EFFECT OF DIFFERENT LEVELS OF SALT AND DROUGHT STRESSES ON GENE EXPRESSION OF TWO TOLERANCE-DIFFERENT TOMATO CULTIVARS *IN VITRO*

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ABSTRACT

A lab experiment was conducted at the Plant Tissue Culture Lab / College of Agricultural Engineering Sciences / University of Baghdad. This experiment was aimed to investigate gene expression index in tomato (*Solanum lycopersicum* L.) after preparation of salt and simulated drought stresses. Two tomato cultivars were selected which claimed to exhibit different levels of tolerance toward abiotic stresses designated as salt-tolerant Yassamine (Y) and salt-sensitive GS12 (G) to assess the test. Seven day-old seedlings from both cultivars were grown in MS media supplemented with four concentrations of NaCl at 0, 50, 100 and 150 mM and four concentrations of PEG at 0, 10, 20, and 30% for 48 hours. The results were showed that Y cultivar exhibited more proline secretion and chlorophyll content when compared with G. In addition, Y cultivar showed less ion leakage and less affected by elevated abiotic stresses in term of seedling weight variation when compared to G counterparts. The SDS-PAGE gel analysis showed that Y cultivar showed more band intensity when compared with G suggested more corresponding gene expression of tolerant protein against abiotic stresses.

Key words: PEG, NaCl, proline, ions leakage, SDS-PAGE, abiotic stress

تاثير مستويات مختلفة من الاجهاد المائي والملحي على التعبير الجيني لصنفين من الطماطة بدرجات تحمل مختلفة للاجهادات خارج الجسم الحي

لمياء خليفة جواد العامري **علي هاني عنون**
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المستخلص

نفذت تجربة مختبرية في مختبر زراعة الانسجة النباتية التابع لكلية علوم الهندسة الزراعية / جامعة بغداد اذ هدفت لدراسة نمط التعبير الجيني لنبات الطماطة (*Solanum lycopersicum* L.) بعد تعريضها للاجهاد الملحي والمائي. تم اختيار صنفين من الطماطة مختلفين في درجة تحملها للاجهادات اذ ان الصنف الاول متحمل للملوحة ويسمى ياسمين بينما الصنف الثاني حساس للملوحة ويسمى GS12. زرعت البادرات بعمر سبعة ايام من كلا الصنفين في وسط MS مضاف له اربعة تراكيز من كلوريد الصوديوم هي 0 و 50 و 100 و 150 ملي مولر واربعة تراكيز من البولي اثيلين كلايكول PEG هي 0 و 10 و 20 و 30% لمدة 48 ساعة. اوضحت النتائج ان الصنف ياسمين اعطى اعلى محتوى برولين وكلوروفيل عند مقارنته مع الصنف GS12 كما اعطى الصنف ياسمين اقل نسبة من الايونات المتسربة وكان اقل تاثرا بارتفاع تراكيز الاجهادات الااحيائية من ناحية الاختلاف في وزن البادرات من الصنف GS12. واظهر تحليل نمط الحزم للبروتين المستخلص ان كثافة الحزم المتكونة في الصنف ياسمين كانت اعلى من الصنف GS12 مما يقترح التعبير العالي لجينات مقاومة الاجهادات الااحيائية في الصنف ياسمين عند مقارنته مع الصنف GS12.

