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Effects of salinity and heat-shock on wheat seedling growth and content of carbohydrates, proteins and amino acids

A. M. HAMADA and E. M. KHULAEF*

Botany Department and Chemistry Center Laboratory, Faculty of Science, Assiut University, Assiut 71516, Egypt*

Abstract

The effects of salinity (0, 50, 100, 150, and 200 mM NaCl) and heat-shock (42 °C) and their interactions on germination, seedling growth, and some relevant metabolic changes of two cultivars (cv. Giza 155 and cv. Stork) of wheat (*Triticum vulgaris* L.) were studied. Germination studies indicate that plants tolerated salinity up to 100 mM NaCl. The lengths of roots and shoots and their water content, as well as fresh and dry matter yield of cv. Giza 155 seedlings remained more or less unchanged up to 100 mM NaCl and of cv. Stork up to 50 mM NaCl. Salinity induced progressive increase in soluble carbohydrates, soluble proteins and proline in cv. Giza 155 and in soluble proteins, proline and other free amino acids in cv. Stork. However, under the higher salinity levels, in cv. Giza 155 increase in soluble carbohydrates was accompanied by lose in other free amino acids, whereas in cv. Stork an opposite effect was obtained. Heat-shock treatment (42 °C for 24 h) induced a significant decrease in the final germination percentage, the shoot and root lengths, fresh matter yield and the water content. The dry matter yield of the two cultivars was considerably increased as compared with the corresponding treatments with NaCl only. Heat-shock treatment resulted in a significant increase in the amount of soluble carbohydrates and proline in salt treated seedlings of both cultivars. The pattern of changes in amino acids was opposite to that of soluble proteins, indicating that the increase in soluble proteins was at the expense of other amino acids in cv. Giza 155 and *vice versa* in cv. Stork.

Key words: germination, NaCl, salinity tolerance, *Triticum vulgaris* L.

Introduction

Under natural conditions plants may be simultaneously exposed to salinity and high temperature. These effects are not well documented although the separate effects of heat-shock and salinity on plant growth have been studied extensively. Vinizky and Ray (1988), Coons *et al.* (1990) and Fowler (1991) showed that seed germination

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was more inhibited by NaCl under higher temperature. Other stages of plant growth may also be affected by the strong interactions between salinity and temperature. For example, salinity injury to azalea became more pronounced when growth temperature was raised (Oertli 1960).

The present study was thus undertaken to investigate the interactions between the heat-shock and NaCl salinity on growth and some metabolic changes in two wheat cultivars.

Materials and methods

The effect of 0 (control), 50, 100, 150 and 200 mM NaCl in 1/10 Hoagland solution on germination and seedling growth of wheat (*Triticum vulgaris* L.) cvs. Giza 155 and Stork were studied. Twenty-five grains of the test cultivars were pretreated with 10 % *Colorx* and 5.25 % sodium hypochlorite for 4 min and then were germinated in Petri dishes at about 23 °C (6 replicate per treatment). Grains were considered to be germinated after the radicle emerged from the testa. After 3 d, half of Petri dishes from each treatment were incubated at 42 °C (heat-shock temperature) for 24 h. After 4 d of germination the length of shoots and roots of germinated seeds were measured. The fresh shoots and roots were then dried in an aerated oven at 70 °C during which successive weighing was carried out until a constant dry mass was reached.

Soluble carbohydrates were extracted with 5 % trichloro acetic acid (TCA) and determined according to the method of Naguib (1964). A calibration curve using pure glucose was made. Soluble proteins were determined according to Lowry *et al.* (1951). A calibration curve was constructed using egg albumin. Free proline was determined according to Bates *et al.* (1973). The proline concentration was determined using a standard curve and calculated on a dry mass basis. Total free amino acids, other than proline, were extracted from the plant tissues and determined according to the method of Moore and Stein (1948). A calibration curve was constructed using L-histidine.

Results

NaCl up to concentration of 100 mM had no inhibitory effect on the germination of both cv. Giza 155 and cv. Stork. The germination was inhibited when higher concentrations of NaCl was applied (Table 1). The inhibitory effects of high concentrations of NaCl on germination were further increased by heat shock.

The lengths of roots and shoots as well as fresh and dry matter of cv. Giza 155 seedlings remained more or less unaffected up to the level of 100 mM NaCl, but above this level growth parameters decreased sharply (Table 1). It is worthy to note that a concentration of 50 and 100 mM NaCl even stimulated root growth, fresh and dry matter of cv. Giza 155. In case of cv. Stork, the lengths of roots and shoots, as well as fresh and dry matter remained unchanged up to 50 mM NaCl, at greater concentrations a decrease was observed (Table 2). Heat-shock resulted in a further decrease in the final germination percentages, the shoot and root lengths as well as fresh and dry matter yield of both cultivars.

