IN-VITRO STUDY ON SALT TOLERANCE IN RICE

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ABSTRACT

In-vitro studies on salt tolerance were carried out with callus and seedlings of two indica varieties, CSR27 (salt tolerant) and HBC19 (salt sensitive). Callus of both the varieties was transferred to salinized MS medium containing 0 (control), 0.5, 1.0, 1.5 and 2.0% NaCl (w/v) for four weeks. Fourteen-day old seedlings were also exposed to salt treatment in half-strength MS salts solution with 0 (control), 0.25, 0.50, 0.75, 1.0 and 1.50% NaCl (w/v) for one week. High salt concentration decreased the relative growth, based on fresh weight of both callus and seedlings, however, the effect was more in the callus of HBC 19. The accumulation of proline was also studied. The proline content was higher in the callus, but lower in the seedlings of CSR27 compared to that in the callus and seedlings of HBC19, respectively. The rate of accumulation of proline, however, was less in both callus and seedlings of CSR27. All of these results suggest that callus can be used as a system for screening salt tolerance in rice, and proline plays some role in salt tolerance mechanism in callus, but not in seedlings of two rice varieties investigated.

Key words: *Oryza sativa*, proline, rice, salinity, salt tolerance

INTRODUCTION

Salinity is widespread soil problem in rice-growing countries (Senadhira, 1987). The area affected by salinity in the world covers about 400 million hectares (Flowers et al., 1977), of which 54 millions are found in South and SouthEast Asia (Akbar and Ponnamperuma, 1982). The need of the improvement of salt tolerance in crop plants, rice in particular, is well-documented (Nabors, 1990; Flowers and Yeo, 1995). Breeding for salt tolerance in rice is difficult due to the involvement of several genes and insufficient knowledge about mechanism (s) controlling the character (Akita and Cabuslay, 1988; Yeo et al., 1990). Accumulation of some compatible solutes has been observed under salt stress conditions and has been suggested as part of the mechanism(s) that controls salt tolerance in plants. Proline is one of the best-known solutes, however, its relative importance for tolerance and precise protective function during stress require further investigations (Bohnert and Jensen, 1996).

In this study we have examined and compared the proline accumulation in callus and seedling of rice varieties with different degree of salt tolerance in order to exploit proline over-production for improving salt tolerance in rice.

MATERIALS AND METHODS

Plant materials

Seeds of two indica rice varieties, CSR27 (salt tolerant) and HBC19 (salt sensitive) were obtained from the

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Growth conditions

Callus culture

Callus was initiated from mature seeds of both varieties on Murashige and Skoog (1962) (MS) medium supplemented with 2 mg/l 2,4-D at 24-26 ^{0}C in dark. Four-week old callus was divided into 50mg pieces. These pieces were transferred onto the same medium supplemented with 0 (control), 0.5, 1.0, 1.5 and 2.0 % (w/v) NaCl and kept at 24-25^{0}C under 10 hours light intensity from two 40W- fluorescent tubes maintained at a level of 30 cm above the culture bottles. At the end of 4-week periods, the callus was taken for growth and proline analysis.

Seedling culture

Seeds from both the varieties were germinated in petri dishes lined with moistened filter papers and the plants were watered with half-strength MS salt solution, pH5.5. Fourteen-day old seedlings were shifted into test tubes containing the same solution but with 0 (control), 0.25, 0.50, 0.75, 1.00 and 1.50 % (w/v) NaCl. The solutions were changed every two days and the cultures were kept at the same conditions as for callus. After 1 week the seedlings were harvested for growth and proline analysis.

Growth analysis

Fresh weight of callus and seedlings and plant height were recorded at the beginning and the end of the culture period. The relative growths were

$$\mathrm{Re}\,lativeGrowth = \frac{FinalGrowth - InitialGrowth}{InitialGrowth}$$

calculated on the basis of the initial and final growth as follows: (Smith and McComb, 1981)

Proline analysis

Proline content in the tissue was estimated by colorimetric method as described earlier (Bates at el., 1973)

Data analysis

The data were subjected to analysis of variance and Duncan's Multiple Range Test (DMRT) for comparing population means (Gomez and Gomez, 1984)

RESULTS

Effect of NaCl on relative growth and proline content of callus

The relative growths of callus of both rice varieties CSR27 and HBC19 were decreased with increasing salt concentration in the medium (Table 1). The relative growth of callus of the salt tolerant variety CSR27 was significantly decreased at 0.5%NaCl while it was at 1.0% NaCl in callus of salt sensitive variety HBC19.

