**WELSON JUNIOR SILVA**

# PHOTOPERIOD-RELATED CHANGES IN PLANT RESOURCE ALLOCATION: a physiological view

Dissertation presented to the Universidade Federal de Viçosa, as part of the requirements of the Botany Graduate Program for obtention of the degree of *Master Scientiae*.

Adviser: Wagner L. Araújo

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# PHOTOPERIOD-RELATED CHANGES IN PLANT RESOURCE ALLOCATION: a physiological view

Dissertation presented to the Universidade Federal de Viçosa, as part of the requirements of the Botany Graduate Program for obtention of the degree of *Magister Scientiae*.

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Assent:

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*I dedicate this work to the African people, whether on the continent or in the diaspora*.

## ACKNOWLEDGEMENTS

First of all, I would like to thank the power of the universe that rules my life. Without my faith in the divine, nothing would be possible. Gratitude to the life of my ancestors for showing me my purpose in this life.

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## ABSTRACT

SILVA, Welson Junior, M.Sc., Universidade Federal de Viçosa, March, 2022. **Photoperiod-related changes in plant resource allocation: a physiological view**. Adviser: Wagner L. Araújo.

Fabaceae is one of the most consumed botanical families in both human and animal diets, due to its high nutritional value of nitrogen and protein. In fact, about 35 tons of leguminous grains are consumed annually revealing its enormous importance. Contrasting environmental conditions drastically affect the allocation of resources to cultivated plants of agronomic interest. Photoperiod is an environmental condition that plays an essential role in modulating resource allocation being also able to influence plant growth, survival and interactions in the environment. Accordingly, our understanding of the details that govern the flow of nutrients and differential patterns of plant development and growth remains fragmented. The present work was carried out to analyze the impacts on the physiology, metabolism, anatomy and resource allocation of plants of the Fabaceae family (*Vigna unguiculata* and *Pisum sativum*) caused by photoperiod variation [short days (8 light/16 dark) or long days (16h light/8h dark)]. Long days negatively affected the growth of both plants and this lower growth was associated with lower photosynthetic rates. By contrast, under short day conditions, high photosynthesis ($A_N$) was associated with higher stomatal density, as well as higher stomatal conductance ($g_s$). Significant variations in morphological characteristics in vegetative organs in different photoperiods were observed among the genotypes. Long days culminated in reductions of growth parameters (e.g., dry weight of roots, stems and leaves and the number of leaves) yet root length and leaf area were higher. The different photoperiod conditions also altered the concentration of macro and micronutrients as revealed by an increase of mineral nutrients observed in short days plants. In conclusion, the resource allocation pattern in the investigated plants is strongly modified in response to fluctuations in light supply. Further detailed molecular and metabolic studies are still needed to better understand the effects of photoperiod on differential resource allocation.



Keywords: Circadian rhythm. Photoperiodism. Biomass partitioning

## RESUMO

SILVA, Welson Junior, M.Sc., Universidade Federal de Viçosa, março de 2022. **Alterações relacionadas ao fotoperíodo na alocação de recursos em plantas: uma visão fisiológica**. Orientador: Wagner L. Araújo.

Devido ao seu alto valor nutricional de nitrogênio e proteína, Fabaceae é uma das famílias botânicas mais consumidas em dietas humanas e animais. Cerca de 35 toneladas de grãos leguminosos são consumidos anualmente. Com efeito, condições contrastantes afetam drasticamente a alocação de recursos de plantas cultivadas de interesse agronômico. O fotoperíodo é uma condição ambiental que desencadeia um papel essencial na regulação da alocação de recursos, e além disso, influencia o crescimento vegetal, sobrevivência e interação no ambiente. Desta forma, o entendimento preciso dos detalhes que modulam o fluxo de nutrientes e padrões diferenciais de desenvolvimento vegetal é crucial. O presente trabalho foi conduzido com o intuito de analisar os impactos causados pela variação do fotoperíodo sobre a fisiologia, metabolismo, anatomia e alocação de recursos de plantas da família Fabaceae (*Vigna unguiculata* e *Pisum sativum*). Os resultados obtidos demonstraram que o aumento do fotoperíodo afetou negativamente o crescimento dessas plantas e este menor crescimento esteve associado a menores taxas fotossintéticas. Não obstante, em dias curtos, a elevada fotossíntese ($A_N$) apresentou-se associada com uma maior densidade estomática, assim como maiores condutâncias estomática ($g_s$). Variações significativas em características morfológicas em órgãos vegetativos em distintos fotoperíodos foram observadas entre os genótipos. As espécies estudadas diferiram entre si em termos de parâmetros de crescimento, como peso seco de raízes, caules e folhas e número de folhas (unidades). Embora as plantas cultivadas em dias longos apresentaram reduções nestes parâmetros, o comprimento das raízes e área foliar foram maiores. As diferentes condições de fotoperíodo também alteraram a concentração de macro e micronutrientes e, assim, plantas em dias longos apresentaram um incremento de nutrientes minerais em relação às plantas em dias curtos. Em suma, o padrão de alocação de recursos nas espécies investigadas é fortemente modificado em resposta a flutuações no suprimento de luz. Estudos metabólicos e moleculares mais

detalhados são ainda necessários para uma melhor compreensão dos efeitos do fotoperíodo na alocação diferencial de recursos.

Palavras-chave: Ritmo circadiano. Fotoperiodismo. Particionamento de biomassa

## SUMMARY

## 1. INTRODUCTION

Members of the Fabaceae family, colloquially referred to as legumes, are of great agronomic interest because of their high protein and nitrogen content (Da Silva, et al., 2019; Malovichko et al., 2020). Due to this, they are one of the most used botanical families in both human and animal diets. According to data from the FAO (2020), 35 tons of leguminous grains are consumed worldwide each year (http://faostat.fao.org/faostat/collections?subset=agriculture). In addition to its usage as food, the abundant diversity of Fabaceae allows for different uses including raw materials for oils, resins, extracts from medicinal plants and soil recovery through green manuring (Gepts et al., 2005). Of these species, pea (*Pisum sativum*) and Vigna (*Vigna unguiculata* L. Walp.), also known as “cowpea”, “black-eyed pea” and “string bean”, are among the most cultivated crops, representing a source of food security and economic value in several countries in the world (Da Silva et al., 2019).

*P. sativum* is a Leguminosae growing mainly in Europe and North and South America. It is an annual plant, with a life cycle between 60 to 110 days, having a hermaphrodite and autogamous flower and it is a short-day plant. It is consumed in the form of green beans, dried beans for rehydration and green beans for freezing and canning (Arriel, 2005). In addition, *V. unguiculata* can grow in abundance in Africa, Europe, Asia, Oceania, the Middle East, the Southeast of the United States as well as in Central and South America. It is an annual cycle plant, autogamous and in relation to the climate, it is widely adapted to be a long-day plant (Arriel, 2005). *V. unguiculata* can be marketed in the form of dried or green beans, seeds, green salad pods, pre-cooked soup and canned beans (Giordano, 1997; Queiroz et al., 2002).

To understand the allocation of the fixed carbon, most of the research efforts have been focused only on the role of the biomass increase (Liu and Su, 2016). Notwithstanding, investigations dealing with biotic traits e.g., light and photoperiodism, clearly deserve attention in understanding the precise details that modulate the flow of nutrients and resource allocation and its overall impact on both plant size and development (Wright et al., 2004).

Resource allocation is one of the central concepts in plant biology, providing the basis for different strategies. Briefly, any plant has a given amount of allocation of resources at any point in time and it allocates these resources to different structures and organs (Weiner, 2004). However, plants may change their allocation patterns in

response to the environmental conditions (e.g., shade, exposure to sunlight, temperature, nutrient availability and soil composition) (Bonser and Aarssen, 2003; Lima et al., 2017). Resources allocated to a function pathway or organ are therefore not available to others any longer. In addition to the plant demand itself, allocation patterns can be influenced by exogenous (environment) and endogenous factors (regulatory mechanisms) (Müller et al., 2000; Knops et al., 2007). Thus, expanding our understanding of environmental factors (e.g., photoperiod) and its potential effects on carbon metabolism, in particular, in the allocation of resources is of critical significance.

Light, a highly heterogeneous environmental factor, plays an essential role in the regulation of carbon partitioning in plants. Furthermore, light influences plant growth, survival and competitive interactions in the environment (Chen and Yang, 2018; Hussain et al., 2019). In plants, light is perceived by photoreceptors molecules (phytochromes and cryptochromes) that mediate a wide range of developmental responses to light quality, quantity and daylength (Thomas, 2016). Photoreceptors can absorb light, allowing the plant not only to identify the photoperiod but also to control its circadian clocks. Plants utilize circadian clocks to synchronize their physiological and developmental events with daily and seasonal changes in the environment (Nohales and Kay, 2016). Circadian clocks have evolved as molecular timekeeping mechanisms that enable organisms in genera to predict and anticipate periodic changes in their surrounding environment, therefore allowing an efficient allocation of resources and ultimately enhancing the fitness (Song et al., 2010).

