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RESEARCH ARTICLE

Irradiation Studies of LED Light Spectra on the Growth and Development of Potato (*Solanum tuberosum* L.)

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ABSTRACT

The study aimed to explore the impacts of distinctive qualities of the LED light (such as to low power consumption, lesser production costs, longer operational lifetime and cool light emission with specific monochromatic wavelength) on potato (*Solanum tuberosum* L.) growth and development including plant height, number of leaves, root length, fresh and dry weight etc. The accumulation of phyto-pigments, soluble proteins and sugars, free radical scavenging activity and overall tuber yield were also evaluated. Enhanced plant height with increased diameter and branching was observed with the plant growing under the B_{100} and $R_{30}B_{70}$ LED light combination. Similarly, total number of leaves, leaf surface area, health index, phyto-pigments and tuber yield of potato was also significantly increased as compared to the plant growing under the W_{100} as control. Soluble proteins and sugar content and free radical scavenging enzyme activity were also significantly enhanced in the $R_{30}B_{70}$ LED light combination. Tubers yield per plants were also enhanced under the RB combination of the LED light. The current study indicated that the combination of R and B LED lights proved better for plant growth and development in a controlled environment and the $R_{30}B_{70}$ is the best combinational spectra for increased growth and tuber yield of potato plants. Therefore, the precise management of the irradiance and wavelength may hold promise in maximizing the economic efficiency of potato production, and quality of this important vegetables grown in controlled environments.

Introduction

The Potato (*Solanum tuberosum* L.) is a significant crop worldwide with higher nutritional as well as economic value. It is cultivated throughout the world with a production of 400 million tons every year (1-4). Irradiation of the light is an essential environmental factors affecting plant development (5). Plants respond to different factors of light including the quality (wavelength), quantity (intensity) and duration (6). Light has a remarkable effect on the seed propagation, leaf magnitude, leaf structure, plant stature, flowering and fruiting as well as the primary and secondary metabolites constituents of the plant (7-10).

The daily light integral (DLI) is a function of photosynthetic light intensity and duration which is usually expressed as moles of light (mol) per square meter (m^{-2}) per day (d^{-1}). An increase in total DLI via supplementary light promotes the growth of greenhouse-grown seedlings. High-pressure sodium (HPS) lamps have been used commonly for the maintenance of plants growing under the greenhouse conditions. The HPS lamp provides the light by emitting the spectral range of 565 to 700 nm which is important for photosynthesis and overall development including crop productivity. The gallium-aluminum-arsenide light-emitting diodes (LEDs) lighting system is modern lighting system has replaced the HPS lamp due to low power consumption, lesser production costs and longer operational lifetime, cool light emission at a specific monochromatic wavelength with user-friendly light intensity/quality that corresponds to the plant photomorphogenesis (11-13). Blue spectra of light were reported to promote the development of plant photosynthetic pigments,

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enhanced photosynthetic product and accumulation of the starch in storage organs (14). The potato plantlets growing under the exposure of red and blue light irradiating from common fluorescent lamps (using appropriate filters) which supported the plant growth and development under *in vitro* conditions (15). However, the reports on the axillary bud proliferation by red-light or far-red light in potato growing under the *in vitro* conditions as well as the increase of the leaf chlorophyll content of potato by red fluorescent light and blue light LEDs also confirm the influence of the varied light spectrum on the plant growth (16, 17). The blue spectrum of LED light also supported potato growth and development under the *in vitro* conditions *via* enhanced number of leaves, stomatal density and enhanced photosynthetic assimilate and biomass (18).

The absorption peak of photosynthetic pigments in plants falls between 440 nm (blue) and 660 nm (red) wavelength of light and therefore, the red, as well as blue light or their grouping, may offer proficient light spectrum for better growth and development (19, 20). Interestingly, the red and blue light combination was also found to enhance the photosynthesis at the seedling stage and overall plant growth and development (21-23). The plant generally harvests the visible electromagnetic spectrum coming from the sun. However, the visible range of light with a wavelength of 400 to 700 nm is effective for the carbohydrate synthesis through the photosynthetic reactions (22, 23). Effects of various LEDs spectrum have been studied in some agricultural and horticultural crops including the potato (*Solanum tuberosum* L. (15, 24)), *lilium* (*Lilium lancifolium* (22)), *Cymbidium lancifolium* (12), lettuce (*Lactuca sativa* (11, 25, 26)), *Eucalyptus globulus* (27)), *Salvia officinalis* bedding plants (28), wheat (*Triticum aestivum* (29)) and spinach (*Spinacia oleracea* (12, 5, 26)). Maximum studies focused on the function of blue light spectrum on chloroplast development, chlorophyll formation and stomata functioning. Though, the crop responses, in general, have not been broadly exploited, however, it was concluded that the red and blue monochromatic LED light or their combination were suitable for the overall plant growth and development at a definite ratio to meet the requirement of the light spectra effective for photosynthesis. Though each wavelength present in the visible range plays an important role in the synthesis of photosynthetic assimilates and plant development, the red and blue light influences most of the developmental pathways and also involved in tuber formation (tuberization) in case of potato. Therefore, the purpose of the current study was to investigate the influence of monochromatic red, blue LED light and their combination on potato growth and development and to select a suitable-LED light for commercial use of potato cultivation with minimum input. This study also discussed the influences of LEDs as red, blue, with its varied combinations on the growth, tuber yield, primary and secondary metabolites as well as free radical scavenging enzyme activity of potato and mechanism behind these morphological and biochemical changes.

