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The effect of saline stress on peroxidase activity in the mezquite (*Prosopis articulata*) leaf

F. L. García-Carreño & J. L. Ochoa O.

División de Biología Experimental, Centro de Investigaciones Biológicas de Baja California Sur, BOX 128, La Paz, 23000, BCS, Mexico

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Peroxidase activity and isoperoxidase electrophoretic patterns are being studied in desert plants as biochemical indicators of physiological stress. Herein we report the peroxidase activity of the mezquite (*Prosopis articulata*) leaf associated with water tables of different electrical conductivities supporting plant growth and survival. The poor correlation found for about 18 plants growing in the conductivity range of 1000–18,000 μmhos confirms the wide ecotype variation reported for this species, suggesting different plant strategies in response to saline stress.

Introduction

Plant biochemical events such as changes in enzyme activity, isoenzyme patterns and metabolite pools are useful indicators of physiological alterations due to environmental conditions. Peroxidase activity has been used for screening different physiological stresses such as drought, cold, infection and salt (Gaspar *et al.*, 1982). Stevens *et al.* (1978) reported a decrease in peroxidase activity with increasing salinity in *Brassica* species, while Kalir *et al.* (1984) found an increase in peroxidase activity in leaves of *Halimione portulacoides* exposed to salinity, and Siegel *et al.* (1986) obtained a fall, followed by a rise, with species of *Zea*, *Brassica* and *Carica*.

The leguminous genus *Prosopis*, commonly known as mezquite or mesquite, is well represented among the various shrub phreatophytes found in the Sonoran desert. The mezquites can produce leaves, flowers and fruits under conditions of severe drought. This is due, primarily, to its deep root system extending down to the phreatic zone (capillary fringe or water table) (Virginia & Jarrell, 1987) which enables the plant to survive in very dry seasons (Felger, 1985). The present work is aimed at determining the peroxidase activity changes in mezquite leaves as a result of variations in the salt concentration of their underground water.

Materials and methods

Study area

Work was conducted on the La Paz–El Carrizal alluvial plain (or Basin) (Hammond, 1954), 24–24°10′N, 110°20′–110°30′W (Fig. 1). The climate is BW(h)hw(e) type, dry or desertic. The average annual air temperature is 23·5°C, with a minimum of 6°C in winter