In *Arabidopsis thaliana,* variation in circadian rhythm is perceived through the expression or repression of rhythmic genes, such as *CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)*, *LATE ELONGATED HYPOCOTYL (LHY)*, *PSEUDO RESPONSE REGULATOR (PRR)* and *TIMING OF CAB EXPRESSION 1 (TOC1)* (Siqueira et al., 2021). Briefly, in the early hours of the day, high levels of *CCA1* and *LHY* gene transcripts are observed. In the last hours of the day, high expression of *TOC1*, *GIGANTEA (GI)*, Early *FLOWERING 3* and *4 (ELF3/4)* and *LUXARRHYTHMO (LUX)* genes are usually observed. These genes are involved in numerous processes such as internal metabolic and hormonal signals, ranging from the control of metabolism, growth, development and stomatal opening to metabolic processes (Inoue et al., 2010; Song et al., 2010; Carré and Veflingstad, 2013; Chen and Yang, 2018). The potential role of light and daylength in the regulation and

maintenance of circadian rhythm, altering the translation of rhythmic genes has been demonstrated (Staiger and Green, 2011; Siqueira et al., 2021). Recently, both photoperiod and circadian rhythm were shown to affect Aluminum (Al) tolerance. It was observed that with increasing distance from the equator, Al tolerance disappears, suggesting a relationship with the photoperiod. This outstanding study also revealed that long-day (LD) species are generally more Al-sensitive than short-day (SD) species, suggesting that photoperiod acts as a selective barrier for Al tolerance across the globe (Siqueira et al., 2021). Moreover, this relationship may be explained because plant species have distinct levels of genetic diversity as observed in eudicots whose genetic diversity increases with distance from the Equator. Accordingly, the distance to the Equator is likely an important moderator of genetic diversity through its association with temperature, productivity and photoperiod (De Kort et al., 2021). Taken together, it seems clear that enhancing our understanding of how and to which extent the photoperiodism modulates biological processes and affects productivity is an important agronomic issue. Here, we postulate that day-length can promote differential impacts on plant growth in distinct plant species. To this end, the main goal of this work is to elucidate the differences in size, metabolic accumulation and physiologic pattern during the development of *V. unguiculata* and *P. sativum* cultivated under either long-day or short-day conditions.

Our results revealed that plants grown under short-day conditions were characterized by a higher increase in resources than those grown under long-day conditions, coupled with differences in photosynthetic assimilation of young leaves and primary metabolism in both roots and shoots. Collectively, the results obtained here suggest that photoperiod changes can differentially alter the resource allocation patterns in both species.

## 2. MATERIAL AND METHODS

### Plant material and experimental conditions

Seeds of pea (*Pisum sativum* L., cv. Bolero) and cowpea (*Vigna unguiculata* (L.) Walp cv. Pingo de Ouro) were used in this study and before any experiments we grew plants under the same conditions to obtain seeds with similar vigour and to avoid photoperiodic impacts in the progenies. Seeds were surface-sterilized with 0.5% (w/v) sodium hypochlorite for 5 minutes, thoroughly rinsed in deionized water and germinated on moistened germination paper rolls and remained in darkness for five days to synchronize germination. Following, seedlings were cultivated for 25 days under either short days (8 light/16 dark) or long days (16h light/8h dark) with temperature at 22ºC±2, under 250 µmol photons $m^{-2}$ $s^{-1}$, 60% relative humidity. Seedlings were cultivated in hydroponics solution by using Hoagland-medium modified and at pH 5.0 (Hoagland and Arnon, 1950) under constant aeration with renewal every 5 days. Throughout the experimental period, root elongation and overall other phenotypical, physiological, anatomical and biochemical analyses were evaluated.

### Characteristics of epidermal cells

Leaves were collected and placed in FAA (Formaldehyde; Alcohol; Acetic Acid, 10%:50%:5% + 35% distilled water) for 48 hours, after which the FAA was replaced by lactic acid and the plant material remained for two days in an oven at 65ºC. After the leaves were completely transparent, it was possible to visualize the cells on the adaxial side. Images visualization was made using a digital camera (AxiocamMRc) attached to the Zeiss microscope model AX10. The number of epidermal cells and stomata was determined using the Axiovision program. With these data, the number of stomata (S) and epidermal cells (E), as well as the S/E ratio were estimated.

### Sample homogenization and metabolite extraction

All sampling procedures were carried out on the expanded leaves from 25-day-old plants, during the vegetative stage. Leaves were harvested at the end of each photoperiod (15h/SD-23h/LD) and immediately flash-frozen in liquid nitrogen, and stored at -80°C until further analysis. Extraction was performed after a rapid

grinding of tissue in liquid nitrogen, by immediate addition of ethanol as described by (Gibon et al., 2004). The ethanol extracts and the precipitated were stored at -20°C for subsequent metabolites quantification.

**Pigments determination**

The content of chlorophyll (*a* and *b*) and carotenoids were determined immediately after ethanolic extraction using aliquots from the supernatant and ethanol mix placed on microplates. The absorbance was measured at 645 and 665 nm. The content of chlorophyll *a* was determined following the equation suggested by (Arnon, 1949). Last, the total content of carotenoids and chlorophyll (*a* + *b*), as well as chlorophyll a/b ratio, were determined.

**Amino acids content**

Total amino acid content was determined as described by (Cross et al., 2006). Briefly, the mix containing 1 M citrate buffer, pH 5.2 with ascorbic acid 0.2% (w / v), 50 µL of ethanol extract and 100 mL of ninhydrin solution at 1% (w/v in 70% ethanol) was added to the microplate and incubated for 20 min at 95 °C. After incubation, the plates were centrifuged for 10 seconds at 10.000 *g* and subsequently, the samples were transferred to a new microplate and readings were performed at 570 nm. For the determination of total amino acid content, a standard curve of Leucine was used.

**Determination of sugars content**

The levels of glucose, fructose and sucrose were determined in the ethanol-soluble fraction as previously described (Fernie et al., 2001). Briefly, 60 µL of ethanol extract were add to a reaction medium containing HEPES/KOH buffer 0,1 M pH 7, $MgCl_2$ (30 mM), ATP (60 mg mL-$^{1}$), NADP (36 mg mL$^{-1}$) and glucose6-phosphate dehydrogenase (G6PDH) (70U mL$^{-1}$). The absorbance was determined at 340 nm in one-minute intervals. Once the absorbance was stabilized, it was added hexokinase (1.5 U/reaction), phosphoglucose isomerase (0.7 U/reaction), and invertase (5U/reaction) to determine glucose, fructose, and sucrose, respectively. To calculate the concentration of the respective sugars the following equation µmol NADPH= ΔOD/(2,85*6,22) was used.

**Determination of starch levels**

Starch level was measured as previously described (Fernie et al., 2001) Briefly, the precipitate was resuspended in 0.1 M NaOH, and neutralized with 1M acetic acid. The mix for degradation of starch containing the enzymes amyloglucosidase and α-amylase diluted in sodium acetate 0.5M pH 4.0 was added to 40 µL of suspension and incubated at 55°C for 60 min. The plates were centrifuged for 10 seconds at 10.000 g and then 50 µL of the suspension was transferred to a new plate where it was added to each well 160 µL of a mix containing HEPES / KOH buffer 1M, pH 7.0, $MgCl_2$ (30 mM), ATP (60mg/mL), 8 NADP (36mg/mL), and glucose-6-phosphate dehydrogenase (0.7 U/µL). The absorbances were read at 340 nm in one-minute intervals. Once the absorbance was stabilized, the reaction was started by adding hexokinase (2U/well). To calculate the concentration of glucose the following equation was used: µmol NADPH= ΔOD/(2,85*6,22).

**Determination of total soluble protein**

Protein content was determined as in (Bradford, 1976). Briefly, it was added to the tubes containing the precipitate NaOH 0.1 M following incubation for 1 hour at 95°C. Subsequently, the tubes were centrifuged at 16.000 *g* for 5 minutes. An aliquot of 3 µL of supernatant was added to a microplate containing in each well 180 µL of Bradford reagent (1/5). The absorbance was determined at a wavelength of 595 nm. The content of protein in each sample was determined using a standard curve of bovine serum albumin (BSA).

**Determination of mineral composition**

The mineral content of whole plants was determined in samples harvested at the end of the experiment (25 DAT). For that, plants were harvested, and their shoots and roots were washed with deionized water. Samples (~0.2g) were then oven-dried at 70 °C for five days, reduced to powder (using a mill CIENLAB CE-430; 8 blades, 1,725 r.p.m., 20 mesh size), and submitted to nitric-perchloric digestion (3:1) (nitric acid – HNO3 70% and then perchloric acid – HClO4 70%). The content of calcium (Ca), potassium (K), magnesium (Mg) and phosphorus (P) was determined by atomic

absorption spectrophotometry coupled to flame emission graphite furnace system (Varian Spectra AA, 220FS, added with graphite oven GTA 110).

**Measurements of photosynthetic parameters**

Gas-exchange measurements were performed with an open-flow infrared gas exchange analyzer system (Li-Cor 6400XT, Li-Cor Inc., Lincoln, NE, USA) with a portable leaf chamber of 2 $cm^2$. Measurements were made on the third leaf and two hours after the start of the photoperiod. Light was supplied from a series of light emitting diodes located above the cuvette, providing an irradiance of either 150 or 1000 µmol photons $m^{-2}s^{-1}$. The reference $CO_2$ concentration was set at 400 µmol $CO_2$ $mol^{-1}$ air. Dark respiration ($R_D$) was measured using the same gas exchange system on the same leaves used for the determination of the photosynthetic parameters after at least 30 min of darkness. All measurements were performed at 25ºC and the vapor pressure deficit was maintained at 2.0 ±0.2 KPa while the amount of blue light was set to 10 % of photon flux density to optimize stomatal aperture. The determination of the photosynthetic parameters was performed in 24-days-old plants. The ratio of *F*v/*F*m, which corresponds to the potential quantum yield of the photochemical reactions of PSII and represents a measure of the photochemical efficiency, was measured as previously described (Oh et al., 1996).

