AJAB
Asian J Agri & Biol. 2018;6(1):21-34.
Original Research Article

Effects of water stress on growth, yield, quality and physiological responses of two stevia (*Stevia rebaudiana* Bertoni) varieties in Rabat region, Morocco

Abderrahmane Benhmimou[1*], Mohammed Ibriz[1], Chaouki Al Faïz[2], Fatima Gaboun[2], Naima Shaimi[2], Fatima Zahra Amchra[3], Mounira Lage[2]
[1]Genetic and Biometry Lab, Faculty of Sciences, Ibn Tofail University, P.O. Box 133, 14000, Kenitra, Morocco
[2]Regional Center of Agricultural Research, P.O. Box 6356, Avenue Mohamed Belarbi Alaoui, 10101 Rabat, Morocco
[3]Laboratory of Materials, Environment and Electrochemistry, Faculty of Sciences, Ibn Tofail University, P.O. Box 133, 14000, Kenitra, Morocco

Received:
July 29, 2017
Accepted:
October 06, 2017
Published:
March 27, 2018

**Corresponding author email:*
abderrahmanebenhmimou@gmail.com

Abstract
Since no information is available about the response of stevia (*Stevia rebaudiana* Bertoni) to water stress under Moroccan conditions. This study was aimed at evaluating the effects of water stress on growth, yield, quality and physiological responses of two stevia varieties (Canada and Candy). Three water regimes were applied: R100 (100% of field capacity (FC)), R80 (80% of FC) and R50 (50% of FC) as determined by pot weight. Water stress significantly reduced stomatal conductance (Gs), photosynthetic rate (A) and transpiration rate (E) by 72.50%, 78.54% and 74.45 % at R50, respectively as compared to R100. With the continuing of water stress time, plant allocated less plant height leading to reductions of 20.27% at R50 as compared to R100. All of the above responses led to reduced dry leaf yield. By contrast, the stevioside, rebaudioside A and total steviol glycoside contents were increased in R50 stevia leaves than R100 (23.58%, 13.50% and 11.73%, respectively). The cv Candy displayed a higher dry leaf yield (34.33 g/plant) and leaf physiological responses compared to cv Canada, which experienced a great decrease. By contrast, the cv Canada recorded a higher total steviol glycoside content (19.63%) than the cv Candy (12.63%). The interaction effects indicate that cv Candy, for R100, displayed a greater dry leaf and SG yields, while these parameters were greatly reduced under R80 and R50. By contrast, the cv Canada always showed a lower dry leaf and steviol glycoside yields and a greater total steviol glycoside contents, that enable it to have a better adaptation to water stress conditions. The variation of cv Canada and cv Candy responses to water stress suggested the higher adaptation of cv Canada to water stress compared to cv Candy.

Keywords: Stevia, Water stress, Steviol glycoside, Leaf physiological responses

Introduction

Stevia (*Stevia rebaudiana* Bertoni) is a perennial plant belongs to the *Asteraceae* family, native to the Rio Monday valley, an area in north-eastern Paraguay (Karimi et al., 2015). Its leaves contain low-calorie sweetening agents called steviol glycosides (SG) that can be used as a natural alternative to artificial sweeteners (Yadav et al., 2011).Therefore, stevia has its own significance in the diet of obese and diabetic people.The SG have been approved for use as sweeteners in Japan, China, Brazil, Paraguay, Mexico, Russia, Indonesia, Korea, United States, India, Tanzania, Canada and Argentina (Pal et al., 2015). Though China is the largest stevia producer in the world market, Japan and Korea are the main consumers (Pal et al., 2015).The major SG in stevia leaf are stevioside (STV) (5–10% of dry leaf weight), which is about 300 times sweeter than sucrose (Crammer and Ikan, 2003) and rebaudioside A (Reb A) (2% - 4%), which is more suited than STV for use in foods and beverages due to its pleasant taste (Tanaka, 1997). Other SG of steviolbioside, Reb B, C, D, E, F and dulcoside A are present in smaller amounts (Abou Arab et al., 2010). Generally, the total SG content ranges from 4–20% of dry leaf weight, depending on many factors (Starratt et al., 2002) such as cultivar variations and water availability (Vasilakoglou et al., 2015; Crammer and Ikan, 2003).

Water stress is one of the main abiotic stress factors, which leads to changes in morphological, physiological, and biochemical responses of plants. Consequently, plant growth and crop production are negatively affected (Yousfi et al. 2016; Stagnari et al., 2014; Chrysargyris et al., 2016). It is also characterized by the reduction of leaf water status, which affects photosynthesis through the limitation of the efficiency of the photosystem II (PSII) activity (Fini et al., 2013). This effect depends largely on plant species and water stress severity (Aref et al., 2013).

Stevia is a drought susceptible species (Shock, 1982), it can survive in areas of continuous moisture but not withstand the prolonged water logging conditions (Donalisio et al., 1982). It has been stated that stevia growth was optimal at soil water content of 43- 47.6% (Goenadi, 1983). Midmore et al. (2012) reported that plant height, leaf biomass and stem yield, increased up to field capacity (FC), but all decreased at 120 % of FC. Under in vitro culture condition and using polyethylene glycol to stimulate drought stress on stevia, it has been reported that fresh and dry leaf weights, water content, chlorophylls were negatively affected by drought stress (Hajihashemi and Ehsanpour, 2013). In addition, some studies reported that water stress limits stevia plant height and dry leaf yield in relation to total biomass produced (Aladakatti et al., 2012; Shi and Ren, 2012; Karimi et al., 2015; Kanta et al., 2016).

The increase of secondary metabolites under water stress has been frequently reported for many plants (Stagnari et al., 2014; Ghane et al., 2012), but this response has not been sufficiently investigated in stevia. Lavini et al. (2008) and Guzman (2010) have reported that SG concentration is not responsive to plant water stress. Karimi et al. (2015) reported that soil moisture reduction up to 60% FC was not harmful to stevia metabolites, while a soil moisture level around the 45% FC represented a stressful condition for stevia, leading to quality reduction. Hajihashemi and Geuns (2016) have reported that polyethylene glycol-induced drought stress has a negative effect on the content of SG. Midmore et al. (2012) also observed an increase in SG content with 120% of FC.

Water stress also impacts physiological responses such as photosynthetic rate (A), transpiration rate (E) and stomatal conductance (Gs) of many plants (Ben Hamed et al., 2016; Mathobo et al 2017). But this effect has been less studied in stevia. It has been reported that an irrigation period at 5-days interval did not significantly influence the A and E, while the highest decrease of these traits was observed with an irrigation at 10-day-intervals (Shi and Ren, 2012). Kafle (2011) found no significant difference in A, E and Gs between the irrigation treatments. However, A, E, and Gs were 39.5%, 30.3% and 50.4% less, at 60% compared to 100% of FC respectively.

