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Effects of pre-harvest light intensity on the enhancement of the nutritional contents and antioxidant properties of wheatgrass

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Abstract This study showed that wheatgrass had the greatest growth with the 50IL treatment, whereas the 150C treatment yielded the slowest growth. Investigating the effect of light stress using the Fv/Fm ratio revealed that no light treatment resulted in stress-induced cell mortality, as all Fv/Fm values were >0.7. Chlorophyll, carotenoids, carotenoids, phenolic compounds, flavonoids, antioxidants, total nitrogen, crude fiber, mineral content, and vitamins B1 and B2 were also analyzed. The results demonstrated that extended exposure to both types of light enhanced the accumulation of antioxidants, carotenoids, and chlorophyll. Simultaneously, 50IL treatment boosted antioxidant levels and increased total nitrogen, crude fiber, mineral, and vitamin B1 levels compared with continuous light. Therefore, 50IL therapy is considered essential for enhancing the production of antioxidants and essential nutrients, thus aiding wheatgrass growth.

Keywords: Wheatgrass, Antioxidant, Nutrition, Photosynthesis, Stress

Introduction

Wheatgrass (*Triticum aestivum*) is a highly nutritious superfood or functional food. Wheatgrass is rich in nutrients that benefit the body. An analysis of its nutritional content shows that wheatgrass contains 70% chlorophyll, with a structure similar to that of hemoglobin but with magnesium at its core instead of iron. Thus, it can be used to treat thalassemia, anemia, and other disorders. Besides its high chlorophyll content, wheatgrass contains amino acids, minerals, vitamins, and phytochemicals (Chauhan, 2014; Ogutu *et al*., 2017). A previous report showed that wheatgrass at 5–20 days has high levels of thiamine (B1), riboflavin (B2), phenolic compounds, and antioxidants (Verma and Dubey, 2003; Niroula *et al*., 2019). Another study investigating the phytochemical constituents and antioxidants of wheatgrass juice found high phenolic compounds, including pyrogallol and vanillic, syringic, and ferulic acids (Chomchan *et al*., 2016).

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In plants, light-dependent processes and carbon dioxide fixation work together continuously during photosynthesis. Light energy is transformed into chemical energy during light-dependent processes, producing adenosine triphosphate (ATP) and reducing agents such as nicotinamide adenine dinucleotide phosphate (NADPH). These are then used during carboxylation to convert carbon dioxide into carbon molecules, including sugars, which plants may consume. Light greatly influences plant growth and development, and its duration, quality, and intensity play an important role in these processes. Various reports have indicated that light stimulates the production of phytochemicals and antioxidants in plants during cultivation, pre-harvest, and post harvest, enhancing the quality and nutritional value of agricultural products, including broccoli, lettuce, and basil. Furthermore, these studies have shown that light plays a significant role in biochemical changes within plants, including the accumulation of antioxidants and phenolic compounds within the cells (Barcena *et al*., 2019; Favre *et al*., 2018; Larsen *et al*., 2020). Phenolic compounds are common secondary metabolites found in plants. These compounds are synthesized through the shikimic acid pathway, starting with phosphoenolpyruvate (PEP) and D-erythrose 4-phosphate, subsequently converted to 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) (Almeida *et al*., 2024). Light-dependent processes are reportedly involved in the accumulation of phenolic compounds within cells, mediated by the shikimic acid pathway in chloroplasts.

Light acts as a stress inducer by generating reactive oxygen species (ROS), causing various cellular changes, including growth, photosynthesis, carbohydrate and lipid metabolism, osmotic balance, and gene expression (Hasegawa *et al*., 2000; Rosa *et al*., 2004). Generally, when plants experience stress, they activate stress tolerance mechanisms to cope, often involving a combination of stress avoidance and endurance mechanisms. Light-induced stress reduces photosynthetic efficiency, which is associated with a decrease in organic and inorganic compounds within plant cells. Stress conditions cause significant physiological and biochemical changes to maintain respiration and metabolic processes (Rosa *et al*., 2009). Therefore, the increase or decrease in organic and inorganic compound levels depends on the plant's mechanisms for stress response. Generally, plant resistance mechanisms can be triggered by short-term light exposure, thereby producing substances, including secondary metabolites and antioxidants, that protect against potential damage. Furthermore, it has been demonstrated that plants exposed to light have higher levels of minerals, vitamins, and chlorophyll (Wouyou *et al*., 2017; Miotto *et al*., 2021). The accumulation of flavonoids and phenolic compounds in foods, including red leaf lettuce and ginger, may result from light stimulation (Ghasemzadeh *et al*., 2010; Becker *et al*., 2014).

Additionally, light intensity is directly related to photosynthesis performance. Higher light intensities generally cause plants to conduct more photosynthesis. There was a report that a light intensity at 50–600 μmol $m^{-2}s^{-1}$ increased the yield and nutritional value of basil (*Ocimum basilicum* L.) cv. Emily and Dolly, and the exposure to light intensity at 150 μmol $m^{-2}s^{-1}$ resulted in the highest yield and nutritional value (Larsen *et al*., 2022). Moreover, the mechanism by which continuous light affects the nutritional quality of Eruca vesicaria L. leaves have been investigated. The use of continuous white light at 300 μmol$m^{-2}s^{-1}$ resulted in higher dry weight, sugar content, antioxidant compounds, fiber, and phytochemical contents (Proietti *et al*., 2021). Therefore, this study investigated the effects of moderate and high light intensities on enhancing the nutritional value of wheatgrass.

