Physiological significance of proline and glycinebetaine: Maintaining photosynthesis during NaCl stress in wheat

L.R. RAJASEKARAN*,**, P.E. KRIEDEMANN***, D. ASPINALL* and L.G. PALEG*

*Department of Plant Physiology, Waite Agricultural Research Institute, The University of Adelaide, Glen Osmond, South Australia 5064**
*Faculty of Forestry, University of Toronto, Toronto, Ontario, Canada M5S 3B3***
*CSIRO Division of Forestry Research, Canberra, ACT, Australia****

Abstract

Experiments on the physiological significance of accumulation of proline and glycinebetaine (GB) in sustaining photosynthesis during salt stress in wheat *in vivo* showed that pre-treatment with GB, but not proline, alleviated NaCl-induced stomatal and non-stomatal inhibition of photosynthesis completely. A permeating and non-dissociating osmoticum, 3-orthomethyl-glucopyranose, also alleviated NaCl-induced perturbations of photosynthesis, suggesting that GB may work by maintaining chloroplast volume and not by specific effects on photosynthetic processes.

Additional key words: assimilation rate; CO_2; intercellular CO_2 partial pressure; 3-orthomethyl-glucopyranose; stomatal conductance.

Introduction

Proline and glycinebetaine accumulate in response to water stress and salt stress in wheat, pea, and in halophytes (Stewart and Lee 1974, Coughlan and Wyn Jones 1980, Wyn Jones and Storey 1981, Rajasekaran 1988, Fedina and Popova 1996). They act in osmoregulation (Stewart and Lee 1974, Storey and Wyn Jones 1977, Jagels 1983, Jolivet *et al.* 1983, Grumet and Hanson 1986), in protecting proteins (Nash *et al.* 1982, Paleg *et al.* 1984), and in protecting enzymes (Pollard and Wyn Jones 1979). The studies of Rajasekaran (1988) indicate that proline and GB may

Received 9 April 1997, *accepted* 25 June 1997.
*Fax: (416) 978 3834, e-mail: lada@larva.forestry.utoronto.ca
Abbreviations: ABA - abscisic acid; c_e - external CO_2 partial pressure; c_i - intercellular CO_2 partial pressure; E - transpiration rate; g_s - stomatal conductance; GB - glycinebetaine; 3-OMG - 3-orthomethyl-glucopyranose; P_N - net photosynthetic rate; RuBPC - ribulose-1,5-biphosphate carboxylase; T_l - leaf temperature, TPU - triose phosphate utilization.
Acknowledgement: This study has been carried out with the financial support in the form of Research Grant Scholarship provided by the University of Adelaide and additional assistance from the Director's fund which are greatly appreciated.

have additional roles. A major proportion of the proline and GB is found in chloroplasts (Noguchi *et al.* 1966, Stewart and Lee 1974, Hanson *et al.* 1985, Robinson and Jones 1986), suggesting that these compounds may have specific role(s) in this organelle. An *in vivo* role for proline and GB has yet to be established for salt stressed glycophytes. The experiments in this study were planned to explore the possible role(s) of these compounds in sustaining photosynthesis in wheat during NaCl stress *in vivo*.

Materials and methods

Seedlings of wheat (*Triticum aestivum* L.) cv. Sun 9 E were sown on different dates, grown in a glasshouse at 20 °C for 70 d, and supplied with full strength Hoagland nutrient solution. CO_2 gas exchange measurements were made on the flag leaf on the 71st d. The net photosynthetic rate (P_N) of an attached flag leaf of wheat was measured with an open gas exchange system constructed as described by Caemmerer and Farquhar (1981). Measurements began usually at 09:00 h, and continued until 17:00 h. NaCl (0, 100, 150 or 200 mM) was fed to the penultimate flag leaf through the cut end of the leaf tip, and the leaf was equilibrated in the gas exchange chamber for 30 min before the gas exchange measurements started. Leaves to be measured were enclosed with an aluminium and glass cuvette kept at a constant temperature of 20.5±0.2 °C. Flow rate through the cuvette was 15 $cm^3\ s^{-1}$. Air passing over the leaf was conditioned by removing CO_2 with soda lime, and then humidified with a dew point set when the air passed through the glass condenser at 18 °C. Water vapour content was determined by a *Vaisala HM* (*Vaisala, OY,* Helsinki, Finland) capacitive sensor. The CO_2 concentration was established by injecting 10 % CO_2 in air into the air stream through a mass flow controller. The CO_2 depletion caused by the leaf was measured with an infra red gas analyzer (*ADC Mark III*). Air was circulated inside the cuvette by a small fan. The irradiation source was a tungsten halogen lamp. The P_N, transpiration rate (E), leaf conductance (g_s), and intercellular CO_2 concentration (c_i) were calculated on a computer (*PPC III*) attached system. Calculation of results followed those set out by Caemmerer and Farquhar (1981).