Due to the short time of stevia introduction as a new crop for Morocco, there is no information on optimal agronomic management practices and especially the influence of water availability on its establishment and production. Therefore, the present work was aimed to evaluate the growth, yield, quality and physiological responses during the growing cycle of two stevia varieties (Canada and Candy) at different irrigation regimes and to establish the extent of drought tolerance in the two stevia varieties.

Materials and Methods

Experimental site and design

The study was carried out during stevia growing period from 31 March to 01 September of 2014, in the Regional Centre of Agronomic Research of Rabat in Morocco (INRA) (34.21 N, 6.40 E, 10.5 m above mean sea-level). The experiment was performed in pots outside in an open area. Weather data were measured by an automatic weather station (iMETOS, Pessl Instruments, Austria), located near the experimental plot. The mean temperature ranged from 22.5 to 24.3 °C and relative humidity (RH) ranged from 73.1 to 86.6%.

Two stevia varieties, Canada and Candy were sown into plug trays filled with land and commercial substrate on 31 March 2014 and watered to field capacity (FC) by tap water. Seedlings were transplanted after 70 days on 09 June 2014 in 20 L plastic pots, with one seedling per pot. The weights of the empty pots were recorded, and they were filled with 2 kg of gravel at bottom for drainage and 15 kg of same soil as for study area. Before filling, the soil was collected from 0 to 30 cm soil depth and was analysed in laboratory of research of environment and conservation of natural resources INRA, RCAR-Rabat. The soil contained 5. 1% clay, 11.4% silt, and 83.5% sand. The organic matter content was 2.2%, the pH was 7.8 and the N, P and K contents were 120, 106.6 and 154 ppm, respectively. All pots were placed in open field and irrigated permanently near FC by frequent manual watering to guarantee uniform plant development. On the 48 days after transplanting (DAT), all pots were watered sufficiently up to water saturation and covered in a plastic bag to prevent water from evaporating from the soil. After 24 hours of water draining total weight of pots was measured which determinate FC of the soil.

Treatments were applied from the 49 to the 84 DAT at three water regimes, as follows: R100 (100% of FC), R80 (80% of FC) and R50 (50% of FC).Water loss was determined by weighing the pots at the interval of two days using an electronic weighing device, and then water was added to re-establish initial weight. Weighing of pots was done between 7:00-8:00 am. All pots were irrigated manually using graduated cylinder 250 ml and beaker 1L based.

There were two stevia varieties, three levels of water stress, 3 replications and 2 pots per variety per replicate of stevia plants totalling 36 experimental pots arranged according to the split plot design with a randomized complete block. Water regime treatments were assigned to main plots, and stevia varieties were assigned to the sub-plots.

The measurements

Growth parameters

Growth parameters were measured non-destructively in-situ starting one week after treatments (WAT) began application and continued up to five weeks from all stevia plants from three replicates. Data were obtained and the mean values (per plant) were computed for each treatment.

The plant height was measured with a meter ruler from ground to the base of the fully opened leaf and the stem diameter was measured with slide calipers up to 0.01 mm accuracy.

Yield parameters

At the end of the growing period (September 2014), just prior to flowering when steviol glycoside contents (SG) in the leaves is maximum (Guzman, 2010), the plants of the whole pots were harvested manually 10 cm above the base of the stem (Megeji et al., 2005) to study the effects of different water regimes and varieties on total biomass yield (total fresh leaf and stem yield), fresh leaf yield, dry leaf yield and leaf to stem ratio (fresh leaf yield/ fresh stem yield). Leaves and stem were separated from all stevia plants on the day of harvest and fresh and dry leaf yield was recorded as total biomass yield and expressed in g/plant. Leaves were dried at 50°C temperatures in hot air dryer for 6 hours. At this temperature, the quality of dried leaves produced in terms of color, sweetness and nutrient content was better compared with drying at 70°C (Samsudin and Aziz, 2013). Dry leaf yield is the economic yield of stevia used for extraction of SG (Grammer and Ikan, 2003).

Quality parameters

Dry leaves of stevia obtained during this experiment were ground in a laboratory grinding mill to produce powder particles of 0.10 mm in size and utilized for the analysis to assess the contents of stevioside (STV), rebaudioside A (Reb A) and total SG (STV; Reb, A, B, C, D and F; steviolbioside; rubudioside and dulcoside A) as influenced by water regime and variety. STV (%), Reb A (%) and total SG (%) were determined in the powdered stevia leaves sent to the STEVIA NATURA Company of France. The SG yield was calculated by multiplying dry leaves yield by the concentration of SG in leaves.

Leaf physiological responses

Photosynthetic rate (A), transpiration rate (E) and stomatal conductance (Gs) were measured weekly with a LI-COR 6400 portable photosynthesis meter (LI-COR Inc, Lincoln, NE) under natural climate on three fully expanded leaves for each combination variety and water regime. LI-COR readings were taken in the morning between 8:00-10:00 am five WAT began to avoid potential stomatal closure during the middle of the day (Liu et al., 2016).

Statistical Analysis

Data obtained were analyzed by the analysis of variance (ANOVA) using Statistical Analysis System ver. 9.1 (SAS Institute Inc., Cary, NC., USA), and means were compared using Least Significant Difference (LSD) at 0.05 level.

Results

Growth parameters

Plant height (cm): The data on plant height at the 4th and 5th week after treatments (WAT) were affected by water regime and variety. However, regardless of observation weeks, variety affect this parameter (Table 1). Plant height was significantly affected by water stress introduced at 5th WAT. Water regime R100 recorded significantly higher plant height (67.83cm) which was on par with the water regime R80 (65.67cm), while water regime R50 resulted in a reduction of plant height of 17.65% and 20.27% compared to R80 and R100, respectively. Similar trend was also observed in the 4th WAT data. Plant height clearly differ in relation to the variety. Cv Candy showed, at the end of the growing cycle, a greater length of plant (74.22 cm) compared to cv Canada (50.83 cm). The interaction effects of irrigation level and variety on this growth parameter was significant (Table 1). Cv Candy plant height decreased with increased water stress, resulting in a decrease in fresh biomass and dry leaf yield. Similarly, cv Canada plant height decreased under deficit irrigation, leading to a decrease in dry leaf yield (Table 3). However, the difference between R100 and R80 for cv Canada was not significant. Final plant height at the week 5 in cv Canada decreased by 28.61% in R50 with respect to R100 while for cv Candy the reduction was less marked (18.29%).

Table – 1: Effect of water stress and variety on plant height of stevia at 5 weeks after stress.