**Statistical analyses**

The data were obtained from at least five independent experiments for each growth condition (Short and long days conditions) with similar phenotypes observed each time, even in different growth facilities, and always conducted in a completely randomized design. Six plants from each replicate were used for plant growth analyses, and five plants from each replicate were used for the determination of gas-exchange parameters. Data were expressed as the average ± standard error (SE) and the term significant was used for $p < 0.05$ with Student's *t* test. All statistical analyses were conducted with Sigma Plot (Systat Software Inc. San Jose, California, USA).

## 3. RESULTS

### Plant growth and biomass partitioning under distinct photoperiod conditions

A highly different and characteristic growth pattern was observed among the genotypes tested from the simple visual inspection of plants from short and long-day species, *P. sativum* and *V. unguiculata* respectively, growing under either short or long days (Fig. 1). In agreement, we notice that day-length is capable of influencing plant growth and development as revealed by the lower leaf area and stem diameter were observed in plants grown under long-days yet they also showed higher root length (Fig. 1).

In general, significant variations in morphological characteristics were observed between genotypes when analyzing vegetative organs in distinct photoperiods. The species studied differed from each other in terms of growth parameters as observed by the lower shoot dry weight (Fig. 2A), leaves dry weight (Fig. 2B); root dry weight (Fig. 2C); and number of leaves (Fig 2D) under long day conditions. Interestingly, root length (Fig. 2E) was higher under this condition, whereas leaf area (Fig. 2F); stem diameter (Fig. 2G) and height (Fig. 2H) were reduced under long days. In agreement, the allocation pattern is strongly modified in response to fluctuations in the light supply (Poorter and Nagel, 2000).


**Figure 1.** Imagens showing allocation patterns in shoot and root of 25-day-old plants of *Vigna unguiculata* and *Pisum sativum* under either long or short-days conditions. Plants were cultivated under either short days (8 light/16 dark) or long days (16h light/8h dark). Scale bars equivalent to 10 cm.

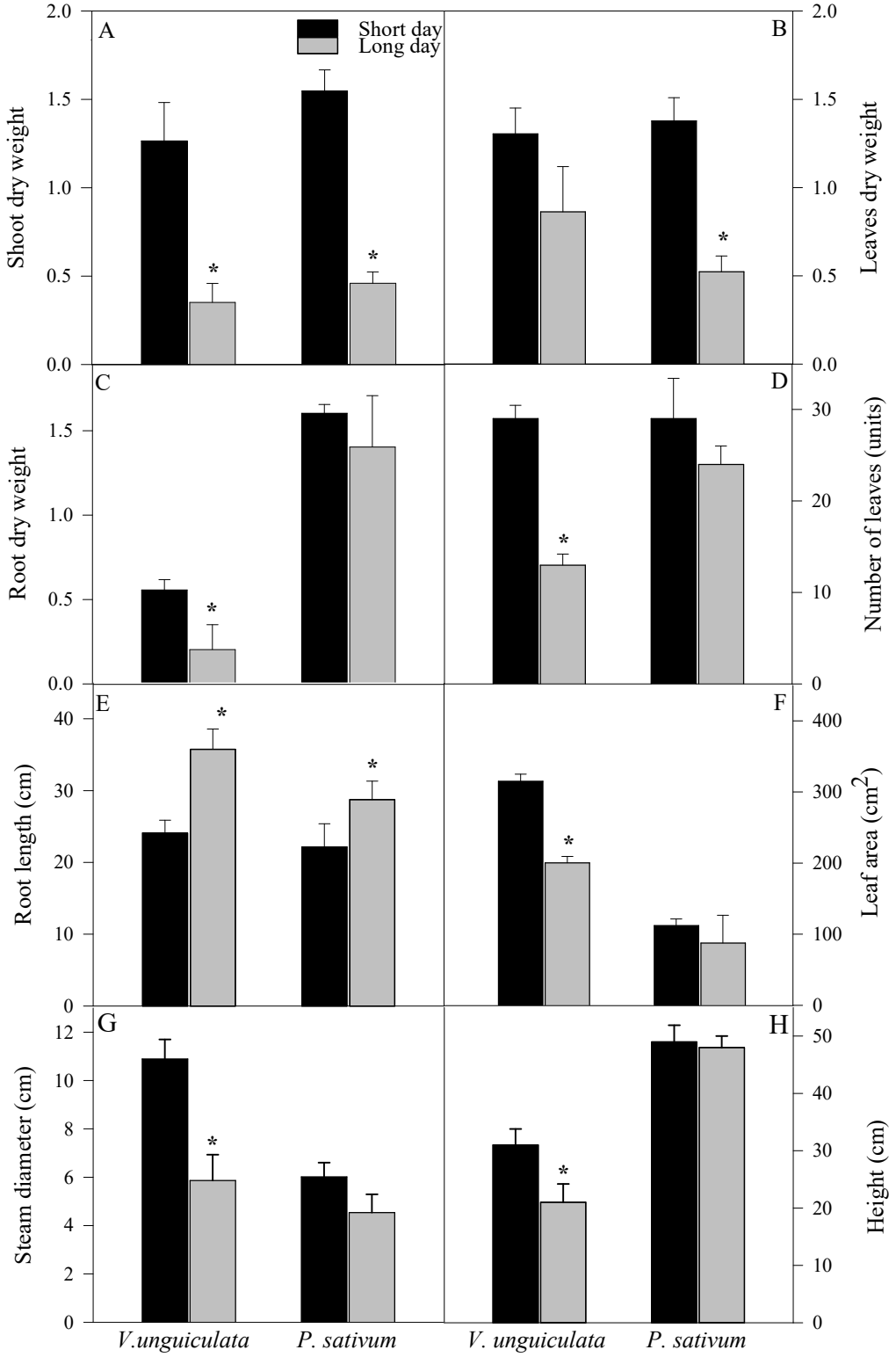


**Figure 2.** Differential growth response of Fabaceae plants. 25-day-old plants of *Vigna unguiculata* and *Pisum sativum* under were cultivated under either short days (8 light/16 dark) or long days (16h light/8h dark). (A) Shoot dry weight; (B) Leaves dry weight; (C) Root dry weight; (D) Number of leaves; (E) Root length; (F) Leaf area; (G) Steam diameter; (H) Height. Values or means ±SE (n=5). Asterisks (*) indicate significant difference as determined by Student's *t* test (*P* <

## Characterization of epidermal cells under distinct photoperiod conditions

*V. unguiculata* plants growing on short-days were characterized by a higher number of stomata than plants growing on long-days (Fig. 3A), and *V. unguiculata* SD presented also the highest number of stomata per leaf area (Fig. 3A). Long-days plants had a higher number of epidermal cells (Figure 3B), yet only statistically different in *P. sativum*. These results culminated with a lower stomata/epidermal cells ratio in *V. unguiculata* plants growing on long-days (Fig. 3C).

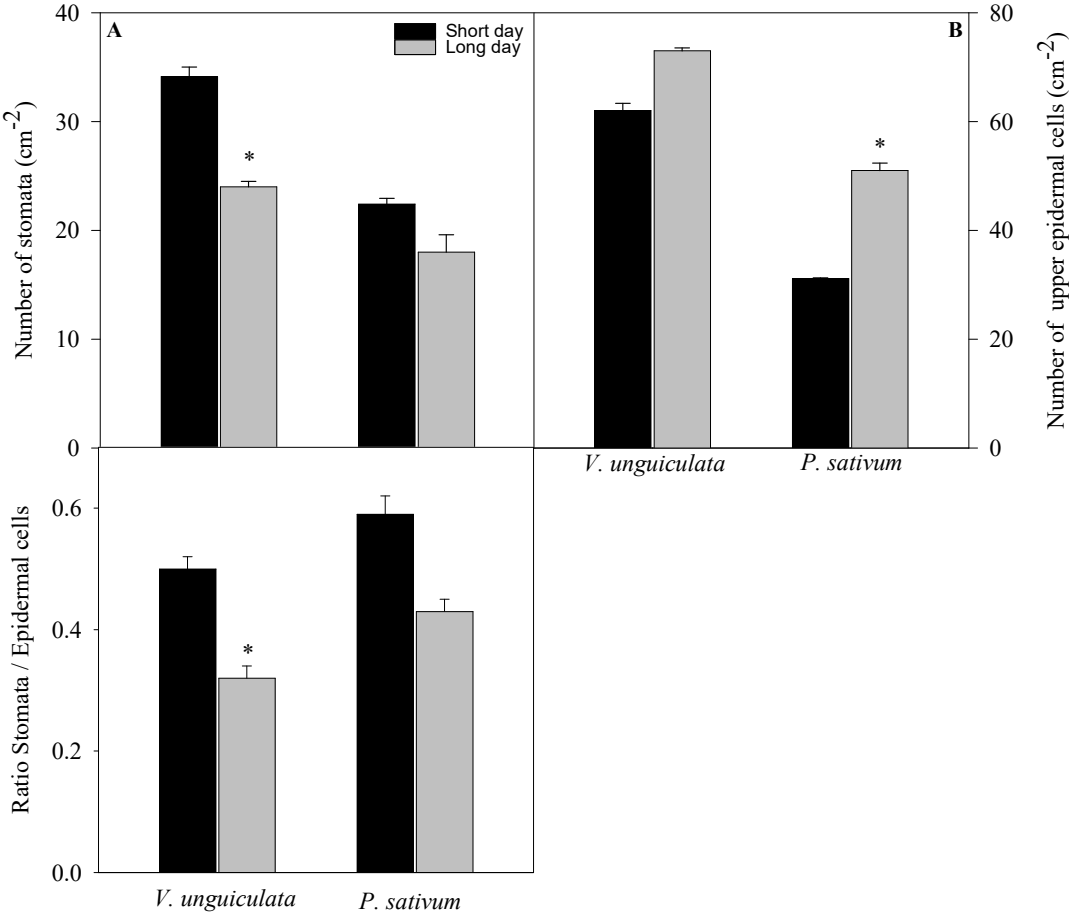


**Figure 3.** Changes in epidermal cells of Fabaceae plants (*V. unguiculata* and *P. sativum*) under distinct photoperiod conditions. 25-day-old plants of *Vigna unguiculata* and *Pisum sativum* under were cultivated under either short days (8 light/16 dark) or long days (16h light/8h dark). (A) Number of stomata; (B) Number of upper epidermal cells; (C) Ratio stomata/epidermal cells. Values or means ±SE (n=5). Asterisks (*) indicate significant difference as determined by Student's *t* test ($P < 0.05$).

