

## The potential of sugarcane bagasse polymer composite for sustainable of *Stevia rebaudiana* productivity under deficit irrigation

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### Abstract

Recycling of ligno-cellulosic residues and economically viable crops production with improved water use efficiency is imperative to secure high-quality sustainable food production and implement the environmental sustainability. Therefore, the objective of this work was to evaluate superabsorbent sugarcane bagasse polymer composite (SBP) on *Stevia rebaudiana* quality and production under deficit irrigation. The experiment was conducted at Sabahia Agricultural Research Station, Alexandria, Egypt during two seasons 2017/2018 and 2018/2019 under the greenhouse conditions. Three SBP treatments (0.0, 2.0 and 4.0 g/5 kg of soils) using three irrigation levels (100%, 70% and 50% of field capacity) were applied. Data revealed that the soil amendment with SBP enhanced and gave the highest values of stevia growth parameters, chlorophyll, total soluble carbohydrate and steviol glycoside (SVglys) under both irrigation systems in both seasons. The SBP at dosage 2 and 4% levels into the soil led to an increase of dry leaves yield by 1.5 and 1.32 fold, respectively compared to yield in untreated soil under 70% field capacity. Calculated SVglys and determined by IR was high in soil amendment with 2.0% SBP (15.60%) under 100% irrigation levels and nearly closed in 70% irrigation level (15.02%). SBP application under deficit irrigation conditions increased of amount WUE and showed improvement in peroxidase isozyme system that can be used as a biomarker for characterizing drought stress tolerance. Hence, it could be recommended that SBP can be used to enhance the stevia productivity and enabled the survival of stevia plant under deficit irrigation conditions.

**Keywords:** Polymer; Composite; Peroxidase Isozyme; Steviol Glycoside

### Introduction

There is considerable interest of recycling and utilization of organic agricultural wastes to maximize their-use efficiencies with minimizes the environmental pollution and economic profitability. These organic agricultural wastes are widely available,

renewable and can be converted into useful resources through their use as an environmentally friendly super absorbent organic composite to improve nutrient status of soils, and hence conserve the environment and enhance food security. According to El Haggag and El Gowini, (2005), sugarcane (*Saccharum officinarum*) bagasse is considered the main by-products and represents nearly 30% of the sugar cane industry. It is available in large quantities in the factories but still largely under-utilized,

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it is used as fuel in Egyptian sugar mills or as fiber in fiberboard and paper manufacturing. Production natural super absorbent polymers fertilizer from lignocellulosic of sugarcane bagasse leads to a decrease the irrigation rate and nutrient percolation with increase in the yield under normal irrigation and water stress condition, and hence is more safe than chemical fertilizers, (Sarvas *et al.*, 2007, Rafiei and Nourmohammadi, 2013). The application of SAB composed as polyelectrolyte gels in agriculture increases irrigation and soil nutritional gaps by retaining water storage capacity, enhances water quality and reduces irrigation costs in arid and semi-arid soils that can be absorbed and thus saves costs and energy. The *stevia rebaudiana* herb is promising renewable medicinal plants as new natural sweetener source or as food additive in the food as well as, its medicinal benefit for diabetic and obese persons. Besides its sweetening properties, it showed antibacterial, antiseptic, anti-inflammatory, anti-fertility, hypotensive, diuretic and cardio tonic properties, (Ghaheri *et al.*, 2018). Quality and productivity of the stevia depends on the concentration of steviol glycosides in the leaves that are 200-300 times sweeter than sugar and effect by environmental conditions agronomical practices and nutritional factors, (Pal *et al.*, 2015 and Ghaheri *et al.*, 2018).

The lack of water is the most effect on agronomic characters and crop productivity in arid regions, (Elewa *et al.*, 2017 and Hassan *et al.*, 2017). As a result of drought-stressed, the plants have developed antioxidant enzymes such as, peroxidase (POD) and catalase (CAT) to protect themselves from reactive oxygen species. The peroxidase isozyme can be used as a marker for drought tolerance, (Hellal *et al.*,

