LED light has a long-distance impact accelerating storage root development and sucrose content in Daucus carota L (carrot)

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Abstract

Light plays a crucial role in several physiological processes in plants, ranging from photosynthesis to the biosynthesis of secondary metabolites such as carotenoids. The emergence of Light-Emitting Diode (LED) technology, has granted researchers unprecedented control over light quality and intensity, enabling detailed research into its effects on plant growth, development, flowering, and secondary metabolite accumulation, such as carotenoids. Carotenoids, vital pigments in plant physiology, serve for pollinator attraction, photoprotective molecules, and precursors for vitamin A biosynthesis. Carrots (*Daucus carota*) known for their high carotenoid levels provide an excellent model for studying the impact of light on carotenoid biosynthesis and taproot development. In a comprehensive study exploring the effects of different light conditions on carrot storage root development and carotenoid content, we employed three lighting regimes: fluorescent light (F) with a high green light content, LED light with a spectrum enriched in blue and red wavelengths (NS12), and LED light with a higher content in Red and Far-Red light and with the highest light intensity (AP67). Our findings revealed that NS12 and AP67 induce an early development of secondary root growth due to carrot storage roots exhibiting wider and heavier storage roots compared to those grown under F light. Together, these findings correlate with an enhancement in carbon fixation, as evidenced by the improved levels of sucrose and starch under LED lighting. Despite these differences, there were no observable variations in carotenoid content. However, there was an induction of *DcPSY1* and *DcPSY2* expression in 8 weeks old storage roots of plants grown under LED lights. Taken together, the quality and intensity of aboveground light in which the carrot is grown significantly affects the development of the storage root but not on carotenoid content. These findings underscore the profound influence of light quality on underground organs and highlight the potential of LED technology to optimize crop production by promoting favourable traits in storage organs.

1. INTRODUCTION

Light is an important factor that regulates metabolites synthesis and morphogenesis in plants, including flowering, fruit coloration, and seed maturity [1]. Light is involved in photosynthesis, photomorphogenesis, metabolites biosynthesis, circadian rhythm regulation, and many other processes including carbohydrate accumulation [2]. Photoreceptors, such as phytochromes (PHYA, PHYB), cryptochromes (CRY), and phototropins, can absorb red (R), far red (FR) and blue light (B) and activate the downstream signal transduction metabolic pathways, thereby modulating gene expression involved in photomorphogenesis, hormone signalling and secondary metabolite synthesis [3]. To study the effect of light on different processes in plants the use of Light-Emitting Diode (LED) lights in artificial lighting conditions has become widespread over the past 20 years [4]. Unlike other types of lights such as incandescent or fluorescent lights, LEDs have characteristics that make them interesting for research, as they allow for precise control of the quality and intensity of the applied light [5, 6] promoting the understanding the spectral effects of light on plants, both at the molecular and morphological level [7]. This type of lighting technology enables the generation of specific light spectra to enhance and study characteristics of interest such as vegetative growth, flowering, accumulation of secondary metabolites,

induction of systemic resistance to pathogenic fungi, and acceleration of crop growth programs "speed breeding" [8–10]. These investigations have driven the use of LED lights for productivity purposes in certain species, leading to the proliferation of vertical farming systems under LED lights. These systems stand out for their efficiency, as they correspond to illumination systems with lower energy consumption, which in turn improve production and quality of crops by regulating the lighting conditions according to the specific requirements of the cultivated species [5, 11]. However, to fully exploit this potential, further studies need to encompass a wider range of species, as currently these systems are primarily research and applied to produce edible leafy vegetables [12].

White light is composed of different wavelengths: UV light (< 400 nm), B light (400–500 nm), green (G) light (500–600 nm), R light (600–700 nm) and FR light (700–800 nm). White LEDs, in addition to being more energy-efficient and economically accessible, provide an enriched spectrum of light with wavelengths ranging from 400 to 700 nm, known as photosynthetically active radiation (PAR). They also offer better human visual perception and resemble natural lighting conditions[5]. In photobiology, the effect of light intensities and light quality on plants such as tomatoes [13], lettuce [14] ,cannabis [15], among others has been studied, mainly driven by the advances in LED technology. Nevertheless, these studies mainly focus on organs which are in direct contact with light such as leaves and fruits. For example, the increase of blue light (400–500 nm) has been shown to inhibit cell division and expansion in lettuce and soybean, resulting in a reduction in leaf area, photon capture and plant growth; however, it has been demonstrated that the presence of a small percentage of blue light improves the photosynthetic performance of plants [16]. Photons within the wavelength range of green (500–600 nm) are less absorbed by plant photopigments. However, a certain fraction of green light (not absorbed by chlorophyll in upper tissue layers) could penetrate the tissue and excite photosystems in deeper cell layers [5, 17]. Photons within the wavelength range of red light (600–700 nm) are photosynthetically efficient [5, 18], while photons within wavelength range of far-red light (700–800 nm), have a positive effect on plant stem growth and elongation, promoting leaf area expansion in lettuce [19], and stem elongation in tomato plants [20]. Despite this, evidence indicates that it is not possible to assert that the same effect is replicated on all plant species under multi-wavelength conditions, making species-specific studies necessary [21].

It is well known that light plays a crucial role in triggering and regulating plant biological processes, including the biosynthesis of primary metabolites such as simple sugars [22] and secondary metabolites, for instance, carotenoids. Carotenoids are lipidic, isoprenoid pigments with important functional roles in plants as pollinator attractant as they confer characteristic colours ranging from yellow, orange, and red colours to organs such as flowers and fruits; they participate as accessory pigments in the antenna complex during photosynthesis and act as photoprotective molecules against oxidative damage. Phytoene synthase (PSY) has been stood out as one of the key enzymes for carotenoid biosynthesis which catalyses the first specific reaction of the carotenoid pathway where the carotenoid precursor phytoene is synthesized ([13, 23], observed that upon supplementation with blue (430 nm) or red LEDs (660 nm) during the anthesis period of tomato plants, the relative expression of *SlPSY1* was induced in both treatments while the developed fruits increased their β-carotene content, an

orange-colored carotenoid. This constitutes a precedent of how the quality of light positively affects the expression of genes involved in the biosynthetic pathway of carotenoids.