**Photosynthetic responses are affected in response to photoperiod**

Due to the observed impacts on plant growth and knowing that it is directly influenced by the balance between photosynthetic and respiratory rates, gas exchange parameters were evaluated. Following 25 days of photoperiod conditions exposure, significant alterations in net $CO_2$ assimilation rate ($A$) were observed (Fig 4A). Plants under SD conditions had higher $A$, whereas plants under LD conditions showed reduced $A$. Accordingly, stomatal conductance ($g_s$) (Fig. 4B) and internal $CO_2$ concentration ($C_i$) (Fig. 4D) were also higher in SD plants (significant only for *V. unguiculata*). This data corroborated with our findings that SD plants had a greater number of stomata per leaf area (Fig. 3B). However, respiration ($R_d$) (Fig. 4C), electron transport rate (ETR) (Fig. 4E) and maximum quantum yield of the PSII ($F_v/F_m$) (Fig. 4F) remained virtually invariant in both species regardless of the photoperiod conditions.

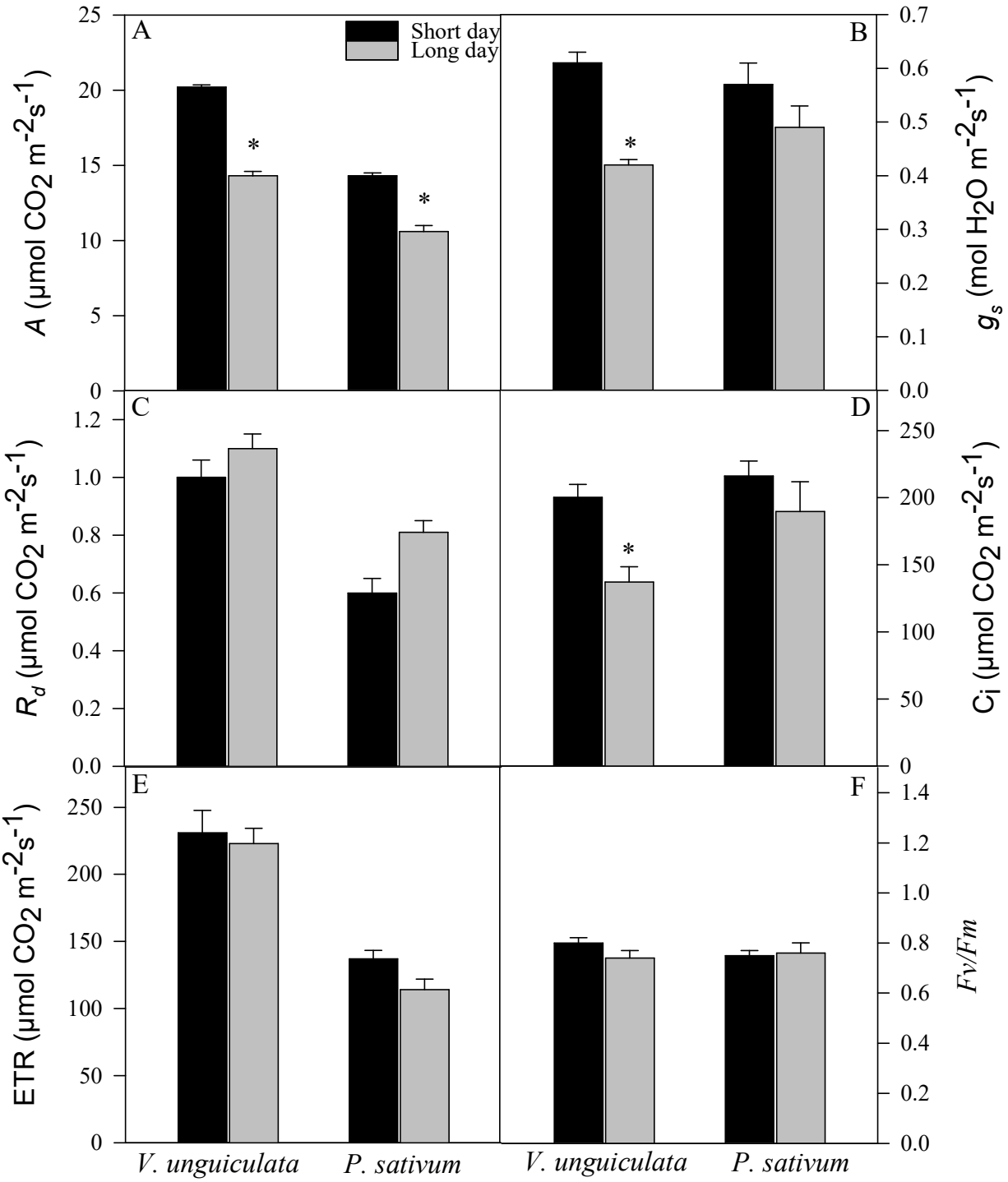


**Figure 4.** Photoperiod modifies gas exchange parameters of 25 days seedlings Fabaceae plants (*V. unguiculata* and *P. sativum*) under short and long-day conditions. (A) Net $CO_2$ assimilation rate; (B) stomatal conductance; (C) respiration; (D) internal $CO_2$ concentration.; (E) electron transport rate; (F) maximum quantum yield of the PSII. Values or means ±SE (n=5). Asterisks (*) indicate significant difference as determined by Student's *t* test ($P < 0.05$).

## Photosynthesis responses as a function of light intensity

To photosynthetically characterize the genotypes under study, gas exchange in response to photosynthetically active photon flux density (PPFD) that ranged from 0 to 1600 µmol m−2 s−1 were analysed. We observed that SD plants exhibited higher light-saturated $A_N$ ($A_{PPFD}$) and compensation irradiance ($I_c$) (Fig. 5). Ic was rather similar between genotypes demonstrating that the photoperiod affected little or nothing this parameter.

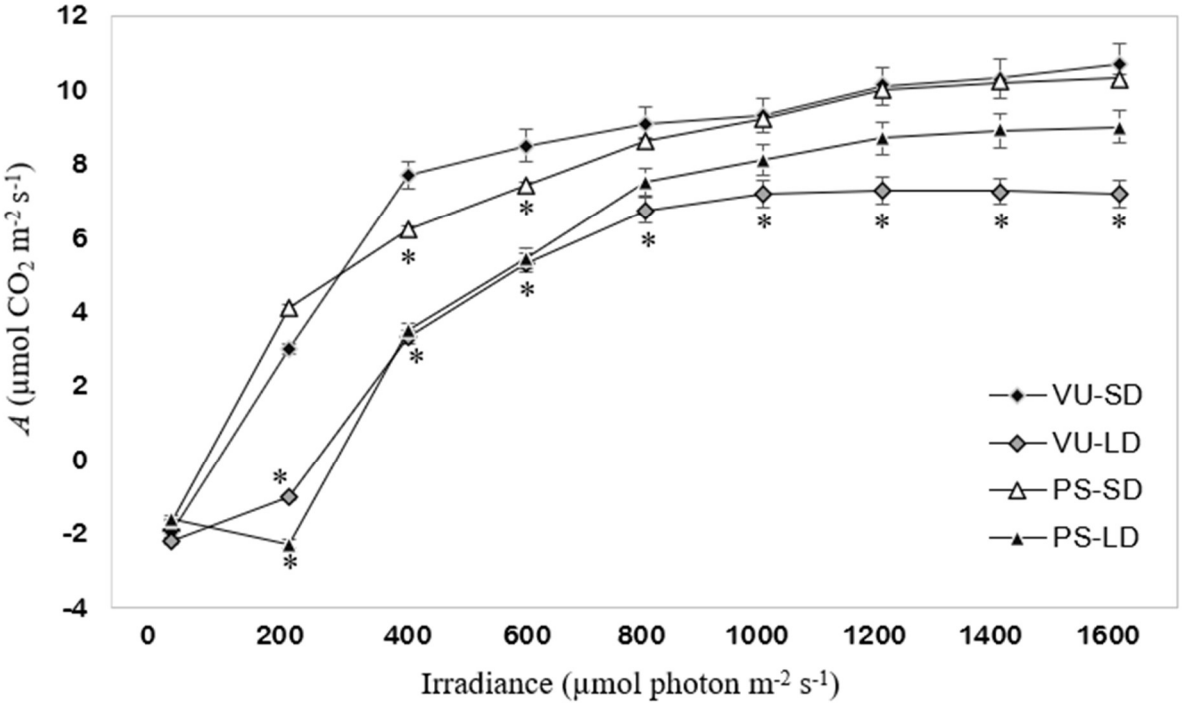


**Figure 5.** Net photosynthesis curves (*A*) in response to changes in irradiance in Fabaceae plants (*V. unguiculata* and *P. sativum*) under short and long-day conditions. Values ±SE (n=5). Asterisks (*) indicate significant difference as determined by Student's *t* test ($P <$ 0.05). (**VU-SD:** *V. unguiculata* short day; **VU-LD:** *V. unguiculata* long-day; **PS-SD:** *P. sativum* short-day; **PS-LD:** *P. sativum* long-day).

