GLYCINEBETAINE ACCUMULATION AND VARIETAL ADAPTABILITY TO SALINITY AS A POTENTIAL METABOLIC MEASURE OF SALT TOLERANCE IN RICE

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THE quaternary ammonium compound (QAC), glycinebetaine is actively accumulated by both halophytic and glycophytic species of the Gramineae during environmental stress periods[1]. Some preliminary reports on cereals indicate that once synthesized, betaine may not further be metabolized and may be mobile within the plant[2,3]. Because of these properties the glycinebetaine content could serve as a cumulative index of the internal water status of the plant[4] with potential applications in both plant breeding and crop management. Recent evidence suggests that salt resistance may be correlated with the accumulation of QAC, particularly glycinebetaine, choline in a number of plant species[1,5,6]. These QAC are considered to act as a non-toxic cytoplasmic osmoticum which maintains the intracellular osmotic balance between the cytoplasm and the NaCl in the vacuole. Our earlier reports on rice have given the relationship of salt tolerance with organic acids[7], enzymes[8], chlorophyll[9] and yield[10]. This paper describes this relationship of salt tolerance with quaternary ammonium compounds in rice cultivars.

Seeds of nine rice cultivars viz. AU 1, Co 43, CSC 1 (salt-resistant), Co 36, CSC 2, GR 3, IR 20, TKM 4 and TKM 9 (salt-sensitive) were obtained from the Tamil Nadu Agricultural University, Coimbatore and the Rice Research Institute, Navagam (Gujarat). Experiments were carried out in earthen pots filled with 7 kg of soil under net-house conditions during the wet season. Salinization was imposed on three-week-old seedlings by addition of 750 ml of sodium chloride solution of EC 10 m mhos/cm once a week. The pots were irrigated with normal water on other days as and when required. Controls received only water. The shoot system was harvested at 2, 4 and 6 weeks after initial salinization for growth studies and biochemical estimations. The total QAC and glycinebetaine were estimated[6] in the third leaf from the top. Salinity index (SI) was calculated[10] using the formula: SI = grain yield of treated plants/grain yield of control × 100. Salt-resistant cultivars AU 1, CSC 1 and Co 43 exhibited less reduction (10–21%) of shoot dry matter accumulation over the control at six weeks after initial salinization and also showed highest values of SI (46–59). The salt-sensitive cultivars Co 36, CSC 2, GR 3, IR 20, TKM 4 and TKM 9 showed high reduction of shoot dry matter accumulation during final harvest and exhibited lowest values of SI

Table 1 *Effect of NaCl on the dry matter accumulation of shoot system in rice cultivars*

Cultivar		Dry weight of shoot system (g/plant)						Salinity Index
		2		4		6		
		C	S	C	S	C	S	
Au 1	SR	1.26	1.04	2.56	1.98	4.46	4.02	45.6
Co 43	SR	0.97	0.63	1.43	1.22	2.03	1.60	58.8
CSC 1	SR	0.94	0.80	1.61	1.04	3.78	3.14	50.9
Mean		1.06	0.82	1.87	1.41	3.42	2.92	
CD								
5%		0.09		0.10		0.13		
CSC 2	SS	0.49	0.36	1.13	0.87	2.95	2.18	5.2
GR 3	SS	0.66	0.41	1.07	0.62	2.09	0.90	42.2
IR 20	SS	0.41	0.26	0.53	0.33	0.91	0.42	13.6
TKM 4	SS	0.40	0.25	0.62	0.37	1.26	0.51	0.9
TKM 9	SS	0.45	0.34	0.80	0.61	1.72	1.09	23.5
Co 36	SS	0.58	0.33	0.70	0.41	1.40	0.83	21.1
Mean		0.50	0.33	0.81	0.54	1.72	0.99	
CD								
5%		0.08		0.10		0.17		

C: control; S: salinized; SR: salt-resistant; SS: salt-sensitive.

