Advances in the performance of stevia (*Stevia rebaudiana* Bertoni): Photoperiod interaction with potassium humate

Maryam Kamali[1], Yahya Selahvarzi[1]*, Atiyeh Oraee[1], Morteza Goldani[2]

1. Department of Horticultural Science and Landscape, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Agronomy, Ferdowsi University of Mashhad, Mashhad, Iran

Abstract

The effect of day lengths (LD_1, LD_2, and LD_3: natural day length, 1.5, and 2.5 h light more than the natural day length, respectively) and potassium humate treatment (PH_1 as control and PH_2: 50% of potassium humate) was studied on photosynthesis traits, chlorophyll fluorescence, carbohydrate and total antioxidant capacity of stevia. Under LD_3, the following parameters significantly improved: root/shoot (R/S), leaf water content per unit leaf area (LWCA), and leaf weight rate (LWR), while R/S increased by 16% under PH_2. A combined application of LD_3 with PH_2 increased the morphological parameters including, shoot dry weight (SDW) and root weight rate (RWR). Furthermore, this combination resulted in higher growth compared with the LD_1/PH_1 combination. Also, an increased day length improved stomatal conductance (gs), transpiration rate (E), WUE_i, photosynthesis pigments; chlorophyll fluorescence (Fo, Fm, Fv, Fm/Fv), and antioxidant activity. Besides, regardless of the photoperiod treatment, these parameters improved compared to PH_1. The impact of LD_3 or PH_2 on carbohydrate content tended to be greater than the expected impact of LD_2 or PH_1. Our results suggest that increasing day length and application of potassium humate, both as individual and combined factors, will change the growth of stevia.

Keywords: antioxidant activity, bio-stimulant, day length, fluorescence, natural sweetener

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Introduction

Stevia rebaudiana Bertoni is an endemic herb native to northeastern Paraguay and Brazil, cultivated in China and Southeast Asia (Khiraoui et al., 2017). Stevia leaves are rich with stevioside, steviolbioside, rebaudioside F, and dulcoside A. These compounds result in the sweet taste of stevia leaves, hence the centuries of being used as a natural sweetener by the indigenous people (Lemus et al., 2012; Lemus Mondaca et al., 2012). The substance responsible is known as stevioside. In recent years, stevioside has enjoyed considerable attention as a natural substitute for synthetic sweeteners, with a few research studies attempting to focus on the plant and stevioside. In addition, as the main compound in plants, stevioside accounts for 3-8% of the weight of dry leaves and is determined to be a calorie-free compound, that is 300 times sweeter than sucrose (Kennelly, 2002; Kroyer et al., 2010). So, it has

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* *Corresponding Author*
E-mail Address: Selahvarzi@um.ac.ir
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great potential in the food industry to reduce sugar consumption (Gardana et al., 2010) and replace artificial sweeteners, such as aspartame and saccharine (Carrera-Lanestosa et al., 2017).

The vegetative and reproductive growth of stevia is influenced by many environmental factors, including light (Simlat et al., 2011; Yoneda et al., 2017; Jarma Orozco et al., 2020), photoperiod (Valio et al., 1997; Zaidan et al., 1980), temperature (Kumar and Sharma, 2012), and soil moisture (Reis et al., 2015; Kurunc et al., 2020). Stevioside production is affected by environmental and agricultural management (Tavarini and Angelini, 2018; Gomes et al., 2018). Studies have shown that temperature, day length, and light intensity affect the yield and quality of stevia (Macchia et al., 2007; Kumar and Sharma, 2012). These effects are evident from the increase in marketability and the summer performance of stevia compared with its winter performance (Allam et al., 2001). Valio and Rocha (1997) determined the effect of photoperiod on the anthesis stage in stevia rebaudiana. The plant flowered in the 8, 10, 12, and 13 h photoperiods, though the highest percentage of flowering occurred in the 13 h photoperiod. While the vegetative behavior such as leaf area and dry weight increased, the interval between leaf pairs in stevia plants decreased in (LD: long day) conditions compared to (SD: short dat) conditions (Yoneda et al., 2017). Under long daylight conditions, the total soluble and insoluble carbohydrate content in stevia leaves increases, and the biosynthesis of some compounds such as steviol (the aglucone present in stevioside) increases (Ceunen and Geuns, 2013).

To improve crop productivity, applying additional mineral elements such as potassium is necessary (Wang et al., 2017a; Xu et al., 2020). Humus is composed of compounds such as humic acid, fulvic acid, and hymatomelonic acid formed due to the decomposition of lignite, peat, soil, and water. Also, humus can be compounded with mineral elements, like potassium (Hays, 1989; Pourkhaneghah et al., 2012). These substances help the soil to be fertile and productive by providing nutrients to plants, increasing water retention capacity, and promoting plant growth (Khaled and Fawy, 2011; Wulandari et al., 2019; Kandil et al., 2020). Proper nutrition enhances the photosynthesis process leading to an increase in biomass (Chen et al., 2018; Simkin et al., 2019). The most cost-effective stevia fertilizing approach is using potassium humate. Potassium humate contains two principal acids: humic acid and fulvic acid. Humic acid, an organic molecule formed by the decomposition of organic matter, is mainly detectable in coal, soil, peat, and dystrophies lakes (Klucáková and Věžníková, 2016; Klucáková, 2018). Stevia plant nutrition with potassium improves photosynthesis. Potassium increases the activity of the enzymes nitrate reductase, the roots activity, and the resistance of plants to stress (Ma and Shi, 2011; Mahajan et al., 2019). Recently, stevia has found commercial importance in advanced countries. The crop is can be cultivated both in gardens and greenhouses. This paper reports a comparative study on the growth behavior and biochemical traits of stevia plants regarding photoperiod and humic acid. Promoting knowledge about these conditions ensures suitable growth of the plant, allowing for more efficient stevioside production.

