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Light Spectrum Effects on the Ions, and Primary and Secondary Metabolites of Red Beets (*Beta vulgaris* L.)

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Abstract: Red beet (*Beta vulgaris* L.) is a root vegetable consumed and cultivated all around the world. It contains plenty of sugars, inorganic ions and a variety of secondary metabolites known to improve human health. The aim of this work was to investigate the effect of light spectra on red beets and their components in a vertical farm (VF) compared to open field (OF). RED (red:blue-white = 4:1)-treated shoots elevated total phenolic contents (TPC) among lights. Sugar content in VF red beets was 4.2 times higher than beets from OF. Betalains in VF red beets were 2.4–2.8 times higher than OF ones, and RED-treated roots had significantly higher betalain levels compared to CON (red:blue-white = 2:1)-treated ones. VF red beets contained a higher level of inorganic nitrates and lower chloride compared to OF beets. In conclusion, the light spectrum alters the concentration of beet components to be higher than that of OF red beets, and RED light elevated TPC, sugars and betalains.

Keywords: red beets; light spectrum; vertical farm; betalains; inorganic nitrates

Citation: Oh, C.; Park, J.-E.; Son, Y.-J.; Nho, C.W.; Park, N.I.; Yoo, G. Light Spectrum Effects on the Ions, and Primary and Secondary Metabolites of Red Beets (*Beta vulgaris* L.). *Agronomy* **2022**, *12*, 1699. https://doi.org/10.3390/agronomy12071699

Academic Editors: Pietro Santamaria and Onofrio Davide Palmitessa

Received: 14 June 2022
Accepted: 16 July 2022
Published: 18 July 2022

1. Introduction

Red beet (*Beta vulgaris* L.) is one of the plants of the *Chenopodiaceae* family, and numerous varieties are grown all over the world. Red beets are commercially important crops because they are widely used as natural dyes [1]. Red beets have been utilized to supply plenty of sugars and inorganic ions [2]. Also, red beets have been used for medicinal purposes due to a variety of secondary metabolites including betalain, flavonoids and polyphenols. Betalain, one of major secondary metabolites in red beets, is a water-soluble, nitrogen-containing natural pigment [3] and is widely used as a colorant in the food industries because of its antibacterial and antiviral properties [4]. It plays an important role in regulating vascular homeostasis and chemoprevention against lung and skin cancer [5]. Betalain is divided into two classes of compounds: betacyanin (red-violet) and betaxanthin (yellow-orange). Betacyanin is a major betalain in red beets and betaxanthin is a major one in yellow beets. The major betacyanins of red beet are betanin (betanidine 5-O-β-glucoside), iso-betanin, prebetanin and neobetanin [6]. The vulgaxanthin1 in the red beet is the major betaxanthin [6]. In addition, phenolic compounds in red beets such as flavonoids, saponins and other phenolic derivatives [7] have antibacterial, antiviral, anti-inflammatory and anti-cancer effects [8]. Nitrate is one of the most important inorganic compounds in red beets. Its content varies from 214–3556 mg/kg, up to 3–10 times higher than that of cabbage (74–1138 mg/kg) or carrot (under 30–525 mg/kg) [9]. Because it is well known that nitrate intake lowers blood pressure and modulates oxidative stress, consumption of red beets is recommended to reduce the risk of cardiovascular disease [10].

A vertical farm is an indoor facility to raise plants in multiple layers with artificial environments. There are several advantages of vertical farms: First, they can grow crops with the same qualities because crops cannot be exposed to the fluctuation of climates such as drought, flood or high temperature. Second, because vertical farms are indoor systems, the risks such as diseases or pests on plants are less than in an open field so that plants can grow without pesticides, leading to eco-friendly products [11]. Third, it is a system capable of the systematic, continuous, high-speed and mass production of plants due to artificial environments such as lights, carbon dioxide and nutrient solution supplements [12].

However, several problems with vertical farms have been pointed out, such as high start-up costs and low economics [13]. Therefore, various studies are being conducted for reaching economics in vertical farms, such as enhancement of secondary metabolites or value in health promotion and medicinal plants [14–16].

In spite of these difficulties, it is possible to control and secure the functional components of plants, so vertical agriculture is being utilized. Still, there are rare trials to grow root vegetables because hydroponic systems have been known not to fit enough root volume [17].

Light is the most important energy source for photosynthesis in plants and plays an important role in plant growth and development. The effect of light on plants can be divided into three main categories: light quality, quantity and photoperiod [18]. Among them, light quality is an important environmental factor that promotes photosynthesis and regulates plant growth and development. Alteration of the light spectrum changes plant growth rate, quality and accumulation of secondary metabolites [19]. Compared to other light sources, LEDs (Light Emitting Diode) enable researchers to precisely control the emission spectrum, because it has a wide wavelength range, providing a new and efficient light source [20]. LEDs are known to affect plant growth and development when used alone or in combination depending on their spectrum [21,22]. Red LEDs (maximum emission at 660–700 nm) are absorbed by phytochromes, which are essential for vegetative development. So, their treatment has been known to decrease the nitrate content of barley, maize leaves and lettuce [23], as well as improve the antioxidant activity and phenol content in lettuce [24]. Blue LEDs (maximum emission at 430–453 nm) are absorbed by crytochromes, which are important for photosynthetic function, chlorophyll formation and chloroplast development [21]. Therefore, using a vertical farm, the application of different light spectra on plants could result in a change on the production of improved plant biomass and phytochemicals.