Table 2 *Effect of NaCl on the glycinebetaine content of the third leaf of rice cultivars*

	Glycinebetaine (μmol/g dry weight)					
	2		4		6	
Cultivar	C	S	C	S	C	S
AU 1	4.55 ± 0.8	23.56 ± 3.7	2.72 ± 0.2	14.16 ± 1.9	4.64 ± 1.5	21.32 ± 1.0
Co 43	3.65 ± 0.4	17.52 ± 2.7	5.57 ± 1.1	17.13 ± 1.8	3.25 ± 0.30	16.41 ± 1.2
CSC 1	5.72 ± 0.6	14.41 ± 1.7	0.80 ± 0.2	13.44 ± 1.1	5.07 ± 0.1	17.68 ± 1.3
CSC 2	2.94 ± 0.9	4.09 ± 1.3	16.79 ± 1.6	16.94 ± 2.3	7.41 ± 0.3	9.57 ± 0.2
GR 3	8.45 ± 2.4	7.16 ± 1.1	14.82 ± 2.9	16.84 ± 1.8	2.42 ± 0.4	5.05 ± 1.3
IR 20	4.79 ± 0.9	9.18 ± 1.3	16.83 ± 0.9	17.57 ± 2.8	5.84 ± 1.0	9.46 ± 0.1
TKM 4	1.50 ± 0.3	5.91 ± 2.5	10.15 ± 0.6	12.72 ± 0.8	8.04 ± 0.8	9.72 ± 1.5
TKM 9	5.44 ± 0.3	5.41 ± 0.4	11.66 ± 1.4	14.71 ± 1.4	5.44 ± 0.3	5.41 ± 0.4
Co 36	1.42 ± 0.2	5.75 ± 0.7	8.49 ± 2.3	11.14 ± 0.3	1.42 ± 0.5	2.28 ± 0.8

C: control; S: salinized; 2, 4 and 6 weeks after initial salinization; The values are the means ± SE for 3 replicates.

(table 1). Varietal dissimilarities in the accumulation of sodium and chloride in the shoot system during salinization have been reported earlier[7].

Generally glycinebetaine content was found to increase in all the cultivars in response to salinity. However there was marked varietal difference in the accumulation of glycinebetaine under saline conditions. The salt-tolerant cultivars CSC 1, AU 1 and Co 43 amassed the glycinebetaine content in the range of 2.5 to 4.1 fold (16.4–21.3 μmol/g dry weight) while salt-sensitive TKM 9, TKM 4, CSC 2, Co 36, IR 20 and GR 3 exhibited less glycinebetaine levels (0 to 1.1 fold) in their leaves at six weeks after initial salinization (table 2). The present results and those reported earlier[5,6] in other plants indicate that salt-tolerant plants accumulate higher levels of glycinebetaine than salt-sensitive plants. The glycinebetaine concentration in the leaves of Co 43, CSC 1 AU 1 was consistently higher (13–23 μmol/g dry weight) than the other cultivars throughout growth under salinization. Glycinebetaine must therefore be synthesized continuously during growth under saline conditions. In wheat, glycinebetaine once formed, constitutes an inert end product that is not further metabolized by the plant during stress[1].

Similarly NaCl treatment highly increased the total QAC (2.0 to 2.9-fold) too, in salt-tolerant Co 43, CSC 1 and AU 1 as compared to other salt-sensitive

Table 3 *Effect of NaCl on the total quaternary ammonium compounds from the third leaf of rice cultivars*

	Total QAC (μmol/g dry weight)					
	2		4		6	
Cultivar	C	S	C	S	C	S
AU 1	7.94 ± 0.7	38.72 ± 3.6	3.53 ± 0.5	30.51 ± 1.7	10.04 ± 1.1	39.01 ± 0.3
Co 43	10.78 ± 0.9	40.37 ± 2.5	14.15 ± 0.5	40.49 ± 2.9	14.96 ± 2.0	42.80 ± 1.3
CSC 1	12.14 ± 0.2	32.60 ± 2.6	10.18 ± 1.1	31.00 ± 1.9	13.13 ± 0.1	43.26 ± 0.6
CSC 2	11.30 ± 1.6	12.14 ± 0.1	24.88 ± 1.6	27.33 ± 3.8	21.33 ± 1.1	23.87 ± 0.6
GR 3	15.68 ± 2.9	19.35 ± 1.4	18.66 ± 3.7	25.36 ± 1.5	7.57 ± 0.7	12.14 ± 1.9
IR 20	18.61 ± 1.0	25.24 ± 1.9	25.66 ± 2.5	28.86 ± 4.1	12.78 ± 1.6	19.65 ± 0.1
TKM 4	17.10 ± 0.5	21.23 ± 0.7	21.60 ± 2.8	22.47 ± 2.6	14.58 ± 1.1	17.78 ± 1.4
TKM 9	21.35 ± 2.0	18.84 ± 1.4	21.12 ± 1.6	23.11 ± 3.5	9.92 ± 1.8	8.99 ± 0.8
Co 36	8.50 ± 0.7	14.05 ± 1.7	16.63 ± 2.1	21.66 ± 0.10	10.70 ± 1.4	12.59 ± 0.9

