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EFFECT OF AMINO ACID PROLINE TREATMENT ON ANATOMICAL CHARACTERISTICS OF LEAVES AND ROOTS OF DATE PALM SEEDLINGS *PHOENIX DACTYLIFERA* L. DEVELOPED UNDER SALINE STRESS CONDITIONS

Mohammed Abdulameer Alnajjar, Wasen Fawzi Alpresem and Majid Abdulhameed Ibrahim*

Department of Horticulture and Landscape Design, College of Agriculture, University of Basrah, Basrah, Iraq.

Abstract

The date palm seedlings were irrigated with water containing sodium chloride at a concentration of 6000 mg L^{-1} for 60 days. These seedlings were sprayed during this period with a proline solution in concentrations 0, 50, 75 and 100 mg L^{-1} once per ten days. The results of the study indicated a significant superiority of proline treatment with a concentration of 100 mg L^{-1}, which recorded the highest values in the thickness of the cuticle layer, the upper and lower epidermis length of palisade cells, diameter of bundle sheath cells, fibrous bundles the length and width of the vascular bundles, the distance between the vascular bundles, the diameter of the primary and secondary xylem and the thickness of the phloem of leaf, and the thickness of the epidermis and cortex, the diameter of the vascular cylinder, primary and secondary xylem and phloem of root. The results showed that the vascular cylinder in the roots of date palm seedlings consisted of radial bundles, which were represented by alternating radiuses between xylem and phloem.

Key words : Cortex, epidermis, germination, phloem, vascular bundles, xylem.

Introduction

Date palm *Phoenix dactylifera* L. is an evergreen fruit tree belonging to the Arecaceae family, a tropical and subtropical plant spread between latitudes 15-30 north of the equator. It is a monocotyledon plant, and the Arabian Gulf region is home to the date palm tree. Its trees are a symbol of the desert environment as it is one of the most adapted plants to the desert environment because of its tolerance of high temperatures, drought and salinity (Govarets and Dransfield, 2005; El-Shibli and Korelainen, 2009). Salt Stress is one of the most influential factors in the growth and development of the plant where this type of stress leads to the exposure of the plant to the second type of stress, water stress. Salinity reduces plant susceptibility to water absorption. In general, most of the stresses on plants cause a clear lack of protein and nucleic acid synthesis in plant tissues (Popp, 1990). Challa and Van Beusichem (2004) explained that the mechanism of direct effect of salinity on the plant by making some morphological, synthetic and anatomical changes in the plant. Martin-Tanguy (2001) found that proline accumulates in plant tissues under different conditions and is one of the amino acids involved in protein synthesis and is created from glutamic acid. The function of the accumulated proline is to regulate the osmosis, maintain cell membranes, stabilize the protein, grow and germinate seeds. The leaf of date palm consists of three main layers: epidermis, which includes upper and lower epidermis, Mesophyll and vascular tissues (Abbas, 2000). The mesophyll is a number of cells between the two epidermis layers where most photosynthesis reactions occur. Vascular bundles are made of xylem and phloem tissues surrounded by a bundle of fibers called a bundle sheath. The seedling resulting from germination of date palm seeds contains one main root and soon branches out from it. This main root decays to form a number of adventitious roots from the seedling base during the first year of life (Salem *et al.*, 2008; Ogburn and Edward,

***Author for correspondence :** E-mail: majid.abdulhameedl@uobasrah.edu.iq

2009). In an anatomical study by Fatima *et al.* (2010) on the adventitious roots of fourteen date palm varieties, epidermis differed among the studied cultivars; with the highest thickness recorded in Berehmi was 59.91 µm. But Halawi-2 and Jansohaar recorded the lowest thickness of 21.78 µm. There are several types of indicators that can be used to describe the types of plant environmental adaptation, such as morphological and anatomical indicators. Dickinson (2000) has shown that the most anatomical adaptations of plants exposed to stress are the small number of stomata in the leaf area, the increase in the thickness of the cuticle layer and the accumulation of wax. These adaptations play an important role in water conservation in plant tissues under stress conditions. Therefore, this study was conducted to investigate the effect of amino acid proline in some anatomical indicators of leaves and roots of date palm seedling growing under salt stress conditions.