In the present study, we investigate the effect of light quality on red beets and its components. We hypothesized that specific light spectra alter the contents of primary and secondary metabolites and biomass of red beets. Also, we estimate whether the improved qualified red beets could be produced in VF using a comparison with red beets from OF. So, we measured the physiological indicators of red beets and analyzed metabolites such as betalains, nitrate and sugars of red beets under a variety of light spectra. Based on these data, we can develop the cultivation methods of red beets in a vertical farm to increase the production of health-beneficial metabolites.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

This experiment was conducted using commercial red beet seeds obtained from William Dam Seed (Detroit Supreme, Dundas, ON, Canada). The red beets were grown in a vertical farm facility (SMART u-FARM) in the Korea Institute of Science and Technology (KIST, Gangneung, Korea) in 2020. Red beet seeds (n = 1000) were sown in moist rockwool cubes (W × L × H, 25 mm × 25 mm × 40 mm; Grodan Co., Roermond, The Netherlands) and placed under 200 ± 11 μmol m^{-2} s^{-1} of PPFD (photosynthetic photon flux density) at a distance of 25 cm from fluorescent lamps (TL5 14 W/865, Philips, Amsterdam, The Netherlands) under a 14:10 h light/dark cycle at 18–26 °C and 50–80% relative humidity conditions. Then, 14 days after sowing, red beet plants which had two true leaves and similar growth rates were selected and transplanted to a cultivation box (W × L × H, 41 cm × 17 cm × 57 cm) filled with pearlite (Newpershine no. 2, GFC. Co. Ltd., South Korea). After transplantation of each of the 6 plants in 6 cultivation boxes, red beets were grown using the ebb and hydroponic system. Commercial red beets grown in an open field (OF) in 2020 were purchased (Jeju Island, South Korea, 33°18′14.0″ N 126°11′50.0″ E) for comparison of growth and chemical properties.

2.2. Light Conditions

Light-emitting diode (LED) bars, which can control the intensity of red and blue-white light, respectively, were used in this study for plant growth and abiotic stress. LED bars for plant growth (KLB-40-2C, red:blue:white = 10:3:2 Ratio, KAST Engineering, Gumi, Korea) have central wavelengths of red (660 nm), blue (440 nm) and warm white (420 nm–790 nm). The spectra of LEDs utilized are shown in Supplementary Data (Figure S1). After transplanting the beets, plants were radiated at 150.2 ± 5.4 µmol m^{-2} s^{-1} of PPFD (photosynthetic photon flux density) at a distance of 37 cm from LED bars to plant canopy on a 14:10 h light/dark cycle at 18–25 °C and 50–80% relative humidity conditions. During the cultivation period, the red beet plants were grown under three kinds of light conditions that are three different light intensity ratios of red and blue-white emitting diodes. They are the basic ratio of red and blue-white (CON, red:blue-white = 2:1), strengthened red (RED, red:blue-white = 4:1) and strengthened blue-white light (BW, red:blue-white = 2:3).

2.3. Harvesting Plants

The red beet samples were collected at 11, 12 and 13 weeks after sowing (WAS, BBCH 39) [25], respectively. For each respective experimental group, five plants were harvested and the fresh weight of their shoot and root parts were measured using a digital balance (W-200, CAS Corp., Yangju, Korea). Harvested plants were freeze-dried to measure dry weight and then extracted to analyze chemical properties.

2.4. Extraction

After freeze-drying (−70 °C, 5 days, FDB-7003, OPERON Co., Ltd., Gimpo, Korea) the harvested red beets, extraction was performed for analysis. An amount of 500 mg of the freeze-dried sample was mixed with 10 mL of distilled water, and was sonicated (UCP-20, JEIO TECH Co., Ltd., Daejeon, Korea) for 90 min. Then, liquid was filtered by filter paper (Quantitative Filter Paper No. 5A, Whatman, UK). The supernatant was collected after centrifugation at 12,000 rpm for 10 min (mikro 200R, Hettich GmbH, Germany) and filtered using a 0.2 µm syringe filter. After extraction, it was stored in a deep freezer (−80 °C) until analysis.

2.5. Total Phenolic Content and Antioxidant Properties

The amount of total phenolics in the extracts was determined using a modified Folin-Ciocalteu method [26]. An amount of 120 µL of diluted extract (extract-water, 1:11 (*v/v*)) was put into a test tube and mixed with 120 µL of 1 N Folin-Ciocalteu's reagent (Sigma Aldrich, St. Louis, MO, USA). After 5 min incubation, 240 µL of 20% Na_2CO_3 solution was added in each tube. After 10 min incubation at room temperature, the absorbance of the supernatant was measured at 730 nm with a Synergy HTX Multi-Mode Reader (BioTek, Winooski, VT, USA). Gallic acid (Sigma Aldrich, St. Louis, MO, USA) was used as a standard. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram dry matter.

Ferric ion reducing antioxidant power (FRAP) assay was performed following a previously described method by Benzie and Strain [27]. Briefly, 2,4,6-tri(2-pyridyl)-s-triazone (TPTZ) reagent (Sigma) was mixed with 20 mM $FeCl_3$ solution in 0.3 M acetate buffer (pH 3.6) to make the reagent solution. After 30 min incubation at 37 °C, 800 µL of reagent solution was mixed with 20 µL of diluted extract (extract-water, 1:4 (*v/v*). The absorbance of the supernatant was measured at 593 nm for 5 min on a Synergy HTX Multi-

Mode Reader (BioTek, Winooski, VT, USA). The absorbance for initial point and 5 min after were measured and their difference was used for calculating the ferric reducing/antioxidant power (FRAP value) of the sample. Trolox (Sigma Aldrich, St. Louis, MO, USA) was used as a standard. The results were expressed as micromole Trolox equivalents (TE) per gram dry weight basis (μmol TE/g DW).

2.6. Determination of Sugar Content

To analyze the sugar content in red beets, 5 mg of extract was mixed with 1 mL distilled water. The solution was centrifuged (mikro 200r, UK) at 12,000 rpm for 10 min, and the extract was filtered using a 0.2 μm syringe filter. The sugar content in samples was analyzed using high-performance liquid chromatography (HPLC; Agilent Technologies, Santa Clara, CA, USA), and a YMC-pack polyamine II column (250 mm × 4.6 mm, 5 μm, YMC, Kyoto, Japan) was used for separation. The evaporative light scattering detector (ELSD) was used for analyzing mono- and di-saccharides in samples. Binary gradient elution was performed with solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid), which were delivered at a flow rate of 1 mL/min as follows: 0–14 min with 60–60% B. The injection volume was 5 μL, and the column temperature was 20 °C. Sucrose, fructose and glucose standards were obtained from Sigma Aldrich (USA).