Table 1. Effect of salinity only and combined with heat-shock on percentage of grain germination, shoot and root lengths [cm seedling^{-1}], fresh and dry matter yields [g seedling^{-1}], and water content [g g^{-1}(d.m.)] of two wheat cultivars.

	NaCl [mM]	Germination	Shoot lengths	Root lengths	Fresh mass	Dry mass	Water content
		salinity					
cv. Giza 155	0	100 Aa	14.80 Aa	12.10 Ba	0.655 Ca	0.110 Ba	4.955 Ba
	50	100 Aa	15.10 Aa	14.60 Aa	0.740 Aa	0.118 Aa	5.271 Aa
	100	100 Aa	15.00 Aa	13.20 Aa	0.707 Ba	0.112 Ba	5.313 Aa
	150	85 Ba	9.70 Ba	10.10 Ca	0.450 Da	0.103 Cb	3.369 Ca
	200	65 Ca	5.40 Ca	6.10 Da	0.262 Eb	0.094 Db	1.787 Db
cv. Stork	0	100 Aa	13.50 Aa	15.20 Aa	0.621 Aa	0.103 Ba	5.029 Aa
	50	100 Aa	13.80 Aa	15.00 Aa	0.552 Ba	0.124 Aa	3.452 Ba
	100	98 Aa	10.90 Ba	11.30 Ba	0.479 Ca	0.121 Ab	2.959 Ca
	150	80 Ba	6.80 Ca	8.40 Ca	0.401 Da	0.108 Bb	2.713 Ca
	200	63 Ca	4.90 Da	5.10 Da	0.397 Da	0.100 Cb	2.970 Ca
		salinity + heat-shock					
cv. Giza 155	0	100 Aa	13.60 Ab	12.00 Ba	0.563 Ab	0.103 Db	4.466 Aa
	50	100 Aa	11.90 Bb	13.80 Ab	0.480 Bb	0.108 Cb	3.444 Bb
	100	95 Aa	10.30 Cb	10.30 Cb	0.412 Cb	0.118 Aa	2.492 Cb
	150	83 Ba	7.70 Db	8.00 Db	0.380 Db	0.111 Ba	2.423 Cb
	200	60 Ca	5.00 Ea	6.20 Ea	0.312 Ea	0.103 Da	2.029 Da
cv. Stork	0	100 Aa	12.10 Ab	13.50 Ab	0.368 Bb	0.103 Ca	2.573 Ab
	50	100 Aa	12.90 Ab	13.60 Ab	0.427 Ab	0.128 Aa	2.336 Bb
	100	92 Aa	9.30 Bb	10.20 Bb	0.298 Cb	0.127 Aa	1.346 Cb
	150	81 Ba	5.20 Cb	7.30 Cb	0.269 Db	0.124 Aa	1.169 Db
	200	55 Cb	3.20 Db	4.90 Da	0.229 Eb	0.117 Ba	0.957 Eb

Means which are not significantly different ($P = 0.05$) are followed by the same letter (capital letters-different concentrations of NaCl, small letters - heat-shock).

The water content of cv. Giza 155 seedlings (Table 1) remained more or less unchanged or even slightly increased up to the 100 mM NaCl. In cv. Stork water content of seedlings decreased as the concentration of NaCl increased. Heat-shock treatment induced a significant decrease in the water content of seedlings of both cultivars.

The content of soluble carbohydrates (Table 2) of cv. Giza 155 increased with the rise in salinity, whereas in cv. Stork an opposite pattern was observed. Heat-shock treatment resulted in a significant increase in the amounts of soluble carbohydrates in salt-treated cv. Giza 155 or cv. Stork seedlings.

Soluble proteins content of salt-treated cv. Giza 155 or cv. Stork seedlings was markedly higher than that of the control. In cv. Giza 155 (Table 2) the increase in salinity in the culture medium was associated with a gradual increase in soluble proteins also under heat-shock treatment, in cv. Stork the different salinity levels under heat-shock treatment failed to induce any stimulation in their content.

Table 2. Effect of salinity only and combined with heat-shock on soluble carbohydrates, soluble proteins, proline and other free amino acids [mg g^{-1}(d.m.)] on two wheat cultivars.

