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Study on spectral response of saffron (*Crocus sativus* L.) at different leaf ages and evaluation on photosynthetic energy efficiency of narrow-band LED spotlights

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Abstract

Leaf photosynthesis largely determines the daughter corm yields in vegetative growth for saffron (*Crocus sativus* L.). Most of previous researches focuses on spectral response in various species, but lacking the study on saffron leaves at different leaf ages. In this study, the action spectrum based on photosynthetic photon flux density (PPFD) and irradiance were distinguished and interpretated. The optical properties and photosynthetic performances of leaves were respectively investigated at two leaf ages, dependence on customized narrow-band LED spotlights from 380 nm to 780 nm with an interval 20 nm and a band width 10 nm. The younger leaves charactered higher reflectance and transmittance at 500−600 nm, resulting in lower absorptance compared to the older leaves. The spectral response curves including action spectrum and quantum yield for younger leaves were higher than the older, but their relative curves displayed coincidence. The spectral response curves exhibited two peaks at 440 and 640 nm, but not low between 500 and 600 nm. Nevertheless, the photosynthetic energy efficiency of spotlights demonstrated very low in green/yellow region. Accordingly, more attentions should be paid to green and yellow LED lighting during the vegetative stage for saffron, as well as improving their manufacturing technology.

Keywords Leaf photosynthesis, Spectral dependence, Absorptance, LED lighting, green light

1 Introduction

The dried stigmas of saffron (*Crocus sativus* L.) has excellent preventive and therapeutic effects on circulatory system diseases, irregular menses, mental depression, tumors, etc (Bhandari 2015; Moratalla-López et al. 2019; Cardone et al. 2020). It can be used as a drug substitute to improve the immune system and self-repair ability for people, resulting in an increasing market demand for saffron (Bagur et al. 2018). Saffron is a perennial herbaceous plant, and vegetatively propagated by corms (Lotfi et al. 2015). The reproduction of high-quality corms has become a crucial factor to increase stigma yields. Nevertheless, the stigma yield is far below market demand at present, which is greatly affected by corm size and environmental conditions (e.g. irrigation methods, growth cycles, and fertilization conditions, etc) in the field (Khorramdel et al. 2015; Koocheki et al. 2014; Yarami & Sepaskhah 2015). The research has shown that the size of mother corm and the interaction of year × corm size

have significant effects on the production of daughter corms (Özel et al. 2017). The corm yield significantly varies year to year, and a two-year growth cycle can maximize the reproduction rate of corms (Douglas et al. 2014). Applying organic and biological fertilizers with high water-absorbing, and irrigating every four weeks can improve the yields of the daughter corms (Shajari et al. 2018). Besides, some studies improve stigma yield by two-segment cropping system (Wang et al. 2021; Zhou et al. 2022). The corms without any culture medium are cultivated in cultivation rooms for dormancy and flowering from June to November, and then are transplanted to the fields for vegetative development from December to May (Renau-Morata et al. 2012). However, this system has not solved the problem about variable environments during the corm development in the field, and thus a controllable environment in the field may be a solution to improve corm yields.

Light is a crucial factor in controllable environment, and can affect crop yield and nutrient storage of root and shoot by spectrum, light intensity and photoperiod (Bantis et al. 2016; Chen et al. 2016). Photosynthesis is the ultimate source of carbohydrates and supplies some ATP and NAD(P)H in photosynthetic tissues (Keller 2010). The vegetative development of daughter corms for saffron is mainly supplied from leaf photosynthesis and the carbon nutrients of mother corms. After flowering, most of the remaining mother corm reserves are depleted by the development of roots and leaves, and only a small portion of the biomass are provided to daughter corms. Entering February, the daughter corms initiate and rapidly develop. The photosynthetic capacity of leaves contributes about 90% of the biomass accumulations to the daughter organs (Renau-Morata et al. 2012). Moradi et al. (2021) have found that a high ratio of blue to red light-emitting diodes (LEDs) can improve the photosynthetic performance of saffron leaves by facilitating electron flow between photosystem I and II, and enhance the daughter corm yields and carbohydrate accumulations for saffron. A higher light intensity and a higher proportion of blue light significant increased leaf area, total yield of daughter corms, and proportion of larger-sized daughter corms, as well as 200 μmol m^{-2} s^{-1} (the ratio of blue and red light was 2 : 3 (namely 2B3R) in spectrum containing red, blue, green, and white LEDs) was regarded as optimal light in comparison to 56 μmol m^{-2} s^{-1} (1B3R), 200 μmol m^{-2} s^{-1} (1B3R), and 200 μmol m^{-2} s^{-1} (1B4R) (Zhou et al. 2022). However, both studies above did not directly investigate the effect of LED lighting on leaf photosynthesis and corm yields during the vegetative development. The corms in these researches were irradiated with LEDs in a cultivation room during the flowering period, and then were investigated the subsequent effects of light spectrum and intensity on corm developments after transplanting to the field. So far, the propagation of saffron corms still relies on traditional field production and a gap still exits about applying LED to the vegetative development in controlled environment. Few studies have been conducted to explore the impact of light, especially spectrum on leaf photosynthesis and corm yields for saffron in the field.

The widely accepted spectral response curve in plant photosynthesis is proposed by McCree (1971/1972), which mainly determines blue (400–500 nm) and red (600–700 nm) regions as the most efficient light in LED controlled environments. Nevertheless, diverse studies exhibit various peaks and proportions of blue and red regions in spectral response (McCree 1971/1972; Paradiso et al. 2011; Zhen et al. 2019; Lee et al. 2017). This phenomenon is not only influenced by species differences, plant growth conditions and experimental designs (Wu et al. 2019; Inada 1976), but more importantly, different photometric units can

also cause differences in the spectral response curves of photosynthesis. Irradiance as the unit of incident light was used to the photosynthetic measurements by McCree (1971/1972), but the results was less accurate than those obtained by photosynthetic photon flux density (PPFD) as the measurement unit. Inada (1976) determined directly the action spectrum from the reciprocal of the irradiance referring from McCree's method, and further obtained the action spectrum for unit incident quanta, but the conversion factor from irradiance to PPFD was not disclosed. The conversion relationship was provided by Balegh & Biddulph (1970), but it was only a conversion between wavelengths, that is, using irradiance at 400 nm and wavelength to calibrate irradiance at other wavelengths. Wu et al. (2019) compared the action spectrum from several early reports, and mentioned the photometric units of W m^{-2} and μmol m^{-2} s^{-1} in different studies, but their conversion was not clearly explained. For this reason, it is vital to quantitatively reinterpret the action spectrum for PPFD and irradiance.

