AAB BIOFLUX
Advances in Agriculture & Botanics-
International Journal of the Bioflux Society

Effect of NaCl stress on protein pattern changes in embryogenic callus of the date palm (*Phoenix dactylifera* L.) cv. Ashkar

[1]Muayed F. Abbas, [1]Abbas M. Jasim, [2]Batool H. Al-Zubaidy

[1] College of Agriculture, Basrah University, Basrah, Iraq; [2] Date Palm Research Center, Basrah University, Basrah, Iraq. Corresponding author: M. F. Abbas, muayedfadhil@yahoo.co.uk

Abstract. Effect of NaCl stress on changes in protein pattern of embryognic callus of the date palm (*Phoenix dactylifera* L.) cv. Ashkar, was investigated. Under salt stress conditions, fresh weight of the embryogenic callus decreased significantly. SDS-PAGE of protein extracted from embryogenic callus subjected to NaCl stress (0, 80, 160, 240, and 320), revealed the synthesis of new proteins. As salinity increased, the number and molecular weight of the new proteins decreased. At 240 mM NaCl and 320 mM NaCl, the following molecular weight proteins appeared on the gel: 35.0, 43.0, 59.0, 31.0, 34.0, and 54.0 kDa for both NaCl levels respectively. It is concluded that salinity stress induced the synthesis of new proteins which may play an important role in salinity tolerance of the date palm.
Key Words: New protein synthesis, molecular weight, salinity stress, SDS-PAGE.

Introduction. Salinity is one of the major abiotic stresses that adversely effect crop productivity and quality. This is due to the fact, that salinity affect adversely plant metabolism, and causes important modification in plant growth, development and gene expression. Such modifications may lead to the accumulation or depletion of certain metabolites, alteration in the behavior of many enzymes, and of particular interest is the synthesis of new proteins (Dubey 1999). Several investigations have shown the synthesis of new proteins in cultured plant cells when subjected to salinity (Erickson & Alfinito 1984; Ben-Hayyim et al 1989; Amini et al 2007; Garcia et al 2008).

The date palm (*Phoenix dactylifera* L.), is a subtropical fruit tree native to Iraq and other parts of the Middle East, and it has been a stable food in those regions since first recorded history. Although, the date palm tree is more tolerant to salinity than citrus and olive trees, the productivity of the tree decreases with increasing salinity. The reduction in productivity starts with salinity level of 45 mM NaCl and there is no production of date fruits at salinity level of 210 mM NaCl (Zaid & Liebenberg 2005). Various strategies have been used to increase crop tolerance to salinity stress, including conventional plant breeding, plant genetic engineering and plant tissue culture. Plant tissue culture has been used by some workers to produce salt tolerant cell lines and plants in several species (Ben-Hayyim & Kochba 1983; El-Hammady et al 1999).

As part of a research project on improving the salt tolerance of date palm cultivars in the Basrah area using *in vitro culture,* the effect of NaCl stress on protein pattern changes in embrogenic callus of the date palm cv. Ashkar is presented. Such information is important to understanding the molecular basis of salinity tolerance in crop plants.

Material and Method

Two-year old offshoots of date palm cv. Ashkar were used as a source of shoot tip explants. Each shoot tip was divided into four equal quarters and surface sterilized with 20% commercial bleach, then rinsed three times with sterilized distilled water. Explants were culture on MS medium (Murashige & Skoog 1962) supplemented with naphthalene

acetic acid (NAA at 30 mg L^{-1} and 2-isopentenyl adenine (2-ip) at 3 mg L^{-1} for callus induction and proliferation (Jasim et al 2010). Well developed embryogenic callus was cultured on different levels of sodium chloride (NaCl) in the culture media (0, 80, 160, 240 and 320 mM NaCl).

The effect of NaCl on fresh weight of the embryogennic callus. Fresh weight of embryogenic callus was determined at 4^{th} and 8^{th} weeks from culture.

Protein extraction and SDS-PAGE. 400 mg of freeze-dried ground sample of embryogenic callus were mixed with 1 mL of extraction buffer (0.1 Tris-HCl, pH 6.8, 2% SDS). The extract was centrifuged at 10000 rpm for six minutes. Characterization of proteins was carried out using one dimensional sodium dodecyl sulphate polyacrylamide as described by Laemmli (1970). Protein samples were prepared by mixing clear supernatant with sample buffer (0.12M Tris- Hcl, pH 6.8, 10% SDS, 10% Sucrose, and 0.1% mercaptoethanol) and denatured by heating at 90°C for 5 minutes, then loaded in equal amounts. Protein bands were separated at constant current, 20 mA for 10 minutes, and the current increased up to 144 mA until the tracking dye (Bromophenol blue, 0.05 %) reached the end of the gel. Protein bands were visualized by staining the gel with 0.01% Comassie brilliant blue R-250. Relative molecular weight of proteins was determined using a standard curve generated from standard proteins. The standard proteins were as follows: Lysozyme (14.4 kDa), Trypsin (23.0 kDa). Pepsin (34.0 kDa), Ova albumin (43.0 kDa), Bovine serum albumin (67.0 kDa) and Aldolase (158.0 kDa).