2018). Peroxidase activity has a role in adjusting the osmotic cell, and its adjustment has been considered as the most important phenomenon to reduce the worst effects caused by the drought stress, (Guo *et al.*, 2018). Hajhashemi *et al.* (2012) proved that stevia needs regular irrigation and their physiological and chemical characters are affected by drought-stressed and salinity of the irrigation water as well as differences in steviol glycosides percent have been shown with irrigation criteria, (Parris *et al.*, 2016). In the coming years, demand for both stevia leaves and SV gly is expected to rise increasingly especially after EFSA Panel on Food Additives and Flavorings and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) approval a SV gly as food additive. Moreover, global climate change leads to a sustainable expansion of reclaimed areas with optimal water usage due to insufficient water availability in many parts of the world as well there is a little information on the impact of steviol glycoside of stevia under stress situation. Ligno-cellulosic of sugarcane bagasse (SBP) based eco-friendly sustainable superabsorbent composites were synthesized by our scientific group at Faculty of Science, Tanta University, Egypt (a part of project entitled: "Superabsorbent polymer composite for agricultural applications". Funded by the Science and Technology Development Fund (STDF). Project ID: 5842). The main objective of this study was to evaluate the effectiveness of a SBP to improve their physico-morphologic characters and steviol glycosides of stevia plant under deficit irrigation. Therefore, peroxidase isozyme analysis as an indicator of tolerance to water deficit were evaluated.

## Materials and Methods

***Stevia rebaudiana* Bertoni**

The planting materials are cuttings of *Stevia rebaudiana* Bert. cultivar which was obtained from the Sabahia Agricultural Research Station (SARS), Sugar Crops Research Institute (SCRI), Agricultural Research Center (ARC), Ministry of Agriculture, Egypt

***Sugarcane bagasse***

The material was provided by a local sugar factory. Bagasse was dried in sunlight, it was ground and sieved under 30 mesh sieves and stored at room temperature in air tight polybag.

***Super absorbent polymer composite (SBP)***

The superabsorbent polymers composite from sugarcane bagasse (SBP) was synthesized using filler sugarcane bagasse, beaching and grafting of acrylamide with N,N' methylene bisacrylamide as cross linker and potassium persulfate and Attapulgitic clay as initiator. The detailed method, characterization and swelling behavior of the resulted composite are previously reported by Kenawy *et al.* (2019).

***The chemical characteristics of the soil***

Soils are mixed a sandy with clay soil (2:1 w/w) and main characteristics are presented : 8.05 soil pH, 2.71 ds/m electrical conductivity soil, 6.1 meq /L Na, 0.3 meq /L K, 13.2meq /L Ca, 8.2 meq /L Mg, 3.2meq /L HCO, 6 meq /L CL and 18.6 meq /L SO<sub>4</sub>.

***Experimental procedures***

A pot experiment was carried out during two seasons 2017/2018 and 2018/2019 in the greenhouse of the Sabahia Agricultural Research Station (SARS), Alexandria, Egypt located at 31°19'N and 29°95'E. Treatments were used the three amounts of super absorbent sugarcane bagasse polymer composite including 0.0, 2.0 and 4.0 g polymer per 5 kg of soils in plot with three

irrigation regimes including irrigation at 100% of field capacity, and two deficit irrigation treatments 70 and 50% of treatment control. The pots were supplied with the chemical fertilizer doses as recommended for each crop by the Ministry of Agriculture and Land Reclamation of Egypt. The experimental design was completely randomized blocks (CRB) and the pots were left to grow under the normal growth conditions of the greenhouse (32/23°C ±2) day/night, day length 12–14 hours, relative humidity 66–73%). Each crop was harvested individually according to its maturity duration. Three cuttings per year were collected of planting. The plants were cut uniformly 10 cm above the ground level. Morphological characters (plant height, number of branches per plant, number of leaves per branch, and fresh leaf yield). The leaves were dried in an oven at 50°C and kept until further analysis.

***Chemical analysis***

Ash and chlorophyll were determined according to the procedures of A.O.A.C. (2005). Total soluble carbohydrates in stevia extract were estimated by using Anthrone reagent according to the method of Yem and Willis (1954). Total steviosides (steviol glycoside) calculated by the equation as follow  $TC = 7.56 + 0.96 TS$ . as described by Nishiyama *et al.* (1992). Dried stevia leaves in different treatment were ground on a 1 mm screen, prepared as pellets with KBr and analyzed using Perkin-Elmer system 1600 FTIR spectrophotometer in the region 650–4000 cm<sup>-1</sup> to identify of steviol glycoside, (Hearn and Subedi, 2009).

***Peroxidase isozyme analysis***

In this work an attempt was carried out to investigate the isozyme patterns of the peroxidase in order to identify biochemical marker in Biotechnology Laboratory,

Sabahia Agricultural Research Station (SARS). Leaf samples at thirteen-week-old were chosen for studying peroxidase isozymes. Electrophoresis was used to separate the peroxidase isozyme patterns in the leaves of the stevia samples according to Mesbah *et al.* (2016). Measurement of bands was carried out using the computer program software TOTALLAB V.1.11.

#### ***Effect of SBP on water use efficiency***

Water use efficiency (WUE) was expressed as the total stevia yield (kg pot) that could be produced from one cubic meter of water as the method described by Howell (2002).  $W.U.E. (g \text{ leaves/one } m^3 \text{ of water}) = \text{total stevia yield (kg pot)}/\text{water regime (m}^3/\text{pot/year)}$ .