On the day of measurement, the plants were taken from the glasshouse to the gas exchange unit. The penultimate leaf was laid across the cuvette. The top of the cuvette was then laid over the leaf, and tightened with clamp screws until the inside cuvette was completely seated from outside air. After 15-30 min equilibration, the partial pressure of CO_2 within the cuvette (c_c) was increased from 35-90 Pa, and triplicate gas exchange measurements were made at an ambient CO_2 concentration of 35 Pa. The c_c was then reduced down to 5 Pa, and measurements were made at low c_c of 5-35 Pa to obtain a curvilinear relationship.

The intact wheat flag leaf was used for examination of the responses of gas exchange parameters to organic solutes as the leaf was long enough to extend beyond the gas exchange chamber. Gas exchange at various c_c was measured. The leaf tip was then cut under water, and solutions fed through the cut tip. E was expected to be

high in the leaf chamber due to high irradiance, so solutions fed through the cut end would have reached the site of gas exchange rapidly.

In experiments to investigate the effects of solutes on photosynthesis, solutes such as proline (20 mM), GB (5, 10 mM), or 3-O-methyl glucopyranose (3-OMG) (*Sigma*, Australia) (5 mM) were pre-fed to the penultimate flag leaf through the cut end of the leaf tip which were then equilibrated in the gas exchange chamber for 30 min. After this period, the solutions were replaced with 200 mM NaCl, and the leaf tips were equilibrated for further 30 min before gas exchange measurements began. The distal end of the leaf tip remained in salt solution until the measurement was completed. The P_N, E, leaf temperature, g_s, and c_i were measured simultaneously over a range of c_c. These measurements were made on a single leaf for each treatment.

Results

The effect of a range of NaCl concentrations on photosynthesis: Increase in the NaCl concentration in the solution bathing the cut end of the leaf tip resulted in a decline in g_s (expressed as H_2O conductance), compared to the control (Fig. 1*A*, Table 1). However, c_i at ambient CO_2 concentration did not show any decline with g_s except at 200 mM NaCl. In the leaf fed with 200 mM NaCl the value of c_e/c_i declined from 0.77 to 0.69 at ambient CO_2 concentration, indicating that stomatal limitation of photosynthesis increased due to NaCl treatment to 9.9 %. Parallel to this response, E declined, accompanied by an increase in T_l (Table 1).

Table 1. The effects of range of NaCl concentrations on net photosynthetic rate (P_N) [μmol m^{-2} s^{-1}], stomatal conductance (g_s) [mmol m^{-2} s^{-1}], ratio of external and intercellular CO_2 concentrations (c_e/c_i), transpiration rate (E) [mol m^{-2} s^{-1}], and leaf temperature (T_l) [°C] at ambient CO_2 partial pressure (35 Pa) (values obtained from Fig. 1*A*,*B*).

NaCl [mM]	P_N	g_s	c_e/c_i	T_l	E
0	23.0	737	0.77	23.1	6.18
100	23.0	705	0.78	23.2	6.05
150	14.8	465	0.78	23.9	4.50
200	9.2	178	0.69	25.6	2.68