**Determination of photosynthetic pigments**

To further explore the consequences of changes in both growth and photosynthetic capacity among the genotypes and growth conditions, we conducted a detailed metabolic analysis in the leaves of 25-day-old plants. In general, the concentration of chlorophylls (*a* and *b*) and carotenoids were similar between plants under either SD or LD conditions (Fig.6). The exception was observed for *V. unguiculata*, where a reduction in chlorophyll *a* was seen in LD plants (Fig. 6A). It should be noted, however, that *V. unguiculata* (SD) had the highest chlorophyll *a* level possibly favoring the highest $A_N$ (Fig. 6A) and the highest number of stomata per leaf area (Fig. 3A).

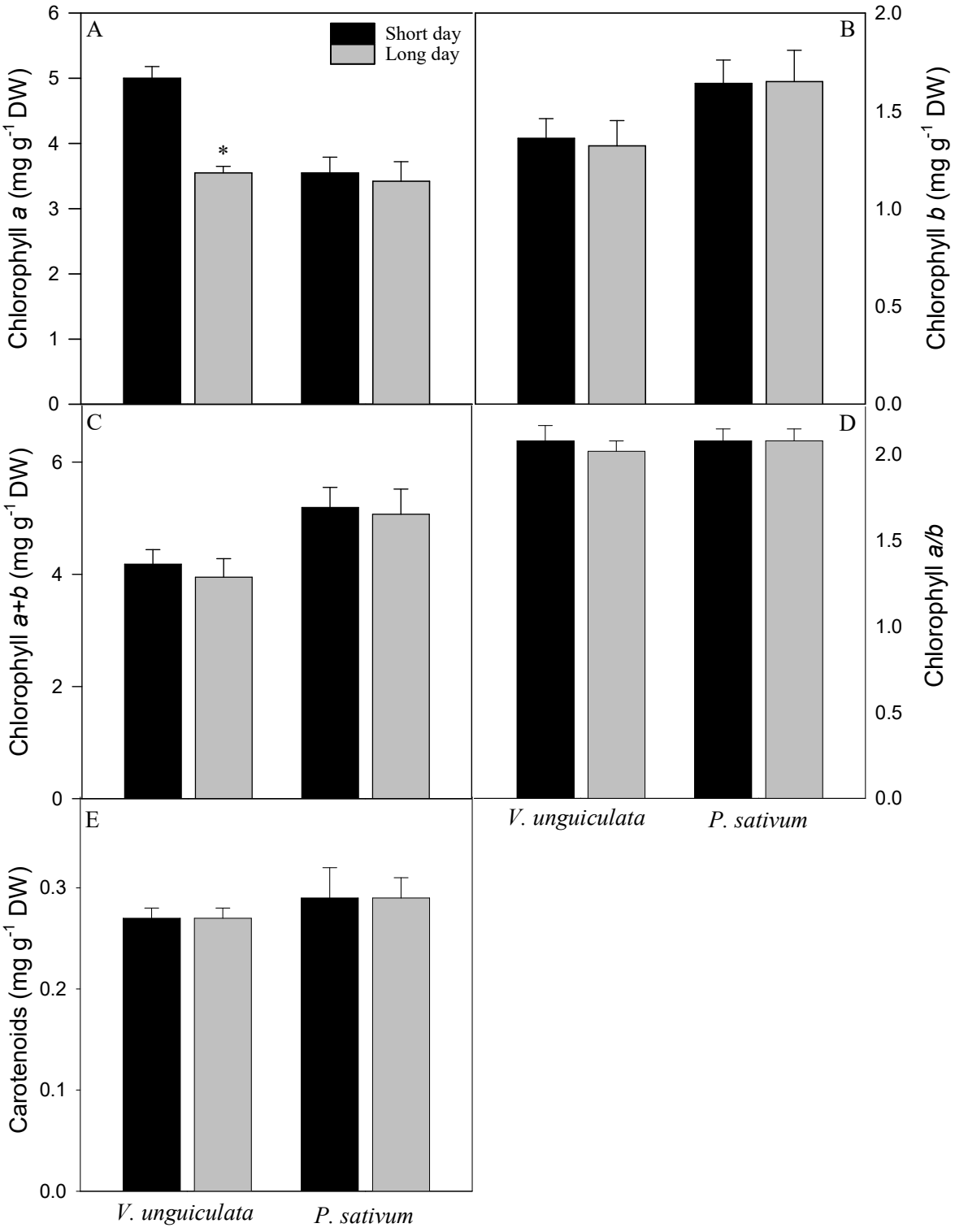


**Figure 6.** Variation in chlorophyll concentration in response to photoperiod of 25 days seedlings Fabaceae plants (*V. unguiculata* and *P. sativum*) under short and long-day conditions. (A) chlorophyll a; (B) chlorophyll b; (C) chlorophyll a + b; (D) ratio chlorophyll a/b.; (E) carotenoids. Values or means ±SE (n=5). Asterisks (*) indicate significant difference as determined by Student's t test ($P < 0.05$).

**Metabolite levels in shoot and root of Fabaceae plants under photoperiod conditions**

We next decided to investigate whether the differential growth response also extends to the metabolic characteristics of these plants by analyzing both shoot and roots. In general, the content of proteins (Fig. 7A), amino acids (Fig. 7C), and proline (Fig. 7E) in the shoots of both species were rather similar regardless of the specie or growth condition. In the roots, there was no difference between genotypes or growth conditions for protein content (Fig. 7B). In terms of amino acids, a contrasting behaviour was observed for *V. unguiculata* and *P. sativum* which showed higher levels under LD and SD, respectively (Fig. 7D). Highest levels of proline were observed for *V. unguiculata* under LD (Fig. 7F) whereas in *P. sativum* the levels of proline were similar in both growth conditions.

The content of both glucose and fructose was reduced was observed in the genotypes under short-day conditions in both species (Fig. 8A and 8C, respectively), with little or no change in glucose and fructose concentrations in the root (Fig. 8B and 8D). In addition, in *P. sativum* the levels of sucrose were similar in roots and shoots regardless of the growth condition (Fig. 8E and 8F) whereas in *V. unguiculata* the levels of sucrose were lower in shoots under LD and higher in roots under LD (Fig. 8F). Regarding the content of soluble sugars (starch), there were no differences in both shoot and root regardless of the photoperiod (Fig. 8G and 8H).

