Indian J. Agric. Res., 53(3) 2019: 348-352
Print ISSN:0367-8245 / Online ISSN:0976-058X

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
www.arccjournals.com

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

Seyyed Fazel Fazeli Kakhki*, Morteza Goldani and Alireza Ghasemi Arian

Khorasan Razavi Agricultural and Natural Resources Research and Education Center, AREEO, Mashhad, Iran.

Received: 31-07-2018 Accepted: 04-03-2019 DOI: 10.18805/IJARe.A-383

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 theb 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 Steviawas 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 (ChlF) is a good indicator of Calvin cycle activity in many fruit species (Smillie and Hetherington, 1983). Various ChlF parameters have been applied in many areas of plant research. These applications have proved that ChlF 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 CO_2 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 CO_2 entering for photosynthesis is increased. This research

*Corresponding author's e-mail: sf_fazeli@yahoo.com

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 (*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; LD2and 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 aplied 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 prometer (Model SC-1, Decagon, USA) on fully expanded leaves.

Photosynthesis parameters: For measuring the photosynthesis rate, the amount of CO_2 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 OS1-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 $\mu molCO_2.m^{-2}.s^{-1}$) and the minimum amount of photosynthesis was found in KH1×LD1 (7.51 $\mu molCO_2.m^{-2}.s^{-1}$).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 $\mu molCO_2.m^{-2}.s^{-1}$, in KH2×LD1, the amount of photosynthesis was 13 $\mu molCO_2.m^{-2}.s^{-1}$. 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 CO_2 in the substomatal chamber reached to

Table 1: Some properties of potassium humate.

Humic acid	Potassium (K_2O)	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, antioxidant, 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*	12996**	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 founding 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

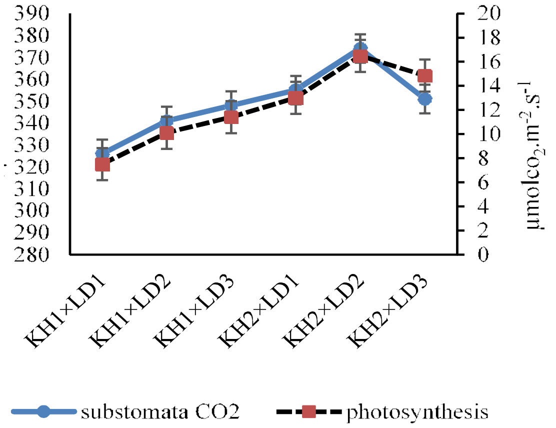

Fig 1: Interaction effects of potassium humate (KH) and light duration (LD) on photosynthesis and sub stomata CO_2 concentration.

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 involve 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 of mesophyll conductance (g_m) leading to a significant draw-down in CO_2 concentrations from the substomatal internal cavities (*C*i) in this condition persent of K^+ ions can be help to increased activities of gard cells for increased gas exchangethat lead to enhancing of carboxylation inside the chloroplast stroma (*C*c) (Weng *et al*., 2007).

Fluorescence chlorophyll: K-humate and the interaction of theK-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 importance

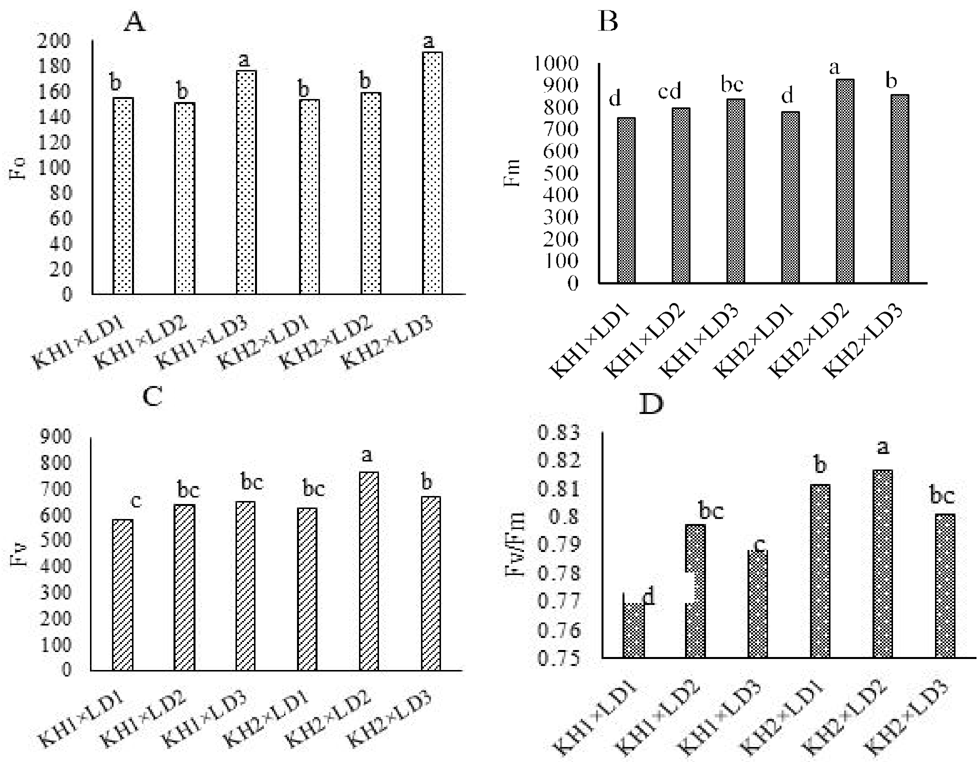

Fig 2: Effect interaction K-humate (KH) and light duration photoperiod on A) Fo (fluorescence original), B) Fm (fluorescence maximum), C) Fv (flourecence variable) and D) Fv/Fm.