Further on, the effect of NaCl on P_N was measured at varying c_i (Fig. 1*B*). The initial slope of the response of c_i at 5 Pa is being used as a measure of the RuBP-carboxylase-limited CO_2 fixation capacity of the leaves (Caemmerer and Farquhar 1981, Seemann and Berry 1982). Determination of the initial slope permits an assessment of photosynthetic capacity, independent on stomatal aperture, since calculation of c_i includes differences in g_s. An increase in NaCl concentration above 100 mM resulted in a decline in P_N at an ambient CO_2 partial pressure of 35 Pa (Table 1): the reduction was 35.7 (at 150 mM NaCl) and 60 (at 200 mM NaCl) %. Furthermore, NaCl inhibited the CO_2-limited (initial slope of P_N:c_i curve) rate of photosynthesis. Test of significance of slopes of the regressions of P_N:c_i at the linear

region of the curve (Fig. 2*A*) showed a significant difference in the slopes between control and NaCl (150, 200 mM) treated leaves, indicating that NaCl influenced RuBP carboxylase (RuBPC) activity significantly. NaCl also influenced the CO_2-saturated P_N (upper portion of the P_N:c_i curve - Fig. 1*B*) at 150 and 200 mM concentrations.

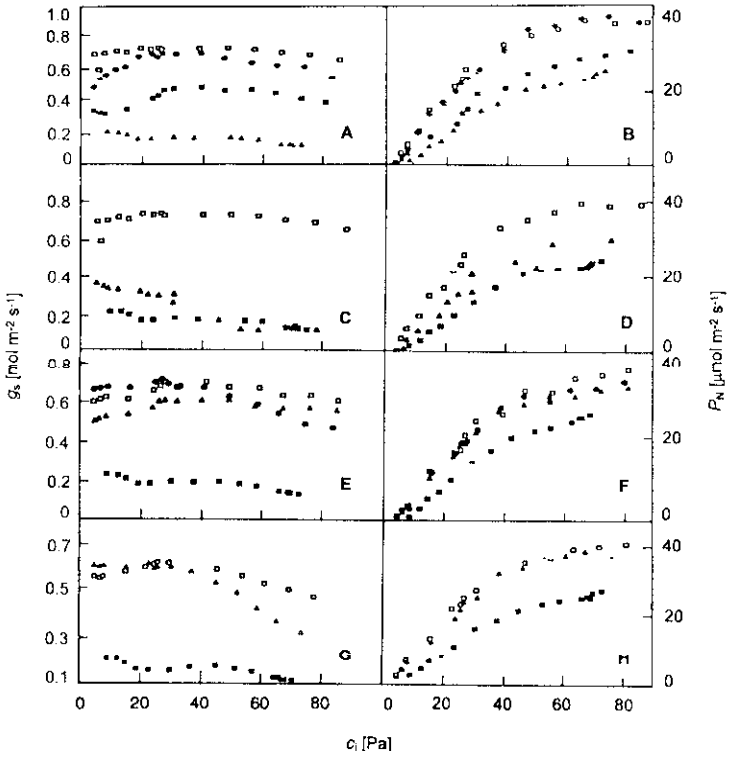

Fig. 1. Effects of NaCl (*A*, *B*) and pre-treatments with proline (*C*, *D*), glycinebetaine (GB) (*E*, *F*), and 3-ortho-methylglucopyranose (3-OMG) (*G*, *H*) on stomatal conductance (*A*, *C*, *E*, *G*) or net photosynthetic rate (P_N) (*B*, *D*, *F*, *H*) at various intercellular CO_2 concentrations (c_i). ☐ - control. *A*, *B*: NaCl [mM] 100 (●), 150 (■), or 200 (▲). *C*, *D*: water → 200 mM NaCl ■, 20 mM proline → 200 mM NaCl ▲. *E*, *F*: water → 200 mM NaCl ■, 5 mM GB → 200 mM NaCl ▲. *G*, *H*: water → 200 mM NaCl ■, 5 mM 3-OMG → 200 mM NaCl ▲.

The effect of pre-treatment with proline: The g_s of control leaves was about 0.74 μmol m^{-2} s^{-1}. Pre-treatment with proline alleviated the NaCl induced effect to a small extent only (17 % at 35 Pa CO_2 - Fig. 1*C*).

As with the previous experiment, feeding NaCl (200 mM) to the leaf substantially reduced both CO_2-limited and CO_2-saturated P_N compared to the control (Fig. 1*D*). Although proline pre-treatment appeared to alleviate NaCl-inhibited P_N in both regions of the P_N:c_i curve to some extent, the test of significance of the slope of the regressions for the CO_2-limited region showed no significant alleviation of NaCl inhibition (Figs. 1*C*, 2*B*).

