Bangladesh J. Bot. **22** (1): 29-36, 1993 (June)

PHYSIOLOGY OF NaCl TOLERANCE IN CALLI OF *VIGNA RADIATA* (L) WILCZEK

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Key words: Salt tolerance, Compatible solute, Osmotic adjustment, $NaCl_{ext}$, Endogenous Na^+, Proline, Free sugars.

Abstract

Calli of two strains of *Vigna radiata*, CUM 82007 and CUM 82037 were cultured on the callus proliferation medium (MS + 2.0 mg l^{-1} BA + 0.2 mg l^{-1} NAA) in presence of NaCl concentrations ranging from 0 to 150 mM. Calli of CUM 82007 exhibited salt tolerance upto 100 mM $NaCl_{ext}$ while CUM 82037 was susceptible to salinity at this concentration. Presence of NaCl in the medium affected various growth parameters differently of the Calli of the two strains. Correlation coefficients between external and internal Na^+ concentrations with various growth parameters as well as soluble sugars and proline contents revealed that proline is a better stress marker than sugars and it accumulates in the cells possibly to withstand situation of salinity stress. However, the salt tolerant strain accumulates relatively less proline than susceptible one. Free sugars appear to have a more important role in salt tolerance of the legumes.

Introduction

Plant tissue culture techniques have been developed to be new and powerful tools for crop improvement including the possibilities of manipulation to induce genetic changes and selection of desirable traits (Carlson 1975, Reinert and Bajaj 1977, Gresshoff and Mahapatra 1981, Rajdan and Cocking 1981). This technique also helps to recover useful variants by using cellular selection agents. The exposure of cultured cells to toxic concentrations of the selection agents (e.g., NaCl) and plant regenerated from the tolerant cell lines are presumptive mutants.

Glycophytic higher plants show variations in their tolerance to excess salt in the growth media (Greenway and Munns 1980). The tolerance of the non-halophytes is concomittant with accumulation of compatible solutes like proline, soluble sugars or other compounds (Weimberg *et al.* 1982). Lowering of internal K:Na ratio has been ascribed to one of the reasons for adverse effect of excess salinity, a ratio considered critical for several metabolic functions (Greenway and Munns 1983). Efforts have been made to determine the flow of Na and K into tissue and its ultimate effect on proline accumulation in various

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parts of salt acclimated legumes (Imamul Huq and Larher 1985) and halophyte *Aster tripolium* (Goas *et al.* 1982). Imamul Huq and Larher (1984) also made a comparative study on the accumulation of proline and free sugars as stress markers in salt acclimated *V. sinensis*.

Reports on Na and K accumulation and its ultimate consequence on proline and/or free sugar accumulation in salt acclimated cells show that in *Nicotiana sylvestris* (Dix and Pearce 1981) and in rice (Reddy and Vaidyanath 1986) proline accumulates as a compatible solute to salt stress. Imamul Huq and Larher (1985) pointed out that in legumes accumulation of proline and sugars in response to salt stress is a function of the water deficit and of the cellular K contents, not of the endogenous Na content. The more a species susceptible to external salinity the higher is the proline content in it, or consequently, the relative accumulation of proline is an indication of the mechanism of the species or the strain to its capability of acclimation to $NaCl_{ext}$. High accumulation of proline in legumes indicated its susceptibility (Imamul Huq and Larher 1985). The present work aims at further clearing the point at cellular level. Evolution of Na and K accumulation and corresponding accumulation of proline and soluble sugars in unorganised callus tissues of *V. radiata* cultured at different levels of salinity were studied.

Materials and Methods

Seeds of *Vigna radiata* (L) Wilczek, CUM-82007 and CUM-82037 were asceptically germinated on sugar (2%) and agar (1%) media. Calli were initiated from hypocotyl explants of 5 days old seedlings in MS medium supplemented with seven different hormonal combinations (Table 1) and were maintained on MS + 2.0 mg l^{-1} BA + 0.2 mg l^{-1} NAA. Calli obtained from hypocotyl explants after three passages of growth were transferred to the callus proliferating medium supplemented with different concentrations of NaCl (0, 10, 25, 50, 100 and 150 mM). Samples of calli were collected carefully from each culture tube so that agar/medium did not adhere to it. The fresh and dry weights of each sample were determined and the water contents calculated. The results presented are the averages of three individual replicates of three samples in each.

Analyses: Plant materials were extracted with cold 80% ethanol. The suspension was evaporated in a rotary evaporator and the residue was dissolved in deionized water. This extract was used for the determination of Na and K by flame photometry, proline and soluble sugars by spectrophotometric analyses following the methods of Troll and Lindslay (1955) and IRRI (1976) respectively. Total soluble sugars are expressed in terms of glucose. The results are presented in terms of dry matter, m mol^{-3} or mM as and when needed. Simple correlations

were calculated on the results to study the relationship between external Na^+, cellular Na^+ and other parameters to determine the relationships of NaCl acclimation of the two strains.