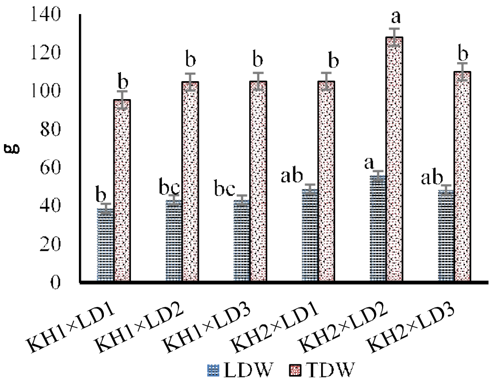

Fig 3: Interaction effects of potassium humate (KH) and light duration (LD) on leaf dry weight (LDW) and total dry weight (TDW).

when it is integrated with natural photoperiod light (Fig 2A). The Fo is the primary chlorophyll fluorescence yield that measures the stability of the light-harvesting complex. The increase in Fo showed the disruption of the photosynthetic apparatus and decrease in Fo was cause increase a speeding up in ETR (electron transfer rate) and CO_2 fixation (Baker and Rosenqvist, 2005, in fact quenching of chlorophyll fluorescence peak (ChFp) to the steady-state level is associated with changes in the photosynthetic apparatus that lead to induction of CO_2 assimilation, many factors can induce modifications in the kinetics of quenching from ChFp, thus application potassium compound can be increased this transient observed in ChFp that aslo was reported in *Pisum sativum* L (Russell *et al*. (2006). In the other hand it seems that an increase in Fo might be attributed to the effect of excess duration light that induces an abiotic stress. When the dark-adapted leaf is exposed to a short actinic light pulse of very high PPFD (generally less than 1 second on several thousand l(mol.m^{-2}.s^{-1}), Fm is generated as the majority of the PSII reaction centers have been closed (thus, incapable of photochemistry). In the present study, spraying K-humate during LD2 has the maximal Fm, which was increased by about 18 percent in comparison to the control treatment (KH1×LD1) (Fig 2B). The maximum of Fv was found of interaction KH2×LD2 treatment (Fig 2C). Hassanpanah (2009) in potato cultivars reported that maximum Fm was observed during the application of K-humate to about 378, which was increased to 12 percent in comparison to the control treatment. He did not see significant differences between the controls with K-humate treatments in the Fo value. (Fv/Fm)=(Fm-Fo)/Fm, the actual efficiency of PSII photochemistry, indicates the proportion of absorbed light (energy) that is used in photochemistry. Since decreases in Fv/Fm can be due to the development of slowly relaxing quenching processes and photo damage to the PSII reaction centers, both of these conditions reduce the maximum quantum efficiency of PSII photochemistry. In our study, 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 CO_2fixation 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.

REFERENCES

Baker, N. and Rosenqvist, E. (2005). Review article: Applications of chlorophyll fluorescence can improve crop production strategies: An examination of future possibilities. *J. Exp. Bot,* **55**:1607–621.

Benlloch-González, M., Arquero, O., Fournier, J.M., Barranco, D., and Benlloch, M., (2008). K^+ starvation inhibits water-stress-induced stomatal closure. *J. Plant. Physiol,* **165**: 623–630.

Canellas, L.P., Olivares, F.L., Aguiar, N.O., Jones, D.L., Nebbioso, A., Mazzei, P., and Piccolo, A. (2015). Humic and fulvic acids as biostimulants in horticulture. *Sci. Hort.* **196**: 15-27.

David, P.P., Nelson, P.V., and Sanders, D.C. (1994). A humic acid improves growth of tomato seedling in solution culture. *J. Plant. Nutr,* **17** (1): 173-184.

El-Bassiony, A.M.; Fawzy, Z. F., El-Samad, E.H, and, Riad, G.S. (2010). Growth, yield and fruit quality of sweet pepper plants (*Capsicum annuum* L.) as affected by potassium fertilization. *J. Am. Sci,* **6**(12): 722-729.

El-Bassiony, A.M., Fawzy, Z.F., Abd El-Baky, M.M.H., and Mahmoud, A.R. (2010). Response of snap bean plants to mineral fertilizers and humic acid application. *Res. J. Agri. Biol. Sci,* **6**(2): 169-175.

Gholamhoseini, M., Ebrahimian, E., Habibzadeh, F., Ataei1, R., and Dezfulizadeh, M.S. (2018). Interactions of shading conditions and irrigation regimes on photosynthetic traits and seed yield of soybean (*Glycine max* L.). *Legume Research,* **41**(2): 230-238

Hassanpanah, D. (2009). *In vitro* and *in vivo* screening of potato cultivars against water stress by polyethylene glycol and potassium humate. *Biotech,* **8**(1): 132-137.

