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ORIGINAL ARTICLE

Light-mediated biosynthesis of phenylpropanoid metabolites and antioxidant potential in callus cultures of purple basil (*Ocimum basilicum* L. var *purpurascens*)

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Abstract

Ocimum basilicum L. var *purpurascens* (purple basil) contains medicinally valuable metabolites. Light greatly influences the physiological processes, including biomass accumulation and secondary metabolites production in medicinal plants. Herein, we investigated the influence of different spectral lights on the biosynthesis of phenylpropanoid metabolites in purple basil callus cultures. Growth kinetics was østudied for a total of 49 days, with 7 days of sampling time. Among the various treatments, blue light resulted in maximum biomass accumulation, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant DPPH, FRAP and ABTS activities, as compared to controls. Moreover, blue light also encouraged higher superoxide dismutase activity while the red light was found effective for enhanced peroxidase activity. HPLC analysis revealed enhanced rosmarinic acid (87.62 mg/g DW) and anthocyanins (cyanidin: 0.15 mg/g DW and peonidin: 0.13 mg/g DW) contents under dark grown callus cultures which were almost 1.55, 1.25 and 1.18–fold greater than controls, respectively. Conversely, red light caused maximum production of cichoric acid (14.65 mg/g DW). Moreover, a positive correlation occurred among the accumulation of phenolic and flavonoids and antioxidant activities. These results suggest that light quality strongly influences medicinally valuable phenylpropanoid metabolites biosynthesis along with antioxidant potential in in vitro cultures of purple basil.

Key message

Light-enhanced precious metabolites in callus of Basil.

Keywords *Ocimum basilicum* · Light quality · Rosmarinic acid · Anthocyanins · Peroxidase · Superoxide dismutase · Flavonoids · Phenolics · Antioxidant activities

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Abbreviations

ABTS	2,2-Azinobis 3-ethylbenzthiazoline-6-sulphonic acid
FRAP	Ferric reducing antioxidant power
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
LEDs	Light-emitting diodes
MS	Murashige and Skoog
DMSO	Dimethyl sulfoxide
NAA	α-Naphthalene acetic acid
TFC	Total flavonoid content
TPC	Total phenolic content
TFP	Total flavonoid production
TPP	Total phenolic production
FW	Fresh weight
DW	Dry weight
FRSA	Free radical scavenging activity

POD	Peroxidase
SOD	Superoxide dismutase
TEAC	Trolox C equivalent antioxidant capacity
RA	Rosmarinic acid

Introduction

Ocimum basilicum L., commonly called "Basil", is a widely recognized plant species of *Lamiaceae* family with diverse medicinal properties. *O. basilicum* is usually found in Africa, India and South Asia. As a herbal medicines, *Ocimum* genus have been cultivated around the globe since ancient times (Hakkim et al. 2013). Food and cosmetic industries provide huge market space for use of essential oils, obtained from basil (Vani et al. 2009).

Most commercial basil cultivars available in the market belong to the species *O. basilicum* (Wang et al. 2004; Iwai et al. 2010). Purple basil (*Ocimum basilicum* L. var *purpurascens*) is one of the cultivars of *Ocimum* with a traditional sweet basil flavour (Lee et al. 2014). Purple basil is known for its ornamental value and is also a good source of secondary metabolites (Phippen and Simon 1998), mainly phenylpropanoid metabolites which include anthocyanins, caffeic acid, chicoric acid and rosmarinic acid (Gang et al. 2001; Kiferle et al. 2011; Allan et al. 2008; Flanigan and Niemeyer 2014; Bertoli et al. 2013). These secondary metabolites play a key role against oxidative damage and variety of environmental stresses (La Camera et al. 2004). Rosmarinic acid is considered to be a one of the most significant caffeic acid ester present in *Ocimum spp*., having various pharmacological and therapeutics properties (Lee and Scagel 2009; Zheng and Wang 2001; Javanmardi et al. 2002; Shan et al. 2005) such as antioxidant, astringent, antibacterial, antiviral and anti-inflammatory (Pereira et al. 2009; Mastaneh et al. 2014), which make it a chief mediator for health endorsing assets, as well as, useful for cosmetic and food industries (Le Claire et al. 2005; Park et al. 2008; Petersen et al. 2009). Plant phytochemicals tend to act as strong antioxidant agents by cession of electron(s) (Aruoma 1998). Hydrogen atom transfer (HAT)-based mechanism and electron transfer (ET)-based mechanism are two main mechanisms through which antioxidants usually neutralize free radicals (Prior et al. 2005). Distinct in vitro-based protocols have been designed to study the mode of action and antioxidant activity of plant phytochemicals. Among these assays, ABTS (2,2-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) assay is considered as a HAT-based reaction, whereas, FRAP (ferric reducing antioxidant power) assay operates via ET mechanism. On the other hand, the most commonly used is DPPH (2,2-diphenyl-1-picrylhydrazyl) assay which is mixed type that operates through both HAT and ET mechanisms (Prior et al. 2005). These assays provide not only antioxidant activity but also information regarding the complex molecular mechanisms involved in free radical scavenging activity. Plants are exposed to harsh environmental conditions on daily basis such as drought, temperature fluctuations, oxidative damage and light intensity. These environmental factors trigger molecular, physiological and morphological changes in plants via activation of cascade of reactions (Ahmad et al. 2012). Influence of stress on plant metabolites production has previously been studied (Trejo-Espino et al. 2011; Nagella and Murthy 2011; Lee and Shuler 2000). Physical factors and availability of suitable nutrients play a key role in optimum productivity of these metabolites (Matsubara et al. 1989; Karwasara and Dixit 2013). Light is an important physical factor, playing fundamental role in plant development, morphogenesis and regulation of phytochemicals biosynthesis (Kim et al. 2004; Shohael et al. 2006; Khan et al. 2013; Abbasi et al. 2007). Various studies have proposed the regulatory effect of light intensity and quality on enhanced metabolites biosynthesis in various plant species cultures (Ellis and Roberts 1978; Senger 1987; Exonomou 1987). High pressure sodium lamps (HPS-light), incandescent light lamps, metal halide lamps and tubular fluorescent lamps are commonly employed as a source of light for in vitro culturing of plants. However, these light sources contain low quality wavelengths that are ineffective for growth stimulation (Kim et al. 2004). Alternatively, LEDs have low thermal emissions, narrow bandwidth, long life, wavelength specificity and low degradation, and are thus considered suitable for in vitro culturing of plant species (Gupta and Jatothu 2013; Yeh and Chung 2009; Yam and Hassan 2005). LEDs have been tested in many plants such as wheat, pepper, lettuce and spinach etc. for improved morphogenesis (Okamoto et al. 1996; Jao and Fang 2004; Jao et al. 2005; Nhut et al. 2000; Yanagi and Okamoto 1994). Studies indicated blue and red lights to be more effective, nevertheless, mode of action and response to light is variable among different plant species. Herein, for the first time, we report light quality effect on antioxidant potential as well as production of valuable metabolites in *O. basilicum* L. var *purpurascens* callus cultures.