The proline content in callus of both varieties was gradually increased with increasing salt concentrations, but dropped below the level of the controls at 2.0% NaCl.

Table 1. Effect of NaCl on relative growth and proline content of callus of rice varieties CSR27 and HBC19.

Treatment (1)	Relative Growth (2) (%)	Proline content (2) (μmol/g F.W.)
CSR27		
Control	187.00b	3.05a
0.5%NaCl	213.57b	4.97b
1.0% NaCl	103.80cd	5.15b
1.5%NaCl	35.83de	6.17c
2.0%NaCl	27.07e	1.92d
HBC19		
Control	312.80a	0.79e
0.5%NaCl	155.93bc	2.26f
1.0% NaCl	62.03de	4.63g
1.5%NaCl	35.63de	6.81h
2.0%NaCl	18.70e	0.62e

(1) Approximately 50mg-pieces callus was cultured on MS medium supplemented with 2mg/l 2,4-D and various salt concentrations as indicated for 4 weeks period.
(2) Average of three replications.
(3) Any two means having a common letter are not significantly different at 5% level of significance by Duncan's Multiple Range Test (DMRT).

Effects of NaCl on relative growth and proline content of seedlings

NaCl decreased the relative growth based on fresh weight, but it non-significantly affected the relative growth based on the plant height of seedlings of both varieties (Table 2). The significant decrease in the relative growth based on fresh weight was observed at 0.75% NaCl in CSR27 and at 1.0% NaCl in HBC19.

The significant increase proline content in seedlings was observed at1.50%NaCl in variety CSR27, while it was only at 0.75% NaCl in variety HBC19.

Table 2. Effects of NaCl on Relative Growth and Proline Content of seedlings of rice varieties CSR27 and HBC19.

Treatment (1)	Relative Growth (%)(2)		Proline Content(3)
	Fresh Weight	Plant Height Plant	(µmol/g F.W.)
CSR27			
Control	51.11a	38.74abc	0.47a
0.25 % NaCl	56.98a	39.68ab	0.52ab
0.50% NaCl	49.45a	36.00abc	0.56ab
0.75% NaCl	48.35b	20.74abc	0.57ab
1.00% NaCl	33.70bc	10.46bc	0.63ab
1.50% NaCl	21.84cd	7.85c	0.67b
HBC19			
Control	49.62bc	18.24abc	0.97c
0.25 % NaCl	71.43bc	18.97a	0.98c
0.50% NaCl	51.11bc	14.49abc	1.30d
0.75% NaCl	46.79cd	8.19abc	1.53e
1.00% NaCl	32.37d	5.94bc	1.64e
1.50% NaCl	25.26d	5.49bc	2.06f

(1) Fourteen-day old seedlings were cultured on half-strength MS salt solution supplemented with various salt concentrations as indicated in 1-week period.
(2) Average of three replications. Any two means having a common letter are not significantly different at 5% level of significance by Duncan's Multiple Range Test (DMRT).
(3) Average of two replications. Any two means having a common letter are not significantly different at 5% level of significance by Duncan's Multiple Range Test (DMRT).

DISCUSSION

Salinity slowed down relative growth of callus and seedlings of both salt tolerant (CSR27) and salt sensitive (HBC19) rice varieties. The reduction of relative growth under salt stress conditions has also been reported in callus (Smith and McComb, 1981 a,b; Suenaga et al., 1982; Mill, 1989; Nabors, 1990) as well as in seedlings (Levitt,1980; Yeo and Flowers, 1986; Kefu and Harris, 1990: Salim, 1990). The reduction of the relative growth is due to osmotic stress as well as salt injury (Levitt, 1980). The relative growth of callus and seedlings of CSR27 based on fresh weight and expressed as percentage of control, however, was always higher than that of the corresponding system of HBC19 except that of seedling at salt concentration above 1.00% (Fig.1). The difference was significant only in callus at 0.5 and 1.0%NaCl in the culture medium.