Materials and Method

Plants, growth conditions, and treatments

The study was designed and performed as a greenhouse experiment at the Faculty of Agricultural in Ferdowsi University of Mashhad, Iran, to investigate the length of day (LD) and potassium humate (PH) in stevia. This research encompassed a split-plot design based on an RCB design and was conducted with four replications in the spring and summer of 2019. Humidity, greenhouse temperature, and CO_2 were 45-70%, 23-27 ℃ day/night, and 400-550 ppm, respectively.

Two main factors were considered for the experiment. The first factor included the length of the day (LD_1, LD_2, and LD_3 with natural day length, 1.5, and 2.5 hours of light more than the natural day length, respectively) for the main plot. The second factor included potassium humate fertilizer (PH_1: control and PH_2: 50% potassium humate) for the subplot. Stevia seedlings were obtained by tissue culture. The seedlings were, at

Table 1
Properties of potassium humate

Humic acid	Potassium (K_2O)	Zn, Fe, Mn, etc	Appearance	pH	Water solubility
80%	10-12%	200ppm	Black powder	5.8-5.9	<98%

Table 2
Physicochemical properties of used soil

EC (ds m^{-1})	pH	Nitrogen (%)	Organic matter (g/kg)	Lime (%)	Magnesium (ppm)	Calcium (ppm)	Potassium (ppm)	Sodium (ppm)	Clay (%)	Silt (%)	Sand (%)
0.98	7	0.049	3.41	9	1.5	3.2	0.59	1.2	24.8	25.4	49.8

first, cultivated in a peat moss medium and then were transferred to the greenhouse after being adapted to the laboratory environment to select uniform and well-established plants. In the 4-6 leaf phase, the seedlings were transferred to boxes (50×30×40 cm) filled with 10 kg substrate, including a mixture of field soil and silt in a 1:1 ratio. For the length-of-day treatment design, the experiment used 400 W. h^{-1} sodium vapor lamps placed one meter above the plants, with an automatic switch mechanism, which switched on the lamps after sunset following the timing of the treatments. Potassium humate was used as foliar application of fertilizer treatment four times with 7-day intervals from the 20-leaf phase. Irrigation was done two times per week using the dripping system. Tables 1 and 2 show the potassium humate fertilizer analysis and the analysis of the medium culture, respectively. Two months after the onset of the experiment and the transfer of seedlings, some morphological, physiological, and biochemical traits of the stevia plants were measured.

Morphological traits assay

To measure the shoot dry weight (SDW), specific leaf area (SLA), leaf area ratio (LAR), leaf water content per unit leaf area (LWCA), leaf weight rate (LWR), and root weight rate (RWR), the plant samples were dried at 72 ℃ for 48 hours and were weighed after drying using a digital scale with a precision of 0.001 g. Then leaf area meter (Li-Cor) Model Li-1300 was used to determine the leaf area.

The SLA value (cm^2 g^{-1}) was expressed as the ratio of leaf area to leaf dry weight. LAR was calculated as the ratio between leaf area and total dry weight (cm^2 g^{-1}). LWCA was determined by dividing the dry and wet leaf weights by the leaf area. The value of LWR was calculated as the ratio between leaf dry weight and total dry weight (g g^{-1}). Also, the value of RWR was obtained as the ratio between root dry weight and the total dry weight (g g^{-1}).

Measurements of transpiration rate, stomatal conductance, and WUE$_i$

The study used a portable infrared gas analyzer (IRGA, LCA4, ADC Bio. Scientific Ltd., Hertfordshire, UK) to measure the stomatal conductance (gs) and transpiration rate (E) for the leaves of approximately one-year-old plants, after which the intrinsic water use efficiency (WUE$_i$) was calculated as follows (Santos et al., 2017):

$$WUE_i = CO_2 \text{ assimilation rate/transpiration rate}$$

Chlorophyll fluorescence assay

Before measurements, leaves were kept in the dark for 30 minutes. Then, they were exposed to a saturating red light pulse (650 nm, 3000 µmol photons m^{-2} s^{-1}) flashed with an array of three light-emitting diodes on a homogeneous irradiation area of 12.5 mm^2. Then, using Fv/Fm ratio, the initial chlorophyll fluorescence (Fo), maximum chlorophyll fluorescence (Fm), variable chlorophyll fluorescence (Fv), and maximum quantum yield of PSII were measured (Krause and Weis, 1991).

Photosynthesis pigment assay

The chlorophyll index was measured by a (chlorophyll meter or SPAD meter) (Model 504). First, 0.5 g of fresh stevia leaves were

Table3
Effect of light periods and potassium humate on stevia growth parameters

Treatments	SDW (g)	SLA (cm^2g^{-1})	LAR (cm^2g^{-1})	LWCA (gcm^{-2})	LWR ($g\ g^{-1}$)	RWR ($g\ g^{-1}$)	R/S
Light periods							
LD_1	375.5b	94.23b	41.6b	0.022b	0.45c	0.19b	0.16b
LD_2	394.9b	130.9a	51.10ab	0.022b	0.49b	0.33a	0.31a
LD_3	480.1a	136.73a	62.92a	0.037a	0.56a	0.33a	0.31a
Potassium humate							
PH_1	361.4b	152.9a	38.66b	0.024a	0.44b	0.27a	0.31a
PH_2	472.3a	88.3b	65.13a	0.024a	0.56a	0.29a	0.21b
Significance							
Light periods	**	**	*	**	**	*	*
Potassium humate	**	**	**	ns	**	ns	**
LD×PH	**	**	**	ns	ns	**	ns

