Evaluation response of photosynthesis of stevia plant (*Stevia rebaudiana* Var. Bertoni) to potassium humate and photoperiod

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**ABSTRACT**

For studying the effect of photoperiod and potassium humate in the photosynthesis apparatus of the Stevia plant, an experiment was carried out in factorial arrangement base on completely randomized design with four replications in Mashhad/Iran during 2017. The experiment consisted of three lengths of the day (LD1 = natural day, LD2 = 1.5 hours of light more than the natural length of day, and LD3 = 2.5 hours light more than natural day) and potassium humate fertilizer [KH1: zero with KH2: potassium humate (50%)]. The results showed that maximum of photosynthesis was found in KH2×LD2. The most of Ci, quantum yield Fv/Fm value was also found from spraying K-humate in LD2 treatment. The maximum amount of leaf dry weight and biomass was observed in KH2×LD2. The maximum biomasses were also gained in KH2×LD2.

**Key words**: Ci, Leaf dry weight, Photosynthesis, Quantum yield, Stomata conductance.

**INTRODUCTION**

*Stevia* (*Stevia rebaudiana* var. Bertoni), a perennial plant, belongs to the Asteraceae family; it is widely cultivated for its sweet leaf. This plant was first discovered in Paraguay, and after phytochemical studies on it, the presence of steviol glycoside (SG) compounds in Stevia was confirmed and its sweetening effect was recognized (Soejarto et al. 1983). The Stevia plant grows under short growing conditions. During the reproductive phase, decreased leaf fresh and dry weight, glycoside content, and increased length of daylight to 16 hours or the night-break technique during long nights with red light (640–700 nm) stimulates vegetative growth and increases leaf dry weight (LDW); sugar is also enhanced by the increased day length to 14 hours (Ramesh, 2006). Zubenko et al. (1991) was reported that the highest biomass obtained from increased day length and light intensity. Metivier and Viana (1979) reported that light intensity of 0.089 cal.cm⁻² kept the plant in a vegetative stage for a long period and cultivated Stevia, over long days, was produced by high leaf: stemratio; this ratio also increased the leaf area and leaf dry weight in comparison to short days.

Humic substances are one of the substances that make up the bulk of humus, which result from organic soil decomposition and constitute 65–75% of organic matter. Potassium has a strong influence on the turgor of growing cells, in maintaining their water content, in osmo regulation and stomata regulation (Benlloch-González et al. 2008). Therefore, humic compounds have high molecular weight and can form compounds with mineral elements like potassium; thus, producing potassium humate is an effective fertilizer that positively affects growth, increases the rate of nutrient uptake, and enhances plant biomass (Canellas et al. 2015). These properties can be attributed to the acceleration of plant-energy metabolism through the enhancement of the photosynthesis process and the formation of starch (Mady, 2009). Results from the researcher showed that potassium humate resulted in a remarkable increase in the total soluble proteins and carbohydrate content of grains; this may be due to the increase of the photosynthesis process, which leads to the formation of starch and/or the increase N⁺, P⁺, and K⁺ uptake as well as an increase the ratios of K⁺/Na⁺ and Ca⁺²/Na⁺, which are necessary for plant growth (EL-Bassiony et al. 2010).

In the photosynthesis process, Fv/Fm ratio is a useful ratio that provides the efficiency of PSII and shows a high degree of the relationship with photosynthesis. Chlorophyll Fluorescence (ChIF) is a good indicator of Calvin cycle activity in many fruit species (Smillie and Hetherington, 1983). Various ChIF parameters have been applied in many areas of plant research. These applications have proved that ChIF is closely related to photosynthesis and contains information about the physiological status of plants and environmental changes. Thus, the increased LD photoperiod increases stomata conductance and enhances CO₂ in the substomatal chamber, which increases photosynthesis assimilation in the plant. Pearce et al. (2006) suggests that there is a positive correlation between the photosynthesis rate and stomata conductance with biomass yield; so, with enhanced stomata conductance, the amount of CO₂ entering for photosynthesis is increased. This research
has been performed with the gained information of the effect of potassium humate and the long length of day on photosynthesis apparatus and biomass production in the Stevia plant.