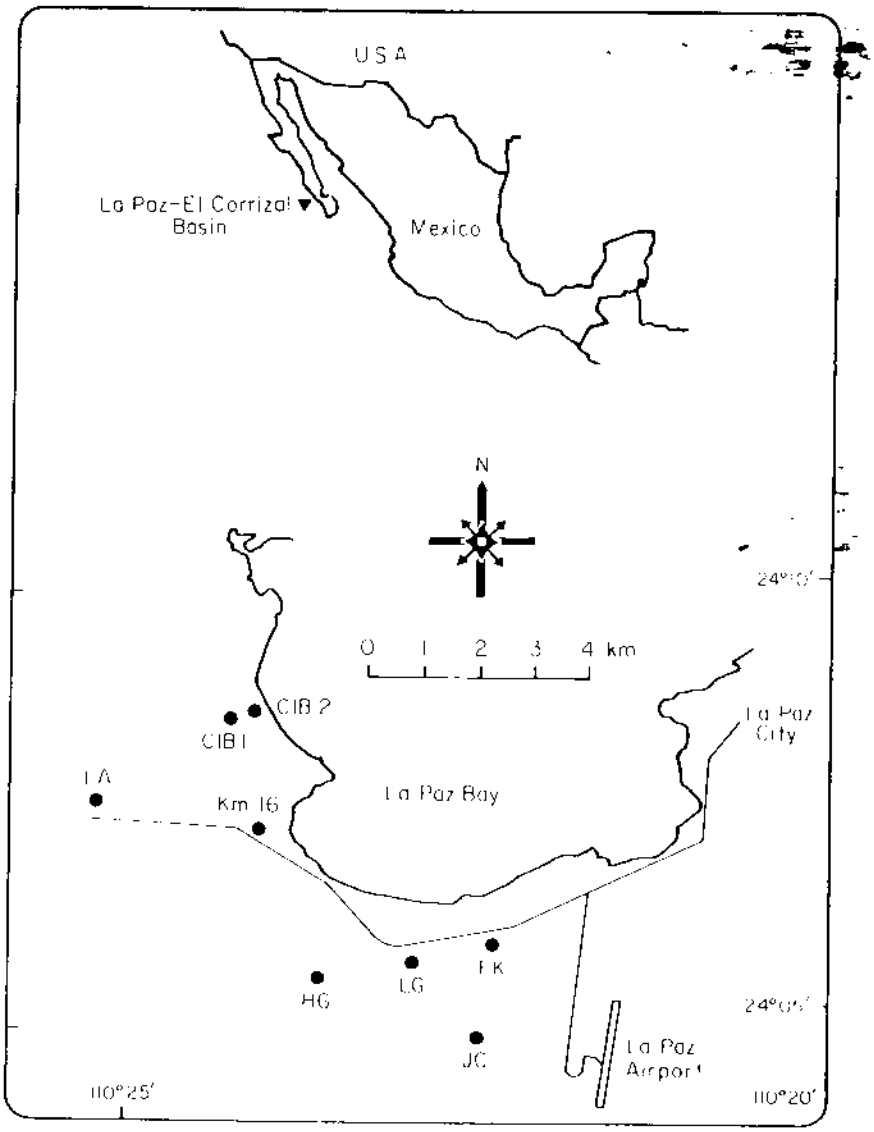

Figure 1. Study area within the La Paz–El Carrizal alluvial plain. The location of selected wells are indicated by the black dots.

and a maximum of 42°C in summer. The average annual rainfall during the summer is 150 mm. The main winds are from the north-west in the fall and winter, and from the south in the spring and summer (Salinas-Zavala, 1986). The biotic community is sarcocaulescent shrub (Shreve & Wiggins, 1954; León de la Luz, 1981). Field sampling took place on 25 November and 6 December 1988, and 10 January and 8 February 1989. The quality of the phreatic tables was determined by measuring the electrical conductivity of water samples from eight wells less than 30 m deep. The corresponding conductivities are expressed as μmhos, which are equivalent to μS. Shrub branches and water samples were also collected at the same time.

Peroxidase activity assay

Separate extracts of 0·5 g of leaves from each of two or three plants were obtained by homogenisation in a mortar with 5 ml of 50 mM acetate buffer, pH 5·1, and centrifuged at 3000 rpm for 10 min. Protein content was assayed according to the method of Bradford (1976). Peroxidase activity was determined by mixing 0·010 ml sample with 2 ml substrate mixture containing 16 mM guaiacol and 2 mM hydrogen peroxide in 50 mM acetate

buffer, pH 5·1, at 25°C. The increase in absorbance as a result of peroxidase activity was recorded at 470 nm.

Isoelectric focusing

Agarose (0·8%) thin-layer horizontal isoelectric focusing was performed according to LKB Application Notes 317, at 15°C on 10 × 11 cm plastic sheets. Mezquite leaf extracts (10 μl) were applied to the gel on filter paper. Electrofocusing separation was carried out at constant power (5 W) for 1·5 h. The gels were immersed in freshly prepared guaiacol solution (16 mM) with 50 mM acetate buffer, pH 5·1. After 5 min, the guaiacol solution was drained off and the gels washed with distilled water before being immersed in 0·03% H_2O_2 solution until isoperoxidase bands were visible. For drying, the gels were covered with a filter paper sheet soaked in ethanol, followed by three dry sheets and pressed for 30 min under a glass plate and a 1 kg weight. The gels were then blown dry with warm air.

Results

The peroxidase activity of mezquite leaves, and the electrical conductivity of the various water tables supplying the plants, sampled at different times, reflect an erratic response to saline stress (Tables 1 and 2). Interestingly, due to a chance rainfall, a reduction in the ionic strength of the water from selected wells was significant at the third sampling

Table 1. *Water table conductivities (C in μmhos) and specific peroxidase activity (Q) in different mezquite leaf samples*

	Sampling	25 Nov 1988		7 Dec 1988		10 Jan 1989		8 Feb 1989	
Well name	Plant code no.	C	Q	C	Q	C	Q	C	Q
LA	LA–1	1100	31	1000	34	900	29	900	31
	LA–2		42		160		34		23
	LA–3		29		ND		20		27
HG	HG–1	1400	12	1400	50	1200	24	1000	36
	HG–2		19		120		28		31
	HG–3		ND		80		33		46
FK	FK–1	2200	51	2000	166	1650	35	1600	66
	FK–2		27		81		16		39
LG	LG–1	2450	36	2500	64	2350	17	2400	37
	LG–2		13		131		20		30
K–16	K–16–1	3200	97	3400	470	1800	75	1800	ND
	K–16–2		57		53		43		50
CIB1	CIB1–1	4200	16	4200	87	3700	28	3900	16
	CIB1–2		17		45		14		17
JC	JC–1	11,000	29	12,000	67	10,000	45	9000	42
	JC–2		34		34		22		37
CIB2	CIB2–1	18,000	ND	17,000	ND	13,500	15	14,000	34
	CIB2		38		115		20		ND

ND = not done.