Furthermore, narrow-band light has been still generated by traditional xenon lamps or tungsten halogen lamps combined with interference filters in the most researches about spectral response curves (McCree 1971/1972; Paradiso et al. 2011; Inada 1976; Nilsen & Mortensen 1978; Evans 1987). The irradiance or PPFD received by leaves at various narrow-band wavelengths is not uniform (McCree 1971/1972; Paradiso et al. 2011; Inada 1976; Evans 1987), especially the ultraviolet region lower than 400 nm due to the low radiant power ratio. Plants receiving diverse light densities may have different photosynthetic rate and further affect their spectral response at the corresponding wavelength (Liu & Iersel 2021). Our research group has measured the spectral response of saffron leaves before, but some data results are not ideal due to the poor stability and significant attenuation of short arc xenon lamps (Ji et al. 2021). LEDs have gained popularity for horticultural applications due to narrow spectrum, cold light source and long lifespan (Kohler & Lopez 2021). The narrow-band LEDs at 400−700 nm was used to investigate the photosynthetic spectral dependance for horticultural crops by Lee et al. (2017), but non-uniform wavelength interval may result in missing key wavelengths during measurements. The previous researches about spectral response in diverse plants are summarized and listed in Table 1.

Table 1. Comparison of spectral response measurement in diverse plants in previous researches

Researcher	Plant	Light source	Filtering technology	Peak wavelength (nm)	Interval (nm)	Band width (nm)	Light intensity
McCree (1971/1972)	22 crops	Xenon lamp	Monochromator	350−750	25	25	30 W m^{-2}@650 nm
Paradiso et al. (2011)	Rose	Tungsten halogen lamp	Interference filter	406−720	about 20	10@460−720, 20@406, 427 & 445	30 & 60 μmol m^{-2} s^{-1}
Inada (1976)	26 herb crops & 7 arbores	Xenon lamp	Interference filter	344−758	about 10−40	8.5−17@400−700, 10−21@others	1.5−2.5 mW cm^{-2}@344 & 368 nm, 3 mW cm^{-2}@others

Nilsen et al. (1978)	Spruce	Xenon lamp	Interference filter	463, 517, 557, 579, 605, 637, 672, 687, 704	Unequal	/	150 μmol m^{-2} s^{-1}
Evans (1987)	Pea & Spinach	Xenon lamp	Interference filter	400−700	about 20−25	/	0−100 μmol m^{-2} s^{-1}
Ji et al. (2021)	Saffron	Xenon lamp	Interference filter	380−760	20	10	100 μmol m^{-2} s^{-1}
Lee et al. (2017)	Lettuce	LED luminaire	/	400−700	10, 20, 30 or 40	10	150 μmol m^{-2} s^{-1}
This work	Saffron	LED spotlight	Interference filter	380−780	20	10	100 μmol m^{-2} s^{-1}

The vegetative growth of saffron corms is mainly supported the carbon reserves by leaf photosynthesis, and thus it is of great significance to study the photosynthesis and spectral response of saffron leaves. This work would like to develop narrow-band LED spotlights with equal peak wavelength interval and PPFD, and to measure the photosynthetic properties of saffron leaves at different leaf ages. Meanwhile, different photometric units in photosynthetic spectral response based on PPFD and irradiance will be distinguished. At last, the photosynthetic energy efficiency for narrow-band LED spotlights will also be evaluated. The field environment is relatively complex and uncontrollable for saffron corms nowadays. The findings in this study can provide a guidance for lighting condition in controlled field environments and further promote the vegetative growth and corm yields.

2 Materials and methods

2.1 Plant materials and growth conditions

Plant samples in this study were obtained from saffron production area in Chongming Island (121° 40' N, 31° 62' E, Shanghai, China). The saffron corms were transplanted in the field in late November 2022, after flowering on the culture shelf indoors. Thirty-six corms with a dry weight of 18−23 g were selected and planted in 3.5-L round flowerpots filled with sandy loam soil at a plant density of 78.6 corms per m^2. The planting conditions in flowerpots such as soil, temperature, humidity, and nutrient management were completely consistent with those in the field. The relative humidity was 60%−90%, the day/night temperature ranged from −10 to 10 ℃. The earthworm organic fertilizer was applied once every month, and the amount were 3 kg m^{-2} every time. During the period of vigorous leaf growth from February to March, liquid organic fertilizer was applied 0.003 kg m^{-2} every 2 weeks. The irrigation was based on the rainfall to keep the soil moist without ponding (Ji et al. 2021). The photosynthetic rates of saffron leaves were beforehand determined in mid-December and mid-January, and gained negative value. For this reason, two treatments with three replicates in the experiment were designed, including corm leaves from mid-February and mid-March. The saffron leaves reached total senescence at the end of March (Renau-Morata et al. 2012).

2.2 Methodology of photosynthetic spectral response based on PPFD and irradiance

Photosynthetic spectral response of leaves includes action spectrum and quantum yield in leaf photosynthesis. Due to the inconsistency in the measurement units of incident light during various experiments, there are mainly two forms of expression about action spectrum (Wu et al. 2019). Action spectrum based on PPFD, can be expressed as $S_P(\lambda)$, refers to the mole numbers of CO_2 assimilated per mole number of incident light. Its expression is

$$S_P(\lambda) = (P_n - P_d)/E_P \,, \tag{1}$$

where P_n and P_d mean net photosynthetic rate and dark respiration rate of leaves (μmol m^{-2} s^{-1}), respectively, and E_p is photosynthetic photon flux density (μmol m^{-2} s^{-1}).

The other action spectrum based on irradiance represents the mole numbers of CO_2 assimilated by incident light per unit irradiance, expressed as

$$S_r(\lambda) = (P_n - P_d)/E_r \,, \tag{2}$$

where $S_r(\lambda)$ represents the action spectrum based on irradiance (μmol W^{-1} s^{-1} or μmol J^{-1}), and E_r is irradiance (W m^{-2}).

Besides, due to the narrow-band incident light used in photosynthetic spectral response, there exists following relationship between PPFD and irradiance (Nelson & Bugbee, 2014):

$$K_{P,r} = E_P/E_r = \int \frac{P_r(\lambda)\lambda \mathrm{d}\lambda}{n_A hc} \Big/ \int P_r(\lambda)\mathrm{d}\lambda = \lambda/(n_A hc) \,, \tag{3}$$

where $K_{P,r}$ represents the conversion coefficient between PPFD and irradiance (μmol W^{-1} s^{-1} or μmol J^{-1}), $P_r(\lambda)$ indicates spectral irradiance of incident light, λ is wavelength (nm), n_A represents Avogadro constant (n_A = 6.02214076×10²³), h signifies Planck constant (h = 6.626×10^{-34} J s), and c expresses the speed of light (c = 2.99792458×10^8 m s^{-1}). Therefore, the action spectrum based on PPFD can also be expressed as:

$$S_P(\lambda) = n_A hc(P_n - P_d)/(\lambda E_r) = (n_A hc/\lambda)S_r(\lambda) \,. \tag{4}$$

In Eq. (4), the action spectrum based on PPFD is inversely proportional to wavelength. When the irradiance was adopted as the unit of incident light, the action spectrum based on PPFD should be obtained through dividing by the wavelength and multiplying by a constant $n_A hc$.