Water regime	Week 1	Week 2	Week 3	Week 4	Week 5
R100	31.92a	38.17a	46.08a	54.92a	67.83a
R80	30.87a	36.42a	43.00a	54.17ab	65.67a
R50	32.33a	39.17a	45.83a	50.67b	54.08b
LSD (5%)	1.52	3.18	3.58	3.85	3.80
Variety					
Canada	23.56b	28.83b	34.83b	42.39b	50.83b
Candy	39.39a	47.00a	55.11a	64.11a	74.22a
LSD (5%)	1.24	2.59	2.93	3.14	3.10
Interaction					
R100 Canada	25.00b	30.17b	37.83b	46.50b	57.67d
R100 Candy	38.83a	46.17a	54.33a	67.00a	82.00a
R80 Canada	20.83c	25.67c	31.00c	41.33c	53.67d
R80 Candy	39.50a	47.17a	55.00a	63.33a	73.67b
R50 Canada	24.83b	30.67b	35.67bc	39.33c	41.17e
R50 Candy	39.83a	47.67a	56.00a	62.00a	67.00c
LSD (5%)	2.00	4.09	4.69	5.15	5.14

* Values in each column with different letters are significantly different at P=0.05.

Stem diameter (mm)

Stem diameter data showed the same trend as plant height (Table 2). Stem diameter at 5th WAT differed significantly due to water regimes. Water regime R100 recorded significantly higher stem diameter (9.96mm) which was on par with the water regime R80 (8.86mm), and water regime R50 recorded significantly lower stem diameter (6.89mm). Similar trend was also observed in the week 4 data. The stem diameter at week 5 differed significantly due to varieties. Cv Candy resulted in higher stem diameter (10.79 mm) as compared to cv Canada (6.34 mm). Both, cv Canada and cv Candy plants grown under irrigation shortage had thinner main stem. However, the highest stem diameter provided by cv Candy when irrigated with R100 (12.60mm), as compared with that in cv Canada (7.12mm).

Table – 2: Effect of water stress and variety on stem diameter of stevia at 5 weeks after stress.

Water regime	Week 1	Week 2	Week 3	Week 4	Week 5
R100	5.69a	6.14a	6.98ab	8.58a	9.96a
R80	5.54a	6.39a	7.31a	8.23a	8.86b
R50	5.51a	6.28a	6.72b	6.86b	6.89c
LSD (5%)	0.20	0.30	0.52	0.55	0.55
Variety					
Canada	4.54b	5.05b	5.57b	5.94b	6.34b
Candy	6.62a	7.48a	8.44a	9.84a	10.79a
LSD (5%)	0.16	0.25	0.43	0.45	0.45
Interaction					
R100 Canada	4.48d	4.94cd	5.53c	6.20c	7.12d
R100 Candy	6.89a	7.34b	8.42ab	10.96a	12.60a
R80 Canada	4.29d	4.87d	5.64c	6.00c	6.28e
R80 Candy	6.79a	7.90a	8.98a	10.46a	11.64b
R50 Canada	4.84c	5.35c	5.54c	5.62c	5.64e
R50 Candy	6.19b	7.20b	7.90b	8.10b	8.14c
LSD (5%)	0.31	0.46	0.70	0.73	0.70

* Values in each column with different letters are significantly different at P=0.05.

Yield parameters

The effects of water stress, variety and their interaction on fresh biomass yield, fresh leaf yield, dry leaf yield and leaf/stem ratio are presented in Table 3. Water regime significantly affected all of the above parameters at the end of the first growing cycle 84 days after transplanting (DAT). Increasing moisture level increased fresh biomass, fresh leaf and dry leaf yields up to FC but they decreased at the lower soil moisture treatment R50. Water regime R100 resulted in greater fresh biomass (174.24 g/plant), fresh leaf yield (97.26 g/plant) and dry leaf yield (31.52 g/plant), while the lower water regime R50 significantly decreased fresh biomass, fresh leaf yield and dry leaf yield until 46.25%, 44.53% and 51.40%, respectively, compared to R100 and resulted in significant reduction in SG yield. Leaf to stem ratio differed significantly between the watering treatments (Table 3), although there was no significant difference between R80 and R50. The highest ratio was obtained at R100 and gets reduced when moisture level was decreased. The least leaf to stem ratio, which was 11.19% lower than the highest at R100, was observed at R80.

All yield parameters were significantly affected by the main effect of variety. The cv Candy provided greater fresh biomass yield, fresh leaf yield and dry leaf yield (200.02, 109.79 and 34.33 g/plant) than those provided by the cv Canada (78.99, 46.68 and 13.49 g/plant, respectively).A significant difference was also noted on leaf to stem ratio which appeared higher in cv Candy (1.42) than in cv Canada (1.26).The result of leaf to stem ratio indicated that the contribution of leaf to the overall fresh biomass yield of stevia is higher in all treatments and varieties.

The interaction effects of water stress and variety was significant on fresh biomass yield, fresh leaf yield, dry leaf yield and leaf/stem ratio. Mean comparison of interaction effects indicated that, at R100, the greatest fresh biomass yield (252.91 g/plant), fresh leaf yield (134.00g/plant), dry leaf yield (45.83g/plant) and the lowest ratio leaf/stem (1.13) were observed from cv Candy. The same trend was observed for R80 and R50 treatments, demonstrating the maximum yields of fresh biomass, fresh leaf and dry leaf for cv Candy than in cv Canada (Table3). However, cv. Candy was less tolerant to water stress, there was a sharp drop in dry leaf yield (56%of reduction in R50) , while the cv Canada showed a significantly lower decrease in dry leaf yield (40% of reduction in R50).

Table – 3: Effect of water stress and variety on yield of stevia collected at final harvest (84 DAT).

Water regime	Fresh biomass yield (g/plant)	Fresh leaf yield (g/plant)	Dry leaf yield (g/plant)	Ratio Leaf/Stem
R100	174.24a	97.26a	31.52a	1.43a
R80	150.63b	83.50b	24.88b	1.27b
R50	93.65c	53.95c	15.32c	1.32b
LSD (5%)	8.16	3.80	1.17	0.10
Variety				
Canada	78.99b	46.68b	13.49b	1.26b
Candy	200.02a	109.79a	34.33a	1.42a
LSD (5%)	6.66	3.10	0.95	0.08
Interaction				
R100 Canada	95.57d	60.52d	17.21c	1.72a
R100 Candy	252.91a	134.00a	45.83a	1.13d
R80 Canada	87.30d	50.02d	12.98d	1.34bc
R80 Candy	213.95b	116.98b	36.78b	1.21cd
R50 Canada	54.11e	29.51e	10.27d	1.20cd
R50 Candy	133.18c	78.40c	20.37c	1.43b
LSD (5%)	18.92	11.18	3.93	0.15

* Values in each column with different letters are significantly different at P=0.05.

