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Trehalose as Osmoprotectant for Maize Under Salinity-Induced Stress

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Abstract: Response of maize grains (Giza 2) to the pre-soaking treatment with 10 mM trehalose was studied under salinity-induced stress conditions. Trehalose treatment induced growth of salt-stressed and unstressed plants. Trehalose pretreatment alleviated the adverse effects of salinity stress on the metabolic activity of maize seedlings. Hill-reaction activity, photosynthetic pigments and nucleic acids content increased in response to trehalose application. Organic solutes e.g., sugars, soluble proteins and proline content increased under salinity stress. This increment was associated with increased hydrolytic activity of amylase and protease enzymes. Trehalose treatment may be ameliorate salinity stress through stabilization of the plasma membranes, since it decreased the rate of ion leakage and the rate of lipid peroxidation of the root cells, and increased the ratio of K/Na ions in the leaves of maize seedlings. Electrophoretic protein banding patterns were also investigated. Trehalose treatment reduced salt expression.

Key words: Hill-reaction, ion leakage, K/Na ratio, lipid peroxidation, organic solutes, photosynthetic pigments, protein.

INTRODUCTION

The water availability and the water movement are very important to seed germination and seedling emergence, and these factors are influenced by soil water potential, soil texture and soil-seed contact surface. Soil salinity limits growth of several plants[30]. Salinity is one of the most common universal problems, it changes the water potential of the cell[13]. It reduces plant growth, may be due to the disturbances in water potential of the plants and the reduction in photosynthesis[38]. Salinity may be lead to accumulation of some cations[6]. The direct effects of salt on plant growth may involve (1) a reduction in the osmotic potential of the soil solution that reduces plant-available water, and (2) toxicity of excessive Na^+ or Cl^- towards the plasma membrane. Osmotic effects are associated with inhibition of cell wall extension and cellular expansion, leading to a reduction in plant growth[45]. Thus, improving crop resistance to osmotic stresses is a long standing goal of agricultural biotechnology. Upon exposure to osmotic stress, plants exhibit a wide range of responses at the whole plant, cellular and molecular levels. Morphological and developmental changes in life cycle, inhibition of shoot growth and enhancement of root growth constitute whole plant level responses. Molecular and cellular level responses include adjustment in ion transport (such as uptake, extrusion and sequestration of ions) and metabolic changes (e.g., carbon metabolism, the synthesis of compatible solutes) which are induced upon regulation of gene expression[33].

Tuna *et al*.[48] found that salt stress reduces the total dry matter, chlorophyll content, relative water content (RWC) in maize, but increases proline accumulation, enzyme activities and electrolyte leakage. Salt stress reduced some macro and micronutrient concentrations. Nitika *et al*.[32] also indicated the enhancement of enzyme activities and lipid peroxidation under salt stress. Tajdoost *et al*.,[47] in their experiment on *Zea mays* seedlings (Var. single cross 704) indicated that the amount of K^+-leakage and malondialdehyde (MDA) have been increased because of salt-induced lipid peroxidation and membrane un-stability. Soluble sugars and proline as osmoregulators has been increased in stress condition and in pretreated plants with NaCl. The rate of Hill reaction was reduced significantly in stressed plants. Therefore, it has been concluded that salt stress causes serious physiological and biochemical damages in plants and salt pretreatment enhances tolerance mechanisms of plants and help them to tolerate salt stress and grow on salty environments.

Many plants accumulate organic osmolytes in response to the imposition of environmental stresses that cause cellular dehydration. Although an adaptive role for these compounds in mediating osmotic adjustment and protecting subcellular structure has

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become a central dogma in stress physiology, the evidence in favour of this hypothsis is largely correlative[19]. They added that transgenic plants engineered to accumulate proline, mannitol, fructans, trehalose, glycine betaine or ononitol exhibit marginal improvements in salt and/or drought tolerance. While these studies do not dimiss causative relationships between osmolyte levels and stress tolerance, the absolute osmolyte concentrations in these plants are unlikely to mediate osmotic adjustment. Metabolic benefits of osmolyte accumulation may augment the classically accepted roles of these compounds. In re-assessing the functional significance of compatible soluble accumulation, it is suggested that proline and glycine betaine synthesis may buffer cellular redox potential. Disturbance in hexose sensing in transgenic plants engineered to produce trehalose, fructans or mannitol may be an important contributory factor to the stress-tolerant phenotypes observed. Associated effects on photoassimilate allocation between root and shoot tissues may also be involved. Whether or not osmolyte transport between subcellular compartments or different organs represents a bottleneck that limits stress tolerance at the whole-plant level is presently unclear. If osmolyte metabolism does impinge on hexose redox signaling, then it may be important in long-range signal transmission throughout the plant.

