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EFFECT OF PROLINE TREATMENT ON THE TRAITS OF VEGETATIVE GROWTH AND FLOWERING OF TOMATO PLANT

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Abstract

The Complete Randomized Design method for the simple experiments was used to variable analysis of the calculated properties by the statistical program SPSS. The significant difference (RLSD) at 0.05 probabilities was used to compare the treatment means of the results. The results were focused on exposed tomato plant that delivered from seeds named Aya, to *S*-proline amino acid, which have traditionally considered as precursor to constituent of protein and play an important role in plant metabolism and growth. A positive correlation between *S*-proline concentration and the vegetative growth of tomato plant was observed. A variety differences in *S*-proline interactions between variety treatments were achieved. The effect of treatment with different concentrations of *S*-proline makes the date of the appearance of the first inflorescence earlier. Owing to, increase the number of the inflorescences and the number of the total flowers in high concentration. Good relation was envisioned between the *S*-proline content in leaves and in the roots with same level in each side of the plant. Further, the total protein content and the total chlorophyll content in leaves were increased. These results confirm that the *S*-proline was a necessary source of nitrogen that effects the formation of chlorophyll due to increase the life time of plant. Also the level of the free *S*-proline in leaves and roots were increased after treatment, which explain the ability of proline in osmoregulation in the cells of the plant. Theoretical studies were showed, that the rotation of atoms about the single bonds in the *S*-proline, which is the subject of the conformational analysis can affect the internal coordinate (ICM) of the amino acid molecule, due to affect their molecular mechanics (MM2) properties which can affect their structure, activity and their action in the plant.

***Key words*:** Vegetative growth, *S*-proline, Internal coordinate mechanics, Conformational analysis, Molecular mechanics, Inflorescence.

Introduction

The plants are subjected to various types of environmental stresses which include salinity, water deficit, temperature extremes, toxic metal ion concentration and UV radiation throughout their life cycle (Hayat *et al.*, 2012). These environmental factors limit the growth and productivity of plants to varying degrees, depending upon severity of stress. A large body of data suggests a positive correlation between proline accumulation and plant stress. Proline, an amino acid, plays a highly beneficial role in plants exposed to various stress conditions (Hayat *et al.*, 2012). Besides acting as an excellent osmolyte, proline plays three major roles during stress, *i.e.*, as a metal chelator, an antioxidative defense molecule and a signaling molecule. Review of the literature indicates that a stressful environment results in an overproduction of proline in plants which in turn imparts stress tolerance by maintaining cell turgor or osmotic balance, stabilizing membranes thereby preventing electrolyte leakage and bringing concentrations of reactive oxygen species (ROS) within normal ranges, thus preventing oxidative burst in plants. Proline accumulation may also be part of the stress signal influencing adaptive responses (Mafakheri *et al.*, 2010). Furthermore, the proline as reported have a role in osmoprotectant during osmotic stress, osmoregulation under drought and salinity stresses might cause stabilization of protein, prevention of heat denaturation of enzymes and conservation of nitrogen and energy for a post-stress period. Proline content was reported to be the highest in leaves and in tomato hybird Calli root (Mohammad *et al.*, 2017). Tomatoes like *Lycopersicon esculentum* Mill (Fariba *et al.*, 2005) and *Solanum lycopersicum* L., are important, popular and nutritious vegetable crop all over

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the world. Tomato is the top source of vitamin A and C in human diet and contains high content of antioxidant compounds which offer a number of benefits to human health. It is also plays a vital role in providing a substantial amount of dietary fiber, lycopene, β-carotene, phenolic compounds, iron, magnesium, niacin, potassium, phosphorus, riboflavin and thiamine, which prevents oxidative changes in the human body (Mohammad *et al.*, 2017, Fariba *et al.*, 2005).

Materials and Methods

This study was focused on tomato plant that delivered from seeds named Aya. The later was cultured on plastic anvils laying, which prepared from mix of 1:3 natural to artificial soil (Peat moss). The best seeds that were homogeneous in size and shape were selected and soaked with distilled water for one day period, then were planted in equal depths (0.5 cm) for all seeds. The resulting plants were sprayed with *S*-proline in (0, 50, 100 and 150 mg/L^2) concentrations using Tween-20 to reduce the surface tension. This step was repeated for three times with leaving two weeks in between each replicate. Add to which, the following studies and tests were achieved after 90 dyes of treatment.