Materials and methods

Seeds germination and callus induction

Seeds of purple basil were taken from National Agriculture Research Center, Pakistan. Superficial sterilization of seeds was done by adopting modified protocol of Abbasi et al. (2010). In brief, seeds were treated with mercuric chloride (0.1%) and ethanol (70%) for 30 s and 1 min, respectively. After that, seeds were washed three times with dH_2O (autoclaved) and placed on MS media (Murashige and Skoog

1962), supplemented with carbon source (sucrose: 3%) and gelling agent (agar: 0.8%) at a pH of 5.6–5.7 prior to autoclave. Seeds were allowed to grow under controlled environment i.e. light/dark cycle (16/8 h) and temperature (25 ± 2 °C). Callus culture was established using leaf explants (0.5 cm^2) from 28 days old plantlets (in vitro derived) cultured on MS media additionally supplied with 2.5 mg/L NAA, as previously optimized by Nazir et al. (2019). Experiment was carried out under controlled conditions i.e. light/dark cycle (16/8 h) and temperature (25 ± 2 °C) in growth room and callus was further sub-cultured after 28 days on same optimized hormonal media.

Spectral lights treatment

On explant-derived callus, different monochromatic lights were used as elicitors which included photoperiod cycle (light/dark i.e. 16/8 h), White light (24 h, wavelength 400–700 nm), Darkness (24 h), Red light (24 h, wavelength 660 nm), Green light (24 h, wavelength 510 nm), Blue light (24 h, wavelength 460 nm) and Yellow light (24 h, wavelength 570 nm). Culture grown under photoperiod condition was considered as control. Optimized hormonal concentration (2.5 mg/L NAA) was used for callus growth under different sources of monochromatic lights (40–50 μMol m^{-2} s^{-1} intensity). Experimentation was done thrice. For estimation of biomass (FW, DW) and analysis of phytochemicals, harvesting was carried out at 7 days interval for each light treatment.

Phytochemical analysis

Total phenolic and flavonoid contents

For the estimation of total phenolic and flavonoid contents (TPC & TFC), the previous procedure was employed with slight modifications (Zahir et al. 2014). Briefly, dried powder (100 mg) of each tested sample was thoroughly mixed with 99.9% methanol (500 μL) by vortexing (5 min) and sonication (half an hour) at room temperature, followed by 10 min centrifugation at 15000 rpm. Supernatant was finally assembled and saved for future use at 4 °C.

TPC was evaluated following a previously modified protocol (Velioglu et al. 1998). In brief, the extracted sample (20 μL), Na_2CO_3 (90 μL) and FC-reagent (90 μL) were mixed. Microplate reader was used to measure absorbance at 630 nm. Phenolic content was expressed as gallic acid equivalents (GAE)/g of DW and gallic acid was also used as a control standard. Total Phenolic Production (TPP) was determined by multiplying the TPC value with a dry weight of the respective sample. For the evaluation of TFC, aluminum chloride assay, as previously described by Ahmad et al. (2010), was used. Potassium acetate (10 μL), distilled water (160 μL) and aluminum chloride (10 μL) were mixed with test sample (20 μL). Absorbance was then taken with microplate reader at 630 nm, after half an hour of incubation. Quercetin was employed as positive control and flavonoid content was expressed as quercetin equivalents per gram (QE)/g DW of sample. Total flavonoid (TFP) was then determined via multiplying TFC value with respective dry weight of the tested sample.

Free radical scavenging activity (FRSA)

FRSA of tested samples was examined via DPPH reagent, as designated earlier (Abbasi et al. 2010). In short, the solution DPPH (180 μL) was added in the test sample (20 μL). Prior to taking absorbance at 517 nm with microplate reader, solution was placed at 37 °C in dark for one hour. Final concentrations of ascorbic acid (40, 20, 10 and 05 μg/mL) and DMSO was used as positive and negative control, respectively. FRSA was then calculated with formula:

$$\% \text{ scavenging DPPH free radical } = 100 \times (1 - AE/AD).$$

where AE is the mixture absorbance at 517 nm with sample addition, while AD is only DPPH solution absorbance.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was also done according to the previously reported procedure (Benzie and Strain 1996). In brief, 190 μL FRAP solution and 10 μL of calli extract were mixed and then placed for 15 min at 25 ± 1 °C. Afterwards, OD was measured with microplate reader at 630 nm. FRAP solution constitute acetate buffer (pH 3.6, 300 mM), $FeCl_3{\cdot}6H_2O$ (20 mM) and 10 mM TPTZ in a ratio of 10:1:1 (v/v/v), respectively. Experiment was performed three times. Antioxidant activity was expressed as TEAC (Trolox C equivalent antioxidant capacity).

Antioxidant ABTS assay

ABTS antioxidant assay (Velioglu et al. 1998) was also done for further screening of antioxidant potential of extracts. Briefly, to make ABTS solution, equal quantity of 7 mM ABTS salt was added to 2.45 mM potassium per sulphate and kept in darkness for 16 h. After that, absorbance was taken, and mixture was then placed again for 15 min in the dark (25 ± 1 °C) and absorbance was again measured at 734

nm with microplate reader. Experiment was repeated three times and the values were expressed as TEAC equivalent.

Enzymatic antioxidant activities

Enzymatic antioxidant activities (peroxidase (POD) and superoxide dismutase (SOD)) were estimated using fresh samples of callus cultures. Procedure designed by Nayyar and Gupta (2006) was employed with minor adjustments for extraction of fresh samples. Buffer (1 mL) of potassium phosphate (50 mM, pH 7.0) having 1% PVP was added to 100 mg of fresh sample. Mixing and grinding of samples was done using Mortar and pestle, followed by half an hour centrifugation at 15000 rpm. Supernatant collected was kept at 4 °C for enzymatic activities. POD activity was performed using previous protocol (Lagrimini 1980). Firstly, fresh sample extract (20 µL), 0.1 mL distilled H_2O, 20 µL hydrogen peroxide (27.5 mM; 10 ×), 20 µL guaiacol (100 mM, 10 ×) and potassium phosphate buffer (40 µL; 50 mM; pH 7) were mixed and then, with the aid of a microplate reader, solution absorbance was measured at 470 nm. To determine SOD activity, Giannopolitis and Ries (1977) method was employed. Briefly, 20 µL EDTA (1 mM), 20 µL methionine (130 mM), 60 µL calli extract (fresh), 2 µL riboflavin (0.02 mM), 78 µL phosphate buffer (50 mM, pH 7) and 20 µL NBT (0.75 mM) were mixed to prepare the reaction mixture. Further, prior to taking absorbance, the fluorescent white light was used for 7 min incubation of reaction mixture. Absorbance was taken by microplate reader at 660 nm. The following formula was then used to calculate POD and SOD values:

$$A = ECL$$

where A = Absorbance; E = Extinction coefficient (6.39 mM^{-1} cm^{-1}; C = enzyme concentration (value calculated in nM/min/mg FW) & L = Length of wall (0.25 cm).

HPLC analysis

Extraction and HPLC separation were performed as described by Nazir et al. (2019). To quantify phenylpropanoid metabolites in callus cultures of purple basil, HPLC with standard grade chemicals (Sigma Aldrich) were used (Takemiya et al. 2005). Hypersil PEP 300 C18 column (250 × 4.6 mm, 5 µm), equipped with a guard column Alltech (10 × 4.1 mm) was utilized to perform separation at 35 °C and compounds separation was observed at 210 and 250 nm wavelength via a Varian HPLC system (equipped with a degasser (Metachem Degassit), Prostar 335 Photodiode Array Detector and autosampler (Varian Prostar 410)). Quantification of the compounds was accomplished on the basis of reliable reference standards and retention times. Two HPLC-grade solvents were used in mobile phase: Solvent A = $HCOOH/H_2O$, pH 2.1 and solvent B = CH_3OH. Throughout 1-hour run, composition of mobile phase varied with a nonlinear gradient 8% B (0 min), 12% B (11 min), 30% B (17 min), 33% B (28 min), 100% B (30–35 min), 8% B (36 min) at a flow rate of 1 mL/min. A 10 min re-equilibration time was used among individual runs. All the tested samples were examined thrice, and the values were expressed as µg/mg DW of the sample.

