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GLUTAMINE AND CUCUMBER TOLERANT TO DROUGHT STRESS

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ABSTRACT

A factorial experiment with Randomized Complete Block Design was carried out in the plastic house of the Department of biology / College of Science/ University of Diyala for the autumn season 2016 to study the effects of drought stress and Glutamine spraying in total chlorophyll concentration, proline concentration, total carbohydrates and relative damage of the plasma membrane. The experiment was conducted using two levels of stress S_1 and S_2 and three levels of Glutamine (0, 1000, 2000 mg l^{-1}) with three replicates. The results showed a significant superiority of the first stress level S_1 in the mean concentration of chlorophyll recorded 0.66 mg g^{-1}, while the total carbohydrate concentration decreased to 350.6 mg g^{-1}, proline to 1.4 mg g^{-1} and plasma membrane damage to 52.7% compared with the second stress level S_2 which recorded values that amounted 0.56 mg g^{-1}, 460.8 mg g^{-1}, 1.6 mg g^{-1} and 61.7% respectively. Glutamine showed significant increase in the concentration of total chlorophyll, total carbohydrate and significant decrease in the relative damage of the plasma membrane. The highest concentration of total chlorophyll was recorded in the treatment of spraying with 2000 mg l^{-1} it amounted to 0.66 mg g^{-1} while the concentration of total carbohydrates 440.3 mg g^{-1} also recorded the lowest value of relative damage amounted to 54.6%.Treatment of spraying 0 mg l^{-1} recorded values amounted to 0.55 mg g^{-1} and 356.7 mg g^{-1} and 60.6% respectively

KEYWORDS: Drought stress, Glutamine, *Cucumis sativus*.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most important economic and desirable crops in Iraq. It is one of the most famous plants of the Cucrbitaceae which includes about 90 genera and more than 750 species (Chakravarty, 1966). Water forms about 95% of the wet weight of the fruit and each 100 g of fruits contains 12 calories, 0.6 g protein, 0.1 g fat, 2.2 g

carbohydrates, 15 mg magnesium, 24 mg phosphorus, 12 mg calcium, 12 g vitamin C, 0.06 g vitamin A, 0.03 g vitamin B_1 and 0.02 g B_2 (Papadopoulos, 2003).

Drought stress is one of the most dangerous types of abiotic stresses (Yasin, 2001). Its symptoms occur when the plant cannot absorb water in sufficient quantity to balance the rate of water loss by transpiration (Vannozzi *et al.*,1999). Drought stress inhibits photosynthesis, carbonation, nitrogen metabolism, while it enhances the production of Reactive Oxygen Species (ROS) that causes damage to proteins, cellular membranes, reduced vegetative growth and yield (Gupta, 2011; Rao *et al.*,2006).

Experiments of spraying plants with nutritions, growth-stimulating solutions, including some amino acids, some organic acids and plant growth hormones, have been shown to improve the plant's ability to withstand biotic and abiotic stress and increase the vegetative and yield of many crops (AL-Sahaf, 1989 ; Lafitte *et al.*, 2007).

Glutamine is an important amino acid in nitrogen metabolism. It is the first amino acid produced by plants, a precursor to the manufacture of other amino acids and is an important source of energy (Vickery *et al.*, 2017) The current study aims is study the role of Glutamine in the improving the growth of cucumber, especially in drought stress.

MATERIALS AND METHODS

The experiment was carried out in the plastic house of the Department of biology/ College of Science/ University of Diyala for the autumn season 2016 in clay loam soil. Table (1) shows some physical and chemical properties of the soil of the experiment site.

Table 1. Some physical and chemical properties of the soil of the experiment site.

Property	value
Sand	**73.98 g.kg^{-1}**
Silt	**9.86 g.kg^{-1}**
Clay	**16.16 g.kg^{-1}**
Texture	**Clay loam**
Electrical conductivity	**2.82**
pH	**7.61**
O.M	**1.449 %**
$CaCO_3$	**260.36 g.kg^{-1}**
N	**24.51 mg.kg^{-1}**
P	**30.62 mg.kg^{-1}**
K	**192.6 mg.kg^{-1}**

The experiment was designed as a factorial experiment according to the design of the Randomized Complete Block with three replicates. The soil was plowed and divided into terraces with a width of 60 cm and leaving 50 cm between the terrace and the other. The soil was fertilized with NPK fertilizer (20:20:20) with micro elements and at a rate of one kilogram per week with the irrigation water through fertilizer tank attached to the irrigation system and using drip irrigation system, cucumber seeds of Wassim cultivar was planted on 10/10/2016 with one seed in each hole and the distance between hole and the other 40 cm.
The experiment included using two levels of water stress S_1 (irrigation for 14 minutes per day) and S_2 (irrigation for 7 minutes per day).