On the other hand, primary metabolites derived directly from the photosynthesis process are affected by changes in light as well. Several studies stipulate that the exposure to different light intensities and spectra can change soluble sugar content [24, 25] in various species. Nevertheless, light effects are studied mainly in leafy vegetables as leaves are in contact with light, harnessing its energy for carbon fixation in photosynthesis. Evidence of the effect in light intensities in sink organs is little, even though it is pivotal to explore as they depend directly on, amongst other factors, the capacity of the plant to fixate carbon into photosynthates that are transported are accumulated in these sink organs such as fruits and edible roots, etc.

Carrot (*Daucus carota*) is a dicotyledonous plant that accumulates large amounts of β-carotene in its edible storage root, which gives the characteristic orange colour and poses it as a novel model for the study of carotenoid biosynthesis [26]. Regarding the taproot development of *D. carota*, three key stages have been reported: an initial stage of development at 4-weeks post-germination, where the root its emerging and has a thin and pale phenotype; at 8-weeks post-germination, where thickening of the taproot and synthesis of carotenoid is noticeable; and at 12-weeks post germination, where storage roots reach maturity, displaying a fully developed taproot of deep orange coloration, attributed to the high accumulation of β-carotene [27–30]. To date, the genes involved in the carrot carotenoid biosynthesis pathway are known. In carrot, the two *DcPSY1* and *DcPSY2* paralogs present an organ specific expression: *DcPSY1* is expressed to a greater extent in leaves, proposing a role in carotenoid synthesis in chloroplasts, while *DcPSY2* is mostly induced in the storage root where there is greater carotenoid synthesis in the chromoplasts [27]. In addition, it has been demonstrated that the direct exposure of white light on the root impairs *DcPSY1* and *DcPSY2* expression and carotenoid content specially at 8 and 12 weeks of development contrary to the root that grows underground. These results demonstrate that the direct incidence of white light represses carotenogenic gene expression and carotenoid content in carrot storage root.

Given the known impact of light and LED technology on plant growth and carotenoid production, a pertinent inquiry arises: How do differing light conditions experienced by aboveground photosynthetic tissue influence the development of underground carrot storage roots and their carotenoid levels? Therefore, we grew carrot plants under three different light conditions: Fluorescent light (F) with a high G light content, LED light with a spectrum enriched in G and R wavelengths (NS12), and LED light with a higher content in R and FR light and with the highest light intensity (AP67) (Table 1) and we determined phenotypic parameters, carotenoid content, relative carotenogenic gene expression, and soluble sugars in the storage root at 4-, 8- and 12-week post germination (WPG). Interestingly we observed that plants grown under NS12 and AP67 present an early development of secondary root growth, presenting wider and heavier storage roots and an increment in soluble sugars (SS), sucrose and starch level regarding F light. Although they did not present any differences in carotenoid content, the expression of *DcPSY1* and

DcPSY2 was induced at 8-WPG. These findings suggest that LEDs, specially NS12, facilitate improved carbon fixation, which is then transported and accumulated in the storage roots.

Table 1
Spectral characteristics of Fluorescent, NS12 (LED) and AP67 (LED) lights. Percent of UV (380–400 nm), blue (400–500 nm), green (500–600 nm), red (600–700 nm) and far-red (700–780 nm) light in each light condition. PFD: Photon Flux Density (380–780 nm).

Light treatment	Spectral Composition (%)					PPFD ($\mu mol\ m^{-2}\ s^{-1}$)
	UV	Blue	Green	Red	Far-red	
F	0.9	29.0	45.9	20.6	3.6	54.5
NS12	0.4	18.3	35.3	38.4	7.6	86.6
AP67	0.1	11.6	18.9	55.3	14.1	165.2

2. MATERIALS AND METHODS

2.1 Plant Growth conditions.

Commercial seeds of *D. carota* var. Nantes were sown in vermiculite and kept under fluorescent lights (F) until cotyledons appeared (~ 15 days). This was defined as 1 day post germination. Since this day, plants were subjected to light treatments. All plants were grown under 16/8 photoperiod and 23–24°C, with a relative humidity of 40%.

2.2 Light treatments

After cotyledons emergence, plantlets were moved under experimental LED light conditions, NS12 and AP67 (PLGL-L series of 28 W, Valoya), while control plants were kept under F light (Table 1, raw spectra Figure S3). Light conditions, light quantity (PPFD: Photosynthetic Photon Flux Density) and spectral quality were measured using a LI-COR 180 spectrometer (between 380–780 nm) and are summarized in Table 1.

2.3 Phenotypic analysis

Phenotypic parameters (root fresh weight, elongation, and diameter) were quantified at 4-, 8- and 12-weeks post germination (WPG) per light condition. For fresh weight quantification, 10 carrot roots from each light condition were weighted on an analytical balance (error ± 0.003 g; WTB 200, Radwag). For root elongation and diameter, a photographic register was taken at 4, 8, 12-week and 8, 12-week, respectively. Image-J software was used to measure the diameter in centimetres. Root elongation was calculated using 20 individual roots per light condition. Carrot root diameter was determined using 10 root discs obtained by crosscutting each root at the thickest section.

2.4 Total carotenoids quantification

Carotenoid total content was determined in roots at 4, 8 and 12-week post germination per light condition following Arias et al., 2022 protocol. For extraction, 50–100 mg of root tissue was pulverized in presence of liquid nitrogen. Then, 4 mL of hexane:acetone:ethanol (2:1:1) was added and samples were incubated on ice for 2 minutes and then centrifuged at 14000 x *g* for 10 minutes at 4°C. The upper phase was recovered and freeze-dried in HyperCOOL (Model 3055, Labogen) lyophilizer for 24 hours. For quantification, the dry extract was resuspended in acetone and measured by spectrophotometry (Jenway 6300). The absorbance was measured at 474 nm (Carotenoid absorption), 645 and 662 nm (chlorophyll a and b absorption, respectively), and 750 nm (turbidity measurement). The equations described in the literature [31] were used to calculate the concentration of pigment and normalized it per gram of dried tissue.