Means in each column and for each factor followed by similar letter(s) are not significantly different ($P \leq 0.05$) using LSD test; * and**: significant at 0.05 and 0.01 probability levele respectively; ns: non-significant; SDW: shoot dry weight; SLA: specific leaf area; LAR: leaf area ratio; LWCA: leaf water content per unit leaf area; LWR: leaf weight rate; RWR: root weight rate; R/S: root/ shoot; LD_1: natural day length; LD_2 and LD_3: 1.5 and 2.5 hours light more than the natural day length, respectively; PH_1: without application of potassium humate; PH_2: foliar spraying of potassium humate (50%)

homogenized in 5 ml of 96% methanol, and the homogenate was centrifuged for 10 minutes at 2500 rpm at 4 ℃ in a cooling centrifuge. After that, the absorbance of the produced material was recorded at the wavelengths of 470, 653, and 666 nm using a spectrophotometer (Bio Quest, England, CE 2502). Different pigments were estimated using the methods proposed by Lichtenthaler and Wellburn (1983).

Leaf total carbohydrate assay

The leaves were homogenized in 30 ml phenol and 150 ml sulfuric acid. The absorbance was recorded at a wavelength of 490 nm after 30 minutes. The standard curve was made by standard glucose (Mecozzi, 2005).

Antioxidant activity assay

DPPH method was employed based on the assessment of the free radical scavenging capacities of stevia extracts (Gil and Tomas, 2000). For this purpose, 0.1 ml of methanol extract of each stevia sample was mixed with DPPH, and the mixture was shaken for 30 minutes. Next, the changes in absorbance were measured at a wavelength of 517 nm. Finally, the antioxidant activity was calculated using the following formula:

$$\text{Antioxidant activity} = \left(1-\frac{Asample(517\ nm)}{Acontrol(517\ nm)}\right)\times 100$$

Statistical Analysis

SAS 9.2 was used for data analysis, and the data were subjected to Two-way ANOVA. The least significant difference (LSD) test was used to compare treatment means.

Results

Effect of light periods and potassium humate on morphological traits of *S. rebaudiana*

The effect of the light period was significant ($P \leq 0.01$) for all of the morphological traits analyzed in the study (Table 3). R/S and LWR traits significantly increased by 93.7% and 24.4%, respectively, when the plants were under LD_3 compared to LD_1. Also under LD_3 conditions, LWCA increased by 40.5% compared to LD_1.

In general, potassium humate provided more favorable conditions for growth parameters, such as LWR, than the control. Besides, PH_2 elevated the LWR but reduced the R/S compared to PH_1.

In this investigation, potassium humate did not affect LWCA and RWR (Table 3). SDW, LAR, and RWR were higher in plants treated with LD_3 than non-treated plants under the PH_2 condition. The maximum (69.7%) and minimum values of SDW were recorded in plants treated with PH_2 under

LD_3 and PH_1 under LD_1 and LD_2 combinations, respectively.

Table 4
Effect of light periods and potassium humate on transpiration rate, WUE_i, stomatal conductance, and chlorophyll fluorescence parameters of stevia

Treatments	Transpiration Rate ($mmH_2O\ m^{-2}s^{-1}$)	WUE_i ($\mu mol\ CO_2 molH_2O^{-1}$)	Stomatal Conductance ($mmol\ m^{-2}\ s^{-1}$)	Fo	Fm	Fv	Fv/Fm
Light periods							
LD_1	4.51b	0.87b	42.62b	0.31a	0.96b	0.62b	0.74b
LD_2	4.68b	0.97ab	60.78a	0.26b	1.11a	0.64b	0.80a
LD_3	6.02a	1.16a	57.69a	0.25b	1.09a	0.70a	0.82a
Potassium humate							
PH_1	4.26b	0.90b	49.14b	0.28a	1.0.b	0.61b	0.76b
PH_2	5.88a	1.09a	58.26a	0.26b	1.08a	0.68a	0.81a
Significance							
Light periods	*	*	**	**	**	**	*
Potassium humate	**	*	**	*	**	*	*
LD×PH	ns	ns	ns	ns	ns	ns	ns

Means in each column and for each factor followed by similar letter(s) are not significantly different ($P \leq 0.05$) using LSD test; * and**: significant at 0.05 and 0.01 probability levele respectively; ns: non-significant; LD_1: natural day length; LD_2 and LD_3: 1.5 and 2.5 hours light more than the natural day length, respectively; PH_1: without application of potassium humate; PH_2: foliar spraying of potassium humate (50%); Fo, Fm, and Fv: initial chlorophyll fluorescence, maximum chlorophyll fluorescence, and variable chlorophyll fluorescence, respectively.

In general, the SLA in all plants increased under LD_3 while the PH_2 treatment did not affect SLA under different photoperiods. Under LD_2 and LD_3 conditions, the LAR increased by 11.6% and 82.4%, respectively, compared with control plants under PH_2 while the mean values at LD_2 and LD_3 conditions were not different compared to LD_1 under PH_1 treatment.

RWR significantly increased in the LD_2- and LD_3-treated plants compared to the LD_1 plants treated with PH_2. Also, the plants treated with LD_2 and LD_3 under PH_2 represented the maximum RWR value. No significant difference was observed between LD_2 and LD_3 compared to the control under PH_1 treatment.