Materials and methods

Plant materials and treatments

Wheat seeds (*Triticum aestivum* L., cv. Fahng 60) with complete sizes and no disease or insects were selected at a quantity of 400 seeds per bag (total of 150 bags). They were soaked in distilled water for 24 h and cultivated in 4-inch pots (400 seeds per pot). The wheat seeds were grown under normal light conditions for 5 days, with the planting material used being peat moss. The experimental group was divided into five groups, with three replicates per group (each group had 30 pots).

Wheat seeds were exposed to light intensities of 50 and 150 μmol. m^{-2}. s^{-1}, with two lighting patterns (continuous and intermittent) for 3 days before harvest. Intermittent light means 2 h of light and 2 h of darkness, alternating with each other. The light source was LED (cool white light). The wheat seedlings that had been growing for 5 days were divided into five experimental groups as follows: normal light (control), continuous lighting at 50 μmol. m^{-2}. s^{-1} (50C), intermittent lighting at 50 μmol. m^{-2}. s^{-1} (50IL), continuous lighting at 150 μmol. m^{-2}. s^{-1} (50C), and intermittent lighting at 150 μmol. m^{-2}. s^{-1} (150IL). The wheatgrass were harvested at the end of the period and baked at 50 ^{o}C for 24 h. The samples were finely ground using a grinder at 10,000 rpm for 2 min (Retsch GM 200). They were analyzed for nutritional and antioxidant contents.

Growth

Plant height was measured daily from the pot's top to the tip of the wheatgrass. The obtained values were used to calculate the growth rate as

follows: growth rate (%) = (beginning value − ending value) × 100 / beginning value

Chlorophyll fluorescence

Chlorophyll fluorescence meters (Hansatech Instruments Ltd., Kings's Lynn Norfolk, UK) are specialized instruments used to measure the fluorescence emitted by chlorophyll molecules in plants. This fluorescence provides valuable information about the efficiency and health of the photosynthetic apparatus in plants. The parameters were measured as follows:

Fv/Fm	=	Variable fluorescence/Maximum fluorescence
PI	=	Performance index
ABS/RC	=	Absorption flux per reaction center
DIo/RC	=	Dissipation flux per reaction center
TRo/RC	=	Trapping flux per reaction center
ETo/RC	=	Electron transport flux per reaction center
REo/RC	=	Relative electron transport rate per reaction center

Chlorophyll and carotenoid contents

To extract and measure chlorophyll and carotenoids, Arnon's method (1949) was used. The solvent used for extraction was 80% acetone.

Phenolic compounds

The concentrations of total phenolic compounds in wheatgrass and its extracts were measured using the Folin-Ciocalteu assay (Ketsa and Atantee, 1998), with gallic acid as the standard. Sample absorbance was measured at 765 nm, and the results are expressed as mg per 100 grams.

Flavonoid content

The total flavonoid content of the wheatgrass was analyzed using the colorimetric method (Wolfe *et al*., 2003). In brief, 0.5 ml of the wheatgrass extract was mixed with 0.75 ml of 0.5% w/v $NaNO_2$, 0.75 ml of 10% w/v $AlCl_3$, and 0.5 ml of 1 N NaOH. The absorbance was measured at 510 nm. A calibration curve was prepared using a standard catechin solution. The results are presented as milligram of catechin per gram.

Antioxidant properties of DPPH, ABTS, and FRAP

The free radical-scavenging activity of wheatgrass was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, a commonly used method in scientific research to measure antioxidant capacity. Trolox was used as the standard substance (Brand-Williams *et al*., 1995).

The ABTS assay was performed using the method described by Stratil *et al*. (2006). The ABTS assay involves the generation of the ABTS radical cation (ABTS•+), which is a blue-green chromophore. In the presence of an antioxidant, it reduces ABTS•+, decreasing color intensity. The results are expressed as milligram equivalents of Trolox per 100 grams.

The FRAP assay (Ferric Reducing Antioxidant Power) was used to assess the antioxidant capacity of the wheatgrass using Trolox as a standard. It measured their ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions with an absorption maximum of 593 nm (Benzie and Strain, 1996).

Total nitrogen

The Kjeldahl method was used to analyze total nitrogen by digesting the sample with sulfuric acid (H_2SO_4) and measuring the ammonia released. The Kjeldahl method involves determining the protein content of food by assessing the total nitrogen content in the sample (AOAC, 2019).

Crude fiber

The analysis of the crude fiber content can involve the use of concentrated H_2SO_4 and NaOH for extraction, as stated in the AOAC method (AOAC, 2019).

Mineral

The minerals in the wheatgrass extract were analyzed using an inductively coupled plasma-optical emission spectrometer (ICP-OES) (AOAC, 2019).

Vitamins B1 and B2

The procedure for analyzing vitamins B1 and B2 was modified from Guenther's method (Santos *et al*., 2012), using PerkinElmer's high-performance liquid chromatography equipment. The analytical conditions included mobile phase A (90) containing 2.4% acetic acid, 15% methanol, 0.1 M potassium dihydrogen phosphate, and mobile phase B (10) containing acetonitrile. A

Quasar SPP C18 column (150 mm × 3.0 mm I.D., 2.6 µm particle size) was used with a column temperature of 25ºC, flow rate of 0.5 ml/min, UV detector set at 240 nm, and an injection volume of 10 µl.