Material and Methods

Plant material

Potato cultivar Kufri jyoti, released from the Central Potato Research Institute (CPRI, Meerut station India) which is widely grown in India, was taken for this study. The characteristics of the cultivar include early time to maturity (90-100 days), medium-size tubers and upright plant type. The potato tubers collected were washed in running water to remove soil attached with it and kept for sprouting under the dark humid chamber at 37^0C for a week. The experiments were done at Potato Phenomics laboratory established at Department of Biotechnology, Dr Harisingh Gour Vishwavidyalaya (A Central University), Sagar, Madhya Pradesh, India (23.8388° N, 78.7378° E). The plantlets sprouted were then transferred to the pots (25 cm) filled with agro peat mixed with coco-peat and sand (3:1 ratio) and maintained in the plant growth chambers (Matrix Eco India, size 1.5 m x 1.2 m x 2 m, providing a total growth area of 4.2 m^2 with 40-cm high clearance) under control temperature (22.2°C±0.8°C), and humid conditions (65%±7%). The experiments were conducted in between year 2016-2019 preferably in the months of October to February which is naturally favourable for the cultivation. The plants were fortified with 50 ml of Hoagland nutrient solution (diluted to 1/10) once a week otherwise plants were watered with tap water as per requirements that is standardized in the laboratory for better plant growth.

The light source and culture conditions

In this study, the LED light source was procured from the supplier (Jay Appliances Sagar, Madhya Pradesh, make Syska, India) with the corresponding R (650 nm) and B (460 nm) wavelength. The LED source comprised of longer stripe (5 × 100 cm) containing 40 LEDs, which created a light-emitting set with 100 µmol $m^{-2}s^{-1}$ total photosynthetic photon flux density (PPFD). The plants were subjected to the monochromatic Red (R) and Blue (B) LED such as 100% R (R_{100}), 100% B (B_{100}), and various combinations of RB such as 30% R- 70% B ($R_{30}B_{70}$), 50% R- 50% B ($R_{50}B_{50}$), 70%R - 30% B ($R_{70}B_{30}$) LED spectra. The W_{100} LED without supplementation of R and B LED used as a control. Wavelengths of R and B LED were procured from the company mentioned at 660 nm and 440 nm respectively. The photo period, photosynthetic photon flux density (PPFD), day/night temperature and relative humidity were 16/8 h (day/night), 210 µmol m^{-2} s^{-1}, 22±2 ^{0}C, 65±5% in the plant growth chambers respectively following protocol (47) with slight modification suitable for the experiments.

Evaluation of plant growth traits

The plant height (cm), stem diameter (in mm, and measured 5 cm above the ground), number of branches, number of leaves, leaf area, plant fresh and dry weight were quantified upon 15-day interval till the harvesting of potato. The plant health index, total phyto-pigments, starch, soluble proteins, soluble sugar, total phenolics, ROS scavenging enzyme and ascorbate contents were quantified as per standard protocols (17, 50). The health index was determined

using the equation: Health index = Stem diameter/Stem height x Dry mass. For each trait, 3 replicates with 5 plants were taken to the experiments. The tubers yield per plants (g), tuber size (mm) and tuber fresh/dry weight (g) were also analyzed upon the full maturity by harvesting potato tubers.

Study of leaf stomata

Fresh tender leaves collected from the plants growing under the LED light and translucent nail polish was applied to it a little apart from the main vein as and kept for drying as standardized in laboratory following the published protocol (30). A clear adhesive tape was pasted onto the leaf which was then softly peeled-off from the leaf which contains the leaf epidermal part. This was observed under a confocal microscope [Nikon LSCM (NIS-Elements AR 4.20.00 64-bit)] and quantification of stomata was done as per the standard procedure (30).

Extraction and estimation of phyto-pigments

Estimation of the chlorophyll content was done following the standard protocol (31) in the leaves of potato grown under the different LED treatments. The leaves (100 mg) were homogenized in 10 ml of ice-cold acetone (80%). The homogenate was centrifuged at 5000 g for 10 min (Hermle, Germany, Model Z326K) and the supernatant collected was used for estimation of Chl a, Chl b, Chl a/b ratio, total Chl and total carotenoids. Absorbance was recorded at 663, 645, 650, 510 and 470 nm in Spectrophotometer (Shimadzu, UV-160). Reference cuvette contained 80% acetone. Chlorophyll assay and carotenoids were estimated as reported earlier (32, 33).

Estimation of total carbohydrates, and soluble proteins

The soluble sugar was extracted as reported earlier and quantified using the phenol sulfuric acid method (34, 35). Concentrated sulfuric acid causes hydrolysis of glycosidic linkages; these hydrolyzed neutral sugars were then partially dehydrated with the elimination of three molecules of water to form furfural or furfural derivatives. The colored compounds developed by the condensation of furfural or furfural derivatives with phenol were measured at 490 nm. Starch from fresh leaf and tuber samples was estimated by the anthrone method (36). Soluble proteins were extracted using phosphate buffer and quantified using Coomassie-Brilliant Blue G-250 (37).

Estimation of total phenolics content

Total phenolics were extracted from leaves of potato using ethanol (80%) and estimated following the Folin–Ciocalteau reagent (38). The total polyphenols were estimated by spectrophotometer (Shimadzu - 1800, Japan) at 660 nm and calculated in comparison with a standard curve of gallic acid. Results were expressed as gallic acid per g FW.

