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IMPROVING TOLERANCE OF *STEVIA REBAUDIANA* TO WATER DEFICIT STRESS THROUGH FOLIAR SPRAY OF POTASSIUM NITRATE

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ABSTRACT

An experiment was performed to study the role of potassium nitrate in improving the tolerance capacity of *Stevia rebaudiana* plants grown under water stress. The *Stevia* plants were subjected to different water regimes viz., 100 ml, 200 ml and 300 ml water and the control plants were watered with 400 ml water daily. The different sets of *Stevia* plants were also foliar sprayed with 300ppm KNO_3 solution on weekly basis. The periodic collection of plant samples were made and analysed for various growth parameters viz. number of leaves, growth rate index, biomass, chlorophyll content, proline content and reducing sugar content. The results of the experiment reveal that potassium nitrate acts as a bioregulator and is effective in promoting the growth and physiological performance of potted *Stevia* plants under water stress.

KEYWORDS: *Stevia*, *Growth* Rate Index, Proline, Water Stress, Potassium

INTRODUCTION

Plants are exposed to a variety of biotic or abiotic stress. The abiotic stresses like temperature (heat, cold chilling/frost), water (drought, flooding), radiation (UV, ionizing radiation), chemicals (mineral deficiency/excess, pollutants heavy metals/pesticides, gaseous toxins), mechanical (wind, soil movement, submergence) are responsible for over 50% reduction in agricultural production. Drought or dehydration results in water deficit stress. Water deficit is defined as an imbalance between soil water availability and evaporative demand (Tardieu *et al*., 2011). The process of photosynthesis and thereby the primary productivity of plants are known to decrease under water stress (Gnaana Saraswathi and Paliwal, 2011). It is one of the major environmental stresses that reduces plant growth and productivity (Zlatev *et al*., 2012).

Status of mineral-nutrient in plants plays a critical role in increasing plant resistance to drought stress (Marschner, 1995). Adverse effect of water stress can be alleviated by the use of bioregulators as it offers a convenient and rapid approach for improvement of stress tolerance. They are known to enhance the source sink relationship, photosynthetic efficiency and stimulate the translocation of photo-assimilates thereby helping in better growth and productivity of crops (Solaimalai *et al*., 2001). Foliar feeding is an effective method for correcting soil deficiencies and overcoming the soil's inability to transfer nutrients to the plant under low moisture conditions (Marchener, 1995 and Stigler, *et al*. 2010).

Stevia rebaudiana (Family Asteraceae) is one of the 154 members of genus *Stevia* and is cultivated for its sweetening compounds. It is native to South America (Alhady, 2011), but now it is grown all over the world. It is a perennial semi-shrub upto 30 cm in height. *Stevia* have versatile medicinal uses without any side effects that focus the interest towards *Stevia* in worldwide. *Stevia* is susceptible to water stress and that results in severe cell damages and growth reduction (Srivastava and Srivastava, 2014).

Plant growth processes are influenced under scarcity of water. Therefore an attempt has been made in the present research to study the difference in morphological and physiological changes in *Stevia* due to water deficit stress and to overcome the changes by application of potassium nitrate as bioregulator.

MATERIALS METHODS

Cuttings of *Stevia* plants were collected from the University campus and were used as test plant. Vegetatively propagated *Stevia* plants (20 days old) were transferred to earthenware pots containing sand, supplied with Hoagland's nutrient solution at 7 days interval and were subjected to different water regimes viz., 100 ml (severe water stress), 200 ml (moderate water stress) and 300 ml (mild water stress) and the control plants were watered with 400 ml water daily. The plants were also foliar sprayed with KNO_3 solution (300ppm) on weekly basis. The potted *Stevia* plants were allowed to grow for a period of 50 days. The periodic collection of plant samples were made and analysed for various growth parameters viz. number of leaves, growth rate index, biomass, chlorophyll content, proline content and reducing sugar content at every 10 days interval from day 30 upto day 70.