Experimental design and statistical analysis

The entire experiment was conducted using a completely randomized design. All data were evaluated using one-way analysis of variance. Tukey's honestly significant difference test was used for mean separations. Significant differences were determined at $p \leq 0.01$.

Results

Impact of light on growth rate and stress response

Wheatgrass growth was studied by observing both height and growth rate (Figure 1). Wheatgrass that received 50 µmol.m^{-2}. s^{-1} of intermittent light (50IL) for 3 days before harvest showed the most growth, followed by continuous light at the same intensity (50C) and the control group. However, the growth rate was lowest under continuous lighting at 150 µmol.m^{-2}. s^{-1} (150C). On the last harvesting day, wheatgrass that received 50IL had the tallest height and fastest growth rate, measuring 16.60 ± 0.38 cm. and 64.55 ± 4.20%, respectively. Conversely, wheatgrass exposed to 150C had the shortest height and slowest growth rate, at 12.68 ± 0.31 cm and 14.06 ± 2.01%, respectively.

In this experiment, wheatgrass exposed to 50C and 50IL had the highest PI value. Plants under normal conditions (control) had the lowest value (0.99 ± 0.09), indicating that 50C and 50IL conditions yielded the highest photosynthesis efficiency. This is consistent with the growth rate of wheatgrass in this study (Figure 1B). The Fv/Fm values of wheatgrass grown under normal light (control) and 50C, 50IL, 150C, and 150IL were 0.75 ± 0.01, 0.79 ± 0.00, 0.80. ± 0.00, 0.77 ± 0.01, and 0.77 ± 0.02, respectively (Table 1).

Chlorophyll fluorescence meters can generally quantify energy fluxes through photosynthetic electron transport in plants and can be used to study ABS/RC, DIo/RC, TRo/RC, ETo/RC, and REo/RC values (Table 1). It was found that wheatgrass of the different light treatment groups had lower ABS/RC, DIo/RC, TRo/RC, ETo/RC, and REo/RC values than those of the control group.

Result showed the chlorophyll and carotenoid levels in wheatgrass grown under regular light conditions (control) and under various light patterns 3 days before harvest (Figure 2). The study revealed that the 50C treatment resulted in the highest levels of chlorophyll and carotenoid at 91.26 ± 1.04 and 46.69 ± 0.48

mg.100g^{-1}, respectively, whereas the control group had the lowest levels a 56.85 ± 2.18 and 30.80 ± 1.34 mg.100g^{-1}, respectively.

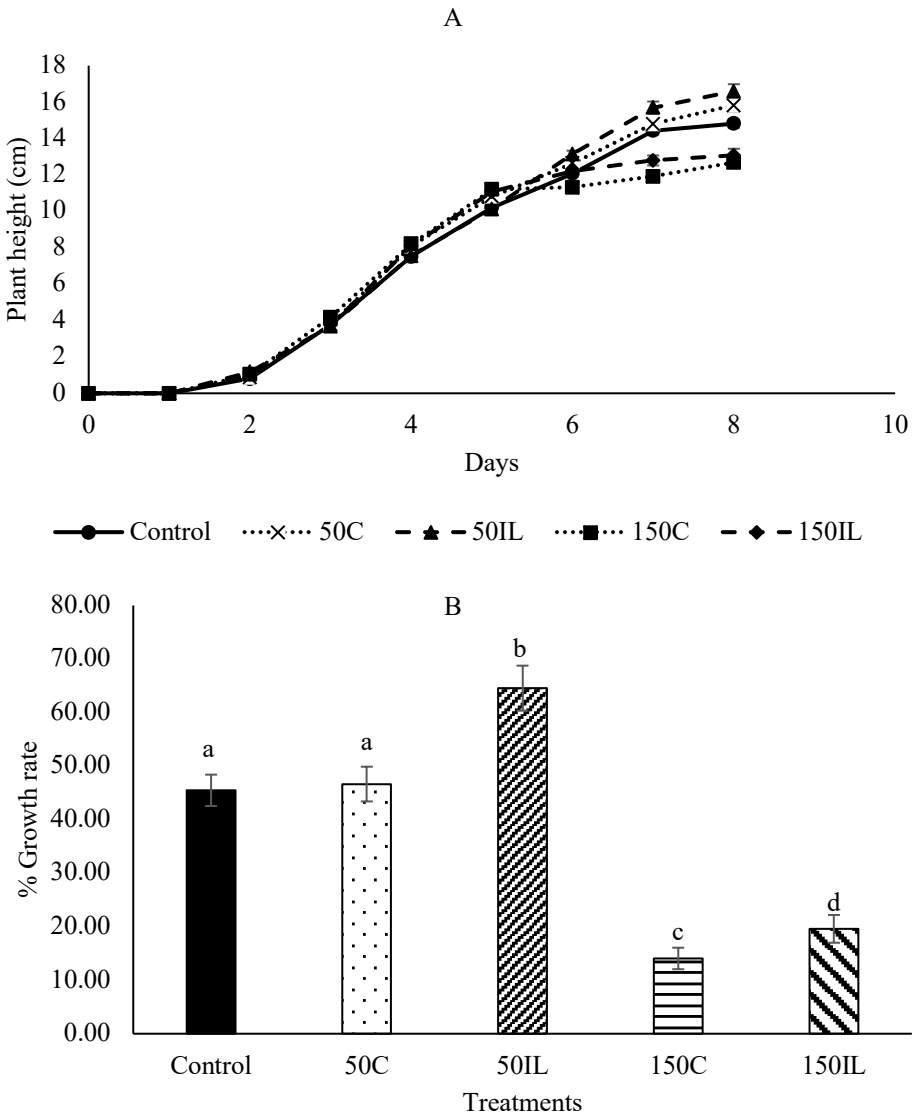

Figure 1. Effects of light on wheatgrass growth. (A) plant height; (B) growth rate. Data are presented as mean ± SE (n = 3). Different lowercase letters above the bars indicate significant differences ($P < 0.01$) between treatment groups