#### ***Statistical analysis***

The data were analyzed by variances analysis (ANOVA), using the R, GLM procedure, (R Software, 2013). Means were compared by Duncan test at 5% probability level. Figures were drawn by Excel (2010) Software.

### **Results and Discussion**

Positive impacts of absorbent polymers composite on reducing the adverse effects of drought stress in crops.

#### ***Morphologic characters and yield***

The mean of yearly values of morphology characters for stevia plant under different SBP and irrigation levels at the two seasons are shown in Table 1. It was noticed that the morphology characters were decreased by decreasing the irrigation level. Drought stress led to the reduction in stem diameter, shorter internodes and stem height, (Lovisololo and Schuber, 1998). This decrease may be either due to the decrease of cell elongation which led to a decrease in each cell turgor, cell volume and, eventually, cell growth. The SBP treatment with under deficit irrigation significant ( $P \leq$

0.05) was enhanced of these characters, resulting in an increased in fresh and dry plant yield. In each cutting, plant height irrigated at 100% was significantly higher than when irrigated at 70, and 50%, but not significant effects were observed in treatment with 2 % or 4% SBP under same irrigation level (Table 1). The maximum height was obtained by 4% SBP treatment at 100% irrigation and its effects were less noticeable under deficit irrigation. Similarly, number of leaves was affected by decreasing the irrigation level that leading to a decrease in leaf yield as shown in Table 1. The SBP treatment at 2 and 4% significantly increased leaves number per branch by 19.18 % and 17.18% respectively as compared to control at 100% irrigation. When using 2% SBP under 70% and 50% deficit irrigation, the stevia leaves were increased by 47.78 and 38.89% respectively, as compared with that no treatment stevia. Increasing SBP to 4% increased the leaves by 44.45 and 62.94% under 70% and 50% deficit irrigation respectively 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<sub>2</sub> to produce more photosynthesis, and thus greater growth, (Souch and Stephens, 1998). Superabsorbent composite application in soil improved plant canopy height, (Yousefian *et al.*, 2018). This is consistent with the results by Rashad *et al.* (2020) found that the application of 1% of superabsorbent polymer with soil resulted in higher growth parameters of sweet pepper and plant capacity to positively respond to water drought conditions. However, 100% irrigation treatment with 2% SBP resulted in greater fresh leaf yield

(96.55g/plant) and dry leaf yield (21.81 g/plant), while the deficit irrigation 70 and 50% decreased fresh leaf yield 4.64 and 28.79% respectively, compared with 100% and resulted reduction in plant yield. Also, under deficit irrigation, SBP application had a positive effect on plant dry weight and increased it 1.5 and 1.32 fold when SBP was used 2 and 4%, respectively, in 70% irrigation treatments as compared with control without SBP Fig.1 (a & b). Tavarini *et al.* (2015) reported that the reduced Canada stevia yield in reduced irrigated treatments can be due to stable accumulation of CO<sub>2</sub> in biochemical photosynthesis reactions and to stable carbohydrates production. Plant dry weight showed higher values at the second cut than first cut in the same season while the plants of the second season were significantly lower than those of the same treatments of the first season and flower Fig (1-c). Results are agreement with the results by Abrisham *et al.* (2018) they stated that SAP enhanced soil and plant properties. Also, Gogo *et al.* (2020) observed that the treatment with superabsorbent polymer enhanced soil moisture, physiological and yield of pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena* L.).

#### **Quality characters**

Deficit irrigation in plants influences many biochemical and physiological processes. Chlorophyll content in plants is an important factor in determining photosynthetic capacity and depends on duration and severity of water stress and hence is affecting the metabolism of carbohydrates in plant, (Bray, 1997 and Kargar *et al.*, 2017). The data in Table (1) shows that the irrigation level had effects on the chlorophyll content, ash and total soluble carbohydrate (TSC) of stevia's leaves. The deficit irrigation at 50% FC was