Table 2. *Water table conductivities (C in μmhos) and protein content (P in mg ml^{-1} of extract) in different mezquite leaf samples*

	Sampling	25 Nov 1988		6 Dec 1988		10 Jan 1989		8 Feb 1989	
Well name	Plant code no.	C	P	C	P	C	P	C	P
LA	LA–1	1100	1·6	1000	0·9	900	1·0	900	1·4
	LA–2		0·6		0·2		0·4		0·3
	LA–3		0·5		ND		0·5		0·7
HG	HG–1	1400	1·5	1400	1·3	1200	1·0	1000	2·0
	HG–2		1·1		0·8		0·8		1·4
	HG–3		ND		0·4		0·9		1·3
FK	FK–1	2200	0·9	2000	0·2	1650	0·5	1600	0·6
	FK–2		0·9		0·6		0·5		0·7
LG	LG–1	2450	1·0	2500	0·4	2350	0·5	2400	0·9
	LG–2		0·8		0·2		0·4		1·0
K–16	K–16–1	3200	0·5	3400	0·1	1800	0·4	1800	ND
	K–16 2		0·3		0·6		0·5		0·9
CIB1	CIB1–1	4200	0·6	4200	0·5	3700	0·9	3900	1·3
	CIB1–2		0·9		0·3		0·9		0·7
JC	JC–1	11,000	0·9	12,000	0·7	10,000	0·7	9000	1·2
	JC–2		0·7		0·8		0·9		1·3
CIB2	CIB2–1	18,000	0·3	17,000	0·1	13,500	0·2	14,000	0·6
	CIB2–2		0·5		0·1		0·3		ND

ND = not done.

(Fig. 2), allowing us to establish a correlation between enzyme activity and the effect of salt within each plant. It is apparent that the enzyme activity of mezquite leaves is not dependent on the influence of salt concentration in the water supply to the plant.

Mezquite plants show a mean peroxidase activity of 31 units in water with an electrical

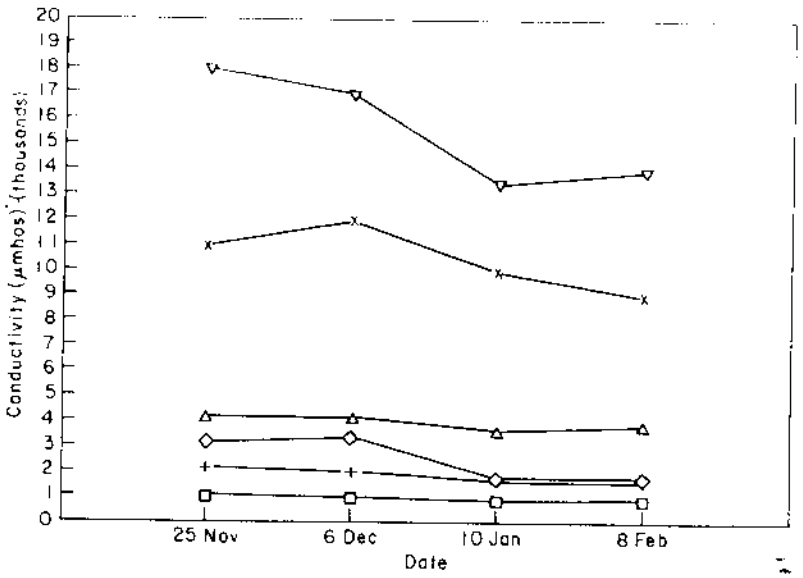

Figure 2. Electrical conductivity of water tables at different sampling dates. Well codes: □ LA; + FK; ◇ K-16; △ CIB1; × JC; ▽ CIB2.