	NaCl [mM]	Salinity soluble carbohydr.	Salinity soluble proteins	Salinity proline	Salinity amino acids	Salinity + heat shock soluble carbohydr.	Salinity + heat shock soluble proteins	Salinity + heat shock proline	Salinity + heat shock amino acids
cv. Giza	0	114.42Eb	26.36Ca	2.41Db	60.83Ab	174.88Ca	25.46Ba	3.08Da	67.50Aa
155	50	154.42Db	30.46Ba	2.58Db	51.67Bb	223.26Ba	23.82Bb	3.92Da	55.00Ba
	100	206.51Cb	34.09Aa	4.26Cb	39.17Cb	245.58Aa	25.00Bb	5.18Ca	43.33Ca
	150	251.16Ba	34.55Aa	5.04Bb	32.50Db	256.28Aa	31.82Ab	6.30Ba	35.83Da
	200	279.07Aa	36.82Aa	6.87Ab	25.00Eb	253.26Ab	34.09Ab	8.74Aa	32.50Da
cv. Stork	0	273.49Aa	28.18Bb	2.56Eb	26.67Ca	240.00Db	33.18Aa	3.23Ea	18.75Cb
	50	269.77Aa	28.41Bb	3.01Db	28.75Ba	245.58Db	31.36Aa	4.22Da	25.00Bb
	100	253.49Bb	30.91Ba	4.56Cb	30.00Ba	311.63Ca	30.00Ba	5.57Ca	31.25Aa
	150	241.86Cb	36.36Aa	6.08Bb	33.33Aa	418.61Ba	28.18Bb	7.12Ba	32.92Aa
	200	223.26Db	37.27Aa	7.12Ab	35.83Aa	497.67Aa	25.46Cb	8.82Aa	35.42Aa

Means which are not significantly different ($P = 0.05$) are followed by the same letter (in a column - capital letter, in a row - small letter).

The proline progressively accumulated, in the both cultivars, with increasing NaCl concentration (Table 2) and heat-shock resulted in a further increase in the content of proline irrespective of the concentration of NaCl.

The free amino acids accumulation decreased in cv. Giza 155 and increased in cv. Stork with the increase in concentration of NaCl. Under heat-shock treatment of cv. Giza 155, the production of free amino acids was gradually reduced (Table 2). In cv. Stork, all the investigated salinity levels stimulated production of free amino acids under heat-shock treatment (Table 2).

Discussion

The data obtained in the present work clearly demonstrate that, cv. Giza 155 can tolerate salinity up to the concentration of 100 mM whereas cv. Stork up to the concentration of 50 mM of NaCl. Tolerance up to this level was closely associated with a relatively stable water contents. However Uhvits (1964), Heikal *et al.* (1982), Shaddad and Zidan (1989) and Shaddad *et al.* (1990) pointed out that the decrease in final germination percentage was always associated with a decrease in water absorption.

Salinity induced a progressive increase in amounts of soluble carbohydrates and soluble proteins in cv. Giza 155. In cv. Stork only the level of soluble proteins was raised. Proline content in both cultivars was progressively raised with the rise of salinity level. These results are in agreement with the results obtained by many other authors (*e.g.* Zoglauer *et al.* 1987, Venekamp *et al.* 1989, Shaddad *et al.* 1990). The increase in soluble components may play an important role in osmotic adjustment; a conclusion which is in accordance with the results obtained *e.g.* by Hellebust (1976) and Flowers *et al.* (1977) working with halophytic plants and Munns *et al.* (1979), Drossopoulos *et al.* (1987) and Shaddad *et al.* (1990) working with glycophytic plants.

In addition proline can serve as a protector of enzyme denaturation (Paleg *et al.* 1984), a reservoir of nitrogen and carbon (Fukutaku and Yamada 1984), or as a stabilizer of the machinery for protein synthesis (Kardpal and Rao 1985).

In cv. Giza 155, the free amino acids accumulation showed marked decrease as the concentration of NaCl was increased. Thus salinity induced a promotion in the conversion of the other amino acids into proline (Boggess *et al.* 1976, Faheed 1987 and Shaddad *et al.* 1990). In cv. Stork, all the investigated salinity levels stimulated production of free amino acids. In this respect, Hsiao (1973) and Stewart and Larher (1980) showed that free amino acids were variously accumulated under salinity stress. The present results showed that heat-shock increased the suppressive effect of salinity on seed germination and seedling growth and further increased the amounts of soluble carbohydrate and proline. The pattern of changes in amino acids was opposite that of soluble proteins, indicating that the increase in soluble protein is at the expense of other amino acids through an effect of salinity and heat-shock in promoting their conversion. Krishnasamy *et al.* (1988) showed that heat-shock proteins (HSP) were only induced after a gradual increase in temperature rather than after heat-shock. HSP were observed in the soluble protein fraction (Clarke and Critchley 1990).

References

Bates, L.S., Waldren, R.P., Teare, I.D.: Rapid determination of free proline for water stress studies. - Plant Soil **39**: 205-207, 1973.

Boggess, S.F., Aspinall, D., Paleg, L.G.: Stress metabolism. IX. The significance of end product inhibition of proline synthesis and of compartmentation in relation to stress-induced proline accumulation. - Aust. J. Plant Physiol. **3**: 513-525, 1976.

Clarke, A.K., Critchley, C.: Synthesis of early heat shock proteins in young leaves of barley and sorghum. - Plant Physiol. **94**: 567-576, 1990.