Total number of leaves was observed by counting the leaf from top to bottom of the plant and was expressed as number per plant from day 30 upto day 70. Growth Rate Index was measured by the amount of plant growth in terms of biomass in a specified time period and was calculated by the following formula:

Growth Rate Index (GRI) = difference of initial and final biomass

Plants were oven dried at $60\pm20^{o}C$ for 48 hours and the dry weight was recorded for biomass of the plant. Proline was estimated by the method of Bates *et al*., (1973). Chlorophyll was measured in primary leaves by the method of Arnon (1949). Reducing sugar was estimated by the method of Somogy (1952).

RESULTS AND DISCUSSIONS

Analysis of data revealed that the number of leaves was more in control plants than in stressed plants with the lowest number in plants under severe stress. However the number of leaves increased in KNO_3 treated *Stevia* plants at all water levels (Figure 1). Our findings are similar to many reports (Nielsen and Nelson, 1998; Boutraa and Sanders, 2001), where low water level reduced the total number of leaves per plant. Reduced number of leaves on a plant greatly reduces the rate of water loss through transpiration, thus plants under water stress have reduced leaf formation. The amount of leaf production in *Crotalaria ochroleuca* also decreased with an increase in water deficit (Sikuku *et al*. 2013).

Growth rate index was measured for all the treatments. The growth rate index decreased with the decrease in water level having the minimum value in plants treated with 100 ml water. However the growth rate index increased on treatment with KNO_3 at all water levels (Figure 2). Many authors have observed that when wheat plants were exposed to water stress, many growth parameters, including plant height, fresh and dry weights of shoot and root, the relative growth rate as well as leaf area tended to decrease (Shao *et al.*, 2008; Aldesuquy, 2014). Crop growth rate decreases in drought stress condition because of increasing respiration intensity and decrease of photosynthesis (Goldani and Rezvani, 2007; Prasad *et al*., 1978). Our results were similar to the results of Royo and Blanco (1999) and Ashraf *et al.*, (2003). The increased plant growth due to foliar potassium might be attributed to increased cell division and cell elongation induced by potassium nitrate. Potassium application under drought moderates the adverse effects of water shortage on plant growth (Sangakkara *et al*., 2001).

Drought stress decreased accumulation of total dry matter in *Stevia* plants. Severe stress resulted in decrease in biomass of *Stevia* plants. Less accumulation of total dry matter in stress condition might be due to decrease in leaf area index and consequently decrease in photosynthetic area. Foliar treatment with KNO_3 increased the biomass of *Stevia* plants and maximum biomass was observed in KNO_3 treated control plants (Figure 3). This increase in biomass of KNO_3 treated lants may be due to the fact that sufficient nutrient is very much essential for plant growth and the plants will have good vegetative growth which in turn helps to utilize light better. Fresh and dry weights of *Ocimum basilicum* L. were decreased as plant water deficit increased (Simon *et al.*, 1992). Plants treated with different potassium levels showed better results under drought conditions resulting in enhanced growth and biomass (Ihsan *et al*., 2013).

A significant variation in proline content in leaves of plants treated with different water levels was observed. Proline content in the leaves of water stressed *Stevia* plants increased from day 30 upto day 60 followed by gradual decrease upto day 70 whereas in untreated control plants, it increased from day 30 upto day 50 and then decreased upto day 70. Highest proline content was found in plants treated with 100 ml water (severe stress) followed by moderate and mild stress plants at 60 DAS. Stressed plants always had more proline accumulation than that of control. KNO_3 treated plants exhibited minimum proline content (Figure 4). This result are similar to the findings of Selahvarzi *et al*., (2008) in ornamental turf grasses and Shooshtarian (2010) in ten species of ground cover plants. Din *et al*., (2011) found that free proline content in leaves increased significantly under severe drought stress. Potassium is an important macronutrient and osmoticum which help plants to adjust to low water potential under drought stress (Bukhsh *et al*., 2012).