MATERIALS AND METHODS

In order to investigate the length of day and potassium humate (KH) in the photosynthesis apparatus of the Stevia plant (\textit{Stevia rebaudiana} var. Bertoni), an experiment was carried out in the factorial management base on a completely randomized design with four replications in Ferdowsi University in Mashhad, Iran, in 2017. The first factor was the three lengths of the day (LD1= natural length of day; LD2 and LD3 are 1.5 and 2.5 hours of light more than the natural length of day respectively). The second factor was potassium humate fertilizer [KH1 = zero and KH2 = with potassium humate (50%)]. The specifics of potassium humate were given in Table 1. At the first, the seeds of the Stevia plant were germinated in trays containing coco peat at 25ºC. After adaptation to the environment and in the 6–7 leaf phase, the seedlings were transferred to boxes (with dimensions of length = 50 cm, width = 30 cm, and depth = 40 cm), which were filled with 10 kg of soil consisting of a mixture of field soil and silt in the ratio 1:1. Light treatment was performed after sunset by turning on sodium light (11 W.hour⁻¹). The lamp was established one meter above the plants. Foliar spraying of potassium humate (50%) was performed four times at an interval of seven days from the 20-leaves phase (1.0 liter/100 water liters/fed). Irrigation was applied two times per week by a dripping system. At the onset of flowering, the planted Stevia was cut from above the ground and transferred to a laboratory, where the leaves and the stem were separated from the three selected plants in each experimental unit. The leaf dry weight and biomass were measured after they were dried in an oven at 70ºC for 48 hours. The physiological parameter assessment was as below:

Stomata conductance was determined by a leaf protriser (Model SC-1, Decagon, USA) on fully expanded leaves.

Photosynthesis parameters: For measuring the photosynthesis rate, the amount of CO₂ in the substomatal chamber was measured by an infrared gas analyzer (LCA-4-ADC Portable Photosynthesis System). Prior to use, the instrument was warmed for 30 minutes and calibrated in the ZERO IRGA mode.

Chlorophyll Fluorescence (ChlF): For measuring ChlF, variables were derived from the fully expanded leaf in the marginal leaf area. Leaves darkened for 30 minutes after their attachment to the leaf clip holder. ChlF was assessed by the OSL-FL Chlorophyll Fluorometer. The fluorometer was connected to a leaf-clip holder. Fo, Fm and Fv were derived from the difference between Fm and Fo. The ratio of the fluorescence variable to the fluorescence maximum (Fv/Fm) which represents the maximum quantum yield of Photosystem II (PSII) in photosynthesis process.

The data was statistically analyzed by using a one-way ANOVA test, carried out by Mstatc and Minitab (Version 13 programs) software. The comparison of the means was performed with the DMRT test at a 5% probability level. The diagrams were drawn by using the Microsoft Excel software.

RESULTS AND DISCUSSION

Physiological traits: The results of the analysis variation showed that the amount of photosynthesis was affected by the light duration and the interaction between the light duration and K-humate (Table 2). The maximum amount of photosynthesis was found in KH2×LD2 (16.4 µmolCO₂.m⁻².s⁻¹) and the minimum amount of photosynthesis was found in KH1×LD1 (7.51 µmolCO₂.m⁻².s⁻¹). The consumption of K-humate increased photosynthesis greater than the zero level of K-humate (KH1) in all light duration treatments (Fig 1).

Increment of the length of day in LD3 caused decreased photosynthesis to 14.8 µmolCO₂.m⁻².s⁻¹, in KH2×LD1, the amount of photosynthesis was 13 µmolCO₂.m⁻².s⁻¹. Our finding showed that with a prolonged length of day, photosynthesis was steadily performed. The same as photosynthesis trait, the interaction of treatments had significant differences on the Ci trait (Table 2); thus, with prolonging length of day of LD2 and in KH2 treatment, the amount of CO₂ in the substomatal chamber reached to

### Table 1: Some properties of potassium humate.

| Humic acid | Potassium (K₂O) | Zn, Fe, Mn | Appearance | pH | Solubility in water |
|------------|----------------|------------|------------|----|---------------------|
| 80%        | 10-12%         | 200 ppm    | Black powder | 8.5-9.5 | <98% |

### Table 2: Analysis of variation (mean-square) of leaf dry weight, biomass, photosynthesis, Ci, Chlo-a, Chlo-b, total Chlo, catrenoid, antioxidative, Fo, Fm, Fv, Fv/Fm.

| S.O.V       | df | Leaf dry weight | Biomass | Anti-oxidant | Fo | Fm | Fv | Fv/Fm | Photo-synthesis |
|-------------|----|-----------------|---------|--------------|----|----|----|-------|-----------------|
| KH          | 1  | ns              | ns      | ns           | ns | 10045** | 8550* | NS | ns               |
| LD          | 2  | 177*            | NS      | 0.016**      | 2205** | 21594** | 17529** | 0.003** | 11.5**           |
| KH×LD       | 2  | 154*            | 819*    | 0.002**      | 121* | 12966** | 10712** | 0.001* | 0.569*           |
| Error       | 18 | 30.5            | 160     | 0.001        | 91.4 | 1172   | 1455   | 0.0001 | 0.130            |
| CV          | 11.9 | 11.6          | 15.0    | 5.82         | 4.15 | 5.78   | 1.60   | 22.3  |