Müller *et al.*[31] cleared that trehalose is a non-reducing disaccharide consisting of two alpha-glycosidically linked glucose units. It accumulates in many microorganisms and invertebrate animals when they are imposed to various forms of stress, and it may serve as a protectant of enzymes and membranes, particularly under conditions of heat and desiccation stress. Most vascular plants lack the capacity to produce trehalose, except for small number of desiccation tolerant plants, such as some ferns and the angiosperm *Myrothamnus flabellifolia*. In contrast, a highly specific trehalase activity has been described in many plants. The enzyme does not cleave other common alpha-glucosides, and it is highly sensitive to the inhibitor validamycin A., particularly high trehalase activities occur in pollen and legume root nodules. Rodriguez *et al.*[41] determined the presence of trehalose in 9 bean (*Phaseolus vulgaris*) cultivars and its correlation with resistance to drought stress. They found that those cultivars exhibiting high nodule trehalose levels and/or a high degree of trehalose stimulation in response to water stress also exhibited a high leaf relative water content and were also the most resistant to water stress. During water stress nodule trehalose levels rose only slightly. Trehalose genes of *Escherichia coli* and *Saccharomyces cerevisiae* have been expessed in tobacco and potatoes, thought only in very small quantities. However, it was observed that these small amounts of trehalose increase the drought resistance of the transformed plants[50]. Schellenbaum *et al.*[42] subjected non-mycorrhizal and mycorrhyzal maize plants to moderate drought stress by reducing the water supply. This stress induced a conspicuous increase in the trehalose pool in the mycorrhizal roots, probably because it was accumulated by the fungal symbiont.

Garcia *et al.*[17] characterized some of the changes in solute accumulation in NaCl-stressed rice (*Oryza sativa*) cv. Taichung Native 1 seedlings, several alternative osmoprotectants to proline were identified. One such substance, trehalose, began to accumulate in small amounts in roots after 3 days. The effects of proline (Pro) and trehalose on ion accumulation were compared to determine whether the two chemicals protect the same physiological processes. Pro either had no effect or, in some cases, exasperated the effect of NaCl on growth inhibition, chlorophyll loss, and induction of a highly sensitive marker for plant stress, the osmotically regulated salt gene. By contrast, low to moderate concentrations of trehalose reduced Na^+ accumulation, salt expression, and growth inhibition. Somewhat, higher concentrations (10 mM) prevented NaCl-induced loss of chlorophyll in blades, prevented root integrity, and enhanced growth. It is suggested that during osmotic stress trehalose or carbohydrates might be more important for rice than proline. Gaff[14] reported that tobacco plants transformed for gene Tps1, which enables transgenic plants to synthesize the disaccharide trehalose, exhibit increased drought tolerance compared to the wild-type. The influence of trehalose on osmoregulation was put forward as an explanation of improved water retention and desiccation tolerance. His data also indicated that stomata in the transgenic plants begin closing at milder drought stress than stomata in non-transgenic plants, thus improving water retention.

The present work aims to elucidate the trehalose application for increasing tolerance of maize grown under salinity-induced stress. Growth of maize seedlings, chlorophyll content, photosynthetic activity, enzyme activity, organic solutes, and electrophoretic protein banding were investigated. Effect of trehalose on permeability of root plasma membrane and lipid peroxidation were also studied.