Quantification of ROS scavenging enzyme activity

The crude proteins isolated from the leaves were used for the enzyme estimation. The SOD activity was evaluated by standard protocol (39). One unit of SOD was defined as the enzyme activity that inhibited the photo-reduction of nitroblue tetrazolium (NBT) to blue formazan by 50%, and SOD activity of the extracts was expressed as NBT units mg protein^{-1} min^{-1}. Estimation of the CAT activity was done following the standard protocol using spectrophotometric method via a decrease in absorbance of H_2O_2 at 240 nm which expressed as nmol of H_2O_2 decomposed mg protein^{-1} min^{-1} (40). The APX was assayed by the method as published and expressed as m mol of guaiacol oxidized mg protein^{-1} min^{-1} (41). One unit of APX activity was defined as an absorbance change of 0.01 units min^{-1}. The spectrophotometric assay of ascorbate content was done following the protocol reported earlier (42), which was based on the oxidation ascorbic acid to dehydroascorbic acid by bromine water in the presence of acetic acid. A coupling reaction with buffer containing the metaphosphoric - acetic acid solution and 2, 4-dinitrophenylhydrazine (DNPH) results in the development of a red complex and absorbance of the complex was measured at 521 nm at a spectrophotometer (Shimadzu1800, Japan).

Statistical analysis

The data were reported as the mean of three replicates (± standard deviations, SD). Data were analyzed by variance and Tukey's test (71) with the statistical package SPSS 15.0, unless otherwise stated.

Results

Effect on plant growth parameters grown under various LEDs light spectrum

The growth parameters such as the plant height, stem diameter number of leaves etc were significantly changed exposed to various LED light spectrum (Fig. 1). The plants exposed to the monochromatic R_{100} LED light spectra exhibited an elongation in plant height parameter (39.25±3.52 cm) with thinner stem diameter (0.57±0.13mm), and lesser number of leaves (52±7.1) while the plants exposed to B_{100} LED spectra stunted morphology (22.11±3 cm) with thicker stem diameter (1.59±0.2 mm) and improved branching (Table 1). Interestingly, the plants exposed to $R_{30}B_{70}$ combination spectra displayed elongated plant height (28.25±3 cm), improved stem diameter (0.543±0.17 mm) with multiple branches (up to 13.6±1 in number), enhanced plant fresh weight (24.5±8), dry weight (4.1±3), and health index (1.88±.2) than those of plants exposed to the W_{100} LED light. Other growth parameters such as number of leaves, and leaf surface area were also enhanced with the plants exposed to the $R_{30}B_{70}$ combination of LED spectra in comparison to the control W_{100} LED light (Table 1).

Stomatal analysis

The stomatal density (number of stomata per mm^2) in the plant leaves exposed to the B_{100} and $R_{30}B_{70}$ LED combination light spectra were improved up to 2.2 and 1.48 times in comparison to the plants exposed to the monochromatic R_{100} and W_{100} LED spectra respectively. Moreover, the stomatal index (%) also significantly enhanced with the plants exposed to the $R_{30}B_{70}$ combination of LED spectra (Table 2).

Fig.1. A representative photograph of the potato plants taken upon 4 weeks growing under different LED spectra.

The growth parameters of potato plants were prominently influenced by various LED light spectrum growing in the plant growth chamber. W_{100} denotes 100% white LED light ; R_{100}, 100% Red light; B_{100}, 100% Blue light; $R_{30}B_{70}$, 30% Red and 70% Blue; $R_{50}B_{50}$, 50% Red and 50% Blue; $R_{70}B_{30}$, 70% Red and 30% Blue.

Table 1. Morphological parameters of potato plantlets growing under various LEDs light spectrum

	LED W_{100}	LED R_{100}	LED B_{100}	LED $R_{50}B_{50}$	LED $R_{30}B_{70}$	$R_{70}B_{30}$
Plant height (cm)	36.37±2.41^{a}	39.25±3.52^{a}	22.11±3.00^{a}	31.75±2.09^{a}	28.25±3.11^{b}	33.25±2.51^{a}
Stem diameter (mm)	1.05±0.14^{a}	0.57±0.13^{a}	1.59±0.24^{a}	0.78±0. 28^{a}	1.22±0.17^{ba}	0.543±0.17^{b}
No. of branches	08.94±1.69^{ab}	09±1.74^{a}	12.05±0.82^{b}	10.5±1.50^{a}	13.61±1.70^{ab}	10.5±1.98^{b}
No. of leaves	67.69±10.25^{b}	52±7.196 ^{ab}	72.22±6.28^{ab}	62.58±4.19^{ab}	76.33±11.81^{b}	52.67±7.85^{ab}
Leaf area (mm^2)	12.15±2.15^{a}	08.88±1.72^{ab}	15.53±2.73^{b}	12.06±1.88^{a}	20.65±2.24^{a}	12.23±1.19^{ab}
Plantlet fresh weight (g)	17.4±7.26^{a}	11.88±6.10^{b}	24±7.11^{ab}	17±75.25^{b}	24.5±8.26^{a}	18.6±7.24^{b}
Plantlet dry weight (g)	2.3±2.20^{ab}	1.78±3.10^{bc}	3.5±3.11^{b}	2.2±2.14^{bb}	4.1±3.22^{a}	2.4±2.20^{a}
Health index	0.75±0.05^{ab}	0.48±0.15^{ab}	1.58±0.20^{ab}	0.65±0.12^{ab}	1.88±0.23^{a}	0.65±0.05^{ab}

Means followed by a different letter within a row are significantly different at P<0.05 according to the according to Tukey's test.
W, 100% White; $R_{100,}$ 100% Red; B_{100}, 100% Blue; $R_{30}B_{70,}$ 30% Red and 70% Blue; $R_{50}B_{50}$, 50% Red and 50% Blue; $R_{70}B_{30}$, 70% Red and 30% Blue
Means followed by a different letter within a row are significantly different at P<0.05 according to the Duncan's multiple range test.

