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INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol.2, Issue, 1, pp. 015-022, January, 2011

ISSN: 0975-833X

RESEARCH ARTICLE

ACCUMULATION OF PROLINE AS A TOOL TO SCREEN RICE GENOTYPES IN THEIR ABILITY TO WITHSTAND OSMOTIC STRESS

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ARTICLE INFO

Article History:

Received 12th September, 2010
Received in revised form
15th October, 2010
Accepted 13th November, 2010
Published online 6th January, 2011

Key words:

Salinity,
Rice genotypes,
Proline,
Osmolytes.

ABSTRACT

Drought and salinity are major abiotic stress factors that severely affect agricultural systems and food production. Many plant species including most important crops are subjected to growth inhibitions under high NaCl conditions. In the present study, seeds of ten different rice genotypes were allowed to germinate and grow in the absence (control) and presence of different concentrations (50mM, 100mM, 150mM and 200mM) of NaCl. The varieties ASD-16, ADT-36 and ADT-43 show higher percentage of reduction in germination than IR-50, JGL-1728 and MDU-5. After 5days, 10days and 15days of salt treatment, the seedlings were harvested and the shoot length and root length measured. Ten days old seedlings were transferred to 50mM and 100mM of NaCl and accumulation of proline was monitored after 12, 24, 48, 72 and 96 hours. The rice varieties IR-50, JGL-1728, MDU-5 and ADT-43 show better tolerance against other varieties.

INTRODUCTION

There are 955 million hectares of saline soil worldwide including arid and semi-arid regions that are limited in their ability to produce crops (Subbarao *et. al*., 1990). Salinity/drought is a major abiotic stress that severely affects at critical stages of cereal crops and may reduce yield (Ludlow and Muchow 1990). Salinity causes additional ion toxicity effects mainly through perturbations in protein and membrane structure. The initial effect of salinity at cellular level is due to its osmotic effects.

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Shoot and root growth rate is reduced, resulting in smaller and fewer leaves and shorter plant stature. The effects of salinity depend on interactions with environmental variables such as water deficit, temperature and solar radiation (Yeo 1999). There are few traits, such as osmotic adjustment and stomatal conductance, considered to be important for drought resistance. Osmotic adjustment is defined as a decrease of osmotic potential within the cells due to active accumulation of compatible osmolytes such as proline, glycine betaine, sorbitol, fructan etc during a period of cell water deficit. Proline is acting as a major osmoprotectant. The transient accumulation of certain metabolites such as proline might have as a safety valve to adjust cellular redox state during stress (Anoop and Gupta 2003; Kavi Kishore *et al.,* 2005, McCue and Hanson 1990). Rice is a semiaquatic plant and does not need standing water for a successful crop. Rice, the most important food crop in many parts of the world is considered to be salt sensitive at young seedlings stages and less so at reproduction. The present study, attempts were made to study the effect of drought/salinity stresses on the growth and development of different genotypes of rice and to quantify free proline accumulated during stress.

MATERIALS AND METHODS

The plant materials used in this study included ten different rice genotypes such as ADT-36, ADT-48, ADT-43, ADT-45, ASD-16, ASD-18, JGL-1728, BPT-5204, MDU-5, IR-50 etc. Seeds of these genotypes are obtained from Rice Research Station at Adothodai, Tamilnadu, India.

Growth Measurements: Various growth parameters such as seed germination shoot lengths and root lengths were measured.

Proline Quantification: Ten days old seedlings were transferred to petridishes with 0mM, 50mM, 100mM sodium chloride and proline content quantified after 12, 24, 48, 72 and 96 hours. The proline was estimated following the method of Bates *et al.,* (1973).The amount of proline in the sample was expressed in µM [proline]/g fresh weight by using the below formula

$$\mu M \text{ of proline } g^{-1}FW = \frac{\mu g \text{ of proline/ml} \times \text{Total volume of toluene}}{115.5} \times \frac{5}{\text{Weight of sample (g)}}$$

where 115.5 is the molecular weight of proline.