Characteristics of vegetative growth

The plants height : The plants height was measured from the place of contact with the soil to the top growing in rate of two plants per pot or by three replicates per treatments per cm.

The diameters of stem: The diameters of stem were measured using Verneirs caliper from the place of contact the fifth real leaf with a modified leg for two plants of each pot per cm.

The number of leaves in the plant: The total number of the whole leaves, that grown on the main stem was calculated at the rate of two plants per pot.

The leaves area: Each leaf was cut off (tomato plant in each pot) from their main contact with the stem. Then, each leaf was put on the paper and shaped and then labeled using a pencil, followed by cut the labeled paper with area equal to 5*5 cm. Further, the weight was taken using accurate balance. Therefore, the leaf's area of each plant was calculated by the relation between the weight of paper and the weight of area unit (the resulting data was in cm^2/ plant).

The fresh weight of the vegetative total: The fresh weight of the vegetative total was measured using accurate balance.

The dry weight of the vegetative total: The vegetative part of the plant was dried using oven at 75°C for 48 hours and the dry weight of the vegetative total was measured also by using the accurate balance.

Characteristics of inflorescence growth

The date of the appearance of the first inflorescence: The number of days of seed cultivation to date has been calculated for all treatments.

The number of the inflorescences: The number of days of seed cultivation was calculated until the first inflorescence of the plant was formed, this process was achieved for all treatments.

The number of the total flowers: The numbers of the total inflorescences for each pant were calculated during its growth period for each experimental unit.

The ratio of the contract for the first inflorescence: The percentage of ripe fruits was estimated from calculating the number the ripe flowers in the first inflorescence according to the following equation:

$$\text{Ripe fruits\%} = \frac{No. of\ the\ ripe\ flowers}{No. of\ the\ total\ open\ flowers} \quad (1)$$

The physiological characteristics of plants

The total chlorophyll content in leaves: The total chlorophyll content in leaves was realized by using a method same to that designated by Porra (Porra *et al*, 2002) as following:

• The leaves of the tomato plant (0.5 g) were dissolved by adding 15cm^3 of acetone (80%) and then were mashed very well. This step was replicated for three times for each treatment.

• The absorption of the resulting symbol was obtained at λ (645) nm and λ (665) nm using the spectrophotometer. Then, the total chlorophyll content in leaves were calculated *via* the following equation:

The total chlorophyll (mg/mg^{-1}) = 20.2*photo-density in λ (645+8.02) nm* photo-density in λ (665) nm (2)

By the changing of the values (mg/mg^{-1}) in equation (2) to the values (mg/100 mg^{-1}) by equation (3):

$$\left(\frac{\text{mg}}{100\ \text{g}^{-1}}\right) = \frac{\frac{mg}{L^{-1}}}{100\,cm^3} + \frac{100}{The\ weight\ of\ symbol\,(g)} \quad (3)$$

The *S*-proline content in leaves: The *S*-proline content in leaves (µg/g dry material) was achieved by using Lindsley and Toll as following:

• The dried plant tissues (0.2g) were dissolved by adding 5 mL of ethanol (95%).

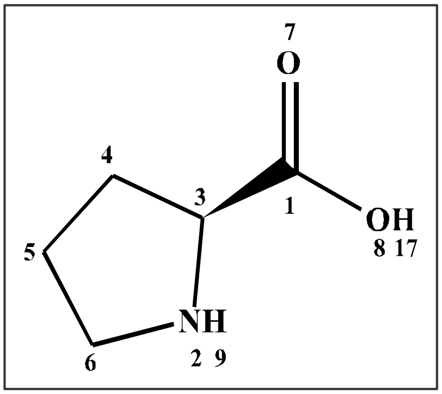

Fig. 1: The structure of *S*-proline.

• The resulting solution was centrifuged and the clear solution was evaporated and dried and the distilled water (2 mL) was added. The resulting solution was centrifuged again.