2.7. Determination of Total Betalain Content

The quantification of betacyanins and betaxanthins in the red beets was determined according to the method of Zin, Marki, and Banvolgyi [28], with slight modification. For this experiment, Synergy HTX Multi-Mode Reader (BioTek, USA) was used. The betalain (betacyanin, betaxanthin) content was calculated by the following equation: mg/g DW = [(A × DF × MW × 100)/(e × l)] * EY; where A is the absorbance at 538 nm (betacyanins) or 476 nm (betaxanthins), DF is the dilution factor and l the pathlength (1 cm) of the cuvette, MW and e are molecular weight and extinction coefficients of betacyanin (MW = 550 g/mol; e = 60,000 L/mol cm in water) and betaxanthin (MW = 339 g/mol; e = 48,000 L/mol cm in H_2O), and EY is an extraction yield. The contents of betacyanins and betaxanthines were expressed as mg/g DW.

2.8. Chemical Analysis of Ion Content

An ion chromatography system (940 Professional IC Vario TWO, Metrohm, Metrohm AG, Switzerland) was used for analyzing some kinds of ion content. A Metrosep A Supp 5–150/4.0 column (Metrohm, Metrohm AG, Switzerland) was equipped with an ion chromatography system to separate ions in samples. The amounts of 3.2 mM Na_2CO_3 and 1 mM $NaHCO_3$ were used for the eluent solution, and 100 mM H_2SO_4 was used for suppressor regeneration. The injection volume was 20 μL, and the column temperature was 20 °C. The flow rate was 0.7 mL/min. IC Multi-Component Anion Standard (IC-2, high-purity standards, USA) was used for determining the contents of six anion components (bromide, chloride, fluoride, nitrate, phosphate, and sulfate ions) in red beet samples.

2.9. Statistical Analysis

GraphPad Prism v. 8.0 (GraphPad Software, San Diego, CA, USA) and IBM SPSS Statistics for Windows Version 25.0 (IBM Corp., Armonk, NY, USA) were used for statistical analysis. The one-way ANOVA and Tukey's multiple range test were used to compare treatment groups. Results are presented as mean ± standard error of the mean (SEM). Significant differences were considered at $p < 0.05$.

3. Results

3.1. The Influence of Light Conditions on the Growth of Red Beets

The effect of various types of lights in the initial phase of plant growth was investigated and the content of dye in red beets in a vertical farm was determined in comparison to cultivation in an open field. Physiological features of red beets including fresh weight and dry weight of the shoots, fresh weight and dry weight of the roots were measured from 11 WAS to 13 WAS (Figure 1). Fresh weights and dry weights of the shoots between groups were not significantly different at any week. The fresh weight of the shoots in average values was the highest at the 13 WAS of the BW treatment (Figure 1A). The dry weight of the shoots in average values was the highest at the OF (Figure 1C). The fresh weight of the roots increased during the 11 to 13 WAS, and the highest value in treatment groups was observed at the 13 WAS of the BW treatment (Figure 1B). However, when comparing root of OF beets with red beets grown in vertical farms, OF beets had about three times higher fresh weight (Figure 1B, $p < 0.05$). The dry weight of the roots in all groups was the highest in the OF group (Figure 1D, $p < 0.05$). While the fresh and dry weights of OF shoots were not different from those in the vertical farm, OF root weights were 2.5–3 times higher than those in the vertical farm.

