Morphological Response of Stevia *Rebaudiana Bertoni* to Organic Fertilizer and Proline

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Abstract. A field experiment was carried out in the wooden canopy - Department of Horticulture and Gardening Engineering - College of Agriculture - University of Anbar to testing the response of *Stevia rebaudiana bertoni* to spraying Organic fertilizer and Proline, three levels of Organic fertilizer (0mg, 50mg and 100mg) used, as well as three levels of Proline used (0mg, 100mg and 200mg). The results showed that the treatments sprayed with the Organic fertilizer (100mg) were significantly superior with the highest ratios of plant height, number of leaves per plant and leaf thickness, recorded (55.20cm) (729 leaf) (0.7370 mm), respectively. Whereas, the best ratios of the above characteristics were recorded when spraying with Proline, (87.04cm) (737 leaf) (0.7620 mm) respectively. The highest rate of plant height, the number of leaves per plant, and the thickness of the leaf were recorded when the interaction between the experiment factors, where the highest rate was recorded at the interaction (100 mg Organic fertilizer and 200 mg Proline) the highest ratios were recorded (97.50cm) (789 leaf) (0.7740 mm), respectively.

1. Introduction

*Stevia rebaudiana* Bertoni, Stevia is known to have many benefits for diabetics, as it helps reduce calories, maintains blood sugar levels, and contains vitamins and antioxidants, but researchers warn against overloading it. The use of stevia leaves is not new, as South Americans discovered it hundreds of years ago. stevia has many benefits, especially for diabetics. Eating them does not affect the level of sugar in the blood, because the particles of stevia are not absorbed into the blood such as glucose. The stevia plant also contains a large amount of antioxidant compounds, such as flavonoids and terpenes, and is rich in protein, iron, potassium, magnesium, sodium, and vitamins A and C.[1]. It should be noted that most of the studies were on extracts of the stevia herb and not on the leaves themselves. Because, as previously mentioned, the US Food and Drug Administration does not classify the use of the leaves and raw extracts of the stevia herb as being generally safe (in English: Generally Recognized as Safe) or what is known as GRAS, a regulation of the US Food and Drug Administration in which experts classify the substances. Since the leaves and raw extracts of the stevia plant do not follow this classification, it is not permitted to market them with the aim of using them as natural sweetening products.[2], [3]. Amino acids are present in large quantities in the organism and are built in mitochondria and chloroplasts to provide ketone acids resulting from the representation of carbohydrates formed by the process of photosynthesis (the Krebs cycle) and are formed as a result of the interaction of ammonia with ketogenic acids.

To organic vegetable or microbial nitrogen, the reaction of + or NH4 - metallic nitrogen (NO3, acid glutaric-keto-α, is transformed with ammonia forming acid glutamic acid in the
presence of the enzyme Nicotinamide adenine (NAD) and the compound NADP (Nicotinamide adenine phosphate_dinucleotide) [4]. The two amino acids, proline and L-arginine, play an important role in many biological processes either in its presence in a free form or one of the components of proteins, therefore, its importance and effectiveness in all stages of growth plants, including their role in reducing the effect of drought and salinity stresses through their different physiological activity, by changing the osmotic stress of the plant tissue. [5]. The increase in amino acids leads to a decrease in the osmotic voltage and in turn reduces the water stress of the cell, thus increasing the ability of the cell to withdraw water and nutrients dissolved in it from the growth medium and then increase the vegetative growth of plants. [6]. Free amino acids, when added, are an essential nitrogen source in building proteins and enzymes and preparing energy that encourages vegetative and root growth.[7]. The objective of this study is testing the response Stevia rebaudiana bertoni to spraying Organic fertilizer and nano Proline,

2. Materials and Method

2.1 Location of the experiment:
Thus experiment was carried out under the wooden canopy for the Department of gardening and landscaping in the Faculty of Agriculture -University of Anbar- it has been planted during the period (10/1/2019) until (1/9/2019). To study to assessing the response of stevia rebaudinna Bertoni for nanoparticle enrichment and nanoproline.