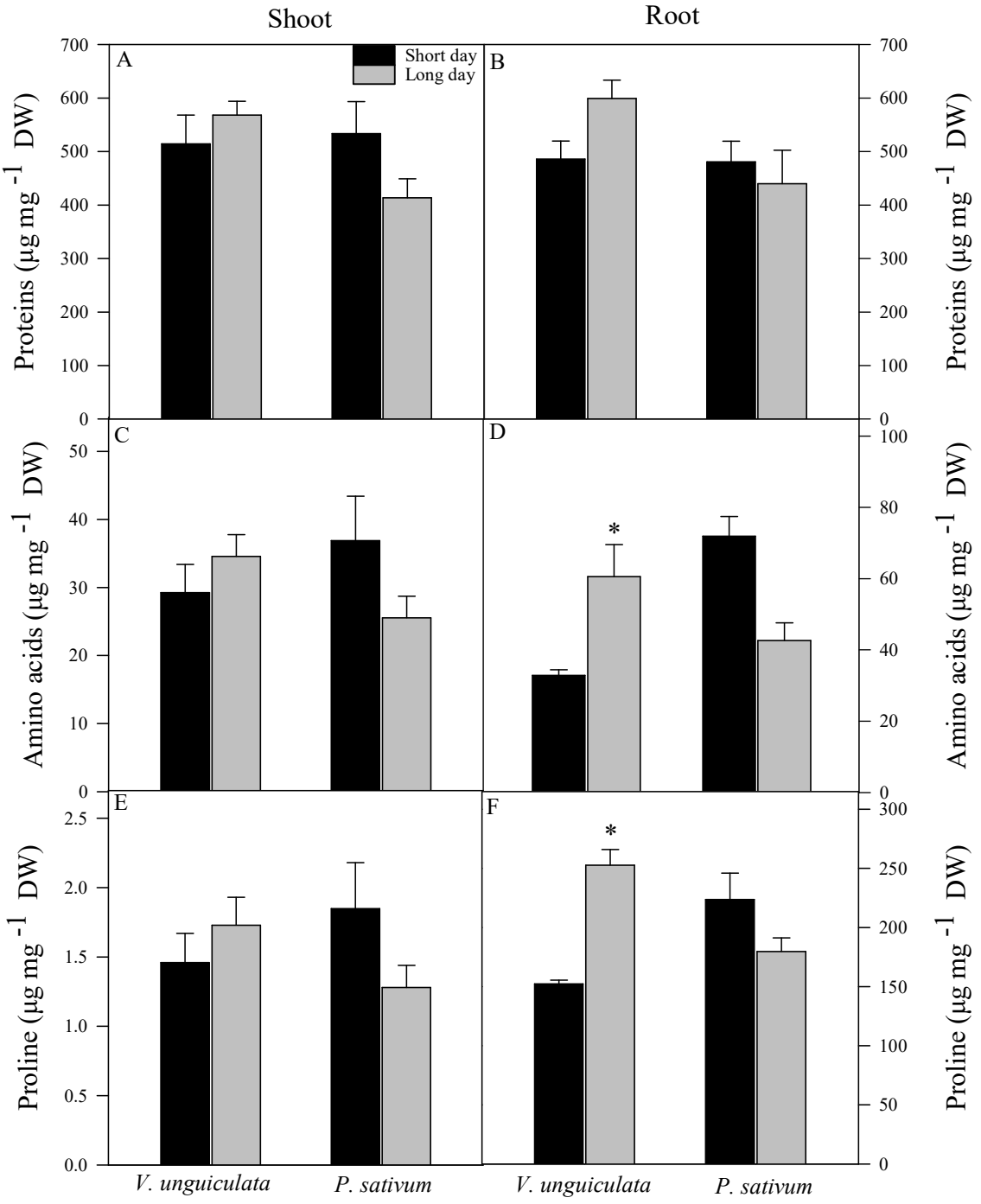


**Figure 7.** Nitrogen related metabolite levels at the end of the light period in Fabaceae plants (*V. unguiculata* and *P. sativum*) under short and long-day conditions. (A) shoot proteins; (B) root proteins; (C) shoot amino acids; (D) root amino acids; (E) shoot proline; (F) root proline. Values or means ±SE (n=5). Asterisks (*) indicate significant difference as determined by Student's *t* test ($P < 0.05$).

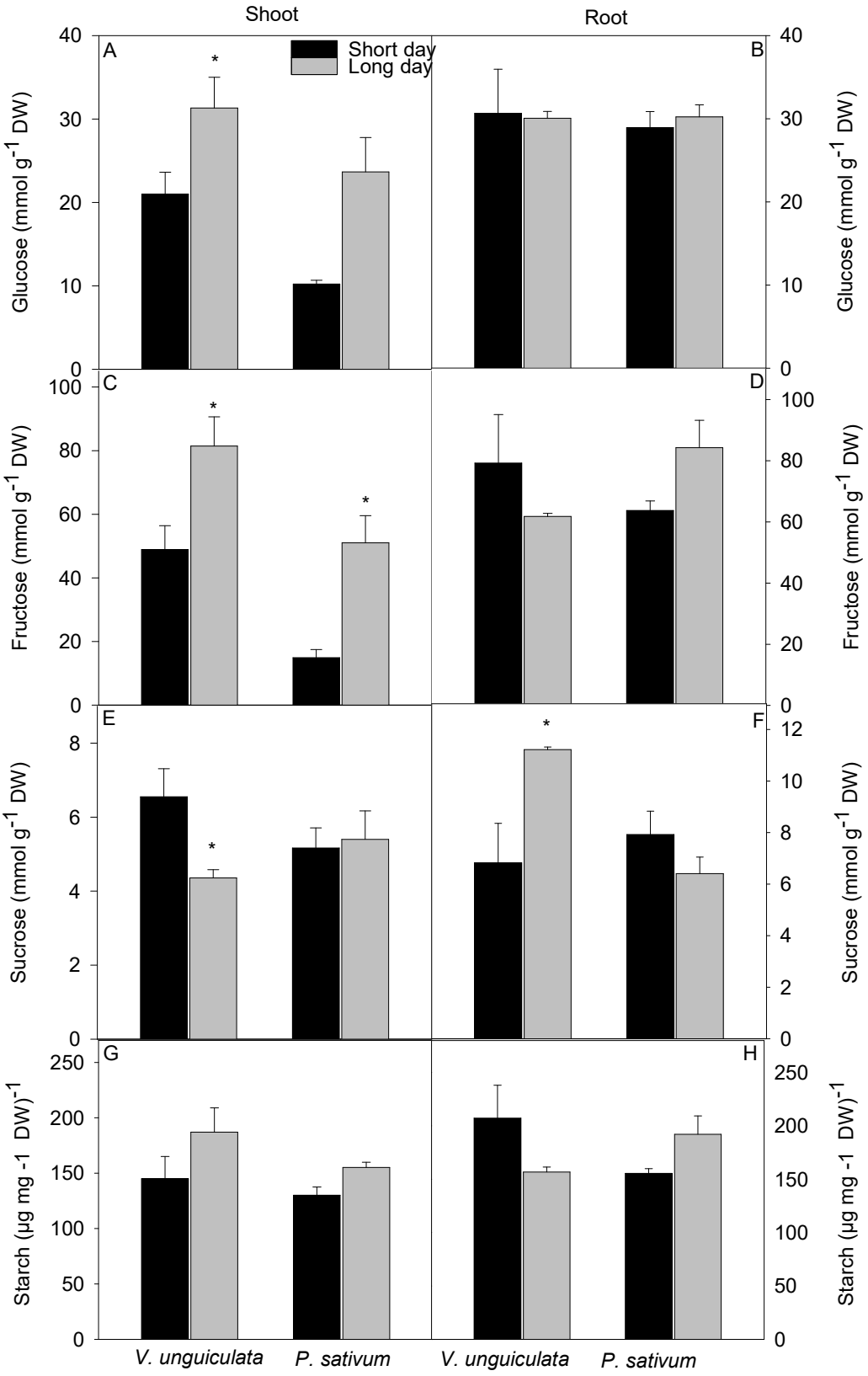


**Figure 8.** Carbon related metabolite levels at the end of the light period in Fabaceae plants (*V. unguiculata* and *P. sativum*) under short and long-day conditions. (A) glucose shoot; (B) glucose root; (C) fructose shoot; (D) fructose root; (E) sucrose shoot; (F) sucrose root; (G) starch shoot; (H) starch root. Values or means ±SE (n=5). Asterisks (*) indicate significant difference as determined by Student's *t* test ($P < 0.05$).

**Mineral composition is affected by photoperiod**

Different concentrations of macro (magnesium, phosphorus, potassium, calcium, nitrogen) and micro (iron) elements were identified (Fig. 9). Overall, when compared to LD plants, a significantly higher content of the quantified mineral nutrients was observed in SD plants. Briefly, *V. unguiculata* (SD) exhibited significantly higher concentrations of potassium, calcium and magnesium. In addition, *P. sativum* (SD) showed a higher content of potassium only. On the other hand, the mineral content of plants under LD conditions was not significantly different except for calcium in *P. sativum* (Fig 9D).

