Enhanced growth and cardenolides production in *Digitalis purpurea* under the influence of different LED exposures in the plant factory

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In this report, we have investigated the influence of different light qualities on *Digitalis purpurea* under a controlled environment. For this purpose, red (R), blue (B), fluorescent lamp (FL, control), along with combined red and blue (R:B) LEDs were used. Interestingly, the plant growth parameters such as number of leaf, longest root, width of leaf, width of trichome, leaf area, leaf or root fresh weight (FW), weight (DW) as well as length of trichome were maximum under R:B (8:2), and significantly larger than control plants. The stomatal conductance or anthocyanin was maximum under B LED than those under FL, however the photosynthesis rate was greater under FL. RuBisCO activity was maximum under R:B (1:1) LEDs while the quantity of the UV absorbing substances was highest under R LED than under FL. The maximum amount of cardenolides were obtained from leaf tissue under R:B (2:8) LED than those under FL. The R:B LEDs light was suitable for *Digitalis* plant growth, development, micro- and macro-elements, as well as cardenolides accumulation in the plant factory system. The adaptation of the growth strategy developed in this study would be useful for the production of optimized secondary metabolites in *Digitalis* spp.

*Digitalis purpurea* (commonly known as purple foxglove or lady’s glove) is a herbaceous biennial or short-lived perennial plant, which belongs to the Scrophulariaceae family. Foxglove has been commonly used to treat congestive heart failure1,2. A recent research has identified the *in vitro* anti-cancerous effects of ‘cardenolides’, a compound that is derived from foxglove; suggesting its possible use in oncology3. Pharmacologically active compounds like cardenolides can hardly be extracted from plants owing to its structural complexity that impedes its smooth chemical synthesis. Utilizing the mechanisms of plant tissue culture and plant factory techniques have become the need of the hour4 for researches, that are being conducted on modern agricultural biotechnology and for large-scale production of cardiotonic glycoside and its derivatives4. Few reports till date are available regarding the cardenolides accumulation from *in vitro* propagation4-6 and temporary immersion bioreactor7 in *D. purpurea*, however the *in vitro* morphophysiological growth parameters and production of cardenolides in a plant factory system (PFS) have not been studied yet.

The production of various secondary metabolites in plants including cardenolides (digoxin and digitoxin) are the results of interaction of plants with various environmental factors5, one of such being light6-9. These phytochemicals are significantly acknowledged for their dynamic pharmacological properties that aids in improving human health, and also their productive use in agricultural and industrial applications, thus increasing the...
controlled environment, the LEDs were placed horizontally, above the bench top, at a height of 20 cm. The average photosynthetic photon flux density (PPFD) (LI-250A, LI-COR Inc., USA) was adjusted to 150 mol m$^{-2}$ s$^{-1}$.

Well-developed seedlings were transplanted in a plant factory system (Fig. S1, Supplementary Information). These seedlings were fed once a week after one month of germination, as well as watered as and when needed. After two months, the plants were grown under the light provided by six different light qualities such as R (R light 100%), B (B light 100%), fluorescent lamp (FL 100%), and R: B light provided by the fluorescent lamps and bar-type LEDs. Light spectral distribution (Fig. S2, Supplementary Information) was scanned using a spectroradiometer (RPS-900R, International Light Co. Ltd., USA). In the PFS, plants were grown on rockwool medium for 35 days under the following conditions: 21 ± 1 °C, 70 ± 10% relative humidity, photoperiod of 18/6 (light/dark), and CO$_2$ concentration of 500 µmol mol$^{-1}$. The details of the nutrient solution used, have been provided in Table S1 (Supplementary Information).

**Growth measurements and water potential ($\Psi_w$).** Hydroponic-grown plants were uprooted with care from trays with rockwool pellets and the same were dehydrated with lint-free wipes, before separating the roots and leaves from each plant. Finally, the separated roots and leaves were kept in blotting paper sheets for further examination of their biomass. Dry biomass was determined after oven drying the leaf and root samples at 65 °C for two days. After determining the fresh- and dry-biomass of the leaf samples, the following formula was used for calculating leaf $\Psi_w$ potential.

$$\text{Relative water content (RWC)\%} = \frac{\text{FW (fresh weight)} - \text{DW (dry weight)}}{\text{TM (turgid weight)} - \text{DW (dry weight)}} \times 100$$
Microscope survey for changes in stomata and trichome. A piece of D. purpurea leaf (approximately 6 mm² in area) was excised, after 35 days of treatment period. Excised leaf segments were fixed in 2.5% (v/v) buffered glutaraldehyde (a fixative solution) for 24 h. Subsequently, the samples were dehydrated in 30, 50, 60, 70, 80, 90, and 100% (v/v) series of ethanol solutions and then they were further incubated in a mixture of isoamyl acetate/ethanol for 1 h at room temperature. The leaf section was then oven dried at 45 °C and coated with gold. The photographs of mounted specimens were taken with a scanning electron microscope (JXA-8530F, JEOL, USA), operated at 15 kV. Stomatal and trichome length, density, and width of the leaves were examined and measured with three replicates, respectively. Stomatal and trichome were observed using a DM4000 light microscope (Leica, Wetzlar, Germany) at different magnifications. For this purpose, fresh leaves were first harvested from PFS, and then very fine layers of leaf tissues were peeled off and transferred to glass slides. Drops of glycerine solution were added to the slides before coverslips were placed onto the surface.

Photosynthetic and stomatal conductance measurements. A portable photosynthesis measurement system (LI-6400, LI-COR Inc., Lincoln, NE) was used for measuring the photosynthesis rate (µmol CO₂ m⁻² s⁻¹) and stomatal conductance (mmol H₂O m⁻² s⁻¹) in plants under PFS (during the photoperiod).

Estimation of chlorophyll and carotenoid pigments. Chlorophyll (Chl) a, (Chl) b, and carotenoids were analyzed and calculated, as described by Lichtenthaler and Welburn. Chlorophyll (Chl) a, (Chl) b, and carotenoids were analyzed and calculated, as described by Lichtenthaler and Welburn. Briefly, fully expanded young leaves of 30-days-old plants were collected and the leaf samples were ground to a fine powder and then transferred to 2-mL Eppendorf tube. Further 1 mL of acetone (80%; w/w) was added and homogenized for 10 min at 4 °C. The absorbance was measured by a UV-Vis spectrophotometer (Biochrom Libra S22) at 479, 649, and 665 nm.