Three levels of Glutamine spray with concentration 0 mg l^{-1} (G_1), 1000 mg l^{-1} (G_2) and 2000 mg l^{-1} (G_3), G_1 as a control treatment (spray with distilled water only). The plants were sprayed with Glutamine aged (32, 42, 52 days).

Total chlorophyll concentration was determined by method (Lichtenthaler, 1987). Proline was estimated by method of Bates *et al*.(1973). The total carbohydrate concentration was determined by method of Joslyn (1970) and the relative damage of the plasma membrane was measured by method of Lutts *et al*. (1996) contained in Valentovic *et al*. 2006). Results were statistically analyzed using SAS program and Duncan's multiple range test was adopted to compare the means at the probability level of 0.05.

RESULT AND DISCUSSION

1- Total chlorophyll concentration: Results in table (2) showed a significant effect of drought stress in reducing the mean concentration of total chlorophyll of leaves, in the second stress level S_2 the mean chlorophyll concentration decreased with a percentage of 15.2 % registered value reached 0.56 mg g^{-1} compared with the first stress level S_1 which scored 0.66 mg g^{-1}. The decrease in chlorophyll concentration can be attributed to the lack of water in the guard cells causes a partial closure of the stomata, which inhibits photosynthesis and chlorophyll synthesis, which destroys chlorophyll molecules,drought stress causes increasing in chlorophyllase enzyme activity, also increasing Dechelalatase enzyme which removes the magnesium from the Chlorophyll molecule, degradation of the porphyrin ring with the help of Dioxygenase enzyme, oxidation of iron by Ironoxidase enzyme and degradation of the residual protein from chlorophyll degradation in the vacuoles produced by the plastids plasmolysis (AL-Anbari, 2007; AL- Dasouki,2008).

Glutamine spraying showed a significant increase in the concentration of chlorophyll. The highest mean chlorophyll concentration was 0.66 mg g^{-1} for treatment G_3 while treatment G_1 was 0.55 mg g^{-1}. This increase can be attributed to the addition of Glutamine has contributed to the supply of nitrogen and increase its percentage, as the 70% of the nitrogen content of the leaf is included in the composition of this pigment and the plastids contain more than half of the total nitrogen content so that chlorophyll is directly related to the nitrogen content of the plant (AL-Sahaf, 1989). Thus, the construction of chlorophyll can be enhanced by the Glutamine as a source of nitrogen (Kazemi and Ameri, 2012).

In the same table (2), there was a significant interaction between Glutamine and drought stress. The highest concentration of chlorophyll in treatment S_1G_3 was 0.70 mg g^{-1} while treatment S_2G_1 showed the lowest concentration of chlorophyll amount to 0.50 mg g^{-1}.

Table. 2: Effect of drought stress, Glutamine and their interaction in the total chlorophyll concentration (mg g^{-1} of wet weight) of Cucumber leaves.

Glutamine mg l-1 G		Drought Stress		mean effect of Glutamine
		Irrigation for 14 minutes S1	Irrigation for 7 minutes S2	
0	**G1**	0.61 **bc**	0.50 **d**	**0.55 b**
1000	**G2**	0.67 **ab**	0.57 **c**	**0.62 a**
2000	**G3**	0.70 **a**	0.61 **bc**	**0.66 a**
mean drought stress		**0.66 a**	**0.56 b**	-----

2- Proline

Drought stress significantly increased the concentration of proline in leaves Table (3), the increase rate was approximately 14.8%. The concentration proline increased from 1.4 mg g^{-1} in the first stress level S_1 treatment to 1.6 mg g^{-1} in the second stress level S_2 treatment. The reason for the increased concentration of proline is that the drought stimulates the enzymatic activity of the protein degradation enzymes, including Proetinase, as the proteins decompose into basic amino acids causing increasing the concentration of those amino acid in plants exposed to drought stress including proline. Increasing proline is one of the important mechanisms drought stress tolerance, it is very important in osmoregulation and thus help the plant to maintain a low water potential that improves the ability of the roots to draw water from the soil (Taylor *et al*., 1982 ; Goring and Plescher, 1986).

The results showed that the Glutamine spraying had no significant effect on the mean concentration of proline. S_1G_1 treatment and S_1G_2 treatment showed the lowest values of proline amount to 1.4 mg g^{-1}, which could be attributed to the role of Glutamine contributes to proline synthesis by providing the Glutamic acid, which is a precursor to proline construction In addition, increased Glutamic acid can enhance the ability to collect intracellular proline (Forde and Lea, 2007), which contributes to reducing the Osmotic potential within the plant cells that improved the plant's drought tolerance (Zhu, 2002; Munns *et al*., 2006).

Table. 3: Effect of drought stress, Glutamine and their interaction in prolin concentration (mg g^{-1} of wet weight) of Cucumber leaves.