Statistical analysis

Whole experiments were carried out thrice. Microsoft Excel was used to calculate means ± standard deviation of replicates and Origin software (Origin Lab Corporation, Wellesley Hills, MA, USA) was used to draw all the graphs. Tukey's multiple comparison test was employed for calculating significant differences. One-way analysis of variance (ANOVA) with significant difference $p < 0.05$ was used to compare the means of different treatments.

Results and discussion

Effect of light quality on biomass accumulation & growth kinetics

Light quality as well as light quantity are necessary factors which affect the development and composition of plant (Bian et al. 2015). Red light is usually considered to be most efficient in the process of photosynthesis while other spectra are essential for plant growth and physiology. Blue light, for instance, controls the opening of stomata, thus improve access to transpiration and carbon dioxide (CO_2) and is also necessary to inhibit "red light syndrome" (Davis and Burns 2016). Light acts as an abiotic elicitor to increase the accumulation of valuable metabolites (Fazal et al. 2016b). Various plant species callus cultures have been previously exposed to light for enhanced metabolites biosynthesis (Fazal et al. 2016a; Nadeem et al. 2018; Shohael et al. 2006). Herein, maximum callus biomass accumulation in purple basil was observed for 35 days period with 7 days sampling time under different LEDs treatments. Morphological variations were observed with visual eye and are presented in Fig. 1. All the spectral lights resulted in shorter lag phases. However, elongated log phases were observed for most of the LEDs treatments as well as control, starting from day 7 up to day 35. Biomass accumulation suddenly increased from day 28 to day 35 during the log phases. Moreover, from day 35 to 42, a shorter stationary phase occurred. From day 42 to 49, a decline phase was observed for all the LEDs treatments and control. Among the various LEDs

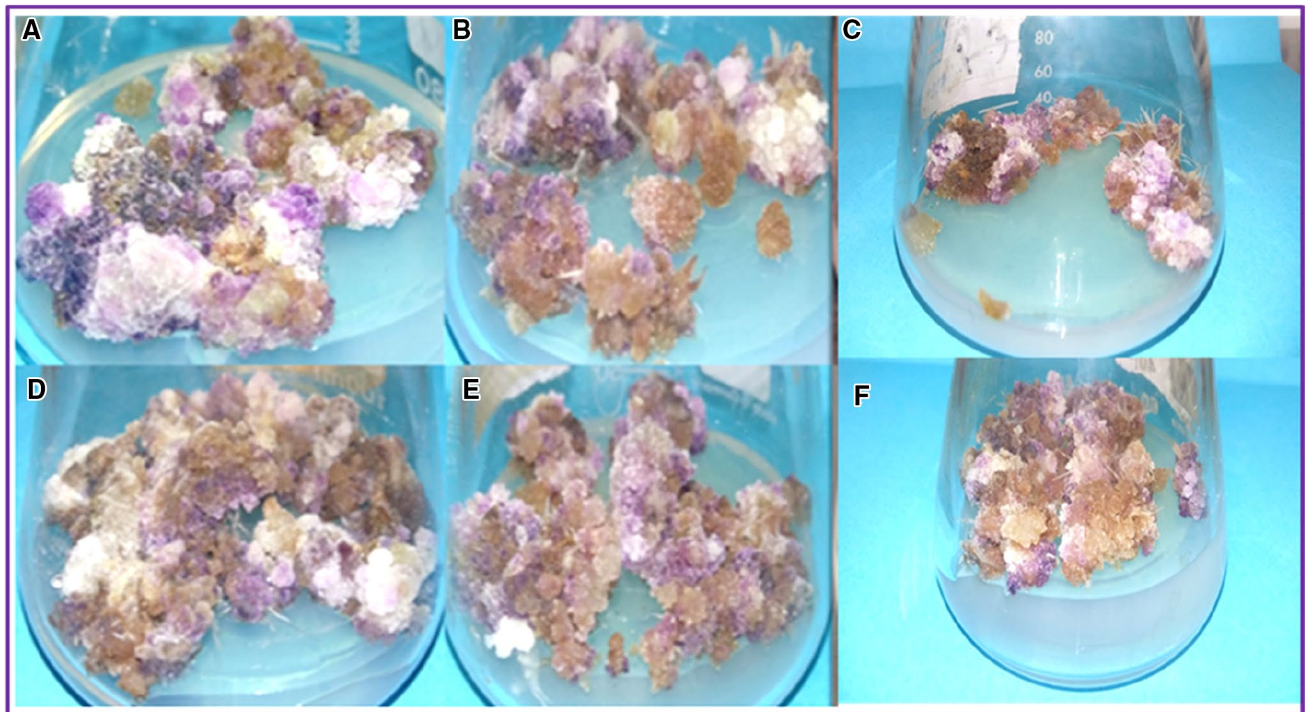

Fig. 1 Morphological variations in purple basil calli at day 35, treated with different spectral lights (A = Blue; B = Control; C = Dark; D = Yellow; E = Green; F = White)

treatments, maximum biomass (18.83 g/L DW) accumulation was shown by blue light, followed by white (18.34 g/L DW) and yellow (18.2 g/L DW) lights at day 35 of growth kinetics (Fig. 2a, b, e). However, biomass accumulation for green light (15.8 g/L DW), darkness (15.5 g/L DW) and red light (14.8 g/L DW) were lower than control (17.7 g/L DW) (Fig. 2g). Overall, blue spectrum was found efficient for enhanced biomass accumulation and callus induction. Blue light has the ability to increase the chlorophyll content which results in increased photosynthesis (Lobiuc et al. 2017; Nadeem et al. 2018) and subsequent increase in carbon and carbohydrate accumulation. Effect of light wavelength is highly dependent on plant species, type of explant and in vitro cell culture (Andi et al. 2018; Liu et al. 2006).

Effect of light quality on phenolic and flavonoids accumulation

Plant phenolic and flavonoids have a powerful biological activity, which makes it necessary to assess them (Sulaiman and Balachandran 2012). Here in, we explored the influence of spectral lights to enhance phenolic and flavonoids in purple basil calli grown under different spectral lights for a duration of 7 weeks with 7 days of sampling time (Fig. 3). Calli grown under blue spectrum increased the TPC (20.1 mg/g DW), followed by red (19.7 mg/g DW), green (18.7 mg/g DW) and dark (18.2 mg/g DW) spectra, respectively (Fig. 3a). Similarly, maximum TPP was also observed for blue light (378.3 mg/L), followed by green (295.63 mg/L) and red (283.9 mg/l) lights, respectively (Fig. 3c). There exists a considerable evidence for light induction and synthesis of phenolic by blue light (Engelsma 1969; Kim et al. 2014; Fazal et al. 2016b). Studies indicate that *PAL* expression is stimulated by blue light, which is a key enzyme involved in phenylpropanoid biosynthetic pathway (Heo et al. 2012; Son et al. 2012).