الكلمات المفتاحية: بولي اثلين كلايكول، كلوريد الصوديوم، برولين، الايونات المتسربة، SDS-PAGE، الاجهاد الااحيائي

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops grown worldwide. Tomatoes differ in their sensitivity towards abiotic stresses but could be considered sensitive to moderate sensitive to such stresses (14). Water deficiency is a major problem influence agricultural world production where most countries are unable to provide adequate amount of water for crop production to convoy the increasing demand of sustenance. Sub-humid, semi-arid, and Arid regions are frequently under drought regions due to their highly variables in inter-annual precipitation. Consequently, agriculture in these regions is often tenuous and it gets more vulnerable under below-normal precipitation during the years. Droughts affect plant growth and productivity through affecting the morphological, physiological, and molecular processes and result in growth inhibition, chlorophyll degradation, and other quality traits such as protein (21, 34, 35). During water deficiency, another problem rising such as the alleviated soil salinity which reduce crop production (27). Knox et al (20) mentioned that nearly 20% of the cultivated areas and half of the world irrigated fields were affected by salinity which could cause reduction in agricultural production especially in poor drained soils. Genes can either directly involved in protecting plants from abiotic stresses or can involve in regulating gene expression during stress (10, 8). Amini et al (3) suggested that in order to adapt abiotic stress, new proteins in tomato seedlings are induced. However, the actual tolerance mechanism was still ambiguous because it can be controlled by multiple genes that also responsible for plant growth and development (19). To understand how abiotic stresses can alter gene expression, two cultivars of tomato plants differ in their sensitivity towards stresses were subjected to simulated drought and salt stresses and the differential gene expression along with some physiological and biochemical analyses was accordingly examined and further presented in this paper. This study was aimed to investigate the effect of different levels of salt and drought stresses on gene expression of two tolerance-different tomato cultivars *in vitro.*

MATERIALS AND METHODS

Plant material and simulated abiotic stresses: Seeds of *Solanum lycopersicum* L. var. Yassamine (Y cultivar, semi-tolerant) and GS12 (G cultivar, sensitive) were surface sterilized in 70% ethanol for 30 seconds followed by three washes with sterile distilled water. The seeds were then soaked in 20% sodium hypochlorite and a drop of Tween-20 for 10 minutes with continuous shaking. After four washes with sterile distilled water, the seeds were germinated on the MS medium (24) for 7 days. Seven day-old seedlings uniform in size from both cultivars were taken, rinsed with distilled water, and transferred to 20 ml tubes contained 10 ml MS media supplemented with 0, 50, 100, and 150 mM NaCl and 0, 10%, 20%, and 30% Polyethylene glycol (PEG 6000) represented the simulated condition for salt and drought stresses, respectively for two days. The experiment composed of 8 treatments and carried out according to the completely random design (CRD) with 3 replications. The treated seedlings were taken for protein extraction and biochemical analyses to evaluate the degree of tolerance and differentially expressed proteins for both cultivars under investigation **Protein extraction and SDS-PAGE protein electrophoresis :** Protein extraction conditions were carried on ice using reagents from Promega, WI, USA. Seedlings were collected after 2 days of salt and drought stress treatments and total soluble protein (TSP) was extracted following Song and Ahn (32) with some modifications. Tomato seedlings were homogenized in protein extraction solution composed of (0.3% SDS, 200 mM DTT, 28 mM Tris-HCl (pH 8), and 22 mM Tris base). The protein-reagent mix was centrifuged at 10000 rpm for 15 minutes at 4°C and supernatant was collected and kept at -80°C. The SDS-PAGE gel electrophoresis was executed following the protocol established by Florina (13).

Estimation of proline content in tomato seedlings: Proline levels in seedlings tissue were determined according to (1). Stressed and unstressed seedlings were homogenized in 1 ml of 3% sulfosalicylic acid (Sigma-Aldrich), collected in 1.5 ml microfuge tubes, and centrifuged at 10000 rpm for 10 minutes. The

collected supernatant was mixed with 1 ml of acid ninhydrin (1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid) and 1 ml of glacial acetic acid in 20 ml tube and incubated at 100°C for 1hour. The reaction was terminated by incubating the tubes on ice. An aliquot of 2 ml of toluene was added to each tube and the mixture was vortexed for 10 seconds. One milliliter of the upper, toluene phase containing the chromophore was collected and read at 520 nm in a quartz cuvette specrophotometrically. Tissue proline concentrations were estimated based on a standard curve (0–100 µg/mL) for proline and are presented as µg proline.g^{-1} FW according to the following equation:

µg proline.g^{-1} FW= [(µg proline/mL x 3.7)/100µg tissue] x10

Determination of chlorophyll content

Chlorophyll content was estimated according to (15) with some modifications. Seedlings (uniform in weight as possible) were taken from each treatment, cut into small pieces, and incubated in 80% acetone overnight in dark. Supernatants from each sample were then taken and the absorbance was recorded at 645 and 663 nm wavelength using spectrophotometer. Total chlorophyll content was calculated according to the formula:

Chlorophyll content (µg.ml^{-1}) = (OD_{645} * 20.2) + (OD_{663} * 8)

Measurement of ion leakage in tomato seedlings: A modified method of Jambunathan (16) was followed to measure the ion leakage of stressed and unstressed tomato seedlings. Five uniform-size tomato seedlings were collected from each treatment in a 20 m1 centrifuge tube containing 5 ml of 0.4 M mannitol. The tubes were incubated (with gentle shaking) at room temperature for 3 hours and the conductivity of the bathing solution was measured using conductivity meter. After this estimation, the samples were boiled for 10 minutes and the total conductivity of the bathing solution was determined. Membrane ion leakage was expressed in terms of initial conductivity of the bathing solution as a percentage of the total conductivity.

Measurement of seedlings weight

Three weeks old seedlings from both cultivars were subjected to the proposed salt and drought stresses. Their weight before and after the stresses were recorded and seedling weight variation were calculated according to the following equation:

((Final weight – initial weight) / initial weight)*100

RESULTS AND DISCUSSION

In order to estimate tolerance potential of selected tomato cultivars, a series of physiological and biochemical analyses were performed. Results in Table 1 show that Y cultivar was significantly high in proline content even before the stress conditions were initiated which gave 0.719 µg.g^{-1}fresh tissue in Y control when compared with G control that gave 0.244 µg.g^{-1}fresh tissue. Consequently, proline content steadily rises in response to the elevated PEG and salt concentrations and always recorded significantly higher in Y compared to G in all concentrations under evaluation. In term of chlorophyll content, Results in Table 1 show that all treatments of Y were higher when compared to G; However, most of the highest recordings in Y cultivar were not significant with two exceptions. In regard, Y cultivar treated with 50 mM NaCl had significantly higher chlorophyll content of 12.67 µg.ml^{-1} when compared with G treated with the same NaCl concentration that had 8.97 µg.ml^{-1}. In addition, Y cultivar treated with 30% PEG recorded significantly higher chlorophyll content and had 7.18 µg.ml^{-1} when compared with G of the same treatment that gave 4.63 µg.ml^{-1}. Plant tissue of G cultivar showed to be more vulnerable to suggested abiotic stresses when compared with Y cultivar which reflected by the increased leakage of metabolites.

Table 1. Proline and chlorophyll contend, and ion leakage of two tomato cultivars under salt stress and simulated drought stress

Stresses	Treatment	Proline content ($\mu g.g^{-1}$)	Chlorophyll content ($\mu g.ml^{-1}$)	Ion leakage (%)	Treatment	Proline content ($\mu g.g^{-1}$)	Chlorophyll content ($\mu g.ml^{-1}$)	Ion leakage (%)
		Yassamine cultivar				GS12 cultivar		
control	Y	0.719	13.98	9.11	G	0.244	13.73	8.52
NaCl	Y50	0.997	12.67	12.33	G50	0.457	8.97	17.83
stress	Y100	1.346	8.87	20.56	G100	1.234	7.97	26.73
(mM)	Y150	1.809	7.23	23.01	G150	1.316	6.88	37.79
PEG	Y10%	1.235	10.11	11.05	G10%	0.789	8.62	14.91
stress	Y20%	1.773	8.71	18.71	G20%	1.067	7.71	25.09
(%)	Y30%	2.0	7.18	25.64	G30%	1.263	4.63	35.59
LSD		Proline LSD5%= 0.1883		Chlorophyll LSD5%= 1.822		Ion leakage LSD5%= 3.801		