Quantum yield $Y_p(\lambda)$ represents the mole number of CO_2 assimilated per mole number of absorbed light, and thus can be expressed as:

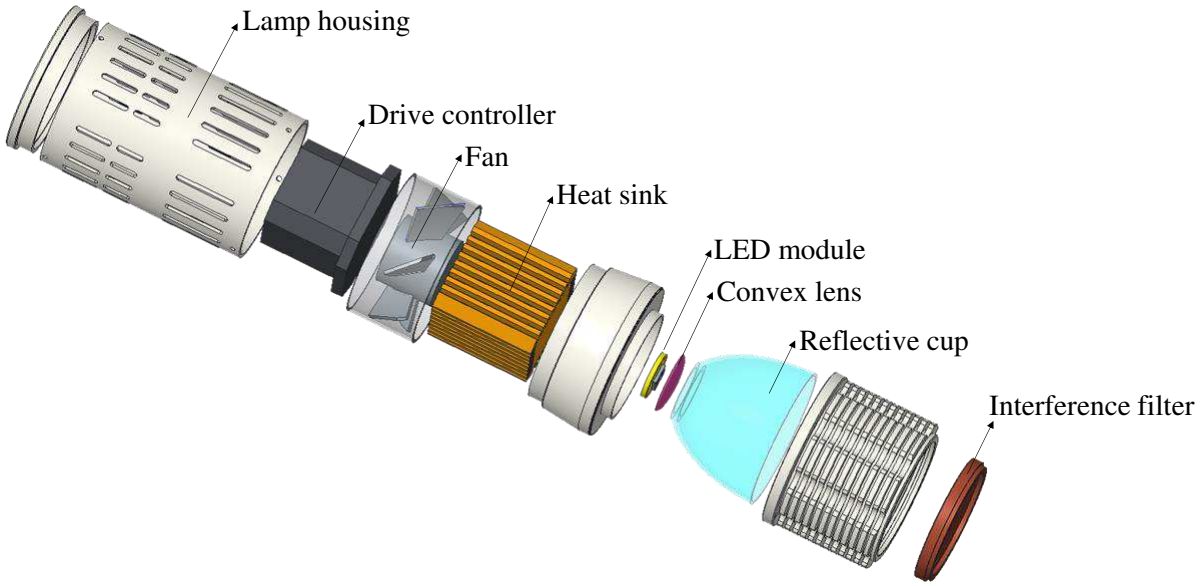
$$Y_P(\lambda) = (P_n - P_d)/(E_P \cdot A) \,, \tag{5}$$

$$A = 1 - (R + T) \,, \tag{6}$$

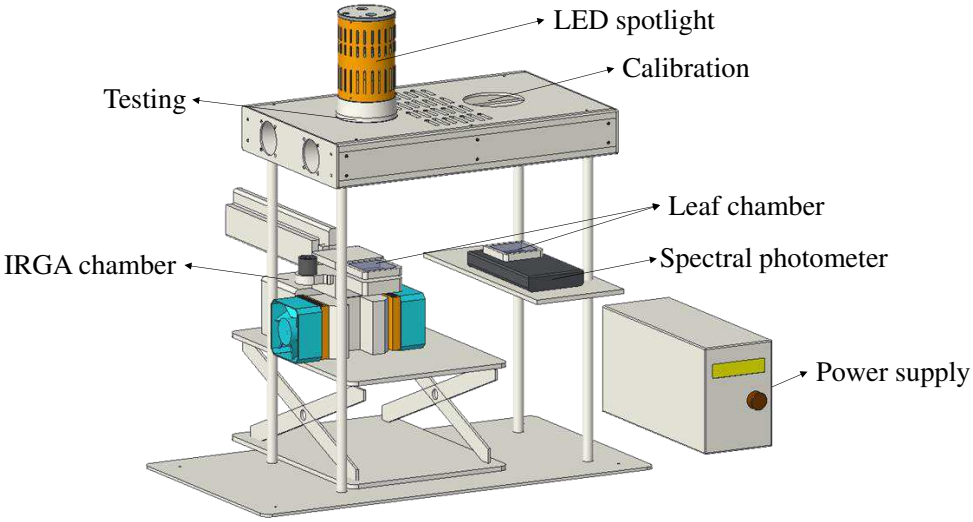
where A, R and T is absorptance, reflectance and transmittance of leaves, respectively.

2.3 Narrow-band LED spotlights and testing platform

The narrow-band light was obtained from 21 LED spotlights for photosynthetic measurements. They were made of LED modules with different peak wavelengths and interference filters with band width of 10 nm. A convex lens, reflective cup and an interference filter were equipped in front of the LED module to concentrate and filter light. The heat sink, fan and drive controller were placed at back of the LED chip module to decline the radiant heat load and adjust the radiant output. All components were covered with a lamp housing, as shown in Fig. 1(a). During photosynthetic measurements, the spotlight was placed on a testing platform, with the transparent leaf chamber 15 cm below the light outlet. There were two positions on the testing platform, one for calibration and the other for testing. To further reduce the radiant heat and avoid the ambient temperature interference around the leaf chamber, the interior of testing platform was designed as a cavity with fans around, as shown in Fig. 1(b).



(a)



(b)

Fig. 1 Schematic diagram of narrow-band LED spotlight and testing platform. (a) Narrow-band LED spotlight. (b) Testing platform

Before photosynthetic measurements, each LED spotlight should be preheated and calibrated. The spotlight was lit at the rated power for 20 min to stabilize on a preheating platform, and then was placed on the testing platform for calibration. A spectral photometer (PLA-20, EVERFINE, China) was utilized to measure spectrum and PPFD received by saffron leaves in the transparent leaf chamber. Its probe was covered with the transparent leaf chamber and placed 15 cm below the calibration position to maintain the same level as the transparent leaf chamber connected to infrared gas analyzer (IRGA) chamber. The PPFD was adjusted to 100 μmol m^{-2} s^{-1} using an adjustable DC regulated power supply (HSPY-200-05, HANSHENG PUYUAN, China) and the dimming controller. Since the spotlight has been preheated, the PPFD reading could stabilize within 5 min. Then the spotlight was placed at the testing position for photosynthetic measurements. The separation of calibration and testing can avoid interference caused by frequently opening the leaf chamber connected with the IRGA chamber for PPFD calibration. After the reading of net photosynthetic rate P_n was recorded, the spotlight was put back again to the calibration position for another testing. Two PPFD tests was conducted to determine the stability of light output from spotlight before and after the net photosynthetic rate measurements.

2.4 Photosynthesis measurements

The photosynthetic measurements were respectively taken on the sunny mornings of mid-February and mid-March. Six saffron corms in the flowerpots for replicate were randomly selected with a completely randomized block design, and then were moved into the laboratory for high PPFD adaptation with broad-band white background light. The laboratory was ventilated to maintain the temperature and humidity consistent with the outside. The net photosynthetic rate P_n was measured using a portable photosynthetic system (LI-6400XT, LI-COR, America). The conditions inside the IRGA chamber were set as a constant leaf temperature of 20 ℃, and a CO_2 concentration of 400 μmol m^{-2} s^{-1} with a flow rate of 500 μmol s^{-1}. Due to the elongated shape of saffron leaves, 8−10 leaves in each corm were arranged and paved side by side without gap, and then the middle part of leaves were fixed to the transparent leaf chamber in photosynthetic system, shown in Fig. 2. The leaves were exposed to 21 narrow-band lights ranging from 380 to 780 nm, in an interval of 20 nm with a band width of 10 nm. Under each light spectrum, five P_n readings were recorded with 10 s intervals, after PPFD and P_n in the transparent leaf chamber were stable (generally about 4−10 min). Then five P_n readings under the spectrum were averaged for analysis. After that, the leaves were exposed to the next spectrum for next photosynthetic measurements. When changing the spectrum and adjusting the PPFD, the white LED spotlight provided a broad-band background light. After all spectra measurements were completed, the dark respiration rate P_d were recorded at the dark. To avoid the potential impact of the order of spectra on net photosynthetic rate of leaves, the spectrum was randomly selected with a Latin square design for each measurement (Liu & Iersel 2021). In addition, the PPFD on the leaf surface in transparent leaf chamber from narrow-band spectra was set as 100 μmol m^{-2} s^{-1}, based on the region where the net photosynthetic rate and PPFD were linearly related (Singsaas et al. 2001).



