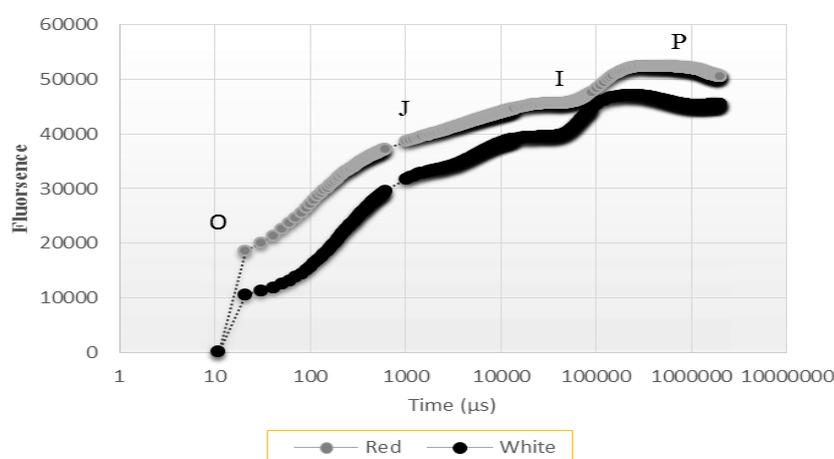
**Fig. 2.** Different growth morphology and vegetative characteristics in English marigold plants following 21 days growth under monochromatic red (A) or white (B) LEDs.



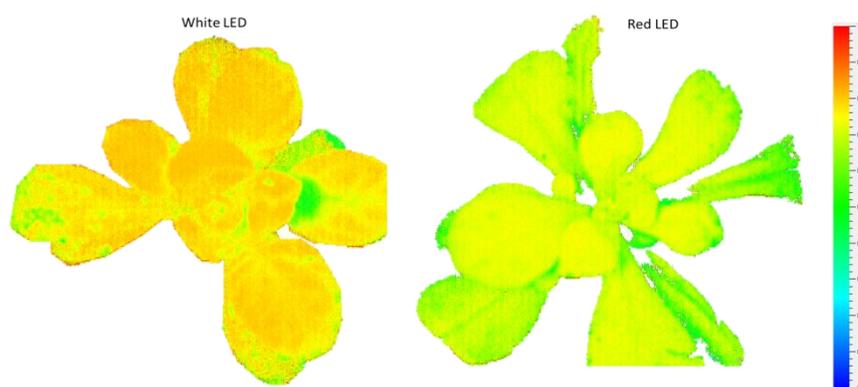
**Fig. 3.** Number of rosette and lateral leaves in English marigold plants following 45 days growth under red and white LEDs. Bars represent means of four replicates  $\pm$  SD.

**Table 1.** Transient chlorophyll fluorescence calculated parameters measured on the leaves of English marigold plants following 45 days growth under red and white LEDs. The data are average values $\pm$ SD

Transient fluorescence Parameters	White LED	Red LED
$F_0$	11769 $\pm$ 401.50	21213.5 $\pm$ 3600.67
$F_j$	33258.75 $\pm$ 826.90	40021.25 $\pm$ 5246.13
$F_i$	39354.75 $\pm$ 2726.65	45401.5 $\pm$ 4810.93
$F_m$	46921.5 $\pm$ 2730.10	56547.33 $\pm$ 4616.61
$F_v$	35152.5 $\pm$ 2345.06	31161.75 $\pm$ 1553.20
$V_j$	0.62 $\pm$ 0.03	0.59 $\pm$ 0.054
$V_i$	0.78 $\pm$ 0.02	0.77 $\pm$ 0.024
$F_m/F_0$	3.9 $\pm$ 0.10	2.5 $\pm$ 0.22
$F_v/F_0$	2.9 $\pm$ 0.10	1.58225 $\pm$ 0.22
$F_v/F_m$	0.74 $\pm$ 0.006	0.60 $\pm$ 0.035