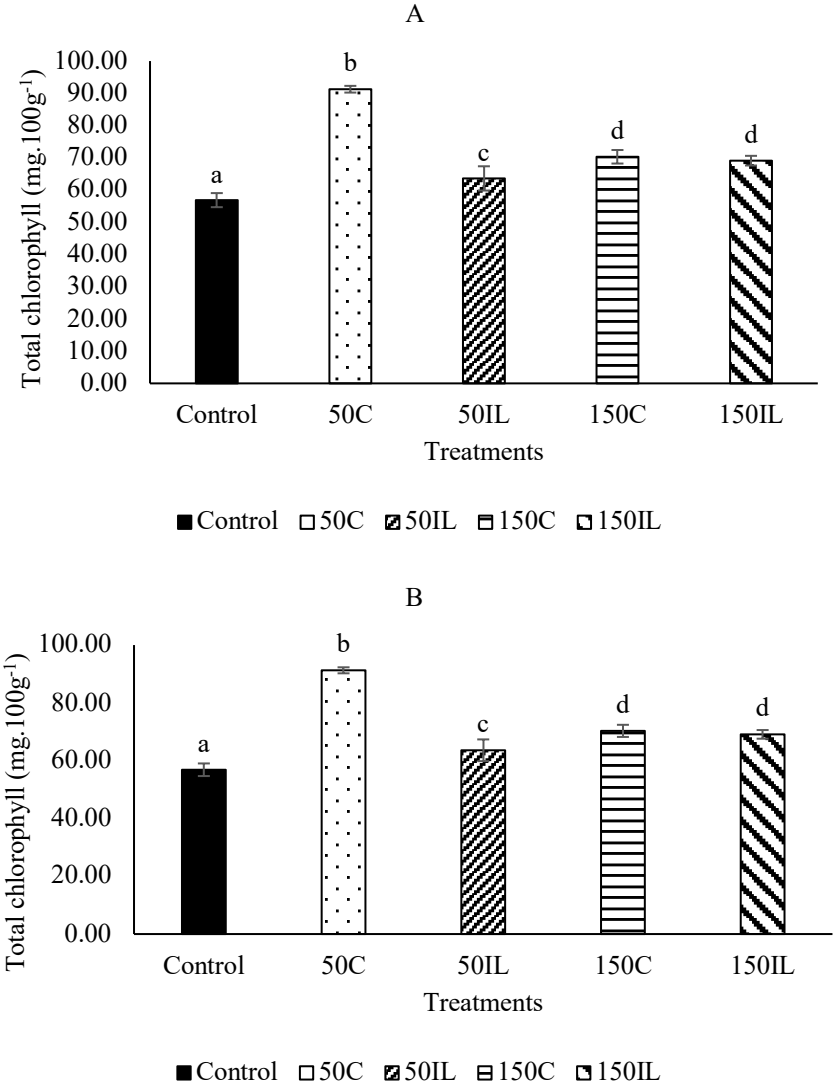

Figure 2. Effects of light on wheatgrass pigment. A) chlorophyll content; B) carotenoid content. Data are presented as mean ± SE (n = 3). Different lowercase letters above the bars indicate significant differences ($P < 0.01$) between treatment groups

Impact of light on antioxidants

The accumulation of phenolic compounds in wheatgrass was highest in the 150C treatment group at 20.90 ± 0.03 mg.100g^{-1}, whereas the phenolic

compounds in the control and the other lighting treatment groups differed insignificantly (Table 2).

In this study, wheatgrass subjected to light under the 50C, 150C, and 150IL conditions showed a notably higher level of flavonoids than the control and 50IL treatment groups (Table 2). No significant difference was found between the light intensity upon continuous light exposure and the flavonoid content. Conversely, intermittent lighting at 150 μmol. m^{-2}. s^{-1} had a stronger impact than that at 50 μmol. m^{-2}. s^{-1}. Therefore, light intensity and pattern both influence flavonoid content. This outcome indicates that the wheatgrass in the 50C, 150C, and 150IL treatment groups had more flavonoids than that in the control and 50IL treatment groups. The findings showed that the 50C and 150C treatments were equally successful in triggering flavonoid production. The findings showed that the 50C and 150C treatments had the same impact on promoting flavonoid production. Although the 150IL treatment had a stronger impact than the same light pattern in the 50IL treatment group, it is evident that the flavonoid quantity is influenced by both light intensity and pattern. The 50IL treatment may not have induced enough stress to facilitate the production of flavonoids or phenolic compounds.