Anatomical analysis of stomata revealed an elliptical stomata with the plant leaves growing under eventually all the LED light spectrum, however, a nearly spherical in shape stomata observed with the plant growing under the white LED (W_{100}). A comparatively small sized with narrower pore stomata observed with the plants exposed to R_{100} monochromatic LED light, while, enlarged with wider pore stomata were observed with the plant exposed to $R_{30}B_{70}$ LED combination spectrum (Fig. 2).

Biochemical Analysis of potato grown under LEDs light spectrum

A remarkable variation in different biochemical traits of the plants were observed with the plants growing under the monochromatic or the combined LED light spectrum. The biochemical analysis revealed a significant increase in the total chlorophyll (Chl a+b) pigments with the plant growing under the monochromatic B_{100} and the combined LED spectra. The $R_{30}B_{70}$ combination of LED light spectra caused 2 to 2.2 times more chlorophyll content in comparison with the plants growing under the R_{100} or W_{100} LED light spectra respectively. Besides, an increase in the ratio of chlorophyll a/b were also observed with the plants exposed to the $R_{30}B_{70}$ combination of LED light spectra (Table 3). A substantial increase in the carotenoids content were also observed with the plant growing under the $R_{30}B_{70}$ combination in comparison with W_{100} LED light, however, the carotenoids content was found to be minimum (0.16 ± 0.02 mg g^{-1} of FW) in the plant exposed to the monochromatic R_{100} LED light spectra.

Accumulation of carbohydrates (soluble sugars and starch) in the leaves and tubers were significantly improved with the plants exposed to the monochromatic R_{100} LED spectrum (160 mg g^{-1} FW)

Table 2. Effects of LED light spectrum on the size, shape and density of stomata in potato

Light Treatment	White	R_{100}	B_{100}	$R_{50}B_{50}$	$R_{30}B_{70}$	$R_{70}B_{30}$
Length (µm)	21.29±0.55 a	23.16±0.87 a	25.08±0.74 ab	25.28±0.47 a	25.97±0.53 ab	23.95±0.53 a
Width (µm)	17.86±0.45 a	17.55±0.55 a	17.79±0.55 ab	17.93±0.44 a	18.02±0.45 a	17.18±0.35 a
Length/Width	1.17±0.02 b	1.37±0.02 ab	1.42±0.02 b	1.41±0.02 a	1.44±0.04 a	1.39±0.02 ab
Stomata density (number per mm^2	168.91±9.06 ab	129.05±6.12 a	195.78±21.80 b	184.23±11.85 ab	249.96±17.50 b	189.58±21.05 b
Stomatal Index %	23.53±4.19 b	20.88±0.68 a	25.79±1.98 a	20.85±2.06 b	26.40±0.87 b	19.75±0.21 a

The RB combination ($R_{30}B_{70}$) improve size, shape and activity of the stomata. Stomata density and index were also higher in B_{100} and RB combination ($R_{30}B_{70}$) LED light in comparison with other LED light treatments.

Mean values followed by a different letter within a row indicate significant differences (P<0.05) according to Tukey's test. Values are mean ±SE of 5 replications of each set.

W, 100% White; $R_{100,}$ 100% Red; B_{100}, 100% Blue; $R_{30}B_{70,}$ 30% Red and 70% Blue; $R_{50}B_{50}$, 50% Red and 50% Blue; $R_{70}B_{30}$, 70% Red and 30% Blue.