Results and Discussion

Calli were readily initiated from hypocotyl explants in MS medium supplemented with seven combinations of different hormones (Table 1). Calli from hypocotyl explants after three passages of growth in MS+2.0 mg l^{-1} BA+0.2 mg l^{-1} NAA were subcultured to the same medium supplemented with different concentrations of NaCl as this medium appeared to be the best.

Table 1. Effect of different combinations of auxin and cytokinin in MS medium on callus induction*.

Supplements mg l^{-1}	Materials	No. of explant initiated callus	% of callus inducted
2.0 BA	*V. radiata*		
+	CUM 82007	22	88
0.2 NAA	CUM 82037	18	72
2.0 BA	CUM 82007	18	72
+			
0.5 NAA	CUM 82037	20	80
2.0 Kn	CUM 82007	16	64
+			
0.2 NAA	CUM 82037	12	48
2.0 Kn	CUM 82007	21	84
+			
0.5 NAA	CUM 82037	16	64
0.5 Kn	CUM 82007	12	48
+			
0.5 2, 4-D	CUM 82037	8	32
0.5 Kn	CUM 82007	16	64
+			
1.0 2,4-D	CUM 82037	12	48
0.5 Kn	CUM 82007	23	92
+			
2.0 2, 4-D	CUM 82037	20	80

* Number of explants inoculated were 25 for each supplement.

Effect of NaCl $_{ext}$ on callus growth : Growth of *V. radiata* CUM 82007 was favoured at low salinity (10 mM) while that of CUM 82037 was affected at the same concentration of NaCl. The former survived upto 100 mM NaCl $_{ext}$ but turned brown within seven days at 150 mM while the latter could tolerate only upto 50 mM $NaCl_{ext}$; it turned brown at 100 mM (Table 2).

Dry weight of CUM 82007 gradually decreased with increasing salt concentration in the growth media. The dry wt. was higher than control at 10 mM $NaCl_{ext}$. Water content was greater in the calli grown at higher $NaCl_{ext}$ than those growing at lower salinity. However, it did not exceed that of control (Table 2). Changes in dry weight of CUM 82037 did not follow any pattern; the water content remained somewhat static for the treatments.

Table 2. Effect of $NaCl_{ext}$ on callus growth, water contents, fresh and dry weights of the Calli.

$NaCl_{ext}$ mM	Strains of *V. radiata*	No. of tubes* in which calli proliferated	Fresh wt. g	Dry wt. g	Water content %
0	CUM 82007	18 (90)	1.98	0.13	93.7
	CUM 82037	18 (90)	3.80	0.23	93.9
10	CUM 82007	20 (100)	1.60	0.21	86.7
	CUM 82037	18 (90)	2.90	0.18	93. 3
25	CUM 82007	17 (85)	1.53	0.16	88.9
	CUM 82037	15 (75)	2.77	0.18	93.3
50	CUM 82007	16 (80)	1.71	0.13	92.3
	CUM 82037	11 (55)	3.00	0.20	93.1
100	CUM 82007	10 (50)	1.49	0.12	91.3
	CUM 82037	0 (00)	-	-	-
150	CUM 82007	0 (00)	-	-	-

* There were 20 culture tubes for each treatment. Figures in parenthesis are the percentage of proliferated calli.

Na and K contents in the callus cells: The contents of cellular Na and K in CUM 82007 and CUM 82037 were different depending upon the concentration of NaCl in the medium. K accumulation was higher than the control for the salt treatments in CUM 82007 while in CUM 82037 it was found to be lower even at 10 mM $NaCl_{ext}$ (Table 3). With increasing salt concentration the cellular K, though decreased, never was below that of control cells in CUM 82007. On the other hand, K accumulation in CUM 82037 decreased with increasing salinity and the content became lower than that of control even at 10 mM $NaCl_{ext}$. The strain CUM 82007 accumulated relatively higher amounts of Na than CUM 82037. Na accumulation increased gradually with increase in salinity of the media. It is apparent that the strain CUM 82007 has capability of absorbing higher amounts of K than CUM 82037 which might contribute to a better osmotic adjustment under similar conditions of salinity as K is involved in

osmotic adjustment in glycophytes (Hellebust 1976). The differential K and Na accumulation by the two strains are indication of their relative salt tolerance. It has been observed with intact plants that species capable of tolerating $NaCl_{ext}$ better have the mechanism of either excluding Na accumulation or incorporation relatively higher K concomittant with Na influx (Imamul Huq and Larher 1983, 1985, Imamul Huq *et al.* 1987).

Table 3. Accumulation of Na^+, K^+, soluble sugars and free proline in NaCl acclimated calli of *Vigna radiata*.