Quality parameters

Results indicated that water stress, variety and their interaction had significant effects on steviol glycosides content and yield in most cases (Table 4). Water stress significantly enhanced steviol glycosides content and resulted in significant reduction in steviol glycosides yield. The highest values of STV, Reb A and total SG contents were obtained in stevia plant irrigated at R50 (6.15, 8.15 and 17.05 % of the leaf dry weight, respectively), while R80 and R100 showed a STV content of 5.60 and 4.70%, a Reb A content of 7.75 and 7.05% and a total SG content of 15.60 and 15.05%, respectively. The water stress caused a significant effect on SG yield (SG production per a plant leaves) and the highest values of STV, Reb A and total SG yields were observed in plants grown under R100 (1.74, 1.82, 4.36 g/plant, respectively).

The effect of variety on steviol glycosides content and yield was significant. The highest values of STV content and yield and total SG yield were 7.00%, 2.36, 4.21 g/plant, respectively and the lowest content of Reb A, total SG and Reb A yield were 3.83%, 12.63% and 1.25 g/plant, respectively, recorded in cv Candy (Table 4).

Means comparison of interaction effects indicated that STV, Reb A and total SG contents as well as for Reb A and total SG yields were significantly affected. However, no significant differences in STV yield was found for cv Canada between R100, R80 and R50 (Table 4). Irrigation levels affected total steviol glycosides yield of two stevia varieties. However, the greatest steviol glycosides yield provided by cv Candy when irrigated with R100, mainly due to greater dry leaf yield in cv Candy (45.83 g/plant), as compared with that in cv Canada (17.21g/plant).

Table – 4: Quality parameters of stevia under irrigation regime and variety.

Water regime	Stevioside (%)	Rebaudioside A (%)	Total SG (%)	Stevioside yield (g/plant)	Rebaudioside yield A (g/plant)	Total SG yield (g/plant)
R100	4.70c	7.05c	15.05c	1.74a	1.82a	4.36a
R80	5.60b	7.75b	15.60b	1.57b	1.39b	3.46b
R50	6.15a	8.15a	17.05a	1.00c	1.09c	2.48c
LSD (5%)	0.39	0.37	0.53	0.15	0.22	0.44
Variety						
Canada	3.97b	11.93a	19.63a	0.51b	1.62a	2.66b
Candy	7.00a	3.83b	12.63b	2.36a	1.25b	4.21a
LSD (5%)	0.32	0.68	0.78	0.12	0.18	0.36
Interaction						
R100 Canada	2.90e	12.10a	20.00a	0.50d	2.08a	3.44c
R100 Candy	6.50b	3.40d	11.50e	2.98a	1.56b	5.27a
R80 Canada	4.10d	12.30a	19.20b	0.53d	1.60b	2.50de
R80 Candy	7.10a	3.20d	12.00d	2.61b	1.18c	4.41b
R50 Canada	4.90c	11.40b	19.70a	0.50d	1.17c	2.03e
R50 Candy	7.40a	4.90c	14.40c	1.51c	1.00c	2.93cd
LSD (5%)	0.36	0.36	0.36	0.21	0.31	0.62

* Values in each column with different letters are significantly different at P=0.05.

Leaf physiological responses

Photosynthetic rate (µmol CO_2/m^2s)

As illustrated in Table 5, the photosynthetic rate (A) varied significantly among the three water treatments and two stevia varieties. The A at 3rd, 4th and 5th WAT differed significantly due to irrigation levels. The A increased with the advancement of crop stage in R100 and R80 while is decreased in R50. At the 5th WAT, the highest values of external CO_2 concentrations were found in R100 and R80 (20.08 and 15.83 µmol $(CO_2)/m^2s$) and the lowest value in R50 (4.31 µmol $(CO_2)/m^2s$). These results suggest that R50 during the 5th WAT can results in serious reductions of A regardless of variety tested. These reductions were 72.77 and 78.54% when compared to R80 and R100, respectively. The A varied significantly between the varieties at all five measurement weeks. The highest A of 16.27 µmol $(CO_2)/m^2s$) was produced by cv Candy while the lowest A value was 10.05 µmol $(CO_2)/m^2s$) from cv Canada at 2 WAT. The interaction effects of irrigation level and variety on this gas exchange parameter was significant in all WAT (Table 5). However, for both varieties, a significant decrease was recorded since the 3thWAT, while the high decrease of A was marked at the end of the water stress period (5th WAT). However, the greatest A provided by cv Candy when irrigated with R100 (23.73µmol $(CO_2)/m^2s$), as compared with that in cv Canada (16.43µmol $(CO_2)/m^2s$).

Table – 5: Effect of water stress and variety on stevia photosynthesis rate at 5 weeks after stress.

Water regime	Week 1	Week 2	Week 3	Week 4	Week 5
R100	13.67a	13.55a	15.47a	18.27a	20.08a
R80	12.67a	13.48ab	14.04b	14.67b	15.83b
R50	13.62a	12.45b	10.47c	6.45c	4.31c
LSD (5%)	1.08	1.08	0.82	0.96	0.99
Variety					
Canada	10.71b	10.05b	10.65b	11.20b	11.13b
Candy	15.92a	16.27a	16.00a	15.06a	15.69a
LSD (5%)	0.88	0.88	0.67	0.79	0.81
Interaction					
R100 Canada	10.43cd	10.43c	13.10b	15.67c	16.43c
R100 Candy	16.90a	16.67ab	17.83a	20.87a	23.73a
R80 Canada	10.04d	10.15c	10.93c	11.93d	13.37d
R80 Candy	15.30b	16.80a	17.14a	17.40b	18.30b
R50 Canada	11.67c	9.57c	7.91d	6.00e	3.58f
R50 Candy	15.57ab	15.33b	13.03b	6.90e	5.03e
LSD (5%)	1.38	1.37	1.03	1.24	1.31

* Values in each column with different letters are significantly different at P=0.05.

Transpiration rate (mmol H_2O / m^2s)

Transpiration rate (E) values revealed the same trend as photosynthetic rate (Table 6). The E decreased with the increase of water stress duration. Results showed that E was always higher under R100 than under R80 and R50 treatments. The high reduction of E was observed mainly in R50. Therefore, it decreased steadily until the end of water stress period (5^{th} WAT), marking the lowest values which were significantly lower than those recorded at the start of measurement (1^{st} WAT). Under R50 treatment, the E value was lower than those of R80 and R100 by 51.05% and 74.45%, respectively. Among the varieties, cv Candy showed significantly higher E values than cv Canada at all WAT. The interaction effects of irrigation level and variety on this gas exchange parameter was significant at the end of growing cycle (week 5) (Table 6). Water regimes affected E of two stevia varieties. However, the greatest E provided by cv Candy when irrigated with R100 (5.28 mmol H_2O / m^2s), as compared with that in cv Canada (3.81mmol H_2O / m^2s).