UV absorbing substances (UAS). UAS was extracted and determined following the previously published spectrophotometric protocol. For this, one 0.5 diameter leaf disc was incubated with 5-mL of methanol (99): HCL (1) mix and allowed digestion at −4 °C for 48 h. UAS was measured from leaf extract at 305 nm. Absorbance was expressed on the basis of the leaf FW.

RuBisCO determination by sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE). For the extraction of RuBisCO, freshly harvested leaves were homogenized in 100 mM Tris buffer (pH = 7.5) containing 5% (v/v) glycerol, 5 mM of DTT, and 2 mM iodoacetate; a leaf (1 g):buffer (10-mL) ratio was used for further extraction. A buffer without potassium (K⁺) or sodium (Na⁺) ions was recommended for RuBisCO analysis by SDS-PAGE, it is because those cations reduce the solubility of dodecyl sulfate (DS). A Triton X 100 was added, before centrifugation (5,000 × g) at 4 °C for 3 min. The supernatant was thoroughly mixed with 2-mercaptoethanol and lithium DS solution (25%, w/v) to final concentrations of 1% (v/v) and 1% (w/v), respectively. The final extraction was rapidly treated at 100 °C for 1 min and then stored at −30 °C, until it was being used for SDS-PAGE analysis. The samples were loaded on a 12% polyacrylamide gel. After completion of electrophoretic run, the gels were stained with silver stain. The stained bands corresponding to larger and smaller subunits of RuBisCO were then cut out from the gels with a razor blade and were eluted in 1–2.5 mL of formamide in s-stoppered amber test tubes and evaluated in the spectrophotometer. RuBisCO content was determined by using the standard curve calculated from the absorbance of a known amount of purified RuBisCO.
Assessment of different elements contents in harvested leaves. For determination of the concentrations of ten elements (B, Ca, Cu, Fe, K, Mg, Mn, P, S, and Zn), around 1 g of fresh leaf sample was oven-dried at 45 °C and then digested with concentrated H2SO4 and perchloric acid (50%, v/v) for 2–5 h at 100–300 °C. After digestion, the samples were then filtered with filter paper (Whatman) and finally diluted up to 100 mL with distilled water. The elemental contents were estimated by inductively coupled plasma optical emission spectrometry (ICP-OPTIMA 4300DV/5300DV/Perkin Elmer, Waltham, MA, USA).

Sample preparation and cardenolide extraction. Shoots of well-developed plants, under closed type PFS, were collected and then freeze-dried at −56 °C, and later used for digitoxin and digoxin extraction. Cardenolides were extracted following the method described by Wiegrebe and Wichtl[30]. Approximately 50 mg dry leaf powder was transferred to 2-mL centrifuge tube and 1 mL of 70% methanol was added. The mixture was kept in an ultrasonic bath for 30 min at 65–70 °C, following which the mixture was cooled on ice for 3–5 min and centrifuged at 13000 rpm for 10 min. The supernatant was collected and mixed with 0.5 mL of 4% (w/v) monosodium phosphate solution and 0.25 mL of 15% (w/v) lead acetate solution. The resultant extract was transferred and diluted with water up to 2 mL and then centrifuged at 12,000 rpm for 8 min at room temperature. The supernatant was collected and mixed with 0.5 mL isopropanol:chloroform (2:3) and centrifuged at 12,000 rpm for 5 min at room temperature. The lower phase was transferred in the new tube as the ‘first extraction’. The remaining methanolic solution was used for the ‘second extraction’ by adding: isopropanol:chloroform and centrifuging at 13,000 rpm for 5 min at room temperature. The first and second extractions were mixed and evaporated under high air for 3 h and finally dissolved in HPLC grade methanol (500 μL).

Quantification of cardenolides via HPLC. The quantitative analysis of cardenolides from leaf samples was performed according to the previously published method of Wiegrebe and Wichtl[30]. Briefly, an aliquot of 20 μL from each sample (n=3 sample for each injection) solution was injected into the HPLC system, at a flow rate of 1.2 mL per min using binary pump solvent system. The selected wavelengths were 220 nm and 350 nm. Separation of the cardenolides was achieved with Reprosil-Pur C18 AQ (5 μm, 250 × 4 mm). For quantification of cardenolides, the mobile phase consisted of acetonitrile (α) and water (β) gradients [20% (α), 80% (β) for 0–20 min; 32% (α), 68% (β) for 20–27 min; 58% (α), 42% (β) 27–35 min; 60% (α), 40% (β) 35–40 min; 0% (α), 100% (β) for 40–60 min; 20% (α), 80% (β) for 60–65 min]. The detection of cardenolides compounds were performed using pure standards purchased from Sigma-Aldrich (St. Louis, MO, USA).

Experimental design, data collection, and statistical analysis. In the controlled environment experiment, a completely randomized design with three replications of each LED treatment (to minimize position effects) with total thirty seedlings per each treatment, were used. Data were collected and statistical analysis was performed with SPSS Version 18 (SPSS Inc., Chicago, IL, USA). The experimental results were subjected to an analysis of variance (ANOVA) and Duncan’s multiple range tests[31]. The mean ± SE (standard error) were subjected to Duncan’s multiple range test at p < 0.05 level.

Results and Discussion. D. purpurea was selected as a model plant since it is a rich source of cardiac glycoside or cardenolides. For the potential biological effects of LED in the plant factory system, the physiological, morphological, and biochemical investigation of LED on Digitalis growth could be of enormous benefit. In this study, a complex ecosystem in a PFS was constructed to create a model growth environment for Digitalis to further assess the effects of LEDs on plant growth (Fig. 1).