caused a highly decline in both of the chlorophyll content and total soluble carbohydrate with increased of ash content in both seasons. Drought stress results in the decrease of the amount of chlorophyll, damage in the cell membrane and lack of balance between nutrient substances which results in the aging of cells of plant, (Santos *et al.*, 2009). On the other hand, treatment of soil with SBP (2 or 4%) enhanced chlorophyll content and total soluble carbohydrate under normal and deficit irrigation. There were significant ( $p < 0.05$ ) increased in chlorophyll content in plant treatment with 2% SBP with 9.15, 13.81 and 14.45 % when compared to the untreated plants under 100, 70 and 50% irrigation treatment, respectively (Table 1). Shangguan *et al.* (1999) reported that the application of SAP increased photosynthesis rate in intercellular carbon dioxide concentration and leaf water potential of plant. TSC and steviol glycoside (SVglys) values showed the same trend as chlorophyll content. Increasing water deficit from 100 to 50%, significantly decreased TSC and SVglys values during the two growing seasons. Average TSC values decrease to 22.92, 17.96 and 14.77 % in the first season and 18.68, 15.67 and 14.82 % in the second season under 100, 70 and 50% irrigation level respectively, which can be regarded as a medium level compared with ranges reported by Barbet-Massin *et al.* (2016). SBP application had a positive effect its values with significantly ( $P \leq 0.05$ ) increased as compared to control without SBP under both irrigation systems. The highest mean values of TSC contents (25.70%) were obtained from stevia plant treated with 2% SBP under 100% irrigation level in the two studied seasons as shown in Table (1). Calculated SVglys was high in soil amendment with 2% SAP (15.60%)

under 100% irrigation level and nearly closed in 70% irrigation level (15.02%). There were no significant differences in SVglys contents between 2 and 4 % SBP samples. According to Benhmimou *et al.* (2018), the water stress significantly reduced yield of steviol glycosides, which it was increased when application of biofertilizer. Similarly, Karimi *et al.* (2015) reported that the highest value of total steviol glycosides content was obtained in plant irrigated at 60% field capacity. Moreover, the means of ash content of stevia as depicted in Table 1 were range from 8.76 to 13.82%. This variation may be due to the difference in SBP and irrigation levels. However, there was non-significant variation in chlorophyll, ash, TSC and SVglys among 2 and 4% SBP doses under both the irrigation level.

#### ***Infrared spectroscopy analysis as a measure of steviol glycoside content***

The infrared (IR) spectroscopy was used to evaluate the efficiency of the SBP on steviol glycoside (SVglys) content in stevia leaves samples. The infrared absorption method is a considered as rapid, sensitive, powerful and accurate analytical methods for investigating structural, functional, and compositional changes in molecules that can be of benefit to determine the steviol glycoside value, (Martono *et al.*, 2018). IR spectra of treatment dried powder stevia leaves were presented in figure (2). IR spectra of stevia showed a strong wide and intense absorption bands at range 3500-3200 $\text{cm}^{-1}$  corresponded to the stretching vibration of the -OH stretching and was associated with the presence of hydrogen bond. Absorption at 2924  $\text{cm}^{-1}$  was characteristic of stretching-CH sp<sup>3</sup> bond. Intense peaks at 1731 and 1634  $\text{cm}^{-1}$  corresponded to C = O stretching in esters. Bending vibrational of -CH bond was

observed at 1570 and 1378  $\text{cm}^{-1}$ . C-O-C stretching in esters (1072–1254  $\text{cm}^{-1}$ ) high intense peaks at 1072 and 1031  $\text{cm}^{-1}$  corresponded to C-O derived from steviol glycoside and was characteristic absorption band of the glycosidic bond. Finally, peaks at 812  $\text{cm}^{-1}$  and 606  $\text{cm}^{-1}$  were recognized to bending vibration of = CH and = CH<sub>2</sub> bonds respectively, (Chranioti *et al.*, 2016). The spectrum displayed a wide and intense absorption at 3500-3200  $\text{cm}^{-1}$  typical corresponded to the stretching vibration of hydroxyl groups associated with the presence of hydrogen bond which can supported by concentration of SVglys. The decreasing or increasing intensities of bands in the IR spectrum in the range 3500–2855  $\text{cm}^{-1}$  can function as a measure for the efficiency of the SBP treatment. These intensities of the biomass spectra was higher in SBP samples compared to the spectrum untreated samples. These results of IR spectrum are supported by the calculated SVglys yield for the same samples. Results indicated that water stress, variety and their interaction had significant effects on steviol glycosides content.

#### ***Water use efficiency***

Results in Figure 3 showed an increase in WUE with SBP treatment under the deficiency water supply, the WUE changed slightly following the application of SBP in soil under 100% irrigation but SBP application was positive on WUE under deficit irrigation conditions (Fig. 3). The amount of WUE increased by 30.14% and 32.97 when applied 2 and 4% SBP in the soil compared to the untreated control under 70% irrigation level. The highest WUE (1.23 g/pot) was recorded with 4% SBP under 50% irrigation closely followed under 70% irrigation (1.22g/pot). The results of the second season showed that, the use 70% irrigation combined with 2 and

4% SBP gave the highest water use efficiency of 1.077 and 1.107 g/m<sup>-3</sup>, respectively (Figure 3). Similar results were obtained by Mahalleh *et al.* (2011) they reported that using SAP under water deficit treatments of corn increased water use efficiency. The superabsorbent polymers may be take up water and gradually release it back to the soil to balance the lower water reservoir and has reduced the adverse influence of deficit irrigation.