2.5 Relative gene expression

Relative expression of *DcPSY1* (DCAR_023043), *DcPSY2* (DCAR_010057), DcARC6 (DCAR_017505) and *DcARF6* (DCAR_003884) in carrot roots were quantified at 4-, 8- and 12-WPG per light condition following Fuentes et al. (2012) procedure. For this purpose, RNA was obtained from 100–200 mg of frozen root tissue by the CTAB (2% w/v CTAB, 2% w/v PVP40, 25 mM ethylenediaminetetraacetic acid (EDTA), 2 M NaCl, 100 mM Tris–HCl (pH 8.0), and 0.05% w/v spermidine trihydrochloride and 2% v/v β-mercaptoethanol) extraction protocol. following an overnight precipitation with 10M LiCl at -20°C. Then, samples were centrifuged at 18000 x *g* for 30 minutes at 4°C, supernatant was discarded, and the pellet was washed with 70% ethanol, dried and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA). RNA quantification was performed by spectrophotometry (Nanovue, Healthcare Biosciences). For cDNA synthesis, 3 µg of high-quality extracted RNA was treated with DNAse I (Thermofisher), following manufacturer instructions. For the synthesis of the complementary strand, 1.5 µL of oligo AP 10 uM (5’ CGC CAC GCG TCG ACT AGT ACT TTT TTT TTT TTT TTT T 3´) was mixed with DNAse I treated RNA and Improm II reverse transcriptase (Promega), following the manufacturer recommended program. Quantitative PCR was performed in a Stratagene Mx3000P thermocycler, and results were visualized by MxPro software. Evagreen Forget-Me-Not Master Mix (Biotium) was used, following manufacturer recommendations. The primers utilized (Table S1) were added to a final concentration of 10 µM, and 4 µL of 12-fold diluted cDNA was added. For the analysis of each gene, four biological and two technical replicates were performed per light condition. Quantification of relative expression was calculated by the method proposed by Pfaffl [32], setting results from fluorescent light condition as calibrator, and *DcUbiquitin* (DCAR_024457) as normalizer gene.

2.6 Soluble sugars extraction and quantification

Soluble Sugars (SS) were extracted from freeze-dried carrot tissue by homogenizing it into fine particles using a mortar and pestle, then transferred to 15 mL conical tubes [33]. Then, 8 mL of 80% v/v ethanol was added to 50 mg of tissue. Samples were then heated to 95°C in a thermoregulated bath and agitated occasionally for 10 minutes. The anthrone method was used for SS quantification, where 50 µL of the extract was diluted with 950 µL of 75% H2SO4 and anthrone reagent (Sigma Aldrich) was added to a final concentration of 2 g/L. Samples were heated to 95°C with agitation for 10 minutes, cooled to

room temperature and absorbance at 620 nm was measured in a spectrophotometer (Jenway 6405, USA). After total soluble sugar quantification, fructose, glucose, and sucrose was determined by HPLC and the respective external standards (Sigma Aldrich). Briefly, 950 µL was taken from the SS extract, and evaporated for 3 hours at 30°C in a rotational vacuum concentrator (Christ RVC 2–18 CD Plus, Germany). Samples were resuspended in 500 µL of mili-Q water and then 500 µL of HPLC-grade acetonitrile was added. Samples were centrifuged at 13000 x *g* for 1 minute and the supernatant was transferred to a HPLC vial. Separation of the three aforementioned analytes was carried out in a Nexera (Shimadzu) UHPLC, equipped with a refractive index detector, in isocratic mode using acetonitrile:water (75:25) as mobile phase with a flow rate of 0.3 mL/min. For each sample, 5 µL were injected in the column (Shodex Asahi pak NH2 P-40 3E), maintaining a constant temperature of 40°C. Chromatograms were analysed with Lab Solutions Software (Shimadzu). For starch quantification, SS samples were dried at 70°C for 16 hours. After this, the precipitate was digested with 20 µl of amylase (A3306, Sigma Aldrich) and amyloglucosidase (A9913, Sigma Aldrich). Finally, starch content was determined from the resultant monomers with the anthrone method [34].

2.7 Statistical analysis

Data reported was analysed and plotted using GraphPAD Prism 8.0.2 software. Bar graphs were used, in which the mean and standard deviation are represented and individual values are present as symbols. One-way ANOVA test with Brown-Forsythe and Welch correction for multiple comparisons was used with a confidence interval of 0,05.

3. RESULTS

3.1 LED light treatments induce an early development of carrot storage roots.

This work started with the observation that the AP67 induces greater stem elongation compared to plants grown in the control condition (F) at 4 and 8 weeks (Figure S2). Therefore, to evaluate if different aboveground light conditions could affect the phenotype of roots developed underground, we grew carrot plants under three different light qualities and intensities: F light with a high content in blue (B) and green (G) light and with a low light intensity (54.5 µmol m^{-2} s^{-1}, Table 1); NS12 with a high content in red (R) and G light and with a middle light intensity (86.6 µmol m^{-2} s^{-1}, Table 1) and AP67 with a high content in R and far red (FR) light and with the highest light intensity (165.2 µmol m^{-2} s^{-1}, Table 1). As described in Methods, all plants were germinated under F and after cotyledons appeared, plants were transferred under the respective lights in a greenhouse at 23ºC and 40% RH. Then, at 4-WPG, 8-WPG, and 12-WPG the roots were harvested for phenotypical, biochemical, and molecular analysis. At 4-WPG, roots are pale and thin under F light, as reported previously (Stange et al., 2008, Fuentes et al., 2012) but slightly wider under LED light treatments. But significant differences could be appreciated visually and statistically at 8-WPG, the developmental stage where thickening of the root and carotenoid synthesis start [27, 35]. At this point, the length and size of the roots appear wider and bigger in plants grown

under LEDs (Fig. 1a). For confirmation that roots were thicker, we cross-sectioned 10 individual roots and compared them to F, being the roots grown under LED treatments at least two times wider than in F (Fig. 1b). We repeated these qualitative measurements at 12-WPG, detecting a similar result (Fig. 1c-d). Then, to be more precise, we quantified these phenotypic parameters. Regarding root elongation we noticed that at 4-WPG those plants grown under the NS12 light have a shorter root than those grown under F and AP67 (Fig. 2a), but this difference disappears at 8-weeks, where roots of plants grown under F are shorter than those grown under LEDs. At 12-weeks, the length of the storage roots is similar as there are no differences between the three light conditions (Fig. 2a). On the other hand, the fresh weight of the roots increased significantly over time in the three light conditions, reaching 2–5 g each. But roots grown under F had a lower fresh weight with respect to the roots of plants grown under NS12 and AP67 for each developing stage (4, 8 and 12-WPG). At 4 WPG, roots growing under AP67 are heavier than those grown under NS12 and F, whereas at 8 WPG plants grown under NS12 and AP67 are 4 times heavier than those grown under F light (Fig. 2b). At 12-WPG, roots grown under NS12 and AP67 conditions had 3.7 and 2.2 times more fresh weight than roots of plants grown in F condition, respectively (Fig. 2b).