Materials and Methods

Date palm seeds were chosen as healthy, disease-free and homogeneous in size and shape, soaked in distilled water for a week and then sown in equal depths (3 cm) in plastic pots containing mixed soil washed with distilled water. Two months after the emergence of seedlings, they were all irrigated with water containing sodium chloride at a concentration of 6000 mg L^{-1} for 60 days. The leaves of seedlings were sprayed during this period with an amino acid proline solution in concentrations at 0, 50, 75 and 100 mg L^{-1} every ten days. One month after the last spray, anatomical sections were prepared according to the method described in Khafaji (2001) and Alnajjar (2014). Leaf samples were collected from the middle of the leaf, and roots from the last 2 cm from the tip of the tap root. Fixation was then carried out in the F.A.A solution for 48 hours. The pieces were then passed in ascending concentrations of ethyl alcohol and the samples were buried with paraffin wax at 58 °C. The pieces were then cut by Rotary Microtome with a thickness of 10 µm, placed on slides and dyed with Safranin dye and then placed in a fast green dye, a few drops of DPX were added and covered with the slide covers (Tomlinson, 1990; Thomas and Franceschi, 2013). The anatomical characteristics of the leaves and roots were then the measurements were recorded as the micrometer units by the ocular micrometer in an Olympus optical microscope equipped with a camera.

Experimental design and statistical analysis

Simple experiments are designed using Randomized Complete Block Design. Data were analyzed statistically according to analysis of variance using SPSS statistical program. The comparison between the mean of the treatments using R-LSD test at a probability level of 0.05 (Snedecor and Cochran, 1986).

Results and Discussion

Anatomical characteristics of leaf

The results of statistical analysis in table 1 and plate 1 indicated the significant effect of amino acid proline in some anatomical characteristics of date palm seedling leaves. The concentration at 100 mg. L^{-1} proline gave the highest value of the thickness of the cuticle layer and the upper and lower epidermis of 4.50, 11.09 and 11.39 µm, respectively, significantly different from other treatments. There was no significant difference between the concentrations of 50 and 75 mg. L^{-1} proline. The control treatment recorded the lowest values in these traits were 1.67, 4.75 and 4.69 µm, respectively.

The results of the same table also indicated the effect of the amino acid proline in the diameter of the tannin cells. The treatment of 100 mg. L^{-1} reduced the diameter of the tannin cells by 17.52 µm, a significant difference over other treatments. The highest mean diameter of the tannin cells in the untreated leaves was 28.92 µm. The length of palisade cells, diameter of bundle sheath cells and diameter of fibrous bundles were significantly affected. Treatment with the amino acid proline at a concentration of 100 mg. L^{-1} significantly increased these characteristics by 17.98, 61.60 and 36.32 µm, respectively when compared to other treatments.

The results in table 2 and plate 2 indicate that the concentration at 100 mg. L^{-1} of proline was significantly superior in anatomical characteristics of vascular bundles when compared with other treatments. This treatment recorded the highest values in the vascular bundle length and width, the distance between the vascular bundles, the diameter of the primary and secondary xylem and the thickness of the phloem tissue at 260.20, 132.00, 500, 29.60, 60.4 and 86.60 µm, respectively. While the control treatment recorded the lowest values in these studied characteristics were 119.95, 90.43, 307, 13.60, 32.90 and 49.80 µm, respectively (table 2).

The reason for the changes in the anatomical characteristics of date palm seedling leaves is due to their exposure to salt stress. This means that these changes are a means of protection to reduce the loss of water from the leaves by increasing the thickness of the cuticle layer. Salt stress causes the plant to be affected by the second type of stress, which is water (drought) stress (Challa and Van Bensichem, 2004).