RESULT

Effect of salinity on plant growth and development

Effect of salt stress on seed germination: It has been reported that abiotic stresses like salinity/drought affect the growth and development of higher plants. Seeds of rice genotypes were germinated in petridishes supplemented with different concentration of NaCl and the percentage of germination was recorded after 5days. It could be observed that there is reduction in seed germination under the different concentration of salt (50-200mM of NaCl) when compared to the control. Out of the 10 genotypes screened ASD-16, ADT-36 and ADT-48 show higher percentage of reduction in seed germination than IR-50, JGL-1728 and MDU-5 (Table 1 and Fig. 1).

Effect of salt stress on shoot length and root length of plants: Seedlings were raised in petridishes supplemented with different concentrations of NaCl viz., 0mM (control), 50mM, 100mM, 150mM and 200mM. Shoot length and Root length were measured after 5, 10 and 15 days of germination. It has been observed that the impact of salinity was concentration dependent as evidenced by the increase in reduction of growth of seedlings with the increase in the concentration of sodium chloride. Genotypes such as IR-50, ADT-48, JGL-1728 and MDU-5 showed less reduction in growth compared other genotypes screened (Table 2 - 7 and Fig. 2-4).

***The effect of salinity on free proline accumulation*:** Different genotypes of crops differ in their ability to withstand both biotic and abiotic stresses. Evaluation of genotypes in their ability to withstand these stresses is essential in order to find out better genotypes to be employed in breeding programmes. Since, higher plants accumulate free proline to counteract the osmotic imbalance caused by salinity and drought, accumulation of proline could serve as a biochemical marker to evaluate the genotypes in their level of tolerance against osmotic stress. 10 days old seedlings of different genotypes of rice were subjected to salt stress by placing the seedlings in petridishes containing 0, 50 and 100mM sodium chloride.

Proline content in stressed and control seedlings were measured after 12, 24, 48, 72 and 96 hours. Accumulation of proline can serve as a level of tolerance against salinity and drought. The proline content increased with the increase in concentration of salt as well as time (Figure 5). Genotypes such as IR-50, ADT-48, JGL-1728 and MDU-5, showed higher rate of proline accumulation (about 400-800µM/g FW) when compared to other genotypes.

Table 1. Effect of salinity on germination of seeds of different genotypes of rice after 5 days of treatment.

S. No	CV	Ctrl % of Germination	50mM NaCl		100mM NaCl		150Mm NaCl		200mM NaCl	
			% of germination	% of reduction	% of germination	% of reduction	% of germination	% of reduction	% of germination	% of reduction
1	MDU-5	94	91	3.19	90	4.26	87	13.82	72	25.53
2	ASD-16	95	87	7.30	77	18.95	74	32.63	46	51.58
3	ADT-48	92	87	5.40	82	10.86	76	17.39	64	30.43
4	ASD-18	98	95	3.06	89	9.19	82	16.33	68	30.61
5	ADT-36	94	89	5.30	85	9.57	73	22.34	50	46.80
6	ADT-43	91	90	1.09	83	8.79	74	18.68	62	31.87
7	IR-50	93	90	3.22	88	5.38	85	8.60	77	17.20
8	JGL-1728	96	93	3.15	87	9.38	83	13.54	74	22.92
9	BPT-5204	96	82	14.58	76	20.83	73	23.96	65	32.29
10	ADT-45	90	81	10.00	70	22.22	58	35.56	42	53.33

Table 2. Impact of salinity on the growth (shoot length) of rice genotypes measured after 5 days of germination.

S .No.	CV	Control	50mM NaCl	% of reduction	100mM NaCl	% of reduction	150mM NaCl	% of reduction	200mM NaCl	%of reduction
1	MDU - 5	4.31±0.65	1.80±.0.16	58.2	1.05±.0.13	75.63	0.35±0	91.88	0.23±0.01	94.66
2	ASD - 16	1.89±0.29	1.12±0.11	40.74	0.42±0.036	77.78	0.35±0.045	81.48%	0.20±0.028	89.2
3	ADT - 48	1.58±0.52	1.01±0.086	34.21	0.19±0.009	87.50	0.13±0.021	91.77%	0.12±0.019	92.41
4	ADT - 18	1.89±0.29	1.67±0.1	11.64	0.59±0.028	68.78	0.45±0.09	76.19%	0.15±0.022	92.06
5	ADT - 36	1.22±0.032	0.53±0.18	56.56	0.44±0.09	68.93	0.19±0.009	84.43%	0.07±0.008	94.26
6	ADT - 43	2.01±0.54	0.38±0.23	81.09	0.21±0.04	89.55	0.15±0.026	92.53%	0.10±0	95.02
7	IR – 50	1.43±0.72	0.96±0.25	32.87	0.66±0.24	53.84	0.42±0.033	70.63%	0.27±0.015	81.12
8	JGL - 1728	3.44±0.18	0.74±0.025	78.49	0.50±0.12	85.47	0.19±0.017	94.47%	0.05±0.016	98.55
9	BPT - 5204	3.65±0.23	1.11±0.01	69.59	0.72±0.067	80.27	0.33±0.026	90.96%	0.27±0.10	92.60
10	ADT - 45	2.18±0.34	1.16±0.02	46.79	0.55±0.098	74.77	0.27±0.042	87.61%	0.23±0.01	89.45