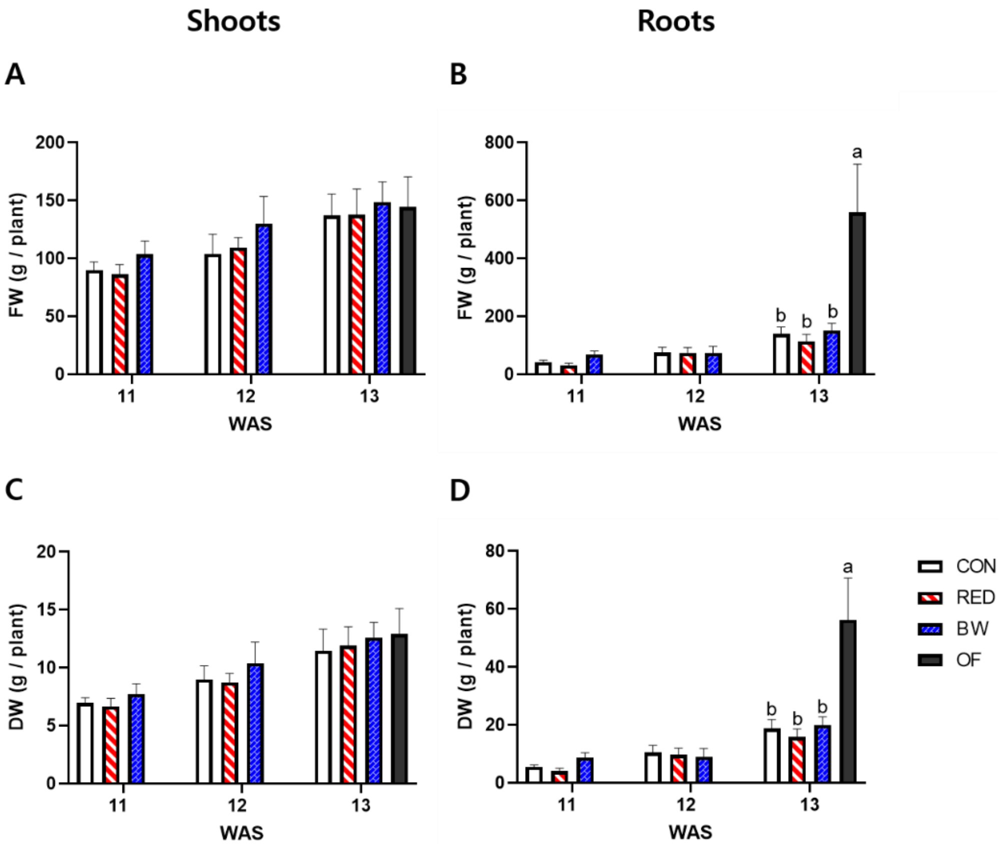

Figure 1. Growth indicators of red beets grown under different light conditions: control (CON, red:blue-white = 2:1), strengthened red (RED, red:blue-white = 4:1) and strengthened blue-white light (BW, red:blue-white = 2:3) in the VF (vertical farm) and natural light condition in the OF (open field). Red beets were harvested and measured at 11, 12 and 13 WAS (weeks after sowing). Fresh weight (FW) of shoots (**A**) and roots (**B**), dry weight (DW) of shoots (**C**), and roots (**D**) were measured. Bars and error bars represent means ± SEM. Different letters indicate significant differences at $p < 0.05$ determined by one-way ANOVA.

3.2. Impact of Light Conditions on the Production of Phenolic Compounds and Anti-Oxidant Capacity in Red Beets

The effect of light conditions on anti-oxidant ability in red beet extract, total phenolic compounds (TPC) and antioxidant ability (FRAP) of red beets were measured (Figure 2). As a result of measuring the TPC content, RED-treated shoot increased TPC concentration while the CON treatment and BW treatment decreased it, leading to a significant difference between RED- and BW-treated beets at 11 WAS (Figure 2A, $p < 0.05$). At 13 WAS, the RED-treated shoots significantly elevated TPC contents compared to other treatments (Figure 2A, $p < 0.05$). In contrast, no significant difference was found in roots between all groups (Figure 2B). TPC content of OF shoots and roots was not different from those from the vertical farm. FRAP content of the shoot increased under RED treatment compared to CON treatment and decreased under BW treatment compared to CON treatment at 11 WAS. (Figure 2C, $p < 0.05$). At 13 WAS, RED treatment induced a significant increase of FRAP activity compared to other groups, which confirmed our TPC data (Figure 2C, $p < 0.05$). In roots, RED treatment elevated FRAP content compared to the CON treatment slightly, and OF roots contained significantly lower FRAP activity than those from the vertical farm at 13 WAS (Figure 2D, $p < 0.05$).

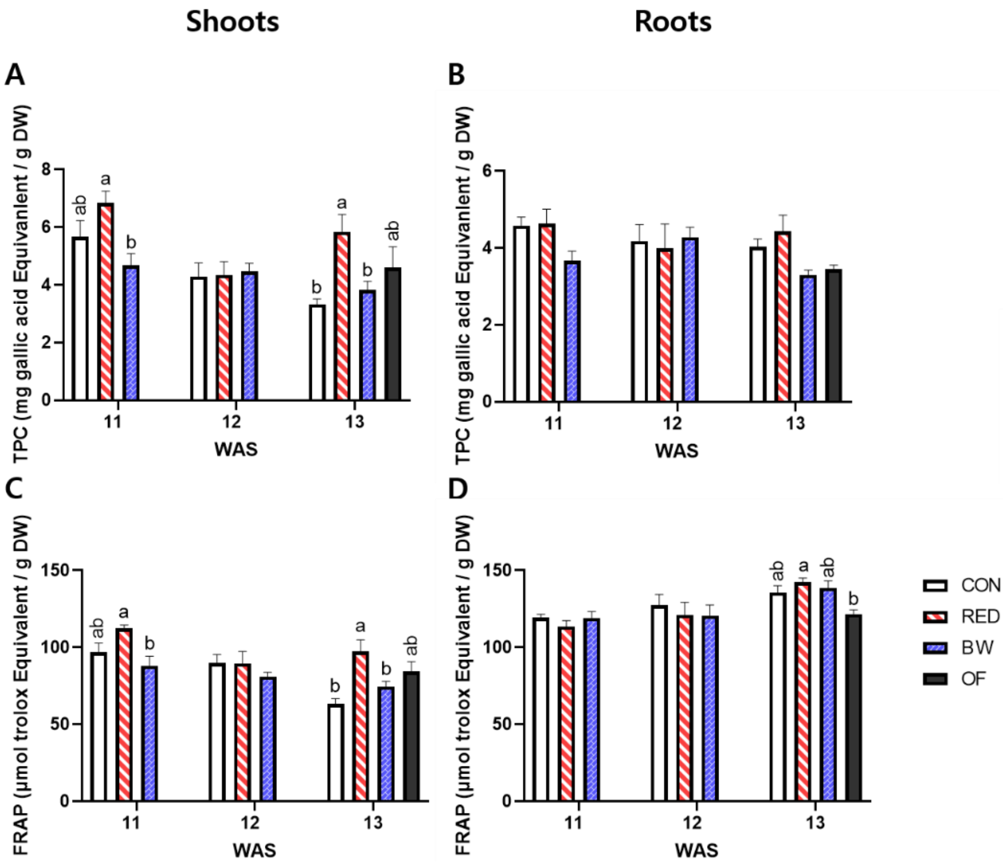

Figure 2. Total phenolic content (TPC) and ferric ion reducing antioxidant power (FRAP) of red beets grown under different light conditions: control (CON, red:blue-white = 2:1), strengthened red (RED, red:blue-white = 4:1) and strengthened blue-white light (BW, red:blue-white = 2:3) in the VF (vertical farm) and natural light condition in the OF (open field). Red beet plants were harvested at 11, 12 and 13 WAS (weeks after sowing), and the TPC of shoots (**A**) and roots (**B**), and FRAP of shoots (**C**) and roots (**D**) were analyzed. Bars and error bars represent means ± SEM. Different letters indicate significant differences at $p < 0.05$ determined by one-way ANOVA.