DPPH, $ABTS^{\bullet+}$, and FRAP assays were used to assess antioxidant properties (Table 2). DPPH is commonly used to assess the ability of chemicals to eliminate free radicals by measuring antioxidant levels. A high DPHH value indicates that the test substance has a strong antioxidant capacity. In this study, wheatgrass treated with 150IL showed the highest DPPH value at 15.47 ± 0.30 mg Trolox.$100g^{-1}$. The 150C, 50IL, 50C, and control groups had Trolox levels of 13.81 ± 0.25, 11.18 ± 0.17, 6.64 ± 0.84, and 5.70 ± 0.43 mg Trolox.$100g^{-1}$, respectively. However, there was no significant difference in the DPPH values between the wheatgrass exposed to 50C and the control group. High-intensity light was found to be more effective in enhancing antioxidant activity than low-intensity light.

The ABTS assay revealed that wheatgrass treated with 50C, 150C, and 150IL had greater $ABTS^{\bullet+}$ values than those treated with 50IL and the control. Applying a strong light intensity of 150 μmol. m^{-2}. s^{-1} triggered antioxidant production, leading to elevated ABTS levels. Continuous light at 50 μmol. m^{-2}. s^{-1} enhanced antioxidant production more effectively than intermittent light at the same light intensity.

FRAP is a method for assessing a substance's antioxidant capacity based on its ability to convert Fe^{3+} to Fe^{2+} through reduction. The 150IL treatment group had the highest FRAP value at 19.08 ± 0.07 mg of 100. g^{-1} Trolox. The FRAP values of the control, 50IL, 50C, and 150C treatment groups differed insignificantly. Regarding antioxidant properties, wheatgrass exposed to 50C,

150C, and 150IL light treatments had elevated ABTS levels compared with those in the 50IL and control groups (Table 2).

Impact of light on nutritional values

The total nitrogen content of any food is the total amount of nitrogen contained in the food. This may originate from different sources, including amino acids, proteins, inorganic nitrogen, and other nitrogen-containing substances (Jones, 1994). In this study, wheatgrass that was exposed to intermittent light at 50 μmol. m^{-2}. s^{-1} had the highest total nitrogen content at 5.76 ± 0.01% (Table 3). Additionally, total nitrogen content correlated positively with wheatgrass growth (Figure 1). Similarly, wheatgrass treated with 50IL showed the highest crude fiber percentage at 52.18 ± 0.10%, followed by treatment with 50C, 150IL, 150C, and control (Table 3).

Crude fiber represents the number of undigestible materials, including cellulose, pentosans, and lignin, as well as similar components found in various foods. This study found that wheatgrass treated with 50IL had the greatest amount of crude fiber, suggesting that light intensity influences fiber production in wheatgrass (Table 3).

Exposure to light affected the levels of vitamins B1 and B2 in wheatgrass, influencing their accumulation. This study showed that the 50IL treatment group had the highest amount of vitamin B1 but the lowest amount of vitamin B2. However, wheatgrass cultivated under normal conditions (control), containd significantly more vitamin B1 and B2. In particular, vitamin B2 had the highest concentration in the different light treatments (Table 3). Vitamin B levels in plants are influenced by the amount of light they receive and the stress they endure. Research on the levels of vitamin B1 and B2 in wheatgrass shows that light influences the levels of these vitamins. The highest vitamin B1 level was found in the 50IL treatment group, whereas the lowest vitamin B2 level was also detected in the same group. It was found that the vitamin B1 and B2 levels were significantly high in wheatgrass grown under normal conditions (control), with vitamin B2 being the most abundant among the different light treatments.

This study examined the composition of eight minerals, including zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), phosphorus (P), magnesium (Mg), calcium (Ca), and potassium (K). It was found that light increased mineral content in wheatgrass compared with the control (Table 4). Wheatgrass exposed to 50IL had high levels of Zn, Fe, Mn, Cu, P, Ca, and K. The abundance of minerals supports wheatgrass development. Treatment with 50IL yielded the highest levels of Zn, Fe, Mn, Cu, P, and Mg: 46.21 mg. kg^{-1}, 62.27 mg. kg^{-1}, 50.55 mg. kg^{-1}, 14.75%, and 1.26%, respectively.

Wheatgrass exposed to 50IL exhibited the highest concentrations of Zn, Fe, Mn, Cu, P, Ca, and K. Wheatgrass in the 50IL treatment group exhibited the most optimal growth directly linked to elevated mineral levels. Moreove, wheatgrass grown in the 50C treatment group exhibited the highest magnesium accumulation at 0.22%, whereas those grown in the control group exhibited the lowest magnesium content at 0.17%. Compared with the other treatments, the 150IL treatment group exhibited the highest potassium content (2.46%), whereas the control group exhibited the lowest potassium content (2.22%).

Discussion

Impact of light on growth

Wheatgrass that received 50IL had the tallest height and fastest growth rate. This demonstrated that bright light and consistent intensity slowed wheatgrass growth. Light is crucial for plant photosynthesis. Plants require different levels of light intensity depending on their variety and growth stage. Moderate-intensity light is ideal for plant growth, improving photosynthesis efficiency. Excessive light intensity can hinder photosynthesis due to cellular damage (Hopkins and Huner, 2008).

