

Ineffectual role of proline metabolism in salt-stressed sugarcane leaves

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Abstract. Sugarcane var. CO 740 is known to be salt-sensitive. Effect of increasing concentrations of NaCl and Na₂SO₄ as prolonged salt stress, and of -10 bar NaCl and PEG as well as 10 EC NaCl, Na₂SO₄, MgCl₂ and MgSO₄ as abrupt stress in base-nutrient solution has been investigated on proline metabolism. Neither of these stress conditions could effectively stimulate proline accumulation in the leaves of treated sugarcane plants, in spite of the abrupt drop in leaf osmotic potential. It can be concluded that the sugarcane var. CO 740 lacks this adaptive mechanism and hence seems to be salt-sensitive.

Keywords. Proline metabolism; salt stress; sugarcane.

1. Introduction

It has been fairly established that iminoacid proline accumulates in many plants when the tissue water potential falls. Such a decrease in tissue water potential and consequent proline accumulation may follow depletion of the water in the rooting medium due to water stress (Singh *et al* 1973) or salt stress (Palfi and Juhasz 1970; Stewart and Lee 1974; Chu *et al* 1976; Nutrit Bar-Nun and Poljakoff-Mayber 1977; Tal *et al* 1979; Weimberg *et al* 1982). The relationship between proline accumulation and salt and drought tolerance in different plants raises the possibility that proline may also be implicated in the response of sugarcane under saline conditions. Sugarcane var. CO 740 is salt-sensitive and shows 50% decline in productivity at 3.5 to 10 EC salt concentration (Joshi and Naik 1977, 1981). There are no reports of studies on proline metabolism in salt-stressed sugarcane plants. The present report opens a new line of investigation in this direction.

2. Experimental

The healthy seed setts of sugarcane var. CO 740 were obtained from the local research station and treated with organomercuric fungicide aretan. The cane setts were grown in aerated Hoagland solution (Hoagland and Arnon 1950) and the salt treatment employed in the diluted nutrient solution as described earlier (Joshi and Naik 1981). After the establishment of seedlings in the pure solution for 60 days the respective salt treatments were adopted both abruptly as well as by gradual increase in concentration (USDA Handbook No. 60, 1954). Increasing concentration of NaCl and Na₂SO₄ (5, 10 and 15 EC) were employed gradually (Black 1956) whereas the NaCl and PEG (-10 bar) concentration was used for the abrupt stress. In addition, the effect of four different salts namely NaCl, Na₂SO₄, MgCl₂ and MgSO₄ was also

studied at 10 EC salinity level by exposing the seedlings to abrupt salt stress. These concentrations have been fixed after several preliminary experiments carried to study salt tolerance limits of sugarcane var. CO 740 (Joshi and Naik 1977, 1981). The experiments were carried out in duplicate and the second and third leaves of treated and control (pure nutrient solution) plants were used for analysis.

Proline has been spectrophotometrically estimated by the method of Bates *et al* (1973). The osmotic potential of the cell sap is determined by the electrical conductivity method described by Janardan *et al* (1975). The analytical experiments were performed in three replicates and the values reported in tables 1 and 2 are the mean of these replicated trials.

3. Results

3.1 Effect of increasing concentration of NaCl and Na₂SO₄ on proline contents

The concentration of NaCl and Na₂SO₄ was raised gradually in the Hoagland nutrient solution in which sugarcane plants were growing. The effect of this prolonged stress is studied on the proline level after 120 days of growth. The values of the leaf osmotic potential were also determined and the results are presented in table 1. It is already reported that the cane growth is strongly inhibited following salt stress except at 5 EC NaCl where a slight stimulation was observed (Joshi and Naik 1977).

The increasing salinity (table 1) lowers the leaf osmotic potential as the salt concentration increases. There is a slight increase of proline in the leaves of the plant treated with 5 EC NaCl. Neither NaCl nor Na₂SO₄ results in any accumulation of proline in the sugarcane leaves. This happens despite the drastic drop in the values of leaf osmotic potential.

3.2 Effect of abrupt stress of NaCl and PEG (-10 bar) on proline accumulation

Data on the change in proline level of sugarcane abruptly exposed to -10 bar NaCl and polyethylene glycol (PEG) solution in Hoagland nutrient solution are presented in table 2. The proline contents have been measured from 12 to 192 hr after the commencement of the stress. The values of the fall in the leaf osmotic potential have also been recorded. It is clear from table 2 that even the abrupt stress of either NaCl or PEG cannot accumulate proline in the sugarcane leaves. There is no stimulated synthesis of proline although osmotic potential decreases following sudden stress conditions at the rooting medium.