Results presented in Table 1 exhibited that all the stress treatments significantly increased ion leakages in G compared to Y with the highest ion leakage in G cultivar treated with 150 mM NaCl that gave 37.79%. Results in Figure 1 illustrate that both cultivars were clearly and significantly affected by the increased concentrations of NaCl and PEG. However, the impact was much severe in G compared to Y. At the salt stress conditions, all treatments in Y were able to tolerate the inclined NaCl concentrations and increased in seedlings weight above stress point while two treatments of the G cultivar at 100 and 150 mM NaCl collapsed and exhibited seedling weight lost below stress point.

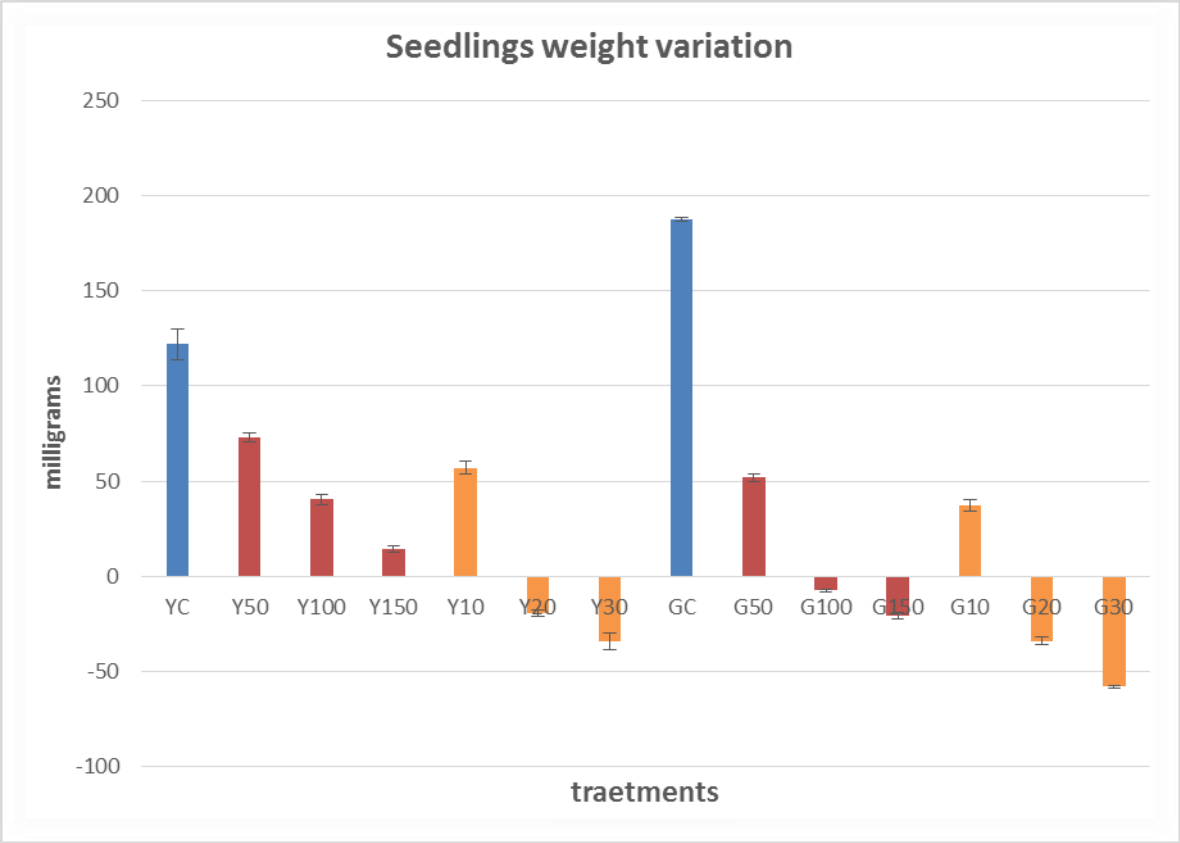

Figure 1. Seedling weight variation of two tomato cultivars as affected by salt and simulated drought stresses