#### ***Peroxidase isozyme changes response to water stress and SBP levels***

The peroxidase isozyme analysis showed that water deficit and different SBP affected peroxidase isozyme and was observed for each parameter band existence, band volume, peak height and R.f. (Fig. 4-a) illustrates six gel electrophoretic isozyme patterns of stevia plant. The data indicated that there were nine different bands migrated towards the cathode (Fig. 4-b); while there were four different bands migrated towards the anode (Fig. 4-c) . Band existence, band volume, peak height and R.f. parameter were found to be different from all samples (Table 2). The interpretation of isozyme band pattern, samples were classified into six categories and coding as follows: (C<sub>1</sub>, C<sub>2</sub> and C<sub>3</sub>) which means control under different water irrigation (100, 70 and 50%, respectively), (T<sub>4</sub> and T<sub>5</sub> were treated stevia with 2 and 4% SBP, respectively) under 70% water irrigation and T<sub>6</sub> was treated stevia with 4% SBP under 50% of water irrigation. Samples were subjected to electrophoresis and the obtained results would be illustrated as follows: band No.1, 2, 4, 7, 8 and 9 in the cathode side were absent in control samples, while band No.3 and 6 in the cathodal side was absent in the treatment sample plants (Table 2), giving accurate evidence that differential gene expression

was achieved. Table 2 shows the band No.9 had R.f. value of about 0.953; while band No.1 had R.f. value of about 0.111 in the cathodal side. On the other hand, band No.1 and 2 in the anodal side was found in almost treated materials, while band No. 4 presented only in most control samples. Data showed that bands No.1 and 2 had R.f. values of about 0.086 and 0.305; respectively while band No.3 and 4 which had R.f. value of about 0.410 and 0.509; respectively as mention in Table 2. Weising *et al.* (1995) reported that isozymes are enzymes that convert the same chemical substrate but are not necessarily products of the same gene. Isozymes may be active at different life stages or in different cell component. Kolodziejczak and Krzakowa (2003) suggested that cathodic peroxidase system is controlled by four independent genes, of which only one is polymorphic as well as anodal bands were used to investigate the differences between bolting and non-bolting plants (Ghonema, 2005). Bai *et al.* (2006) also observed that level of antioxidants and the activities of antioxidant enzymes such as POD increased in plants under stressed conditions and in several cases their

**Table1.** Effect of sugarcane bagasse polymer (SBP) on some morphologic and quality characters of stevia plant under deficit irrigation