Coons, J.M., Kuehl, R.O., Simons, N.R.: Tolerance of ten lettuce cultivars to high temperature combined with NaCl during germination. - J. amer. Soc. hort. Sci. **115**: 1004-1007, 1990.

Drossopoulos, J.B., Karamanos, A.J., Niavis, C.A.: Changes in ethanol soluble carbohydrates during the development of two wheat cultivars subjected to different degrees of water stress. - Ann. Bot. **59**: 173-180, 1987.

Faheed, F.A.: Combined effect of salinity and some plant hormones on growth and chemical composition of some plants. - M.Sc. Thesis, Assiut Univ., Sohag 1987.

Flowers, T.J., Troke, P.F., Yeo, A.R.: The mechanism of salt tolerance in halophytes. - Annu. Rev. Plant Physiol. **28**: 89-121, 1977.

Fowler, J.L.: Interaction of salinity and temperature on the germination of crambe. - Agron. J. **83**: 169-172, 1991.

Fukutaku, Y., Yamada, Y.: Sources of proline nitrogen in water-stressed soybean (*Glycine max*). II

Fate of ^{15}N-labelled protein. - Physiol. Plant. **61**: 622-628, 1984.

Heikal, M.M., Shaddad, M.A., Ahmed, A.M.: Effect of water stress and gibberelic acid on germination of flax, sesame and onion seeds. - Biol. Plant. **24**: 124-129, 1982.

Hellebust, J.A.: Osmoregulation. - Annu. Rev. Plant Physiol. **27**: 485- 505, 1976.

Hsiao, T.C.: Plant response to water stress. - Annu. Rev. Plant Physiol. **24**: 519-570, 1973.

Kardpal, R.P., Rao, N.A.: Alterations in the biosynthesis of proteins and nucleic acids in finger millet (*Eleucine coracana*) seedling during water stress and the effect of proline on protein biosynthesis. - Plant Sci. **40**: 73-79, 1985.

Krishnasamy, S., Mannar Mannan, R., Krishnan, M. Gnanam, A.: Heat shock response of the chloroplast gnome in *Vigna sinensis*. - J. biol. Chem. **263**: 5104-5109, 1988.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. Randall, R.J.: Protein measurement with the Folin phenol reagent. - J. biol. Chem. **193**: 265-275, 1951.

Moore, S., Stein, W.W.: Photometric ninhydrin method for use in the chromatography of amino acids. - J. biol. Chem. **176**: 367-388, 1948.

Munns, R., Brady, C.I., Barlow, E.W.R.: Solutes accumulation in the apex and leaves of wheat during water stress. - Aust. J. Plant Physiol. **6**: 379-389, 1979.

Naguib, M.I.: Effect of sevin on the carbohydrate and nitrogen metabolism during the germination of cotton seeds. - Indian J. exp. Biol. **2**: 149-154, 1964.

Oertli, J.J.: Der Einfluss der Temperature auf die Salzempfindlichkeit der Azaleen. - Agrochimica **4**: 314-323, 1960.

Paleg, L.G., Stewart, G.R., Bradbeer, J.W.: Proline and glycine betaine influence protein salvation. - Plant Physiol. **75**: 974- 978, 1984.

Shaddad, M.A., Zidan, M.A.: Effect of NaCl salinity on the rate of germination, seedling growth, and some metabolic changes in *Raphanus sativus* L. and *Trigonella foenum-graecum* L. - Beitr. trop. Landwirtsch. vet. Med. **27**: 187-194, 1989.

Shaddad, M.A., Radi, A.F., Abdel-Rahman, A.M. Azooz, M.M.: Response of seeds of *Lupinus termis* and *Vicia faba* to the interactive effect of salinity and ascorbic acid or pyridoxine. - Plant Soil **122**: 177-183, 1990.

Stewart, G.R., Larher, F.: Accumulation of amino acids and related compounds in relation to environmental stress. - In: Miflin, B.J. (ed.): The Biochemistry of Plants. Vol. 5. Pp. 609-635. Academic Press, New York 1980.

Uhvits, R.: Effect of osmotic pressure on water absorption and germination of alfalfa seeds. - Amer. J. Bot. **33**: 278-285, 1964.

Venekamp, J.H., Lamppe, J.E.M., Koot, T.M.: Organic acids as sources for drought-induced proline synthesis in field bean plants, *Vicia faba* L. - J. Plant physiol. **133**: 654-659, 1989.

Vinizky, I., Ray, D.T.: Germination of guar seed under salt and temperature stress. - J. amer. Soc. hort. Sci. **113**: 437-440, 1988.

Zoglauer, K., Dembny, H., Göring, H.: Inhibition of IAA-induced ethylene production and proline accumulation in wheat coleoptiles by fusicoccin. - Biochem. Physiol. Pflanz. **182**: 23-29, 1987.