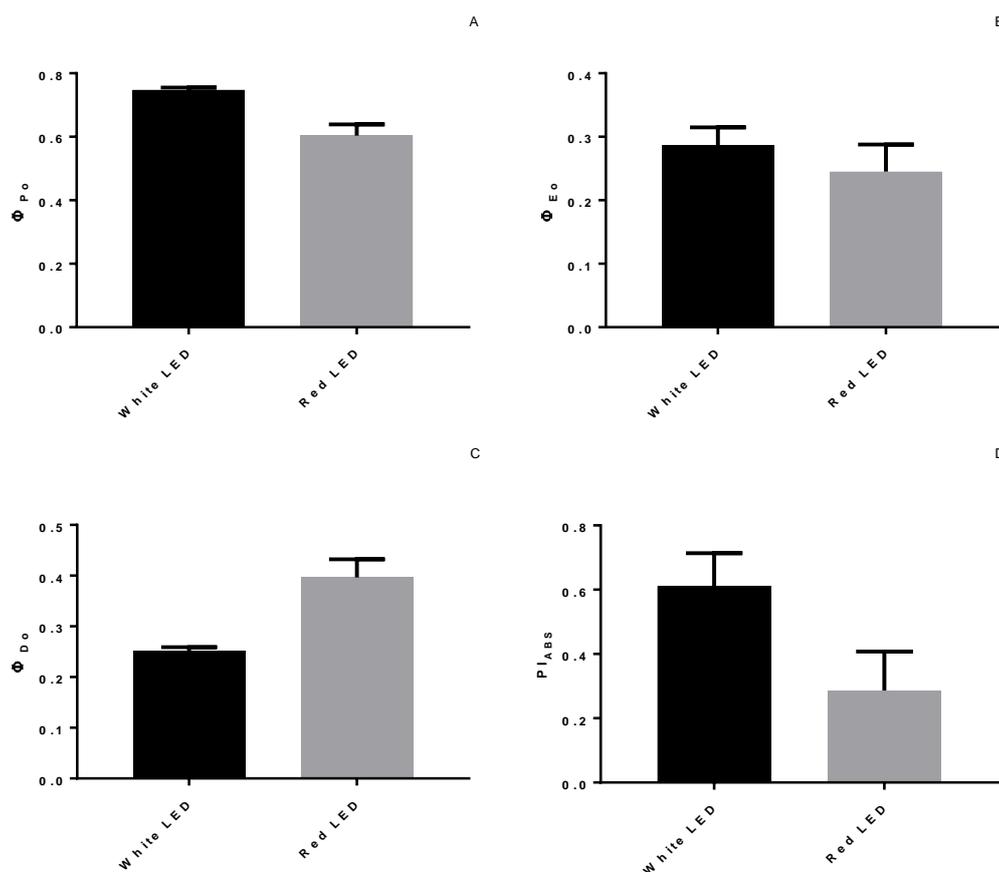
**Fig. 4.** OJIP steps in English marigold plants following 45 days growth under red and white LEDs.



**Fig. 5.** Image of maximum efficiency of photosystem II ( $F_v/F_m$ ) in English marigold plants following 21 days growth under white and red LEDs

Analyzing the parameters that estimate the yields and efficiency of the electron transport chain showed that in comparison with white light,  $\phi_{P_0}$  (Fig. 6A),  $\phi_{E_0}$  (Fig. 6B) and  $PI_{ABS}$  (Fig. 6D) were decreased by growing plants under monochromatic red light. On the other hand,  $\phi_{D_0}$  was

considerably higher in red light-grown plants in comparison with the  $\phi_{D_0}$  of the plants that were grown under white light (Fig. 6C). The  $\phi_{D_0}$  was doubled by growing plants under red light when compared its value in white light-grown plants.



**Fig. 6.** Maximum quantum yield of primary photochemistry ( $\phi_{P_0}$ ), quantum yield of electron transport ( $\phi_{E_0}$ ), Performance index on the absorption basis ( $PI_{ABS}$ ), quantum yield of energy dissipation ( $\phi_{D_0}$ ) calculated from the fluorescence transient exhibited by leaves of English marigold plants following 45 days growth under red and white LEDs. Bars represent means of four replicates  $\pm$  SD.

## Discussion

In our experiment, growing plants under red light promoted growth and accelerated flowering in English marigold plants (Fig. 2 and 3). It has been shown that plants that have been grown under light with huge proportion of red spectrum are usually large and tall with many branches. Absorption of large quantity of red light by plant photoreceptor can result in production of a plant hormone with the name of metatopolin (Steele, 2004). This hormone is similar to cytokinins (example.g. Benzlyaminopurine) which can stimulate cell division as well as branching.