MATERIALS AND METHODS

Plant Materials and Growth Conditions: Maize grains (*Zea mays,* cv. Giza 2) were obtained from the Agriculture Research Center, Giza, Egypt. The grains were sterilized by using 2.5% sodium hypochlorite for 3 minutes, and then washed with distilled water. Maize grains were soaked for 8 hours in different concentrations of trehalose (2-20 mM), or in distilled

water, as a pre-sowing treatment. The seeds were then washed and sown in sand culture and irrigated with Hoagland's nutrient solution. Salinity was induced by the addition of NaCl and $CaCl_2$ to adjust the nutrient solution at -0.2 MPa[52], since the osmotic potentials of -0.4 and -0.6 MPa greatly inhibited seedling growth. 10 mM trehalose was the most effective concentration on seed germination and early growth. Growth criteria (shoot and root length, shoot and root fresh and dry mass, and leaf area) and chemical analyses were measured after 14 days from sowing. Relative water content was measured as:

$$\frac{\text{Fresh weight - Dry weight}}{\text{Fresh weight}} \times 100$$

Enzyme Assay: Activity of the hydrolytic enzymes, amylase and protease was assayed in the fresh leaves after 14 days from sowing. The cell-free extract of the plant material was prepared at 0-4 °C by macerating the leaves with a chilled pestle and mortar. The tissue homogenate was centrifuged at 10,000g for 20 min and the supernatant obtained was used directly for determining enzyme activity. For assaying the activity of amylase, soluble starch was used as the enzyme substrate in phosphate buffer (pH 7.0) and the produced maltose was measured by using 3,5-dinitrosalicylic acid reagent as described by Rick and Stegbauer[40]. Protease activity was assayed according to the method described by Gallop *et al.*[15] using casein as substrate in phosphate buffer (pH 7.2) and Folin phenol as the color reagent for measuring the produced peptides.

Chemical Analysis: Photosynthetic pigments [chlorophyll (Chl *a*, Chl *b*) and carotenoids (Car.)] were estimated in 85% acetone-extracted leaves according to Metzner *et al.*[29]. Photosynthetic activity was measured as Hill-reaction activity of the isolated chloroplasts. For isolation of chloroplasts, the method of Aronoff[1] and Osman *et al.*[37] was followed. Hill-reaction of the isolated chloroplasts was measured by using potassium ferricyanide as electron acceptor in the light reaction. Fresh seedling leaves were extracted in 70% ethanol and completed to a known volume with distilled water and used for estimation of total soluble sugars using anthrone reagent[49] and total soluble proteins by using Folin phenol reagent[26]. Nucleic acids were extracted by the method of Marmur[27] in Tris-EDTA buffer (pH 8.5). DNA was quantitatively determined using diphenylamine by the method adopted by Dische and Schwartz[12]. RNA was quantitatively determined using $FeCl_3$-orcinol reagent by the method adopted by Ashwell[3]. Fresh leaves was homogenized in salfosalisylic acid, and acid-ninhydrin reagent was used for determination of proline according to Bates *et al.*,[5]. Electrophoretic protein banding was carried out at the Central Laboratory of Agricultural Research center, Giza, Egypt. Separation of proteins was performed using Sodium Dodicyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE), according to the method of Laemmli[24]. Potassium and sodium ions were measured photometrically in acid-digested samples using a Corning-400 Flame Photometer. Fresh root segments were used for measuring of lipid-peroxidation by the thiobarbituric acid (TBA) color reaction according to Bernheim *et al.*,[7]. The relative permeability of the root membranes was calculated as described by Zwiazek and Blake[56]. For total electrolyte leakage assay, 10 embryonic axes, primary root tips or hypocotyls were excited and washed briefly with deionized water to remove adhering electrolytes. The tissue sections were then immersed in test tubes at 28 °C. After 5 h, the electrolyte leakage was estimated by a conductivity meter. The samples were then boiled for 30 min and the conductivity measured again. The percentage of leakage was calculated as:

$$\frac{\text{conductivity before boiling}}{\text{conductivity after boiling}} \times 100$$

Statistical Analysis: Statistical analysis was carried out according to Snedecor and Cochran[44] using analysis of variance and the significance was determined using LSD values at P = 0.05 and 0.01.