Moreover, blue light is also involved in *cis* form hydroxycinnamic acids biosynthesis which has minimal inhibitory potential as compared to *trans*-form (Billett et al. 1981). Thus, blue light has a role in stimulating the biosynthetic pathways of metabolites, resulting in high amount of phenolic content and their production (Nam et al. 2018; Nadeem et al. 2018). Similar observations were previously reported for the accumulation of phenolic compounds under the influence of blue and red lights in buck wheat (Lee and Chen 2014). Maximum total flavonoid content (TFC) was also detected in log phase (day 35) for blue light (13.9 mg/g DW), and subsequently by red (13.4 mg/g DW), dark (12.9 mg/g DW) and green (11.8 mg/g DW) spectra, respectively (Fig. 3b). Similarly, higher total phenolic production (TFP) was observed for blue spectrum (263.3 mg/L), followed by white (207.2 mg/L) and red (199.9 mg/L) spectra, respectively (Fig. 3d). Likewise, it was stated that blue spectrum

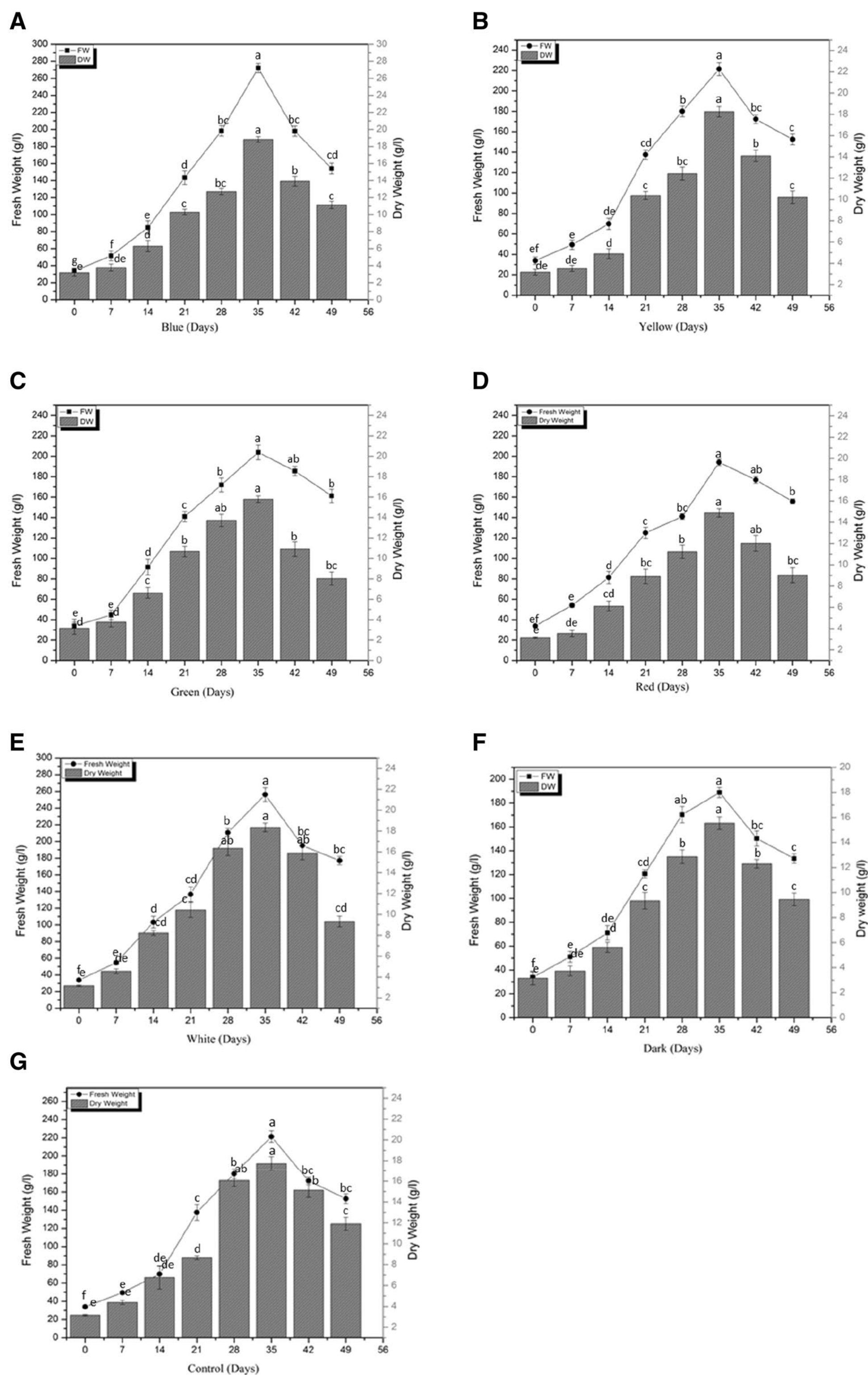

Fig. 2 Growth kinetics and biomass (Fresh Weight and Dry Weight) accumulation at optimized hormonal conditions under different spectral lights. **a** Blue light treatment, **b** Yellow light treatment, **c** Green light treatment, **d** Red light treatment, **e** White light treatment, **f** Dark treatment, **g** Control. Values are means ± SE from triplicates. Columns with similar alphabets are not significantly different ($p < 0.05$) according to Tukey's test

encourages maximum flavonoid accumulation in *O. basilicum* callus cultures (Nadeem et al. 2018).

The results of our study indicated that blue spectrum is more efficient in phenolic and flavonoids accumulation, as compared to other spectra. Possible link exists between blue light and phenylpropanoids production by the process of photosynthesis. Phototrophins and cryptochromes are specific photoreceptors of blue light. These receptors perceive specific wavelengths of light and thus regulate functional adaptations and photo-morphogenic responses (Takemiya