Plant growth parameter measurements and Ψw potential. The effect of LEDs on the shoot and root growth of D. purpurea have been presented in Fig. 2, with the plant growth data (in Table 1). It is evident that the D. purpurea plants grown under different individual LEDs and in combinations, exhibited distinct growth responses and biomass productions. The growth parameters, such as number of leaves (43.33, length of longest root (29.40 cm), leaf width (6.50 cm), leaf area (608.92 cm2 plant−1) (Table 1), leaf (21.1 g) or root (4.706 g) fresh weight (FW), leaf (2.422 g) or root (0.411 g) dry weight (DW) (Fig. 3) significantly increased in the plants grown under R:B LED (8:2), whereas leaf width was maximum in the plants grown under R LED alone as compared to control plants. The present results showed that R:B LED (8:2) was beneficial for plant growth and development as shown in Table 1. Earlier work has shown that rapeseed plantlets grown under R:B LED showed maximum root values as compared to the plants cultivated under FL LED or under R LED[32]. In chrysanthemum and rose plants, total biomass and total dry weight increased with higher B light and R light ratio, respectively[33]. In addition, studies on lettuce plants, grown on hydroponic system also exhibited greater leaf area for the plants cultivated under FL than the plants those were grown under R:B LED[34]. Although FL lamps (cool white) influence the formation of larger leaves when compared to R:B treatment, however, the size of the leaves remained smaller when compared to the RG(green)/B LED[35]. The effects and mechanisms associated with light quality in plants may be specific to plant species or cultivars. In the present study, R:B LED light is a novel illumination system for Digitalis cultivation in a PFS.

RWC reached a maximum of 89% in plants grown under R:B (1:1) and a minimum of 77.55% in leaves of plants grown under R LEDs (Table 1). Yorio et al.[36] reported that lettuce plants grown under R:B light had higher water accumulation than in plants grown under R light alone. All these outcomes show that plant responses to LEDs lighting under PFS are cultivar and/or species dependent.

Changes in stomatal and trichome characters. Stomatal density, length, width, and a length-to-width ratio of the Digitalis leaves were measured using the scanning electron microscopic images (Figs 4 and 5). It was recorded that the stomatal density and stomatal length:width ratio were the highest in the plants grown under
Figure 2. The effect of LED's on *Digitalis purpurea* L. grown under a closed type plant factory (a) Red; (b) Blue; (c) Fluorescent light; (d–f) combined LEDs of red and blue light [RB (1:1, 8:2, and 2:8) respectively].

| LED treatment | Plant height (cm) | No. of leaf (n) | Leaf length (cm) | Leaf width (cm) | Leaf area (cm²) | Length of longest root (cm) | Leaf relative water content (%) |
|---------------|-------------------|----------------|-----------------|-----------------|-----------------|---------------------------|-------------------------------|
| R             | 17.73 ± 0.64b     | 26.33 ± 2.03b  | 17.07 ± 0.72b   | 6.47 ± 0.50a    | 318.04 ± 49.66b | 24.57 ± 1.88a            | 77.55 ± 0.58d                 |
| B             | 22.43 ± 0.28a     | 24.67 ± 1.45b  | 20.57 ± 1.26a   | 5.20 ± 0.36a    | 322.32 ± 29.92b | 28.17 ± 2.88a            | 88.02 ± 1.00ab                |
| FL            | 13.10 ± 1.55c     | 22.33 ± 1.86b  | 15.40 ± 1.33c   | 5.73 ± 0.77a    | 330.66 ± 64.05b | 29.20 ± 2.40a            | 83.59 ± 0.58c                 |
| RB (1:1)      | 12.67 ± 0.38c     | 27.67 ± 1.67b  | 15.71 ± 0.37b   | 5.10 ± 0.45a    | 375.08 ± 73.65b | 25.60 ± 1.21a            | 89.00 ± 1.00a                 |
| RB (8:2)      | 15.53 ± 0.24b     | 27.67 ± 1.45b  | 11.16 ± 0.49d   | 5.73 ± 0.43a    | 368.24 ± 38.44b | 27.83 ± 1.73a            | 83.59 ± 0.88c                 |
| RB (2:8)      | 12.13 ± 0.49c     | 27.67 ± 1.45b  | 11.16 ± 0.49d   | 5.73 ± 0.43a    | 368.24 ± 38.44b | 27.83 ± 1.73a            | 83.59 ± 0.88c                 |

Table 1. The effect of Red, Blue, Fluorescent light, and combined LEDs of red and blue light [RB (1:1, 8:2, and 2:8) respectively] under a closed type plant factory. Data for each column followed by different alphabets are significantly different according to Duncan's multiple range test at p < 0.05.
that trichome number and size is partly regulated by light 40. In this case, stomatal and trichome density may
to FL lamps, as reported by Vieira et al.
equally in both the abaxial and adaxial leaf surfaces, under white (W) LED and deep R/W LED, when compared
to FL lamps, as reported by Vieira et al. 38. In the present study, length and width of trichome were the highest in
the plants grown under R:B (8:2) treatment. The number of trichome was recorded to be the highest in R:B (2:8)
treated plants and the lowest were recorded in the plants grown under FL treatment (Table 2). It is well known
that trichome number and size is partly regulated by light 40. In this case, stomatal and trichome density may
increase or decrease in response to the environmental variations caused by the light intensity, quality or duration.

**Influence on photosynthesis rates and stomatal conductance.** The photosynthesis rates in the
leaves of D. purpurea, under different light treatments have been presented in Fig. 6. Photosynthesis rate increased
during the FL treatment, followed by treatment with R:B (8:2). The highest photosynthesis rate was recorded to
be 7.6 ± 0.12 µmol CO₂ m⁻² s⁻¹ at 1,500 µmol mol⁻¹ of CO₂ concentration in FL treatment, while 7.1 ± 1.3 µmol
CO₂ m⁻² s⁻¹ at 1,200 µmol mol⁻¹ of CO₂ concentration was recorded in the R:B (8:2) treated plants, respectively
(Fig. 6a). This result is consistent with the findings of Goins et al. 46 who reported that wheat plants when grown
under R:B LEDs had higher photosynthesis rates. These photosynthesis rates were higher due to increased stoma-
tal conductance (stomata opening) under more B light 42. Light-saturated maximum stomatal conductance and
photosynthesis are closely associated in many plant species 43,44. It is well known that a higher B light amount is
mostly related to the development of ‘sun-type’ leaves, which are characterized by a higher leaf mass per unit leaf
area and a high photosynthetic capacity 43,44.