3.3. The Effect of Light Conditions on Sugar Production in Red Beets

To estimate the effect of light conditions on sugar production, the main soluble sugars fructose, glucose and sucrose were analyzed in shoots and roots of red beets. Only fructose and glucose were detected in the shoots of red beets, whereas fructose, glucose and sucrose were detected in the roots (Figure 3). The major sugar was glucose (5–148 mg/g DW) in the shoots, while it was sucrose (83–296 mg/g DW) in the roots.

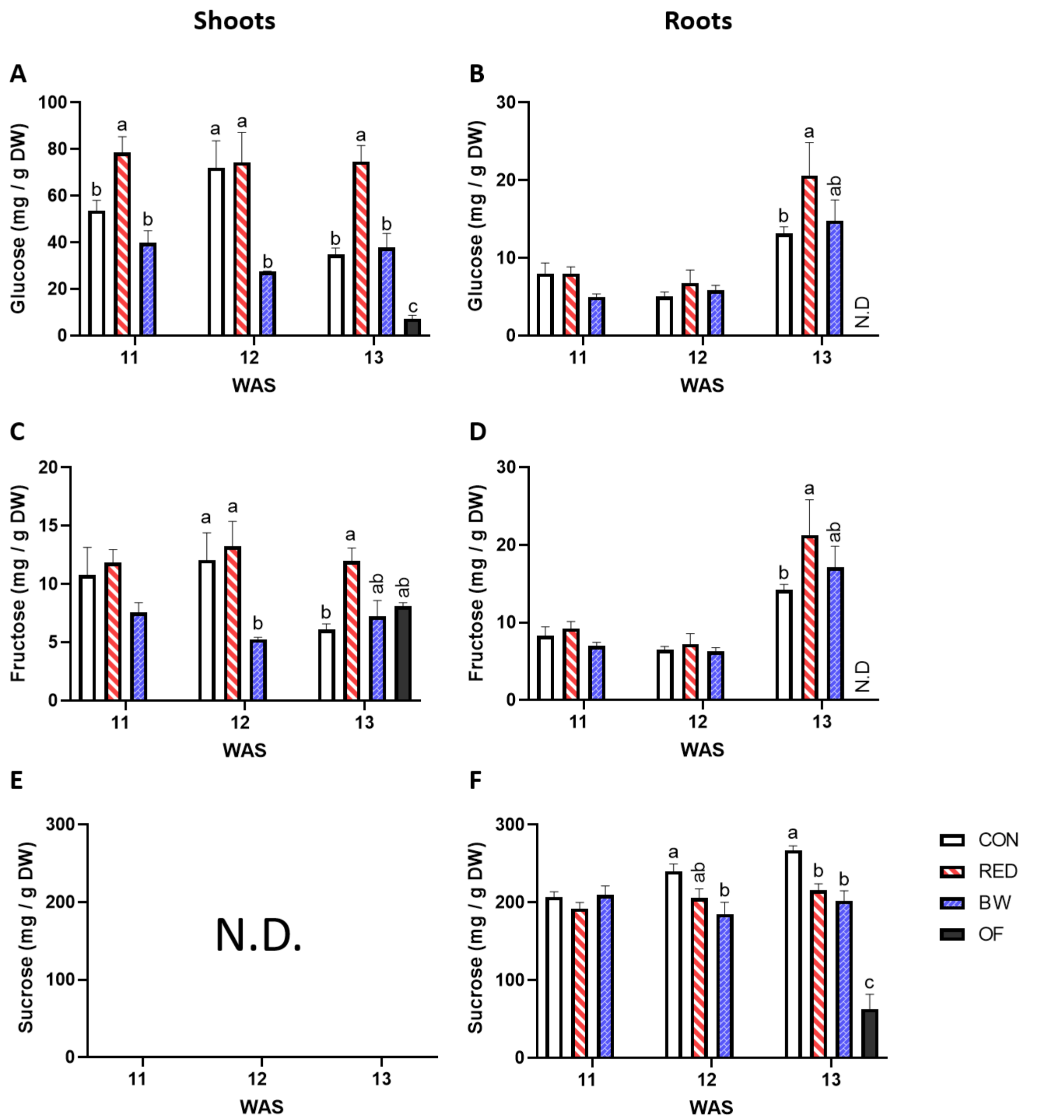

Figure 3. Sugar content of red beets grown under different light conditions: control (CON, red:blue-white = 2:1), strengthened red (RED, red:blue-white = 4:1) and strengthened blue-white light (BW, red:blue-white = 2:3) in the VF (vertical farm) and natural light condition in the OF (open field). Red beet plants were harvested at 11, 12 and 13 WAS (weeks after sowing) and glucose contents of shoots (**A**) and roots (**B**), fructose contents of shoots (**C**) and roots (**D**), and sucrose contents of shoots (**E**) and roots (**F**) were analyzed. Bars and error bars represent means ± SEM. Different letters indicate significant differences at $p < 0.05$ determined by one-way ANOVA.