Glutamine mg l-1 G		Drought Stress		mean effect of Glutamine
		Irrigation for 14 minutes S1	Irrigation for 7 minutes S2	
0	**G1**	1.4 **c**	1.5 **bc**	**1.5 a**
1000	**G2**	1.4 **c**	1.7 **ab**	**1.5 a**
2000	**G3**	1.5 **bc**	1.7 **a**	**1.6 a**
mean drought stress		**1.4 b**	**1.6 a**	-----

3- Total carbohydrate concentration:

Drought stress significantly increased carbohydrate concentration in leaves Table (4). The concentration increased from 350.6 mg g^{-1} in S_1 treatment to 460.8 mg g^{-1} in S_2 treatment. High carbohydrate concentration helps plants ability to absorb water from the soil (AL-Shammari, 2001).

This is confirmed by Gill and Tuteja (2010) that drought stress can cause increased cells content of carbohydrates and other combinations of compounds to be used as a response to adaptation to drought conditions where carbohydrate and sugars work to maintain a Osmotic potential that allows preservation of the integrity of the cell and its components (Cales *et al*., 1990). Results table (4) showed the mean carbohydrate concentration was significantly increased by increasing the concentration of Glutamine. The highest mean carbohydrate concentration was 440.3 mg g^{-1} in G_3 treatment and the percentage of increase was 23.5% compared with G_1 treatment, which recorded 356.7 mg g^{-1}, The increase in carbohydrate concentration when spraying with Glutamine has been attributed to the role of Glutamine in increasing the leaf content of total chlorophyll, which contributes to the increased efficiency of photosynthesis and thus the production of carbohydrates (Kazemi and Ameri, 2012).

The results showed a significant interaction between Glutamine and drought stress. The S_2G_3 treatment was given the highest mean of 488.3 mg g^{-1}. The lowest mean value of carbohydrates was 288.9 mg g^{-1} for control S_1G_1 treatment with the percentage of increase was 69.1%.

Table. 4: Effect of drought stress, Glutamine and their interaction in Carbohydrate concentration (mg g $^{-1}$ of dry weight) of Cucumber leaves.

Glutamine mg l-1 G		Drought Stress		mean effect of Glutamine
		Irrigation for 14 minutes S1	Irrigation for 7 minutes S2	
0	**G1**	288.9 **d**	424.5 **bc**	**356.7 b**
1000	**G2**	370.5 **c**	469.5 **ab**	**420.0 a**
2000	**G3**	392.3 **c**	488.3 **a**	**440.3 a**
mean drought stress		**350.6 b**	**460.8 a**	-----

4- Relative damage of the plasma membrane

Drought stress caused a significant increase in plasma membrane damage from 52.7% in S_1 treatment to 61.7% in S_2 treatment and the percentage of increase was 17.0%. The increase in membrane damage may be due to the drought stress stimulates oxidative stress and Reactive Oxygen Species production especially(H_2O_2 and O_2^-), which cause oxidation of cytoblasmic lipids, resulting in damage and changes in membrane properties such as liquidity, electoral capacity and transfer of ions (Sharma *et al*., 2012).

The addition of Glutamine to the plant significantly reduced the percentage of damage in the plasma membrane. The ratio decreased from 60.6% in G_1 treatment to 54.6% in G_3 treatment The effect interaction between Glutamine spraying and drought stress was significant in the percentage of damage in the plasma membrane of cucumber leaves. S_1G_3 treatment recorded the lowest damage rate in the plasma membrane, which is 50.2%. The highest value recorded in the S_2G_1 treatment was 64.7% and the percentage of decrease was 22.4%, In this way, Glutamine spraying has contributed positively to reducing the damage of the plasma membrane due to the effect of amino acids in increasing the content of soluble sugars in the shoot system, and assemblage these sugars with the amino acids inside the cells working on osmoregulation for cells constantly, thus preventing the cells plasmolysis during the drought stress, in addition to the involvement of amino acids in the construction of proteins that make up the plasma membrane and thus maintain the integrity of the membranes and their stability, especially in drought conditions (Rajasekaran and Blake, 1999; Rife and Zeinali, 2003).

Table 5. Effect of drought stress, Glutamine and their interaction in percentage of damage in the plasma membrane of leaf tissue cells (%) of Cucumber leaves

Glutamine mg l-1 G		Drought Stress		mean effect of Glutamine
		Irrigation for 14 minutes S1	Irrigation for 7 minutes S2	
0	**G1**	56.5 **bc**	64.7 **a**	**60.6 a**
1000	**G2**	51.4 **c**	61.4 **ab**	**56.4 ab**
2000	**G3**	50.2 **c**	58.9 **ab**	**54.6 b**
mean drought stress		**52.7 b**	**61.7 a**	-----

From the previous results we can conclude that the Glutamine acid can have a positive and direct role in improving the growth of cucumber plant in drought conditions.

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