Stomatal conductance in leaves was the highest under B (861.5 ± 108.5 at 300 mmol H₂O m⁻² S⁻¹) LED treat-
ments (Fig. 6b). Terfa et al. 42 and van Ieperen 49 reported that higher stomatal conductance in LED-grown plants
might at least partly be due to a higher number and frequency of stomata per area of epidermal cells. As reported
in some studies, stomatal conductance in cucumber plants proportionally increased with increasing B light and
it was related to both the aperture of stomata and a higher number of stomata 50. In another study, Wang et al. 51
observed that stomatal conductance increased in cucumber plants that were grown under B monochromatic
light, when compared to plants that were grown under white, R, G, and yellow monochromatic lights.

**Influence on chlorophyll, carotenoid content, and Fv/Fm.** The results of the chlorophyll and carot-
enoid contents in the leaves of Digitalis are presented in Fig. 7. Chlorophyll a (Chl-a) concentration significantly
increased in the plants grown under B (3.0 µg mL⁻¹) and R (2.5 µg mL⁻¹) LEDs, in comparison to that with the FL
(1.5 µg mL⁻¹)-treated plants. In contrast, chlorophyll b (Chl-b) concentration was found to be the highest in the
plants grown under R LED (1.4 µg mL⁻¹), while the B LED (0.4 µg mL⁻¹)-grown plants yielded the lowest con-
centration. Similar observations were reported on edible bananas by Vieira et al. 50 who investigated the effects of
W-LED and deep RW LED light treatments. These authors reported increased levels of Chl-a and Chl-b in the in
vitro plantlets using W-LED and deep RW LED lights, which were higher than those in the FL-treated plantlets.
With respect to the carotenoid content, the B LED treatment (0.23 µg mL⁻¹) and the R:B (2:8) LED treatment
(0.23 µg mL⁻¹) showed equal concentrations of Chl-b, while under the FL treatment (0.125 µg mL⁻¹) the concen-
tration was recorded to be much lower. Recent studies of Brazaitytė et al. 52 suggested that influence of light quality
and light intensity on Brassicaceae microgreens produced more total carotenoids. With respect to the Fv/Fm ratio,
the B and R:B (2:8) LED treatments resulted in comparable concentrations, while under the R:B (8:2) LED treat-
ment, the concentration was much lower. A maximum photochemical yield of PS II in the dark-adapted state of
the plant is also known as the maximum quantum efficiency of photosystem II (PS II). Fv/Fm ratio is often used to

![Figure 4](https://www.nature.com/scientificreports/)

**Figure 4.** Scanning electron micrographs of stomata in leaves of Digitalis purpurea L. as affected by (a) Red, (b)
Blue, (c) Fluorescent light, and (d-f) combined LEDs of red and blue light [RB (1:1, 8:2, and 2:8 respectively] under a closed type plant factory. (g) Stomata under a light microscope (bar = 4 µm) and (h) under scanning electron micrographs.
Figure 5. Scanning electron micrographs of trichomes in leaves of *Digitalis purpurea* L. as affected by (a) Red, (b) Blue, (c) Fluorescent light, and combined LEDs of red and blue light, (d) RB (1:1), (e) R:B (8:2), and (f) R:B (2:8) respectively; (g,h) and (j,k) multicellular glandular trichomes under a light microscope (Leica CME) at 20x magnification (bar = 75 μm), (i) single spherical secreting cell (trichome head) (bar = 4 μm), (l) unicellular stalk with single spherical secreting cell at the apex (bar = 30 μm).

| LED Treatment | Density of stomata (no. of stomata/mm²) | Length of stomata | Width of stomata | Length-to-width ratio of stomata | Density of trichome (no. of stomata mm⁻²) | Length of trichome | Width of trichome |
|---------------|-----------------------------------------|------------------|-----------------|----------------------------------|--------------------------------------------|-------------------|------------------|
| R             | 24.80 ± 2.84ab                         | 17.40 ± 2.30a    | 11.65 ± 0.49b   | 1.51 ± 0.26a                     | 36.00 ± 24.58b                             | 37.93 ± 0.81a     |
| B             | 23.50 ± 2.59ab                         | 16.90 ± 3.96a    | 13.17 ± 1.09b   | 1.25 ± 0.20a                     | 36.00 ± 37.58b                             | 41.30 ± 5.11a     |
| FL            | 41.04 ± 10.74a                         | 16.47 ± 3.75a    | 9.07 ± 2.11b    | 2.28 ± 1.03a                     | 343.33 ± 33.65b                            | 44.03 ± 2.26a     |
| RB (1:1)      | 21.73 ± 1.68b                         | 18.43 ± 4.56a    | 12.73 ± 2.17b   | 1.67 ± 0.67a                     | 360.67 ± 44.73b                            | 35.87 ± 5.76a     |
| RB (8:2)      | 26.07 ± 2.28ab                         | 16.63 ± 1.01a    | 18.83 ± 0.66a   | 0.89 ± 0.08a                     | 565.33 ± 25.30a                            | 46.30 ± 1.90a     |
| RB (2:8)      | 25.58 ± 5.55ab                         | 15.40 ± 1.44a    | 12.13 ± 1.76b   | 1.29 ± 0.07a                     | 307.00 ± 20.88b                            | 38.57 ± 5.06a     |

Table 2. The effect of Red, Blue, Fluorescent light, and combined LEDs of red and blue light [RB (1:1, 8:2, and 2:8) respectively] on stomata and trichome characters in *Digitalis purpurea* L., under a closed type plant factory. The data represents mean ± standard error. Data for each column followed by the different alphabets are significantly different according to Duncan’s multiple range test at p < 0.05.
indicate this phenomenon. Most healthy plants have an Fv/Fm value of 0.85 or 0.83\textsuperscript{53,54}. Since 0.83 ratio of Fv/Fm has been reported for unstressed plants\textsuperscript{55}, reduction of the Fv/Fm ratio in the current study may not be so severe. However, Fv/Fm ratio may only reflect the increased levels of photoprotection.