The effect of pre-treatment with GB: A substantial reduction in g_s due to the exposure to NaCl, similar as observed in the previous experiment, was found. Here, however, pre-treatment with GB substantially alleviated this response, a 10 mM concentration giving g_s similar to control values (Fig. 1*E*).

As in the previous experiments, NaCl substantially reduced the CO_2-limited and CO_2-saturated P_N. Pre-treatment with GB completely alleviated this inhibition in both regions of the P_N:c_i curve irrespective of the concentration range tested (Fig. 1*F*). Test of significance of the slopes of the regressions of the linearly related regions of the P_N:c_i curve showed no significant difference between control and GB pre-treated leaves (Fig. 2*C*), indicating that GB protected RuBPC activity from NaCl inhibition.

The effect of pre-treatment with 3-OMG: This pre-treatment completely eliminated the stomatal barrier consequent upon exposure to NaCl, as did GB (Fig. 1*G*).

The responses of the NaCl exposed leaf in photosynthesis were consistent with the previous experiments. Pre-treatment with 3-OMG alleviated NaCl-induced inhibition of both CO_2-limited and CO_2-saturated P_N substantially as did GB (Fig. 1*H*), and the test of significance of slopes of the regressions (Fig. 2*D*) for the linear region of the P_N:c_i curves showed that 3-OMG also produced complete recovery from NaCl-inhibition.

Discussion

Salt effects on photosynthesis have been reported for many plant species (Gale *et al.* 1967, Downton and Millhouse 1983, Seemann and Critchley 1985, Yeo *et al.* 1985, West *et al.* 1986). However, in all these studies the direct effects of salt were indistinguishable from alteration in photosynthesis induced by perturbations in root metabolism. NaCl influences root metabolism, particularly that of cytokinins and abscisic acid (ABA) (Itai and Vaadia 1965, Walker and Dumbroof 1981). An increase in ABA concentration and a reduction in cytokinins influence both stomatal and non-stomatal factors controlling photosynthesis (Jones and Mansfield 1972, Raschke 1979, Davis *et al.* 1986). Feeding salt through the leaf tip facilitated investigation of the effects of salt on photosynthesis with less likelihood of perturbations at root level. The response we observed indicated that solutes fed through the cut leaf reached the site of measurement.

Salt effects on photosynthetic processes fall into two major categories: (*1*) response of stomata to salinization, and (*2*) effects of salt on the capacity of the plant for CO_2 fixation, independent of altered diffusion limitations. Stomatal closure is generally associated with salinization of glycophytes (Gale *et al.* 1967, Downton 1977, Walker *et al.* 1983). Our results showed that g_s was reduced by NaCl; however, the extent to which stomatal closure affects photosynthetic capacity is indicated by the magnitude of reduction in c_i (Berry and Downton 1982, Farquhar and Sharkey 1982). Our results showed no decline in c_i (at an ambient CO_2 concentration of 35 Pa) upto 150 mM NaCl, and a reduction of only 9.9 % at 200

mM NaCl (Table 1). Thus the decline in P_N was not solely due to limitation in CO_2 diffusion.

The extent of non-stomatal limitations on P_N can be assessed *in vivo* by analyzing the response of P_N to varying c_i since the initial slope at CO_2 limiting region is dependent on the kinetics and concentration of RuBPC, and the response at high CO_2 concentration in CO_2-saturating region is a function of the capacity for the regeneration of RuBP (Caemmerer and Farquhar 1981, Seemann and Berry 1982, Badger *et al.* 1984). Using such an approach, Ball and Farquhar (1984) found that salinity brought about a reduction in both the initial slope and the CO_2-saturated portion of the P_N:c_i curve. The response of P_N in wheat, in the present study (Fig. 1*C,D*), was similar to that found in a number of salt sensitive glycophytes (Gale *et al.* 1967, Downton 1977, Walker *et al.* 1983). The biochemical basis for such alterations in photosynthetic capacity as a result of stress is yet to be understood. Changes in P_N of the leaf that are not a consequence of increased diffusional limitations, may be the result of either reduction in capacity or efficiency of RuBPC, or of reduction in regeneration capacity. This conclusion, however, rests on uniformity of responses in stomatal aperture. Terashima *et al.* (1988) with *Vicia faba* found that the apparent non-stomatal inhibition of P_N by ABA, deduced from the depression of P_N:c_i relationship, is an artifact which they have attributed to the non-uniform distribution of E and P_N over the leaves. However, no such variation in wheat has been reported so far.