Table – 6: Effect of water stress and variety on stevia transpiration rate at 5 weeks after stress.

Water regime	Week 1	Week 2	Week 3	Week 4	Week 5
R100	3.66a	3.69a	3.79a	4.07a	4.54a
R80	3.64a	3.62b	3.32b	2.90b	2.37b
R50	3.61a	3.54c	2.34c	1.79c	1.16c
LSD (5%)	0.04	0.05	0.08	0.08	0.09
Variety					
Canada	2.91b	2.90b	2.69b	2.48b	2.41b
Candy	4.36a	4.33a	3.61a	3.36a	2.96a
LSD (5%)	0.04	0.04	0.06	0.07	0.07
Interaction					

R100 Canada	2.95c	2.98c	3.10c	3.20b	3.81b
R100 Candy	4.37a	4.40a	4.48a	4.94a	5.28a
R80 Canada	2.91cd	2.89d	2.76d	2.73d	2.36c
R80 Candy	4.38a	4.35a	3.87b	3.07c	2.37c
R50 Canada	2.87d	2.83d	2.21f	1.50f	1.07e
R50 Candy	4.31b	4.25b	2.46e	2.08e	1.24d
LSD (5%)	0.06	0.07	0.09	0.10	0.12

* Values in each column with different letters are significantly different at P=0.05.

Stomatal conductance (mol /m^{2}s)

Stomatal conductance (Gs) is closely related to the A and E. The Gs was significantly affected by water stress and variety (Table 7). The results indicated that Gs values were significantly decreased with increasing water stress duration, with the most significant reductions in R50 at 5th WAT regardless the variety tested, with decreases of 63.33% and 72.50% when compared to R80 and R100, respectively. The differences in Gs among the varieties were significant on all weeks after treatment, with the maximum values of cv Candy that are higher than those of cv Canada. The interaction effects of irrigation level and variety on this gas exchange parameter was significant at the end of growing cycle (week 5) (Table 7). Water regimes affected Gs of two stevia varieties. However, the greatest Gs provided by cv Candy when irrigated with R100 (0.44mol /m^{2}s), as compared with that in cv Canada (0.36mol /m^{2}s). In general, stevia varieties maintain lower A, Gs, and E under water stress.

Table – 7: Effect of water stress and variety on stevia stomatal conductance at 5 weeks after stress.

Water regime	Week 1	Week 2	Week 3	Week 4	Week 5
R100	0.28a	0.29a	0.31a	0.35a	0.40a
R80	0.27a	0.28a	0.28b	0.30b	0.30b
R50	0.26a	0.25b	0.21c	0.14c	0.11c
LSD (5%)	0.03	0.03	0.02	0.02	0.02
Variety					
Canada	0.19b	0.20b	0.21b	0.23b	0.24b
Candy	0.35a	0.34a	0.32a	0.30a	0.30a
LSD (5%)	0.02	0.03	0.02	0.02	0.01
Interaction					
R100 Canada	0.19c	0.19c	0.23c	0.30b	0.36b
R100 Candy	0.37a	0.38a	0.39a	0.41a	0.44a
R80 Canada	0.19c	0.21c	0.24c	0.28b	0.31c
R80 Candy	0.35ab	0.35a	0.32b	0.31b	0.30c
R50 Canada	0.19c	0.19c	0.16d	0.10d	0.06e
R50 Candy	0.33b	0.30b	0.26c	0.19c	0.16d
LSD (5%)	0.03	0.04	0.03	0.03	0.02

* Values in each column with different letters are significantly different at P=0.05.

Discussion

In our trial, stevia growth, yield and gas exchange were found to be mutually associated with the time, intensity of stress and varieties.

The growth parameters were higher with the water regime R100 (100% of FC), this might be due to more availability of water which facilitate nutrient accumulation, maintained cell turgidity and increased number of leaves which converted more solar energy and fixed more CO_2 to produce more photosynthates, and thus greater growth (Souch and Stephens, 1998). In R50 (50 % of FC), plant height and stem diameter were less. This indicates irrigation at level 50 % of FC was not enough to provide sufficient water supply for the growth of stevia plants. When the demand of water exceeds the available amount, it creates water stress which reduces plant growth through the effects on plant (reduction of cell water content, diminished leaf water potential and turgor loss), stomatal closure, transpiration rate, photosynthesis (photosynthetic activity declines because of decreased stomatal opening and the inhibition of chloroplast activity), and leaf expansion (Lisar et al., 2012). Shock (1982) reported that the stevia crop is much sensitive and susceptible to water stress during the crop growth period. The value obtained in this study of plant height is higher than the value reported by Shilpi and Malvika (2014) who measured an average plant height of 35cm in an experiment conducted in India. Karimi et al (2015) found a significant reduction in plant height from 75% FC experiment carried out at the Agricultural Biotechnology Research Institute of Iran. Lidia et al (2015) report that inside the greenhouse the stevia plants had a greater development, due the application of drip irrigation. The maximum stem diameter in this study was detected in the more irrigated treatment. Benhmimou et al. (2016) found that stevia stem diameter was 12.33 mm in well-watered. Najla et al. (2012) observed suppression of stem length and diameter due to water stress in two parsley (*Petroselinum crispum* L.) hybrids. Alavi-Samani et al. (2013) also observed reduced plant height in two species of thyme in response to water deficit conditions.

In R80 cv Canada plant height was not significantly affected as compared to R100 as the cv Canada was able to tolerate moderate water stress (R80) at the end of growing cycle. Similar results were found by Shilpi and Malvika (2014) in mild and moderate stress stevia plants the plant height was not much reduced as compared to control. In contrast, Midmore et al. (2012) have reported no significant difference in plant height between the stevia varieties.

The lower fresh biomass and dry leaf yields as a result of the reduction in water availability would be explained by inhibition of leaf growth and plant height, reduction nutrient uptake, transport and distribution (Rouphael et al., 2012) and considerable decrease photosynthesis and canopy structure (Bhatt and Rao, 2005).

A decrease in the biomass and dry leaf weight under water stress was found in some previous works in stevia plants (Vasilakoglou et al., 2015; Aladakatti et al., 2012; Shi and Ren, 2012; Kanta et al., 2016) and in various plants such as *Aloe vera* (Hazrati et al., 2017) and lavender (*L. angustifolia*) (Chrysargyris et al., 2016).