The glucose content in the shoots was elevated under the RED treatment compared to the CON and BW treatment and it was significant at 11 WAS (Figure 3A, $p < 0.05$). At 12 WAS, the shoot of the BW treatment showed a lower glucose level than the CON and RED treatments (Figure 3A, $p < 0.05$). The RED treatment elevated glucose contents of the shoot at 13 WAS and there was a significant difference with other groups (Figure 3A, $p < 0.05$). Glucose content of roots was increased in the RED treatment compared to the CON treatment at 13 WAS (Figure 3B, $p < 0.05$). Shoots of the vertical farm contained a significantly higher glucose content compared to OF and glucose in the root of OF beets was not detected (Figure 3A,B). Overall, the glucose content of red beets grown in vertical farms was higher than that of in OF, and among them, the RED treatment was about 14 times higher than that of the OF (Figure 3A, $p < 0.05$). The fructose content in the shoots under the RED treatment was detected as the highest among all groups (Figure 3C). The BW treatment decreased fructose content of shoot and there was a significant difference with other groups at 12 WAS (Figure 3C, $p < 0.05$). At 13 WAS, the RED treatment had a significantly elevated fructose level compared to the CON treatment (Figure 3C, $p < 0.05$). In roots, the fructose content was the highest in the RED treatment at 13 WAS, which showed a significant increase from the CON (Figure 3D, $p < 0.05$). Fructose also was not detected in the root of OF (Figure 3D). In both vertical farm and OF, no sucrose was detected in the shoots. The sucrose content in the roots was decreased by the RED and BW treatment compared to the CON treatment at 12 and 13 WAS (Figure 3F). The BW treatment showed a significant reduction of sucrose level in the roots at 12 WAS (Figure 3F, $p < 0.05$). Red beets grown on vertical farms also had a higher sucrose content, and especially the CON treatment was about 4 times higher than OF (Figure 3F, $p < 0.05$).

3.4. Alteration of Betalain Production by Light Conditions

The effect of light types on pigments betalains, betanin (red-violet) and vulgaxanthin (yellow-orange) were specified (Figure 4). Betacyanin content of the shoot was significantly increased under the RED treatment compared to the CON treatment and BW treatment at 11 WAS. (Figure 4A, $p < 0.05$). At 13 WAS, RED-treated roots showed a significantly higher betacyanin level compared to the CON treatment and BW treatment (Figure 4A, $p < 0.05$). The RED treatment had about a three times higher betacyanin level than OF (Figure 4A, $p < 0.05$). In roots, the CON treatment and RED treatment elevated the betacyanin level compared to OF at 13 WAS (Figure 4B, $p < 0.05$). Overall, betacyanin levels in vertical farms were higher than in OF (Figure 4B). In particular, the betacyanin levels of the CON and RED treatments were about two times higher than that of OF (Figure 4B, $p < 0.05$).

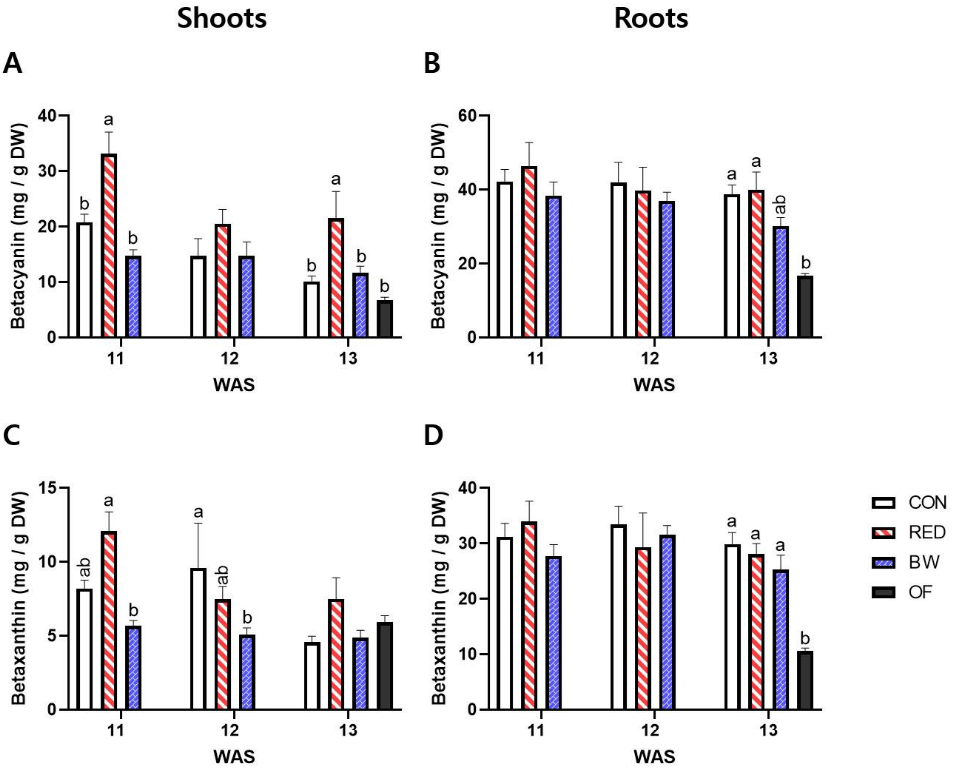

Figure 4. Betalains (betacyanin, betaxanthin) content of red beets grown under different light conditions: control (CON, red:blue-white = 2:1), strengthened red (RED, red:blue-white = 4:1) and strengthened blue-white light (BW, red:blue-white = 2:3) in the VF (vertical farm) and natural light condition in the OF (open field). Red beet plants were harvested at 11, 12 and 13 WAS (weeks after sowing), and betacyanin of shoots (**A**) and roots (**B**), and betaxanthin of shoots (**C**) and roots (**D**) were analyzed. Bars and error bars represent means ± SEM. Different letters indicate significant differences at $p < 0.05$ determined by one-way ANOVA.

The bataxanthin level of shoots slightly increased in plants subjected to the RED treatment compared to the CON treatment and decreased in plants influenced by the BW treatment compared to CON treatment at 11 WAS (Figure 4C, $p < 0.05$). At 12 WAS, the betaxanthin levels in BW-treated shoots were significantly decreased compared to the CON treatment (Figure 4C, $p < 0.05$). In roots, the betaxanthin content of vertical farm beets was significantly higher compared to OF at 13 WAS (Figure 4D, $p < 0.05$).