Chlorophyll content ('chl a' and 'chl b') of *Stevia* plants increased from day 30 upto day 50 and then decreased under water stress treatments and lowest amount was found to be present in severely stressed plants treated with 100 ml water. KNO_3 application to *Stevia* plants increased the leaf chlorophyll content Table 1. Water deficit can destroy the chlorophyll and prevent making it (Lessani and Mojtahedi, 2002). A reason for decrease in chlorophyll content as affected by water deficit is that drought stress produce reactive oxygen species (ROS) such as O^{2-} and H_2O_2, which can lead to lipid per oxidation and consequently, chlorophyll destruction (Smirnoff, 1993; Foyer *et al*., 1994).The amount of chlorophyll decreased significantly in the leaves with the increase in water stress (Shinde and Thakur, 2015).

A significant variation in reducing sugar content in leaves of plants treated with different water levels was observed. The reducing sugar content was more in stress plants with maximum in plants treated with 100 ml and 200 ml water (Figure 5). Foliar application of KNO_3 decreased the reducing sugar content in stress as well as control plants with a maximum decrease in KNO_3 treated control plants. These results are in accordance with Irigoyen *et al*., (1992) that the sugar content in leaves of the plant can increase under drought conditions. The accumulated soluble sugars in the cell under stress, balances the osmotic strength of the cytosol with that of the vacuole and the external environment (Abdalla, 2011). Foliar treatment with different concentrations of potassium chloride (KCl) to mulberry plants resulted in higher level of total chlorophyll, total sugars and soluble protein (Das *et al*., 2003).

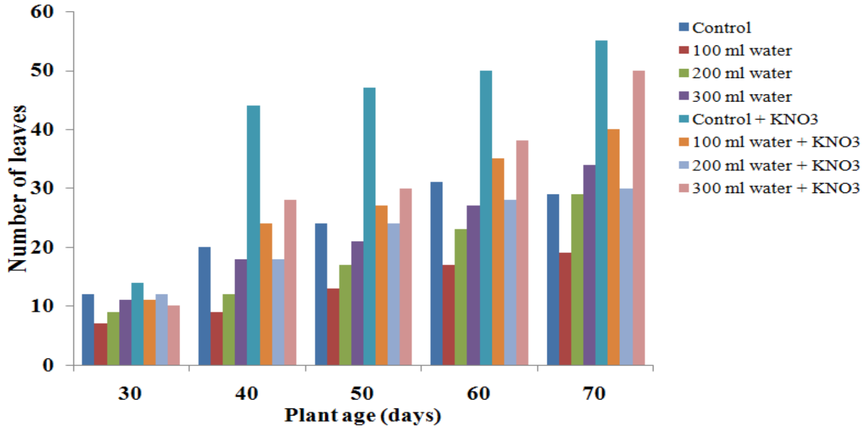

Figure 1: ***Stevia Rebaudiana:*** **Number of Leaves at Different Days of Growth under Different Water Regimes Alone and in Combination with Kno$_3$ (LSD = 2.42)**