In this study, water regime R100 resulted in higher dry leaf yield which was higher than the data reported by Midmore et al. (2012) who recorded a dry leaf weight of 3.0 g/plant for the December harvest. Similarly, Shilpi and malvika (2014) obseved that stevia total biomass was highest in well watered control plants, followed by mild and moderate, while the severely stressed plants had lowest biomass. Karimi et al (2015) found that dry leaf weight showed a reduction trend to progressive soil water depletion, a significant decrease was recorded only in 45% FC treatment.

The greater dry leaf yield for cv Candy could be attributed to longer stems produced by the cv Candy (ranged from 67 to 82 cm), as compared with those of the cv Canada (ranged from 41 to 57 cm). In contrast, Vasilakoglou et al. (2015) observed that stevia cv Candy achieved lower dry leaf yield as compared with cv Morita, while Midmore et al. (2012) have reported no significant difference in total biomass yield and leaf weight between the stevia varieties.

The lack of biomass yield reduction for cv Canada in reduced irrigated treatments may be attributed to stable CO_2 accumulation in biochemical reactions of photosynthesis and to stable carbohydrates production (Tavarini et al., 2015).

The leaf to stem ratio were higher in all treatments and varieties because of the higher temperature (22.5 to 24.3 °C) and longer photo periods prevailed during the crop growth period, resulting in advanced growth with high number of leaves/plant. This had positive impact on the biomass yield and dry leaf yield. Several research revealed that temperature, length and intensity of photoperiod significantly affected stevia biomass production as evident from the remarkable increase in yield during summer cuttings than that of winter cuttings (Aladakatti, 2011). In this experiment stevia leaf/stem ratio ranged from 1.13 to 1.72 and was slightly greater than those reported by Kumar et al. (2014) and Megeji et al. (2005), who studied the productivity of three stevia accessions in India, and they found that this ratio ranged from 0.9 to 2.0 and 0.79 to 1.14, respectively. These differences could be attributed to different varieties (Ceunen and Genus, 2013).

The SG contents of stevia leaves increased under water stress. This increase in SG contents could be a parameter for adapting to water stress conditions, thus maintaining high cellular integrity in plant tissues. This would allow the plant to maintain water at the cell level as a result of an increase in the intracellular osmotic potential (Ceunen and Geuns, 2013). Similary, Karimi et al (2015) reported that the highest value of total SG content was obtained in plant irrigated at 60% FC. In contrast, Lavini et al. (2008); Aladakatti et al. (2012) have reported that SG content is not responsive to water stress.

The SG content of stevia plants also increased under the effect of varieties. The highest value was observed in cv Canada, while the lowest was recorded for the cv Candy. In contrast, Vasilakoglou et al. (2015) stated that the highest SG Contents were recorded in cv Candy leaves than the cv Morita. However, Starratt et al. (2002) analysed stevia samples from different world cultivation areas showing data of stevioside content ranging between 4 and 20% on leaf dry weight, depending on the varieties. Lavini et al. (2008) found that stevioside and rebaudioside-A contents in stevia leaves were 8.36% and 5.72%, respectively, with a significant differences among varieties. However, Yadav et al. (2011) also suggested that this trait is constutive, with a genetic variation.

The glycoside contents of the present study were higher than those reported by Karimi et al. (2015) and lower than those reported by Megeji et al. (2005) who recorded a value of 9.94% for stevioside content in the leaves. Kovylyaeva et al. (2007) reported that levels of stevioside ranging from a minimum of 4.6% in Paraguay to a maximum of 15.5% in Viet Nam, while rebaudioside A ranged from 0.3% in Canada to 3.8% in Viet Nam and Paraguay.

Our results indicated that water stress was not deterrent for cv Canada because no significant reduction in STV yield and lower reductions in SG contents were recorded between water regimes. Similary, Guzman (2010) also observed that moderate water-deficit stress did not significantly affect the SG content. Moreover, it has also been reported that stevia has modest water needs, as growing in sandy soils in native habitat, Paraguay (Madan et al., 2010). However, cv Candy SG contents shows a tendency to increase with less water availability.

Irrigation levels affected SG yield of two stevia varieties. However, the greatest SG yield provided by cv Candy achieved when irrigated with the 100% of Hcc, mainly due to greater dry leaf yield in cv Candy (45.83 g/plant), as compared with that in cv Canada (17.21g/plant). In similar, Karimi et al. (2015) reported that the soil water depletion caused a significant effect on SG yield.

The effect of water stress on photosynthetic rate (A) has been reported in stevia varieties (Guzman, 2010; Shi and Ren, 2012), in oriental lily (Zhang et al., 2011) and four tropical plants (common bean, cowpea, lablab, and maize) (Sohrawardy and Hossain, 2014). In contrast, Kafle (2011) found no significant difference in A, E and Gs in stevia plants between the irrigation treatments. However, A, E, and Gs were 39.5%, 30.3% and 50.4% less, at 60% compared to 100% of FC respectively. The present study indicated stomatal closure as one of the factors that affect the stevia photosynthetic rates during water stress (Jaleel et al., 2008). In fact, the reduction in CO_2 uptake rates in water stressed stevia reduced CO_2 diffusion through the leaf mesophyll (Flexas et al., 2012). Tang et al. (2002) argued that a combination of stomatal and non-stomatal effects on photosynthesis exists, depending on the extent of drought stress (Osakabe et al., 2014).The stomatal effect of photosynthesis, through stomatal closure, is often considered the primary physiological response followed by respective decreases in photochemical (i.e. decrease in NADPH and ATP supply) and/or biochemical (i.e. reduced RuBP regeneration and carboxylation efficiency) reactions with the increase in water stress (Kannan and Kulandaivelu, 2011). Non-stomatal effects has been assigned to the reduced ribulose-1,5-bisphosphate (RuBp) supply caused by low ATP synthesis, reduced carboxylation efficiency (Jia and Gray, 2003), and also increased the oxygenase activity of the RuBP carboxylase/oxygenase (Rubisco) (Tezera et al.,1999).The increase in photosynthesis in R100 during the five-week measurements was mainly related to increase in leaf area.

Under water stress conditions stevia plants tend to close their stomata to conserve water via reducing transpiration losses, which may limit the CO2 intake into the leaf for photosynthesis (Shi and Ren, 2012; Flexas et al., 2012). Control of the entry of CO2 and water loss is performed by continuously setting the opening of the stomata distributed across the leaves. The decrease in transpiration due to water stress is in agreement with previous reports observed in dry beans (Mathobo et al., 2017) and Madagascar periwinkle cultivars (Jaleel et al., 2008). The reduction in stomatal conductance is a consequence of stomatal closure (Chaves et al., 2003). In addition, stomatal conductance has been used in several studies to detect the effects of water deficit on the functioning of the photosynthetic system (Souza et al., 2004).