Fig. 2 Photos of saffron leaves fixed to the transparent leaf chamber in photosynthetic system

2.5 Optical properties

After photosynthetic determinations, the optical properties of leaves per corms were measured including spectral reflectance, transmittance, and absorptance at 380−780 nm. The reflectance and transmittance were determined by means of a high accuracy array spectroradiometer (HASS-2000, EVERFINE, China) coupled with a spectrum reflection/transmittance testing box (TR80, EVERFINE, China). And a precision digital display DC stabilized current power supply (WY, EVERFINE, China) was adopted to supply power for standard tungsten halogen lamps in the reflection/transmittance testing box. Before testing, a standard whiteboard was used for calibration. And then 8−10 leaves in each corm were fixed to the light outlet and inlet of the testing box for reflectance and transmittance measurements, shown in Fig. 3. Leaves of each corm were measured three times in total, and the results were averaged for analysis.



(a) (b)

Fig. 3 Testing Photos of optical properties of saffron leaves in mid-March. (a) Reflectance measurement. (b) Transmittance measurement

2.6 Statistical analysis

One-way analysis of variances (ANOVA) was performed using IBM SPSS Statistics software (version 25, USA), and significant differences among leaf optical properties were assessed using the LSD multiple range analysis test ($P \leq 0.05$).

3 Results and discussion

3.1 Leaf optical characteristic at different leaf ages

The reflectance, transmittance, and absorptance curves of saffron leaves in mid-February and mid-March were shown in Fig. 4. In the ultraviolet/blue regions of 380−500 nm, the reflectance and transmittance of leaves at different leaf ages were observed very low and relatively flat, around 5.2% and 2.6% in average, respectively. In the green/yellow region of 500−600 nm, the reflectance/transmittance reached a peak at about 550 nm, with an average of about 12.2% and 6.9% at 550 nm. Then the two curves decreased slightly from 600 to 680 nm, and reached a minimum of 7.0% and 3.0% at 680 nm, respectively. After exceeding 680 nm, the reflectance and transmittance increased rapidly, reaching 51.6% and 34.8% at 750 nm, respectively. At last, the reflectance basically remained stable, while the transmittance slightly increased above 750 nm.

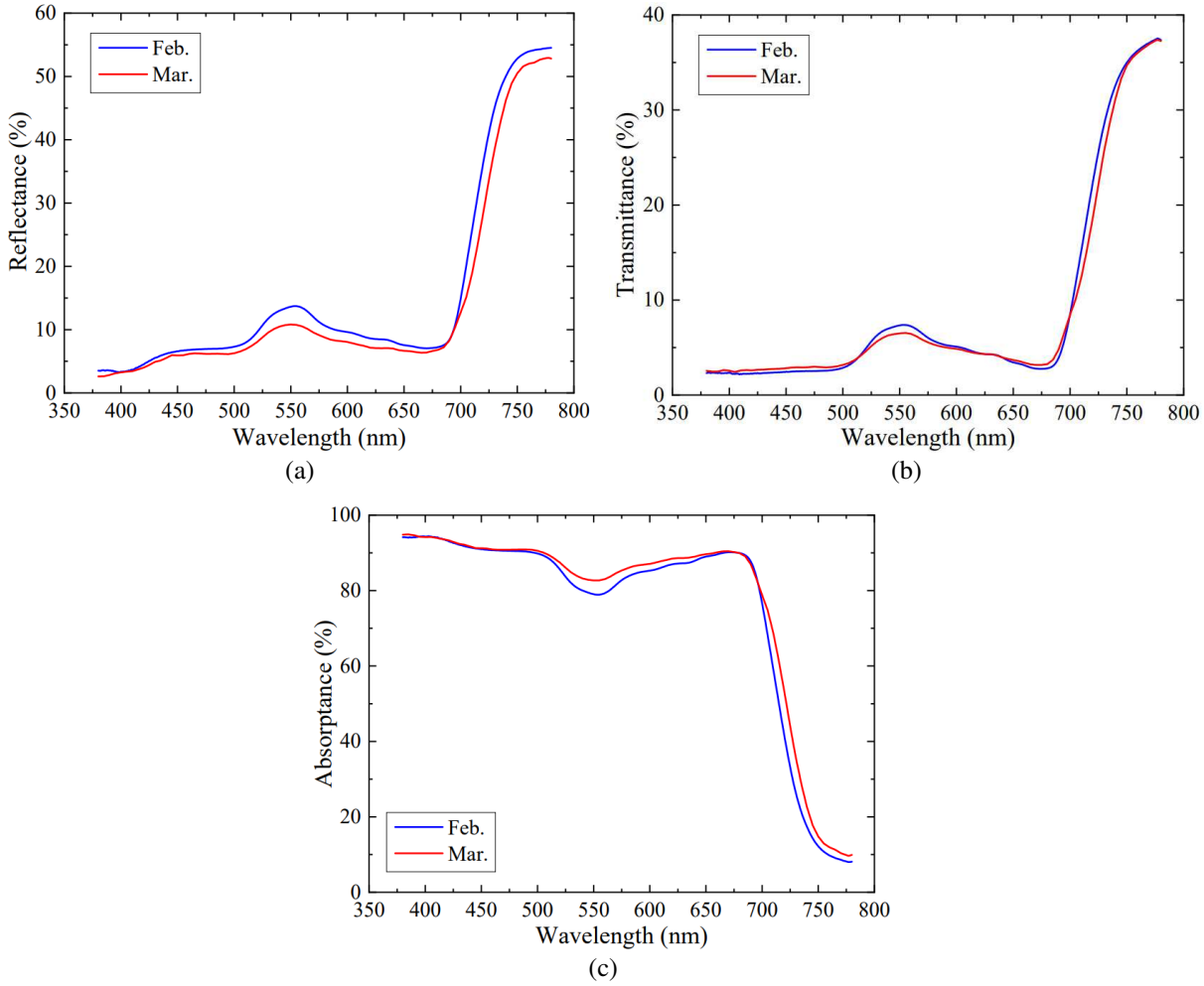


Fig. 4. Leaf optical properties at different leaf ages. (a) Reflectance. (b) Transmittance. (c) Absorptance. Each line represents the mean of 6 plants with three replicates

The tendencies of absorptances in saffron leaves was shown similar with that of other species [19]. They displayed a higher absorptance in the ultraviolet/blue region of 380−500 nm with average 92.2%, and red region of 600−680 nm with about 88.6% in average. In the green/yellow region of 500−600 nm, there existed a small trough in the absorptance, and about 80.9% at 550 nm. After exceeding 680 nm, the absorptance decreased rapidly, down to 13.5% at 750 nm, and then slightly declined to 9.0% at 780 nm.