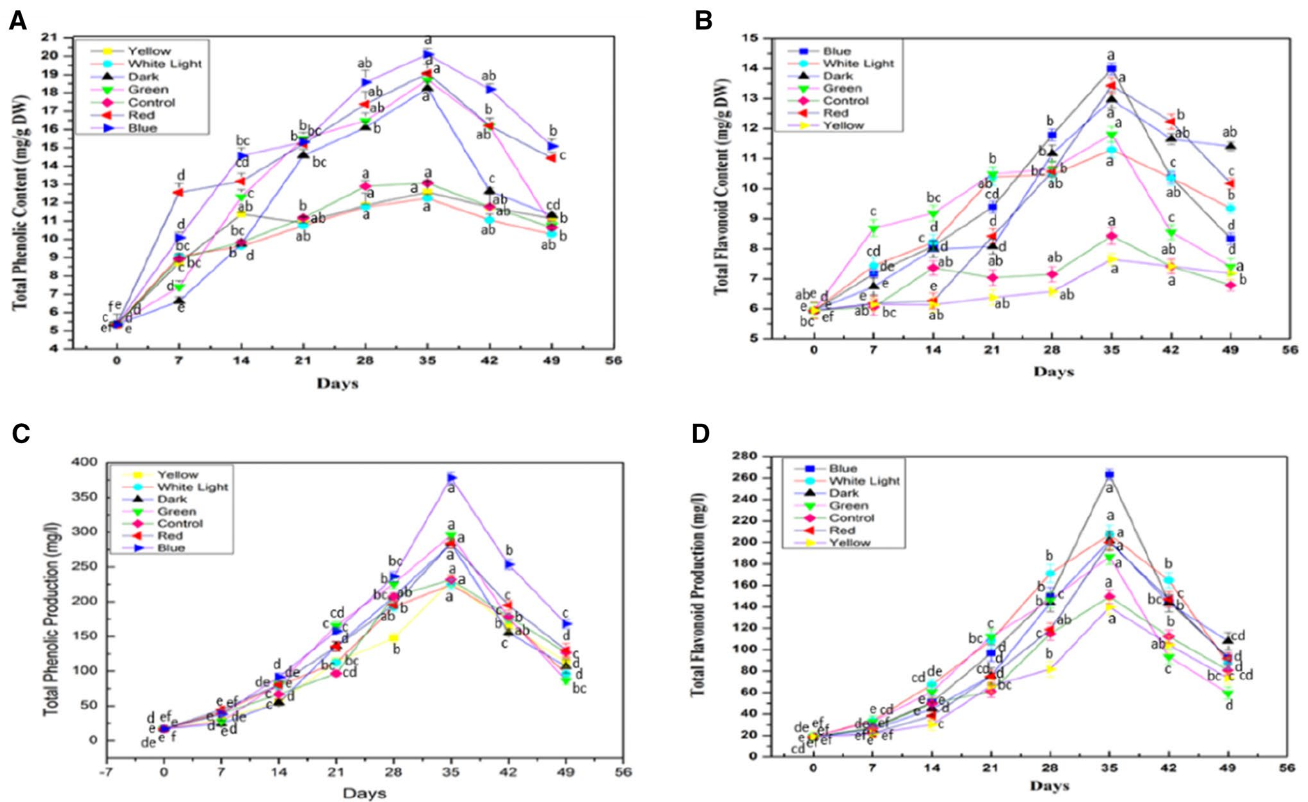

Fig. 3 Growth kinetics and total phenolic content (TPC), total flavonoid content (TFC), along with their productions (TPP & TFP) at optimized hormonal conditions under different spectral lights. **a** TPC (mg/g DW), **b** TFC (mg/g DW), **c** TPP (mg/L), **d** TFP (mg/L). Values are means ± SE from triplicates. Columns with similar alphabets are not significantly different ($p < 0.05$) according to Tukey's test

et al. 2005). Cryptochromes have been shown to induce the expression of genes associated with flavonoids biosynthesis (Wade et al. 2001; Liu et al. 2018). Lights of shorter wavelength, including the blue light, have a vital role in flavonoids biosynthesis in buck wheat seedling (Nam et al. 2018). Likewise, blue light has been shown to enhance the contents of flavonoids and phenolic acids in numerous other plant species (Wang et al. 2010; Liu et al. 2016).

Effect of light quality on antioxidant capacity

The antioxidant capacity of callus culture was estimated using DPPH, ABTS and FRAP assays, due to their ease of use, sensitivity, inexpensiveness and simplicity. The results of these antioxidant activities are reported in Fig. 4. As the accumulation of biomass was found optimal at log phase, therefore, harvesting was done for all the treated cultures at the end of log phase (day 35) to assess the antioxidant potential. DPPH antioxidant activity provided the overall antioxidant ability of purple basil callus. Here, higher free radical activity (DPPH) was demonstrated in culture treated with blue light (94%), followed by white (90%) and dark (87%) treatments, respectively (Fig. 4a). The antioxidant power of callus cultured under different spectral lights was also measured using FRAP assay. Extracts total antioxidant and single antioxidant power was analyzed by using this assay (Siddhuraju et al. 2002). The FRAP assay showed a large difference in antioxidant reduction profile of purple basil in different LEDs treatments. As shown in Fig. 4b, the reducing ability of blue light has highest activity than other lights. The FRAP values (TEAC μM) were in the order: blue (630.6) > white (592.0) > dark (504.9) > green (507.9) > red (501.5). The FRAP assay for blue spectrum was 1.4-folds higher than control, while, yellow light displayed the lowest activity. Wu et al. (2007) noticed that blue light has positive significant effect to improve the antioxidant capacity of the sprouted pea seed. Similarly, higher DPPH and FRAP activities in soybean, treated with blue light, was also reported (Azad et al. 2018). Likewise, blue light also resulted in highest antioxidant ABTS assay (Fig. 4b). Callus cultures of purple basil treated with different LEDs displayed the ABTS activities in the following order: blue (937.3 TEAC μM) > white (879.0 TEAC μM) > dark (807.1 TEAC μM) > green (800.1 TEAC μM) > red (790.7 TEAC

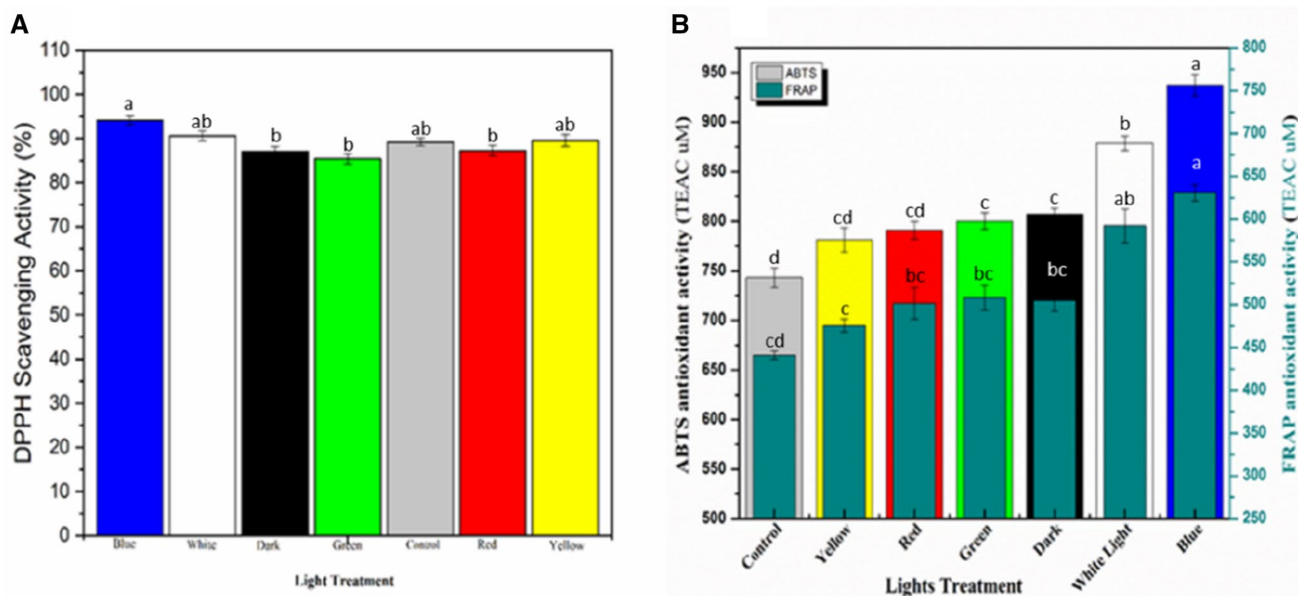

Fig. 4 **a** % Free radical scavenging, and **b** antioxidant activities (ABTS and FRAP (TEAC µM)) in purple basil calli in response to different spectral lights at day 35. Values are means ± SE from triplicates. Columns with similar alphabets are not significantly different ($p < 0.05$) according to Tukey's test

µM) > yellow (781.1 TEAC µM). To assess the antioxidant potential of plant extracts, ABTS and DPPH assays have previously been employed, which depends on the capability of electron transfer antioxidant to decrease oxidants (Li et al. 2008). It has been shown that blue light results in highest ABTS assay in buckwheat sprouts, which might be linked to maximum TPC and TFC (Nam et al. 2018), which is in harmony with the results of our study. Furthermore, a correlation occurred between the accumulation of phenolic and flavonoids and antioxidant assays in our study. Therefore, the enhanced antioxidant activities (DPPH, FRAP, ABTS) in cultures treated with blue light might be due to increased contents of phenolic and flavonoid (Table 1).