3.5. Anion Changes in Red Beets with Light Conditions

Changes in the content of anion levels, F, Cl, NO_3, PO_4 and SO_4, influence different light treatments in the red beets, presented in Figure 5. Overall, analyzed ions were higher in the shoots than the roots, and especially Cl, NO_3 and PO_4 levels in the shoots were three to five times higher than in the roots (Figure 5). F was analyzed but not detected in shoots and roots.

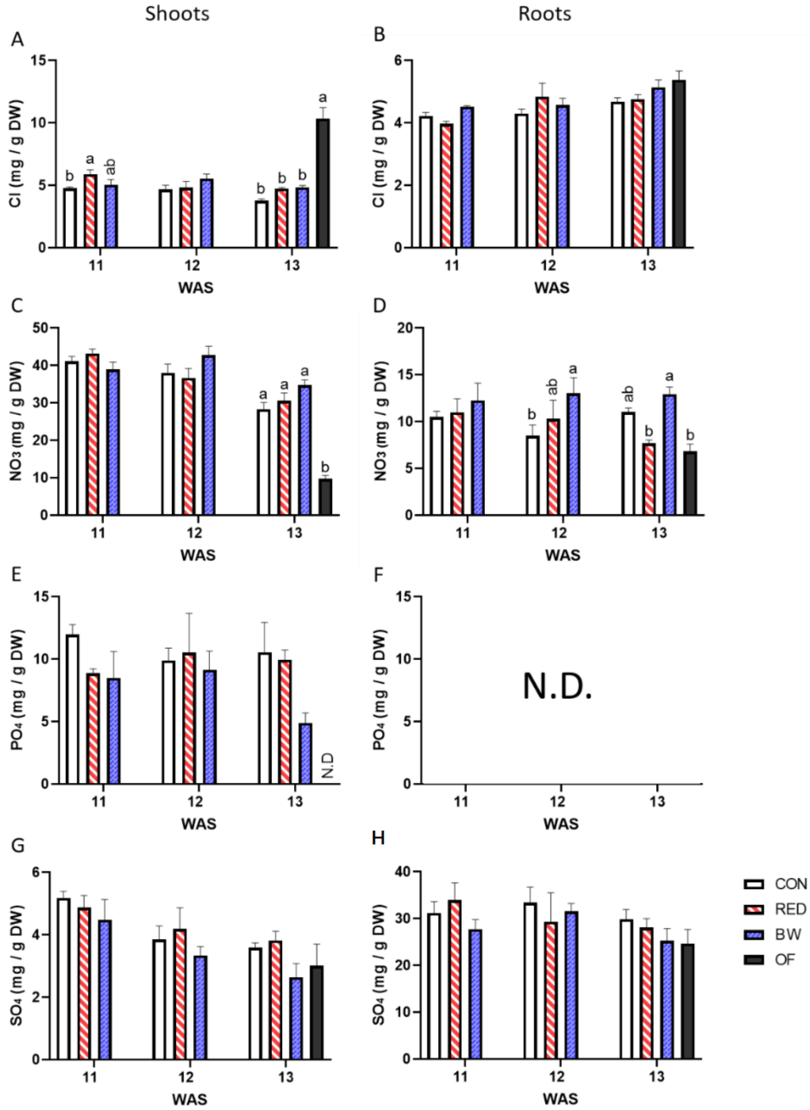

Figure 5. Anions in red beets grown under different light conditions: control (CON, red:blue-white = 2:1), strengthened red (RED, red:blue-white = 4:1) and strengthened blue-white light (BW, red:blue-white = 2:3) in the VF (vertical farm) and natural light condition in the OF (open field). Red beet plants were harvested at 11, 12 and 13 WAS (weeks after sowing), and chlorine of shoots (**A**) and roots (**B**), nitrate of shoots (**C**) and roots (**D**), and phosphate of shoots (**E**) and roots (**F**), and sulfate of shoots (**G**) and roots (**H**) were analyzed. Bars and error bars represent means ± SEM. Different letters indicate significant differences at $p < 0.05$ determined by one-way ANOVA.

In shoots, the Cl level was increased by the RED treatment compared to the CON, and it was significant under RED at 11 WAS (Figure 5A, $p < 0.05$). Cl level was higher in OF than CON, and the RED and BW treatment significantly elevated the Cl level at 13 WAS in shoots (Figure 5A, $p < 0.05$). In contrast, no significant difference in Cl level was found in roots between all groups (Figure 5B). As a result of NO_3 analysis, NO_3 content of shoots with all treatments at 13 WAS was three times higher than OF (Figure 5B, $p < 0.05$). NO_3 level in roots was elevated in the BW treatment compared to the CON treatment and it was significant at 12 WAS (Figure 5D, $p < 0.05$). At 13 WAS, the BW treatment elevated NO_3 level compared to the RED treatment and OF (Figure 5D, $p < 0.05$). The PO4 level of shoots did not have a significant difference among all groups (Figure 5E, $p < 0.05$) and was not detected in OF shoots. In roots, PO4 was not detected. SO_4 levels were not changed by any light treatment and OF in both shoots and roots (Figure 5G,H).

4. Discussion

There are a variety of studies on how different ratios of light spectra impact leafy vegetables and their components in vertical farms [29]. However, data of VF with root plants such as red beets are rare because hydroponic systems generally utilized in vertical farms have a limit to grow plants with a large-sized roots [17]. Here, we investigated the influence of various ratios of strengthened red (RED, red:blue-white = 4:1) and strengthened blue-white light (BW, red:blue-white = 2:3) on the growth and functional component changes of red beets. It is known that the appropriate combination of red and blue LEDs causes an increase in the fresh and dry weight of plants [30]. Therefore, when cultivating plants in a vertical farm, it is important to adjust the light environment optimized for cultivation in order to increase the yield. The fresh and dry weight of red beets was not changed by different LED conditions. Although red beets grown in vertical farms were still insufficient in terms of the growth of red beet roots compared to those grown in the open field, cultivation of red beets in vertical farms was possible and was effective in increasing the production of primary and secondary metabolites in red beets.