No pronounced differences in reflectance (P=0.099), transmittance (P=0.741), and absorptance (P=0.269) were found at different leaf ages within 380−780 nm. However, in the green/yellow region of 500−600 nm, the leaf ages significantly affected reflectivity (P=0.000 $<$0.01), transmittance (P=0.008$<$0.01), and absorptance (P=0.000$<$0.01) for saffron leaves. As the leaves changes from younger to older, the leaf color varies from green to dark green. The leaf age and color affect variations in leaf optical properties (Lee et al. 2017; Paradiso et al. 2020). In previous researches, red leaves have a higher absorptance than green leaves in lettuce, but their absorptances are very close and both exceed 90% in blue band of 400−500 nm and red band at 600−700 nm (Lee et al. 2017). The photosynthetic pigment chlorophyll has a high absorptance in both regions, which contributes to the high leaf absorptance for many species including saffron leaves. The great differences in terms of the absorptances in lettuces with two colors mainly exhibits in the green/yellow region of 500−600 nm. This is mainly affected by the contents of anthocyanins in the leaves whose absorptive peak was at 530 nm (Steele et al. 2009). For *Eucalyptus tereticornis*, the concentrations of anthocyanin in new leaves are relatively lower than that in older leaves. The carbon assimilation and photorespiration rates are enhanced, resulting in a descending requirement for photoprotection and lower absorptance in green region for new leaves (Wujeska-Klause et al. 2019). In the present study, the younger leaves in mid-February exhibited the green color, and their reflectance was higher in 500−600 nm. The contents of anthocyanin were lower, leading to a lower absorptance. On the contrary, the older leaves showed dark green and contained a higher content of anthocyanins, resulting in a higher absorptance in green/yellow region. In addition, the spongy tissue of the leaves in the older leaves is mature, and green light can vertically penetrate deeper spongy tissue and be absorbed by the chlorophyll in the lowest layer of chloroplasts (Terashima et al. 2009).

3.2 Leaf action spectrum at different leaf ages

The curve shapes of action spectrum were similar in saffron leaves at two leaf ages, as shown in Fig. 5(a). They both exhibited two obvious peaks, respectively, the main red peak at 640 nm and the second blue peak at 440 nm. The leaves in mid-February displayed an average 1.29 times higher action spectrum than those in mid-March. For younger leaves in mid-February, the action spectrum at red peak reached 0.048, 1.36 times of that at blue peak. The curve descended significantly in ultraviolet/blue region below 440 nm, only 0.008 at 380 nm. In blue/cyan region from 440 nm to 500 nm, the curve reached a lower value at 480 nm, and then slowly ascended from 500 to 600 nm. After exceeding 640 nm, the action spectrum first slowly decreased to 0.043 at 680 nm, and then drastically declined above 700 nm. By normalizing the action spectrum, the relative action spectrum curves at two leaf ages were

obtained in Fig. 5(b). The two curves showed basically coincident, and no significant difference in each wavelength region.

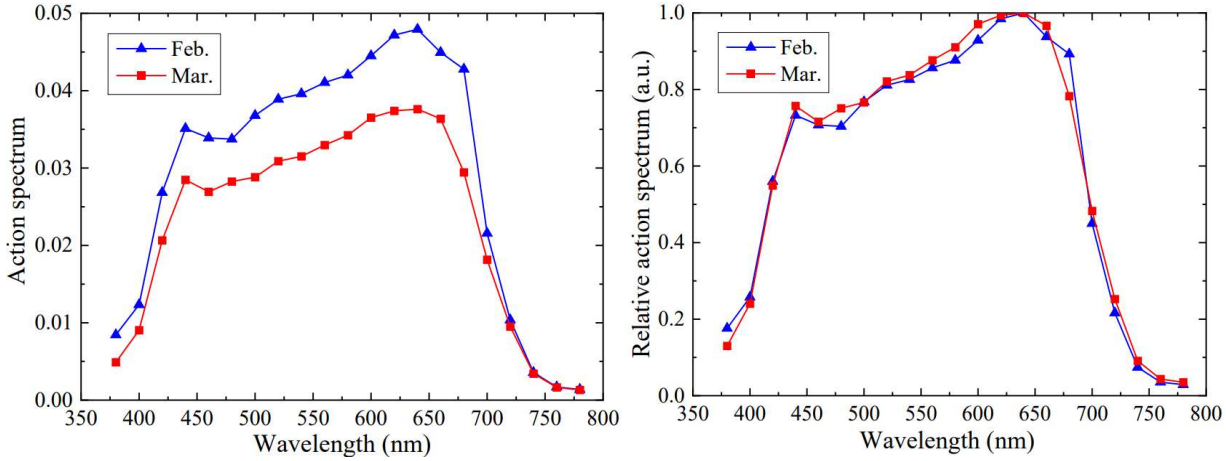


Fig. 5 Action spectrum based on PPFD of saffron leaves at different leaf ages. (a) Action spectrum. (b) Relative action spectrum. Each symbol represents the mean of 6 plants with three replicates

The saffron leaves in mid- February were green, and entered the most active stage of photosynthesis. After entering March, the leaf tips begun to turn yellow, but most of them presented still dark green. For this reason, the younger leaves appeared higher absolute action spectrum compared to older leaves. Nevertheless, the relative action spectra at different growth stage were coincidence in general, which agreed with McCree's analysis about the effect of leaf age on action spectrum (McCree 1971/1972).

Relative action spectrum data from different reports were compared and replotted in Fig. 6(a), respectively 8 field crops proposed by McCree (1971/1972), herb species and arbor species from Inada (1976), and peanut leaves (Inada 1976), as well as the saffron leaves in mid-February in this study. It was worth noting that the curve by McCree was recalculated with Eq. (4) and the data of the action spectrum based on irradiance. The peaks of all curves fell in blue and red regions, but their corresponding wavelengths and shapes of peaks were slightly different, especially the blue peaks. The blue peak in the McCree curve was 450 nm, and was 74.5% of the red peak 625 nm. The blue peak of both herbaceous and arborous plants was located at 437 nm, and their values were 100% and 68.3% of that at red peak 646 nm, respectively. For saffron leaves, the blue peak at 440 nm was observed not very distinct, but still 73.3% of the red peak at 640 nm. Its wavelength and height of blue peak were relatively close to those of the McCree curve, and fell between herbaceous and arborous plants.