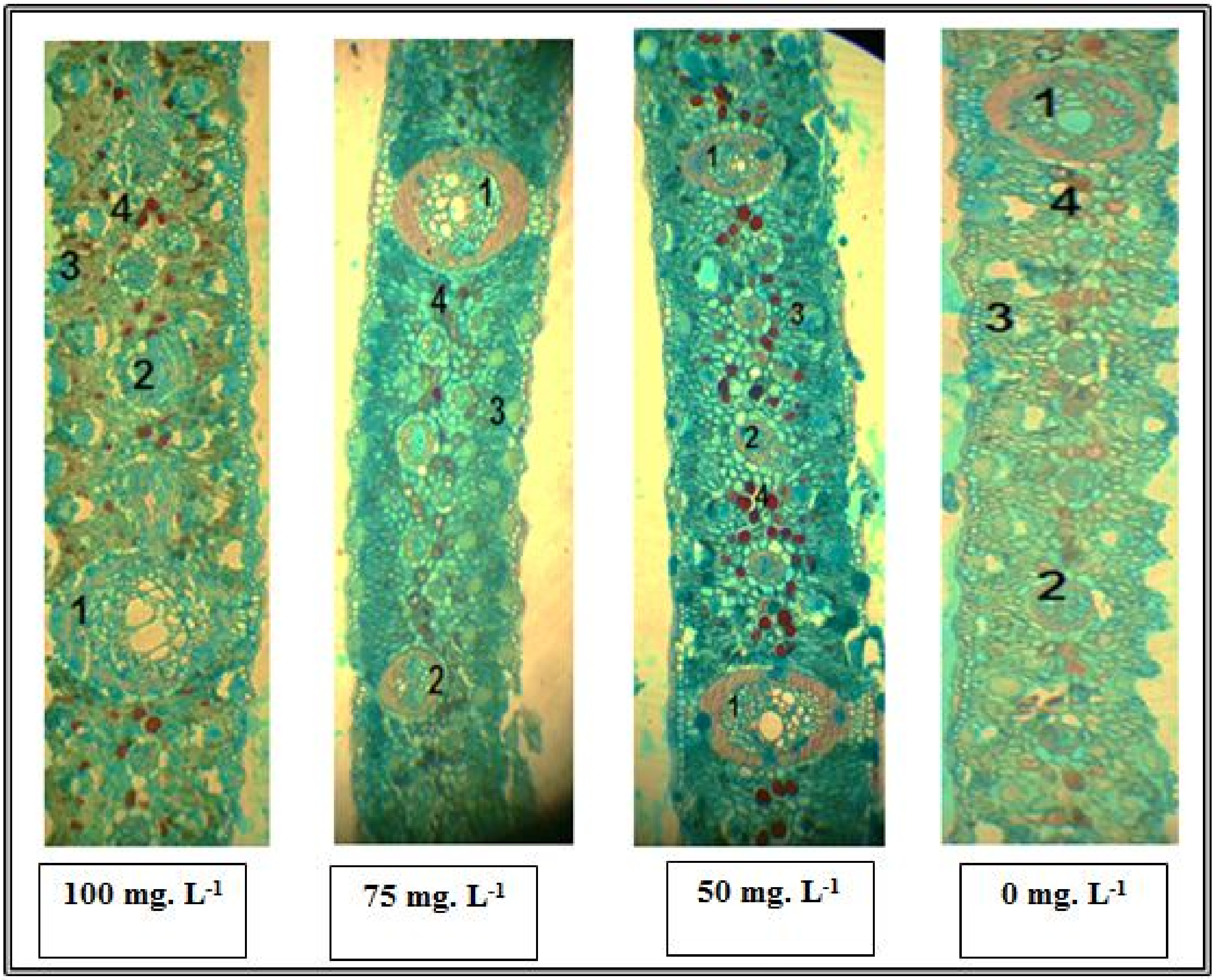

Plate 1 : Some anatomical characteristics of leaf of date palm seedling that sprayed with different concentrations of proline (0, 50, 75 and 100 mg. L^{-1}): 1-Vascular bundle 2- Bundle sheath cells 3- Fibrous bundle 4- Tannin cells (10X).

Table 1 : Effect of spraying date palm seedlings with different proline concentrations on some anatomical characteristics of the leaf under salt stress.