Effect of light quality on enzymatic antioxidant activities

Herein, we also determined the influence of spectral lights on POD (peroxidase) and SOD (superoxide dismutase) activities in purple basil callus cultures to measure their role in stress situations. Highest POD activity was shown by red light which was 1.5-fold higher than control, while dark grown cultures displayed minimum POD activity (Fig. 5b). The highest POD activity for cultures grown under red spectrum might be because of harmful effects of increased production of H_2O_2 or over production of its poisonous active oxygen derivatives, as due to oxidative stress (Younas et al. 2018). It was also concluded that red light enhanced POD besides other antioxidant enzymes in strawberry plantlets (Yonghua et al. 2005). Similarly, red light resulted in increased protease and peroxidase activities in *Artemisia* (Tariq et al. 2014). On the other hand, blue light resulted in highest SOD activity (0.246 nM/min/mg FW), followed by red spectrum (0.194 nM/min/mg FW), while minimal SOD activity was found for yellow light (Fig. 5a). Previously, Khan et al. (2018) also concluded that blue LED causes optimum POD activity in *Fagonia indica* callus cultures, whereas, Younas et al. (2018) reported that yellow light results in lowest SOD in *S. marianum* L. cultures. Lee et al. (2009) concluded that peroxidase is a reference enzyme which is mainly used for the blanching treatment due to its high thermal resistance and is mainly involved in the decomposition of polyphenols (Bhat and Sharma 2016). During this oxidation, highly reactive oxidant species such as H_2O_2 are produced. It is considered that blue light treatment leads to less degradation of polyphenols that might be the reason that blue light showed a high phenolic content but less POD activity.

Effect of light quality on accumulation of phenylpropanoid metabolites

As an abiotic elicitor, light directly or indirectly influences the growth as well as development of plant species (OuYang et al. 2015; Adams et al. 1996; Stefano and Rosario 2003). Not only the presence or absence of light but also the

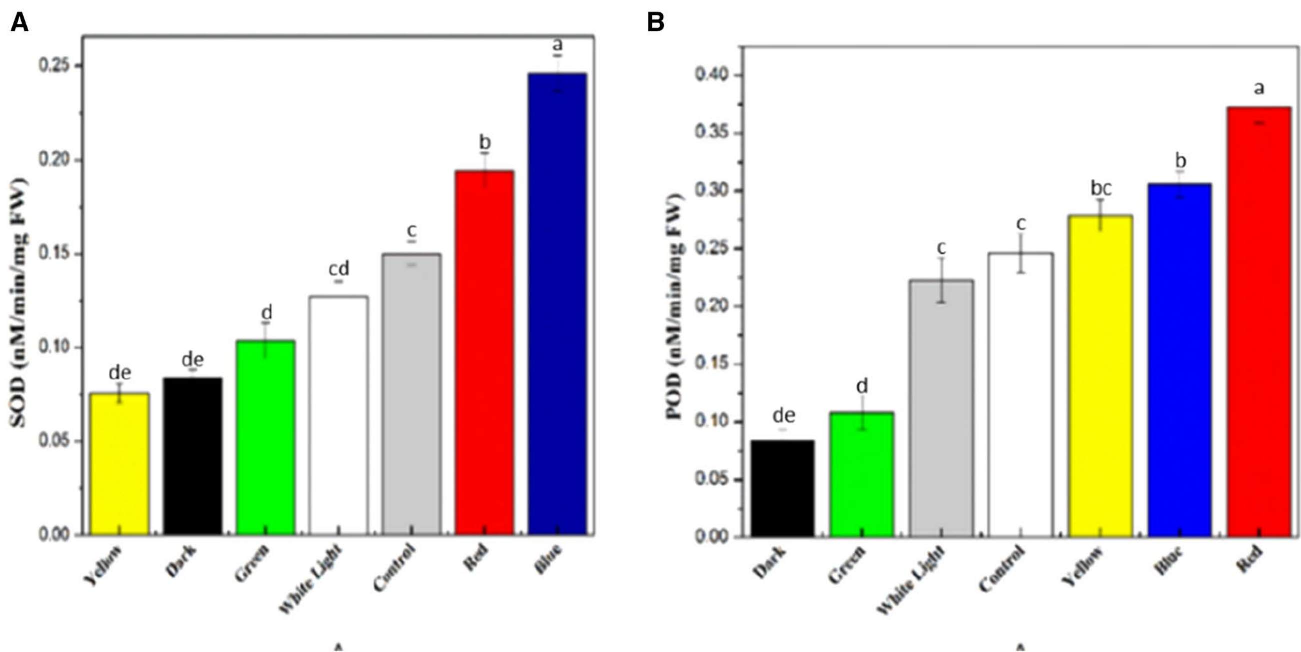

Fig. 5 **a** Superoxide dismutase (SOD), and **b** peroxidase (POD) activities in response to different spectral lights at day 35. Values are means ± SE from triplicates. Columns with similar alphabets are not significantly different ($p < 0.05$) according to Tukey's test

Table 1 Treatment conditions for callus cultures of *Ocimum basilicum* L. var *purpurascens*

Treatments	MS + PGR	Light Regimes	Incubation Period
T1	MS + 2.5 NAA	Dark	24-h Dark (7 weeks)
T2	MS + 2.5 NAA	Green	24-h light (7 weeks)
T3	MS + 2.5 NAA	Yellow	24-h light (7 weeks)
T4	MS + 2.5 NAA	Control	16-h light, 8-h dark (7 Weeks)
T5	MS + 2.5 NAA	Red	24-h light (7 weeks)
T6	MS + 2.5 NAA	Blue	24-h light (7 weeks
T7	MS + 2.5 NAA	White	24-h light (7 weeks)

changes in light quality influence plant responses (Felippe 1979). Previously, light has been shown to stimulate metabolites production in numerous callus culture systems (Shohael et al. 2006; Exonomou 1987; Fazal et al. 2016b). Rosmarinic acid (RA) is a key phenolic compound of basil. Therefore, we also examined the light quality effect on callus cultures of purple basil for RA biosynthesis. All spectral lights exhibited maximum biosynthesis of RA in day 35 (log phase) of growth kinetics, followed by a shorter stationary phase (from day 35 to 42) and then decline phase (from day 42 onwards), as shown in Table 2. Maximum biosynthesis of RA in log phase was observed for darkness (87.62 mg/g DW), followed by red (85.33 mg/g DW), white (82.16 mg/g DW) and blue (77.57 mg/g DW) spectra, respectively. RA content under control was 56.30 mg/g DW. Light and dark periods play a significant role in provoking responses in plants, particularly the synthesis of secondary metabolites (Mir et al. 2017). As a defense strategy, plants accumulate secondary metabolites (Ferrandino and Lovisolo 2014), synthesized via phenylpropanoid pathway (Iriti and Faoro 2009). RA is the key metabolite of basil plant, synthesised through phenylpropanoid pathway (Shiga et al. 2009). In current study, high RA accumulation in dark-grown callus cultures may be due to the fact that continuous dark creates stress conditions and hence, stimulates signal transduction cascade of phenylpropanoid pathway for increment in production of RA. The secondary metabolites undergo photo-block in continuous light conditions, resulting in photoconversion (Mir et al. 2017). The increase in RA might be because of the accumulation of higher levels of reactive oxygen species (ROS) by cytochrome P450. Non-enzymatic scavengers of ROS, like RA, are produced as a defense mechanism (Iwai et al. 2010).