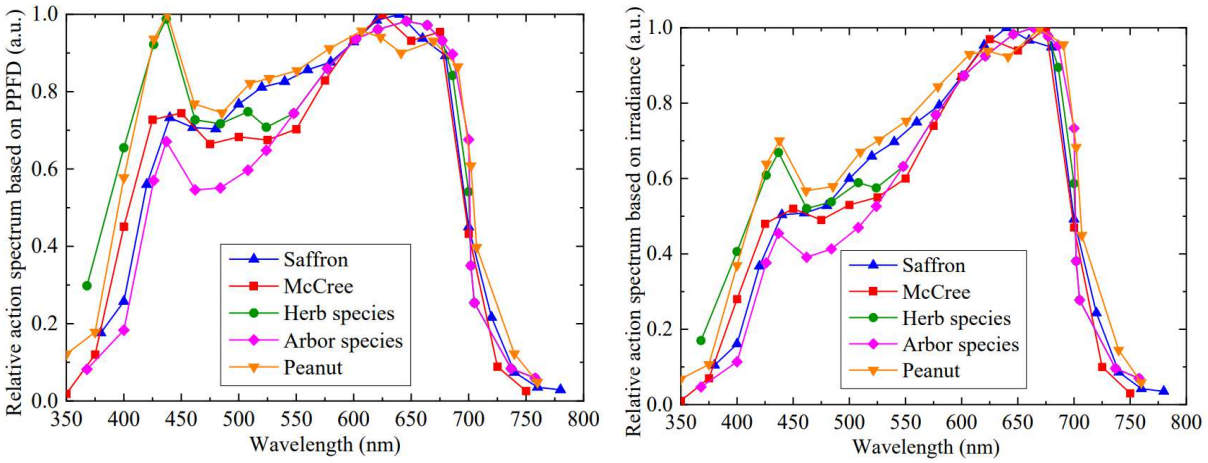


Fig. 6 Relative action spectrum of different plant leaves (McCree 1971/1972; Inada 1976). (a) Relative action spectrum based on PPFD. (b) Relative action spectrum based on irradiance. All curves are redrew using original data and Eq. (4)

Different blue peaks were influenced by the peak wavelength and band width of narrow-band light used in various spectral response studies, but basically located at the absorption peak region of chlorophyll a and chlorophyll b (Balegh & Biddulph 1970). The diversity in the proportion of blue peaks among different species may be affected by various factors, such as growth conditions and green degrees of leaves, an increase or decrease of inactive absorption from photo-screened compounds such as carotenoids or anthocyanins in leaves, an ascending leaf absorptance within leaf tissue, and an incremental blue light reflection caused by wax deposition on the leaf epidermis (Inada 1976). Additionally, a blue light irradiation with high PPFD may trigger an avoidance reaction of chloroplasts in leaves, resulting in a descending net photosynthetic rate by staying away from strong light (Zhou et al. 2022). If the PPFD from narrow-band blue light is higher than that of blue light in natural light, it may cause a reduction in the action spectrum of blue light (Wu et al. 2019). In the ultraviolet/purple region of 350−440 nm, the relative action spectrum curve of saffron leaves fell between the McCree curve and arborous plants, which may be due to these plants growing in the field or outdoors. As for the far-red region above 700 nm, the relative action spectrum curves among various species were observed basically consistent.

Furthermore, in contrast to the McCree curve, herbaceous and arborous plants, the relative action spectrum of saffron leaves was higher in green/yellow region from 500 to 600 nm, but slightly lower than that of peanut leaves (Fig. 6(a)). In fact, the McCree curve was mostly determined with light green leaves, while the saffron leaves exhibited a darker green state, leading to a higher action spectrum in green/yellow region (Inada 1976). Therefore, in addition to red and blue light irradiation for driving photosynthesis, adding green and yellow lights may be conducive to the photosynthetic accumulation for saffron leaves during the vegetative development in the field.

In order to intuitively illustrate the effect of different photometric units on action spectrum, the action spectra based on irradiance were obtained in Fig. 6(b) for different plants. The values in longer wavelength range were raised but that in shorter wavelength range was lowered comparing to Fig. 6(a), due to the weighted wavelengths in Eq. (4). A decrease in the

proportion of blue to red peaks was observed in relative action spectrum for irradiance, demonstrating that irradiance as the photometric unit would underestimate the action spectrum in blue region. Consequently, it is noteworthy to distinguishing between irradiance and PPFD when the determination of action spectrum.

3.3 Leaf quantum yield at different leaf ages

Similarity to action spectrum, the quantum yield curves at two leaf ages also demonstrated the same shapes, with two peaks respectively located at 440 nm and 640 nm, shown in Fig. 7(a). The quantum yield in mid-February was observed higher than that in mid-March. Nevertheless, different from action spectrum (Fig. 5(a)), the quantum yield in far-red region above 700 nm was significantly raised, and higher than that in ultraviolet region of 380−400 nm. This was mainly affected by the fact that the absorptance drastically decreased in far-red region (Fig. 4(c)). The quantum yield for younger leaves reached 0.028 at 700 nm, 1.3 times greater than action spectrum. Once over 700 nm, the quantum yield slowly decreased, down to 0.017 at 780 nm, 12.3 times more than action spectrum. Besides, the relative quantum yield curves for saffron leaves at two leaf ages basically coincide, demonstrating that the quantum yield was mainly influenced by the action spectrum, but little by the absorptance of the green/yellow region (Fig. 4(c)), which was consistent with the results by McCree (1971/1972). Plant photosynthesis are mainly adjusted by photosynthetic organs of leaves, not the absorption of pigments from leaves (Liu & Iersel 2021).

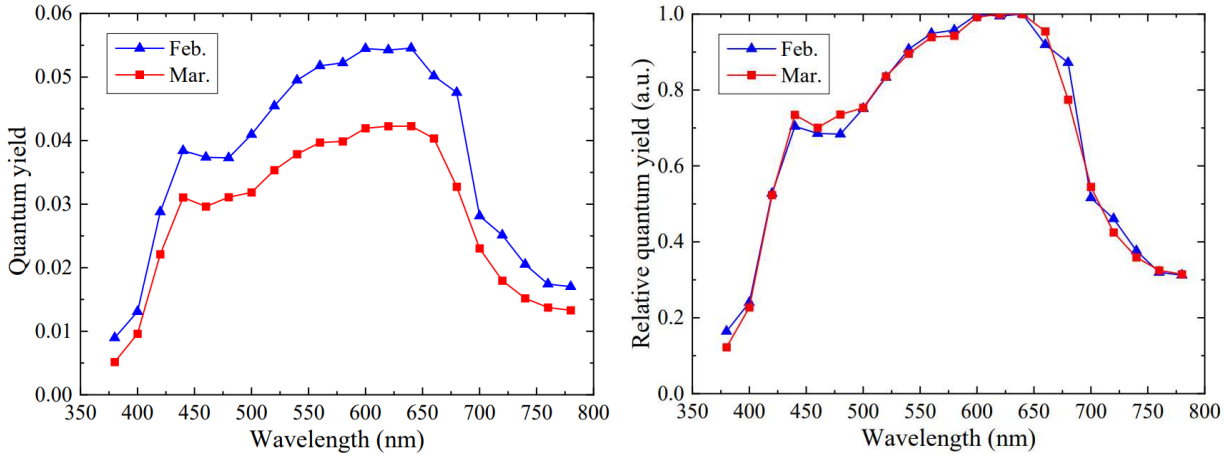


Fig. 7 Quantum yield of saffron leaves at different leaf ages. (a) Quantum yield. (b) Relative quantum yield. Each symbol represents the mean of 6 plants with three replicates

The differences observed in the relative action spectrum among diverse species were diminution in the relative quantum yield curve, displayed in Fig. 8(a). The reduced differences existed in the height and profiles of blue peak, owing to a higher absorptance in blue region than others. The red peak had shifted among various plants, 602 nm for herb species and 621 nm for arbor species, respectively, but the relative quantum yield was close to 1 from 600 to 640 nm. The shift in red peak is not only related to the absorptance, but also affected by the wavelength and band width of narrow-band light in various researches. When the band width exceeds 15 nm, narrow-band light at adjacent measurement wavelengths may cause

unpredictable wavelength interference to photosynthesis, further resulting in incorrect respond to the corresponding wavelength for plants (Wu et al. 2019).