Statistical design and analysis. For the effect of NaCl on fresh weight of embryogenic callus, a completely randomized design was used with 10 replicates. Revised LSD at 5% level was used to compare mean values (Gomez & Gomez 1984).

Results and Discussion. Table 1 shows the effect of NaCl concentration on the fresh weight of embryogenic callus of the date palm cv. Ashkar, at 4^{th} and 8^{th} weeks from culture. It is clear, that NaCl at 80 mM caused a significant increase in fresh weight. This NaCl concentration is probably optimal for growth, which allows maximum possible absorption of water and essential mineral elements, with the resultant increase in fresh weight. Similar results were reported by Al-Khayri (2002) in the date palm cv. Barhi.

However, fresh weight of the embryogenic callus significantly decreased at high concentrations of NaCl, particularly at 240 and 320 mM NaCl. Such effects are probably due to osmotic effect and ion toxicity. Furthermore, it has been suggested, that the reduction of growth in response to salinity is the result of great portion of respiratory energy being diverted toward processes resulting in salt tolerance rather than growth (Munns & Tester 2008).

Table 1

Effect of NaCl concentration (mM) on fresh weight of embryogenic callus of the date palm, cv. Ashkar, each value is the mean of 10 replicates

Treatment (mM NaCl)	*Fresh weight (g) 4 and 8 weeks from culture**	
	4 weeks	*8 weeks*
Control	0.295^{b}	0.790^{b}
80 mM	0.348^{a}	1.240^{a}
160 mM	0.138^{c}	0.530^{c}
240 mM	0.064^{d}	0.101^{d}
320 mM	0.021^{e}	0.058^{e}

*Mean separation in each column by LSD at 0.05 probability level.

Figure 1 and Table 2 shows the SDS-PAGE of protein extracted from the embryogenic callus of date palm subjected to various concentrations of NaCl. It is obvious, that in control callus, seven proteins appeared on the gel, with molecular weights of 14.0, 23.0, 37.0, 66.0, 76.0, 120.0 and 151.0 kDa. However, when the embryogenic calls was

subjected to 80 mM NaCl, five proteins appeared with molecular weights of 14.0, 43.0, 79.0 93.0 and 141.0 kDa. At 160 mM NaCl the following protein appeared in the gel 14, 23.0, 32.0, 65.0 and 89.0 kDa. However, as the concentration of NaCl in the culture media increased to 240 and 320 mM, only three proteins were present on the gel with moleculer weight of 35.0, 43.0, 59.0 kDa and 31.0, 34.0 and 54.0 kDa for both concentrations of NaCl respectively. The results obtained in the present work are similar to those reported by other workers for other callus tissues and cell lines, in which salinity stress induced the synthesis of new proteins, or the disappearance of others (Ericson & Alfinito 1984; Singh et al 1985; Ben-Hayyim et al 1989; Amini et al 2007).