RESULTS AND DISCUSSION

Salinity at -0.2 MPa significantly reduced shoot height (Table 1). Trehalose pre-sowing treatment at 10 mM concentration stimulated shoot height of maize (Giza 2) under normal and salinity-induced stress conditions. Salinity also affected both fresh and dry mass of shoot (Table 1). However, trehalose treatment significantly increased shoot fresh and dry mass of salt-stressed seedlings. Leaf area, which represent the photosynthetic area, indicated a marked reduction in response to salinity stress (Table 1), but the trehalose treatment significantly increased this value of salt-stressed and unstressed seedlings. It was interesting to observe a considerable increment in root length of trehalose-treated plants more than the control and the salt-stressed plants. Fresh and dry mass of roots also increased in response to trehalose treatment under stress and un-stress conditions (Table 1). Trehalose reduced the

Table 1: Effect of trehalose treatment on growth criteria [shoot and root length (cm), fresh and dry mass of shoot and root (g), leaf area (cm^2)], and relative water content of shoot and root cells (%) of maize seedlings (14-d-old) under salinity-induced stress conditions.

Treatment	Shoot				Leaf area	Root			
	height	fresh mass	dry mass	relative water content		height	fresh mass	dry mass	relative water content
Control	26.17	1.34	0.098	92.69	19.74	13.80	1.157	0.118	89.8
Trehalose(10 mM)	28.55	1.47	0.102	93.06	23.44	16.75	1.256	0.127	89.89
Salinity(-0.2 MPa)	23.51	0.80	0.070	91.25	17.42	12.75	0.928	0.094	89.87
Salinity + Trehalose	25.50	0.95	0.076	92	20.89	15.95	1.126	0.114	89.88
LSD at 0.05	0.31	0.01	0.005		0.10	0.08	0.08	0.006	
LSD at 0.01	0.41	0.02	0.007		0.13	0.11	0.11	0.008	

inhibitory effects of salinity on growth may be through improving the water status of the plant tissues, since the relative water content of the shoot increased (Table 1). On the other hand, the relative water content of the root did not affected by salinity and trehalose treatment may be due to the increased osmotic potential of the root cells by the excess of ion accumulation. Gaff[14] indicated that trehalose in transgenic plants improves water retention and desiccation tolerance through osmoregulation and stomatal closing at milder drought stress.

hyll (Chl. *a*, *b*), carotenoids (Car), and total pigments content of maize leaves was significantly decreased by salinity stress (Table 2). Trehalose treatment stimulated chlorophyll synthesis under normal and salinity-stress conditions. One visible symptom of ion accumulation in leaves is a concomitant loss of chlorophyll[51], indicating some form of disruption of the chloroplasts. The reduction in growth criteria as a result of reducing the anabolic processes by the influence of salinity could be attributed to the limiting effect of salinity-induced stress on the chlorophyll content and photosynthetic activity. Photosynthetic activity was indicated by Hill-reaction activity (Table 2). It was markedly decreased in salt-stressed leaves. Trehalose treatment exhibited a positive effect on the activity of the Hill-reaction in salt stressed and unstressed leaves. Salinity limits the photosynthetic activity may be through lowering the cellular water potential, the increased leaf content of sodium and chloride ions, and/or disturbance in the electron transport chain. Stepien, and Klobus[46] revealed a considerable decrease in the efficiency of PS II and electron-transport chain (ETC) under salinity stress. Similar results have been obtained by Sepehr and Ghorbanli[43] and Liu, *et al*.[25]. Trehalose may be preserved the stability of the chloroplast envelope and maintained the osmotic potential of the chloroplast.

Nucleic acids content was in parallel with growth parameters. Leaf content of DNA and RNA decreased under salinity stress (Table 2). However, their content increased in the seedling leaves in response to trehalose treatment. Growth which is the integral of cell division and cell elongation was negatively affected by salinity which reduced the cellular water content and decreased content of nucleic acids required for anabolic processes. Zeid[53] suggested that the decrease in nucleic acids content concurrently with the increase in RNase activity caused by the increase in salinity level might be involved in inhibiting nucleic acids biosynthsis and/or stimulating their degradation. Kabanov and Aziyashvili[22] attributed the depression of nucleic acid metabolism to the cation imbalance (in particular to the abundance of Na^+ cations in the cell) and also to intensify the activity of cytoplasmic RNase or to the reduction of phosphorus incorporation into nucleic acids. The increased content of nucleic acids by the influence of trehalose treatment indicates rise in activity of anabolic processes.