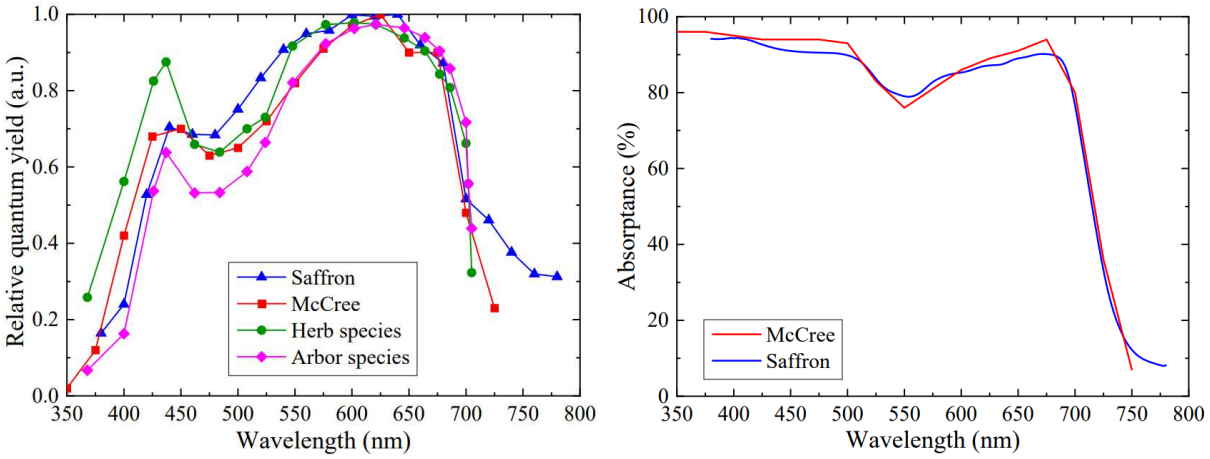


Fig. 8 Quantum yield and absorptance of different plant leaves (McCree 1971/1972; Inada 1976). (a) Relative quantum yield of different plant leaves. (b) Comparison of absorptance between saffron leaves and the leaves by McCree. All curves are redrew using original data

Moreover, the relative quantum yield of saffron leaves was higher than that of other plants above 700 nm, which was largely affected by the ratio of absorptance in far-red region to red or blue region. The leaf absorptances obtained by McCree are higher in blue region (400−500 nm) and red region (600−700 nm), but lower in far-red region (740−780 nm) compared to those of saffron leaves, as shown in Fig. 8(b). The average absorptance of leaves by McCree in far-red region accounts for 43.6% and 46.6% in blue and red region, respectively, while only 29.5% and 31.0% in saffron leaves. For this reason, when the relative action spectrum of McCree's curve and saffron leaves basically coincide in far-red region, the relative quantum yield from McCree is lower than that of saffron in this region. Some studies have not measured the absorptance and action spectrum above 750 nm, and cannot make comparison in further detail here.

3.4 Assessment of photosynthetic energy efficiency for narrow-band LED spotlights

The absolute spectra of narrow-band LED spotlights were shown in Fig. 9(a), with a peak wavelength from 380 to 780 nm at an interval of 20 nm, as well as band width of about 10 nm. The average PPFD received by leaves in the transparent leaf chamber was measured as 100.0 ± 1.3 μmol m^{-2} s^{-1}, demonstrating the spotlights had reliable light outputs during photosynthesis measurements. There were a few differences at the maximum absolute spectrum among diverse peaks, due to band width of interference filters within an error range of 10 ± 2 nm, but they all closed to 10 μmol m^{-2} s^{-1} nm^{-1}. Additionally, only a small overlapping area existed between adjacent wavelengths, which will not interfere with leaf photosynthesis at the corresponding wavelength. The PPFD received by leaves remained invariant before and after photosynthesis measurements at the same wavelength, which can further avoid inaccurate response from varying light output and attenuation by xenon lamps or tungsten halogen lamps in the previous studies (McCree 1971/1972; Paradiso et al. 2011; Inada 1976; Nilsen & Mortensen 1978; Evans 1987). The narrow-band LED spotlights in this study had an excellent

thermal management and can reduce the ambient temperature around the leaf chamber, making it more suitable for spectral response determination.