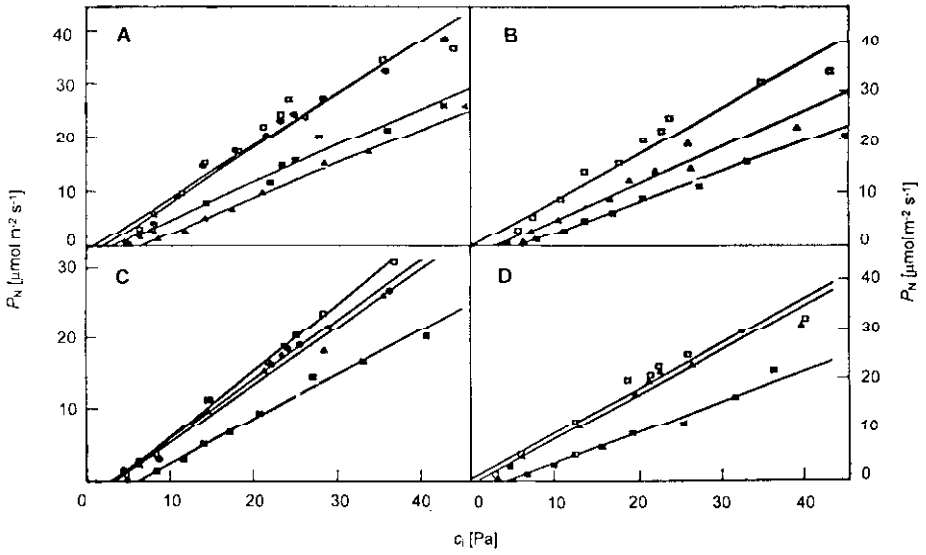

Fig. 2. Relationship between intercellular CO_2 partial pressure (c_i) and net photosynthetic rate (P_N) affected by NaCl (*A*) or pre-treatments with proline (*B*), glycinebetaine (GB) (*C*), or 3-orthomethyl glucopyranose (3-OMG) (*D*). Values obtained from linear portion of P_N/c_i curves of Fig. 1*B,D,F,G*. Standard deviations (SD), standard errors of mean differences of slopes (SES), least square deviations at $t_{0.05}$ (LSD), and significance (***p = 0.01 %) of slopes R of the regression between c_i and P_N was as follows:

	Symbol	Treatment	Regression equation	R
A	❑	control	Y = 0.1018 + 0.0818X	0.97***
	●	100 mM NaCl	Y= -0.9927 + 0.0842X	0.99***
	■	150 mM NaCl	Y= -1.3441 + 0.0576X	0.99***
	▲	200 mM NaCl	Y= -3.3321 + 0.0539X	1.00***
		SD	0.0158	
		SES	0.079	
		LSD	0.0161	
B	❑	control	Y= 0.1018 + 0.0818X	0.97***
	■	water → 200 mM NaCl	Y= -3.3321 + 0.0539X	1.00***
	▲	20 mM proline → 200 mM NaCl	Y= -2.0963 + 0.0645X	0.98***
		SD	0.0141	
		SES	0.0081	
		LSD	0.0219	
C	❑	control	Y = -2.4040 + 0.0801X	1.00***
	■	water → 200 mM NaCl	Y = -3.3321 + 0.0539X	1.00***
	▲	5 mM GB → 200 mM NaCl	Y = -1.9732 + 0.0696X	0.99***
	●	10 mM GB → 200 mM NaCl	Y = -1.7037 + 0.0716X	0.99***
		SD	0.0109	
		SES	0.0055	
		LSD	0.0113	
D	❑	control	Y = -0.4175 + 0.0791X	0.98***
	■	water → 200 mM NaCl	Y = -3.3321 + 0.0539X	1.00***
	▲	5 mM 3-OMG → 200 mM NaCl	Y = -1.5668 + 0.0788X	0.99***
		SD	0.0145	
		SES	0.0084	
		LSD	0.0174	