Anthocyanins are pigments, produced in plants, which are affected by numerous environmental factors, mainly light (Gould 2006). Cyanidin and peonidin are major anthocyanins that have previously been identified in these purple basil

Table 2 Accumulation of phenylpropanoid metabolites under different spectral lights at optimized hormonal conditions

No. of days	Treatment	Rosmarinic acid (mg/g DW)	Cichoric acid (mg/g DW)	Caffeic acid (mg/g DW)	Cyanidin (mg/g DW)	Peonidin (mg/g DW)
7	Control	35.91 ± 2.54^{cd}	9.71 ± 1.19^{ab}	0.23 ± 0.06^{ab}	0.10 ± 0.002^{ab}	0.09 ± 0.003^{ab}
14		42.57 ± 1.39^{bc}	9.30 ± 1.43^{ab}	0.24 ± 0.04^{ab}	0.09 ± 0.004^{ab}	0.08 ± 0.006^{ab}
21		45.31 ± 2.77^{b}	11.03 ± 0.98^{a}	0.27 ± 0.03^{a}	0.09 ± 0.001^{ab}	0.08 ± 0.009^{ab}
28		56.30 ± 3.85^{a}	12.33 ± 1.05^{a}	0.28 ± 0.08^{a}	0.12 ± 0.006^{a}	0.11 ± 0.001^{a}
35		51.73 ± 2.61^{ab}	11.28 ± 1.59^{a}	0.23 ± 0.03^{ab}	0.10 ± 0.008^{ab}	0.10 ± 0.004^{a}
42		49.84 ± 1.44^{ab}	11.01 ± 0.77^{a}	0.19 ± 0.01^{b}	0.07 ± 0.003^{b}	0.06 ± 0.002^{b}
49		41.93 ± 4.62^{bc}	8.46 ± 0.71^{ab}	0.17 ± 0.07^{b}	0.07 ± 0.001^{b}	0.07 ± 0.008^{b}
7	Dark	69.04 ± 3.05^{bc}	8.53 ± 1.31^{b}	0.28 ± 0.05^{ab}	0.10 ± 0.003^{ab}	0.09 ± 0.005^{ab}
14		62.85 ± 2.95^{cd}	11.04 ± 1.14^{a}	0.22 ± 0.02^{b}	0.08 ± 0.006^{b}	0.07 ± 0.009^{b}
21		70.96 ± 4.55^{bc}	11.07 ± 2.91^{a}	0.27 ± 0.04^{ab}	0.10 ± 0.002^{a}	0.09 ± 0.003^{ab}
28		73.69 ± 1.57^{b}	11.04 ± 0.34^{a}	0.28 ± 0.07^{ab}	0.11 ± 0.004^{ab}	0.10 ± 0.001^{ab}
35		87.62 ± 2.05^{a}	12.52 ± 0.55^{a}	0.32 ± 0.04^{a}	0.15 ± 0.001^{a}	0.13 ± 0.008^{a}
42		80.39 ± 1.67^{ab}	10.58 ± 1.89^{ab}	0.31 ± 0.06^{a}	0.13 ± 0.005^{a}	0.11 ± 0.004^{a}
49		72.85 ± 6.38^{b}	7.93 ± 1.41^{b}	0.24 ± 0.09^{b}	0.10 ± 0.003^{ab}	0.06 ± 0.007^{b}
7	Red	63.89 ± 1.41^{cd}	9.43 ± 1.11^{b}	$0.23 \pm 0.04\text{b}^{c}$	0.08 ± 0.001^{b}	0.07 ± 0.002^{b}
14		67.42 ± 3.57^{c}	10.02 ± 0.78^{ab}	0.25 ± 0.05^{b}	0.09 ± 0.001^{ab}	0.08 ± 0.005^{b}
21		62.86 ± 2.10^{cd}	12.31 ± 1.05^{a}	0.32 ± 0.02^{ab}	0.08 ± 0.001^{b}	0.07 ± 0.003^{b}
28		74.24 ± 1.64^{b}	12.88 ± 2.57^{a}	0.37 ± 0.07^{a}	0.12 ± 0.004^{a}	0.10 ± 0.007^{ab}
35		85.33 ± 3.66^{a}	14.65 ± 1.05^{a}	0.31 ± 0.01^{ab}	0.14 ± 0.001^{a}	0.12 ± 0.004^{a}
42		79.74 ± 1.43^{ab}	11.74 ± 0.91^{ab}	0.26 ± 0.03^{b}	0.10 ± 0.003^{ab}	0.07 ± 0.009^{b}
49		69.33 ± 1.91^{bc}	8.62 ± 0.88^{b}	0.22 ± 0.06^{bc}	0.07 ± 0.005^{b}	0.08 ± 0.006^{b}
7	Yellow	70.55 ± 6.42^{ab}	9.59 ± 1.67^{ab}	0.27 ± 0.02^{ab}	0.11 ± 0.001^{a}	0.09 ± 0.004^{ab}
14		64.31 ± 5.38^{b}	11.15 ± 1.27^{a}	0.28 ± 0.09^{a}	0.09 ± 0.004^{ab}	0.08 ± 0.002^{ab}
21		68.43 ± 1.06^{ab}	10.19 ± 0.74^{a}	0.24 ± 0.01^{b}	0.12 ± 0.007^{a}	0.11 ± 0.008^{a}
28		72.03 ± 2.86^{a}	10.87 ± 0.96^{a}	0.26 ± 0.06^{ab}	0.11 ± 0.005^{a}	0.10 ± 0.005^{a}
35		73.36 ± 3.03^{a}	11.41 ± 1.55^{a}	0.29 ± 0.07^{a}	0.11 ± 0.009^{a}	0.10 ± 0.001^{a}
42		67.89 ± 2.45^{ab}	8.33 ± 1.83^{ab}	0.25 ± 0.05^{ab}	0.06 ± 0.004^{b}	0.08 ± 0.003^{ab}
49		61.35 ± 1.97^{bc}	7.19 ± 1.39^{b}	0.27 ± 0.01^{ab}	0.08 ± 0.003^{ab}	0.09 ± 0.006^{ab}
7	Blue	68.90 ± 0.94^{b}	8.68 ± 0.99^{ab}	0.26 ± 0.08^{a}	0.10 ± 0.007^{ab}	0.09 ± 0.008^{ab}
14		76.29 ± 1.18^{a}	9.88 ± 0.38^{a}	0.24 ± 0.02^{a}	0.12 ± 0.004^{a}	0.10 ± 0.002^{a}
21		$75.63 \pm 3{,}13^{a}$	8.76 ± 1.66^{ab}	0.22 ± 0.06^{ab}	0.12 ± 0.006^{a}	0.11 ± 0.004^{a}
28		75.80 ± 1.55^{a}	10.16 ± 1.05^{a}	0.21 ± 0.03^{ab}	0.13 ± 0.003^{a}	0.12 ± 0.007^{a}
35		77.57 ± 3.57^{a}	10.25 ± 1.24^{a}	0.20 ± 0.05^{b}	0.12 ± 0.008^{a}	0.10 ± 0.009^{a}
42		69.44 ± 2.85^{b}	8.42 ± 0.77^{ab}	0.24 ± 0.09^{a}	0.10 ± 0.002^{ab}	0.07 ± 0.003^{ab}
49		60.97 ± 2.43^{c}	6.33 ± 0.49^{b}	0.19 ± 0.07^{b}	0.08 ± 0.007^{b}	0.05 ± 0.005^{b}
7	Green	70.78 ± 2.67^{ab}	11.86 ± 1.59^{a}	0.25 ± 0.01^{ab}	0.10 ± 0.001^{ab}	0.09 ± 0.008^{ab}
14		72.79 ± 3.98^{ab}	9.80 ± 0.63^{ab}	0.27 ± 0.08^{a}	0.11 ± 0.006^{a}	0.10 ± 0.001^{a}
21		68.17 ± 1.55^{b}	10.44 ± 1.36^{a}	0.26 ± 0.05^{ab}	0.10 ± 0.009^{ab}	0.09 ± 0.004^{ab}
28		73.31 ± 4.93^{a}	10.58 ± 0.66^{a}	0.30 ± 0.01^{a}	0.11 ± 0.004^{a}	0.10 ± 0.007^{a}
35		75.11 ± 1.77^{a}	9.50 ± 1.69^{ab}	0.24 ± 0.02^{ab}	0.12 ± 0.007^{a}	0.11 ± 0.009^{a}
42		71.52 ± 3.52^{ab}	9.86 ± 0.70^{ab}	0.21 ± 0.06^{b}	0.06 ± 0.002^{bc}	0.07 ± 0.003^{b}
49		62.19 ± 2.75^{b}	8.47 ± 1.72^{ab}	0.23 ± 0.04^{ab}	0.04 ± 0.008^{c}	0.09 ± 0.001^{ab}
7	White light	73.46 ± 3.28^{b}	7.96 ± 1.32^{b}	0.23 ± 0.03^{b}	0.11 ± 0.004^{ab}	0.11 ± 0.007^{a}
14		64.38 ± 1.77^{c}	8.82 ± 1.14^{b}	0.20 ± 0.07^{bc}	0.09 ± 0.001^{ab}	0.08 ± 0.002^{ab}
21		71.19 ± 1.42^{b}	9.19 ± 1.53^{ab}	0.26 ± 0.01^{ab}	0.11 ± 0.005^{ab}	0.10 ± 0.004^{ab}
28		73.46 ± 0.96^{b}	10.46 ± 1.39^{ab}	0.22 ± 0.06^{b}	0.12 ± 0.008^{a}	0.11 ± 0.008^{a}
35		82.16 ± 2.47^{a}	12.76 ± 0.79^{a}	0.32 ± 0.03^{a}	0.13 ± 0.003^{a}	0.12 ± 0.005^{a}
42		72.99 ± 1.05^{b}	10.33 ± 1.22^{ab}	0.25 ± 0.08^{ab}	0.11 ± 0.007^{ab}	0.10 ± 0.009^{ab}
49		63.55 ± 3.85^{c}	11.75 ± 1.90^{a}	0.18 ± 0.09^{c}	0.09 ± 0.009^{ab}	0.07 ± 0.003^{b}