Transpiration rate, stomatal conductance, and WUE_i

As shown in Table 4, the combined effects of light periods and potassium humate on stevia did not differ significantly in transpiration, stomatal conductance, and WUE_i. Although there was no significant difference between control treatment and 1.5 hours of light supplement, with increasing day length for 2.5 hours, transpiration and WUE_i significantly increased by 33.5% and 33.3%, respectively. Also, an increase in the length of the day enhanced the stomatal conductance trend by 35.3%. Based on the results presented in Table 4, foliar application of potassium humate significantly increased transpiration, stomatal conductance, and WUE_i by 67.1, 18.5, and 38.2%, respectively compared to control (Table 4).

Chlorophyll fluorescence parameters

Although chlorophyll fluorescence parameters were not affected by the light period and potassium humate, except for Fv/Fm, an increase in the photoperiod significantly decreased the Fo trait compared to natural day length; however, the same trend was not observed for the Fm and Fv. Based on the study results, Fo significantly decreased by 19.3% when plants were under LD_3 compared to LD_1. Besides, an increase in the photoperiod length respectively elevated the Fm, and the Fv traits by 13.5%, and 12.8%, compared to the natural day length, respectively. On the other hand, foliar application of potassium humate reduced Fo by 7%, increasing Fm, and Fv by 8 and 11.4%, respectively, compared to control (Table. 4).

SPAD, chlorophyll pigments, and carotenoid contents

Table 5
Effect of light periods and potassium humate on photosynthesis pigments, total carbohydrates, and antoxidant activity of stevia

Treatments	SPAD	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total Chlorophyll (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)	Total carbohydrate (μg ml^{-1})	Antioxidant activities (%)
Light periods							
LD_1	44.19b	2.39b	1.44b	3.84b	1.34b	298.13b	62.98c
LD_2	50.79a	2.85a	1.81a	4.67a	1.44ab	318.44b	72.33b
LD_3	51.80a	3.25a	1.75a	5.01a	1.60a	367.19a	79.13a
Potassium humate							
PH_1	44.31b	2.21b	1.43b	3.65b	1.32b	303.46b	61.70b
PH_2	53.54a	3.46a	1.90a	5.36a	1.60a	352.38a	81.26a
Significance							
Light periods	**	*	**	**	**	**	**
Potassium humate	**	**	**	*	**	**	*
LD×PH	**	ns	ns	ns	ns	**	ns

Means in each column and for each factor followed by similar letter(s) are not significantly different ($P \leq 0.05$) using LSD test; * and**: significant at 0.05 and 0.01 probability levele respectively; ns: non-significant; Means in each column and for each factor followed by similar letter(s) are not significantly different at 5% probability level using LSD test. LD_1: natural day length; LD_2 and LD_3: 1.5 and 2.5 hours light more than the natural day length, respectively; PH_1: without application of potassium humate; PH_2: foliar spraying of potassium humate (50%); Fo, Fm, and Fv represent initial, maximum, and variable chlorophyll fluorescence, respectively

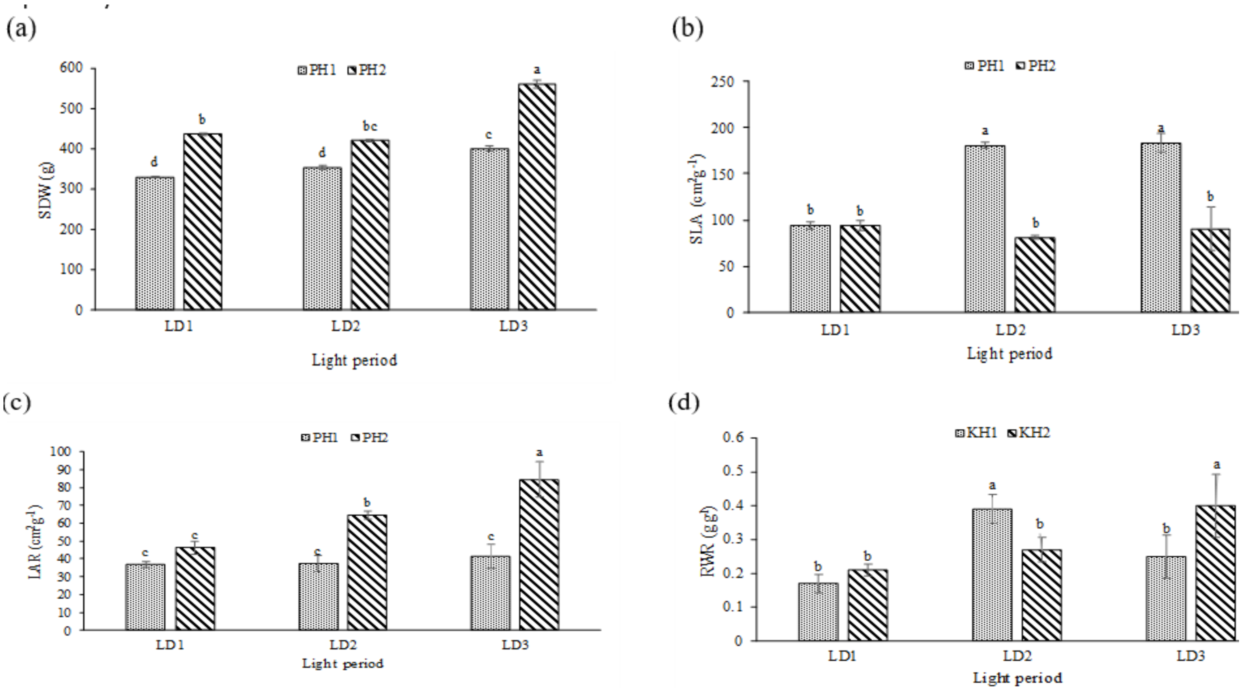

Fig. I. Effect of light periods (LD_1: natural day length, LD_2 and LD_3:1.5 and 2.5 hours light more than the natural day length) and potassium humate on SDW (a), SLA (B), LAR (C), and RWR (D) of stevia; Means followed by different small letters above the bars (standard deviations) indicate a significant difference at $P \leq 0.05$. (PH_1, control; PH_2, 50% of potassium humate; SDW, shoot dry weight; SLA, specific leaf area; LAR, leaf area ratio; and LWCA, leaf water content per unit leaf area)

The value of SPAD and concentrations of photosynthesis pigments (chlorophyll *a*, *b*, and total chlorophyll as well as carotenoids) significantly varied in response to the foliar application of photoperiod treatments and potassium humate; however, the interactive effects of these treatments (LD×PH) were not significant on these traits except for the SPAD.

