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BIOCHEMICAL CHANGES IN STEVIA REBAUDIANA UNDER DROUGHT STRESS

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ABSTRACT

A pot experiment was conducted to determine the biochemical changes in sugar free plant *Stevia rebaudiana* under drought stress condition. The plants were grown in earthenware pots containing sterilized sand. The pots were divided into different sets on the basis of variation in water regime to induce different levels of drought stress. The treated plants were compared with control plants and there was a marked variation in the amount of observed biochemicals viz. proline, total nitrogen, protein and reducing sugar.

INTRODUCTION

Stevia rebaudiana is a perennial medicinal shrub of Asteraceae family. The native of *S. rebaudiana* is in South America (Alhady, 2011) but now it is grown all over the world. It is cultivated for its sweetening compounds (the steviol glycosides). The two main glycosides of *Stevia* are stevioside (5% - 10% of dry leaves) and rebaudioside- A (2% - 4%). Although there are about 230 species in the genus, only *S. rebaudiana* have the sweetest essence (Soejarto *et al.* 1983), while other species contain other biochemicals of interest (Frederico *et al.* 1996). The leaves are mid green and intensely sweet. *Stevia* leaves can be used due to its antifungal and antibacterial properties. After several years of economic cultivation, yield of *Stevia rebaudiana* is still low due to lack of proper knowledge on water requirements during the cultivation period.

Drought, an abiotic stress, is multidimensional in nature, and it affects plant at various levels of their organization. The lack of adequate moisture leading to drought stress is of common occurrence in rainfed areas, brought about by infrequent rains and poor irrigation (Wang *et al*, 2005). Water being an integral part plays a vital role in the maintenance of plant life. Water stress decreases growth of some medicinal plants, including *Hypericum brasiliense* (Nacif de

Abreu and Mazzafera, 2005) and *Bupleurum chinense* (Zhu *et al*., 2009). Availability of water is one of the limiting factors determining plant distribution and survival in natural ecosystem. Under severe water stress conditions caused by high drought, plant growth is affected and the plant cells accumulate solutes such as sugars and amino acids for osmotic adjustment.

Thus an attempt has been made in our experiment to study the variation in the amount of some biochemicals of *Stevia rebaudiana* under drought stress.

MATERIALS AND 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 sterilized sand, supplied with Hoagland's nutrient solution at 7 days interval and were subjected to different water regimes viz., 100 ml(severe stress), 200 ml (moderate stress) and 300 ml (mild stress) and the control plants were watered with 400 ml water daily. Then the plants were allowed to grow for a period of 50 days. The periodic collection of plant samples were made and analysed for various biochemical parameters viz proline total nitrogen protein and reducing sugar content at every 10 days interval from day 30 upto day 70. Proline was estimated by the method of Bates *et al*., (1973). Total nitrogen and protein was calculated by Doneen's (1932) micro-kjeldahl's method. Reducing sugar was estimated by the method of Somogy (1952).

RESULT AND DISCUSSION

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. Baher *et al*. (2002) reported that proline accumulation in *S. hortensis* L. increased under drought stress. Bahreininezad *et al* (2013) observed reduced proline content under moderate water stress but proline increased under severe water stress in *Thymus daenensis.* Therefore, proline accumulation rate increases under drought conditions in medicinal and aromatic plants.

Total nitrogen in the leaves of severe and moderate stress plants increased from day 30 upto day 70 whereas in mild stress plants and in control plants, it increased from day 30 upto day 60 followed by gradual decrease upto day 70. Severe and moderate stress plants significantly reduced the nitrogen content of plants. Ghanbari *et al*., (2013) reported marked decrease in leaf nitrogen content in *Phaseolus vulgaris* L. under water stress. The decline in leaf nitrogen is associated with reduction in

photosynthesis (Wollenweber *et al*., 2003). Drought adversely affects the maximal efficiency of photosystem 2 (PS2) photochemistry (Lu and Zhang, 1999) and decreases the leaf N content (Sinclair *et al*., 2000). Drought can decrease plant nitrogen uptake and leaf nitrogen (Llorens *et al*., 2003).