C: control; S: salinized; 2, 4 and 6 weeks after initial salinization; The values are the means ± SE for 3 replicates.

cultivars CSC 2, Co 36, TKM 4, IR 20 and GR 3 in the leaves due to salinization (table 3). We had earlier reported that the salt-tolerant cultivars AU 1, Co 43 and CSC 1 maintain high levels of K^+ in their shoots than the other salt-sensitive cultivars to saline treatment[10]. The capacity to increase ion uptake, particularly of K^+ and to synthesize small molecules, like glycinebetaine and total QAC, might represent an important adaptation by rice to a shortage of water under saline conditions. Moreover these total QAC could indicate a sparing of K^+ in the cytoplasm by some of these compounds like glycinebetaine and act as a cytoplasmic osmoticum[11].

It is evident from the results that the salt-tolerant cultivars AU1, Co 43 and CSC 1 exhibited less reduction in the dry matter accumulation of shoot, higher values of SI and higher magnitude of increase in glycinebetaine and total QAC in their leaves than the salt-sensitive cultivars under saline conditions. The accumulation of glycinebetaine was consistently associated with salt tolerance of rice and may be used as a cumulative index for evaluation of salt-tolerant rice cultivars.

The authors acknowledge CSIR, New Delhi and ISRO, Bangalore for financial assistance.

6 May 1987; Revised 20 October 1987

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A METHOD FOR THE ISOLATION OF MEGAPLASMID FROM *PSEUDOMONAS SOLANACEARUM*

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A rapid and sensitive 'out gel' lysis technique is described to isolate mega plasmid from *Pseudomonas solanacearum*. Screening of *Azotobacter* sp., *Bacillus subtilis* and *Xanthomonas* spp. for plasmids by this method gave reproducible results. Our procedure can be employed routinely to rapidly screen the strains for plasmids.

Occurrence of plasmids in phytopathogenic bacteria is ubiquitous and they carry genes, implicated in pathogenesis[1]. *P. solanacearum* is an important plant pathogen infecting a variety of plants. Currier and Morgan[2] detected plasmids in 6 out of 20 strains. Rosenberg *et al*[3] observed a slowly migrating plasmid DNA band in 8 out of 9 strains of *P. solanacearum*. Morales and Sequeira[4] screened 39 strains of this bacterium, collected from different geographic regions and noted 1 or 2 plasmids with relative masses from 7.5 kb to about 750 kb in 22 strains. In *P. solanacearum* involvement of plasmid pAMB1 in catechin dissimilation was shown[5]. Both preparative and analytical methods have been used to visualize plasmid DNA. These are not only time-consuming but result in doubling of bands. Morales and Sequeira[4] observed the duplication of plasmid band of *P. solanacearum* strain pps13 when isolated by 'in gel' lysis technique. Often the results are not reproducible. In this communication, we report a simple and rapid method to detect megaplasmid in *P. solanacearum*.

P. solanacearum isolated from infected pseudostems of banana was subcultured in BG medium[6] for 12 h. Cells, (5 ml, 0.6 to 0.8 OD) were harvested by centrifugation at 10,000 *g* for 10 min at 4°C. The pellet was washed with TES buffer (sucrose, 0.3 M; tris-HCl, pH 8, 25 mM; EDTA pH 8, 25 mM) and suspended in 400 μl TES buffer to get 8×10^7 cells/ml. The suspension was transferred to a microfuge tube (1.5 ml) and kept in ice. Lysozyme (100 μl, 1 mg) was added to the suspension and incubated for 20 min, with mixing at 2 min interval. Lysis was completed by the addition of 30 μl SDS (2%). To the lysate, 30 μl loading dye (10 × bromophenol blue) were added and mixed. Aliquots (24 μl) were loaded on to 0.7% agarose gel submerged in tris acetate EDTA buffer. Elec-