The leaf content of the organic solutes e.g., total soluble sugars and proteins, and the amino acid proline showed a great accumulation in response to salinity stress which was associated with increased activity of amylase and protease enzymes (Table 3). Meanwhile, leaves of the trehalose-treated seedlings showed a more accumulation of total soluble sugars and proteins and proline in both stressed and unstressed seedlings. This increment was also paralleled with increased hydrolytic activity of amylase and protease enzymes. The increased content of organic solutes is an important phenomenon for cellular osmoregulation[54]. Proline is one of the most common compatible solutes that contribute to osmotic adjustment[18,39,20,2], and stabilization and protection of membranes, proteins and enzymes[36,28,2] from damaging effects of salt-osmotic stresses. Proline improve salt tolerance by up regulating stress-protective proteins[23] and reducing oxidation of lipid membranes[11.34].

K^+/Na^+ ratio in seedling roots and leaves indicated a great reduction with increasing salt concentration in

Table 2: Effect of trehalose treatment on photosynthetic pigments content (mg g^{-1} d.m.), photosynthetic activity (µg ferricyanide mg^{-1} chl. g^{-1} f.m.), and nucleic acids content (mg g^{-1} d.m.) of maize seedling leaves (14-d-old) under salinity-induced stress conditions.

Treatment	Chl *a*	Chl *b*	Carotenoids	Total pigments	Photosynthetic activity	DNA	RNA
Control	21.28	12.26	16.24	49.78	23.68	18.09	24.81
Trehalose(10 mM)	24.87	13.03	16.72	54.62	26.97	19.56	26.78
Salinity(-0.2 MPa)	18.21	10.37	14.83	43.41	17.62	14.58	19.68
Salinity + Trehalose	20.98	11.68	15.75	48.41	21.61	15.96	23.31
LSD at 0.05	0.02	0.02	0.06		1.17	0.06	0.05
LSD at 0.01	0.04	0.04	0.08		1.53	0.08	0.07

Table 3: Effect of trehalose treatment on amylase and protease activity (units g^{-1} f.m. s^{-1}), total soluble sugars and proteins content (mg g^{-1} d.m.), proline content (µ mole g^{-1} f.m.), and K/Na ion ratio in maize seedling leaves (14-d-old) under salinity-induced stress conditions.

Treatment	Amylase	Protease	Total soluble sugars	Total soluble proteins	Proline	K^+/Na^+	
						in root	in shoot
Control	22.45	2.48	32.82	44.17	6.68	1.695	9.027
Trehalose(10 mM)	25.44	3.35	38.96	51.25	10.68	0.614	4.944
Salinity(-0.2 MPa)	26.74	4.66	43.58	71.16	8.74	0.153	3.71
Salinity + Trehalose	29.28	5.68	48.26	80.22	14.5	0.159	4.61
LSD at 0.05	0.01	0.005	0.06	0.08	0.014		
LSD at 0.01	0.02	0.007	0.08	0.11	0.018		

the soil solution (Table 3). Trehalose treatment increased this ratio in the leaves indicating alleviation of the adverse effects of Na^+ ions in the leaves. Garcia *et al*.[16] noticed that the increased soil salinity promoted by irrigation with saline water increased the sodium content and also the relationships Na^+/Ca^{2+}, Na^+/Mg^{2+}, Na^+/K^+, but decreased the contents of Ca, Mg and K, therefore, characterizing the unbalance and the nutritional stress consequent to the progressive saline stress. Zhang *et al*.[55] indicated that under high salt stress the maize transgenic plants compartmentalized more Na^+ in the roots and kept a relative high K^+/Na^+ ratio in the leaves compared with wild-type plants. Trehalose, on the other hand, did not prevent plants from taking up excess NaCl, but it did reduce Na^+ accumulation in laminae. This alone may have allowed plants to continue growing without loss of chlorophyll. One possibility is that by preserving the integrity and native state of proteins and lipid bilayers[9,10,21,8], it maintains the pumps specially needed to exclude excess NaCl from the photosynthetic organelles.

Relative permeability of root membranes was measured as the amount of ion leakage from the root cells, and expressed as the percent of increase or decrease from the control (Fig. 1). The results indicated a great increase in relative permeability of salt-stressed roots. The increased relative permeability of the root plasma membranes was associated with increased rate of lipid peroxidation. The rate of lipid peroxidation was measured in terms of thiobarbituric acid-reactive substances (TBARS) content, and expressed as the percent of increase or decrease from the control (Fig. 1). Trehalose treatment decreased the relative permeability of salt-stressed and unstressed seedlings may be through preserving the stability of the plasma membrane. The reducing effect of trehalose on lipid peroxidation may indicate that trehalose is an antioxidant agent, or at least play a role in this respect. Stepien and Klobus[43] and Azevedo *et al*.[4] indicated a similar results in response to salinity stress.