When we calculated the metabolites produced by plants, the highest biomass production was found in open field beets. However, the deviation of metabolites of beets grown in vertical farms is small compared to open field, so it can be confirmed that uniform production is possible in vertical farms. Although the yield per individual is important, in vertical farms, unlike open fields, a strategy to increase production can be utilized through multi-stage cultivation and continuous production.

A plant's high sugar concentration is beneficial for plant growth, but it is also effective in building a strong plant defense system [31]. Sugars produced in plants are derived from photosynthesis as an energy source [31]. There are a variety of studies showing that the spectral energy distribution of red and blue light can alter photosynthesis and growth mediated by the absorption spectrum of chlorophylls [32]. In particular, it has been reported that the plant reaches the maximum photosynthetic efficiency when red light supplemented with blue light (red:blue = 4:1) was applied [33]. A high percentage of red light increases nitrogen accumulation, leading to an increasing rate of photosynthesis in rice [33]. Li and his colleagues have reported that glucose and fructose are accumulated under red light, more effective than blue in *Gossypium hirsutum* L. [34]. Also, sucrose was elevated when the red light was high in tomatoes [35]. In this study, red beets grown on VF contained more sugars than red beets grown in an OF. In addition, RED treatment among VF red beets increased the content of large amounts of glucose and fructose. The RED treatment used in this experiment consists of a ratio of red:blue-white = 4:1, in which the ratio of red to blue light is high. These data indicate that high supplementation of red light induces sugar accumulation on red beets, suggesting red light is beneficial to produce red beets.

Although it is generally accepted that light has a positive effect on betalain accumulation [36], the biosynthetic pathway by which betalain accumulates has not been elucidated [36]. Only some enzymes such as tyrosinase, DOPA oxidase and glucosyltransferases have been known for their involvement in the short biosynthetic pathway from tyrosine to betanin [37], but it is unclear whether any of these enzymes are induced by light. Blue light is known to increase betalain production in *Portulaca* callus [38] and hairy roots of red beets [39]. In contrast, red light conditions also increased betalain content in *Amaranthus* seedlings [40] and *Hylocereus costaricensis* [41]. Here, the results of this study showed red light elevated betalains in shoots of red beets, not roots. These data suggest that the light spectrum has more impact on shoots compared to roots, because shoots are the tissues in direct contact with lights. Also, it suggests that the ratio of red to blue we utilized has better conditions to increase betalain production. Still, further study is required to elucidate enzymes related to biosynthesis of betalains and the impact of various light spectra on betalain synthesis.

Nitrates have been known for their activity in preventing high blood pressure and cardiovascular disease [10]. More than 80% of the nitrates consumed by humans everyday come from vegetables and red beets are one of recommended vegetables for nitrate consumption [20]. Nitrates are found in all plant tissues because they are essential for plant growth and development [20]. Many studies have shown that light and nitrogen are two factors that affect nitrate production in plants [42]. Red light has a high ability to stimulate the activity of nitrate reductase, indicating that it can effectively reduce the nitrate concentration in plants [20]. On the other hand, blue light was shown to be effective in increasing total nitrogen concentration because it reduces the activation of nitrate reductase [33]. These results are consistent with the findings of this study because our data showed that red beets treated with high blue light have more nitrates in their roots, though light difference is not critical in the shoots. In addition, artificial light was more effective for nitrate accumulation compared to open field. These data suggest that the combination of specific spectra of lights could be a better option than the whole spectrum of light from the sun for nitrate production.

Phenolic compounds such as flavonoids, saponins and other phenolic derivatives are one group of secondary metabolites in most fruits and vegetables, and have been studied for strong antioxidant properties [43]. A variety of articles have been reported the impact of light on the production of phenolic compounds [44]; a high percentage of blue increased total phenolic content [45] and antioxidant capacity [30]. When red light was used and blue light was added to plants, an increase in antioxidant capacity and accumulation of phenolic compounds was confirmed [46]. The RED treatment used in this study increased the total phenolic content and antioxidant capacity of red beets. The reason is that the red wavelength belonging to the visible light band has the effect of increasing the activation of photosynthesis in plants, preventing the accumulation of reactive oxygen species [47].

5. Conclusions

When growing red beets in a vertical farm, sugars, betalains and nitrates were increased compared to the open field specimens. Among light spectra, a high ratio of red to blue was more effective on the production of phenolic compounds, sugars and betalains in red beet roots. Therefore, cultivation of red beets in a vertical farm could be a good option to produce red beets that have enhanced functional activity, though its productivity should be improved.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12071699/s1, Figure S1: Spectral distributions of LED chipsets that red (A), blue (B) and warm white (C).

Author Contributions: Conceptualization, J.-E.P. and G.Y.; Data curation, G.Y.; Formal analysis, C.O., J.-E.P. and G.Y.; Investigation, C.O., J.-E.P. and Y.-J.S.; Methodology, C.O., J.-E.P., C.W.N., N.I.P. and G.Y.; Project administration, G.Y.; Supervision, G.Y.; Visualization, C.O. and J.-E.P.; Writing—original draft, C.O., J.-E.P., Y.-J.S., C.W.N., N.I.P. and G.Y.; Writing—review and editing, C.O., J.-E.P. and G.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by KIAT-NRC Collaborative R&D project of Ministry of Trade, Industry and Energy, Republic of Korea (Grant number N062000013). Also, this work was supported by the Korea Institute of Science and Technology (Intramural grant, grant number 2Z06670).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

Abbreviations

VF: vertical farm; OF, open field; CON, basic ratio of red and blue-white light (red:blue-white = 2:1); RED, strengthened red light (red:blue-white = 4:1); BW, strengthened blue-white light (red:blue-white = 2:3); FW, fresh weight; DW, dry weight; HPLC, high performance liquid chromatography; WAS, weeks after sowing.

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