Many studies attribute stomatal closure to a single chemical substance, such as cytokinin or abscisic acid, transported from the roots in the drying zones of the soil to the leaves by means of xylem flux (Flexas and Medrano, 2002).
There was a close relation between tolerance to water stress and levels of gas exchange has been found in many species. In some cases the most tolerant plants may show greater gas exchange than others at the same water potential (Lavini et al., 2008).

Conclusion

In conclusion, our results bring to light the response of two stevia varieties to water stress conditions, based on frequent measurements of growth and physiological responses and final measurements of the stevia dry leaf biomass and steviol glycosides yield and demonstrate the biochemical and physiological mechanism involved in protection to stress conditions. Our data demonstrated that cv Candy, for R100, displayed a greater dry leaf and SG yields as well as growth and physiological responses (Gs, A and E), while these parameters were greatly reduced under the other water regimes (T80 and T50). By contrast, the cv Canada always showed a lower dry leaf and SG yields and a greater Reb A and total SG contents, that enable it to have a better adaptation to water availability in the soil. Thus, the high yield in SG may help to those suffering from obesity, diabetes mellitus, heart disease and dental caries.

References

Abou Arab EA, Abou Arab AA and Abu Salem M F, 2010. Physico-chemical assessment of natural sweeteners stevioside produced from *Stevia rebaudiana* Bertoni plant. African. J. Food Sci. 4: 269-281.

Aladakatti YR, Palled YB, Chetti MB, Halikatti SI, Alagundagi SC, Patil PL, Patil VC and Janawade AD, 2012. Effect of irrigation schedule and planting geometry on growth and yield of stevia (*Stevia rebaudiana* Bertoni.). Karnataka J. Agric. Sci. 25: 30–35.

Aladakatti YR, 2011. Response of stevia (*Stevia rebaudiana* Bertoni.) to irrigation schedule, planting geometry and nutrient levels, doctoral Thesis, Univ of Agric Sci, Dept of Agron, Dharwad.

Alavi-Samani SM, Pirbalouti AG, Kachouei MA and Hamedi B, 2013. The Influence of Reduced Irrigation on Herbage, Essential Oil Yield and Quality of *Thymus vulgaris* and *Thymus daenensis*. J. Herb. Drug. 4: 109-113.

Aref I, El Atta H, El Obeid M, Ahmed A, Khan P and Iqbal M, 2013. Effect of water stress on relative water and chlorophyll contents of *Juniperus procera* Hochst. Ex Endlicher in Saudi Arabia. Life Sci. J. 10: 681-685.

Ben Hameda S, Lefia E and Chaieb M, 2016. Physiological responses of *Pistacia vera* L. versus *Pistacia atlantica* Desf. to water stress conditions under arid bioclimate in Tunisia. Sci. Hortic. 203: 224–230.

Benhmimou A, Ibriz M, Al Faïz C, Douaik A, Zouahri A, Moussadek R and Lage M, 2016. Estimating water requirements and reference evapotranspiration of stevia in north-western Morocco. Int. J. Recent. Sci. Res. 11:14395-14400.

Bhatt RM and Rao NK, 2005. Influence of pod load on response of okra to water stress. Indian J. Plant Physiol. 10: 54–59.

Ceunen S and Geuns JM, 2013. Steviol glycosides: chemical diversity, metabolism, and function. J. Nat. Prod. 76: 1201-1228.

Chaves MM, Maroco JP and Pereira J, 2003. Understanding plant responses to drought from genes to the whole plant. Funct. Plant Biol. 30: 239–264.

Chrysargyris A, Laoutari S, Litskas VD, Stavrinides MC and Tzortzakis DN, 2016. Effects of water stress on lavender and sage biomass production, essential oil composition and biocidal properties against *Tetranychus urticae* (Koch). Sci. Hortic. 213:96–103.

Donalisio MG, Duarte FR and Souza CJ, 1982. Estevia (*Stevia rebaudiana*). Agronomico, Campinas (Brazil). 34: 65-68.

Fini A, Bellasio C, Pollastri S, Tattini M and Ferrini F, 2013. Water relations, growth, and leaf gas exchange as affected by water stress in *Jatropha curcas*. J. Arid Environ. 89: 21–29.

Flexas J, Barbour MM, Brendel O, Cabrera HM, Carriquí M, Díaz-Espejo, A, Douthe C, Dreyer E, Ferrio JP, Gago J, Gallé A, Galmés J, Kodama N, Medrano H, Niinemets Ü, Peguero-Pina JJ, Pou A, Ribas-Carbó M, Tomás M, Tosens T and Warren CR, 2012. Mesophyll diffusion conductance to

CO_2: an unappreciated central player in photosynthesis. Plant Sci. 193: 70-84.

Flexas J and Medrano H, 2002. Drought-inhibition of Photosynthesis in C3 plants:stomatal and non stomatal limitations. Ann. Bot. 89: 183–189.

Ghane SG, Lokhande VH and Nikam TD, 2012. Differential growth, physiological and biochemical responses of niger (*Guizotia abyssinica* Cass.) cultivars to water-deficit (drought) stress. Acta Physiol. Plant. 34:215–225.

Goenadi DH, 1983. Water tension and fertilization of *Stevia rebaudiana* on oxic tropudalf soil. Menara Perkebunan. 51:85-90.

Grammer B and Ikan R, 2003. Sweet glycosides from stevia plant. Chem. Br. 23:915–916.

Guzman RD, 2010. Autoecological role of steviol glycosides in *Stevia rebaudiana* Bertoni. Doctoral thesis, CQ University Australia, Rockhampton. http://hdl.cqu.edu.au/10018/913886.

Hajihashemi S and Geuns JMC, 2016. Gene transcription and steviol glycoside accumulation in *Stevia rebaudiana* under polyethylene glycol-induced drought stress in greenhouse cultivation. FEBS Openbio. 6: 937–944.

Hajihashemi S and Ehsanpour AA, 2013. Influence of exogenously applied paclobutrazol on some physiological traits and growth of *Stevia rebaudiana* under in vitro drought stress. Biologia. 68: 414–420.

Hazrati ZT, Sarvestani AM, Bidgoli SA, Sanavya MM, Mohammadi H and Nicolac S, 2017. Effects of zeolite and water stress on growth, yield and chemical compositions of *Aloe vera* L. Agric. Water Manag. 181: 66–72.

Jaleel CA, Gopi R, Sankar B, Gomathinayagam M and Panneerselvam R, 2008. Differential reponses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress. C. R. Biologies. 331: 42–47.

Jia Y and Gray VM, 2003. Interrelationships between Nitrogen Supply and Photosynthetic Parameters in *Vicia faba* L. Photosynthetica. 41:605–610.