Proline concentration (mg. L^{-1})	Thickness of cuticle layer (µm)	Thickness of upper epidermis layer (µm)	Thickness of lower epidermis layer (µm)	Diameter of tannin cell (µm)	Diameter of palisade cell (µm)	Diameter of bundle sheath cells(µm)	Diameter of fibrous bundle (µm)
0	1.67	4.75	4.69	28.92	8.00	22.99	14.00
50	2.10	7.59	7.33	22.53	9.11	35.20	22.60
75	2.90	8.11	8.35	17.52	15.80	59.40	33.12
100	4.50	11.09	11.39	16.52	17.98	61.60	36.32
R-LSD* ($p \geq 0.05$)	1.13	1.23	1.17	2.03	3.19	5.07	3.16

* R-LSD: Revised least significant difference.

Anatomical characteristics of roots

Different zones of the root were measured and the results of the study showed significant differences in the effect of spraying at different concentrations of proline (table 3 and plate 3). There was a significant difference in epidermis thickness of 22 µm in control treatment and 35 µm at 100 mg. L^{-1} concentration of proline. But the thickness of the cortex was 710 µm in the control

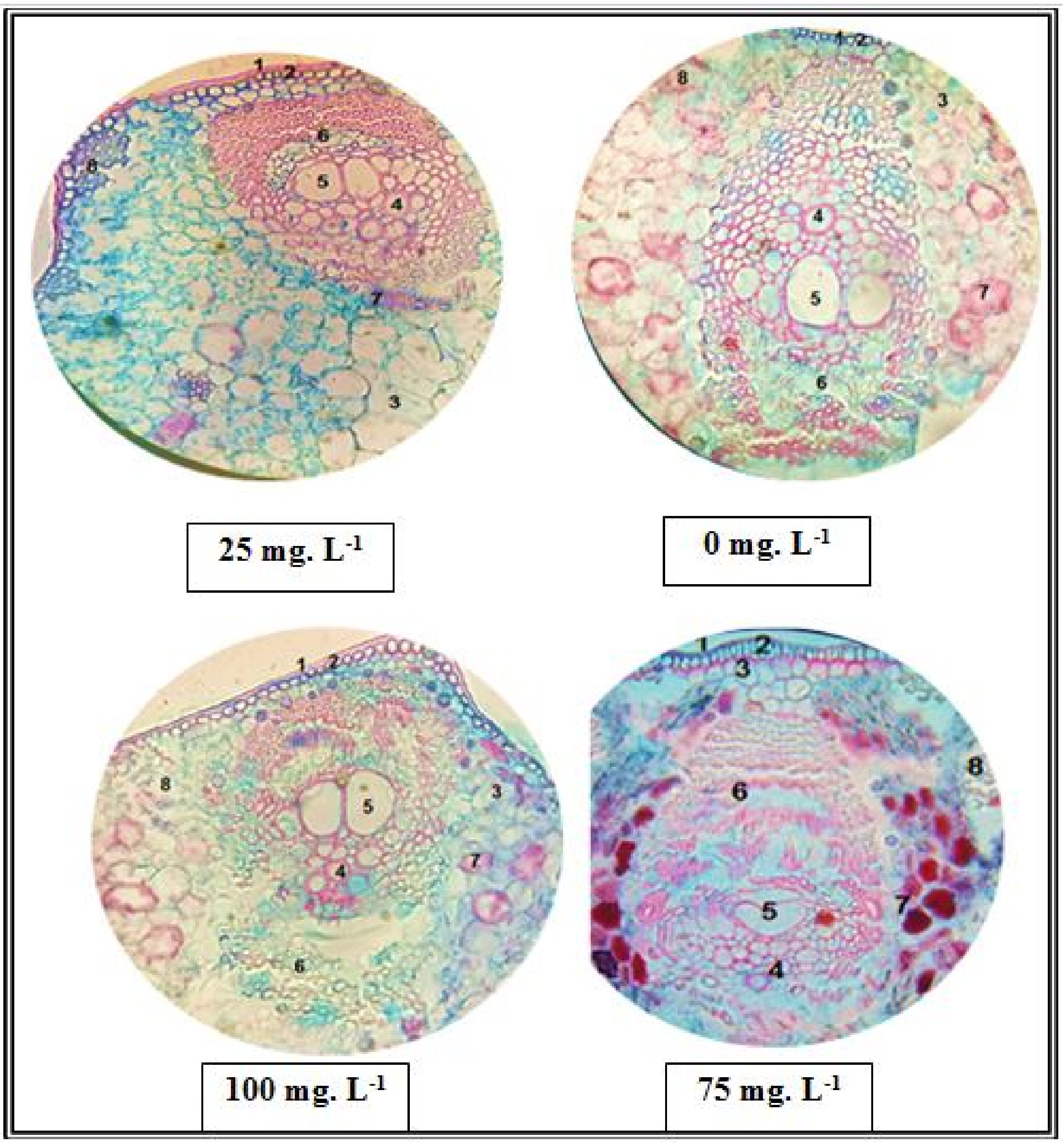

Plate 2 : Some anatomical characteristics of vascular bundle of leaf of date palm seedling that sprayed with different concentrations of proline (0, 50, 75 and 100 mg. L^{-1}): 1- Cuticle 2- Epidermis 3- Palisade cells 4- Primary xylem 5- Secondary xylem 6- Phloem 7- Tannin cells 8- Fibrous bundle (40X).

Table 2 : Effect of spraying date palm seedlings with different proline concentrations on some anatomical characteristics of a vascular bundle of the leaf under salt stress.

Proline concentration (mg.L^{-1})	Length of vascular bundle (µm)	Width of vascular bundle (µm)	Distance between vascular bundle (µm)	Diameter of primary xylem(µm)	Diameter of secondary xylem(µm)	Thickness of phloem (µm)
0	119.95	90.43	307.00	13.60	32.90	49.80
50	173.45	110.46	358.00	17.91	38.12	66.80
75	238.00	128.00	418.00	25.00	55.40	70.20
100	260.20	132.00	500.00	29.60	60.40	86.60
R-LSD* ($p \geq 0.05$)	9.85	6.64	11.02	2.67	5.48	6.03

* R-LSD: Revised least significant difference.