Additionally, we found that at 8-WPG the roots of plants grown in NS12 are 3 and 1.5 times wider than those grown under F and AP67, respectively (Fig. 2c). In turn, LED-grown roots are significantly wider than those grown in F, confirming the phenotype shown in Fig. 1. At 12-WPG however, storage roots of plants growing under NS12 and AP67 lights are 1.5 and 1.3 wider with respect to the roots of plants grown in F light, but there is no difference between LEDs treatments. At the same time, it is noticeable that the NS12 and AP67 induce the same root biomass accumulation at 8 WPG (Fig. 2a-b) that is reached in F only at 12 weeks (Fig. 2c). These analyses lead us to conclude that, NS12 and AP67 treatments induce an early development of secondary roots growth regarding F, starting at 8-WPG, showing that LEDs conditions are favourable for carrot storage root development.

3.2 LED light treatments do not affect carotenoid content in carrot storage roots.

Since the literature reports a correlation between the induction of carotenogenic genes expression and an increase in carotenoid content in plants grown under white light [3, 36] and that the direct light impairs carotenogenic genes expression and carotenoid content in carrot storage roots [27, 35], it was proposed to quantify the carotenoid content in carrot storage roots of plants grown in F, NS12 and AP67 light conditions at 4-, 8- and 12-WPG. We found that there is no difference in total carotenoid content among the roots of plants grown under the three light conditions (Fig. 3a) in the three developmental stages. Therefore, different light intensities nor spectra do not induce a greater accumulation of carotenoids in carrot storage roots. However, there are differences in the accumulation of carotenoids through time; during the development of roots grown under F and AP67, carotenoid concentration shows a gradual increase in pigment content. In contrast, storage roots from NS12 have no significant difference between 4-WPG and 8-WPG; nevertheless, a significant increase between 8- and 12-WPG was observed, duplicating its carotenoid content.

Despite carotenoid abundance is unaffected by the different light treatments, the relative expression of *DcPSY1* and *DcPSY2* was determined to know if they remain unaltered as well. In the case of 4-WPG, we found no significant differences in the expression of *DcPSY1* and *DcPSY2* in roots (Fig. 3b-c). However, at 8-WPG (Fig. 3b-c), both *DcPSY1* and *DcPSY2* are significantly induced in the storage roots of carrot plants grown under NS12 and AP67 light conditions with respect to F. Strikingly, the relative transcript abundance increment ranges between 30 to 50 times under LED treatments. Once again, at 12-WPG no statistical differences in the expression of *DcPSY1* and *DcPSY2* between roots of carrot plants grown under F, NS12 and AP67 light conditions was observed (Fig. 3b-c). It is interesting that differences in relative expression occur only at 8-WPG, which was described as a key time of the development of carrot storage [27]. Even more puzzling is the fact that the induction of the relative expression of *PSY*s doesn't translate into a higher carotenoid content.

3.3 LED light treatments improve starch and sucrose accumulation in carrot storage roots.

Considering that a) the different light treatments did not elicit a higher synthesis nor accumulation of carotenoids during the carrot taproot development but b) did induce a biomass accumulation resulting in heavier and wider roots, we wanted to find an explanation for this improved yield. Considering that carrot storage roots are a starch sink [37] and that photosynthesis is tightly related to biomass production [22], we quantified the non-structural sugar content in mature roots. We found that, at 12-WPG, roots grown under AP67 have around 39% more soluble sugars (SS) than those grown under F and NS12 (Fig. 4a). Interestingly, when specific sugars were quantified (Fructose, Sucrose and Glucose), Sucrose had a higher concentration in storage roots grown under both LEDs than those grown in F: NS12 promoted an increment of around 110% Sucrose and AP67 an 150% increment (Fig. 4b). Fructose and Glucose were not affected by the light conditions (Fig. 4c-d). In the case of the starch content, storage roots grown under F only had around 37 mg/g, while roots grown under AP67 had 68 mg/g, an increment of 83% (Fig. 4e). Strikingly, storage roots grown under NS12 were able to store around 155 mg/g, a 318% increase (Fig. 4e). Together, these results point out to an improvement in fixating carbon as Sucrose and Starch levels are improved under LEDs (being Sucrose one of the main photoassimilates and starch the main source of energy reserves found in sink tissues).

4. DISCUSSION

The study aimed to investigate the effects of different aboveground light treatments on carrot storage root biomass and carotenoid content. Three lighting conditions were compared: fluorescent light (F) with a spectrum enriched in G wavelengths, LED light with a spectrum enriched in G and R wavelengths (NS12), and LED light with a spectrum enriched in R and FR wavelengths and with almost double of light intensity (AP67). It is important to note that the results obtained were due to the synergy between the wavelengths that make up the spectrum and the light intensity and that this effect varies between species [5].

Previously, we determined that at 8 weeks starts the secondary development of storage root in carrots grown under F light, with a small increase in the thickness of the root and the beginning of the acquisition of the orange tone due to the accumulation of carotenoids, reaching a state of maturity at 12 weeks [27, 30, 38, 39]. It is interesting to note that at 8 weeks the most notable differences between the control condition (F) and the experimental conditions (NS12 and AP67) are seen at the phenotypic level (Figs. 1 and 2) and relative gene expression (Fig. 3).