Table 1. Effect of NaCl and Na₂SO₄ on proline and leaf osmotic potential in leaves of sugarcane var. CO 740.

Treatment	Proline mg/100 g. fresh weight	Leaf osmotic potential
Control	7.2 (± 1.1)	-7.8 (± 1.6)
NaCl 5 EC	8.0 (± 0.9)	-7.0 (± 1.5)
NaCl 10 EC	6.0 (± 1.3)	-12.0 (± 1.6)
NaCl 15 EC	5.0 (± 1.15)	-20.0 (± 1.37)
Na ₂ SO ₄ 5 EC	7.2 (± 1)	-6.0 (± 1.3)
Na ₂ SO ₄ 10 EC	7.2 (± 0.85)	-18.0 (± 2.6)
Na ₂ SO ₄ 15 EC	7.2 (± 0.5)	-21.0 (± 0.7)

Table 2. Effect of NaCl and polyethylene glycol (PEG)–10 bar stress on proline accumulation in sugarcane var CO 740. (abrupt salt stress**)

Treatment	12 hr		24 hr		36 hr		192 hr	
	Proline*	Leaf osmotic potential	Proline*	Leaf osmotic potential	Proline*	Leaf osmotic potential	Proline*	Leaf osmotic potential
Control	3.85 (± 0.21)	-8.6 (± 1.2)	3.93 (± 0.18)	-8.3 (± 2.2)	2.72 (± 0.32)	-8.3 (± 1.7)	4.5 (± 0.16)	-9.0 (± 2.6)
NaCl (-10 bar)	4.76 (± 0.10)	-10.0 (± 0.8)	4.82 (± 0.21)	-15.0 (± 2.8)	2.28 (± 0.28)	-16.3 (± 2.4)	3.7 (± 0.3)	-18.0 (± 0.8)
PEG (-10 bar)	3.95 (± 0.07)	-8.5 (± 2.1)	5.42 (± 0.12)	-13.3 (± 3.4)	1.68 (± 0.27)	-15.0 (± 0.9)	3.5 (± 0.21)	-16.2 (± 1.1)

** The analysis was done after 12, 24, 36 and 192 hr of commencement of stress.

* Proline values expressed as mg proline 100 g^{-1} fresh weight.

3.3 Effect of different salts on proline accumulation

Experiment was performed by exposing 100-day old sugarcane plants to NaCl, Na₂SO₄, MgCl₂ and MgSO₄ salts (EC 10), in Hoagland nutrient solution. The proline concentration in the leaves was analysed 96 hr after the commencement of stress. The proline values obtained were 3(±0.6), 4(±2.1), 3.5(±0.8), 4.35(±1.1) and 4.5(±0.8) mg per 100 g fresh weight in the control (non-stressed), NaCl, Na₂SO₄, MgCl₂ and MgSO₄ salt stressed leaves respectively. The results indicate no significant change in the proline level of the sugarcane treated with different salts.

4. Discussion

The results on salt-stressed sugarcane plants in response to proline metabolism differ from that of the general trend established in other plants where a significant higher level of proline accumulation is noted during salt stress (Strogonov 1964; Palfi and Juhasz 1970; Mukherjee 1974; Stewart and Lee 1974; Uday Kumar *et al* 1976; Anthony and Anthony 1978; Weimberg *et al* 1982). Neither the gradual nor the abrupt stress of varying salts could trigger the accumulation of proline in sugarcane var. CO 740 in response to drop in leaf osmotic potential. These results are different from those established for metabolism of proline and its role in salt and water-stress period (Chu *et al* 1976). Contrary to these observations, Kahane (1967) and Kahane and Poljakoff-Mayber (1968) have shown that in pea roots exposed to salinity amino acid composition of the protein changes but proline contents did not significantly increase. Shevyakova and Komizerko (1969) reported decreased concentration of proline in NaCl and Na₂SO₄ stressed callus from cabbage leaves. Totawat and Saxena (1971) also noted decreased proline contents in *Phaseolus aureus* with increasing salinity of irrigation water. Stewart and Lee (1974) also did not reveal any change in the individual amino acids or group of amino acids from plants of *plantago* grown on saline media which might be important in osmotic adjustment. Weigel and Jaegen (1979) also reported no marked increase in proline level of the lichen, *Pseudevermia furtuaces* during water stress. Our results also indicate inability of sugarcane var. CO 740 to accumulate large amounts of proline in response to fall in osmotic potential during salt stress even under lower salt concentrations. The results exhibit that the sugarcane var. CO 740 is not equipped to overcome the stress effect by accumulating higher concentration of proline.

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