Total protein in the leaves increased from day 30 upto day 60 followed by gradual decrease upto day 70. The plants treated with 100 ml water had minimum protein content as compared to other treatments. Many studies showed that protein concentration decreased in many plant species under drought stress (Hussein *et al*., 2007) due to decreasing protein biosynthesis (Amin *et al*., 2009) and enhancing degradation (Black and Prithard, 2002). Some investigators reported that reducing protein content was a result of the negative effect of drought on nitrate reductase activity and nitrogen metabolism (Ahmad *et al*., 2003). A common effect of drought stress is to cause oxidative damage (Smirnoff 1998). The reactive oxygen species (ROS), produced at water stress conditions, enhance decomposition of proteins (Yazdanpanah *et al*., 2011). The accumulation of ROS during drought stress, along with increasing H_2O_2, often enhances protein oxidation in plant species.

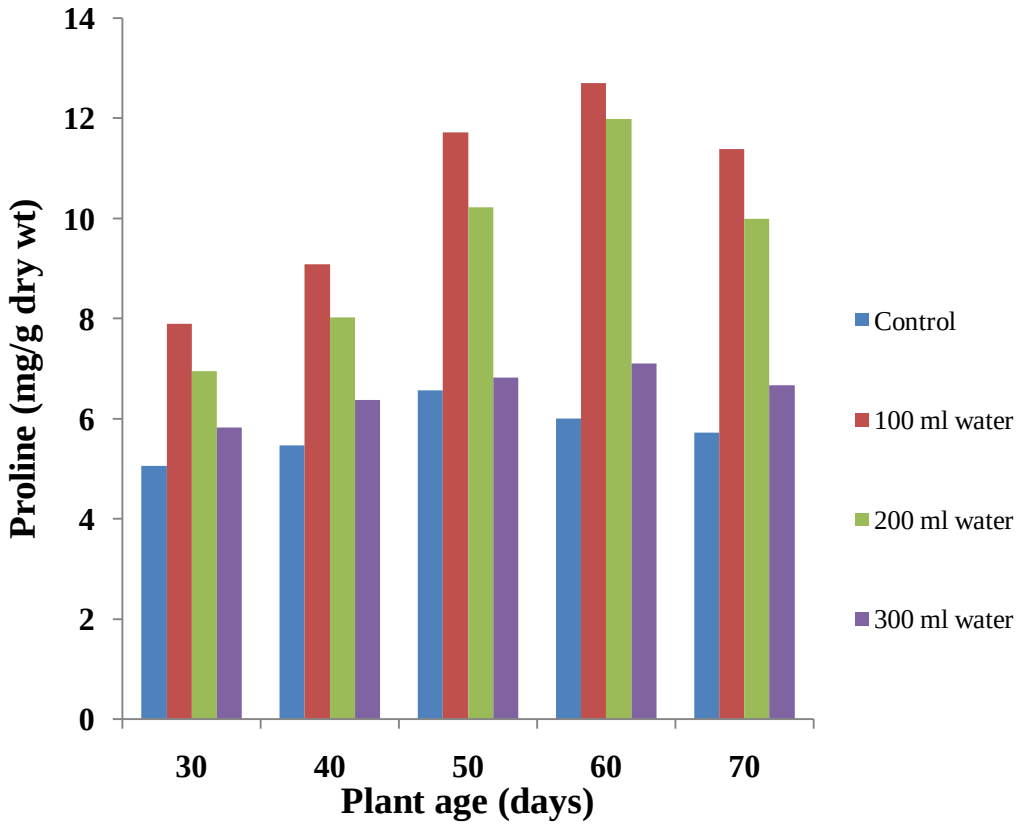

Fig 1: *Stevia rebaudiana* : Proline at different days of growth under different water regimes.