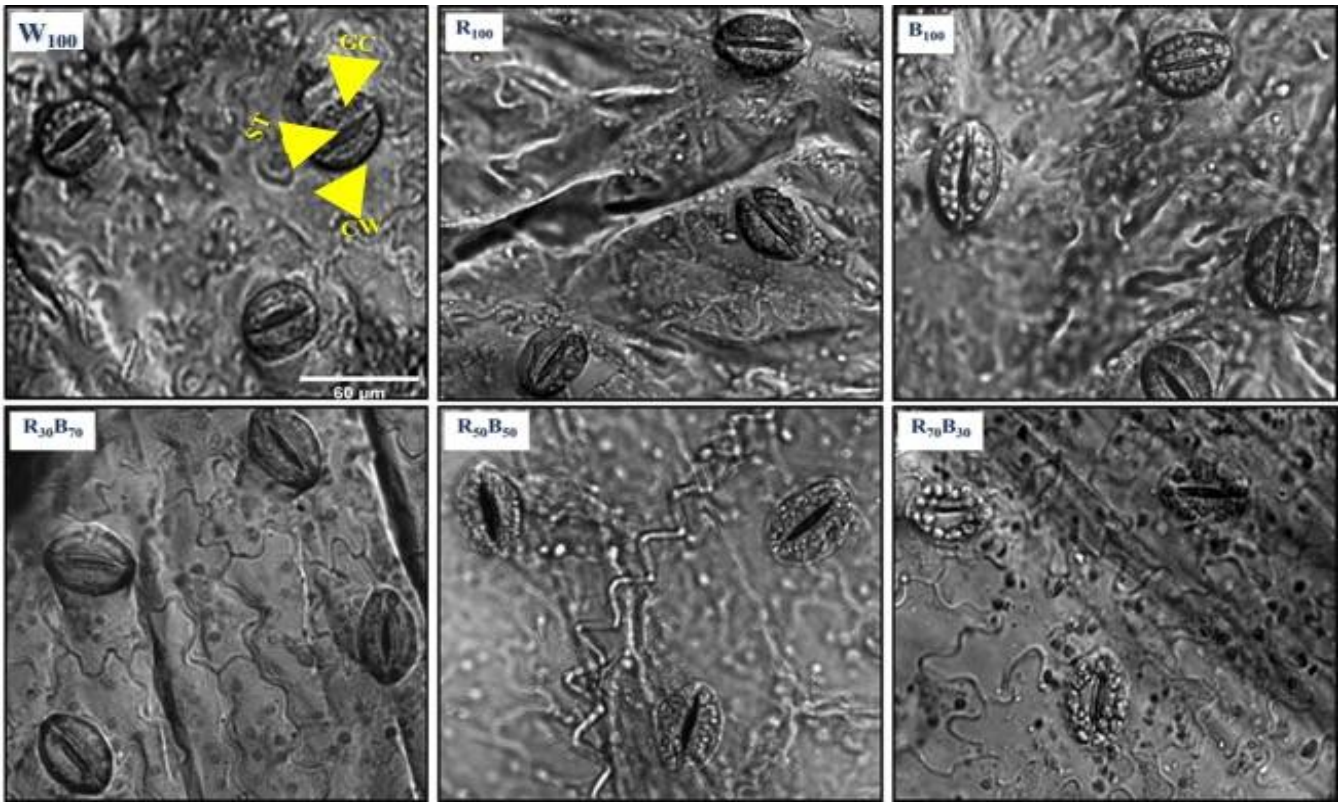

Fig.2. Effect of LED light spectra on leaf stomata of potato plants.

An elliptical-shaped stomata were observed with the plant leaves growing under all the LED spectra except W_{100} LED light. Stomata with small and narrow pore were observed with the plant growing under the R_{100} LED, while slightly bigger with a wider pore stomata were observed with those exposed to $R_{30}B_{70}$ LED combination.

Bar – 60 µm. ST, stomata; CW, cell wall; GC, guard cell.

Table 3. Effects of LEDs light spectrum on phyto-pigment content in potato

Light Treatment	White	R_{100}	B_{100}	$R_{50}B_{50}$	$R_{30}B_{70}$	$R_{70}B_{30}$
Chlorophyll- a (mg/g of FW)	0.64±0.07 a	0.59±0.08 b	0.89±0.10 ab	0.96±0.13 a	1.21±0.16 a	0.69±0.10 a
Chlorophyll-b (mg/g of FW)	0.22±0.03 a	0.20±0.04 b	0.28±0.05 a	0.33±0.05 b	0.38±0.08 a	0.22±0.04 b
Total chlorophyll-a+b (mg/g of FW)	0.86±0.10 b	0.79±0.12 ab	1.17±0.15 a	1.29±0.19 ab	1.59±0.24 a	0.91±0.14 a
Ratio (a/b)	2.01±0.15 b	1.92±0.16 a	3.23±0.18 b	2.22±0.09 b	3.19±0.21 ab	2.77±0.07 a
Carotenoids (mg/g of FW)	0.32±0.02 ab	0.16±0.02 a	0.31±0.02 ab	0.27±0.02 ab	0.37±0.03 b	0.19±0.01 ab

The photosynthetic pigment contents including the total chlorophyll (Chl a+b), carotenoids and Chl a/b ratio were significantly enhanced with the plant growing under the $R_{30}B_{70}$ combination of LED light spectra compared other light treatments.

Mean values followed by a different letter within a row indicate significant differences (P<0.05) according to Tukey's test. Values are mean ±SE of 15 plants.

and $R_{70}B_{30}$ combination of LED light (138.55 mg g^{-1} FW). However, a considerably reduced quantity of the carbohydrates was observed with the plants exposed to the monochromatic B_{100} LED light, followed by $R_{30}B_{70}$ combined LED light spectra (Fig. 3 A). Biochemical investigations of the soluble protein showed a significantly enhanced content with the plants exposed to in B_{100} LED light (8.45 mg g^{-1} FW), and combination of $R_{30}B_{70}$ light (7.42 mg g^{-1} FW), however minimum quantity of proteins were observed with the plants exposed to the monochromatic in R_{100} Led light (Fig. 3 B). The quantification of the total phenolic content in the leaf samples of the plants exposed to B_{100} and $R_{30}B_{70}$ LED light spectra showed enhanced quantity (4.82 mg g^{-1} FW and 4.55 mg g^{-1} FW respectively) as compared to all other LED treatments as represented in Fig. 3 C. It is important to emphasize that the overall soluble carbohydrates and proteins contents were enhanced with the plants maintained under the $R_{30}B_{70}$ combination of LED light.

Estimation of reactive oxygen species (ROS) scavenging enzyme

All the LED light spectrum significantly affected the ROS scavenging antioxidant enzymes which required for removal of the reactive free radicals produced in the cells. The plants exposed to $R_{30}B_{70}$ LED light combination showed enhanced enzyme activity of superoxide-dismutase (SOD, 33.32± 0.22 U mg^{-1} protein min^{-1}), catalase peroxidase (CAT, 6.77± 0.02 µM H_2O_2 mg^{-1} protein min^{-1}), and ascorbate-peroxidase (APX, 33.34± 0.15 U mg^{-1} protein min^{-1}) and total ascorbate content (AsA, 1.06± 0.02 mg g^{-1} FW) in comparison to all other LED light (Fig. 4 A-D). However, the monochromatic B_{100} LED also caused an increase in the enzyme activity in comparison with other monochromatic and W_{100} LED light as shown in the Fig. 4.