Table 3. Impact of salinity on the growth (shoot length) of rice genotypes measured after 10 days of germination.

S.No	CV	Control	50mM NaCl	% of reduction	100mM NaCl	% of reduction	150mM NaCl	% of reduction	200mM NaCl	%of reduction
1	MDU – 5	7.2±0.40	2.34±0.18	67.50	1.34±0.16	81.39	0.46±0.06	93.61	0.29±0.035	95.97
2	ASD – 16	4.08±0.48	1.83±0.17	55.14	0.52±0.13	87.25	0.43±0.058	89.46	0.23±0.015	94.36
3	ADT - 48	4.37±0.18	2.03±0.10	58.32	1.05±0.054	78.44	0.48±0.033	90.14	0.29±0.050	94.05
4	ADT - 18	4.55±0.15	2.17±0.22	52.31	1.34±0.14	70.55	0.82±0.051	81.97	0.27±0.018	94.07
5	ADT - 36	3.01±0.14	1.1±0.043	63.46	0.91±0.04	69.77	0.4±0.022	86.71	0.22±0.020	91.36
6	ADT - 43	4.68±0.33	1.16±0.23	75.21	0.41±0.02	91.23	0.34±0.08	92.74	0.16±0.09	96.58
7	IR – 50	5.72±0.70	2.37±0.11	58.57	1.51±0.05	73.60	0.71±0.063	87.59	0.34±0.02	94.06
8	JGL - 1728	4.96±0.14	1.07±0.09	78.43	0.79±0.04	84.07	0.38±0.020	92.34	0.25±0.023	94.96
9	BPT - 5204	7.57±0.30	1.21±0.08	84.02	0.92±0.19	87.85	0.36±0.045	95.24	0.29±0.045	96.17
10	ADT - 45	4.77±0.18	1.96±0.15	58.90	1.03±0.15	78.41	0.47±0.073	90.15	0.29±0.022	93.92

Table.4: Impact of salinity on the growth (shoot length) of rice genotypes measured after 15 days of germination

S.No	CV	Control	50mM NaCl	% of reduction	100mM NaCl	% of reduction	150mM NaCl	% of reduction	200mM NaCl	%of reduction
1	MDU – 5	10.09±0.6	2.61±0.11	74.13	1.59±0.07	84.24	0.55±0.040	94.05	0.28±0.020	97.22
2	ASD – 16	8.14±0.13	2±0.11	75.43	0.58±0.09	92.87	0.43±0.07	94.72	0.24±0.022	97.o5
3	ADT – 48	8.8±0.32	2.16±0.16	75.45	1.04±0.200	88.18	0.43±0.036	95.11	0.27±0.018	96.93
4	ADT – 18	5.42±0.14	3.06±0.10	43.54	1.08±0.18	69.00	0.74±0.022	86.35	0.23±0.020	95.76
5	ADT – 36	7.05±0.18	1.21±0.048	82.84	1.47±0.036	79.15	0.62±0.028	91.21	0.21±0.022	97.02
6	ADT – 43	6.99±0.22	1.26±0.15	81.97	0.4±0.027	94.28	0.4±0.24	94.28	0.17±0.018	97.58
7	IR – 50	9.7±0.23	2.56±0.13	73.61	1.61±0.42	83.46	0.72±0.055	92.58	0.31±0.020	96.80
8	JGL - 1728	6.26±0.40	1.52±0.054	75.72	0.88±0.049	85.94	0.44±0.018	92.97	0.28±0.011	95.53
9	BPT - 5204	8.69±0.64	1.22±0.060	85.96	1.01±0.10	88.38	0.47±0.048	94.59	0.27±0.09	96.89
10	ADT – 45	5.64±0.40	2.275±0.039	59.66	1.18±0.14	79.08	0.65±0.083	88.48	0.27±0.025	95.21