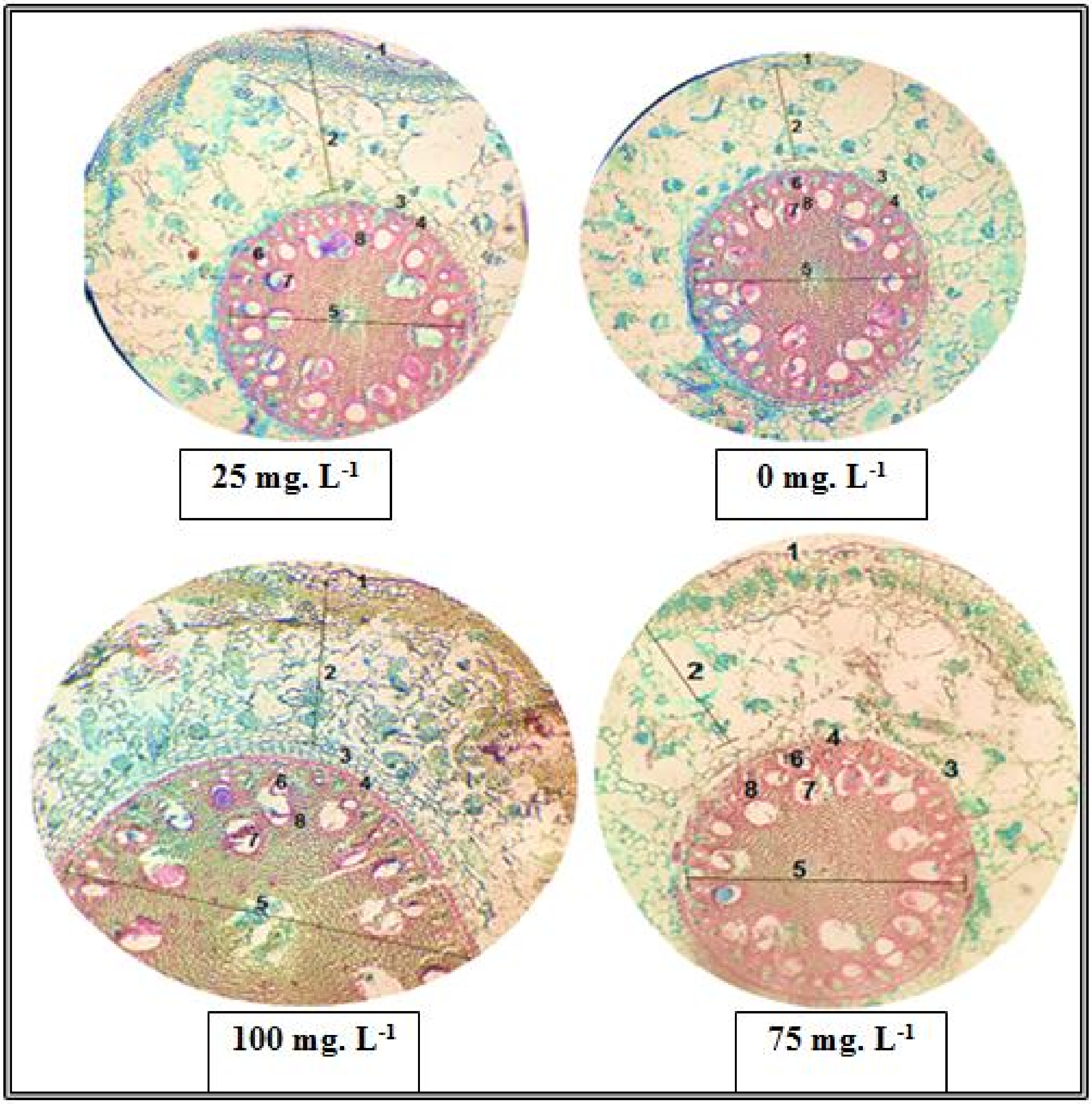

Plate 3 : Some anatomical characteristics of root of date palm seedling that sprayed with different concentrations of proline (0, 50, 75 and 100 mg. L^{-1}): 1- Epidermis 2- Cortex 3- Endodermis 4- Pericycle 5- Vascular cylinder 6- Primary xylem 7- Secondary xylem 8- Phloem (10X).

Table 3 : Effect of spraying date palm seedlings with different proline concentrations on some anatomical characteristics of roots under salt stress.

Proline concentration (mg. L^{-1})	Thickness of Epidermis (µm)	Thickness of cortex (µm)	Thickness of endodermis (µm)	Thickness of pericycle (µm)	Diameter of vascular bundle (µm)	Diameter of primary xylem (µm)	Diameter of secondary xylem (µm)	Diameter of phloem (µm)
0	22	710	18	15	980	40	80	25
50	28	760	20	17	1240	45	120	31
75	30	850	20	20	1360	60	130	35
100	35	900	20	20	1550	70	160	40
R-LSD* ($p \geq 0.05$)	3.13	23.23	2.25	1.27	38.29	7.28	9.57	2.11

* R-LSD: Revised least significant difference.

treatment and 900 μm in the proline treatment at 100 mg. L^{-1}. There was no significant effect of amino acid proline in the thickness of the endodermis and pericycle layers. The concentration at 100 mg. L^{-1} proline had the highest values of 20 μm in each of the endodermis and pericycle layers.

The results shown in table 3 indicate the significant effect of the amino acid proline. Proline treatment with a concentration of 100 mg. L^{-1} recorded a significant superiority over other treatments. This treatment recorded the highest values in the diameter of the vascular cylinder, primary and secondary xylem and phloem, reaching 1550, 70, 160 and 40 μm, respectively. But the control treatment recorded the lowest values in the same studied characteristics. This may be due to the effect of amino acid proline in increasing the growth, number of cells and chlorophyll pigment in the plant. In general, amino acids contribute to increased respiration, which leads to the production of energy compounds (ATP), necessary for the occurrence of cell division and growth (Taha *et al*., 2001; Alnajjar *et al*., 2011).

In general, most stresses on plants cause a reduction of the proteins and nucleic acids synthesis in plant tissues (Popp, 1990). This reduction of proteins synthesis leads to a change in transcription and translation, which in turn leads to the production of new proteins in the Gene expression process as a result of response to the type of stress that the plant is exposed to ensure tolerance to these abnormal conditions (David and Nilsen, 2000).

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