$NaCl_{ext}$ mM	Strain of *V. radiata*	Na^+ in callus cells, mg/g d. w.	K^+ in callus cells, mg/g d. w.	Soluble sugars in callus cells, mM	Free proline in callus cells mM
0	CUM 82007	11.60	49.00	19.16	0.09
	CUM 82037	1.88	20.20	182.06	2.00
10	CUM 82007	46.31	103.15	75.21	0.03
	CUM 82037	6.14	19.04	244.73	3.21
25	CUM 82007	28.33	77.53	29.22	0.03
	CUM 82037	12. 91	19.14	259.35	5.75
50	CUM 82007	60.80	59.71	22.96	0.08
	CUM 82037	18.79	18.78	234.55	5.61
100	CUM 82007	77.08	54.72	32.30	0.08
	CUM 82037	-	-	-	-

Effect of $NaCl_{ext}$ on proline and soluble sugars accumulation: The accumulation of proline and soluble sugars by the two strains of *V. radiata* in response to salinity stress were different. The relative amount of both proline and soluble sugars was higher in CUM 82037 than CUM 82007.

CUM 82007 accumulated lesser amount of proline at 10 mM NaCl than at control. However, with increase in external salinity proline accumulation gradually increased beyond this $NaCl_{ext}$ concentration. Sugar accumulation in this strain was higher at 10 mM $NaCl_{ext}$ than the control. This accumulation continued to increase with increasing salinity with a drop however, at 25 mM $NaCl_{ext}$ (Table 3).

In CUM 82037, proline content was lower at control than at any salt treatments. The amino acid showed increased accumulation with increasing salinity in the media. Sugar accumulation in this strain increased gradually with salinity though at 50 mM $NaCl_{ext}$ it showed a decrease (Table 3). Correlation coefficients were calculated between Na^+_{ext} and fresh weight, dry weight, water content, internal Na^+, K^+, proline and soluble sugars as well as

between endogenous Na^+ and fresh weight, dry weight, water content, endogenous K^+, proline and sugar. The values are presented in Tables 4 (a) and 4 (b) respectively.

Table 4. Correlation coefficient values (r) calculated between (a) $NaCl_{ext}$ and various parameters; (b) cellular Na^+ and various parameters.

(a)

Parameters	r_1	r_2
Fresh weight	-0.4233	-0.4739
Dry weight	-0.3928	-0.2413
Water content	-0.3674	-0.5014
K content	-0.1034	-0.4981
Na content	+0.9873**	+0.9823**
Proline content	-0.2824	+0.8068*
Soluble sugar content	+ 0.1408	+0.2909

(b)

Fresh weight	-0.6886	-0.4335
Dry weight	-0.2944	-0.3759
Water content	-0.7004	-0.8477*
K content	-0.1233	-0. 4431
Proline content	+0.1262	+0.8876*
Soluble sugars content	+0.5353	+0.3897

r_1=*V. radiata* strain CUM 82007, r_2=*V. radiata* strain CUM 82037

*=significant at 5% and **=significant at 1% confidence limit

Na^+_{ext} negatively affected the growth parameters of callus and the accumulation of K^+ in the tissues. However, Na^+ influx in the tissues was significantly affected by the presence of the ion in the medium in both strains of *V. radiata*. Proline accumulation was positively correlated with Na^+_{ext} in the strain CUM 82037 while it was negatively correlated for the CUM 82007 strain. Soluble sugar accumulation, though positively correlated, however was not significant (Table 4a). Correlation coefficients between endogenous Na^+ and other growth parameters and endogenous K^+ content were negative. Proline content in CUM 82037 was significantly and directly correlated with the cellular Na^+ contents. This value for CUM 82007, though positively correlated, was however, not significant. Soluble sugar contents were better correlated with

endogenous Na^+ content in both the strains, indicating possibly that in legumes sugars play an important role in osmotic adjustments.

Proline is known to be accumulated in plants subjected to environmental stress (Hsiao 1973, Stewart and Larher 1980, Greenway and Munns 1983). So, it is probable that the strain CUM 82037, that was more susceptible, suffered a greater stress thereby accumulating more proline than the tolerant strain CUM 82007. Although proline is known to be accumulated in glycophytes acclimated to saline environments, the real significance or consequence of such an accumulation in salt acclimated legumes is still obscure (Imamul Huq and Larher 1985). It is apparent from the present study that the appearance or accumulation of this amino acid in the two strains is gradual and the concentration is higher where cellular Na^+ accumulation is relatively greater than cellular K^+ content. The strain accumulating lesser proline was found to be a better salt tolerant strain. Similar results were also observed by Imamul Huq and Larher (1985) while studying the physiology of salt tolerance of two legume species. The present observation further confirms the doubt put forward by these authors (1985) about the possible role of proline in the cytoplasmic adjustment in legumes. So far the soluble sugars are concerned, these solutes appear to play a more important role in osmotic adjustments in legumes under salinity stress (Imamul Huq and Larher 1983). The present observations lead to such a conclusion. A possible involvement of Na^+ in cytoplasmic adjustment of legumes when they acclimate to NaCl salinity is there too (Imamul Huq 1984). Mozafar and Oertli (1984) have shown that 4.4% of the tissue's total Na^+ can be present in the cytoplasm of cotton leaf cells. Proline accumulation in the calli of the two strains of *V. radiata* acclimated to salinity could be either symptomatic of a salt induced water deficit or a temporary deviation of the metabolic pathway of NH_4 assimilation at the level of glutamic acid (Imamul Huq and Larher 1985).

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(Manuscript received on 16 September, 1992 ; revised on 4 October, 1992)