Kaur, G., and Sharma, M. 2018. Effect of nitrogen doses and inter crops on growth and yield of broccoli (*Brassica oleracea* L. var. italica**)**. *Indian J. Agric. Res.,* **52**(5): 566-570.

Lotfi, R., Kalaji, H.M., Valizadeh, G.R., Khalilvand Behrozyar, E., Hemati, A., Gharvi-kochebagh, P and Ghassemi, A. (2018). Effects of humic acid on photosynthetic efficiency of rapeseed plants growing under different watering conditions. *Photosynthetica,* **56**(3): 962-970,

Mady, M.A. (2009). Effect of foliar application with yeast extract and zinc on fruit setting of Faba bean (*Vicia faba* L.). *J. Biol. Chem. Environ. Sci,* **4**(2): 109-127.0

Marschner, H. (1995). Functions of mineral nutrients: micronutrients. In: Mineral Nutrition of Higher Plants. 2nd Ed., Academic Press, London, pp. 313-404.

Metivier, J., and Viana, A. M. (1979). Changes in levels of total soluble proteins and sugars during leaf ontogeny in Stevia rebaudiana. *Annual. Bot,* **45**: 469-474.

Nardi, S., Pizzeghello, D., Muscolo, A., and Vianello, A. (2002). Physiological effects of humic substances on higher plants. *Soil. Biol. Biochem,* **34**: 1527-1536.

Pearce, D. W., Millard, S., Bray, D. F., and Rood, S. B. (2006). Stomatal characteristics of riparian poplar species in a semi-arid environment. *Tree. Physiol,* **26**: 211-218.

Prakash, P., Dhanalakshmi, P.K., and Anusha, B. (2011). Effect of Humic Acid On Spirulina plantensis Production and Analysis of Nutrient Contents. *Recent Research in Science and Technology,* **3:** 87-89

Rady, M.M., Abd El-Mageed, T.A., Abdurrahman, H.A., and Mahdi, A.H. (2016). Humic acid application improves field performance of cotton (*Gossypium barbadense* L.) under saline condition. *J. Anim. Plant. Sci,* **26**(2): 487-493.

Ramesh K., Virendra Singh., and Megeji, N.W. (2006). Cultivation of stevia (*Stevia rebaudiana* var. Bertoni). *A comprehensive review. Adv. Agron,* **89**: 137-177.

Rashwan, Basma, R.A., Reem, M., Abd-El Raouf, Nagwa, R., Ahmed, and Ferweez, H. (2016). Efficacy of K-Humate, compost and biofertilizer application as well as cutting number on yield and quality of stevia (*Stevia rebaudiana* var. Bertoni) as natural sweetener. *Assiut J. Agri. Sci,* **48**: 251-268.

Russell, L., Stokes A.R., Macdonald H. *et al.* (2006). Stomatal responses to humic substances and auxin are sensitive to inhibitors of phospholipase A2. *Plant Soil,* **283**: 175-185.

Smillie, R.M., and Hetherington, S.E. (1983). Stress tolerance and stress induced injury in crop plants measured by chlorophyll fluorescence *in vivo. Plant. Physiol,* **72**: 1043-1050.

Soejarto, D. D., Compadre, C. M., Medon, P. J., Kamath, S. K., and Kinghorn, A. D. (1983). Potential sweetening agents of plant origin. II. Field search for sweet-tasting Stevia species. *Econ. Bot,* **37**: 71-79.

Wang, X.G., Zhao, X.H., Jiang, C.J., Wang, C. (2015). Effects of potassium deficiency on photosynthesis and photo protection mechanisms in soybean (*Glycine max* L. Merr.). *J. Integ. Agri,* **14**(5): DOI:10.1016/S2095-3119(14)60848-0.

Weng, X.Y., Zheng, C.J. Xu, H.X., Sun J.Y. (2007). Characteristics of photosynthesis and functions of the water-water cycle in rice (*Oryza sativa*) leaves in response to potassium deficiency. *Physiol. Plant,* **131:** 614-621.

Yan, C.J., Song, S.H., Wang, W.B., Miao, S.J. Cao, Y.Q., Wang, C.L., and Zhang, L.J. (2015). Impacts of fertilization on photosynthesis, growth and yield of two soybean cultivars (*Glycine max* L. Merr) in Northeast China. *Legume Research,* **38** (1): 77-84.

Zanandrea, I., Bacarina, M.A., Schmitz, D.D., Bloace, E.J., Jose, B.M., and Peters, A. (2006). Chlorophyll fluore scence *in vitro* cultivated apple, R. Bras. *Agrocieencia Pelotas,* **12**: 305-308.

Zubenko, V. F., Rogovskii, S. V., and Chudnovskii, B. D. (1991). Stimulation of rooting of Stevia cuttings and growth of the transplants by phytohormones. *Doklady Vsesoyuznoi Ordena Leninai Ordena Trudovogo Krasnogo Znameni,* **2**: 16-18.