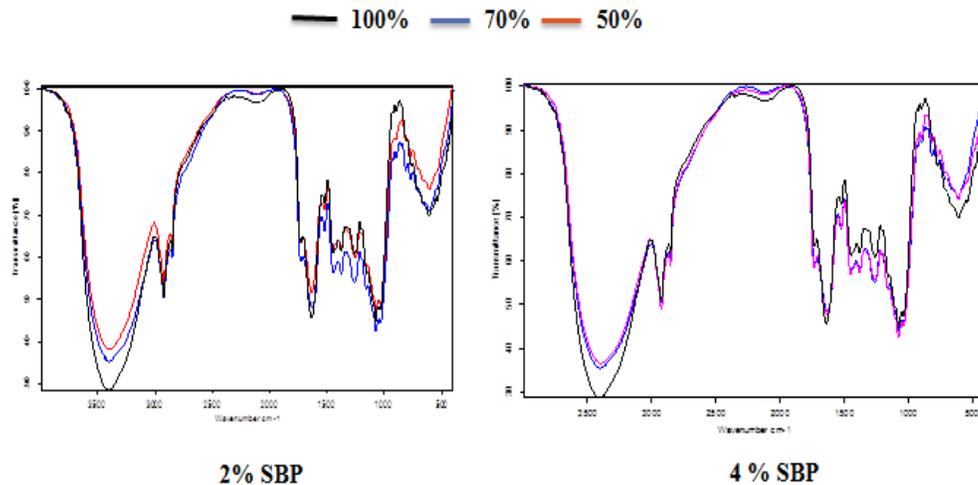
Season	Treatment		Morphologic characters					Quality characters				
	Irrigation levels	SBP	Plant height (cm)	Number of branches /plant	Number of leaves/branch	Plant fresh weight (g)	Plant dry weigh (g)	Chlorophyll %	Ash %	Total soluble carbohydrate	Steviol glycoside %	
2017/18	100	0	58.2 <sup>b</sup>	39.33 <sup>b</sup>	33 <sup>b</sup>	83.33 <sup>b</sup>	18.29 <sup>b</sup>	38.7 <sup>b</sup>	8.93 <sup>b</sup>	22.92 <sup>b</sup>	16.00 <sup>b</sup>	
		2	62.5 <sup>a</sup>	45 <sup>a</sup>	39.33 <sup>a</sup>	96.55 <sup>a</sup>	21.81 <sup>a</sup>	42.24 <sup>a</sup>	8.58 <sup>a</sup>	25.70 <sup>a</sup>	18.89 <sup>a</sup>	
		4	64.1 <sup>a</sup>	45.33 <sup>a</sup>	38.67 <sup>a</sup>	94.74 <sup>a</sup>	20.67 <sup>a</sup>	42.63 <sup>a</sup>	8.78 <sup>a</sup>	24.96 <sup>a</sup>	18.12 <sup>a</sup>	
		Mean	61.6	43.22	37	91.54	20.26	41.19	8.76	22.79	17.67	
	70	0	42.75 <sup>b</sup>	36 <sup>b</sup>	22.33 <sup>b</sup>	70.82 <sup>b</sup>	16.26 <sup>b</sup>	37.22 <sup>b</sup>	9.57 <sup>b</sup>	17.96 <sup>b</sup>	10.83 <sup>b</sup>	
		2	58.5 <sup>a</sup>	43.33 <sup>a</sup>	33 <sup>a</sup>	92.07 <sup>a</sup>	19.89 <sup>a</sup>	42.36 <sup>a</sup>	10.86 <sup>a</sup>	24.50 <sup>a</sup>	17.65 <sup>a</sup>	
		4	59.1 <sup>a</sup>	44 <sup>a</sup>	37 <sup>a</sup>	94.13 <sup>a</sup>	19.84 <sup>a</sup>	41.33 <sup>a</sup>	10.99 <sup>a</sup>	23.89 <sup>a</sup>	17.01 <sup>a</sup>	
		Mean	53.45	41.11	30.78	85.67	18.66	40.30	10.47	22.31	15.16	
	50	0	38.5 <sup>b</sup>	20.67 <sup>b</sup>	18 <sup>b</sup>	46.3 <sup>b</sup>	9.95 <sup>b</sup>	35.35 <sup>b</sup>	10.7 <sup>b</sup>	14.77 <sup>b</sup>	7.51 <sup>b</sup>	
		2	40.45 <sup>a</sup>	31.67 <sup>a</sup>	25 <sup>a</sup>	62.22 <sup>a</sup>	13.22 <sup>a</sup>	40.46 <sup>a</sup>	10.88 <sup>a</sup>	17.21 <sup>a</sup>	10.05 <sup>a</sup>	
		4	42.8 <sup>a</sup>	39.33 <sup>a</sup>	29.33	67.75 <sup>a</sup>	15.25 <sup>a</sup>	36.85 <sup>a</sup>	10.71 <sup>a</sup>	18.84 <sup>a</sup>	14.08 <sup>a</sup>	
		Mean	40.58	30.56	24.11	58.76	12.81	37.55	10.76	16.94	10.54	
			LSD mean	1.808	2.215	1.789	2.463	1.027	4.349	0.868	6.009	4.837
	2018/19	100	0	51.7 <sup>b</sup>	34 <sup>b</sup>	31.33 <sup>b</sup>	79.45 <sup>b</sup>	16.64 <sup>b</sup>	38.4 <sup>b</sup>	11.58 <sup>b</sup>	18.69 <sup>b</sup>	11.59 <sup>b</sup>
2			53.9 <sup>a</sup>	39.67 <sup>a</sup>	32 <sup>a</sup>	87.43 <sup>a</sup>	19.24 <sup>a</sup>	41.53 <sup>a</sup>	12.48 <sup>a</sup>	22.54 <sup>a</sup>	15.60 <sup>a</sup>	
4			55.3 <sup>a</sup>	42 <sup>a</sup>	33.33 <sup>a</sup>	88.55 <sup>a</sup>	17.68 <sup>a</sup>	42.19 <sup>a</sup>	12.62 <sup>a</sup>	21.72 <sup>a</sup>	14.75 <sup>a</sup>	
Mean			53.63	38.56	32.22	85.14	17.85	41.04	12.22	20.65	13.98	
70		0	40.6 <sup>b</sup>	28 <sup>b</sup>	21 <sup>b</sup>	60.68 <sup>b</sup>	13.8 <sup>b</sup>	37.77 <sup>b</sup>	12.52 <sup>b</sup>	15.67 <sup>b</sup>	8.45 <sup>b</sup>	
		2	53.2 <sup>a</sup>	37 <sup>a</sup>	29 <sup>a</sup>	82.9 <sup>a</sup>	17.16 <sup>a</sup>	41.86 <sup>a</sup>	13.97 <sup>a</sup>	21.98 <sup>a</sup>	15.02 <sup>a</sup>	
		4	51.9 <sup>a</sup>	40.33 <sup>a</sup>	32 <sup>a</sup>	85.31 <sup>a</sup>	17.22 <sup>a</sup>	41.9 <sup>a</sup>	13.62 <sup>a</sup>	22.09 <sup>a</sup>	15.13 <sup>a</sup>	
		Mean	48.57	35.11	27.33	76.29	16.06	40.84	13.37	19.91	12.87	
50		0	26 <sup>b</sup>	19 <sup>b</sup>	16.67 <sup>b</sup>	43.58 <sup>b</sup>	9.74 <sup>b</sup>	36.04 <sup>b</sup>	14.97 <sup>b</sup>	14.82 <sup>b</sup>	7.56 <sup>b</sup>	
		2	33.6 <sup>a</sup>	31.67 <sup>a</sup>	21.33 <sup>a</sup>	57.07 <sup>a</sup>	11.88 <sup>a</sup>	42.25 <sup>a</sup>	13.48 <sup>a</sup>	16.55 <sup>a</sup>	9.36 <sup>a</sup>	
		4	37.5 <sup>a</sup>	34.33 <sup>a</sup>	22.67 <sup>a</sup>	59.28 <sup>a</sup>	12.57 <sup>a</sup>	36.83 <sup>a</sup>	13.01 <sup>a</sup>	16.18 <sup>a</sup>	8.98 <sup>a</sup>	
		Mean	32.37	28.33	20.22	53.31	11.39	38.7	13.82	15.85	8.63	
			LSD mean	2.173	1.489	2.478	3.026	3.711	4.439	1.588	4.711	4.393