**Influence on anthocyanin content and UV absorbing substances (UAS).** A significantly higher concentration of anthocyanin was measured in leaf tissues of the plants grown under B LED (0.0028 μmol mL\textsuperscript{-1}), followed by (0.0018 μmol mL\textsuperscript{-1}) R LED. Anthocyanin content was not significantly affected, neither under FL nor under the combinations of R:B LEDs (1:1, 8:2, and 2:8); and as a result, anthocyanin production was recorded to be lower than the plants grown under B and R LEDs individually. It was previously reported that B LEDs is effective in anthocyanin production in most of the plant species, including tomato and cabbage seedlings\textsuperscript{56}, strawbery cells\textsuperscript{57}, roses\textsuperscript{58}, and Chinese bayberry fruit\textsuperscript{59}. With regard to UAS absorbing substances in Digitalis leaves, a decrease of 50% was observed in the plants grown under R:B LED combinations (1:1, 8:2, and 2:8); as well as a 75% decrease was observed in B or FL LED-grown plants (Fig. 7). It is widely known that if the level of UV-B...
radiation increases, plants also increase the concentrations of UAS in order to protect themselves from direct exposure of UV-B.\(^\text{[21]}\)

**Influence on RuBisCO activity.** RuBisCO content was measured using SDS-PAGE (Fig. 8a). The intensity of RuBisCO was recorded to be the highest under R:B (1:1) LED and was recorded to be relatively low under R, B, FL, and R:B (8:2) LED. The intensity of RuBisCO was strongly reduced in the plants grown under R:B (2:8) LEDs. The changes in RuBisCO activity differs with light intensity, over the same range as photosynthesis via changes in the proportional amounts of inactive or active forms of the RuBisCO.\(^\text{[22]}\)

**Influence on different macro- and micro-element content.** The results of the micro- and macro-element analysis of leaf samples of *D. purpurea* plant have been presented in Table 3. The levels of macro- (Ca, K, Mg, P, and S) and micro- (B, Cu, Fe, Mn, and Zn) elements were quantified in mg g\(^{-1}\) of leaf DW. Ca ranged from 7.333 mg g\(^{-1}\) for R:B (8:2) to 11.741 mg g\(^{-1}\) for R:B (2:8). Mg ranged from 2.159 mg g\(^{-1}\) for R-LEDs to 3.243 mg g\(^{-1}\) for R:B (2:8). K ranged from 39.644 mg g\(^{-1}\) for R:B (8:2) to 50.645 mg g\(^{-1}\) for FL-LEDs. P ranged from 4.960 mg g\(^{-1}\) for R:B (2:8) to 7.148 mg g\(^{-1}\) for R:B (2:8). S ranged from 1.245 mg g\(^{-1}\) for R:B (2:8) to 1.720 mg g\(^{-1}\) for R:B (2:8). For micro elements; B ranged from 0.120 mg g\(^{-1}\) for R:B (2:8) to 0.169 mg g\(^{-1}\) for R:B (2:8). Cu ranged from 0.010 mg g\(^{-1}\) for R:B (8:2) to 0.126 mg g\(^{-1}\) for R:B (8:2). Mn ranged from 0.092 mg g\(^{-1}\) to 0.100 mg g\(^{-1}\) for R:B (2:8). Zn ranged from 0.100 mg g\(^{-1}\) for R:B (8:2) to 0.169 mg g\(^{-1}\) for R:B (2:8) (Table 3). Vaštakaitė et al.\(^\text{[23]}\) investigated the effects of LED light spectra and irradiance level, on *Brassica* species and concluded that LEDS light spectra and intensity may improve the micro- and macro-elements contents in the plants grown under controlled environmental conditions.

**Influence on cardenolides accumulation.** Accumulation of cardenolides was significantly influenced by different regimes of LEDs. As shown in Table 3, leaves obtained (in a PFS) from those plants that were grown under R:B (2:8) LEDs, produced the highest amount of digitoxin (0.427 mg g\(^{-1}\)DW) and digoxin (0.16 mg g\(^{-1}\)DW), followed by B-LEDs treated plants, wherein the recorded amount of digitoxin and digoxin were

### Table 3. Total contents of different macro- and micro-elements (mg g\(^{-1}\) of leaf DW) in harvested leaf and Cardenolides accumulations in *Digitalis purpurea* L. grown under a closed type plant factory with different LEDs Red (R), Blue (B), Fluorescent light (FL), and combined LEDs of red and blue light [RB (1:1, 8:2, and 2:8) respectively]. The data represents mean ± standard error. Data columns with different alphabets are significantly different according to Duncan's multiple range test at p < 0.05.

| LED treatment | Macro elements (mg g\(^{-1}\) of leaf DW) | Micro elements (mg g\(^{-1}\) of leaf DW) | Cardenolides accumulation (mg g\(^{-1}\) DW) |
|---------------|----------------------------------------|----------------------------------------|----------------------------------------|
| R             | 7.629 ± 0.037c 2.159 ± 0.031c 42.923 ± 0.092c 5.747 ± 0.048b 1.605 ± 0.013b | 0.034 ± 0.001d 0.014 ± 0.001bc 0.093 ± 0.001c 0.092 ± 0.002a 0.149 ± 0.001c | 0.1033 ± 0.022c 0.059 ± 0.036bcd |
| B             | 7.803 ± 0.062c 2.516 ± 0.031c 44.778 ± 0.206b 5.857 ± 0.035b 1.223 ± 0.028b | 0.041 ± 0.001b 0.011 ± 0.001de 0.070 ± 0.001e 0.100 ± 0.003d 0.150 ± 0.001c | 0.313 ± 0.032b 0.100 ± 0.01b |
| FL            | 9.759 ± 0.043b 2.745 ± 0.003b 50.465 ± 0.685a 7.148 ± 0.322a 1.445 ± 0.036c | 0.063 ± 0.006c 0.016 ± 0.001b 0.106 ± 0.002b 0.117 ± 0.001c 0.162 ± 0.002b | 0.233 ± 0.041b 0.018 ± 0.003d |
| R:B (1:1)     | 9.614 ± 0.048b 2.320 ± 0.007b 43.469 ± 0.401c 6.974 ± 0.130a 1.245 ± 0.030d | 0.022 ± 0.000e 0.012 ± 0.001cd 0.084 ± 0.002d 0.126 ± 0.001b 0.131 ± 0.001d | 0.283 ± 0.033b 0.053 ± 0.006cd |
| R:B (8:2)     | 7.333 ± 0.031c 2.410 ± 0.026d 39.644 ± 0.333d 4.960 ± 0.119c 1.313 ± 0.012d | 0.028 ± 0.001e 0.010 ± 0.001e 0.108 ± 0.001c 0.100 ± 0.001d 0.100 ± 0.002e | 0.253 ± 0.050b 0.08 ± 0.026bc |
| R:B (2:8)     | 11.741 ± 0.074a 9.243 ± 0.030a 43.476 ± 0.367c 7.280 ± 0.174a 1.720 ± 0.047a | 0.043 ± 0.001a 0.024 ± 0.001a 0.201 ± 0.003a 0.145 ± 0.001a 0.169 ± 0.001a | 0.427 ± 0.064a 0.16 ± 0.034a |