Kafle GG, 2011. Some studies on the physiology of *Stevia rebaudiana* (Bertoni).Doctoral thesis, CQ University Australia, Rockhampton.

Kannan ND and Kulandaivelu G, 2011. Drought induced changes in physiological, biochemical and phytochemical properties of *Withania somnifera* Dun. J. Med. Plant Res. 5: 3929–3935.

Kanta C, Sharma IP and Rao PB, 2016. Influence of water deficit stress on morpho-physiological and biochemical traits of four medicinal plant species in Tarai region. Res. Environ. 9: 1391-1396.

Karimi M, Ahmadi A, Hashemi J, Abbasi A, Tavarini S, Guglielminetti L and Angelini LG, 2015. The effect of soil moisture depletion on stevia (*Stevia rebaudiana* Bertoni) grown in greenhouse conditions: Growth, steviol glycosides content, soluble sugars and total antioxidant capacity. Sci. Hortic. 183: 93–99.

Kovylyaeva GI, Bakaleinik GA, Strobykina IYu, Gubskaya VI, Sharipova RR, Al’fonsov VA, Kataev VE and Tolstikov AG, 2007. Glycosides from *Stevia rebaudiana*. Chem. Nat. Comp. 43:81-85.

Kumar R, Sharma S and Sood S, 2014. Yield components, light interception and marker compound accumulation of stevia (*Stevia rebaudiana* Bertoni) affected by planting material and plant density under western Himalayan conditions. Arch. Agron. Soil Sci. 60:1731–1744.

Lavini A, Riccardi M, Pulvento C, De Luca S, Scamosci M and d’Andria R, 2008. Yield, quality and water consumption of *Stevia rebaudiana* Bertoni grown under different irrigation regimes in Southern Italy. Ital. J. Agron. 3: 135–143.

Lisar SYS, Motafakkerazad R, Hossain MM and Rahman IMM, 2012. Water stress in plants: Causes, effects and responses. InTech.pp:1–14.

Liu EK, Mei XR, Yan CR, Gong DZ and Zhang YQ, 2016. Effects of water stress on photosynthetic characteristics, dry matter translocation and WUE in two winter wheat genotypes. Agric. Water Manag. 167:75–85.

Mathobo R, Marais D and Steyn JM, 2017. The effect of drought stress on yield, leaf gaseous exchange and chlorophyll fluorescence of dry beans (*Phaseolus vulgaris* L.). Agric. Water Manag. 180:118–125.

Megeji NW, Kumar JK, Singh V, Kaul VK and Ahuja PS, 2005. Introducing *Stevia rebaudiana*, a natural zero-calorie sweetener. Curr. Sci. 5:801-804.

Midmore DJ, Rank A, Walsh KB, Reyes R, Gautam G and HopkinsKC, 2012. Further development of the stevia natural sweetener industry. Report for RIRDC. 12/051: 55 p.

Najla S, Sanoubar R and Murshed R, 2012. Morphological and biochemical changes in two parsley varieties upon water stress. Physiol. Mol. Biol. Plants. 18:133–139.

Osakabe Y, Osakabe K, Shinozaki K and Tran LSP, 2014. Response of plants to water stress. Front. Plant Sci. 5: 86.

Pal PK, Kumar R, Guleria V, Mahajan M, Prasad M, Pathania V, Gill BS, Singh D, Chand G, Singh B, Singh RD and Ahuja PV, 2015. Crop-ecology and nutritional variability influence growth and secondary metabolites of *Stevia rebaudiana* Bertoni. BMC Plant Biol. 15:67.

Rouphael Y, Cardarelli M, Schwarz D, Franken P and Colla G, 2012. Effect of drought on nutrient uptake and assimilation in vegetable crops. Plant Responses to Drought Stress. pp:171-195.

Samsudin A and Aziz IA, 2013. Drying of stevia leaves using laboratory and pilot scale dryers. J. Trop. Agric. Fd. Sci. 41: 137–147.

Shi Y and Ren GX, 2012. The effects of drought stress on the photosynthetic parameters and dry leaf yield of *Stevia rebaudiana* Bertoni. Adv. Mater. Res. 518: 4786–4789.

Shilpi S and Malvika S, 2014. Morphological changes and antioxidant activity of *Stevia Rebaudiana* under water stress. Am. J. Plant Sci. 5: 3417-3422.

Shock CC, 1982. Experimental cultivation of Rebaudis Stevia in California. Agron. Prog. Rep. 122: 250-258.

Sohrawardy H and Hossain ML, 2014. Response of short duration tropical legumes and maize to water stress: A glasshouse study. J. Adv. Agric. 12 p.

Souch CA and Stephens W, 1998. Growth, productivity and water use in three hybrid poplar clones. Tree Physiol. 18: 829-835.

Souza RP, Machado EC, Silva JAB, Lagoa MMA and Silveira JAG, 2004. Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vigna unguiculata*) during water stress and recovery. Environ. Exp. Bot. 51: 45-56.

Stagnari F, Galieni A, Speca S and Pisante M, 2014. Water stress effects on growth, yield and quality traits of red beet. Sci. Hortic. 165: 13–22.

Starratt AN, Kirby CW, Pocs R and Brandle JE, 2002. Rebaudioside F, a diterpene glycoside from *Stevia rebaudiana*. Phytochem.. 59: 367–370.

Tanaka O, 1997. Improvement of taste of natural sweeteners. Pure Appl. Chem. 69:675–83.

Tang AC, Kawamitsu Y, Kanechi M and Boyer JS, 2002. Photosynthetic oxygen evolution at low water potential in leaf discs lacking an epidermis. Ann. Bot. 89: 861–870.

Tavarini S, Pagano I, Guidi L and Angelini LG, 2015. Impact of nitrogen supply on growth, steviol glycosides and photosynthesis in *Stevia rebaudiana* Bertoni. Plant Biosyst. 150: 953-962.

Vasilakoglou I, Kalfountzos D, Gougoulias N and Reppas C, 2015. Productivity of two stevia varieties under reduced irrigation and fertilization inputs. Arch. Agron. Soil Sci. http://www.tandfonline.com/loi/gags20.

Yadav AK, Singh S, Dhyani D and Ahuja PS, 2011. A review on the improvement of stevia [*Stevia rebaudiana* (Bertoni)]. Can. J. Plant Sci. 91: 1–27.

Yousfi S, Ma´rquez AJ, Betti M, Araus JL and Serret MD, 2016. Gene expression and physiological responses to salinity and water stress of contrasting durum wheat genotypes. J Integr. Plant Biol. 58:48–66.

Zhang YJ, Xie ZK, Wang YJ, Su PX, An LP and Gao H, 2011. Effect of water stress on leaf photosynthesis, chlorophyll content, and growth of oriental lily. Russ. J. Plant Physiol. 58:844.