DISCUSSION

The responses of plants to environmental stresses have been important to students of agronomy, ecology and physiology ever since the disciplines were first defined. Salinity/drought result in osmotic stress that may lead to reduction in growth and salinity causes additional ion toxicity. Growth inhibition by water stress is accompanied by an

Table 5. Impact of salinity on the growth (shoot length) of rice genotypes measured after 5 days of germination.

S. No	CV	Control	50mM NaCl	% of reduction	100mM NaCl	% of reduction	150mM NaCl	% of reduction	200mM NaCl	%of reduction
1	MDU – 5	4.28±1.0	1.46±0.1	65.89	0.55±0.03	87.14	0.28±0.019	93.46	0.14±0.019	96.73
2	ASD – 16	2.64±1.1	1.15±0.14	56.44	0.5±0.11	78.79	0.275±0.06	89.58	0.10±0.014	96
3	ADT – 48	3.11±0.8	1.16±0.23	62.70	0.7±0.06	77.17	0.36±0.034	88.42	0±0	100
4	ADT – 18	2.65±.35	0.86±0.9	67.55	0.32±0.04	87.93	0.29±0.036	89.06	0±0	100
5	ADT – 36	0.63±0.1	0.58±0.063	79.40	0.32±0.05	44.80	0.23±0.025	63.49	0±0	100
6	ADT – 43	3.41±0.5	0.85±0.081	75.07	0.40±0.014	88.27	0.1±0.029	97.07	0±0	100
7	IR – 50	2.18±0.7	1.61±0.27	26.15	0.42±0.30	80.73	0.31±0.044	85.78	0.02±0.008	99.08
8	JGL - 1728	3.98±.32	1.31±0.051	67.09	0.72±0.05	81.91	0.39±0.037	90.20	0.02±0.008	99.49
9	BPT - 5204	4.6±0.32	1.17±0.09	74.56	0.68±0.05	85.22	0.28±0.050	93.91	0.13±0.036	97.17
10	ADT – 45	3.57±0.5	0.60±0.087	83.19	0.45±0.12	87.39	0.22±0.048	95.51	0.12±0.025	96.63

Table 6. Impact of salinity on the growth (shoot length) of rice genotypes measured after 10 days of germination

S. No.	CV	Control	50Mm NaCl	% of reduction	100mM NaCl	% of reduction	150mM NaCl	% of reduction	200mM NaCl	%of reduction
1	MDU – 5	6±1.12	2.45±0.13	59.17	0.64±0.10	89.33	0.37±0.038	93.83	0.26±0.09	95.67
2	ASD – 16	3.37±0.43	1.46±0.14	56.68	0.62±0.024	81.60	0.35±0.037	89.61	0.18±0.028	94.66
3	ADT - 48	4.12±0.27	1.54±0.50	62.62	0.86±0.055	79.13	0.47±0.025	88.59	0.15±0.020	96.36
4	ADT - 18	4.18±0.53	2.3±0.39	44.98	0.59±0.056	85.89	0.32±0.033	92.34	0.13±0.07	96.89
5	ADT - 36	2.64±0.20	1.17±0.09	55.68	0.65±0.031	75.38	0.38±0.020	85.61	0.2±0.022	92.42
6	ADT - 43	4.54±0.70	0.86±0.21	81.06	0.60±0.055	86.78	0.29±0.029	93.61	0.16±0.010	96.48
7	IR – 50	4.48±0.28	2.65±0.10	40.85	0.71±0.10	84.15	0.62±0.052	86.16	0.16±0.028	96.43
8	JGL 1728	4.5±0.42	1.33±0.07	70.44	0.84±0.069	81.33	0.54±0.029	88.00	0.30±0.022	93.33
9	BPT 5204	4.7±0.42	0.89±0.08	81.06	0.71±0.16	84.89	0.34±0.032	92.76	0.28±0.018	94.04
10	ADT - 45	4.58±0.29	1.23±0.10	73.14	0.74±0.12	83.84	0.33±0.067	92.79	0.17±0.020	96.27