Protein banding patterns in the seedling leaves showed a wide variation in response to salinity-induced stress and trehalose treatment (Table 4 and Fig. 2). Protein bands of control leaves were 19 in number and ranged between 113.93 and 15.95 KDa molecular weights. Trehalose treatment resulted in the appearance of one new band (14.79 KDa) to increase the total number to 20 bands. Seven bands with molecular weights 113.93, 108.89, 106.24, 76.58, 53.89, 42.01 and 34.49 KDa were replaced by lower molecular weights (111.15, 106.67, 98.86, 74.74, 52.92, 41.13 and 33.42 KDa); while another five bands (30.56, 27.75, 23.48, 21.46 and 20.95 KDa) were replaced by higher molecular weights (32.03, 30.6, 28.04, 23.68 and 21.72 KDa). Salt-stressed leaves showed 24 bands with the

Table 4: Protein banding pattern of maize seedling leaves (14-d-old) in response to trehalose treatment under salinity stress conditions.

Band number	ControlKDa	TrehaloseKDa	SalinityKDa	Salinity + TrehaloseKDa
1	113.93	111.15	112.99	111.61
2	108.89	106.67	108.44	107.99
3	106.24	98.86	103.01	104.72
4	76.58	74.74	99.27	100.71
5	53.89	52.92	82.85	82.85
6	42.01	41.13	71.21	68.67
7	35.96	35.78	58.66	59.38
8	34.85	34.53	44.03	43.84
9	34.49	33.42	37.29	36.66
10	33.47	33.01	35.23	35.27
11	30.56	32.03	34.79	34.81
12	27.75	30.6	33.93	33.63
13	23.48	28.04	33.38	30.78
14	21.46	23.68	30.81	27.99
15	20.95	21.72	28.53	23.31
16	19.69	19.99	23.72	21.65
17	17.81	17.97	22.25	20.1
18	17.03	17.09	21.35	17.51
19	15.95	16.01	20.27	16.03
20	0	14.79	18.25	15.23
21	0	0	17.36	0
22	0	0	16.2	0
23	0	0	15.2	0
24	0	0	14.74	0
Total	19	20	24	20

appearance of five new bands having molecular weights of 18.25, 17.36, 16.2, 15.2 and 14.74 KDa. Two bands (113.93 and 106.24 KDa) were replaced by lower molecular weights (112.99 and 103.01KDa); while another 16 bands with molecular weights 76.58, 53.89, 42.01, 35.96, 34.85, 34.49, 33.47, 30.56, 27.75, 23.48, 21.46, 20.95, 19.69, 17.81, 17.03 and 15.95 KDa were replaced by higher molecular weights (99.27, 82.85, 71.21, 58.66, 44.03, 37.29, 35.23, 34.79, 33.93, 33.38, 30.81, 28.53, 23.72, 22.25, 21.35 and 20.27 KDa). Trehalose treatment restored the total number of bands to become 20 bands under salinity stress, with the appearance of one new band. Three bands (113.93, 108.89 and 106.24 KDa) were replaced by lower molecular weights (111.61, 107.99 and 104.72); while bands (76.58, 53.89, 42.01, 35.96, 34.85, 34.49, 33.47, 30.56, 27.75, 23.48, 21.46, 20.95, 19.69 and 17.81 KDa) were replaced by higher molecular weights (100.71, 82.85, 68.67, 59.38, 43.84, 36.66, 35.27, 34.81, 33.63, 30.78, 27.99, 23.31, 21.65 and 20.1 KDa). Therefore, trehalose treatment reduced salt expression as also indicated by Garcia *et al.*[17].

