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# Morphological Changes and Antioxidant Activity of *Stevia rebaudiana* under Water Stress

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## Abstract

***Stevia rebaudiana*, a herbaceous perennial shrub contains steviol glycosides, as an alternative source of sugar for diabetic patients. Water being an integral part plays a vital role in the maintenance of plant life. Availability of water is one of the limiting factors determining plant distribution and survival in natural ecosystem. The objective of this study was to investigate the ability of tolerance of *Stevia* plants to water stress. Potted plants of *Stevia* were subjected to different levels of water regimes (100 ml, 200 ml, 300 ml) per day, whereas control plants were watered daily with about 400 ml water. Plant height, leaf area, electrolyte leakage and antioxidant enzyme activity (peroxidase and catalase) were assayed during the experimental period. All these parameters were severely affected under water stress condition. Stress treatment caused an increase in electrolyte leakage compared to control. Plant height decreased under severe stress condition whereas a sharp increase in antioxidant enzyme activity was observed in stressed plants as compared to untreated control plants. Our experiment emphasizes the importance of proper watering schedule for the cultivation of *Stevia* as an agricultural crop to meet the challenges for sugar and energy crisis.**

## Keywords

***Stevia*, Water Stress, Growth, Antioxidant Enzymes**

## 1. Introduction

Stress is an altered physiological condition caused by factors that tend to disrupt the equilibrium. Plants are fre-

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quently exposed to many stresses such as drought and low temperature, salt, flooding and heat, which severely affect plant growth and food productivity. Water, comprising 80% - 90% of the biomass of plants, is the central molecule in all the physiological processes of plants by being the major medium for transporting metabolites and nutrients. Drought is the most severe abiotic stress factor limiting plant growth and crop production (Rohbakhsh, [1]). Water deficit is considered as a major environmental factor affecting many aspects of plant physiology and biochemistry (Charles *et al.*, [2]).

The effect of drought stress on growth and development of medicinal and aromatic plants has been less studied. However, the antioxidant defense system is one of major drought defense and adaptive mechanisms in plants (An and Liang, [3]). Several studies have pointed out that drought-tolerant species increased their antioxidant enzyme activities in response to drought treatment, whereas drought-sensitive species failed to do so (Masoumi *et al.*, [4]).

*Stevia rebaudiana*, a non-caloric sweetener (family—Asteraceae) 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%). Due to the non-caloric and sweetening properties, stevioside has gained attention with the rise in demand for low-carbohydrate, and low-sugar food alternatives (Kalpana *et al.*, [5]). Srivastava and Srivastava [6] reported marked alterations in physiology and biochemistry of *Stevia* plants grown under water stress.

Hence the objective of this work was to evaluate the influence of variable regimes of water on growth performance and antioxidant activity of *Stevia*.

## 2. Materials and Methods

Vegetatively propagated *Stevia* plants were grown in sand and regularly supplied with Hoagland's nutrient solution every 5$^{th}$ day. 20 days after transplantation, plants were treated with different water levels viz. 100 ml, 200 ml, 300 ml water per day. The unstressed plants were kept as control and supplied with 400 ml water daily.

All the studied parameters were recorded at every ten days interval from day 30 up to day 70. Plant height was measured from the attachment point of root and stem up to the slip of uppermost fully opened leaf. Leaf area was calculated by the formula given by Muller, [7]. The electrolyte leakage was calculated by the formula described by Sullivan and Ross, [8]. The peroxidase activity was determined in the primary leaves by the method of Shannon *et al*. [9]. The catalase activity was determined in the primary leaves by the method of Chance and Maehly, [10].

The data presented are means of three replicates and were analysed with two way ANOVA using the minitab statistical package. The least significant difference (LSD) was calculated to verify the significance of difference between the means, Brunning and Kinz, [11].

## 3. Result and Discussion

*Stevia* plants showed greater variability in plant height under different water levels (**Figure 1**). The well watered plants exhibited maximum growth than stressed plants and the plant height recorded during the experimental period was highest in well watered plants and decreased with decreasing water levels. In mild and moderate stress *Stevia* plants the plant height was not much reduced as compared to control as the plants were able to tolerate mild and moderate water deficits at the initial growth stage. Water stress greatly suppresses cell expansion and cell growth due to low turgor pressure. Similar results were found in water stressed Citrus plants, Wu *et al.* [12] and soyabean, Zhang *et al*. [13]. Alavi-Samani *et al.* [14] also observed reduced plant height in two species of thyme in response to water deficit conditions. Water stress as a very important limiting factor for plant growth and development affects both elongation and expansion growth, Shao *et al.* [15].

Leaf area was greatly influenced under water stress. Leaf area increased with the plant age in all the water levels but it decreased with the severity of stress (**Figure 2**). Leaf area increased in control plants due to more retention of water in the leaves in spite of increase in electrolyte leakage which generally increase slightly with plant age. Severe stressed plants had minimum leaf area and the control plants had maximum leaf area. Our result is similar to the findings of Barrios *et al.* [16] and Ghanbari *et al.* [17].