In addition, PEG treatments showed to have the most deleterious effect on seedling weight although Y cultivar exhibited much tolerance to such stress when compared to G as shown in Figure 1. Gel analysis in Figure 2 show the banding pattern of Y and G cultivars after subjected to the proposed abiotic stresses. The interested band is located between the 29 and 20 KDa molecular weight indicated by the molecular ladder on both sides of the gel. The intensity of this band varied among treatments suggesting differential expression of corresponding band. Figure 2A showed the banding pattern of both cultivars after PEG

treatments in which Y cultivar subjected to 20% PEG gave the most intense band indicating relatively higher protein expression. Similarly, G cultivar treated with 20% PEG also gave intense band but relatively lower than the Y20 band.

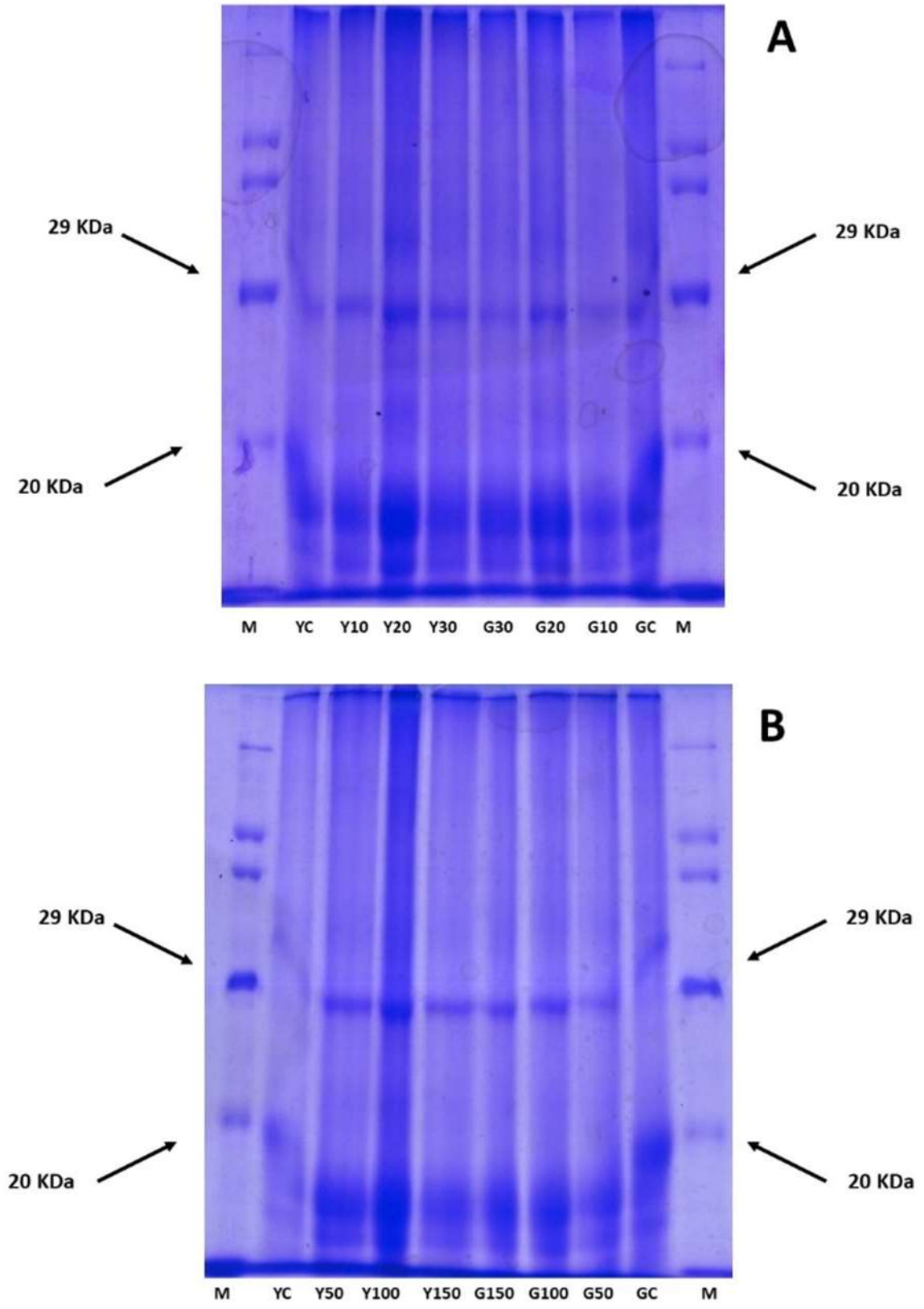

Figure 2. Protein expression banding pattern in SDS-PAGE gel for PEG stress at 0, 10, 20, and 30% (A) and Nacl stress at 0, 50, 100, and 150 mM (B) for two tomato cultivars, Yassamine (Y) and GS12 (G). M= molecular ladder, YC, GC = control

The band intensity of Y10 and Y30 were slightly denser when compared to G10 and G30. Moreover, same banding pattern shown in Figure 2B where salt stress is dominated. Therein, Y100 gave the most intense band when compared with all other treatments in Y and G cultivars. However, the intensity of Y50 and Y150 bands were more pronounced when compared to their counterparts in G50 and G150 bands. The two tested tomato cultivars showed a variation in withstanding salt and drought stress conditions which was proven with the aid of some physiological and morphological parameters. Y cultivar had significant increases in proline accumulation, chlorophyll content, and reduced expel of electrolytes as a result of imposed abiotic stresses. These findings were in line with results of other researchers (12, 18, 30). Proline could play a vital role in scavenging ROS accumulation and protects enzyme structure during