Figure 8. RuBisCO determination by (a) SDS-PAGE and (b) its quantification as affected by affected by Red, Blue, Fluorescent light, and combined LEDs of red and blue light [RB (1:1, 8:2, and 2:8) respectively] under a closed type plant factory. The data represents mean ± standard error. Data columns with different alphabets are significantly different according to Duncan's multiple range test at p < 0.05.
0.313 mg g⁻¹DW and 0.100 mg g⁻¹DW, respectively. Among the different types and combinations of LEDs i.e. B, R, R:B (1:1), R:B (8:2), and R:B (2:8), R:B (8:2) exhibited higher shoot fresh weight (FW) and dry weight (DW) (Fig. 3). However, R:B (2:8) produced less shoot FW and DW, with highest digitoxin and digoxin accumulation. The highest cardenolide accumulation have been reported in D. lanata by Ohlsson et al., using light in the blue region. Similarly other authors observed that irradiation with large doses of B or R-light produced higher contents of cardenolides than irradiation with yellow-green light in somatic embryos of D. lanata. In the present study, this is a crucial factor that favours cardenolides synthesis in D. purpurea, when grown in a PFS under R:B combination or B alone LEDs light. It is well known that a blue light photoreceptor and protochlorophyllide-holochrome, or phytochrome, are involved in the regulation of cardenolides biosynthesis and accumulation. On the other hand, it is a commonly agreed fact that light intensity can positively influence the accumulation of phytochemicals; accordingly, the influence of light quality are observed to be more complicated and usually reported with variable outcomes. A previous study indicated that variations in the light spectrum could cause fluctuations in secondary metabolite content. In our case, the light spectrum not only modulated the amount but also showed variations in cardenolides accumulations in Digitalis.

In conclusion, an important finding of the present study is that the combination of R:B (8:2) LEDs promoted plant growth, while micro- and macro-elements and cardenolides accumulation was enhanced under R:B (2:8) LEDs in the PFS system. These should be taken into consideration as a beneficial component and the plant extract may successfully cure various human diseases. In addition, the present study shows that it is possible to modify the growth parameters and secondary metabolites accumulation and their composition, in D. purpurea, by applying appropriate light quality in a PFS system. The current study also reveals that growing D. purpurea plants under-regulated environments could be regarded as an alternative approach to improve the production of biomass and secondary metabolites, which would aid in an extensive point of view of cardenolides biosynthesis in D. purpurea. The highest cardenolide accumulation have been reported in Digitalis purpurea L. grown in liquid media. Plant Physiol. 69, 653–656 (1982).