Chlorophylls (a, b, and total) and carotenoids showed a trend regarding light periods. Table 5 shows that the chlorophyll and carotenoid levels increased in response to both LD_2 and LD_3

conditions, and the lowest level was related to LD_1.

The use of potassium humate significantly improved the chlorophyll and carotenoid contents compared to the non-fertilizer treatment. In fact, chlorophyll (a, b, and total) contents reached the peak by 56.6%, 32.9%, and 46.8%, respectively. when stevia was treated with PH2 compared to the non-fertilizer condition. PH_2 treatment significantly increased carotenoid contents in the stevia by approximately 21.2%, compared to the PH_1 condition. The highest SPAD value in this study was obtained in the PH_2-treated plants under LD_3 conditions, and in contrast, the control plants under LD_1 represented the lowest SPAD level (Fig. II).

Total carbohydrate content and antioxidant capacity

Although an increase in the light periods and potassium humate application did not improve the carbohydrate accumulation and antioxidant capacity, as illustrated in Table 5, the LD_3 significantly elevated the carbohydrate accumulation by 23.1% and antioxidant capacity by 25.6%. Besides, the highest carbohydrate accumulation and antioxidant capacity were recorded under the PH_1 treatment (Table 5).

Discussion

The study examined the effects of photoperiods and potassium humate to obtain some information on the vegetative growth of stevia. Seasonal pattern in radiation is a result of modifications in both irradiation intensity and day length. Day length increments during spring in Iran. Given the favorable situation, *stevia rebaudiana* Bertoni should develop quickly due to extra photosynthesis. This study revealed that stevia also develops quicker when the photoperiod was prolonged. The vegetative growth of stevia was significantly affected by the light period or potassium humate.

In this study, growth parameters were enhanced through modification in LAR and one of its ingredients, SLA. The part of dry matter maintained by the leaves was applied more frequently to enhance leaf surface and this, in turn, improved the growth rate by rising light reception. Similar improvements in leaf enlargement have been reported in *phleum pretense* due to similar light prolongation of photoperiods (Wu et al., 2004). Moe et al. (2006) showed that supplementing the fluorescent light of the main photoperiod with tungsten lamps placed within the growing space increased leaf growth of some species by increasing leaf temperature. Increases in fresh weight due to increasing PPF or lengthening the photoperiod is in line with the findings of Kang et al. (2013). Thomas et al. (2020) noted the importance of light in determining the production cycle length. This phenomenon was because of the faster plant growth rate resulting from the increase in integrated photosynthesis (photosynthetic rate duration of photosynthesis) in leaves of plants grown under longer photoperiods (Weraduwage et al., 2015). Under full sunlight, a larger quantity of assimilates was distributed between the leaves, which shows that higher leaf dry weight plants^{-1} contained more photosynthetically active cells and had a higher photosynthesis rate (Long et al., 2006; Li et al., 2014).

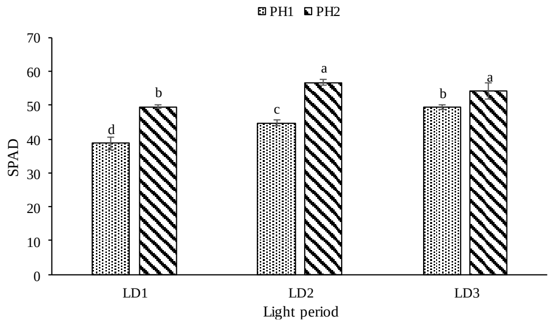

Fig. II. Effect of light periods (LD_1: natural day length, LD_2 and LD_3:1.5 and 2.5 hours light more than the natural day length) and potassium humate on SPAD of stevia; means followed by different letters above the bars(standard deviations) indicate a significant difference at $P \leq 0.05$.

Results indicate that growth parameters, including SDW, SLA, LAR, and RWR increased under LD_2 and LD_3 with potassium humate application (Table 3). Yoneda et al. (2017) also found that all morphological characteristics improved when stevia plants were exposed to more light, and the photosynthetic outputs were enhanced. Increase in leaf characteristics under increased light period is a result of cell expansion over an extended period. This result is also in complete agreement with the study by Liu et al. (2016). A long day photoperiod indicates that daily light integral is high, meaning stevia can improve photosynthetic activity (Elkins and Van Iersel, 2020). Stevia is an (SD) plant for flowering. This result is confirmed by treatments of SD and SD with the interrupted night. Anthesis in plants with 4 to 12 pairs of leaves is inducible in photoperiods shorter than 13 hours, but such plants remain vegetative in photoperiods longer than 13 hours (Evancs et al., 2015). LD cycle was partially effective in increasing growth parameters, including R/S, LWR, and RWR. The increasing number of LD cycles enhanced leaf and weight percentages in plants. These observations are explained by the association between an increase in the total light hours and an acceleration in reactions leading the plants to grow or overcome the inhibitory effect of short days (Sulpice et al., 2014).