• The absorption of 1 mL of the resulting clear solution was obtained using the spectrophotometer.

• The proline content in leaves was calculated (equation 4), depend on the standard curve that achieved using the results of proline (mg/mg^{-1} dry material).

$$\text{The proline content in leaves (mg/mg}^{-1}) = \frac{\textit{The data from sandard curve}}{\textit{The weight of symbol}\ (g)} + \textit{The dilution} \qquad (4)$$

The total protein content in leaves: The total protein content in leaves was attended by using a method same to that elected by A. (Page *et al.*, 1982). The percentage of the total protein content in leaves was also calculated by using equation (5) below:

$$\text{The total protein content} = N\% * 6.25 \qquad (5)$$

The statistical studies

The Complete Randomized Design method for the simple experiments was used to variable analysis of the calculated properties by the statistical program SPSS. The significant difference (RLSD) at 0.05 probabilities was used to compare the treatment means.

Table 1: The effect of treatment with *S*-proline in the vegetative growth of tomato plant.

Treatment mg/ L	Height of plant	The diameter of stem (cm)	Leaves area	Number of leaves	Fresh weight of the vegetative total	Dry weight of the vegetative total
0	14.22	0.25	33.12	8	4.17	0.33
50	21.52	0.53	55.41	10	8.21	0.71
100	29.11	0.72	79.34	12	11.31	0.97
200	35.12	0.91	90.11	15	15.15	1.9
RLS	4.13	0.19	9.35	2.21	3.23	0.23

Result and Discussion

The statistical analysis results as presented in table 1, below were showed the effect of *S*-proline in the vegetative growth of tomato plant. The best growth was observed using 200 mg/L which gave the best height of plant equal to 35.12 mg/L in contrast with control (1.22 mg/L). Proline, an amino acid, plays an important role in plants. It protects the plants from various stresses and also helps plants to recover from stress more rapidly (Hayat *et al.*, 2012). When, the proline applied exogenously to plants exposed to stress. The proline enhanced the growth and other physiological characteristics of the plants (Hayat *et al.*, 2012).

A variety differences in *S*-proline interactions between variety treatments were observed in table 1. above. The content of *S*-proline in leaf, leaf area and stem was increased at growth stages. The best results of the diameter of stem, the number of leaves and the leaves area were also obtained using 200 mg/L which equal to 0.91 cm, 15 leaves and 9.11 cm^2 respectively. These results were compared with that gained by control which equal to 0.25 cm, 8 leaves and 33.12 cm^2 respectively. Further, the treatment with *S*-proline was gave same effect in the fresh weight of the vegetative total and the dry weight of the vegetative total (Table 1). These results can explain by the high relationship in between the fresh weight and the dry weight of the vegetative total in the tomato plant and the structure effect of *S*-proline amino acid Fig. 1.

The increase of the level of *S*-proline content in the plant can decrease the osmotic potential due to affect the aqueous potential of the cell subsequently. Proline acts as an osmolite beside enzymes and other macromolecules and therefore, protects the plant against low water potential and causes osmotic regulation in plant organs (Jureková *et al.*, 2011). Add to which, the main reason of increase in the area of leaf belong to osmotic concentration of the growth media which allow the different cells and tissues of the plant to be naturally worked. Our observation that the ability of *S*-proline to increase the photosynthesis and facilitate the gas exchange by stomata and stopped the analysis of the chlorophyll dyes due to monopolies the CO_2 concentration and water loos by transpiration. The treatment with *S*-proline also lead to increase the number of leaves in the plant depends on their ability to produce the energy for respiration.

The effect of treatment with proline

Table 2: The effect of treatment with *S*-proline in characteristics of inflorescence growth.