4.1 LED lights promote an early development of carrot storage roots.

At the beginning, we noticed that the AP67 induces greater stem elongation compared to plants grown in the control condition (F) at 4 and 8 weeks (Fig. S1). This observation is consistent with the characteristics of the spectrum described by AP67, which is offered on the market to enhance vegetative growth, and which has the highest level of PFD (165.2 µmol m^{-2} s^{-1}), higher percentage of red light (55%) and lower R/RL (3.92) (Table 1). Nagel (2006) observed in *N. tabacum* that when the light intensity increased from 60 to 300 µmol m^{-2} s^{-1}, an increase in the elongation of the stem and roots occurred, associating it with the higher photosynthetic rate achieved at a higher intensity of light. On the other hand, quality of light is related to the wavelength at which chlorophyll absorbs energy; in this case being photons with a wavelength in the ranges of red-light spectrum (600–700 nm) and Blue (400–500 nm). Photons with these wavelength ranges allow the excitation of chlorophyll, where electrons rise to a higher energy state and are transferred to form part of the photosynthetic electron chain (Reol, 2014). Considering this background, the AP67 has two elements that allow us to suggest an appropriate photosynthetic rate: the highest light intensity (165.2 µmol $m^{-2}s^{-1}$) compared to NS12 and F, a high content of R (55%), and low B (11%) (Table 1). Consequently, these favourable elements for photosynthesis could translate into greater elongation of aerial tissue (Fig. S1). On the other hand, the highest content of FR light (14%) with respect to F and NS12, could also be the reason for greater stem elongation under AP67. It has been reported that in response to the low R/FR ratio, or the increase in FR light through supplementation with LED lights, stem elongation occurs [18, 20, 40, 41]. Specifically, in *S. Lycopersicum*, supplementation with LED lights that emit photons in the FR light range promotes stem elongation, while blue light, antagonistically, promotes a decrease in plant size [18, 20]. Hence, the sum of all these characteristics of AP67 could promote the effect in stem elongation. However, carrot plants grown under AP67, acquired a purple tone due to the accumulation of anthocyanins (Fig. S2) that could indicate that these plants are stressed [42] because anthocyanins have a key role in protecting the plant through its antioxidant capacity [8, 43].

Regarding NS12 at aerial tissue, there are no robust differences with respect to F. This would be related to the fact that, as reported by the manufacturer, the NS12 spectrum corresponds to white LED light, providing similar light conditions with natural lighting and photon abundance in the PAR range [5, 6].

Considering the effect of these lights on stem length, we investigated if these LED lights could influence the carrot storage root (sink organ) that grows underground and is not in direct contact with the aboveground light qualities. Interestingly, this study revealed notable differences in carrot root morphology, biomass accumulation and sucrose content among the different light treatments.

At 8- and 12-weeks post germination (WPG), carrots grown under LED lights exhibited storage roots at least two times wider and heavier compared to F light-grown roots at each developmental stage. Interestingly, roots grown at 8-WPG under LED lights accumulated biomass comparable to that achieved by F light-grown roots at 12-WPG, suggesting an accelerated development under LED conditions. Although root length varied among light treatments at 4-WPG and 8-WPG, the length of roots was similar across all light conditions by 12-WPG (Fig. 2).

A greater biomass is usually related to a greater photosynthetic capacity [12, 44]. Similar suggestions have been proposed in radish, where the highest storage root biomass achieved is related to the effect of light intensity on photosynthesis [12].

One of the products of photosynthesis corresponds to sugars (sucrose) that promotes cellular metabolism [45] and is translocated to sink organs such as roots. Sucrose is needed for root growth and development and serves as a long-distance signal from the shoot to inform the root system about light availability above ground. Evidence in *A. thaliana* shows that sucrose can act at the root level as a signalling molecule derived from leaves, thus triggering the morphogenesis and development of the root system [46–48]. Plants grown under LED lights exhibited storage roots with higher levels of soluble sugars (SS), sucrose and starch compared to F light-grown roots, indicating improved carbon fixation and storage under LED conditions (Fig. 4). Specifically, AP67, which has a higher proportion of red light (55.4%, Table 1), induced a 1.2-fold increment in SS and a 2.7-fold increase in sucrose compared to F light. AP67 almost doubled the starch content, while NS12, which has 38% R light and 7.4% FR light (Table 1), quadrupled it compared to F light. The increase in sucrose content may indicate improved photosynthesis processes under both LEDs, as sucrose is a mobile photosynthate. However, the significant increase in starch suggests that starch turnover is likely accelerated in F, as SS levels are like those in NS12. The breakdown of starch may occur at an intermediate level under AP67, as starch concentration is lower than in NS12, but higher than in F.

On the other hand, we analyzed the proportions of R and B lights since a positive effect was observed on radish root development (diameter, volume, and fresh weight) dependent on the proportions of R and B lights, where a 2R:1B ratio promoted better root development compared to 1R:1B (Zha & Liu, 2018). In this work, the R and B range in the lighting conditions were also different, ranging from 2:3 in F, 2:1 in NS12 and 5:1in AP67, which also agrees with the storage root biomass obtained in each condition, showing the highest diameter and fresh weight in NS12, suggesting that the proportion 2:1 of R:B is the best for these purposes in carrot as well (Fig. 1 and Fig. 2).

These results allow us to suggest that LEDs, preferably of NS12, contain an adequate spectral quality enriched in R light (38,4%) and an intensity of 86,6 μmol m^{-2} s^{-1}, two-fold greater than that provided by F

(Table 1). This permits plants to develop a more efficient photosynthesis with the concurrent production and translocation of sucrose to the root, that can be accumulated into starch.

4.2 Different aboveground LED light treatments did not influence carotenoid content in carrot storage roots despite the induction of gene expression.

Despite the significant effects on root biomass, different aboveground light treatments did not influence carotenoid content in carrot storage roots in all light conditions, despite the relative expression of *DcPSY1* and *DcPSY2* were significantly induced in 8 weeks old-storage roots of plants grown under LED lights compared to F light. This suggests a complex regulatory mechanism governing carotenoid biosynthesis, which is not regulated by photosynthesis or by the same pathway as sucrose synthesis. Although various components are transported from the shoot to the root, only a few of them have been directly linked to light signalling in the shoot. These include sugars derived from photosynthesis, plant hormones (auxin), and HY5 [49].