On the other hand, the present study showed that potassium humate influences vegetative traits, including R/S and LWR. Based on the results presented in Table 3, foliar application of potassium humate led to a 30.6% increase in R/S and 27.27% in LWR, compared to the control, since potassium humate supplies essential nutrients, like organic carbon and nitrogen sources for the plant and also fulvic acid, which transports nutrients and improves the growth parameter (Abou-Sreea et al., 2017; Klucáková, 2018). Potassium humate enhances soil health and environmental quality during crop cultivation, which is made possible by releasing nutrients from humic acid and their absorption by plants. Khan et al. (2019) in a field experiment reported that morphological traits such as plant height, leaf numbers, and leaf area increased significantly with the application of humic acid. Likewise, El Mogy et al. (2019) evaluated some vegetative characteristics of wheat under 50 ppm of potassium humate and found that shoot and root dry weights and nutrient contents significantly increased under potassium humate treatment compared to the control. Relevant research studies reported similar increases in plant height (Chen et al., 2004). Studies also revealed that K-humate, as a foliar or in a nutrient solution, positively influenced the growth of plant shoots in various samples (Chen et al., 2004; Aboelsoud, 2020). Ibrahim and Ali (2018) reported the potential of K-humate to increase crop yield and nutrient uptake and also to reduce the amounts of applied chemical fertilizers in calcareous soils.

The study also assayed photosynthesis traits of stevia plants with 4-12 pairs of leaves for a relatively long period. Results clearly demonstrated that the transpiration rate and WUE_i increased immediately following the LD_2 and LD_3 treatments. Day-length extension through the light of high photosynthetic flux density from fluorescent and tungsten lamps improved growth. The growth improvement is due to the increase in assimilation rate and the modification of LAR and its LWR and SLA components. Generally, applying additional lighting is a frequent method in greenhouse arrangements, as the application of additional lighting and increased day-length enhances the photosynthesis traits of many plants (Taylor et al., 2017; Idris et al., 2018; Wan et al., 2020).

At LD_3, an increase in growth parameters was accompanied by an increase in transpiration rate and stomatal conductance under LD_2 and LD_3 (Table 4). Increased gross photosynthesis or decreased respiratory losses relatively enhanced these parameters. Plants under light conditions show the highest respiration achievement compared to dark conditions (Grande et al., 1989). The higher mitochondrial respiration rates observed in plants under optimal light conditions are probably associated with an increase in substrate supply from photosynthesis (Weger et al., 1989).

The correlation analysis (data not shown) revealed that the PH_2 treatment enhanced WUE_i in stevia (Table 4), suggesting that improved growth of stevia in PH_2 conditions was a response to improved WUE_i under this situation, as depicted in Table 3. Also, increasing water use efficiency shows that plants can maintain photosynthesis by preventing reduced loss of water. The results of this experiment correlate with other studies, indicating that an increase in humate substance improves the WUE and growth parameters (Abd-All et al., 2017; Howladar, 2018) and the WUE for total tuber yield of sweet potato as affected by different humic application rates (Abd-All et al., 2017). Idress et al. (2020) stated that among the measured physiological parameters, the combined application of K humate and NPK significantly improved transpiration rate, stomatal conductance, and WUE. It is believed that leaf K nutrient primarily increases CO_2 stomatal conductance by increasing stomatal turgor pressure (Wang et al., 2014); however, further K supply, after CO_2 conductance reaches the maximum level, enhances the vapor conductance, leading to a decrease in the sensitivity of maximum CO_2 uptake to increasing vapor conductance (Brodribb and Holbrook, 2006). These findings regarding the exponential CO_2 conductance rates confirm the earlier established role of K in enhancing the physiological attributes of the leaf, such as gas exchange characteristics, in most plant types (Brodribb and McAdam, 2011).

The improvement of plant growth after potassium is added to the soil is related to its high mobility in the soil solution and plant roots/stems/leaves (Kanbi and Bhatnagar, 2005). It plays a vital role in regulating Pr and Tr (Véry and Sentenac, 2003), which, in turn, are essential factors for controlling WUE (Tuzet et al., 2003). Moreover, stevia plants under K-humate treatments showed the highest gs and Ci values. Furthermore, in pepper, photosynthetic parameters, such as gs and Ci positively correlated with leaf K content, suggesting that K can trigger an increase in gs under foliar K implementation (El Mogy et al., 2019). This improved Ci inside the leaves (Ibrahim et al. 2011) by the following mechanism: K fertilizer improved photosynthesis rate by regulating stomata opening and allowing for gas exchanges with the atmosphere, improving Ci rate and water use efficiency (Coskun et al., 2014; Zahoor et al., 2017).

Previous studies revealed that photosynthetic capacity is affected by day length due to seasonal changes (Bauerle et al., 2012; Ali et al., 2015; Way et al., 2017). Based on the light hypothesis, it can be indicated that the general scheme in chlorophyll fluorescence (Fo, Fv, and Fv/Fm) is that they increased under a long photoperiod when the flow of assimilates from the leaves, as a result of photosynthesis, is usable by plants (Xu et al., 2020b).

Table 4 shows that the stevia plants treated with LD_3 have significantly higher stomatal conductivity than the control plants, which is related to the high light intensity that enhances the gas exchange process (Chinchilla et al., 2018).

Granda et al. (2019) stated that controlling the length of daylight in regulating stomatal conductivity may be an important function of Quercus species. The decrease in stomatal conductance of *Populus cathayana* under a short-day was related to the restriction of photosynthesis (Zhao et al., 2009). The stomatal conductance pattern is controlled by circadian clock guard cells entrained to photoperiod (Wheeler et al., 2019). Likewise, the CO/FT regulatory module, a component of the photoperiod pathway that regulates stomatal aperture in a day-length-dependent manner (Hassidim et al., 2017). The maximum stomatal conductance throughout the peak of day has been associated with high production through maximizing carbon capture, which is stimulated when conditions are more desirable for carbon assimilation (Granda et al., 2019).