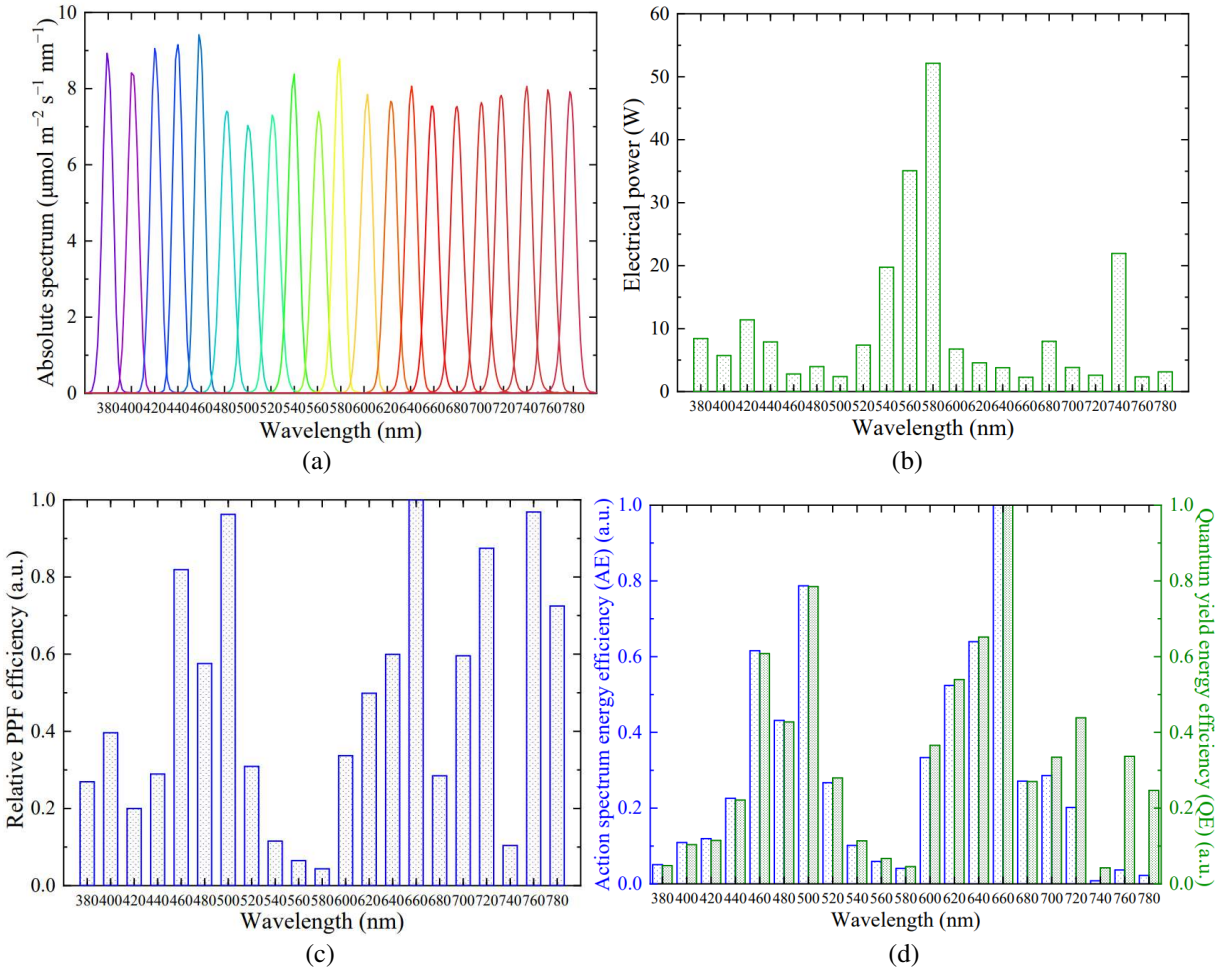


Fig. 9 Narrow-band LED spotlights. (a) Absolute spectrum with a peak wavelength from 380 to 780 nm with an interval of 20 nm and band width of 10 nm, PPFD =100 μmol m^{-2} s^{-1}. (b) Electrical power. (c) Relative PPF efficiency. (d) Relative action spectrum energy efficiency (AE) and relative quantum yield energy efficiency (QE)

The electrical power of narrow-band LED spotlights from 380 nm to 780 nm with an interval 20 nm was described in Fig. 9(b). To further evaluate the electrical efficiency of narrow-band LED spotlights, relative PPF efficiency of spotlights (Fig. 9(c)) were obtained through dividing the PPFD by the electrical power and then normalized. There were significant differences in the relative PPF efficiency at various peak wavelengths. The higher values located at partial wavelength from blue region of 460−500 nm and red/far-red region above 620 nm, while a lower value fell in green/yellow region of 540−580 nm, similarity to the results reported by Lee et al. (2017). This was affected by various factors, such as radiant efficiency of LED modules, overlapping degree of spectrum range between LED modules and target measurement narrow-band light, as well as band width and transmittance of interference filters.

Owing to the demands for red and blue light in LED controlled environment, the process technology for blue and red LED chips and modules has been developed mature nowadays (Stutte & Edney 2009). The higher external quantum efficiency in blue and red LEDs is the direct reason for affecting PPF efficiency at these wavelengths. Band width and transmittance of interference filters are not consistent at different wavelengths, further leading to differences in PPF efficiency. However, even if no differences mentioned above, a higher PPF efficiency will exist in longer wavelengths as a result of less photon energy, compared to shorter wavelengths (Yeh & Chung 2009). Besides, the central wavelength and band width of LED modules also have an impact on target measurement wavelength. If the central wavelength exactly falls in the target peak wavelength, as well as band width is very small to close to 10 nm, then the light output by LED modules will be utilized to the maximum extent. Conversely, if the central wavelength only slightly overlaps with the target, a portion of electrical power will be sacrificed in exchange for the target PPFD. As for the green/yellow LED modules at 540−580 nm, the production materials and process technology limited their external quantum efficiency, resulting in a lower radiant efficiency (Nelson & Bugbee 2014). The LED modules at 580 nm had a quiet low radiant efficiency, only 2.5%. The narrow-band LED spotlight at 580 nm was thus actually developed using broad-band white light with an interference filter at corresponding wavelength, but still very low about PPF efficiency.

In addition to PPF efficiency, relative action spectrum energy efficiency (AE) and relative quantum yield energy efficiency (QE) of narrow-band LED spotlights may more intuitively evaluate the energy efficiency of spotlights based on photosynthesis. The action spectrum and quantum yield of saffron leaves in mid-February were respectively divided by the electrical power of narrow-band LED spotlights and then normalized, as shown in Fig. 9(d), which can represent the photosynthetic rate of leaves under unit electrical power. The relative distribution of AE and QE at each wavelength was similar, only slightly differences in far-red region above 700 nm, which depended on discrepancies in action spectrum and quantum yield above 700 nm. The maximum values lied in blue region of 460−500 nm and red region of 640−660 nm for AE and QE, because of high photosynthetic rates, leaf absorptances and PPF efficiency in these regions. The low photosynthetic rate of saffron leaves in ultraviolet region of 380−400 nm and far-red region above 740 nm contributed to low AE values in these regions. However, the limited technology of green/yellow LED chips and modules resulted in a lower AE and QE in the region of 540−580 nm, although the action spectrum and quantum yield of saffron leaves were not low in this band.

4 Conclusion

A narrow-band LED lighting system with stable 100 μmol m^{-2} s^{-1} light outputs and 20 nm wavelength interval was developed for photosynthetic measurements of saffron leaves at different leaf ages. The action spectrum and quantum yield of younger leaves in February were higher than those of older leaves in March, but their relative curves at two leaf ages were basically the same. In addition to blue and red regions, saffron leaves displayed not low action spectrum and quantum yield in green/yellow regions. February is critical for the development of daughter corms and leaf photosynthesis, so it is recommended to provide additional LED lighting containing blue, yellow, green and red LEDs. After entering March, saffron leaves begun to turn dark green and elder, leading to a decrease in leaf photosynthetic performance.

Perhaps it is possible to appropriately reduce the radiant time by luminaires to save energy consumption.

Furthermore, the action spectrum energy efficiency (AE) and quantum yield energy efficiency (QE) were evaluated to analyze the photosynthetic energy efficiency of LED spotlights. More attentions should be paid to the effects of green and yellow bands on the photosynthetic performance of saffron leaves, and efforts should be made to improve the manufacturing technology of green and yellow LED chips and modules. Last but not least, the conversion of action spectrum based on irradiance and PPFD in this study have established a clear theoretical basis for subsequent researches on spectral response for other species. The narrow-band LED spotlights with equal wavelength interval has solved the problems of partial wavelength loss and interference, which can be considered for more photosynthetic spectral dependence studies in future.

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Data availability: The data presented in this study are available on request from the corresponding authors.

Declarations

Conflicts of Interest: The authors declare no conflicts of interest.

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