From the results it is clear that membrane integrity was not conserved in stressed plants and the electrolyte leakage increased with the severity of stress (**Figure 3**). Valentovic *et al.* [18] reported an increase in electrolyte leakage of sensitive maize cultivars under water stress. Electrolyte leakage was correlated to the sensitivity of plants to stressed conditions. The leakage was due to damage to cell membrane which becomes more permeable,

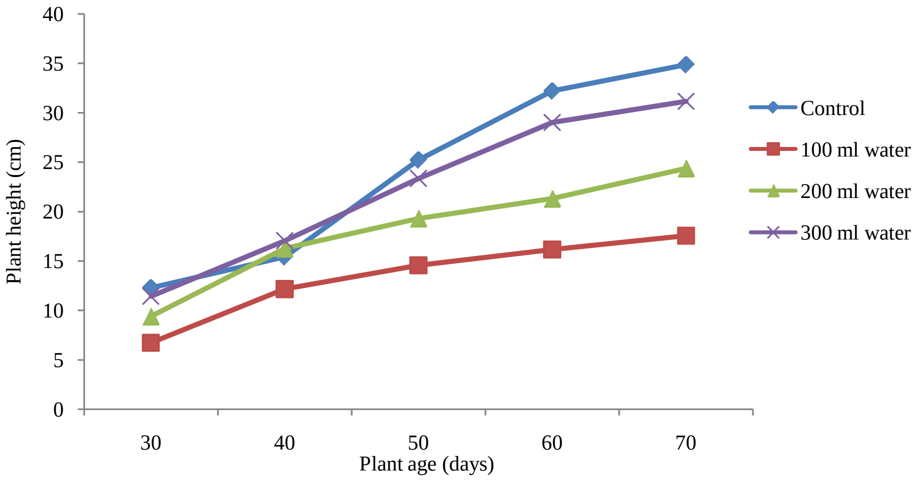


**Figure 1.** *Stevia rebaudiana*: plant height at different days of plant growth under different water regimes (LSD = 7.38).

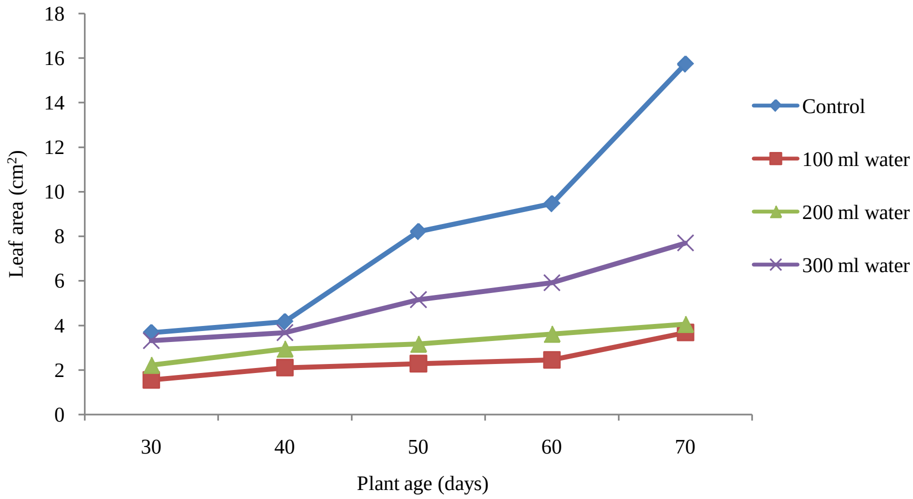


**Figure 2.** *Stevia rebaudiana*: leaf area at different days of plant growth under different water regimes (LSD = 5.153).

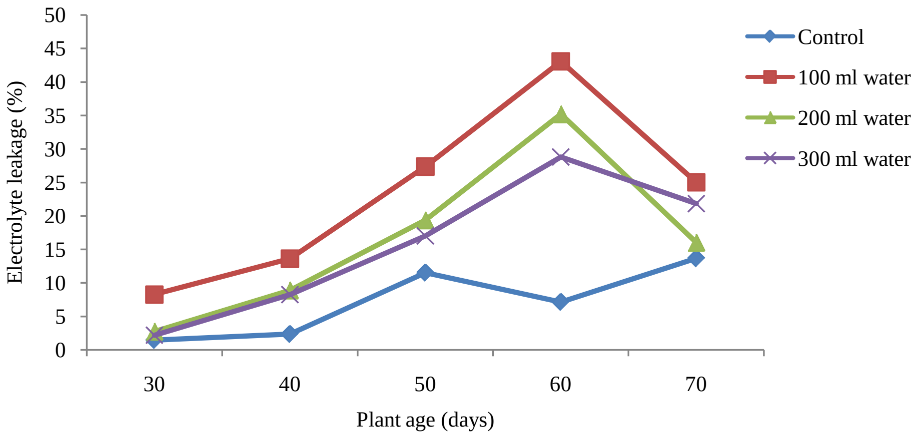


**Figure 3.** *Stevia rebaudiana*: electrolyte leakage at different days of plant growth under different water regimes (LSD = 14.09).