Reduction in CO_2 saturated non-linear region of P_N suggests that NaCl influences either the capacity to regenerate RuBP (Farquhar and Caemmerer 1982) or to utilize triose phosphate (Badger *et al.* 1984). Reduction in regeneration capacity indicates that limitations in rate of photosynthetic electron transport processes are not as sensitive as enzyme reactions. In another glycophyte, tomato, subjected to NaCl there was no reduction in electron transport (Rajasekaran 1988 and unpublished). In halophytes, the electron transport is considerably enhanced *in vitro* during salt stress (Critchley 1982), while the enzymes in halophytes are as salt sensitive as glycophyte enzymes *in vitro* (Flowers *et al.* 1977). It is easier to envisage how triose phosphate utilization (TPU) could limit photosynthesis of NaCl-stressed leaves. TPU involves the conversion of triose phosphates, products of phosphorylation of 3-phosphoglycerate, into starch and sucrose, with a resultant release of inorganic phosphate. If TPU proceeds at a rate lesser than P_N, the inorganic phosphates released will be insufficient to maintain phosphorylation, and P_N would decline.

Pre-treatment with proline in the present study showed some effect in alleviating NaCl-induced photosynthesis (Fig. 1*C,D*), but a complete recovery was not observed. Incharoensakdi *et al.* (1986) have also observed ineffectiveness of proline in alleviating KCl-induced inhibition of RuBPC. However, this may be due to the

proline being rapidly metabolized. Exogenous labelled proline is oxidized in turgid leaves (Oaks *et al.* 1970, Stewart 1972), resulting in carbon being fed into Krebs cycle and respired.

Pre-treatment with GB virtually abolished the NaCl-induced decline in P_N in both the CO_2 limited and saturated regions (Fig. 1*E,F*), suggesting that GB acts as a compatible osmoticum in protecting "photofunction" during salt stress. Such protective roles of GB have been established *in vitro* (Paleg *et al.* 1981, 1984, Incharoensakdi *et al.* 1986). Pre-treatment with GB also ameliorated NaCl effects on g_s. The alleviation of NaCl-inhibited photosynthesis was not solely due to the alleviated stomatal response because c_i was not greatly limited by g_s, suggesting that protection of the activity of RuBPC and capacity to regenerate RuBP were involved. A similar effect of GB in protecting RuBPC activity against inhibition by KCl has been demonstrated in the cyanobacterium *Aphanothece halophytica* (Incharoensakdi *et al.* 1986).

These ameliorative effects of GB may be due simply to the maintenance of the osmotic volume of the chloroplast as 3-OMG, a permeating, non-dissociating osmoticum (Greenway *et al.* 1982) also alleviated NaCl-inhibited P_N (Fig. 1*G,H*). In spinach, a major proportion (30-40 %) of GB accumulates in chloroplasts, in response to NaCl stress, contributing 64 % of the decrease in osmotic potential of the chloroplast. There was no decline in P_N (Robinson and Jones 1986). Alternatively, Incharoensakdi *et al.* (1986) claim that the alleviating nature of GB comes from the methyl group of this compound. They have shown many methylated compounds such as glycerol, sucrose and sarcosine protect RuBPC activity as effectively as GB, and suggest that these methylated compounds protect the enzyme-protein activity by acting at the protein-water interface, decreasing the effects of concentrated salts on the enzyme proteins and other macromolecules. However, as similar protective effects are observed (Incharoensakdi *et al.* 1986) with 300 mM KCl on heat and cold inactivated RuBP-carboxylase activity, the possibility that a specific effect of the methyl group of the GB molecule is involved seems to be ruled out.

References

Badger, M.R., Sharkey, T.D., Caemmerer, S. von: The relationship between steady-state gas exchange of bean leaves and the levels of carbon-reducing-cycle intermediates. - Planta **160**: 305-313, 1984.