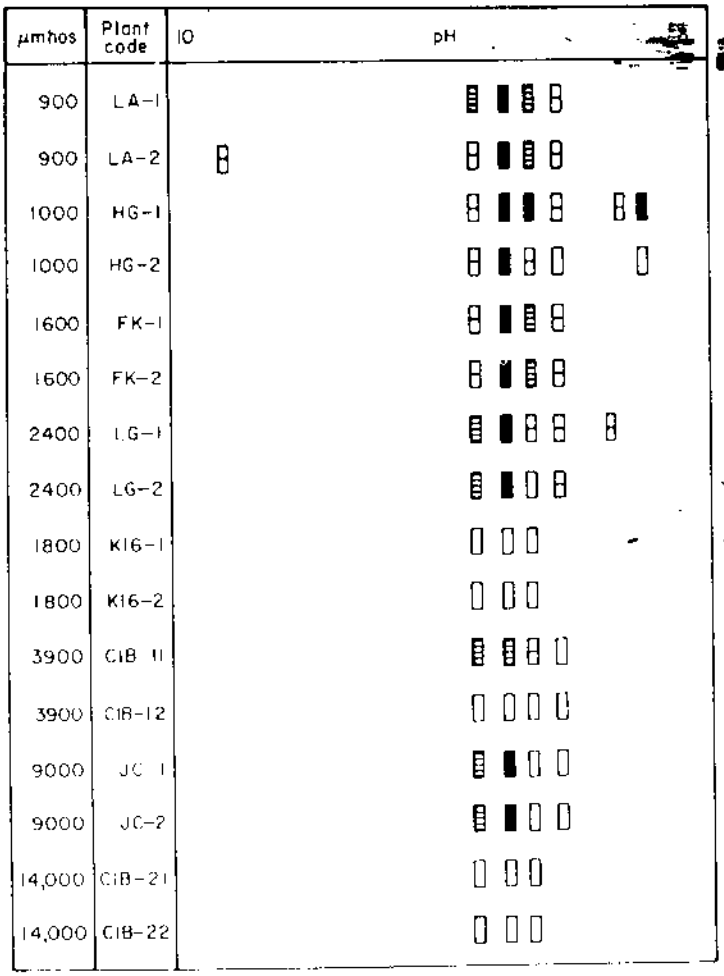

Figure 3. Isoperoxidase pattern from mezquite leaves by isoelectrofocusing (pH 3–10). Intensity of staining is suggested using bars from weak (no bar shown) to very intense (dark square).

conductivity value of 1100 μmhos, which is practically the same as when the conductivity is 10 times higher. Moreover, there are some examples where the enzyme activity is either higher or lower than 31 units in the range of conductivities hitherto found.

In addition, the isoperoxidase zymogram of the different extracts from mezquite leaves does not reveal any particular feature, or isoperoxidase enzyme bands, that could be associated with salt stress (Fig. 3); they only appear unusual in composition, indicating mainly an anionic character, with one exception where a faint cathodic isoperoxidase band was observed.

Discussion

The water used for general consumption in Baja California Sur State is supplied from wells. About 5×10^6 m^3 of rain water drain away to the sea each year in the La Paz–El Carrizal Basin system alone (Garcia-Monarrez *et al.*, 1986). Part of this water infiltrates the soil before draining into the sea, giving rise to very diverse quality phreatic tables, from drinkable to salty, supporting various plant communities. Although there are a number of physical methods employed to localise underground water, it is often necessary to dig in order to determine the water quality.

Peroxidase activity and isoperoxidase zymograms have been used as screening parameters for salt stress in plants from different genera including *Brassica*, *Halimione*, *Beta*

and *Suaceda*, cultivated in greenhouses or under cell culture conditions (Stevens *et al.*, 1978; Kalir *et al.*, 1984; Hagege *et al.*, 1988). This report is the first attempt to establish the relationship between mezquite leaf peroxidase activity with water table salinity. Mezquite plants were chosen because they are almost always dependent on phreatic water for survival and are widely distributed in the northern half of Mexico. The results obtained suggest a lack of correlation between mezquite leaf peroxidase activity, and/or isoperoxidase pattern, and the salt concentration in their supplying water tables.