Treatment mg/ L	Date of the appearance of the first inflorescence	Number of the inflorescences	Number of the total flowers	Percentage of rips
0	90	1	1.9	30.41
50	82	1.55	4.43	40.11
100	77	2.11	7.81	50.71
200	64	3	13.11	60.51
RLS	5.25	1.44	3.37	8.35

Table 3: The effect of treatment with *S*-proline in chemical content in leaves of tomato plant

Treatment mg/ L	Total chlorophyll content in leaves mg / 100 g fresh	*S*-proline content in leaves	*S*-proline content in roots µg/ g dry	Total protein content in leaves
0	1.37	290	330	10.93
50	3.21	479	490	19.21
100	4.63	797	820	28.73
200	6.11	1210	1280	35.12
RLS	1.71	92.19	98.92	5.44

Table 4: Internal coordinate (ICM) of *S*-proline.

No.	Atom	Bond Atom	Bond Length (Å)	Angle Atom	Angle (º)	2nd Angle Atom	2nd Angle (º)	2nd Angle Type
1	C(1)							
2	C(3)	C(1)	1.509					
3	N(2)	C(3)	1.46	C(1)	110.852			
4	C(4)	C(3)	1.519	C(1)	110.852	N(2)	103.776	Pro-S
5	H(10)	C(3)	1.113	C(1)	105.944	N(2)	112.771	Pro-R
6	C(5)	C(4)	1.524	C(3)	105.107	C(1)	-94.649	Dihedral
7	H(11)	C(4)	1.113	C(3)	110.528	C(5)	110.528	Pro-R
8	H(12)	C(4)	1.113	C(3)	112.007	C(5)	112.007	Pro-S
9	C(6)	N(2)	1.46	C(3)	104.967	C(1)	78.507	Dihedral
10	H(9)	N(2)	1.02	C(3)	110.775	C(6)	109.47	Pro-S
11	H(13)	C(5)	1.113	C(4)	110.523	C(6)	110.523	Pro-S
12	H(14)	C(5)	1.113	C(4)	111.996	C(6)	111.996	Pro-R
13	H(15)	C(6)	1.113	N(2)	110.838	C(5)	110.838	Pro-S
14	H(16)	C(6)	1.113	N(2)	112.738	C(5)	112.738	Pro-R
15	O(8)	C(1)	1.338	C(3)	120	N(2)	0	Dihedral
16	O(7)	C(1)	1.208	C(3)	120	O(8)	120	Pro-S
17	H(17)	O(8)	0.972	C(1)	106.1	C(3)	180	Dihedral

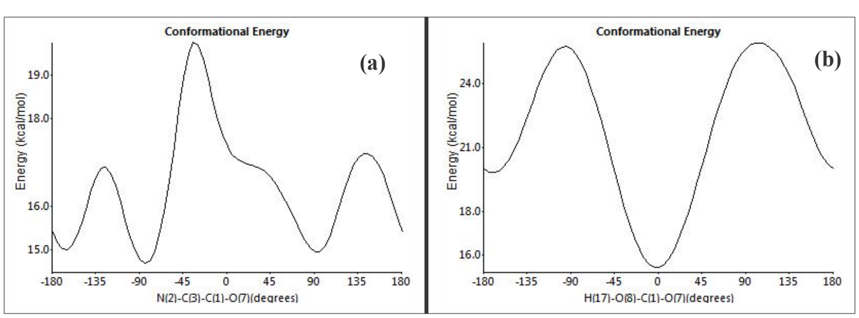

Fig. 2: The conformational energy.

in characteristics of inflorescence growth was also studied (Table 2). This effect leads to make the date of the appearance of the first inflorescence earlier especially in 200 mg/L^{-1}. The period of apparent was in 64 days instead of 90 days in control. Further, the number of the inflorescences and the number of the total flowers were also affected by treatment with *S*-proline. The best increase was observed in 200 mg/L^{-1} which equal to 3 inflorescence and 13.11 flowers in contrast with other treatments. Furthermore, the treatment with *S*-proline also affected the percentage of rips values which equal to 60.51% in 200 mg/ L^{-1} in contrast with control equal to 30.41.