The measurement of Fo and Fv/Fm helps determine the photosynthetic functional state. Fo and Fv/Fm represent the PSII center's efficiency for capturing light energy (Zhang et al., 2020). The Fv/Fm value in light-adapted leaves is a sensitive indicator of the plant's photosynthetic performance, with optimal values around 0.83 for many plant species (Murchie and Lawson, 2013; Moustakas et al., 2020). The maximum rate of Rubisco carboxylase activity and the maximum rate of photosynthetic electron transport increased over a long period (Pettersen et al., 2010). Also, the photosynthesis of plants slightly improved, possibly due to the increase in nitrogen resources for investing in photosynthetic tissues (Qing et al., 2012).

Thus, chlorophyll fluorescence (Fo, Fv, Fv/Fm) potentially correlates with the growth parameters. Consequently, the photosynthetic activity must have increased to enhance shoot weight or leaf characteristics. Moreover, the extra matter produced was apportioned for leaf and further growth so that leaf area increased proportionately with the increase in plant dry matter. The findings of several studies are in line with this study's results concerning the impacts of day length on chlorophyll fluorescence (Tewolde et al., 2016; Rhys et al., 2017). Also, chlorophyll fluorescence improved due to an increase in transpiration rate and the WUE_i of plants in LD_3. The majority of other photosynthetic performance of this leaf is associable with chlorophyll differences, suggesting that changes in photosynthetic traits may have originated from changes in respiration rather than photosynthesis. Research approaches, though, hardly confirm the validity of relying on the short-term measurement of a single leaf's photosynthetic production as a criterion for determining the photosynthetic production of the whole plant. Besides, Fv/Fm regulation impacted light use and heat energy loss efficiency (Sharma et al., 2015; Moustakas et al., 2020).

Humic substances have played a crucial role in stimulating plant growth by promoting photosynthesis, chlorophyll fluorescence, respiration, and chlorophyll content (Fan et al., 2014; Lotfi et al., 2018). Peymaninia et al. (2013) have demonstrated that Fo and Fm in different wheat genotypes increased under Leonardite conditions.

In this experiment, stevia leaves under PH_2 represented higher values of Fo, and Fv/Fm, indicating the enhancement of photosynthesis in genotypes under PH_2 compared to those under PH_1. An accurate probe of photosynthesis *in vivo* is chlorophyll fluorescence. As depicted in Table 4, this study attributes the significant increase in chlorophyll fluorescence to the application of PH_2 to a decline heat dissipation and improve light energy absorbance by the PSII. Therefore, it can be concluded that the PH_2 has a significant influence on PSII to capture light energy, reducing chlorophyll degradation and the excess excitation energy in PSII and protects PSII components, especially in D1 protein, which was also a result of the photosynthesis traits improvement in the stevia plants treated with PH_2.

The chlorophyll pigments are adjustable by day length since a short day reduces pigment accumulation in some plants (Becker et al., 2006). Besides, this study demonstrated that the SPAD and photosynthetic pigments, including chlorophyll *a*, *b,* and total, as well as carotenoids depend on the photoperiod duration. Also, LD_3-treated stevia plants had significantly higher SPAD and photosynthetic pigment accumulation than the LD_1 condition. The increase in SPAD under extended day lengths was accompanied by a rise in light absorption by chlorophyll. Relevant research studies have also reported the association between increased light absorption and day-length (Bayat et al., 2018; Rezai et al., 2018). This improvement in chlorophyll content can enhance photosynthesis and constitute a second mechanism that increases dry weight. Also, an increase in the absorbed light levels led to an increase in the photosynthetic levels, the formation of hydrocarbon substances in leaves, and an increase in the total chlorophyll content. The chlorophyll content significantly influences the light-harvesting capacity of leaves, positively correlating with rubisco enzyme activity. This is because of the increase in light-harvesting reactions in photosystems through enhancing chlorophyll activity and activating rubisco by stimulating rubisco activase, which is regulated by light reactions (Zhang et al., 1999; Suganami et al., 2020).

Results indicated that potassium effectively increased chlorophyll content and SPAD in stevia leaves (Table 5). These findings are in line with the findings of the studies by Iheshiulo et al. (2017) and Al-Karwi Al-Ameer (2018). Two main elements can justify this improvement: nutrient absorption and physiological processes. Application of the PH may has additional effects on chlorophyll content by increasing absorption of N and K by stevia, improving K and N metabolism, and generating protein (Ibrahim et al., 2012; Mohsen et al., 2017). Soares de Melo et al. (2014) reported that the total chlorophyll content in potassium application treatments increased after transplantation. It seems that potassium humate improves soil structure and soil aggregation (Imbufe et al., 2005). Ertani et al. (2011) have shown that an increase in the chlorophyll content improved photosynthesis due to lignosulfonate-humate, subsequently stimulating rubisco enzyme activity by potassium humate.

This enhancing effect is attributable to the role of potassium in plant physiological processes, such as photosynthesis, transpiration, and stomatal conductance (Widaryanto, 2008). Potassium was observed to activate chlorophyll synthesis, ALA dehydratase, and δ-aminolevulinic acid (ALA) and prevented the decomposition of newly-synthesized chlorophyll. Also, this improving effect is attributable to the high efficiency of PH in increasing chlorophyll content, stimulating the enzymes related to the photosynthetic process, reducing electrolyte leakage, and increasing the uptake of magnesium and iron that is necessary for chlorophyll biosynthesis (El-Ghamry et al., 2009; Arafa et al., 2011).