2.2 Experimental Design:
The experiment was carried out, according to the design of Randomized Complete Block Design (RCBD) within three replicates for vegetative and chemical properties.
The experiment included two factors:
The first factor: enriched organic bio nanoparticles and included three treatments.
F0 = without adding
F1 = Add 50 milligram
F2 = Add 100 milligram
The second factor: the addition of nanoproline and includes three additions.
P0 = without adding
P1 = Add 100 milligram
P2 = Add 200 milligram
Each experimental unit contained three plants

2.3 Preparing of the Pots:
Plastic Pots of 30 * 30 dimensions of 15 kg were used. The soil was prepared using an agricultural medium consisting of a mixture of soil, organic matter and peatmoss at a mixing ratio of 1: 1: 2 sequentially and organic and chemical fertilizers were added as needed. A drip irrigation system was used for plant watering [9].

2.4 The planting of Stevea:
After the plants were obtained from Palm Paradise Company for Textile Agriculture was equipped plastic potting soil prepared for planting and was treated with fungicide to prevent fungal infection and was planted inside the greenhouse protected for two months from the date (10/3/2019) and that To preserve plants due to low temperatures during this period. Then it was transferred to the wooden canopy on (2019/4/14) where the service of the plant was carried out hoeing and ventilation and follow-up along the experiment season.

2.5 Preparation of Bio-Fertilizer
Plants were sprayed with nano fertilizer containing 30% organic matter, 3% organic nitrogen, 5% mineral nitrogen, and made compost according to the Malaysian origin nanotechnology. A surface analysis examination of the nano fertilizer was carried out with the AFM, To know
the average granular volume and the surface roughness root mean square (Fig. 1, 2, and 3). As for the comparison treatment, it was fertilized with compound fertilizer (N:P:K 48-24-36) according to the recommendation that was mentioned in [10] with three batches. Note that all the treatments included in the experiment were fertilized with the first batch of compound fertilizer in the stage of the eruption to stimulate the plants to grow, and the nanofertilizing was sprayed in two stages, the first stage after the arrival of the plant 10 cm and the second at the stage of 100% female flowering, then the process of spraying the organic fertilizer in Early morning with a back sprinkler with a capacity of 15 liters, after calculating the appropriate size till the experimental unit is completely wetted.

2.6 Method:
Plants were sprayed in the early morning until the full weight of leaves after 20 days from planting. It was added 1 cm³ spreading agents to the final solution before spraying to increase the space to spread out. It has used the manual sprayer 2-liter capacity for the completion of the spraying process. The spraying process in the early morning and even get fully wet of plants while it was sprayed a distilled water to the comparison treatments, it was used sheets of polyethylene as a barrier to avoid spray mist between treatments. Nano fertilizer and Proline preparation to spray were as mentioned previously.

2.7 Characteristics of the study:
A. Plant Height (cm):
Measure the length of the plant by measuring tape from the soil surface to the top of the plant after the completion of spraying treatments for the total vegetation of each of the plants of the selected experimental unit and divided by the number of measured plants.
B. Leaves number per plant:
The total leaves of each plant were calculated from the first green leaf near the soil surface to the top leaf.
C. Leaf thickness (mm)

3. Results and Discussion
3.1. Plant height (cm):
Results in table 1 indicated the apparent excellence of the treatments sprayed with the Organic fertilizer (ppm) compared with the treatments that weren’t sprayed with the Nano fertilizer, where the highest rate of plant height was recorded when plants sprayed with the highest percentage of fertilizer (55.20cm) with a significant decrease from other treatments reached to (48.50cm), while the lowest rate of plant height (45.30cm) was recorded at the plants that weren’t sprayed with the Organic fertilizer (ppm) along the growing season, where recorded a significant decrease from the other study treatments. As for the Proline, a clear response to plant height was recorded, where the highest rate of plant height was recorded in the plants that were sprayed with the highest rate of Proline (87.04cm) where reached (64.50cm) with a significant increase to other treatments. The lowest plant height recorded at the comparison treatments reached (45.30cm).