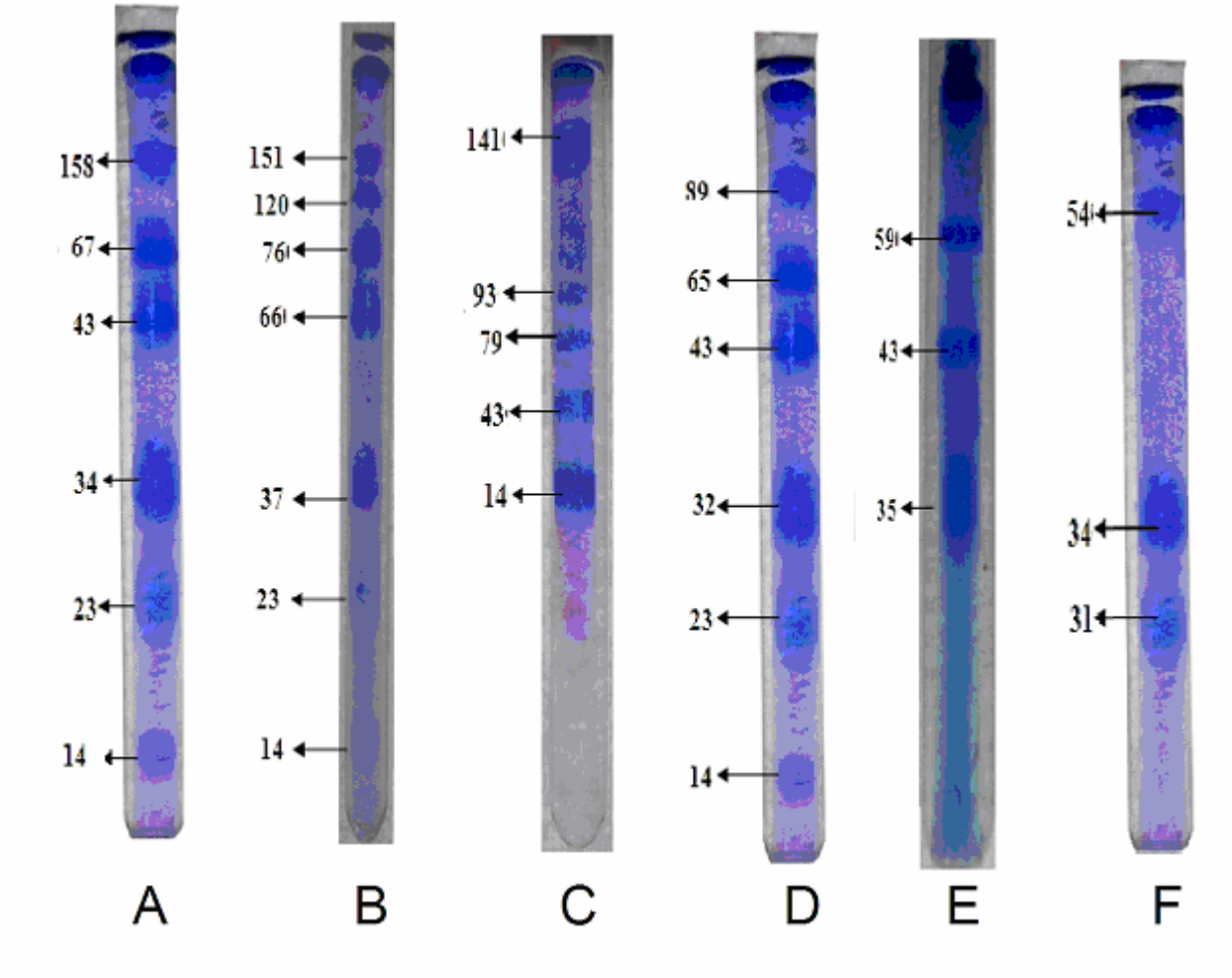

Figure 1. SDS-PAGE of protein extracted from embryogenic callus of date palm cv. Ashkar subjected to various levels of NaCl. A: Standard, B: control, C: 80 mM NaCl, D: 160 mM NaCl, E: 240 mM NaCl, F: 320 mM NaCl.

Table 2

Effect of NaCl concentration (mM) on molecular weight (kDa) of proteins extracted from the embryogenic callus of the date palm, cv. Ashkar

Treatment (mM NaCl)	*Molecular weight of proteins (kDa)*
Control	14.0, 23.0, 37.0, 66.0, 76.0, 120.0, 151.0
80 mM	14.0, 43.0, 79.0, 93.0, 141.0
160 mM	14.0, 23.0, 32.0, 43.0, 65.0, 89.0
240 mM	35.0, 43.0, 59.0
320 mM	31.0, 34.0, 54.0

Salinity induced the synthesis of new protein via its effects on the process of gene expression. Such new proteins probably play an important role in improving salinity tolerance in the date palm. It is also clear, from the results presented in this paper that subjecting the embryogenic callus to salinity reduced both the number and molecular weight of the proteins which appeared on the gel. Several authors reported that salinity induced the formation low molecular weight protein (26 kDa) and they called it osmotin (Bressan et al 1988; Singh et al 1985; Ben-Hayyim et al 1989; Amini et al 2007). With olives Garcia et al (2008) found that two proteins with molecular weights of 24.0 and 40.0 kDa are accumulated under NaCl stress.