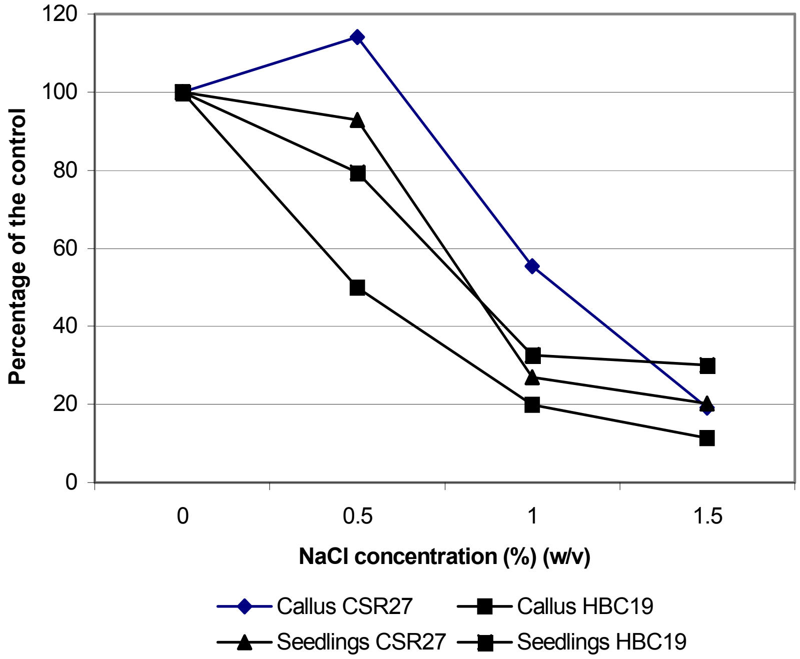

Fig.1. Effect of NaCl on the relative growth based on fresh weight of rice varieties CSR27 and HBC19 and their corresponding calli expressed as percentage of the control.

The accumulation of proline in plants under salt stress has been reported in pea (Williamson and Slicum, 1992), *Arabidopsis thaliana* (Verbruggen et al., 1993; Mizoguchi et al., 1996) and rice (Igarashi et al., 1997). The accumulation of proline was also confirmed by the results of this study in both callus and seedlings of salt tolerant variety CSR27 as well as salt sensitive one HBC19. Although the proline content of callus of CSR27 was higher than that of HBC19 at all salt concentrations except 1.5%NaCl and lower in seedlings, the rate of proline accumulation expressed as percentage of the control of CSR27 was always lower than that of HBC19 in both callus and seedlings (Fig.2)

A compound is of adaptive significance only if (a) it is constitutively accumulated in tolerant but not in sensitive varieties, (b) its accumulation should be enhanced by moderate stress, i.e., 50-200mM and (c) exogenous application of the compound might promote in-vitro the tolerance of the whole-organism or in-vitro enhance the tolerance of specific metabolic process (Jones, 1980). If we accept the criteria, proline seems to be involved in salt tolerance mechanism in callus system, but not in that of seedlings. The role of proline was also investigated in other rice varieties. In Pokkali (salt tolerant) and Hrswa (salt sensitive), the proline content was similar in both callus and seedling under salt stress conditions by using sea water as a stress agent (Sabu et al., 1995). In other two varieties, namely, Dee-gee-woo-gene (salt tolerant) and IR28 (salt sensitive) proline, however, accumulated rapidly in seedlings of the tolerant variety and slowly in that of the salt sensitive one (Igarashi et al., 1997). All of these results indicate that the role of proline may ary among genotypes.

The need for improvement of salt tolerance in crop plants, rice in particular, is well-documented (Nabors, 1990; Flowers and Yeo, 1995). New strategies for increasing salt tolerance in plants have been suggested including "metabolic engineering". Success of its application, however, requires full understanding of the biochemical mechanisms of salt tolerance (Bohnert and Jensen, 1996). Such information, however, is still limited (Nabors,