The importance of light quality on plant growth characteristics such as plant elongation, leaf area, dry and fresh weight has been previously reported ( Dougher

and Bugbee, 2004; Shin et al., 2008; Kami et al., 2010; Wang et al., 2016). Previous studies confirmed that red and far red lights can increase plant growth and development. Improvement of growth and development by red and far red lights has been reported in *Azorina vidalii* (da Silva and Debergh, 1997), *Cymbidium* (Tanaka et al., 1998) and *Rehmannia glutinosa* (Hahn et al., 2000) plants.

Chlorophyll (Chl) fluorescence induction kinetics provides a useful, fast, highly sensitive and non-invasive protocol to study electron transfer kinetics among different components of PSII. In this experiment, photochemical efficiency was measured by Chl fluorescence induction kinetics by means of OJIP transients. When dark-adapted leaves are illuminated with

actinic light, Chl fluorescence shows complex induction kinetics named as kautsky curve (Strasser et al., 2004; Papageorgiou et al., 2007). The OJIP fluorescence transient in Figure 4 reflects successive reduction of acceptor pools of PSII (Strasser et al., 2000). The O-J induction fluorescence is the photochemical phase and is related to plastoquinone (PQ) and  $Q_A$  reduction or reduction of acceptor side of PSII. Increase in the value of minimal fluorescence intensity ( $F_0$ ) or in the O-J phase were observed in red light treatment indicating altered antenna complex of PSII and an increase in oxygen evolving capability (Joliot, 1965; Papageorgiou et al., 2007). Increase in J-I phase in the transient fluorescence test is due to accumulation of  $Q_A^-$ ,  $Q_B^-$  and subsequent  $Q_A^-$  to  $Q_A^{2-}$  ( $Q_B$  is plastoquinone molecule which is loosely bound). Higher Chl fluorescence efficiency in the I-P phase exhibits depletion of subsequent acceptors in PSII. Using the calculated parameters of transient fluorescence induction we can understand the perfect balance between incident light and utilization rate of potential energy as well as the rate of heat dissipation (Kalaji et al., 2017; Kalhor et al., 2018).

In the transient Chl fluorescence protocol, the intensity for the first recorded fluorescence signal is known as  $F_0$ . In this step,  $Q_A$  is maximally oxidized and the rate of primary charge transfer in the reaction centers is also maximum (Papageorgiou et al., 2007).  $F_0$  values were increased in the plants grown under white LED. This showed a significant change in antenna complex of PSII following exposure to red light. The parameter  $F_v/F_m$ , area between  $F_0$  and  $F_m$ ,  $F_m/F_0$  and  $F_v/F_0$  (Table 1) were decreased in the plants grown under red light. This indicated the loss of functional reaction centers resulting in decreased electron transport from reaction centers to PQ pool (Strasser et al., 2000). Change in  $F_v/F_m$  is also caused by non-photochemical quenching (Fricke and Peters, 2002). The

parameters such as  $F_j$ ,  $F_i$  and  $F_m$  were increased in red light treatment (Table 1). On the other hand, the  $F_v$  and  $V_j$  was decreased in red light (Table 1). This indicated altered antenna complex of PSII under red light treatment.

The values of  $\Phi_{P0}$ , maximum quantum yield for primary photochemistry and  $\Phi_{E0}$ , quantum yield of electron transport were decreased in plants that were grown in red light which is in correlation with the decreased values of  $F_v/F_m$ . The  $\Phi_{P0}$  was significantly increased in plants grown under white light in comparison with its value in red light-grown plants.

The parameter  $\Phi_{D0}$ , which shows the efficiency of non-photochemical de-excitation processes, was significantly higher in the plants that were grown under red lights. This indicated that most of the light that absorbed by the photosystems was not used for photochemical yield of the electron transport chain and it was dissipated as heat from the electron transport system.

In conclusion, our result showed that growing plants under monochromatic red light can promote growth and development of English marigold plants. However, plants that were grown under this light showed some morphological defects such as leaf epinasty. Using OJIP protocol confirmed that plants grown under monochromatic red light were less efficient to successfully transfer the excitons and most of the absorbed energy by the photosystems was dissipated as heat.

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