**Table 2.** Analysis of electrophoretic data obtained from six leaf samples control and treated of stevia plant for cathodal and anodal band.

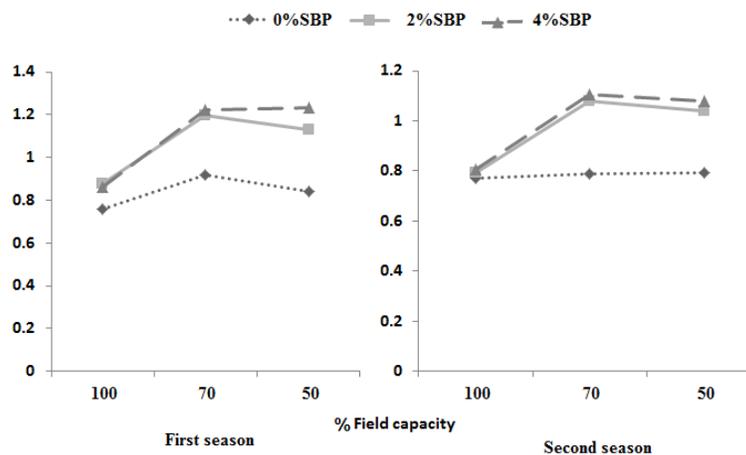
Sample	Band 1 (-)			Band 2 (-)			Band 3 (-)			Band 4 (-)			Band 5 (-)			Band 6 (-)			Band 7 (-)			Band 8 (-)			Band 9 (-)			Band 1 (+)			Band 2 (+)			Band 3 (+)			Band 4 (+)		
	Vol.	Peak height.	R.f	Vol.	Peak height	R.f.	Vol.	Peak height.	R.f.	Vol.	Peak height.	R.f.	Vol.	Peak height	R.f.	Vol.	Peak height	R.f.	Vol.	Peak height	R.f.																		
C1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	51.8	30.35	0.680	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	35.2	6.55	0.548
C2	-	-	-	-	-	-	-	-	-	-	-	38.67	14.4	0.502	127.	62.39	0.706	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	31.2	3.15	0.509
C3	-	-	-	-	-	284.4	65.6	0.32	-	-	-	-	-	35.9	16.86	0.716	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	101.3	3.26	0.410	-	-	-	
T4	-	-	-	-	-	-	-	-	226.1	43.1	0.361	-	-	-	-	-	43.7	13.81	0.763	-	-	-	-	-	-	-	-	-	-	-	-	175.4	3.71	0.423	-	-	-		
T5	52.2	19.7	0.111	-	-	-	-	-	-	-	-	448.5	80.2	0.475	-	-	-	-	-	85.2	29.60	0.916	-	-	-	20.1	0.00	0.123	56.6	1.29	0.341	-	-	-	-	-	-		
T6	-	-	-	55.4	15.3	0.175	-	-	-	-	-	174.0	38.7	0.468	-	-	-	-	-	-	-	-	43.8	26.8	0.953	19.0	0.00	0.086	64.4	9.51	0.305	-	-	-	-	-	-		