Senaratia and Kirsie [19]. Quan *et al.* [20] also reported high electrolyte leakage in drought stressed maize plants than in well watered plants.

There was a higher variability in enzyme activities observed in the leaves of well watered and stress plants of *Stevia*. The POD and CAT activity gradually declined in control plants where an ascending pattern was observed in stress plants with the greatest increase in moderately and mildly stressed plants (**Figure 4** and **Figure 5**) at 30 DAS, accompanied by a gradual decrease in all conditions at 40 and 50 DAS. This result was similar to the observations of Ping *et al.* [21]. The level of antioxidants and the activities of antioxidant enzymes such as POD and CAT generally increased in plants under stressed conditions and in several cases their activity correlated well with enhanced tolerance, Prasad *et al.* [22], Foyer *et al.* [23]. Mohamed and Samia [24] also observed that drought stress resulted in considerable increase in the activity of GR, SOD and APX in shoots of soybean cv. Giza 22 and 111 as compared with control plants. The antioxidant potential of *Stevia* plants was high under mild and moderate stress but in severe stress it was low due to the fact that the plants were not able to defend against the extreme conditions of water stress.

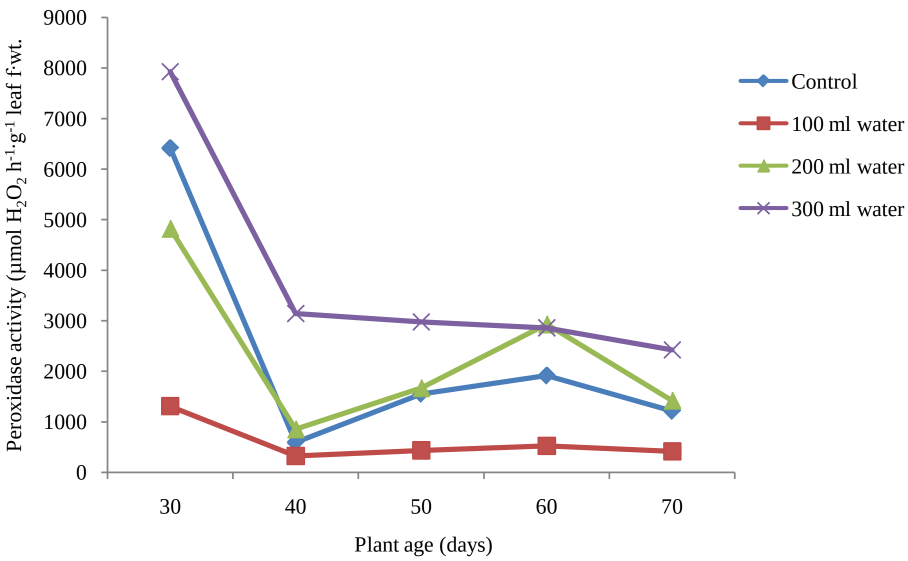


**Figure 4.** *Stevia rebaudiana*: peroxidase activity at different days of plant growth under different water regimes (LSD = 2549.65).

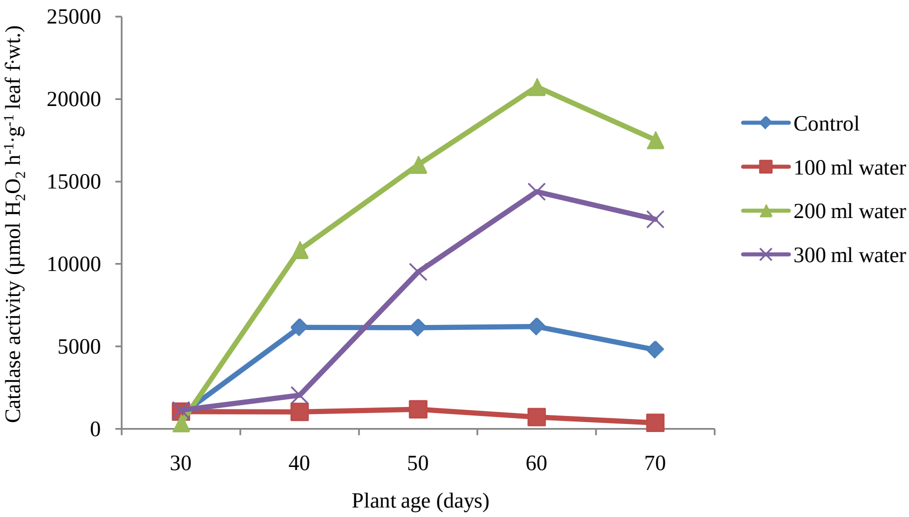


**Figure 5.** *Stevia rebaudiana*: catalase activity at different days of plant growth under different water regimes (LSD = 10309.21).

## 4. Conclusion

*Stevia* is susceptible to water stress and that results in severe cell damages and growth reduction. The antioxidative capacity is also suppressed under severe stress. The necessity of carrying out this work was concerned as there is little knowledge of *Stevia* as an agricultural crop and our further research will highlight the strategy adopted for development of drought resistant *Stevia* plants which will alleviate the future threats for proper growth of *Stevia* plants in water deficit areas.

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