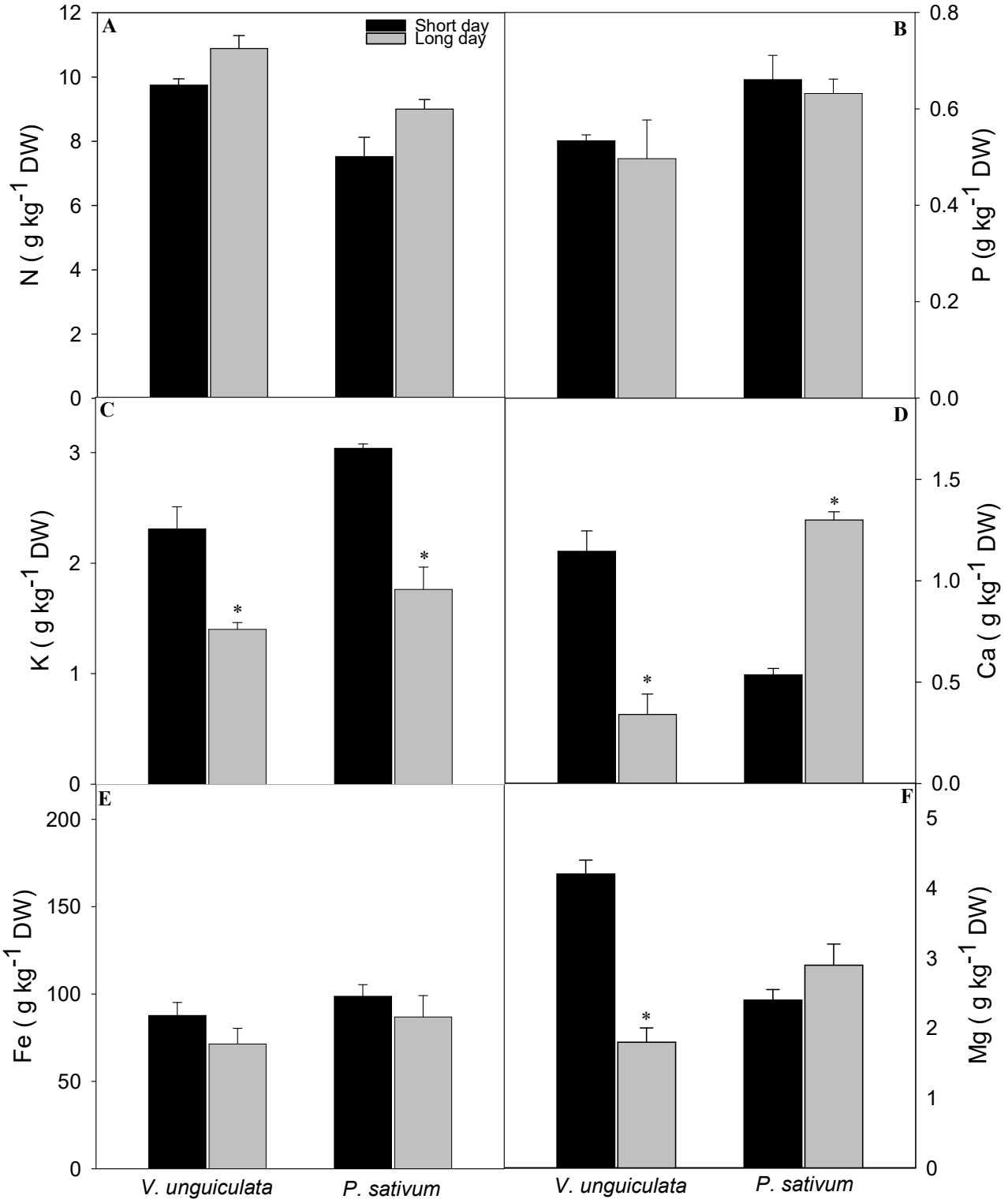


**Figure 9.** Differential mineral composition of Fabaceae plants under either short or long-day conditions. 25-day-old plants of *Vigna unguiculata* and *Pisum sativum* under were cultivated under either short days (8 light/16 dark) or long days (16h light/8h dark). (A) nitrogen; (B) phosphorus; (C) potassium; (D) calcium; (E) iron; (F) magnesium. **N:** nitrogen; **P:** phosphorus; **K:** potassium; **Ca:** calcium; **Fe:** iron; **Mg:** magnesium. Values or means ±SE (n=5). Asterisks (*) indicate significant difference as determined by Student's *t* test ($P < 0.05$).

## 4. DISCUSSION

Given the evident effects of the photoperiod on the development and allocation of resources in plants, this work sought to address, in two species of agronomic interest, the general impact of photoperiod fluctuation on metabolism and growth. Photoperiod is directly involved not only in the growth and development of plants but also in plant adaptation to the surrounding environment (Lanoue et al., 2019; Micallef, 2019). Surprisingly, under short day conditions, both *P. sativum* and *V. unguiculata* were characterized by higher growth in height, being, therefore, considerably different from plants growing under long-day conditions for the vast majority of the growth parameters evaluated here (Fig. 1 and 2). Taken together, these provide further evidence for the role of photoperiod as a pivotal agent modulating not only growth and development but also, consequently, the allocation of resources.

It should also be mentioned that the lower growth observed in long-day plants (Figs. 1 and 2) is likely associated with the lower photosynthetic rates per leaf area unit. Recently, similar results were found in plants of *A. thaliana* in the presence of Aluminum as revealed by their lower growth rates and root elongation under long-day conditions (Siqueira et al., 2021); notwithstanding, the association between growth and photosynthetic rates was not be made since the former was not measured.

As expected, epidermal cells analysis indicated a high correlation between an increased number of stomata and higher photosynthetic rates ($A$) and stomatal conductance ($g_s$) (Fig. 4). Stomata are functionally specialized microscopic pores that control the essential exchange of $CO_2$ and $H_2O$ to the intercellular leaf spaces and exit of water vapor, respectively, with the environment in land plants (Hetherington and Woodward, 2003). The exchange of the aforementioned gases is finely controlled by the opening and closing of the stomata. High stomatal density favours the supply of $CO_2$ to chloroplasts and photosynthetic enzymes (Edwards et al., 1998), yet the maintenance of an adequate water balance through stomatal control is crucial to plants because cell expansion and growth require tissues to remain turgid (Sablowski and Carnier Dornelas, 2014). The high stomatal density in plants under short days suggests that photoperiod-induced genetic mechanisms are likely responsible for the alteration in the stomatal density pattern. However, the molecular

hierarchy regulating the development of those highly specialized cell types in response to photoperiod changes, as yet, remains elusive.

Although photosynthetic differences were observed (Fig. 4) the photoperiod had little or no effect on the photosynthetic apparatus, since no differences were observed in several parameters, including $F_v/F_m$, ETR and $R_d$ as well as in the content of photosynthetic pigments between the species (Fig. 6A). Photosynthetic variations in response to fluctuations in light irradiance indicated that the photoperiod was able to, a certain extent, modulate a higher photosynthetic rate in plants growing on short days (Fig. 6). In this way, it seems mandatory the current need for deep molecular investigations in an attempt to fully elucidate the behavior of other crops to contrasting photoperiods, in order to improve agricultural productivity.

Metabolite concentration was also altered in response to photoperiod conditions, despite relatively minor changes in protein content in shoots (Fig. 7A) and roots (Fig. 7B), amino acids (Fig. 7C) and proline (Fig. 7E) in shoots. In roots, *V. unguiculata* (LD) had higher amino acid content (22%) compared to the short-day condition (Fig. 7D), while *P. sativum* (SD) had higher amino acid content (28%) compared to short-day conditions (Fig. 8D). In plants, amino acids are not only essential components involved in protein synthesis, but they are also associated with growth related hormones and secondary metabolites with a multitude of biological and adaptive fitness-promoting functions, including protection against abiotic stress (Batista-Silva et al., 2019). Although the investigated species are phylogenetically close, the difference in amino acid content may be due to either intraspecific biological differences or different metabolic response. Further studies are clearly required to fully understand how metabolite profile is modified in response to either short or long-day conditions. For instance, despite the small changes observed only for *V. unguiculata* (LD) that had a high proline concentration in roots (Fig. 7F), it is important to mention that proline may be the protection of developing cells. Indeed, in meristematic cells, the protection offered by proline against osmotic damage, especially in developmental processes, is rather necessary since in tissues that undergo dehydration this damage is likely to be neutralized by the proline accumulation (Husen, 2021; Mundada et al., 2021). Although it clearly requires further investigation, it seems reasonable to assume that the high concentration of this amino acid indicates that the long-day conditions may act as a stressor.

Increased content of soluble sugars in shoots and roots of plants under long-day conditions were observed (Fig. 8A, C, E, F and G). In fact, higher sugar content can act as feedback inhibition for the photosynthesis (Sami et al., 2016). As previously discussed here, photosynthesis is a vital process associated with the production of sugars that governs growth and resource allocation. Accordingly, the accumulation of higher concentrations of sugars significantly inhibits photosynthesis and leads to stunted growth and necrotic leaves (Jang et al., 1997). On the other hand, low sugar enhances photosynthesis, reserve mobilization and export (Shi et al., 2016). Given the changes in both *A* and $g_s$, our results coupled with those obtained elsewhere (Medeiros et al., 2016) provided circumstantial evidence for the importance of sugar and organic acid metabolism in photoperiod adaptation. The exact mechanism by which changes in the transport and synthesis of sugars and organic acids induced simultaneous changes in stomatal density, *A* and $g_s$ remains as yet unclear. However, it seems reasonable to anticipate this might be related to an as-yet-unknown signaling compound associated with fluctuations in photoperiod.

We observed that under LD, there was a decrease in the content of Mg (Fig. 9F), an important component of chlorophyll and other chloroplasts enzymes. It can explain, at least partially, the reduced *A* observed here. Moreover, this reduction in *A* could be triggered by Mg reduction in leaves (Huber and Jones, 2012). We also observed a decrease in potassium (K) concentration in plants grown on long-days (Fig. 9C). The plants that were cultivated under short days were the ones that had the highest content of K and were the ones that had the highest rates of resource allocation. K is the most abundant inorganic cation, and it is important for ensuring optimal plant growth. In plants, it regulates several physiological processes such as building and strengthening of the plant, improving the movement of photosynthates in the plant as well as regulating water status and increasing drought tolerance in the plants (Xu et al., 2020). Inside the cells, K is responsible for ion homeostasis, protein synthesis, osmoregulation, enzyme activation, regulating membrane potential and charge balance in plant cells (Cakmak, 2005).

We also observed differences in the concentration of Calcium (Ca) as revealed by the showed higher content of this element in *V. unguiculata* SD (Fig. 9D). Ca plays an extremely important role in producing plant tissues and it enables plants to grow better (White and Broadley, 2003). Ca is responsible for holding together plant cell walls. It is also crucial in activating certain enzymes and sending signals

that coordinate cellular activities. Although *P. sativum* LD showed higher content of this element when compared to SD plants, we did not observe a favorable phenotype with the increase of Ca. Accordingly, our understanding of plant responses to fluctuations in photoperiod is still fragmentary, most likely due to the complex responses involving adaptive changes and /or deleterious effects. It should be borne in mind that caution should be taken when interpreting the results described here since, under field conditions, the responses can be synergistically or antagonistically modified by the interaction with other plants and/or superimposition of other stresses. Therefore, further investigation should be performed within the context of understanding the significance of photoperiod fluctuations to mineral content in crop plants.