Ball, M.C., Farquhar, G.D.: Photosynthetic and stomatal responses of two mangrove species, *Aegiceras corniculatum* and *Avicennia maritima*, to long term salinity and humidity conditions. - Plant Physiol. **74**: 1-6, 1984.

Berry, J.A., Downton, W.J.S.: Environmental regulation of photosynthesis. - In: Govinjee (ed.): Photosynthesis: Development, Carbon Metabolism and Plant Productivity. Vol. II. Pp. 263-343. Academic Press, New York - London - Paris - San Diego - San Francisco - São Paulo - Sydney - Tokyo - Toronto 1982.

Caemmerer, S. von, Farquhar, G.D.: Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. - Planta **153**: 376-378, 1981.

Coughlan, J.S., Wyn Jones, R.G.: Some responses of *Spinacia oleraceae* to salt stress. - J. exp. Bot. **31**: 883-893, 1980.

Critchley, C.: Stimulation of photosynthetic electron transport in a salt tolerant plant by high chloride concentrations. - Nature **298**: 483-485,1982.

Davis, W.J., Metcalfe, J., Lodge, T.A., Da Costa, A.R.: Plant growth substances and the regulation of growth under drought. - Aust. J. Plant Physiol. **13**: 105-126, 1986.

Downton, W.J.S.: Photosynthesis in salt stressed grapevines. - Aust. J. Plant Physiol. **4**: 183-192, 1977.

Downton, W.J.S., Millhouse, J.: Turgor maintenance during salt stress prevents loss of variable fluorescence in grapevine leaves. - Plant Sci. Lett. **31**: 1-7, 1983.

Farquhar, G.D., Caemmerer, S. von: Modeling of photosynthetic response to environmental conditions. - In: Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H. (ed.): Physiological Plant Ecology II. Water Relations and Carbon Assimilation. Pp. 549-587. Springer-Verlag, Berlin - Heidelberg - New York 1982.

Farquhar, G.D., Sharkey, T.D.: Stomatal conductance and photosynthesis. - Annu. Rev. Plant Physiol. **33**: 317-345, 1982.

Fedina, I.S., Popova, A.V.: Photosynthesis, photorespiration and proline accumulation in water-stressed pea leaves. - Photosynthetica **32**: 213-220, 1996.

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

Gale, J., Kohl, H.C., Hagan, R.M.: Changes in the water balance and photosynthesis of onion, bean and cotton plants under saline conditions. - Physiol. Plant. **20**: 408-420, 1967.

Greenway, H., Munns, R., Gibbs, J.: Effects of accumulation of 3-O-methyl glucose levels of endogenous osmotic solutes in *Chlorella emersonii*. - Plant Cell Environ. **5**: 405-412, 1982.

Grumet, R., Hanson, A.D.: Genetic evidence for an osmo regulatory function of glycinebetaine accumulation in barley. - Aust. J. Plant Physiol. **13**: 353-364,1986.

Hanson, A.D., May, A.M., Grumet, R., Bode, J., Jamieson, G.C., Rhodes, D.: Betaine synthesis in chenopods: localization in chloroplasts. - Proc. nat. Acad. Sci. USA **82**: 3678-3682, 1985.

Incharoensakdi, A., Takabe, T., Akazawa, T.: Effect of betain on enzyme activity and subunit interaction of ribulose-1,5-biphosphate carboxylase/oxygenase from *Aphanothece halophytica*. - Plant Physiol. **81**: 1044-1049, 1986.

Itai, C., Vaadia, Y.: Kinetin like activity in root exudate of water stressed sunflower plants. - Physiol. Plant. **18**: 941-944, 1965.

Jagels, R.: Further evidence for osmoregulation in epidermal leaf cells of seagrasses. - Amer. J. Bot. **70**: 327-333, 1983.

Jolivet, Y., Haelin, J., Larher, F.: Osmoregulation in halophytic higher plant: The protective effects of glycinebetaine and other related sources against oxalate destabilization of membranes in beet root cells. - Z. Pflanzenphysiol. **109**: 171-230, 1983.

Jones, R.J., Mansfield, T.A.: Effects of abscisic acid and its esters on stomatal aperture and the transpiration ratio. - Physiol. Plant. **26**: 321-327, 1972.