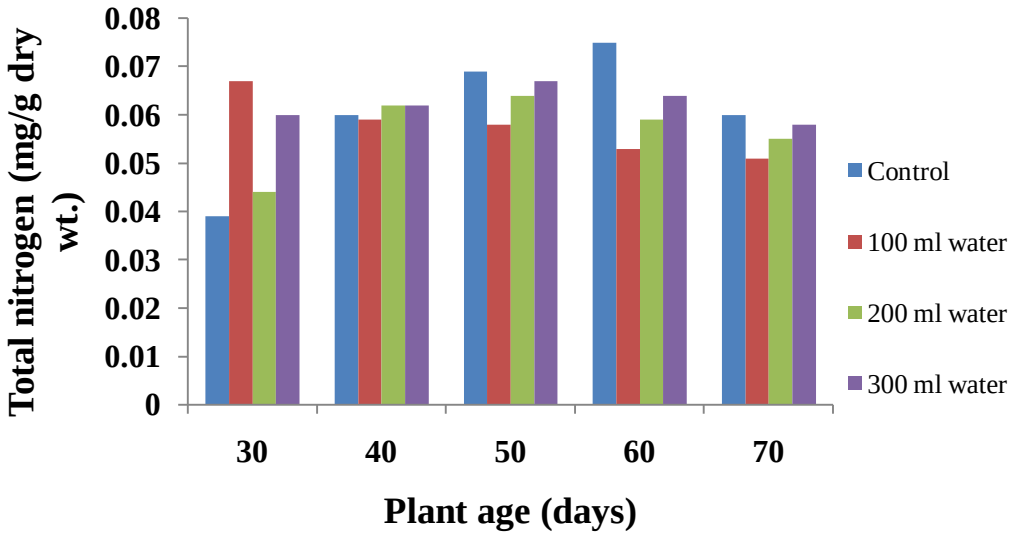

Fig 2: ***Stevia rebaudiana :*** Total nitrogen content at different days of growth under different water regimes.

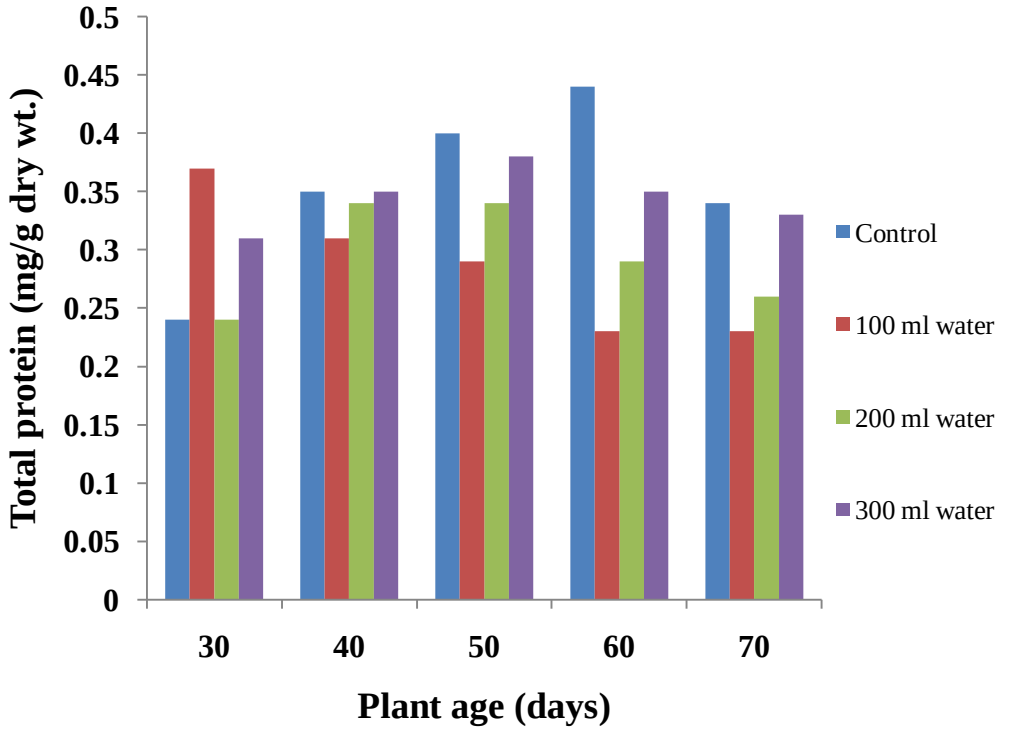

Fig 3 : ***Stevia rebaudiana :*** Total protein content at different days of growth under different water regimes.