## 5. CONCLUDING REMARKS

Here, we demonstrate that the photoperiod is capable of impacting the pattern of resource allocation in vegetative organs in the Fabaceae species studied. Plants grown under short-day conditions were characterized by greater allocation than those grown under long-day conditions. This suggests that short photoperiod conditions played a key role in modulating the increment of resources than those grown in long day conditions. The increase of vegetative organs positively affected the vast majority of physiological and biochemical parameters investigated here. Anatomical analyzes indicated an increase in the number of stomata is correlated with the increase in photosynthetic rates, indicating that the photoperiod can modulated also morphological responses that influenced carbon metabolism. However, more in-depth metabolic studies as well as molecular studies will still be necessary for a better understanding of the effect of different photoperiod conditions on the allocation of these plants. It seems reasonable to suggest that future studies must associate the photoperiod with expected changes in circadian rhythm and their impacts on overall plant metabolism. Together, the information obtained with the present work will lead to a better understanding of the physiological and metabolic mechanisms associated with photoperiod.

## 6. BIBLIOGRAPHY

**Arnon DI** (1949) Copper Enzymes in Isolated Chloroplasts. Polyphenoloxidase in Beta Vulgaris . Plant Physiol **24**: 1–15

**Batista-Silva W, Heinemann B, Rugen N, Nunes-Nesi A, Araújo WL, Braun HP, Hildebrandt TM** (2019) The role of amino acid metabolism during abiotic stress release. Plant Cell Environ **42**: 1630–1644

**Bonser SP, Aarssen LW** (2003) Allometry and development in herbaceous plants: Functional responses of meristem allocation to light and nutrient availability. Am J Bot **90**: 404–412

**Bradford MM** (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248-254

**Cakmak I** (2005) The role of potassium in alleviating detrimental effects of abiotic stresses in plants. J Plant Nutr Soil Sci **168**: 521–530

**Carré I, Veflingstad SR** (2013) Emerging design principles in the Arabidopsis circadian clock. Semin Cell Dev Biol **24**: 393–398

**Chen X li, Yang Q chang** (2018) Effects of intermittent light exposure with red and blue light emitting diodes on growth and carbohydrate accumulation of lettuce. Sci Hortic (Amsterdam) **234**: 220–226

**Cross JM, Von Korff M, Altmann T, Bartzetko L, Sulpice R, Gibon Y, Palacios N, Stitt M** (2006) Variation of enzyme activities and metabolite levels in 24 Arabidopsis accessions growing in carbon-limited conditions. Plant Physiol **142**: 1574–1588

**Edwards D, Kerp H, Hass H** (1998) Stomata in early land plants: an anatomical and ecophysiological approach. J Exp Bot **49**: 255–278

**Fernie AR, Roscher A, Ratcliffe RG, Kruger NJ** (2001) Fructose 2,6-bisphosphate activates pyrophosphate: fructose-6-phosphate 1-phosphotransferase and increases triose phosphate to hexose phosphate cycling in heterotrophic cells. Planta **212**: 250–263

**Gepts P, Beavis WD, Brummer EC, Shoemaker RC, Stalker HT, Weeden NE, Young ND** (2005) Legumes as a Model Plant Family. Genomics for Food and Feed Report of the Cross-Legume Advances through Genomics Conference. Plant Physiol **137**: 1228

**Gibon Y, Blaesing OE, Hannemann J, Carillo P, Höhne M, Hendriks JHM, Palacios N, Cross J, Selbig J, Stitt M** (2004) A Robot-based platform to measure multiple enzyme activities in Arabidopsis using a set of cycling assays: comparison of changes of enzyme activities and transcript levels during diurnal cycles and in prolonged darkness. Plant Cell **16**: 3304–3325

**Giordano L de B** (1997) Cultivo da ervilha (Pisum sativum L.). Embrapa Hortaliças-Outras oublicações técnicas (INFOTECA-E) **1997**: 20

**Helena Castro Arriel N** OLEAGINOSAS, FIBROSAS E LEGUMINOSAS.

**Hetherington AM, Woodward FI** (2003) The role of stomata in sensing and driving environmental change. Nat 2003 4246951 **424**: 901–908

**Huber DM, Jones JB** (1965) The role of magnesium in plant disease. doi: 10.1007/s11104-012-1476-0

**Inoue S ichiro, Takemiya A, Shimazaki K ichiro** (2010) Phototropin signaling and stomatal opening as a model case. Curr Opin Plant Biol **13**: 587–593

**Jang JC, León P, Zhou L, Sheen J** (1997) Hexokinase as a sugar sensor in higher plants. Plant Cell **9**: 5–19

**Knops JMH, Koenig WD, Carmen WJ** (2007) Negative correlation does not imply a tradeoff between growth and reproduction in California oaks. Proc Natl Acad Sci U S A **104**: 16982

**De Kort H, Prunier JG, Ducatez S, Honnay O, Baguette M, Stevens VM, Blanchet S** Life history, climate and biogeography interactively affect worldwide genetic diversity of plant and animal populations. doi: 10.1038/s41467-021-20958-2

**Lanoue J, Leonardos ED, Grodzinski B** (2019) Artificial lighting technologies for agricultural production. Compr Biotechnol 818–832

**Lima M de F, Eloy NB, Siqueira JAB de, Inzé D, Hemerly AS, Ferreira PCG** (2017) Molecular mechanisms of biomass increase in plants. Biotechnol Res Innov **1**: 14–25

**Liu W, Su J** (2016) Effects of light acclimation on shoot morphology, structure, and biomass allocation of two Taxus species in southwestern China OPEN. doi: 10.1038/srep35384

**Malovichko Y V., Shtark OY, Vasileva EN, Nizhnikov AA, Antonets KS** (2020) Transcriptomic Insights into Mechanisms of Early Seed Maturation in the Garden Pea (Pisum sativum L.). Cells. doi: 10.3390/cells9030779

**Medeiros DB, Martins SCV, Cavalcanti JHF, Daloso DM, Martinoia E, Nunes-Nesi A, DaMatta FM, Fernie AR, Araújo WL** (2016) Enhanced Photosynthesis and Growth in *atquac1* Knockout Mutants Are Due to Altered Organic Acid Accumulation and an Increase in Both Stomatal and Mesophyll Conductance. Plant Physiol **170**: 86–101

**Micallef BJ** (2019) Circadian clocks/photoperiodism and crop quality. Compr Biotechnol 237–252

**Müller I, Schmid B, Weiner J** The effect of nutrient availability on biomass allocation patterns in 27 species of herbaceous plants.

**Mundada PS, Jadhav S V., Salunkhe SS, Gurme ST, Umdale SD, Nikam TD, Ahire ML** (2021) Plant Performance and Defensive Role of Proline Under Environmental Stress. Plant Perform Under Environ Stress 201–223

**Nohales MA, Kay SA** (2016) Molecular mechanisms at the core of the plant circadian oscillator. Nat Struct Mol Biol **23**: 1061–1069

**Oh SA, Lee SY, Chung IK, Lee C-H, Nam HG** (1996) A senescence-associated gene of Arabidopsis thaliana is distinctively regulated during natural and artificially induced leaf senescence. Plant Mol Biol **30**: 739–754

**Poorter H, Nagel O** (2000) The role of biomass allocation in the growth response of plants to different levels of light, CO2, nutrients and water: A quantitative review. Aust J Plant Physiol **27**: 595–607

**Queiroz V, Técnico RE, Pi T** (2002) Cultivo do Feijão-caupi (Vigna unguiculata (L.) Walp) Sistemas de Produção 2.

**Sablowski R, Carnier Dornelas M** (2014) Interplay between cell growth and cell cycle in plants. J Exp Bot **65**: 2703–2714

**Sami F, Yusuf M, Faizan M, Faraz A, Hayat S** (2016) Role of sugars under abiotic stress. Plant Physiol Biochem **109**: 54–61

**Shi H, Wang B, Yang P, Li Y, Miao F** (2016) Differences in Sugar Accumulation and Mobilization between Sequential and Non-Sequential Senescence Wheat Cultivars under Natural and Drought Conditions. PLoS One **11**: e0166155

**Da Silva, Jéssica Sueli Pereira da, Negrão, Leticia Maria Viana, Reis, Denilson do Nascimento J, Rodrigo Oliveira do N, Joice Nunes F** (2019) Potencial De Uso De Espécies Da Família Fabaceae Em Uma Floresta Secundária No Leste Da Amazônia Paraense. doi: 10.31692/2526-7701.iiicointerpdvagro.2018.00611

**Siqueira JA, Wakin T, Batista-Silva W, Silva JCF, Vicente MH, Silva JC, Clarindo WR, Zsögön A, Peres LEP, Veylder L De, et al** (2021) Photoperiod shapes aluminium tolerance in plants. bioRxiv 2021.02.12.430934

**Song YH, Ito S, Imaizumi T** (2010) Similarities in the circadian clock and photoperiodism in plants. Curr Opin Plant Biol **13**: 594–603

**Staiger D, Green R** (2011) RNA-based regulation in the plant circadian clock. Trends Plant Sci **16**: 517–523

**Thomas B** (2016) Photoperiodism. Encycl Appl Plant Sci **1**: 448–454

**Weiner J** (2004) Allocation, plasticity and allometry in plants. Perspect Plant Ecol Evol Syst **6**: 207–215

**White PJ, Broadley MR** (2003) Calcium in Plants. Ann Bot **92**: 487

**Xu X, Du X, Wang F, Sha J, Chen Q, Tian G, Zhu Z, Ge S, Jiang Y** (2020) Effects of Potassium Levels on Plant Growth, Accumulation and Distribution of Carbon, and Nitrate Metabolism in Apple Dwarf Rootstock Seedlings. Front Plant Sci **11**: 904