Tubers yield with the plants growing under various LED light spectra

The potato plants growing under various LED light spectrum also impacted on the tuberization efficiency and total yield in potato. The potato plants exposed to the monochromatic B_{100} LED light showed bigger size (>26 mm) and lower number of tubers per plant (4.67±1.20) with enhanced fresh and dry weight of tubers (Table 4). The plants growing under the monochromatic R_{100} LED light yielded small sized (<12 mm) and slightly increased number tubers (6.00±1.00) with decreased fresh and dry weight of tubers. However, the potato plants exposed to the $R_{30}B_{70}$ combination of LED spectra showed larger tubers (16-21 mm), increased number of tubers per plant (9.33±0.88) with enhanced fresh and dry weight

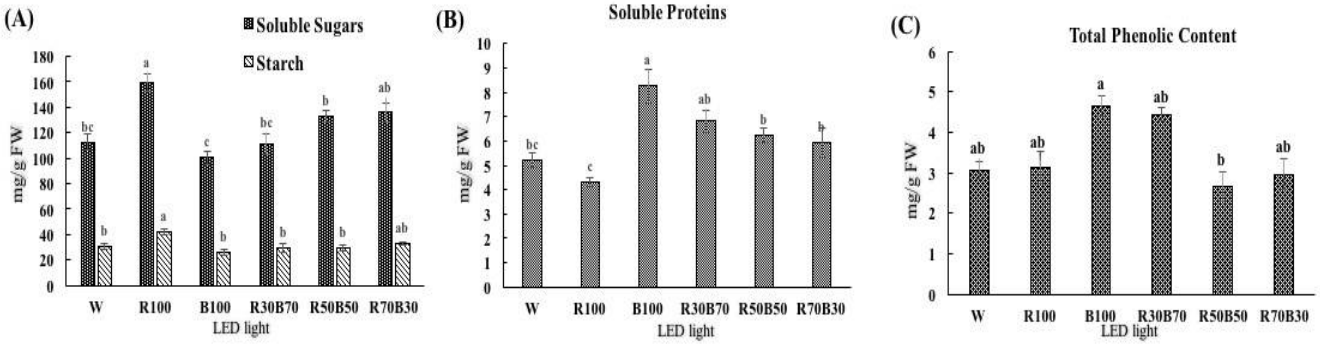

Fig. 3. Soluble carbohydrates, protein and phenolic content of the potato plants grown under the various LED light spectrum. Different letters in each bar graph indicate significant differences (P<0.05).

A. Soluble sugar and starch increased prominently with R_{100} and $R_{70}B_{30}$ combined LED light by 40% and 35% in comparison to the W_{100} .
B. Soluble proteins enhanced with B_{100} and $R_{30}B_{70}$ combined LED light by 75% and 55% in comparison to the W_{100}.
C. Total phenolic content also increased with B_{100} and $R_{30}B_{70}$ combined LED light by 76% and 71 % in comparison to the W_{100}.

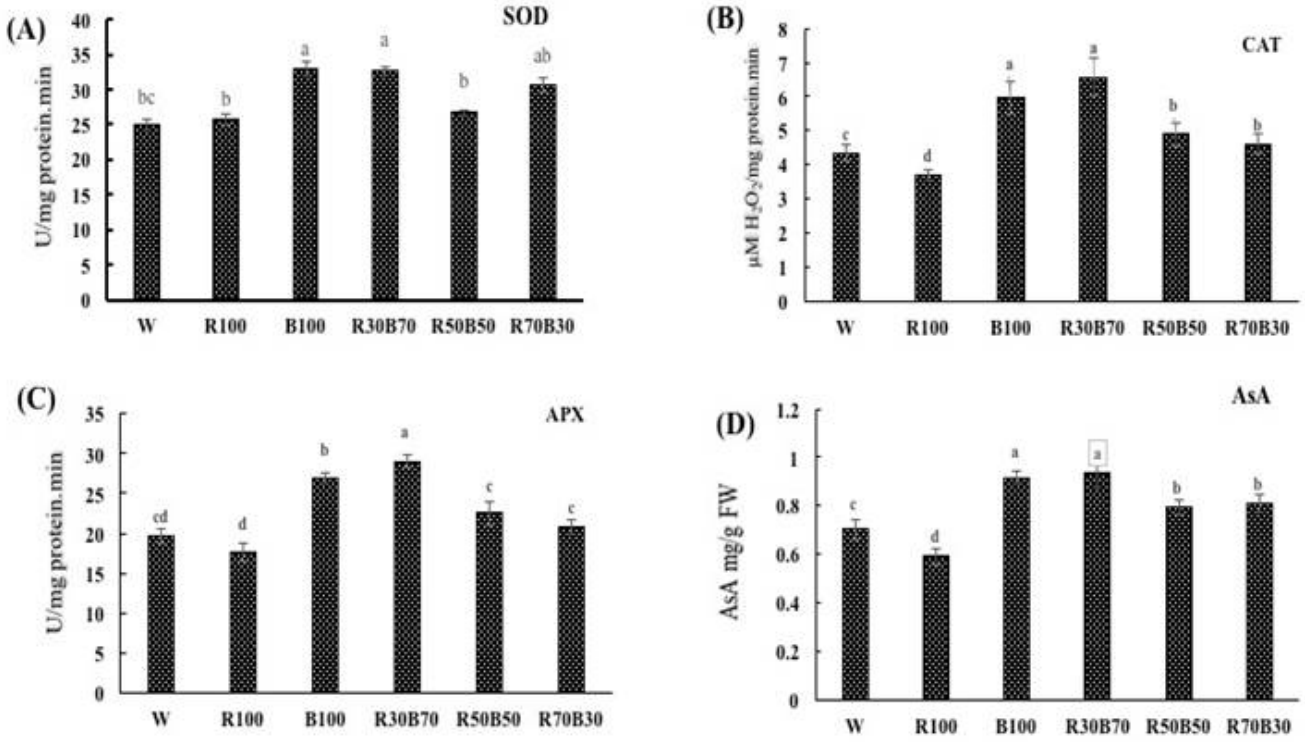

Fig. 4. Specific enzyme activity of ROS scavenging in potato plants grown under the various LED light spectrum.