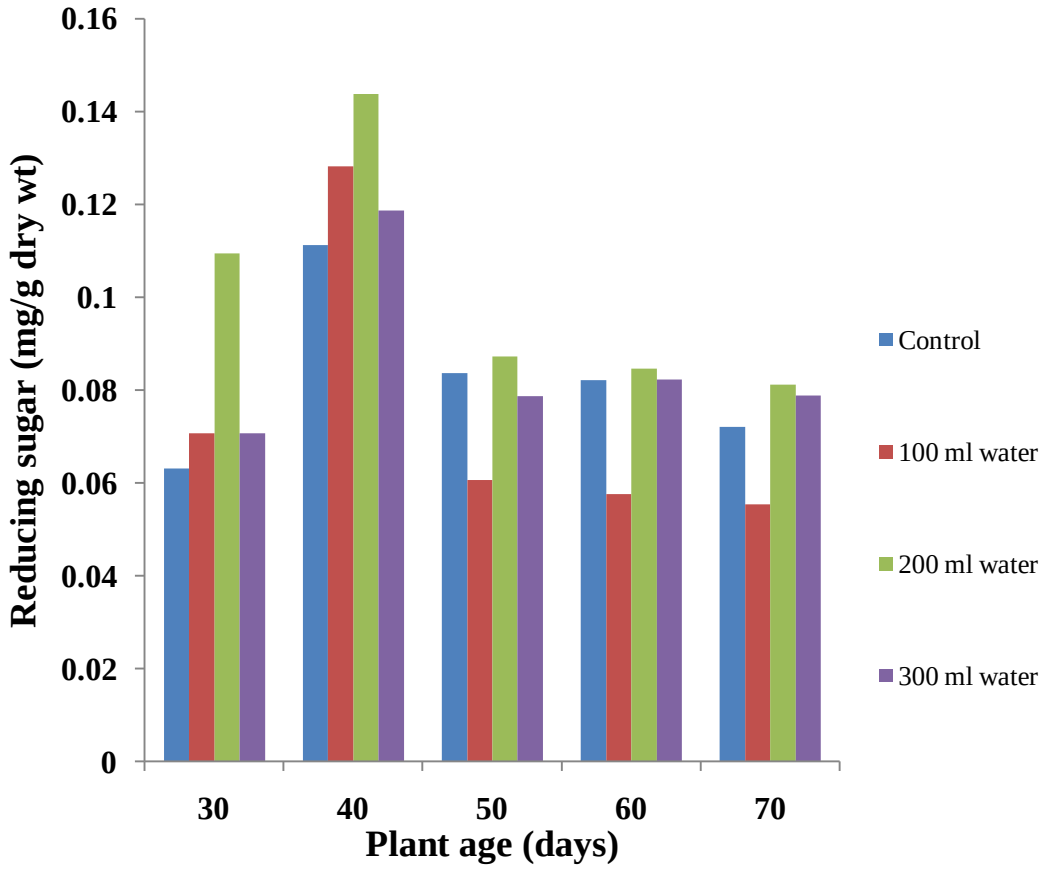

Fig 4 : *Stevia rebaudiana :* Reducing sugar content at different days of growth under different water regimes.

A significant variation in reducing sugar content in leaves of plants treated with different water levels was observed. Total reducing sugar content in the leaves increased from day 30 upto day 40 followed by gradual decrease upto day 70. The reducing sugar content was more in drought stress plants with maximum in plants treated with 100 ml and 200 ml water (Fig. 4). Sinay and Kuruwal, (2014) reported that total soluble sugar content of corn cultivars increased on drought stress treatment as compared to control plants. Some of the organic material that accumulates in plant tissues including corn as a response to drought stress, are proline and soluble sugar (Farhad *et al*., 2011; Liu *et al*., 2011; Moaveni, 2011). Soluble sugar accumulation has been reported with salt and drought stresses (Pinheiro *et al*., 2004; Munns, 2005).

CONCLUSION

From this research, it is concluded that *Stevia* is susceptible to water stress. The amount of biochemicals is significantly affected by water stress in comparison to non stressed *Stevia* plants. It is imperative to improve the drought tolerance of plants under changing circumstances. However, development of *Stevia* plants tolerant to drought stress might be a promising approach which can help in meeting food demands.

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