Values are means of three independent replicates, and similar alphabets are not significantly different at $p < 0.05$

cultures (Phippen and Simon 1998). In this study, anthocyanins and RA were accumulated in correlated manner. Maximum biosynthesis of cyanidin (0.152 mg/g DW) and peonidin (0.135 mg/g DW) were noted in the dark treatment, followed by red (cyanidin: 0.147 mg/g DW & peonidin: 0.1217 mg/g DW) and white (cyanidin: 0.133 mg/g DW & peonidin: 0.127 mg/g DW) lights, respectively (Table 2). Conversely, yellow and green spectra showed lower anthocyanins, as compared to control. From the point of process scale-up, necessity of light irradiation is a problem. However, several types of photo-bioreactors have been designed but it is expensive to operate a photo-bioreactor with light of high intensity. Therefore, production of anthocyanins in the dark is preferable for commercial applications. In literature, reports are available for few plant cell cultures to produce anthocyanins in the dark such as *Fragaria ananassa* (Nakamura et al. 1999), *Aralia cordata* (Sakamoto et al. 1994), *Bupleurum falcatum* (Hiraoka et al. 1986), *Vitis* hybrid (Yamakawa et al. 1983) and *Daucus carota* (Dougall et al. 1980). Reasons for this increase is yet not clear. Nakamura et al. (1999) proposed that high anthocyanin in strawberry callus grown in the dark might be due to genetic mutation that changed the way of expression in an anthocyanin synthetic pathway. In plant cells, anthocyanins are biosynthesized through the phenylpropanoid metabolic pathway and flavonoid metabolic pathway. Another reason may be that, in callus cultured on solid medium, cells on the surface of a clump were always irradiated with strong light intensity. Continuous light irradiation might damage the cells on the surface of clumps, resulting in low anthocyanin productivity (Nakamura et al. 1999). Same will be true for the current findings in our study.

Current study indicated that the total anthocyanin and RA contents increased in response to different LEDs treatments and this might be due to the fact that their initial biosynthetic pathways are common (Petersen, 2003). Nadeem et al. (2018) reported higher anthocyanin contents in basil cultures grown under red spectrum. Another major metabolite found in basil species is cichoric acid which carries diverse medicinal benefits (Lee and Scagel 2009). Here, higher production of cichoric acid was noted during the log phase (day 35) in callus treated with red light (14.65 mg/g DW), followed by white (12.76 mg/g DW) and dark (12.52 mg/g DW) treatments, respectively. Studies indicate that the expression of genes including *CYP98A*6, *PAL* and *C4H* are induced by white light. These genes are thought to be involved in cichoric acid biosynthesis (Shoji et al. 2009; Shiga et al. 2009).

Caffeic acid is another valuable metabolite produced in basil. Here, we also evaluated the influence of spectral lights on caffeic acid accumulation in purple basil callus cultures. No significant effect was observed in caffeic acid production, as presented in Table 2. Since caffeic acid is considered as a precursor of RA, therefore, lower caffeic acid accumulation in our study might be because of its turnover to RA (Petersen and Simmonds 2003; Nazir et al. 2019; Petersen et al. 2009).

Conclusions

Light quality strongly influences the biosynthesis of valuable metabolites in purple basil callus cultures. Among the different LEDs used in this study, blue spectrum is the best for biomass, as well as, phenolic and flavonoids accumulation. Additionally, blue light also encourages superoxide dismutase as well as other antioxidant activities (DPPH, FRAP and ABTS). Conversely, red light is effective in peroxidase activity. Moreover, there is a strong correlation between the accumulation of phenolic and flavonoids and antioxidant activities in purple basil calli grown under different spectral lights. HPLC analysis indicated that red light is effective for cichoric acid accumulation while darkness favors enhanced RA and anthocyanins (peonidin and cyanidin) contents in callus cultures of purple basil. These results demonstrate that LEDs provide a promising elicitation approach for increased accumulation of valuable phenylpropanoid metabolites in in vitro cultures of *Ocimum basilicum* L. var *purpurascens*.

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Author Contribution MN performed the experiments, analysed and compiled data and prepared the manuscript. MA and MY assisted in sub-culturing and write-up. AS and MS helped in phytochemical analysis. CH and NG performed HPLC and antioxidant assays. BHA apprehended the idea, provided platform to complete this research, supervised the research, and reviewed the paper critically.

Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

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