In conclusion, pretreatment with trehalose enhanced tolerance mechanisms to enable plants to grow under salinity stress conditions. Trehalose treatment decreased the rate of electrolyte leakage and the rate of lipid peroxidation of maize root cells, indicating a more stabilization of the cell membranes and a stimulation of

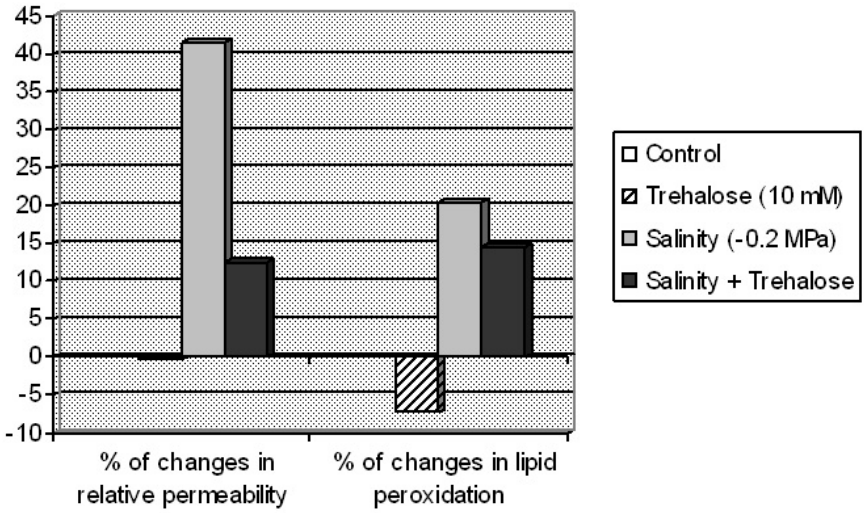

Fig. 1: Effect of trehalose treatment on the relative permeability of the root membranes, and the rate of lipid peroxidation of maize roots under salinity stress conditions. The values were expressed as percent of increase or decrease from the control.

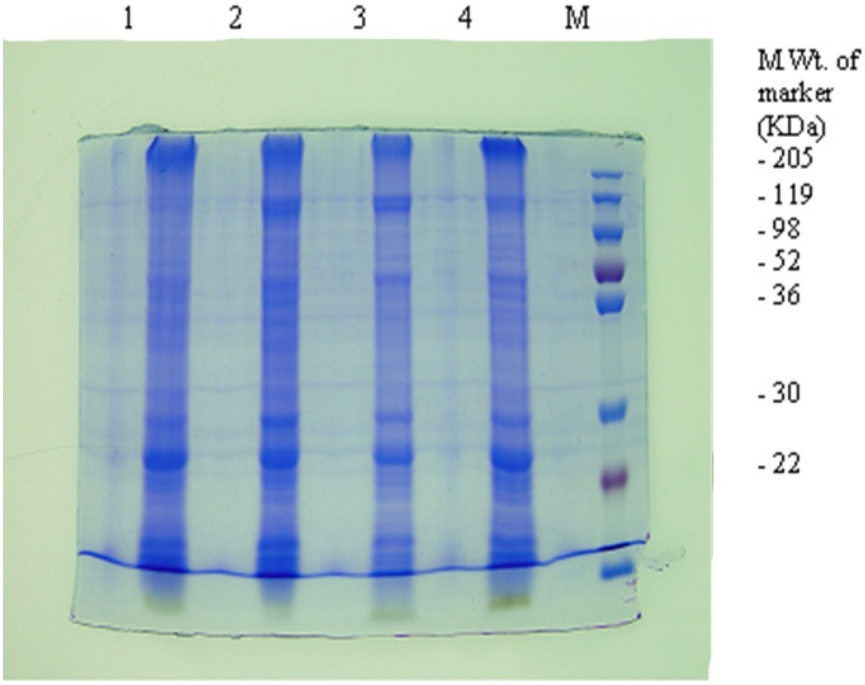

Fig. 2: Protein banding pattern of maize seedling leaves in response to trehalose treatment under salinity stress conditions. M: marker; 1: control; 2: trehalose; 3: salinity; 4: salinity + trehalose.

antioxidation processes. Trehalose treatment led to a retention of leaf water content, enhanced growth, particularly root length and leaf area, and stimulated metabolic activity. K^+/Na^+ ratio increased in leaves of trehalose-treated plants, alleviating the adverse effects of salinity on chlorophyll, photosynthetic activity and nucleic acids content. Protein banding pattern, which indicate the gene expression exhibited a reduction in salt expression by the influence of trehalose treatment.

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