SOD, CAT, APX, and ascorbate activities are represented from A–E. The values are presented as the mean ± SEM of three replicates. *, Mean was significantly different at P\0.01

as well as tuber yield per plants (252.61±27.73 g) in comparison with other combined or W_{100} LED light (Table 4).

Discussion

Improvement of growth parameters with combined RB spectra of LED light

This study was done to evaluate the changes in morphological and biochemical characteristics of potato plants maintained constantly under various LEDs light spectrum. It was observed that the plants grown under the monochromatic R_{100} LED light showed an thin and elongated stems, while stunted morphology with thicker stem and increased number of branches were observed with the plants grown under monochromatic B_{100} LED spectra. Similar observations were recorded in Norway Spruce (*Picea abies*) seedlings where red light illumination prompted elongated stems and blue light subdued the phenomenon (43). Biochemical analysis revealed that the red light spectra induced the biosynthesis of endogenous gibberellin (GA) level in plant twice to that of the plant growing under the blue light spectra. Changes in the stem elongation growing under the R_{100} LED or shortening of plant under the B_{100} LED might be due to alteration in the cellular GA level via the red and blue light-mediated GA signaling pathway. However, the combined $R_{30}B_{70}$ light spectra produced a balanced GA level in the plants and, therefore, the plant attained better growth parameters in terms of elongated stem length, diameter and enhanced branching. As reported earlier, the combined spectrum of RB LEDs was found to be more conducive for stem elongation than the normal white light (23, 44, 45). Overall, the results of this study also exhibited that the combined spectrum of B and R LED (at 30:70 combination) had a synergistic effect on stem growth.

The potato plants exposed to B_{100} LED spectrum exhibited an enhanced number of leaves with increased leaf area (Table 1). This might be due to increase in the plant hormone cytokinin growing

Table 4. Potato tuber yield grown under various LEDs light spectrum after 12 weeks period

Light Treatment	White	R_{100}	B_{100}	$R_{50}B_{50}$	$R_{30}B_{70}$	$R_{70}B_{30}$
Tuber size (mm)	18.26±1.54 a	15.59±0.91 a	33.75±1.91 ab	20.81±1.23 a	25.36±1.25 b	21.32±0.58 a
Total Tuber No. per plant	5.67±0.33 a	6.00±1.00 a	4.67±1.20 b	6.33±0.88 a	9.33±0.88 b	7.33±0.88 b
Tuber F.W. (gm)	20.72±2.07 b	13.34±1.45 a	26.81±1.10 b	23.88±2.12 a	28.97±4.23 ab	19.91±1.12 ab
Tuber D.W. (gm)	7.00±1.06 b	5.79±1.03 b	10.94±1.09 ab	10.25±1.06 b	13.67±1.03 b	7.00±1.02 a
Tuber Yield per plant (gm)	117.92±15.97 ab	77.75±9.77 ab	146.84±27.76 b	134.40±11.94 a	252.61±27.73 a	146.22±19.34 a

The data in table clearly indicates that $R_{30}B_{70}$ larger sized with increased tuber number and fresh and dry weight.

Mean values followed by a different letters within a row indicate significant differences (P<0.05) according to Tukey's test. Values are mean ± SE of 15 plants.

under the blue light spectrum, which also has been reported to augment the overall plant growth via increasing the plant leaf area and number (46, 47). Similar observations were also recorded in *Phaeodactylum tricornutum* where the blue light spectra augmented in the leaf development (48). The combined spectra of $R_{30}B_{70}$ showed enhanced number of leaves with better leaf area than the W_{100} due to the synergistic effect and balanced hormone biosynthesis in the plants. Similar observations were also recorded in other plant species such as grape (23), strawberry (45), upland cotton (49) and tomato (50). Since the plant leaf has the capacity to absorb R and B-light with a greater extend, hence, the combined spectrum of RB LEDs might deliver essential energy for photosynthesis to synthesize enhanced content of soluble protein, sugar and starch (51, 52).

The guard cells found in stomata surround the stomatal pore that regulates the gaseous exchange. Stomata also serve as hydraulic valves on the aerial parts of plants. light intensity affects the development of stomata as well as stomatal conductivity. Results showed the presence of an elliptical stomata with the plants grown under eventually all the LED light except with the plants grown under the W_{100} LED light a circular non-functional stomata observed (Fig. 2). This might be due to presence of fewer blue light spectra in the white LED light (W_{100}) leading to the development of spherical nonfunctioanl stomata while the blue light-induced the functionally better stomata in the leaves. Similar observations of round nonfunctional stomata with the potato growing under the while LED has been reported earlier (53). Smaller stomata with the plant growing under the R_{100} is also due to lack of blue spectra which has been reported to received directly by phototropin thus activating a signal cascade for the development of elliptically functional stomata in leaves (54-56). The result also showed that enhanced percentage of blue spectra augmented the development of functional stomata, therefore, presence of balanced spectrum was crucial for functional stomata which was observed with the plant maintained under the $R_{30}B_{70}$ combination of LED light. Similarly, the plant fresh and dry weight were also improved with the plant developed under the continuous presence of combined $R_{30}B_{70}$ light signifying that the collective spectrum or broad spectrum added maximum to biomass buildup in potato plants. A comparable result was also observed in other *in-vitro* cultured plantlets including the potato (13, 17, 57).