Nash, D., Paleg, L.G.,Wiskich, J.T.: The effect of proline, betain and some other solutes on the heat stability of mitochondrial enzyme. - Aust. J. Plant Physiol. **9**: 47-57, 1982.

Noguchi, M., Kowai, A., Tanaki, E.: Studies on nitrogen metabolism in tobacco plants. VIII. Δ' pyrroline-5-carboxylate reductase from tobacco leaves. - Agr. biol. Chem. **30**: 492-496, 1966.

Oaks, A., Michael, D.J., Barnard, R.A., Johnson, E.J.: The regulation of proline biosynthesis in maize roots. - Can. J. Bot. **48**: 2249-2258, 1970.

Paleg, L.G., Douglas, T.J., Van Dall, A., Keech, D.B.: Proline and betaine protect enzymes against heat inactivation. - Aust. J. Plant Physiol. **8**: 107-114, 1981.

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

Pollard, A., Wyn Jones, R.G.: Enzyme activities in concentrated solutions of glycinebetaine and other solutes. - Planta **144**: 291-298, 1979.

Rajasekaran, L.R.: The Mechanism of Accumulation of Proline and Quaternary Ammonium Compounds and Their Adaptive Significance in Tomato (*Lycopersicon esculentum* Mill) and

Wheat (*Triticum aestivum*) Exposed to Salt Stress. - Ph.D. Thesis. University of Adelaide, Glen Osmond 1988.

Raschke, K.: Movements of stomata. - In: Haupt, W., Feinleib, M.E. (ed.): Physiology of Movements. Pp. 383-441. Springer-Verlag, Berlin - Heidelberg - New York 1979.

Robinson, S.P., Jones, G.P.: Accumulation of glycinebetaine in chloroplasts provides osmotic adjustment during salt stress. - Aust. J. Plant Physiol. **13**: 659-668, 1986.

Seemann, J.R., Berry, J.A.: Interspecific differences in the kinetic properties of RuBP carboxylase protein. - Carnegie Inst. Washington Year Book **81**: 78-83, 1982.

Seemann, J.R., Critchley, C.: Effects of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a salt sensitive species, *Phaseolus vulgaris* L. - Planta **164**: 151-162, 1985.

Stewart, C.R.: Effect of proline and carbohydrates on metabolism of exogeneous proline by excised bean leaves in the dark. - Plant Physiol. **50**: 551-555, 1972.

Stewart, C.R., Lee, E.: Δ¹-pyroline-5-carboxylic acid DH in mitochondria preparations from plant seedlings. - Plant Sci. Lett. **3**: 173-181, 1974.

Storey, R., Wyn Jones, R.G.: Quarternary ammonium compounds in plants in relation to salt resistance. - Phytochemistry **66**: 447-453, 1977.

Terashima, I., Wong, S.-C., Osmond, C.B., Farquhar, G.D.: Characterisation of non-uniform photosynthesis induced by abscisic acid in leaves having different mesophyll anatomies. - Plant Cell Physiol. **29**: 385-394, 1988.

Walker, M.A., Dumbroof, E.B.: Effects of salt stress on abscisic acid and cytokinin levels in tomato. - Z. Pflanzenphysiol. **101**: 461-470, 1981.

Walker, R.A., Törökfalvy, E., Downton, W.J.S.: Photosynthetic responses of the citrus varieties rangpur lime and etong citron to salt treatment. - Aust. J. Plant Physiol. **9**: 783-790, 1983.

West, D.W., Hoffman, G.J., Fisher, M.J.: Photosynthesis, leaf conductance, and water relations of cowpea under saline conditions. - Irrig. Sci. **7**: 183-193, 1986.

Wyn Jones, R.G., Storey, R.: Betains. - In: Paleg, L.G., Aspinall, D. (ed.): The Physiology and Biochemistry of Drought Resistance in Plants. Pp. 171-204. Academic Press, Sydney 1981.

Yeo, A.R., Capron, S.J.M., Flowers, T.J.: The effect of salinity upon photosynthesis in rice (*Oryza sativa*, L.): gas exchange by individual leaves in relation to their salt content. - J. exp. Bot. **36**: 1240-1248, 1985.