Table 7. Impact of salinity on the growth (shoot length) of rice genotypes measured after 15 days of germination

S. No	Varieties	Control	50mM NaCl	% of reduction	100mM NaCl	% of reduction	150mM NaCl	% of reduction	200mM NaCl	%of reduction
1	MDU – 5	7.43±0.78	2.82±0.04	62.64	0.85±0.050	88.55	0.43±0.042	94.21	0.21±0.009	97.17
2	ASD – 16	4.31±0.19	1.65±0.10	61.72	0.68±0.007	84.22	0.37±0.031	91.41	0.18±0.028	95.02
3	ADT – 48	4.31±0.22	1.49±0.22	65.43	0.71±0.055	83.53	0.39±0.015	90.95	0.13±0.021	96.98
4	ADT – 18	4.6±0.43	2.32±0.36	49.56	0.61±0.040	86.74	0.32±0.020	93.04	0.08±0.007	98.26
5	ADT – 36	2.86±0.20	1.26±0.09	55.94	0.69±0.033	75.87	0.38±0.020	86.71	0.16±0.022	94.41
6	ADT – 43	4.67±0.67	1.1±0.22	76.45	0.65±0.050	86.08	0.34±0.023	92.72	0.125±0.007	97.32
7	IR – 50	4.79±0.22	2.85±0.19	40.50	0.78±0.110	83.72	0.52±0.066	89.14	0.09±0.013	98.12
8	JGL – 1728	4.79±0.28	1.51±0.11	68.48	0.8±0.016	83.30	0.43±0.019	91.02	0.14±0.013	97.08
9	BPT – 5204	4.98±0.23	1.28±0.10	74.29	0.73±0.044	85.34	0.27±0.025	94.58	0.1±0.020	97.99
10	ADT – 45	4.78±0.36	1.32±0.10	72.38	0.44±0.13	90.79	0.31±0.044	93.51	0.19±0.020	96.03

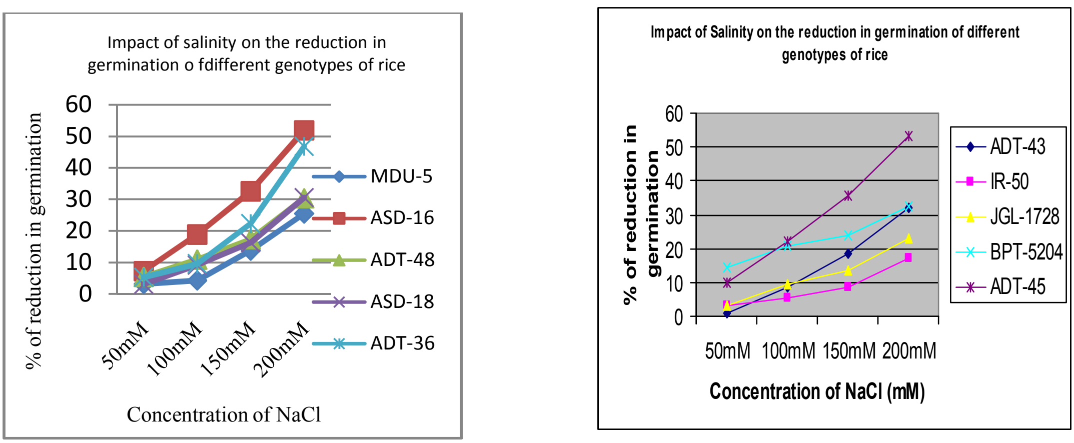

Fig. 1. Effect of salinity on germination of seeds of different genotypes of rice after 5 days of treatment