The results from table 2, above were indicated that the treatment with *S*-proline make the date of the appearance of the first inflorescence earlier because of their ability in produce the equilibrium between the nitrogen and carbohydrate. The major physiological properties of the proline in the hormone equilibrium can increase the number of flowers. The treatment with *S*-proline also improved the total chlorophyll content in leaves as presented in table 3, below. Good relation between the *S*-proline content in leaves and in the roots of the plant, due to same increase in the level of *S*-proline was observed in each side of plant. The highest results in leave and root were equal to (1210 and 1280) µg/g^{-1} dry respectively using 200 mg/ L^{-1}. However, the total protein content in leaves was affected with treatment and the high value was equal to 35.12% using 200 mg/L^{-1} in contrast with control which equal to 10.93%.

The results from table 3, above were showed that the treatment with *S*-proline lead to increase the total chlorophyll content in leaves, especially in 200 mg/L^{-1}. The later was equal to 6.11 mg/100 g fresh in contrast with control which equal to 1.37 mg/100 g fresh. These results confirm that the *S*-proline was a necessary source of nitrogen that can affect the formation of chlorophyll. The treatment with *S*-proline also improved

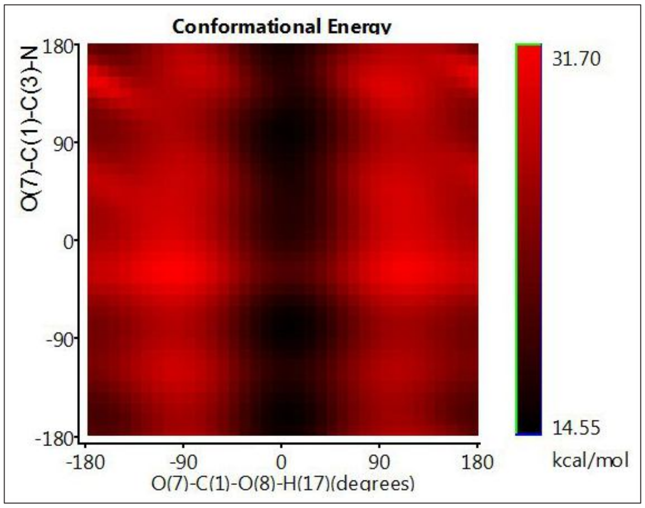

Fig. 3: The double angle plot.

the total chlorophyll content in leaves due to increase the life time of the plant. Also the level of free *S*-proline in leaves and roots were increased after treatment, which explain the ability of proline in osmoregulation in the cells of the plant. Theoretical studies carried on *S*-proline that have a chiral centre C(3): (*S*) in order to study the effect of their structure in their activity. The MM2 properties was envisioned for *S*-proline, the results were revealed that the stretch, cubic stretch, quartic stretch, bend, stretch-bend, torsion, non-1,4 VDW, 1,4 VDW, dipole/dipole and the total energy were equal to 0.4675, -2.0000, 2.3330, 7.5088, -0.0254, 4.0908, -1.7480, 7.6103, 35.6800 and 53.5840 kcal/mol respectively. But, the total energy using MMFF94 was equal to31.449 kcal/mol. The close contact of atoms was also studied the results were showed that the close contact of Lp(21)-H(16), C(1)-H(12) and N(2)-O(8) atoms were equal to 1.939, 2.565 and 2.706 respectively. Further, the ICM was calculated for *S*-proline as seen in table 4.

The conformational analysis of *S*-proline was also studied theoretically. The results were showed that the conformational energy around single bond C1-C3 (a) and C1-O8 (b) was generating eclipsed and staggered conformers as seen in fig. 2.

The rotation of atoms about the single bonds is the subject of conformational analysis. This can affect the internal coordinate due to effect the binding of the amino acid molecule. The double angle plot of C3-C1-O8 was also calculated as seen in fig. 3.

The figure shows that each point in the plot was represented two dimension of energy with respect that the low and high energy in the black and red colour respectively. When we take the two upper sides in the two different directions we find that the energies E(-180°, 170°) and E(170°, 170°), were equal to 21.48 kcal/mole and 22.63 kcal/mole respectively. But, when we take the two lower sides in the two different directions we find that the energies E(-180°, -180°) and E(170°, -180°), were equal to 19.98 kcal/mole and 20.48 kcal/mole respectively. These results with the earlier results were displayed that the conformational analysis and the structure of *S*-proline can affect their action in the tomato plant.

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