The Fv/Fm values of wheatgrass grown under normal light (control) and 50C, 50IL, 150C, and 150IL were 0.75 ± 0.01, 0.79 ± 0.00, 0.80. ± 0.00, 0.77 ± 0.01, and 0.77 ± 0.02, respectively. This indicated that the various types of lighting did not affect stress or mortality because the Fv/Fm value was >0.7. The chlorophyll fluorescence value is a measurement of the fluorescence of chlorophyll molecules in plants. According to Maxwell and Johnson (2000), Fv/Fm and PI values can be used to assess the stress level and photosynthesis efficiency of plants, respectively. The parameter commonly used to assess Photosystem II (PSII) efficiency in photosynthesis is the Fv/Fm value, which can provide insights into plant stress levels. Fv/Fm values below 0.7 indicate severe impairment of the PSII system and a state of stress leading to death. The Fv/Fm ratio is typically 0.7–0.8 (Bhagoolia *et al*., 2021). The Fv/Fm ratio of wheatgrass under different treatment conditions was at least 0.7. The experiment revealed that lighting did not impact stress or mortality. Moreover, the DIo/RC values can also indicate stress levels. High DIo/RC values suggest that plants are stressed because they are emitting additional heat energy, which is a sign of stress. In every light treatment, wheatgrass showed low DIo/RC compared with the control unit. Therefore, we can confirm that lighting did not influence plant stress levels in this study. Wheatgrass grown under low-light conditions (control) exhibited increased stress levels and a decrease in growth rate.

Table 1. Chlorophyll fluorescence of wheatgrass

Treatments	Chlorophyll fluorescence						
	Fv/Fm	PI	ABS/RC	DIo/RC	TRo/RC	ETo/RC	REo/RC
Control	0.75±0.01a	0.99±0.09a	2.87±0.06a	0.72±0.04a	2.15±0.03a	1.11±0.01a	0.56±0.02a
50C	0.79±0.00b	1.60±0.08b	2.36±0.06b	0.50±0.02b	1.86±0.04b	1.01±0.02b	0.44±0.01b
50IL	0.80±0.00c	1.67±0.06b	2.38±0.03b	0.48±0.01b	1.90±0.02b	1.03±0.01b	0.45±0.01b
150C	0.77±0.01a	1.17±0.11a	2.47±0.09b	0.57±0.04c	1.89±0.06b	0.91±0.03c	0.41±0.01c
150IL	0.77±0.02a	1.60±0.12b	2.37±0.19b	0.61±0.14d	1.76±0.06b	0.97±0.03b	0.47±0.03b

The values are presented as mean ± SE, n = 15. Lowercase letters in the same row indicate significant differences among different treatments of wheatgrass.

Table 2. Antioxidant properties of wheatgrass

Treatments	Phenolic compounds (mg.100g^{-1})	Flavonoids (mg of catechin.g^{-1})	DPPH (mg of Trolox)	ABTS•+ (mg of Trolox)	FRAP (mg of Trolox)
Control	19.23±0.18a	1045.70±40.92a	5.70±0.43a	154.14±0.84a	17.52±0.26a
50C	18.94±1.21a	1403.52±22.37b	6.64±0.84a	171.01±0.03b	18.19±0.12b
50IL	20.19±0.10a	1134.62±47.25a	11.18±0.17b	141.95±4.32a	17.49±0.24a
150C	20.90±0.03b	1360.67±47.32b	13.81±0.25c	174.06±4.77b	18.08±0.05b
150IL	18.89±0.37a	1454.32±21.72b	15.47±0.30d	163.49±8.43b	19.08±0.07c

Note: The values are presented as mean ± SE, n = 3. Lowercase letters in the same row indicate significant differences among different treatments of wheatgrass.

Table 3. Nutritional composition of wheatgrass

Treatments	Nutrition in wheatgrass			
	Crude fiber (%)	Total nitrogen (%)	Vitamin B_1 (μg. g^{-1})	Vitamin B_2 (μg. g^{-1})
Control	22.23± 0.28a	5.46±0.02a	0.049±0.001a	0.047±0.000a
50C	41.19±0.17b	5.36±0.03b	0.040±0.000b	0.044±0.002b
50IL	52.18±0.10c	5.76±0.01c	0.056±0.001c	0.037±0.002c
150C	25.96±0.29d	5.46±0.00a	0.040±0.000b	0.036±0.002c
150IL	28.76±0.32e	5.52±0.03d	0.040±0.002b	0.034±0.002c

Note: The values are presented as mean ± SE, n = 3. The lowercase letters in the same row indicate significant differences among different treatments of wheatgrass.

Table 4. Mineral content of wheatgrass

Treatments	Mineral content							
	Zn ($mg.kg^{-1}$)	Fe ($mg.kg^{-1}$)	Mn ($mg.kg^{-1}$)	Cu ($mg.kg^{-1}$)	P (%)	Mg (%)	Ca (%)	K (%)
Control	42.47a	59.15a	33.44a	12.56a	1.14a	0.17a	0.15a	2.22a
50C	44.59b	63.13b	44.23b	13.68b	1.19b	0.22b	0.20b	2.36b
50IL	46.21c	62.27b	50.55c	14.75c	1.26c	0.21c	0.20b	2.46c
150C	38.50d	55.70c	44.72b	11.60d	1.13a	0.20d	0.18c	2.43d
150IL	40.74e	59.70a	42.88b	11.96e	1.18b	0.20d	0.19b	2.44e

Note: Lowercase letters in the same row indicate significant differences among different treatments of wheatgrass.