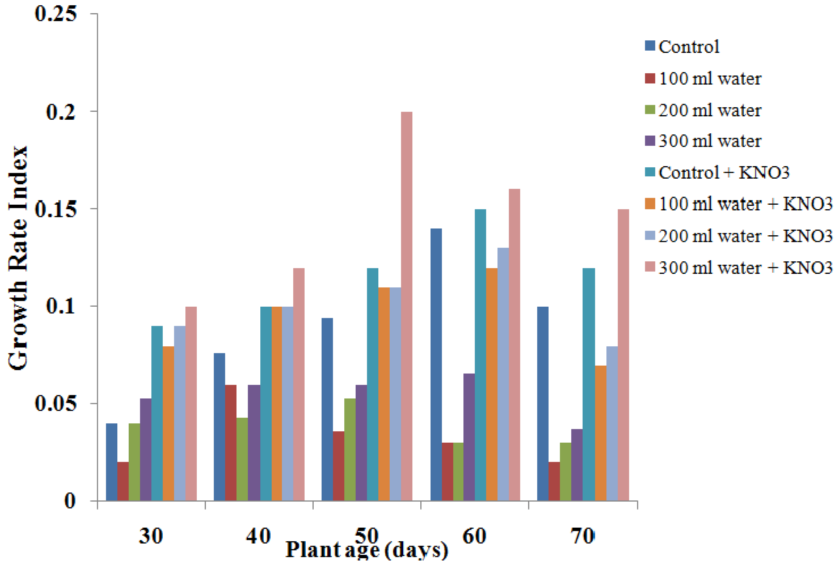

Figure 2: ***Stevia Rebaudiana:*** **Growth Rate Index at Different Days of Growth under Different Water Regimes Alone and in Combination with Kno$_3$ (LSD = 0.013)**

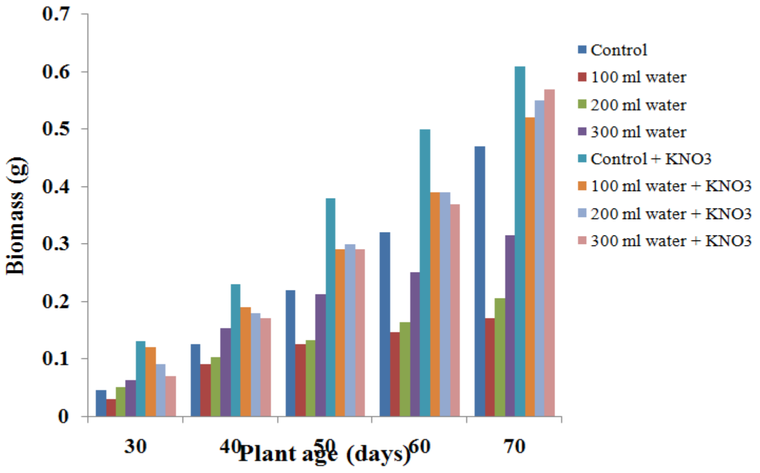

Figure 3: ***Stevia Rebaudiana:*** **Biomass at Different Days of Growth under Different Water Regimes Alone and in Combination with Kno$_3$ (LSD = 0.044)**

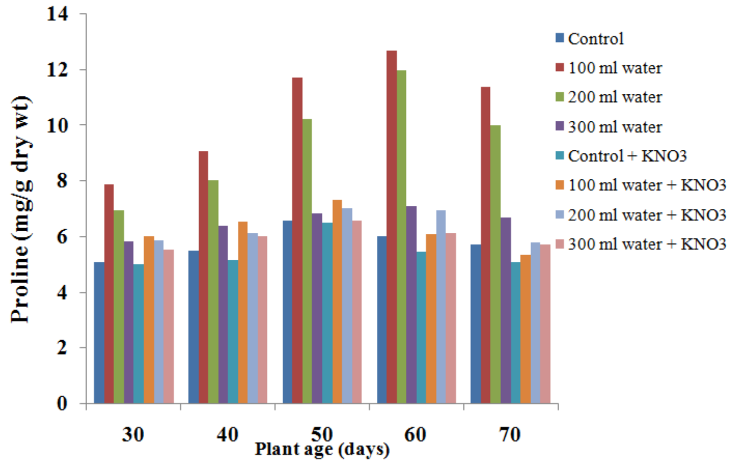

Figure 4: *Stevia Rebaudiana:* Proline at Different Days of Growth under Different Water Regimes Alone and in Combination with Kno_3 (LSD = 0.06)

Table 1: *Stevia Rebaudiana:* Effect of Water Stress on Chlorophyll Content (mg/g Dry Wt. $\times 10^{-4}$) at Different Days of Plant Growth