ns, * and ** are non-significant and significant at the 5 and 1% probability level respectively; KH: potassium humate; LD: light duration.
374 µmol.mol⁻¹ in comparison to the control treatment (326 µmol.mol⁻¹), which was increased about 12.8 percent (Fig 1). There is a similar trend in the curve of Ci and the photosynthesis curve in Fig 1. This finding is similar to Golamhosini et al. (2018) that reported increased light was enhancing Ci in soybean (*Glycine max* L.). At a high level of K-humate (KH2) and LD3, Ci decreased (351 ppm) and the amount of photosynthesis also declined (Fig 1). Some researchers reported that the photosynthesis process was promoted and enhanced by humate application (El-Bassiony et al., 2010; Yan et al., 2015). David et al. (1994) observed that humic acid treatments increased P, K, Ca, Mg, Mn, and Zn contents in stems, and N, Ca, Fe, Zn, and Cu contents in the roots of tomato seedlings. Therefore, with increased availability of essential elements for photosynthesis (light and nutrients), all the processes involved in photosynthesis were accelerated, and the assimilated material was increased and led to increased growth. On the other hand, the exit of K in the humic compound was helped to transport the photosynthetic product from the leaf to the growth regions in soybean (Wang et al., 2015) the Kelvin cycle works better in this condition. It seems that activity of Rubisco depended on the mesophyll conductance (gₘ) leading to a significant draw-down in CO₂ concentrations from the substomatal internal cavities (Ci) in this condition percent of K⁺ ions can be help to increased activities of guard cells for increased gas exchange that lead to enhancing of carboxylation inside the chloroplast stroma (Cc) (Weng et al., 2007).

**Fluorescence chlorophyll:** K-humate and the interaction of the K-humate×Long Duration of light had significantly different effects on the ChF parameters (Fo, Fm, Fv and Fv/Fm) (Table 2). The minimal fluorescence (Fo) value was observed in the KH1×LD2 treatment and the maximum of Fo (190) was observed after spraying K-humate (KH2) during LD3 treatment. While using KH2 in LD2 was not significantly different in comparison to the KH1×LD2 treatment, this showed that K-humate can increase the primary fluorescence, but it is not significantly important.
the results of the interaction between treatments showed that the range of Fv/Fm was 0.773 until 0.817 and spraying K-humate in LD2 had the greatest Fv/Fm value (0.817); the lowest Fv/Fm value was observed in KH1×LD1 (0.773) (Fig 2D). Rady et al. (2016) suggested the use of 15 kg/ha K-humate to obtain the maximum Fv/Fm value (0.81), which increased to about 6 per cent in comparison to the control. Hassanpanah (2009) reported that under a normal condition, using K-humate obtained the greatest Fv/Fm value in potato (var. Caesar) (0.799) in comparison to the absence of K-humate in the same condition. It seems that rate of Fv/Fm can be attributed to a change in the capacity of exploitation of photochemical energy in the dark adapted state; the lower value of Fo and higher Fm indicated that there is lower energy dissipation in the PSII antenna system and increased yield quantum in Photosystem II in apple plant (Zanandrea et al. 2006).

**Biomass:** The use of potassium humate in all the light treatments and the amount of LDW (leaf dry weight) were greater than 48 g. plant⁻¹; thus, the maximum LDW was observed in KH2×LD2 treatment and the amount of LDW was under 43 g. plant⁻¹ in all the interactions of light duration in the KH1 treatment (Fig 3). Rashwan et al. (2016) reported that in the stevia plant, the consumption of K-humate increased LDW to 81 g. plant⁻¹ during the two-year study (2014 and 2015). The total biomass (128.9 g. plant⁻¹) was also observed from consuming of KH2×LD2 in comparison of the control, whose increment was about 25 per cent.

Data also showed that except KH2×LD2 treatment, other treatments there were no significant difference in TDW (Fig 3). However, the use of K-humate in LD3 after KH2×LD2 had the greatest TDW. The study of Prakash et al. (2011) showed that the maximum growth rate was recorded in the application of 4-percent K-humate in Stevia rebaudiana. It seems that the humic compound, with efficacy on cellule metabolism, having hormone effects, was provided a nutritional condition follows the increased photosynthesis and plant growth (Nardi et al. 2002; Kaur and Sharma, 2018), K-humate with associated other factors were changes in some PSII components, electron transport chain components, and light-dependent photo-chemical reactions that lead to increased CO₂ fixation in mesophyll cell, in the other hand, K⁺ as a osmotic ion can be facilitating transport assimilation materials to need plant site and was also be increased TDW. Lotfi et al (2018) was reported that humic substance have auxin activities that stimulating stomatal opening and increased photosynthetic activity in rapeseed plants. In conclusion, the of present study results indicate that better photosynthetic performance parameters, such as photosynthesis and quantum yield (Fv/Fm) were gained from the interaction spraying of K-humate alongside an increase of 1.5 hours light prolong more than the natural day. It was remarkable that the maximum biomass was also recorded from this treatment.