stresses (29) while membrane stability is measured by the amount of leaked electrolytes in the surrounding solution (25). Beside the physiological capabilities of plants to endure environmental stresses, the defense mechanism is also triggered at the molecular level. In regard, stress tolerance mechanism could be enhanced either by stimulating gene expression of plant genome or via genetic modification (4, 7, 33). There are different kinds of proteins that will up regulated in response to stress phenomena including signaling pathways proteins, functional metabolites regulatory proteins, and stress-resistance proteins (17, 23, 26, 31). Figure 2 (A and B) shows a unique band differed in its intensity among treatments and approximately aligned with 28-26 KDa molecular weight. According to the literature, this molecular weight corresponds with a group of tolerant proteins known as pathogenesis related (PR-5) proteins which include the osmotin and osmotin-like proteins (5, 10). We noticed that the band intensity in Y cultivar was higher in comparison with the G cultivar especially at the 100 mM NaCl and 20% PEG concentrations. The reason why the intensity drooped with the higher concentrations might be due to protein degradation in the highest level meaning that the stresses exceeded to threshold level of tolerance in both cultivars. Similar results were obtained by others (5, 9, 28) suggesting the role of osmotin protein in conferring tolerance against biotic and abiotic stresses. Several hypotheses have been proposed suggesting osmotin's mode of action, either by facilitating the confinement of solutes in the vacuoles (6), or by its involvement in altering the plant structure and metabolism during the osmotic adjustment (10). It is also believed to protect the proteins' native structure and repair denatured proteins during stress (2). However, the actual protective mechanisms of osmotin against abiotic stress are still not very clear and are under investigation.

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