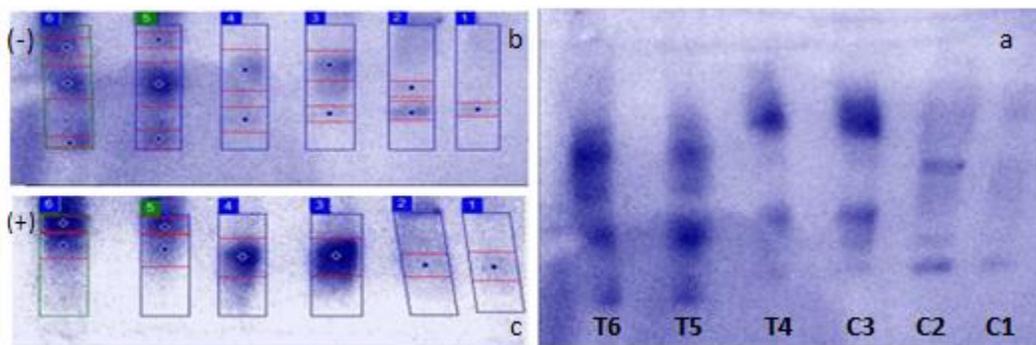
**Figure 1.** The effect of the superabsorbent polymer composite on the morphological characters of *Stevia rebaudiana* Bert under 70% water level 2018/19 season after 90 day (a) first cut (b) second cut in first season (c) second cut in second season.



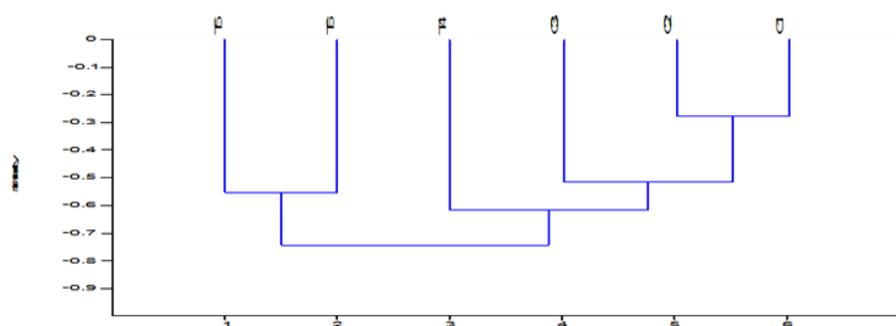
**Figure 2.** IR spectrum of stevia leaves at 4months planting in soil amendment with SBP under different irrigation levels.



**Figure 3.** Effect of deficit irrigation on water use efficiency on stevia grown in soil amendment with SBP during two seasons.



**Figure 4.** Isozyme patterns (a), and their analysis of electrophoretic cathodal peroxidase bands (b) & anodal bands (c) obtained from the six leaf samples control and treated of stevia plant.



**Figure 5.** Dendrogram of cluster analysis for stevia variety based on (0 and 1) data. Whereas: (C): Control – (T): Treatment.

activity correlated well with enhanced tolerance. Helmy *et al.* (2016) investigate antioxidant activity of *Stevia rebaudiana* under salt stress and the ability of tolerance of stevia plants to salt stress. Antioxidant enzyme activity (peroxidase) was assayed during the different cutting times. There is an increase in antioxidant enzyme activity under the high level of salt compared to untreated control plants. Figure 5 shows dendrogram of cluster analysis based on (0 and 1) data employing the figure shows that there were two main different clusters in the dendrogram tree.

The analysis was capable to classify the studied six plant leaf samples into two clusters, cluster No.1 contained two plant samples (T<sub>5</sub> and T<sub>6</sub>) these samples belong to the treated plants in stevia variety. Cluster No. 2 contained four plant samples (T<sub>4</sub>, C<sub>3</sub>, C<sub>2</sub>, and C<sub>1</sub>), this cluster contain all three-control plant sample in a small branch together and treatment sample number four on the other side which close to the other two treatments. Abdelsalam *et al.* (2020) studied the activities of antioxidant enzymes intensively and the results provide strong similarities between the Acacia species were examined, and the obtained data were subjected to cluster analysis.

### Conclusion

The superabsorbent polymers composite from sugarcane bagasse (SBP) was improved of stevia productivity under deficit irrigation conditions, and it showed enhance in peroxidase isozyme system which can be used as a biomarker for characterizing drought stress tolerance.

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