Biochemical analysis of potato plants grown under various LED light spectra

Biochemical assay of chlorophyll revealed an enriched total Chl (a+b) content in the plants maintained under the $R_{30}B_{70}$ combination and W_{100} LED light. Comparatively, a lower content of total Chl (a+b) were observed with the plants maintained under the R_{100} LED light spectra. In a similar experiment (58), the grapes plantlets were grown in presence of various spectra of LED light under *in-vitro* conditions and a significant inhibition in the chlorophyll (Chl) biosynthesis were observed with the plants exposed to the red LED light, while the blue light eradiation augmented the chlorophyll content in the leaves of the grapes. These results were also in consistent with our observations in the case of potato grown under various LED spectra. However, there are reports on increase in the total chlorophyll content with the plants grown under the monochromatic R_{100} light and decrease in B_{100} LED and vice versa also in different genotype of potato (59). Therefore, it seems that impact of the light spectrum on Chl biosynthesis is prominently species/ cultivar specific. A conclusion was drawn that a combined spectrum of $R_{30}B_{70}$ LED light spectra facilitated Chl biosynthesis in potato leaves which also induced the plants to growth with enhanced health index.

The light mediated regulation of carbohydrate and protein metabolism in plants has already been established. The monochromatic red light reported to play an important role in the synthesis and accumulation of starch, while blue light enabled the plant for enhanced biosynthesis of soluble proteins in grapes (58). Therefore, the combination of $R_{30}B_{70}$ light spectra was expected to induce the biosynthesis of both, the soluble carbohydrates and proteins as was observed in this study also which was in agreement of earlier report of induction of the cellular proteins and carbohydrates in alga, diatoms and in potato cultivar maintained under a light condition with the maximum percentage of blue light irradiation (17, 60, 61).

Protection from the photo-oxidative damage in the plant is reported to be achieved via synthesis of various antioxidants, carotenoids, tocopherols, ascorbate, and phenolic compounds (62, 63). Therefore, quantification of phenolic compounds was also done to find-out the light-mediated change in the antioxidant capacity of potato plant growing under various LED spectra. The content of total phenolic compounds in leaves was significantly increased in the plants growing under B_{100} and $R_{30}B_{70}$ combination of LED light as compared to that of W_{100} LED light (Fig. 3 C). On the other hand, the content of total phenolic compounds in leaves showed a decreasing trends under other LED light conditions. This result is also in consistent with earlier studies in the red lettuce where an enhanced phenolic content were observed with the plants maintained under the higher irradiation of the Blue light (64, 65). In our result, the content of polyphenols shown to be greatly increased in the presence of combined $R_{30}B_{70}$ (with enhanced percentage of Blue light) in vegetative tissues in plants supports earlier findings.

The antioxidant enzymes such as SOD, CAT, APX, and total ascorbate were also assayed through the spectrophotometric analysis with the potato maintained under various LED light conditions. The enzyme activities of the SOD, CAT and APX together with the cellular ascorbate pool was significantly enhanced in leaf tissues of potato grown under continuous B100 LED and combined $R_{30}B_{70}$ LED spectra in comparison to the W_{100} or R_{100} LED light. Report on the activation of antioxidant enzymes in winter rye leaves grown under the blue LED light treatment than that the red or far-red light treatment

has already been published (66). Similarly reports on increase in the activities of catalase (CAT), ascorbate peroxidase (APX) and other ROS scavenging enzymes in several plants grown under B and R LED light has been published in literature (67, 68). Moreover, an increase in the APX activity at the cellular level also indicated the increase in the total cellular ascorbate content (69). Taken together, the results also corroborated with earlier study that the potato grown under continuous presence LED light with increased Blue light percentage ($R_{30}B_{70}$ combination in present study) exhibited a positive effect on the activity of antioxidant enzymes in the potato which ultimately helped the plant to grow better and produced maximum tubers per plant in comparison with all other LED light irradiation.

Enhanced tuber yield with plant under RB LED spectra

It was estimated that the potato plants growing under the B_{100} LED spectra of light and combined spectrum of $R_{30}B_{70}$ produced enhanced size, increased tuber numbers and enhanced fresh and dry weight, in comparison with other combined or monochromatic R_{100} and W_{100} LED light. Since the assimilates distribution is also an important factor for tuber size or weight (70), it is possible that more assimilates efficiently partitioned to underground tubers in plant growing under the combined LED $B_{30}B_{70}$ spectra of light.

Conclusion

The current study showed that the combination of R and B spectra of LED light irradiation at a ratio of 30:70 exhibited promotional effect on the growth and development via augmenting the synthesis of total chlorophyll, soluble proteins and carbohydrates as well as ROS removing antioxidant enzymes in potato. The potato plants maintained under the combined spectrum of $R_{30}B_{70}$ also promoted tuber numbers per plant, larger size tuber with enhanced fresh weight and dry weight, in comparison with the plants grown under other LED light. In summary, the combined spectrum of red and blue ($R_{30}B_{70}$) LEDs was found suitable for plant development and recommended for the better growth and yield of early-maturing potato cultivated under the contained conditions.

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Authors' contributions

Conceptualization and designing of the research work by RKP and CPU; Execution of field/lab experiments and data collection by RKP and NJ; Analysis of data and interpretation by AP and NJ; Preparation of manuscript by CPU and DSB.

Conflict of interests

The authors declare that they do not have any conflict of interest.

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