Treatment	DAS	Control		100 ml Water		200 ml Water		300 ml Water	
		Chl a	Chl b	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b
Untreated	30	4.43 ±0.40*	4.31 ±0.05	4.43±0.35	6.72 ±0.25	3.62±0.20	5.23±0.60	5.8 ±0.60	5.18 ±0.15
	40	7.10 ±0.10	5.2 ±0.81	3.22±0.15	4.25 ±0.20	3.24±0.05	6.20±0.4	5.15±0.20	4.30 ±0.05
	50	7.52 ±0.60	5.52 ±0.20	2.03±0.30	4.11 ±0.26	2.60±0.25	2.63±0.15	3.62±0.35	3.65±0.36
	60	5.42 ±0.3	7.26 ±0.20	1.84±0.10	1.23 ±0.20	1.54±0.36	2.43±0.25	3.49±0.25	1.53±0.40
	70	4.93 ±0.3	13.8 ±0.05	0.75±0.15	0.94 ±0.41	1.8 ±0.70	1.90 ±0.20	2.4 ±0.45	12.6 ±0.35
KNO_3 treated	30	6.24 ±0.05	6.43 ±0.40	6.8 ±0.10	3.43 ±0.36	6.43±0.25	5.23 ±0.60	7.79±0.15	4.23 ±0.15
	40	9.36 ±0.05	5.32 ±0.10	7.23±0.25	4.63 ±0.43	8.23±0.15	6.20 ±0.40	9.81±0.30	3.30 ±0.25
	50	10.13 ±0.60	2.18 ±0.25	7.66±0.05	1.45 ±0.70	9.09±0.15	2.63 ±0.15	10.84±0.2	3.23 ±0.35
	60	7.32 ±0.30	2.36 ±0.30	5.11±0.05	1.30 ±0.32	6.43±0.40	2.43 ±0.25	7.31±0.15	2.04 ±0.20
	70	6.36 ±0.10	2.13 ±0.10	4.79±0.84	0.96 ±0.43	4.03±0.35	1.90 ±0.20	6.2 ±0.40	2.11 ±0.60

*Mean ±Standard Deviation (n=3)

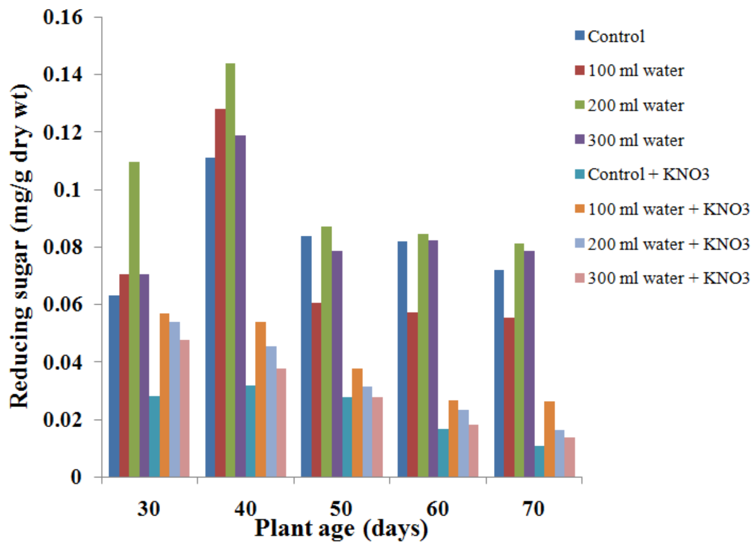

Figure 5: *Stevia Rebaudiana:* Reducing Sugar at Different Days of Growth under Different Water Regimes Alone and in Combination with KNO_3 (LSD = 0.023)

CONCLUSIONS

Stevia rabaudiana is an important medicinal plant cultivated for its sweetening compounds (the glycosides) and other biochemicals of interest. From our research it can be concluded that *Stevia* plants are susceptible to water stress. However, foliar application of potassium nitrate to *Stevia* plants enhances the growth and physiology of *Stevia* plants and can be used as an efficient strategy for cultivation of drought resistant *Stevia* plants.

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