Additionally, wheatgrass of the different light treatment groups had lower ABS/RC, DIo/RC, TRo/RC, ETo/RC, and REo/RC values than those of the control group. The DIO/RC values played a vital role in determining the equilibrium between using photochemical energy and dissipating non-photochemical energy in plants, providing valuable information on plant well-being and responses to stress. If the plant has a high DIo/RC value, it indicates that it is stressed because more of the absorbed light energy is released as heat or fluorescence instead of being used for photosynthesis reactions. According to the results, wheatgrass that was exposed to light had lower values than the control group. Reduced energy flow may signal different stress conditions that affect photosynthesis efficiency and plant overall health. The decreased electron transport rate in the photosynthetic electron transport chain is indicated by the low values, possibly due to issues in electron transport between PSII and PSI or constraints in the electron acceptors like $NADP^+$. However, the short duration of light exposure did not impact the stress-induced deaths in these experiments. Plants make physiological and molecular adjustments to defend themselves against damage during short-term stress, including heat, cold, drought, or flooding. It is used different methods, including the production of stress response proteins, alteration of leaf surface cells, and regulation of the opening and closing of stomata (Chaves *et al*., 2009; Bita and Gerats, 2013; Dong *et al*., 2014).

Chlorophyll and carotenoids play significant roles as pigments in plants, algae, and certain bacteria. They are crucial during photosynthesis, as light is absorbed and transformed into chemical energy (Hopkins and Huner, 2008). This process is crucial for plant growth and function and plays a vital role in supporting human health by maintaining balance in the body. Plant antioxidants can lower the risk of various illnesses and enhance the immune system (Martins *et al*., 2023). The group treated with 50C had the highest chlorophyll and carotenoid levels, in contrast to the control group. Light intensity directly affects the production of chlorophyll and carotenoids, with higher intensity promoting increased synthesis of these pigments (Niroula *et al*., 2019; Elango *et al*., 2023).

Impact of light on antioxidants

Regarding phenolic compounds in wheatgrass, the 150C treatment group had the greatest accumulation of phenolic compounds compared with the other treatments. Therefore, it was proposed that phenolic compounds are involved in plant stress, with sustained high-intensity light exerting the greatest effect on plant stress. Stress triggers the production of phenolic compounds in cells as a defense mechanism. Phenolic compounds are crucial for various plant biological functions, including growth, pest defense, and response to stressful conditions.

Biochemical changes occur in seeds during germination, along with alterations in the nutrients as they transform into seedlings. Enzymes in the seeds are activated to break down molecular compounds into smaller ones, increasing the levels of vital minerals and decreasing those of unnecessary nutrients. Additionally, antioxidant and phenolic compounds are generated during germination (Benincasa *et al*., 2020). According to previous reports, stress in plants can boost the production of phenolic compounds via biological pathways such as phenylpropanoid metabolism (Dixon and Paiva, 1995). Light is crucial for triggering the production of phenolic compounds, including ferulic acid, quercetin, and caffeic acid, in plants. Phenolic compounds found in plants offer various advantages to humans, including antioxidative activities and reducing the risk of cancer (Lai *et al*., 2022; Kotton *et al*., 2022). A previous study revealed that basil leaves (*Ocimum basilicum* L.) had increased levels of antioxidants, including vitamin C, and phenolic compounds, including rosmarinic and chicoric acids, when subjected to LED light at intensities of 50–600 μmol. m^{-2}. s^{-1} for 5 days after harvesting (Larsen *et al*., 2022).

Wheatgrass in the 50C, 150C, and 150IL treatment groups contained higher levels of flavonoids compared to the control and 50IL treatment groups The results indicated that both the 50C and 150C treatments were equally effective in inducing flavonoid production. Flavonoids are present in various plants, and their concentrations are influenced by both the species and the environmental factors during development. Flavonoids are classified as antioxidants that protect plants against sunlight damage, have antioxidant properties, and have anti-pathogenic properties in some plants (Cushnie and Lamb, 2005; Ullah *et al*., 2020). Flavonoid production starts with the phenylpropanoid pathway, in which the enzyme PAL converts phenylalanine into 4-coumaroyl-CoA. It then begins to participate in the biosynthesis pathway of flavonoids (Martins *et al*., 2023). Conversely, the 50IL treatment might not have triggered sufficient stress to promote the synthesis of flavonoids or phenolic compounds. Reports indicate that light induces intracellular stress and triggers ROS production. Plants possess innate defenses that shield them from different stressors. Flavonoids are produced by plants to enable them to adjust and react to varying surroundings, enhancing their ability to withstand unfavorable conditions and ultimately aiding their survival (Winkel-Shirley, 2002; Treutter, 2005).

The antioxidant properties were evaluated using the DPPH method, a common technique for assessing the ability of to neutralize free radicals. A high DPPH value signifies a substance's capacity to counteract free radicals, suggesting strong antioxidant properties. The highest DPPH value was found in wheatgrass treated with 150IL, followed by treatment with 150C. This outcome

suggested that antioxidants were increased more effectively by high-intensity light than low-intensity light. Reports have indicated that photosynthesis and respiration in organisms release energy and produce ROS in cells. To protect against ROS-induced damage, plants activate a protective mechanism known as tolerance metabolism, which involves antioxidant production to manage stress (Rosa *et al*., 2009). Antioxidants play a crucial role in human health by providing protection and minimizing free radical damage. Cell damage is caused by free radicals, elevating the likelihood of chronic conditions, including cardiovascular diseases and cancer. Cell damage occurs because of the generation of free radicals and oxidants, leading to a condition known as oxidative stress. Consequently, the antioxidant properties in wheatgrass can be boosted by light, enhancing its nutritional content.