On the other hand, light from the aboveground enters the stem of herbaceous and woody plants, and via an internal light-conducting system a low proportion of FR light is conducted axially towards the roots underground up to approximately 3 cm [50, 51]. The wavelengths transmitted most efficiently by the stems or roots were generally between 710 nm and 940 nm independent of the light angle and light source [50]. In 8 weeks-old storage roots, genes such as *PHYA, PHYB, PAR1* and *PIF3* are expressed [38, 39] and most of them are regulated by FR light. Indeed, DcPAR1 was proven to be required for carotenoid synthesis and carrot storage root development [38]. It is possible that independent of the light perceived aboveground, the same low quality FR light is translocated, which activates at similar levels the genes regulated by FR light (such as *PHYA* and *PAR1*) required for carotenoid synthesis, thus explaining the fact that the carotenoid level does not differ between the lights used.

Overall, the findings highlight the significant influence of aboveground light treatments on carrot storage root biomass and non-structural sugar accumulation, providing insights into optimizing growth conditions for enhanced crop yield and quality. Our study contributes to a deeper understanding of the complex interplay between light and plant development, paving the way for more efficient and sustainable agricultural practices in the future.

5. CONCLUSIONS

The quality and intensity of aboveground light in which the carrot is grown significantly affects the development of the storage root and sucrose content but not on carotenoid content.

It is proposed to grow carrots in NS12 to induce earlier development and an increase in sucrose content.

Abbreviations

All abbreviations are included in the text.

Declarations

Ethics approval and consent to participate: The work was approved by the Biosecurity Committee of the Faculty of Science from the University of Chile. CERTIFICADO DE BIOSEGURIDAD 2022_CLB_FC_UCH_016

Consent for publication: Not applicable.

Data Availability Statement: All data generated or analysed during this study are included in this published article and its supplementary information files. Additional analyses are available from the corresponding author on reasonable request.

Competing interest: All authors declare that they have no competing interests

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Authors' contributions: C.S and C.G-C conceived and designed the experiments and wrote the manuscript, F.M performed assays for Figure 1-3 under C.G-C and C.S supervision. C.G-C prepared supplementary material. A.S, L.V and PP designed and carried out assays for Figure 4. All authors reviewed the manuscript.

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Figures

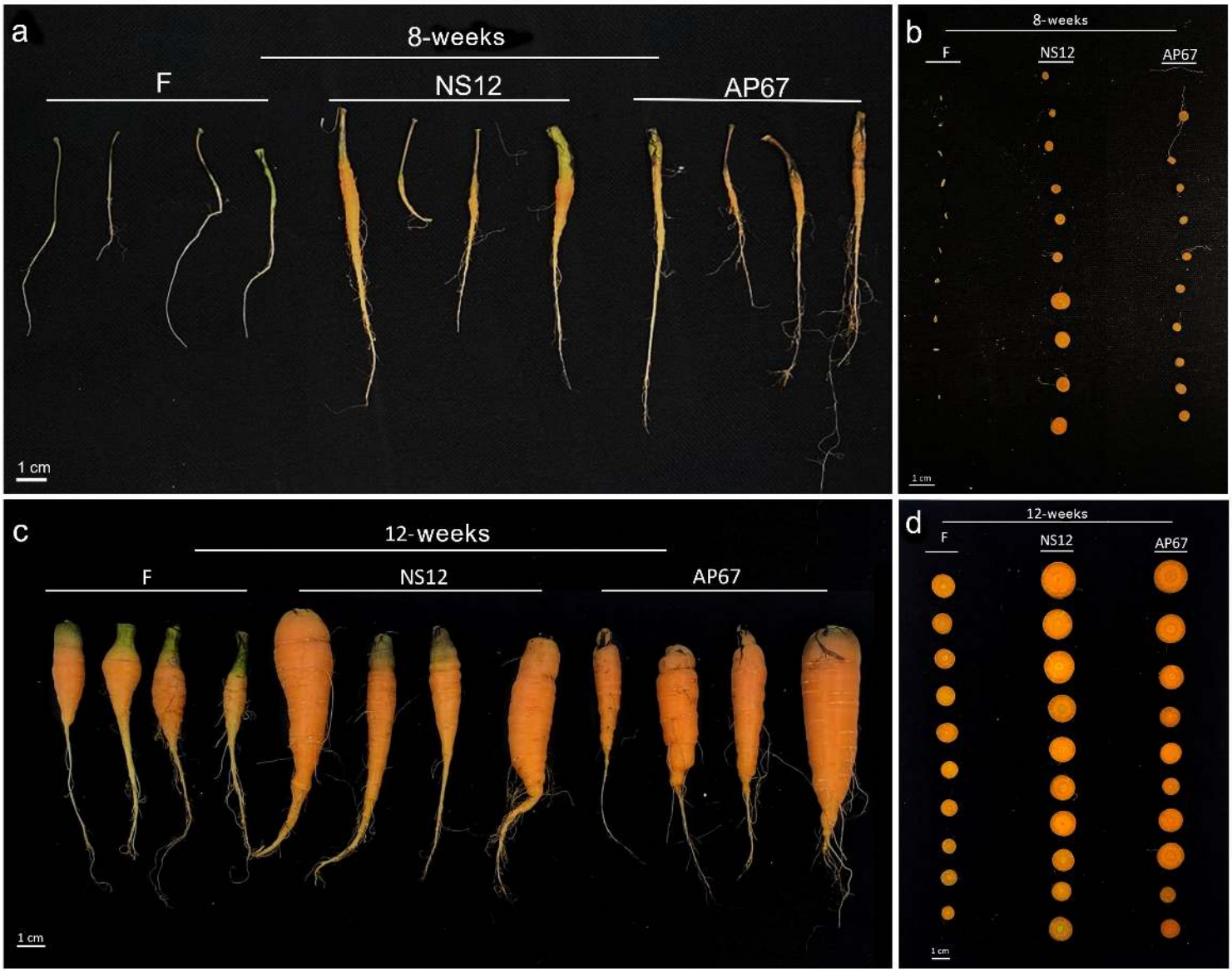

Figure 1

Carrot taproot phenotypes from plants grown under different light conditions at 8- and 12-weeks post germination (WPG). a) 8-WPG unearthed carrot storage roots of plants grown under different light conditions (F, NS12 and AP67), b) cross-sections from an individual storage root from (a). c) 12-WPG unearthed carrot storage roots of plants grown under different light conditions (F, NS12 and AP67), d) cross-sections from an individual storage root from (b)