In the present work, NaCl at concentrations of 160-320 mM, resulted formation of low molecular weight proteins (23.0, 31.0, 34.0, and 35.0 kDa) which are probably very close to osmotin and may play an important role in the adaptation of the embryogenic callus cells of the date palm to salinity stress. The physiological functions of the newly formed proteins under salt stress are not completely understood, but it has been suggested that such proteins may have one or more of the following functions, protecting the integrity of the membranes and macro molecules, some new proteins may bind to excess salts, there by reducing their toxic effects, some of the newly formed proteins participate in the scavenging of free radicals and removal of proteins which are inactivated by salinity stress (Dubey 1999).

Conclusions. The results presented in this communication shows that under salt stress conditions the embryogenic callus fresh weight decreased significantly, SDS-PAGE of extracted proteins revealed that subjecting embryogenic callus to various levels of NaCl (0, 80, 160, 240 and 320 mM) induced the synthesis of new proteins. As salinity levels increased, the number and molecular weight of the new proteins decreased. It is very likely, that salinity induced proteins appear to endow date palm plants with the capacity to adapt to salinity by physiological and biochemical adjustments.

References

Al-Khayri J. M., 2002 Growth, proline accumulation and ion content in sodium chloride stressed callus of date palm. In Vitro Cell Dev Biol Plant 38:79-82.

Amini F., Ehsanpour A. A., Hoang O. T., Shin J. S., 2007 Protein pattern changes in tomato under in vitro salt stress. Russ J Plant Physiol 54(4):464-471.

Ben-Hayyim G., Kochba J., 1983 Aspect of salt tolerance in a NaCl- selected stable cell lines of citrus sinensis. Plant Physiol 75:985-690.

Ben-Hayyim G., Vaadia Y., Williams B. G., 1989 Protein associated with salt adaptation in citrus and tomato cells: involvement of 26kDa polypeptide. Physiol Plant 77:332-340.

Bressan R. A., Singh N. K., Handa A. K., Kononowicz A., Hasegawa P. M., 1988 Stable NaCl tolerance of tobacco cell is associated with enhanced accumulation of osmotin. Plant Physiol 91:855-861.

Dubey R. S., 1999 Protein synthesis by plants under stressful conditions. In: Handbook of plant crop stress. Pessarakil M. (ed), pp. 365-397, 2nd edition, Marcel Dekker, Inc.

El-Hammady A. F., Wanes W. H., El-Saidi M. T., Sahahin M. F. M., 1999 Impact of proline application on the growth of grape plantlets under salt in vitro. Arab University Journal of Agricultural Sciences 7:191-202.

Ericson M. C., Alfinito, S. H., 1984 Proteins produced during salt stress in tobacco cell tissue. Plant Physiol 74:506-509.

Garcia J. L., Troncoso J., Cantos M., Troncoso A., 2008 Differential protein expression in olive tissues due to salt treatment. Acta Hortic 791:117-120.

Gomez K. A., Gomez A. A., 1984 Statistical procedures for agricultural research. Wiley-Science publications, John Wiley and Sons, New York.

Jasim A. M., Abbas M. F., Al-Zubiady B. H., 2010 Effect of salt stress and proline on chemical content of embryogenic callus and somatic embryos of date palm (*Phoenix dactylifera* L.). Acta Hortic 882:219-224.

Laemmli U. K., 1970 Cleavage of structural proteins during assembly of the heat of the bacteriophage T4. Nature 227:680-685.
Munns R., Tester M., 2008 Mechanism of salinity tolerance. Annu Rev Plant Biol 59:651-681.
Murashige T., Skoog F., 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473-497.
Singh N. K., Handa A. K., Hasegawa P. M, Bressan R. N., 1985 Protein associated with adaptation of cultured tobacco cells to NaCL. Plant Physiol 79:126-137.
Zaid A., Liebenberg P. J., 2005 Date palm irrigation. In: Date palm cultivation. Pp.164-197, FAO, Rome, Italy.

Received: 24 August 2014. Accepted: 21 January 2015. Published online: 01 February 2015.
Authors:
Muayed Fadhil Abbas, Basrah University, College of Agriculture, Iraq, Basrah, 0964319128, Garmat Ali Campus, e-mail: muayedfadhil@yahoo.co.uk
Abbas Mehdi Jasim, Basrah University, College of Agriculture, Iraq, Basrah, 0964319128, Garmat Ali Campus, e-mail: abbasjasim5@yahoo.com
Batool Hanoon Al-Zubaidy, Basrah University, Date Palm Research Center, Iraq, Basrah. 0964319128, Garmat Ali Campus.

How to cite this article:
Abbas M. F., Jasim A. M., Al-Zubaidy B. H., 2015 Effect of NaCl stress on protein pattern changes in embryogenic callus of the date palm (*Phoenix dactylifera* L.) cv. Ashkar. AAB Bioflux 7(1):7-11.