The chlorophyll content on the lamellar structure of thylakoids was reported to correspond to electronic transmission and photosynthetic capacity (Zhao et al., 2020); improving the structure of the thylakoids with PH_2 fertilizer enhanced the rate of absorption, transfer, and conversion of photons in the chloroplast, and accelerated the photosynthesis (Hazrati et al., 2016). The effect of potassium humate on photosynthetic pigments is related to CO_2 assimilation and photosynthesis rate, which is dependent on the leaf photosynthetic gas exchange status (Khafagy et al., 2019). Yang et al. (2001) stated the chlorophylls associated with the photosynthesis process, including photosynthetic rate.

Stevia plants exposed to high humate potassium or longer photosynthetic photoperiods exhibited higher carbohydrate accumulation rates than plants grown under shorter photoperiods with high or low humate potassium (Table 5). The results of relevant studies correlate with those of this study, confirming the role of photoperiod length as a primary stimulus for differences in carbohydrate levels (Sulpice et al., 2014; Baerenfaller et al., 2015). The increase in carbohydrate content in stevia grown under longer photoperiods may be a result of enhanced leaf photosynthesis (Sirtutas et al., 2011). The widespread increase in glucose and sucrose within the leaves of *Stevia rebaudiana* increased two-fold after the onset of flower bud formation (Ceunen and Geuns, 2013). Presumably, a genetic response is one of the reasons for the accumulation of carbohydrates in long-day conditions. Duration of photosynthetic period considerably influenced the sugars in the leaves (Eckstein et al., 2012). Moreover, sucrose synthesized from carbohydrates produced by the fixation of atmospheric CO_2 via the reductive pentose phosphate (Calvin Benson) pathway, represents the starting compound for the respiratory pathway, and its increase may justify the improved activity of rubisco. Photosynthesis can impact rubisco activity. The positive correlation between growth and carbohydrate indicated that a long photosynthetic period controlled the allocation of carbon and stimulated an increased allocation of carbohydrates to leaf growth (Aspinwall et al., 2018). The photosynthetic period can trigger the start of this process. A factor explaining this phenomenon is the plants access to sufficient carbon, and that existing shoots of stevia operate the relation between sink and source, and photoperiod regime-controlled would suffice to explain the increased growth production (Regier et al., 2010). The decrease in carbohydrate content in plants under LD_1 is probably related to the depleting carbohydrate reserves before the start of the next photoperiod. Similar results were reported in cucumber under short days were controlled genetically (Robbins et al., 1987).

The findings of this study indicated that photoperiod length had a more significant effect than humate potassium on the carbohydrate content of stevia. An increase in carbohydrate content under PH_2 was reported previously (Khafagy et al., 2019; El-Naqma, 2020). Foliar application of k-humate increased the carbohydrate content of *Thuja orientals* shoots (Abou-Sreea et al., 2017). Activation of several biochemical processes resulted in an increase in enzyme synthesis, protein, and carbohydrate contents, as found by Neri et al. (2003). Improving plant growth processes within the leaves increases leaf and stem carbohydrate contents. Enhanced carbohydrate production can result in higher product quality or yield (Laila et al., 2009). Potassium humate is associated with accelerating plant energy metabolism through enhancing the photosynthesis process and forming starch (Mady, 2009). Application of PH_2 stimulated the accumulation of soluble carbohydrates in plants either by increasing endogenous levels of certain phytohormones or by acting as activators of carbohydrate synthesis and generally of photosynthetic systems (Hemida et al., 2017).

The findings of this study suggested that stevia plants grown in LD_3 have a higher antioxidative capacity than LD_1 plants. In our work, LD plants with higher antioxidative capacity might be preparing for summer and the associated intense high light and water deficit conditions. This implies different regulations of non-enzymatic antioxidant components, which should be studied more thoroughly. Potassium humate has been used in the configuration of bio-stimulants to enhance the antioxidant system in plants (El-Nwehy et al., 2020). Bio-stimulant applications of the PH_2 treatments contribute to the alleviation of oxidative damage and may balance ROS production, playing a protective role in plant cells and the PH-induced antioxidant enzyme activities in Russian olive (*Elaeagnus angustifolia* L.). Osman et al. (2017) indicated clearly that potassium humate significantly inhibited catalase and peroxidase activities and reduced MDA and ascorbic acid contents in salt-stressed plants. This ameliorating effect is attributable to the protection of cell membranes from lipid peroxidation through increasing metallic ions uptake and cell, which is related to the activity of both hydrophilic and hydrophobic sites on the surface of humic substances (Haghighi et al., 2010). Presumably, potassium treatment stimulates a defense mechanism, thus preventing ROS production in stevia leaves (Ahangar et al., 2017). Through various structural groups, such as phenols and carboxylic acid PH_2, OH/OOH can be deprotonated, thereby providing metal chelation, anti-oxidation properties, and free radical scavenging properties (Khil'ko et al., 2011). Although its quinones produce ROS (García-Santamarina et al., 2014), PH has excellent potential as a natural antioxidant. Also, this result may be attributed to the PH_2 as the most effective fraction in the regulation of the O2•− production by the regulation of the xanthine/xanthine oxidase system, which has a crucial part in enhancing antioxidant defense systems (Ahmad et al., 2014; Hemida et al., 2017).

Conclusion

This study demonstrated that stevia plants exposed to both high humate potassium and longer photosynthetic photoperiods exhibited higher growth than plants grown under natural photoperiod with high or low humate potassium. Besides, increasing day length enhanced the growth of stevia by improving the photosynthesis process. The results suggest that increasing day length and the application of potassium humate, both as individual and combined factors, will change the growth of stevia.

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