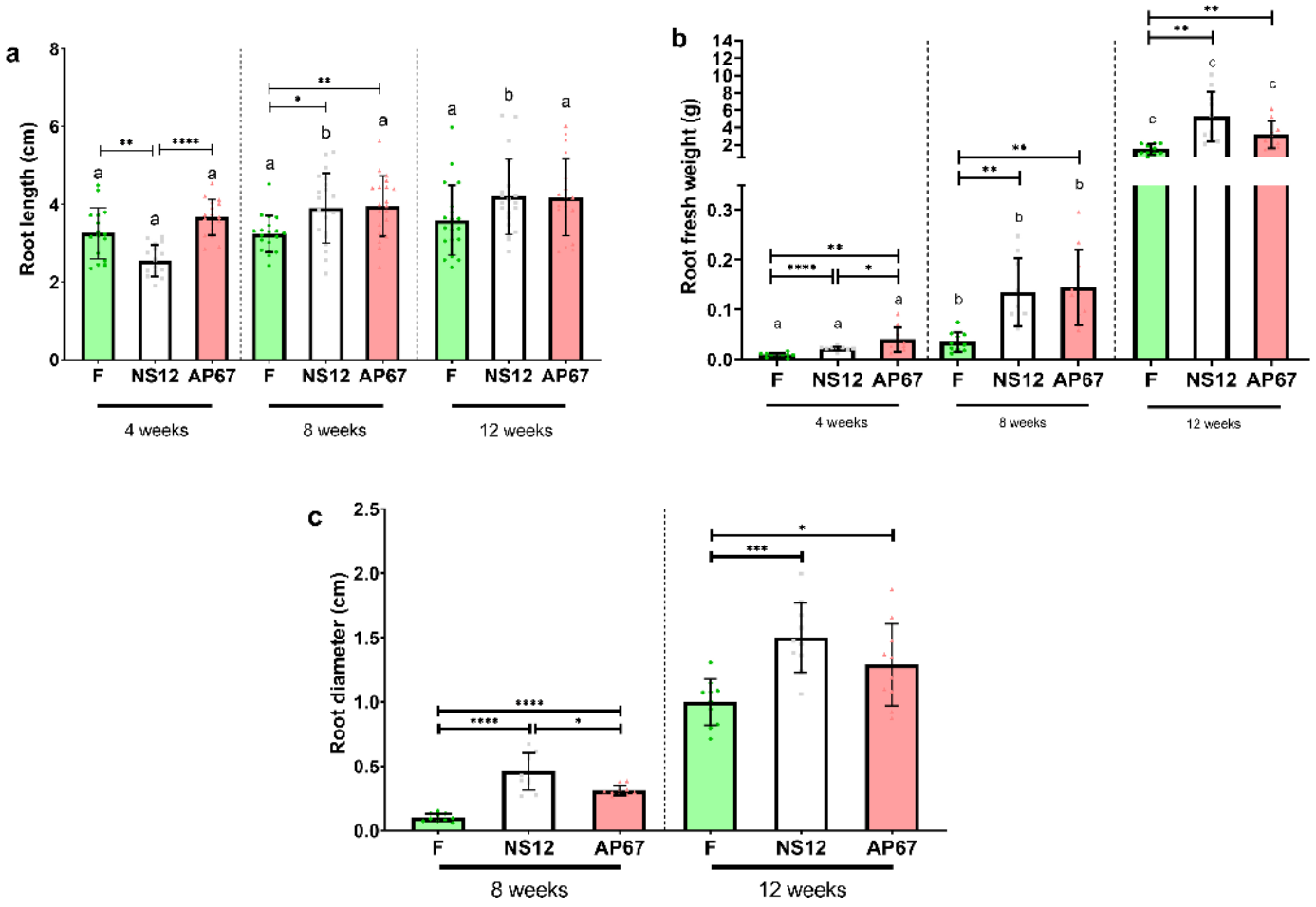

Figure 2

Phenotypic parameters of carrot storage roots from plants grown under different light conditions through development. a) Root length, b) root fresh weight, and c) root diameter of carrot storage roots of plants grown under F, NS12 and AP67 light conditions. Root fresh weight and length parameters were registered at 4-, 8- and 12-WPG. Root diameter was registered at 8- and 12-WPG. Each bar of the graph represents the mean of ten data points and individual values are plotted in front of the bar. Letters denote significant differences within a condition over time, and asterisks denote significant differences between conditions at a specific number of weeks. The differences were calculated using one-way ANOVA ($p<0.05$) with Brown-Forsythe and Welch correction. **** $p<0.0001$, *** $p<0.001$, ** $p<0.01$, * $p<0.1$