References
1. Verma, S. K., Yucesan, B., Sahin, G. & Gurel, E. Embryogenesis, plant regeneration and cardiac glycoside determination in Digitalis ferruginea subsp. ferruginea L. Plant Cell Tiss. Org. Cult. 119, 625–634, https://doi.org/10.1007/s11240-014-0562-9 (2014).
2. Verma, S. K., Das, A. K., Gantait, S., Gurel, S. & Gurel, E. Influence of auxin and its polar transport inhibitor on the development of somatic embryos in Digitalis ferruginea L. J. Biotechnol. 89, 1–19, https://doi.org/10.1016/S0168-1656(00)00240-8 (2001).
3. Verma, S. K., Das, A. K., Gantait, S., Gurel, S. & Gurel, E. In vitro culture of Digitalis ferruginea L. and the production of cardenolides: An up-to-date review. Ind. Crop. Prod. 94, 20–51, https://doi.org/10.1016/j.indcrop.2016.08.031 (2016).
4. Hagimori, M., Matsumoto, T. & Obi, Y. Studies on the production of Digitalis cardenolides by plant tissue culture: II. Effect of light and plant growth substances on digitoxin formation by undifferentiated cells and shoot-forming cultures of Digitalis purpurea L. in liquid media. Plant Physiol. 69, 653–656 (1982).
5. Patil, J. G. et al. In vitro propagation and production of cardenolides glycosides in shoot cultures of Digitalis purpurea L. by elicitation and precursor feeding. Appl. Microbiol. Biotechnol. 97, 2379–2393, https://doi.org/10.1007/s00253-014-4489-y (2013).
6. Pérez-Alonso, N. et al. Efficient direct shoot organogenesis, genetic stability and secondary metabolite production of micropropagated Digitalis purpurea L. Industrial Crops and Products 116, 259–266, https://doi.org/10.1016/j.indcrop.2018.02.067 (2018).
7. Pérez-Alonso, N. et al. Cardiac glycosides from biomass of Digitalis purpurea L. cultured in temporary immersion systems. Plant Cell Tiss. Org. Cult. 99, 151–156, https://doi.org/10.1007/s11240-009-9587-x (2009).
8. Scheibner, H., Björkz, L., Schulz, U., Diettrich, B. & Luckner, M. Influence of light on cardenolide accumulation in somatic embryos of Digitalis lanata. J. Plant Physiol. 130, 211–219, https://doi.org/10.1016/S0176-1617(87)80225-0 (1987).
9. Eisenbeiß, M., Kreis, W. & Reinhard, E. Cardenolide biosynthesis in light- and dark-grown Digitalis lanata shoot cultures. Plant Physiol. Biochem. 39, 91–99, https://doi.org/10.1016/S0981-9428(01)00062-4 (1999).
10. Ohlsson, N., Björkz, L. & Gatenbeck, S. Effect of light on cardenolide production by Digitalis lanata tissue cultures. Phytochemistry 22, 2447–2450, https://doi.org/10.1016/0031-9422(83)80137-X (1983).
11. Cacho, M., Morán, M., Corchete, P. & Fernández-Tarragó, J. Effect of calcium restriction on cardenolide accumulation in two cell lines of Digitalis lanata grown under different light regimes. Acta Physiol. Plant. 21, 335–340, https://doi.org/10.1007/s11738-999-9003-2 (1999).
12. Hemm, M. R., Rider, S. D., Ogas, J., Murry, D. J. & Chappelle, C. Light induces phenylpropanoid metabolism in Arabidopsis roots. Plant J. 38, 765–778, https://doi.org/10.1046/j.1365-313X.2004.02089.x (2004).
13. Ikek, A. et al. Environmental control and operation monitoring in a plant factory using artificial light. Acta Horticult. 304, 151–158, https://doi.org/10.17660/ActaHortic.1992.304.16 (1992).
14. Um, Y. S., Oy, S. S., Lee, J. G., Kim, S. Y. & Jang, Y. A. The development of container-type plant factory and growth of leafy vegetables as affected by different light sources. J. Bio-Environ. Control 19(4), 333–342 (in Korean) (2010).
15. Raskin, I. et al. Plants and human health in the twenty-first century. TRENDS Biotechnol. 20, 522–531, https://doi.org/10.1016/S0167-7799(02)02080-2 (2002).
16. Igbavboa, U. et al. Alternative formation of anthraquinones and lipoquinones in heterotrophic and photoautotrophic cell suspension cultures of Morinda lucida Benth. Planta 166, 537–544, https://doi.org/10.1007/BF00391279 (1983).
17. Hokkanen, J., Mattila, S., Jaakola, L., Pirttilä, A. M. & Tolonen, A. Identification of phenolic compounds from lindenberry (Vaccinium vitis-idaea L.), bilberry (Vaccinium myrtillus L.) and hybrid bilberry (Vaccinium × intermedium Ruthe L.) leaves. J. Agric. Food Chem. 57, 9437–9447, https://doi.org/10.1021/jf0122542 (2009).
18. Kami, C., Lorrain, S., Hornitschek, P. & Fankhauser, C. Light-regulated plant growth and development. Curr. Top. Dev. Biol. 91, 79–96, https://doi.org/10.1016/S0070-2153(10)31002-6 (2010).
19. Han, T. et al. Improving “color rendering” of LED lighting for the growth of lettuce. Sci. Rep. 7, 45944, https://doi.org/10.1038/srep45944 (2017).
20. Ouzounis, T., Razi Parjikolaei, B., Fretti, X., Rosenqvist, E. & Ottosen, C.-O. Predawn and high intensity application of supplemental blue light decreases the quantum yield of PSII and enhances the amount of phenolic acids, flavonoids, and pigments in Lactuca sativa. Front. Plant Sci. 6, 19, https://doi.org/10.3389/fpls.2015.000019 (2015).
21. Morrow, R. C. LED lighting in horticulture. Hort Sci. 43, 1947–1950 (2008).
22. Briggs, W. R. & Huala, E. Blue-light photoreceptors in higher plants. Annu. Rev. Cell Dev. Biol. 15, 33–62, https://doi.org/10.1146/annurev.cellbio.15.1.33 (1999).
33. Ouzounis, T., Fretté, X., Rosenqvist, E. & Ottosen, C.-O. Spectral effects of supplementary lighting on the secondary metabolites in roses, chrysanthemums, and campanulas. *Plant Physiol.* 171, 1491–1499, https://doi.org/10.1095/biophys.101.008315 (2012).

34. Lin, K.-H. The effects of red light and blue light-emitting diodes on the growth, development, and edible quality of hydrponically grown lettuce (*Lactuca sativa* L. var. capitata). *Sci. Hort.* 150, 86–91, https://doi.org/10.1016/j.scienta.2012.10.002 (2013).

35. Kim, H.-H., Goins, G. D., Wheeler, R. M. & Sager, J. C. Stomatal conductance of lettuce grown under or exposed to different light qualities. *Ann. Bot.* 94, 691–697, https://doi.org/10.1093/aob/mch192 (2004).

36. Yorio, N. C., Goins, G. D., Kagie, H. R., Wheeler, R. M. & Sager, J. C. Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. *HortSci.* 36, 380–383 (2001).

37. Lee, S.-H., Tewari, R. K., Hahn, E.-J. & Paek, K.-Y. Photon flux density and light quality induce changes in growth, stomatal development, photosynthesis and transpiration of *Wittomania sommfera* (L.) Dumal. plantlets. *Plant Cell Tiss. Org.* 90, 141–151, https://doi.org/10.1007/s11240-006-9191-2 (2007).

38. Klermund, C. et al. LLM-domain B-GATA transcription factors promote stomatal development downstream from light signaling in *Arabidopsis thaliana* hypocotyls. *Plant Cell* 28, 646–660, https://doi.org/10.1105/tpc.110.10.057083 (2016).

39. Do Nascimento Vieira, L. et al. Light-emitting diodes (LED) increase the stomata formation and chlorophyll content in *Musa acuminate* (AAA) ‘Nanica Corup’ in vitro plantlets. *Theor. Exp. Plant Physiol.* 27, 91–98, https://doi.org/10.1007/s11240-015-9335-5 (2015).

40. Booth, J. K., Page, J. E. & Bohlmann, J. Terpene synthases from *Cannabis sativa*. *Plon e 12*, e0173911, https://doi.org/10.1371/journal.pone.0173911 (2017).

41. Goins, G. D., Yorio, N., Sanwo, M. & Brown, C. Photomorphogenesis, photosynthesis, and seed yield of wheat plants grown under red light-emitting diodes (LEDs) with and without supplemental blue lighting. *J. Exp. Bot.* 48, 1407–1413 (1997).

42. Terfa, M. et al. The effects of solar ultraviolet-B radiation on the growth and yield of barley are accompanied by increased DNA damage and antioxidant responses. *Photochem. Photobiol. B* 9, 53–59 (2000).