It is known that, in addition to peroxidase activity, saline stress may induce changes in several other biochemical parameters in plants such as photosynthetic potential (Robinson *et al.*, 1983), specific protein production (Ericson & Alfinido, 1984), activities of isocitrate dehydrogenase and glutamate dehydrogenase (Rabe *et al.*, 1982), abscisic acid, zeatin riboside, cis and trans zeatin, adenine and adenosine (Dumbroff & Walker, 1981), membrane lipid ratio (Hirayama & Mihara, 1987) and ethylene production (Hagege *et al.*, 1988). In the case of mezquite, there appears to be a complex response to saline stress which remains to be elucidated. Further screening of other biochemical indicators of saline stress in mezquite may help to estimate the water quality of phreatic deposits.

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References

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram sensitivities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248–54.

Dumbroff, E. B. & Walker, M. A. (1981). Effects of salt stress on abscisic acid and cytokinin levels in tomato. *Zeitschrift für Pflanzenphysiol*, **101**: 461–70.

Ericson, M. C. & Alfinido, S. H. (1984). Proteins produced during salt stress in tobacco cell culture. *Plant Physiology*, **74**: 506–509.

Felger, R. S. (1985). New arid land crops from México: three examples for immediate development. In: Ochoa, J. L. & Moreno-Lopez, J. (Eds), *Uso y Preservación de los Recursos Biológicos Marinos y de Zonas Aridas*. pp. 13–24. Uppsala: Uppsala University Press.

García-Monarrez, H., Pimentel-Hernandez, R., Perez-Venzor, J. F. & Flores, E. Z. (1986). Estudio geohidrológico complementario de la Cuenca La Paz–El Carrizal para proporcionar agua en bloque a la Ciudad de La Paz, BCS. *Reporte Técnico Geociencias*. México: Universidad Autónoma de Baja California Sur. 300 pp.

Gaspar, Th., Penel, Cl., Thorpe, T. & Greepin, H. (Eds) (1982). *Peroxidases*. Geneva: Geneva University Press. 324 pp.

Hammond, E. H. (1954). A geomorphic study of the cape region of Baja California. *University of California Publications in Geography*. pp. 45–112.

Hagege, D., Kevers, C., Grandin, M., Boucaud, J. & Gaspar, Th. (1988). Peroxidase activities and ethylene production by calli of *Beta vulgaris* (halotolerant plant) and *Suaceda maritima* (halophilic plant), obtained in absence of NaCl. In: Dhir, K. K. (Ed.), *Growth and Differentiation in Plants* (in press).

Hirayama, O. & Mihara, M. (1987). Characterization of membrane lipids of higher plants: differences in salt tolerance. *Agricultural Biological Chemistry*, **51**: 3215–21.

Kalir, A., Omri, G. & Poljakoff-Mayber, A. (1984). Peroxidase and catalase activity in leaves of *Halimione portulacoides* exposed to salinity. *Physiologia Plantarum*, **62**: 238–44.

León de la Luz, J. L. (1981). Informe de actividades. *Centro de Investigaciones Biológicas de Baja California Sur*. México. pp. 271–278.

Rabe, R., Noble, W. & Kohler, A. (1982). Effects of sodium chloride on photosynthesis and some enzyme activities of *Potamogeton alpinus*. *Aquatic Botany*, **14**: 159–66.

Robinson, S. P., Downton, J. S. & Millhuse, J. A. (1983). Photosynthesis and ion content of leaves and isolated chloroplasts of salt-stressed spinach. *Plant Physiology*, **73**: 238–42.

Salinas-Zavala, C. (1986). Memorias de curso especial de titulación. *Análisis de Sistemas Marinos*. UABCS. México.

Shreve, F. & Wiggins, I. L. (1954). *Vegetation and Flora of the Sonoran Desert*. Stanford, California: Stanford University Press. 1740 pp.

Siegel, B. Z., Siegel, S. M., Shun, J. & Chang, D. (1986). Peroxidase activity and stress: a complex relationship. In: Greppin, H., Penel, C. & Gaspar, Th. (Eds), *Molecular and Physiological Aspects of Plant Peroxidases*. pp. 427–32. Geneva: Geneva University Press.

Stevens, H. C., Calvan, M., Lee, K. & Siegel, Z. (1978). Peroxidase activity as a screening parameter for salt stress in *Brassica* species. *Phytochemistry*, **17**: 1521–22.

Virginia, R. A. & Jarrell, W. M. (1987). Approaches for studying the function of deep root systems. In: Tenhunen, J. D. (Ed.), *Plant Response to Stress*. pp. 107–127. Berlin: Springer-Verlag.