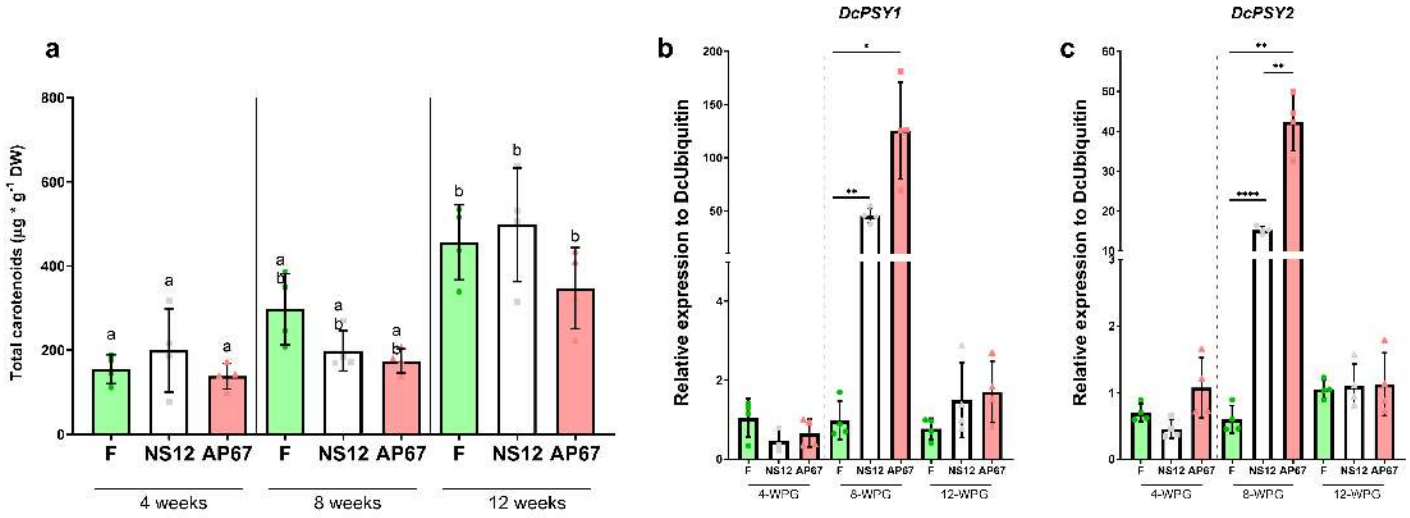

Figure 3

Total carotenoid content and relative expression level of *DcPSY1* and *DcPSY2*in carrot taproot of plants grown under different light conditions. a) Carotenoid content in carrot storage root of plants grown under F, NS12 and AP67 lights at 4-, 8- and 12-WPG. Relative expression of b) *DcPSY1* and c) *DcPSY2* in carrot storage roots of plants grown under F, NS12 and AP67 lights at 4-WPG, 8-WPG and 12-WPG. Each bar represents the mean of three biological replicates. Letters denote significant differences within the same condition over time, and asterisks denote significant differences between conditions at one developmental stage. Statistical differences were calculated using one-way ANOVA ($p<0.05$) with Brown-Forsythe and Welch correction. ****: $p<0.0001$, ***: $p<0.001$, **: $p<0.01$, *: $p<0.1$

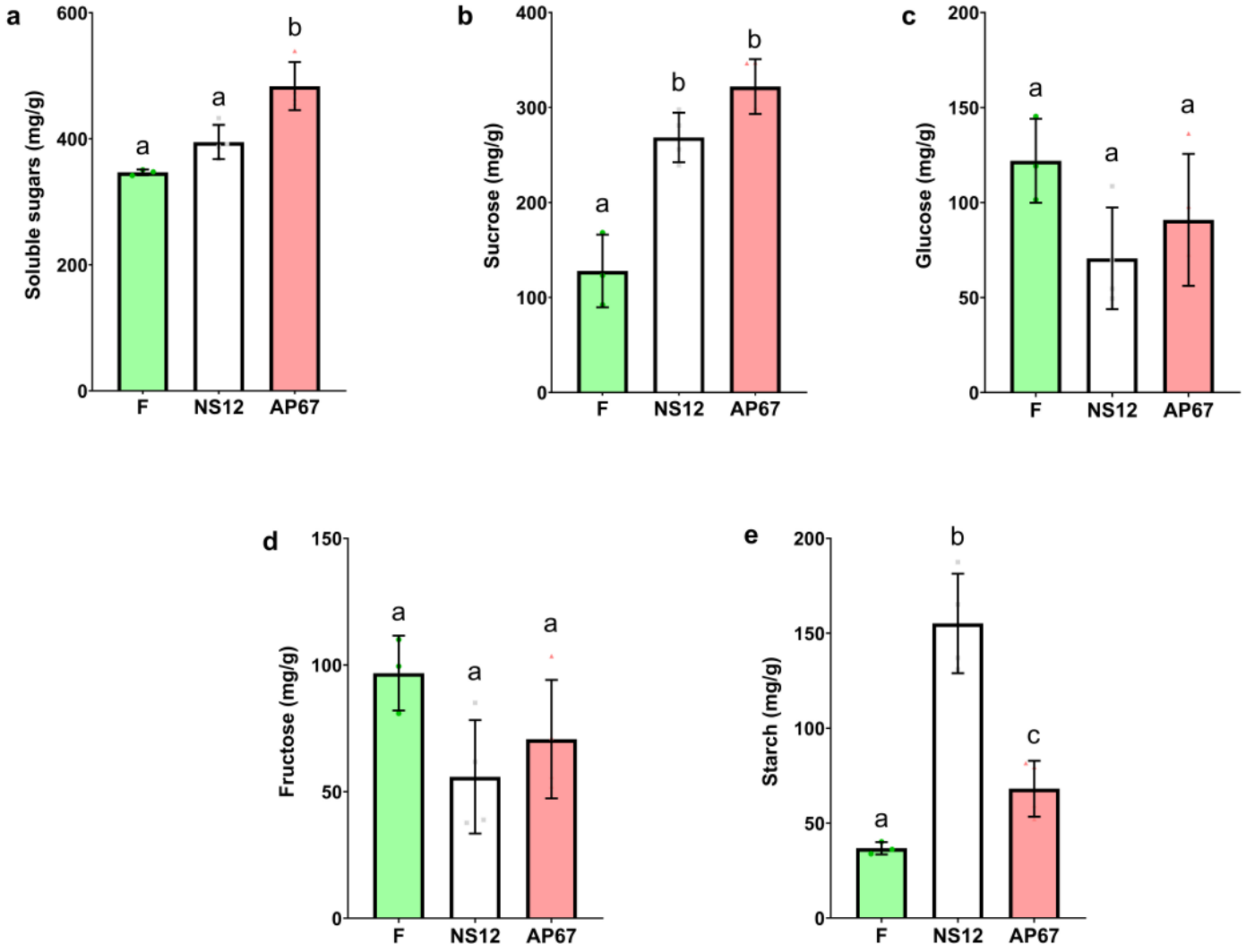

Figure 4

Total sugars content in carrot storage roots of 12-WPG plants grown under different light conditions. a) Total Soluble Sugars, b) Sucrose, c) Glucose, d) Fructose and e) Starch content. Each bar represents the mean of four biological replicates, except for F values which are composed of 3 biological replicates. Different letters denote significant differences. These were calculated using one-way ANOVA ($p<0.05$) with Brown-Forsythe and Welch correction.

Supplementary Files

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