Continuous and intermittent high-intensity light can stimulate an increase in the level of antioxidants, leading to elevated ABTS levels. The values of antioxidant activity measured using the DPPH and ABTS methods differed due to potential variations in the limitations of the methods. DPPH is appropriate for examining substances with lower polarity, whereas ABTS is appropriate for examining substances with higher polarity, as well as both polar and non-polar substances (Re *et al*., 1999). Similarly, the FRAP value is an additional indicator of antioxidant capacity. The highest FRAP values were observed in the wheatgrass treated with 150IL. The analysis of the three antioxidant methods (DPPH, ABTS, and FARP) demonstrated that high-intensity light had a more antioxidant-stimulating effect than low-intensity light. This result aligns with the increased levels of phenolic compounds and flavonoids observed in this study.

Impact of light on nutritional values

The total nitrogen content was highest with 50IL treatments, showing a direct relationship with wheatgrass growth, as nitrogen is crucial for processes, including synthesis of protein, nucleic acid, and chlorophyll in plants. High growth rates lead to a higher nitrogen percentage, consistent with previous findings that the overall nitrogen content in wheatgrass changes according to growth factors, including soil type, nutrient management, and environmental conditions (Li *et al*., 2024). Light is the primary factor for the generation of energy in the form of ATP and NADPH, which are necessary for various cellular functions, including protein synthesis. Therefore, light plays a vital role in the growth and development of leaves and roots, which are the parts with the highest nitrogen accumulation. According to Taiz and Zeiger (2002), nitrogen is stored in leaves as protein and chlorophyll.

This research revealed that wheatgrass exposed to 50IL exhibited the highest levels of crude fiber, indicating that light intensity affects fiber synthesis in wheatgrass. The 50IL treatment group exhibited the highest photosynthesis efficiency due to its correlation with elevated Pindex values. Reports indicate that light affects plant stress and that stress influences fiber content in plants. Plants under high stress levels produce more fiber to reinforce their cells or defend against damage in a changed environment. Fiber levels vary with the stress intensity (Sarker *et al*., 2018).

Wheatgrass exposed to 50IL had high levels of Zn, Fe, Mn, Cu, P, Ca, and K. Light is crucial for promoting and controlling plant root growth by influencing hormone synthesis and the movement of minerals and nutrients (Miotto *et al*., 2021). Light plays an important role in stimulating and regulating plant root growth through various processes related to energy synthesis, including hormone production and the transport of minerals and nutrients (Miotto *et al*., 2021). According to, light triggers photosynthesis, a process that produces food that serves as a vital energy source for plant development. Sufficient light exposure promotes root growth and development. Well-developed roots lead to a sturdy plant structure that can withstand harsh environmental conditions (Gelderen *et al*., 2018; Yun *et al*., 2023). Plants with sturdy roots thrive, leading to enhanced soil nutrient uptake and potentially higher mineral levels. Similarly, wheatgrass exposed to 50IL light exhibited the greatest growth and mineral content among the other experimental groups. This indicates that light plays a role in mineral accumulation, potentially affecting wheatgrass growth.

Wheatgrass grown in the 50C treatment group exhibited the highest magnesium accumulation, whereas those grown in the control group exhibited the lowest magnesium content. The magnesium content in wheatgrass showed a similar trend to the chlorophyll content in this study, possibly due to magnesium playing a crucial role in chlorophyll's structure. Chlorophyll contains a porphyrin ring with magnesium at its core. Magnesium in the ring's center is crucial for chlorophyll to effectively capture and transmit light energy (Taiz and Zeiger, 2002). Therforce, various light intensities affect the intake of nutrients by various plant species (Kowalczyk *et al*., 2020).

This study examined how wheatgrass growth is impacted by light intensity at 50 and 150 μmol. m^{-2}. s^{-1} and varying lighting patterns (intermittent and continuous) over a three-day period before harvest. The results show that seedlings in the 50IL treatment group exhibited the best growth and high PI values, suggesting effective photosynthesis. Conversely, plants showed a decrease in growth with sustained exposure to 150 μmol. m^{-2}. s^{-1} (150C). The Fv/Fm ratio was used to analyze the stress response of the wheat seedlings. The

results showed that the various lighting patterns did not significantly affect Photosystem II, and there was no stress-induced mortality in the seedlings, as indicated by the Fv/Fm values at >0.7. Additionally, the nutritional and antioxidant characteristics were examined by assessing the levels of chlorophyll, carotenoids, phenolic compounds, flavonoids, minerals, total nitrogen, fiber, and vitamins B1 and B2. The light treatment with 50C yielded the greatest effect in increasing the accumulation of chlorophyll, carotenoids, and flavonoids, whereas the treatment with 150C favored the accumulation of phenolic compounds. Antioxidant capacity, assessed using DPPH, ABTS, and FRAP assays, increased in the different lighting groups compared with that in the control group. The highest levels of total nitrogen, fiber, minerals, and vitamin B1 were found in the 50IL treatment group, positively impacting seedling growth. Therefore, 50IL was considered most ideal for stimulating the highest growth rate, nutritional value, and antioxidant activity in wheatgrass.

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Conflicts of interest

The authors declare no conflict of interest.

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