43. Romero, P., Navarro, J. M., García, F. & Ordaz, P. B. Effects of regulated deficit irrigation during the pre-harvest period on gas exchange, leaf development and crop yield of mature almond trees. *Horticulturae* 3, 1–42, https://doi.org/10.3390/horticulturae3010011 (2017).

44. Rosati, A., Metcalf, S., Buchner, R., Fulton, A. & Lampinen, B. Tree water status and gas exchange in walnut under drought, high temperature and vapour pressure deficit. *The J. Hortic. Sci. Biotechnol.* 81, 415–420, https://doi.org/10.1080/14620316.2006.11512082 (2006).

45. Buschmann, C., Meier, D., Kleudgen, H. & Lichtenthaler, H. The effect of cytokinins on growth and pigment accumulation of radish seedlings (*Raphanus sativus* L.) grown in the dark and at different light quanta fluence rates. *Photochem. Photobiol.* 35, 217–221, https://doi.org/10.1111/j.1751-2091.1982.tb03835.x (1982).

46. Lichtenthaler, H., Buschmann, C. & Rahmsdorf, U. The importance of blue light for the development of sun-type chloroplasts. In: Senger, H. (eds) *The blue light syndrome. Proceedings in Life Sciences*. Springer, Berlin, Heidelberg, 485–494, https://doi.org/10.1007/978-3-642-67648-2_45 (Springer, 1980).

47. Matsuda, R., Ohashi-Kaneko, K., Fujiwara, K., Goto, E. & Kurata, K. Photosynthetic characteristics of rice leaves grown under red light with or without supplemental blue light. *Plant Cell Physiol.* 45, 1870–1874 (2004).

48. Matsuda, R., Ohashi-Kaneko, K., Fujiwara, K. & Kurata, K. Effects of blue light deficiency on acclimation of light energy partitioning in PSII and CO2 assimilation capacity to high irradiance in spinach leaves. *Plant Cell Physiol.* 49, 664–670, https://doi.org/10.1093/pcp/pcn041 (2008).

49. van Ieperen, W. Plant morphological and developmental responses to light quality in a horticultural context. *Acta Hortic.* 956, 313–339, https://doi.org/10.17660/ActaHortic.2012.956.12.

50. Hogewoning, S. W. et al. Blue light dose–responses of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. *J. Exp. Bot.* 61, 3107–3117, https://doi.org/10.1093/jxb/erq132 (2010).

51. Wang, H. et al. Effects of light quality on CO2 assimilation, chlorophyll-fluorescence quenching, expression of Calvin cycle genes and carbohydrate accumulation in *Cucumis sativus*. *J. Photochem. Photobiol. B* 96, 30–37, https://doi.org/10.1016/j.jphotobiol.2009.03.010 (2009).

52. Krause, G. & Weiss, E. Chlorophyll fluorescence and photosynthesis: the basics. *Ann. Rev. Plant Bio.* 42, 313–349, https://doi.org/10.1146/annurev.pp.42.060191.001525 (1991).

53. Krause, G. & Weiss, E. Chlorophyll fluorescence and photosynthesis: the basics. *Ann. Rev. Plant Bio.* 42, 313–349, https://doi.org/10.1146/annurev.pp.42.060191.001525 (1991).

54. Hoogeveen, S. W. et al. Light-emitting diodes with a high proportion of blue light affects external and internal quality parameters of potato roots differently than the traditional high pressure sodium lamp. *Acta Hortic.* 956, 635–642 (2012).

55. Krause, G. & Weis, E. Chlorophyll fluorescence and photosynthesis: the basics. *Ann. Rev. Plant Bio.* 42, 313–349, https://doi.org/10.1146/annurev.pp.42.060191.001525 (1991).
59. Shi, L., Cao, S., Chen, W. & Yang, Z. Blue light induced anthocyanin accumulation and expression of associated genes in Chinese bayberry fruit. *Sci. Hortic.* **179**, 98–102, https://doi.org/10.1016/j.scienta.2014.09.022 (2014).

60. Newsham, K. K. & Robinson, S. A. Responses of plants in polar regions to UVB exposure: a meta-analysis. *Global Change Biol.* **15**, 2574–2589, https://doi.org/10.1111/j.1365-2486.2009.01944.x (2009).

61. Kataria, S. & Guruprasad, K. Exclusion of solar UV radiation improves photosynthetic performance and yield of wheat varieties. *Plant Physiol. Biochem.* **97**, 400–411, https://doi.org/10.1016/j.plaphy.2015.10.001 (2015).

62. Zhang, N. & Portis, A. R. Mechanism of light regulation of Rubisco: a specific role for the larger Rubisco activase isoform involving reductive activation by thioredoxin-f. *Proc. Natl. Acad. Sci.* **96**, 9438–9443 (1999).

63. Vaštakaitė, V. *et al.* Effects of LED light spectra and irradiance level on mineral elements content in microgreens. XI International scientific and technical conference: Element cycle in the environment bioaccumulation-toxicity-Prevention, Warsaw, Poland (2015).

64. Li, Q. & Kubota, C. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environ. Exp. Bot.* **67**, 59–64, https://doi.org/10.1016/j.envexpbot.2009.06.011 (2009).

65. Samuoliéni, G. *et al.* LED illumination affects bioactive compounds in romaine baby leaf lettuce. *J. Sci. Food Agr.* **93**, 3286–3291, https://doi.org/10.1002/jsfa.6173 (2013).

66. Xie, B. & Wang, H. Effects of light spectrum and photoperiod on contents of flavonoid and terpene in leaves of *Ginkgo biloba* L. *J. Nanjing Forestry University* **30**, 51–54 (2006).

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Author Contributions
S.K.V. conceived the idea, carried out experiments, data analysis, prepared tables, figures, and wrote the manuscript. S.G. carried out data analysis, edited tables, figures, and wrote the manuscript. B.J.R. revised the manuscript. S.J.H. participated in the study design, coordinates and supervised the experiment, and revised the manuscript. We thanks Y.J. Kim and Dr. S. Muneer for their assistance with plant factory and RuBiSCo analysis, respectively. All authors read and approved the final manuscript.

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