The interaction between the two factors of the experiment led to a significant impact on the plant height, where the highest rate of plant height was recorded at the combination (Organic fertilizer (ppm) and Proline) with a plant height was (97.50cm), while the lowest rate of plant height was recorded in plants that weren’t sprayed with Organic fertilizer (ppm) and the Proline, where the plant height reached (45.30cm) with a significantly reduced compared with the other treatments.
Table (1) Effect of Organic fertilizer (ppm) and nano Proline (ppm) on the plant height (cm)

| Organic fertilizer concentrations (ppm) | Proline concentrations (ppm) | Mean  |
|----------------------------------------|-------------------------------|-------|
|                                        | 0                             | 100   | 200   |       |
| 0                                      | 45.30                         | 64.50 | 87.04 | 65.73 |
| 50                                     | 48.50                         | 71.40 | 88.50 | 69.47 |
| 100                                    | 55.20                         | 81.40 | 97.50 | 78.03 |

LSD (0.05) Nano-fertilizers = 3.764  
Proline = 3.764  
Organic fertilizer × Proline = 6.519

3.2 Number of leaves per plant.
The results shown in a table 2 showed the Excellence of the transactions that were sprayed at the high level of Organic fertilizer (ppm) (729 leaf) with the highest rate of Number of leaves per plant with a significant Excellence compared to other treatments. Whereas, the lower average of Leaves number per plant was recorded at the treatments that weren't sprayed the Organic fertilizer (702 leaf) (comparison), while the lowest rate recorded in the plants that wasn't sprayed with Organic fertilizer where reached (695 leaf) with a significant decrease compared with other treatments.
The results shown in table 2 showed the excellence of the treatments that were sprayed with the highest rate of Proline (200 ppm), where it gave a rate of Number of leaves per plant reached (765 leaf) with a significant difference from the other treatments. Lowest rate of Number of leaves per plant was recorded at treatments that weren't sprayed with Proline (the comparison), where it gave a rate of number of leaves per plant (695 leaf) with a significant decrease compared with the other treatments. As for the interaction between the Nano-Bio-Enriched and the Proline, the highest rate of number of leaves per plant was recorded at the combination (789 leaf) which gave the highest rate of number of leaves per plant reached (695 leaf) while lowest rate of Leaves number per plant was recorded at the treatments that weren't sprayed with the Organic fertilizer and the Proline (comparison), with a significant decrease compared to other combinations.

Table (2) Effect of Organic fertilizer (ppm) and Proline (ppm) on the number of leaves per plant

| Organic fertilizer concentrations (ppm) | Proline concentrations (ppm) | Mean  |
|----------------------------------------|-------------------------------|-------|
|                                        | 0                             | 100   | 200   |       |
| 0                                      | 695                           | 765   | 737   | 732   |
| 50                                     | 702                           | 755   | 765   | 740   |
| 100                                    | 729                           | 767   | 789   | 761   |

LSD (0.05) Nano-fertilizers = 6.31  
Proline = 6.31  
Organic fertilizer × Proline = 10.93

3.3 Leaf thickness (mm).
The results shown in table 3 showed the excellence of the transactions that sprayed at the high level of enriched (100mg) with the highest rate of the Leaf thickness with a significant
Excellence compared to other treatments. Whereas, the lower rate of the Leaf thickness was recorded at the treatments that weren’t sprayed with the Organic fertilizer (0.330mm) (comparison), while the lowest rate recorded in the plants that wasn’t sprayed with Organic fertilizer where reached (0.210mm) with a significant decrease compared with other treatments. The results of the study showed the Excellence of the coefficients that were sprayed with Proline at the highest rate (0.640mm) of the Leaf thickness and significantly higher than the comparison treatment reached (0.620mm) and recorded the lowest rate of the Leaf thickness the lowest rate of reached (0.210mm) with a significant increase when compared on the other treatments.

In the case of interference between the factors of the experiment, the highest rate of the Leaf thickness recorded in the plants that sprayed (0.740mm), while the lowest rate recorded in e plants that weren't sprayed with any of the factors of the experiment that gave the rate the Leaf thickness reached (0.210mm).

Table (3) Effect of Organic fertilizer (ppm) and Proline (ppm) on the Leaf thickness (mm)

| Organic fertilizer concentrations (ppm) | Proline concentrations (mg) | Mean |
|----------------------------------------|-----------------------------|------|
| 0                                      | 0.210                       | 0.490|
| 50                                     | 0.330                       | 0.486|
| 100                                    | 0.370                       | 0.563|

LSD (0.05) Nano-fertilizers = 0.0344
Proline = 0.0344
Organic fertilizer × Proline = 0.0597

4. Conclusion:
It was concluded that proline and Organic fertilizer (ppm) has a significant and clear effect on all plant vital activities, including the traits that were previously measured in the research. Therefore, we recommend that it be widely used of proline and Organic fertilizer (ppm) on plants in order to obtain good results.

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