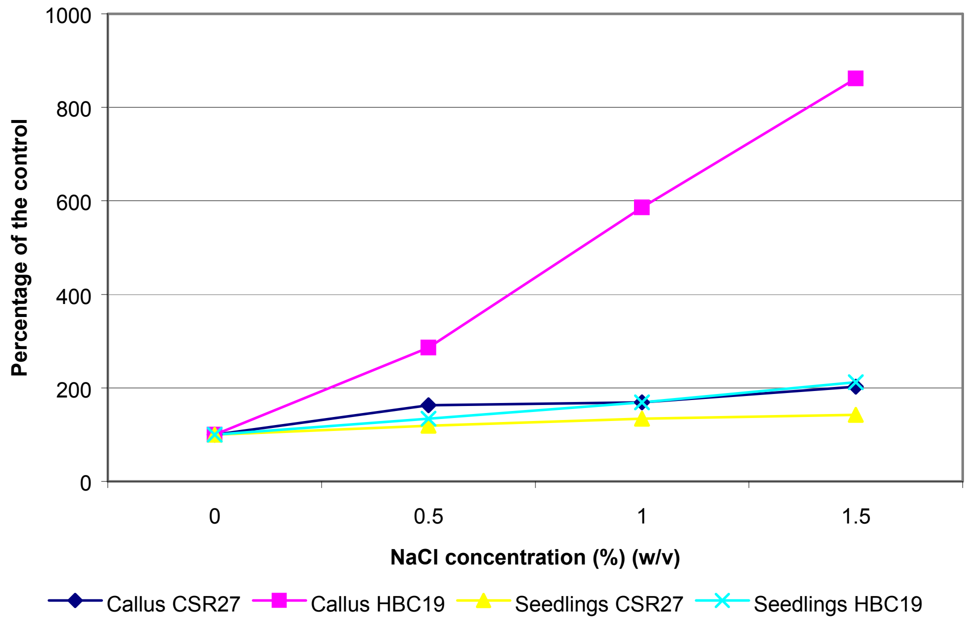

Fig.2. Effect of NaCl on the proline content of seedlings of rice varieties CSR27 and HBC19 and their corresponding calli expressed as percentage of the control.

1990; Vajrabhaya and Vajrabhaya, 1991). Proline, like glycinebetaine is among the best known compatible solutes in plants (Bohnert and Jensen, 1996). While glycinebetaine is not accumulated in many crop plants such as potato and rice (Palva et al., 1996), proline has been proline has been reported to be accumulated in many plants including rice (Sabu et al., 1995, Igarashi et al., 1997). Biosynthesis of proline has been suggested to be responsible by a key enzyme, namely, Δ^1-pyrroline-5-carboxylc acid synthetase (P5CS), which has both γ-glutamyl kinase and glutamic-γ-semialdehyde dehydrogenase activities and is due by dehydration, high salt and treatment with abscisic acid (ABA) (Mizoguchi et al., 1996). Thus, the use of metabolic engineering of proline metabolism is a prospective way to improve salt tolerance in rice as demonstrated in tobacco (Kishor et al., 1995).

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physiological characters contributing to salinity resistance and their relationship to overall performance.

TÓM TẮT

Tính kháng mặn trong điều kiện in vitro của lúa

Nghiên cứu ính kháng mặn trong điều kiện in-vitro được tiến hành trên hai giống lúa indica CSR27 (kháng mặn) và HBC19 (nhiễm mặn). Mô sẹo của các giống này được nuôi trên môi trường MS có chứa 0 (đối chứng), 0,5, 1,0, 1,5 và 2,0% NaCl trong 4 tuần. Cây mạ 14 ngày tuổi cũng được trồng trong điều kiện mặn trong dung dịch chứa 50% muối MS và 0 (đối chứng), 0,25, 0,50, 0,75, 1,00 và 1,50% NaCl trong 1 tuần. Nồng độ muối NaCl cao làm giảm tốc độ phát triển trọng lượng tươi của cả mô sẹo và cây mạ, đặc biệt đối với mô sẹo của giống nhiễm mặn HBC19. Sự tích lũy của proline cũng được nghiện cứu. Hàm lượng proline trong mô sẹo của giống CSR27 cao hơn so với trong mô sẹo của giống HBC19, nhưng ngược lại trong cây ma của hai giống. Tuy vậy tốc độ tích lũy proline trong mô sẹo cũng như trong cây mạ của giống CSR27 đều thấp hơn so với hệ thống tương ứng ở giống BHC19. Từ kết quả nghiên cứu này cho thấy mô sẹo có thể dùng như vật liệu cho thanh lọc tính kháng mặn và proline đóng một vai trò nào đó trong tính kháng mặn của lúa ở múc độ mô sẹo chứ không ở cây mạ của hai giống nghiên cứu.