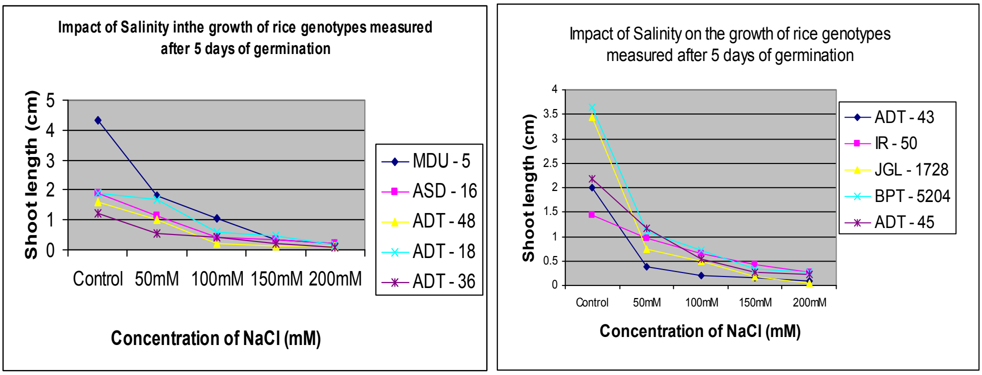

Fig. 2. Impact of salinity on the growth (shoot length) of rice genotypes measured after 5 days of germination

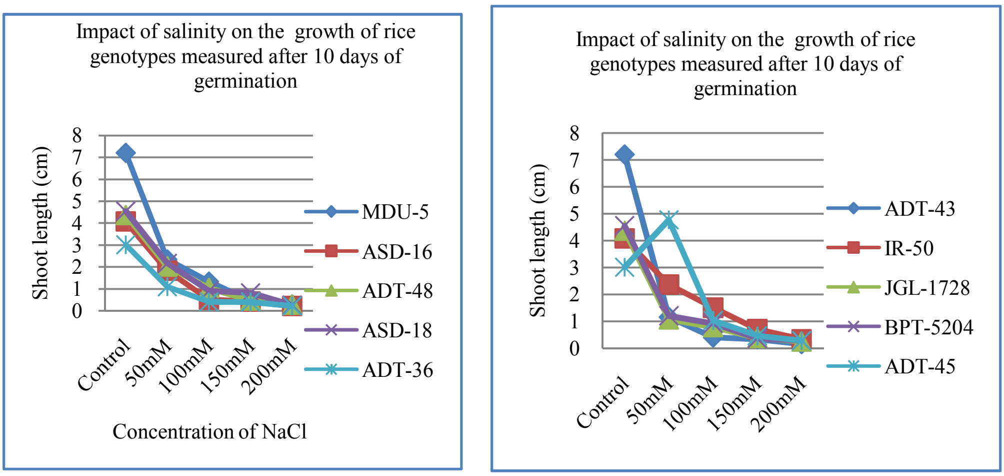

Figure 3. Impact of salinity on the growth (shoot length) of rice genotypes measured after 10 days of germination

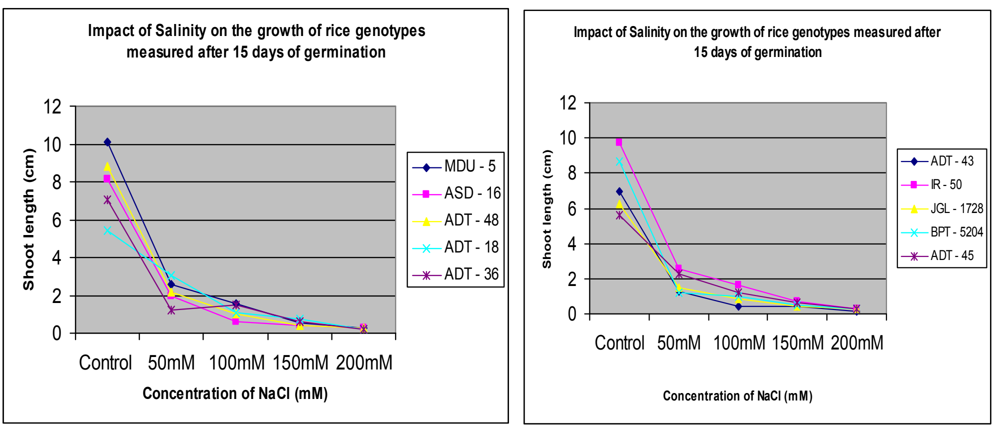

Fig. 4. Impact of salinity on the growth (shoot length) of rice genotypes measured after 15 days of germination

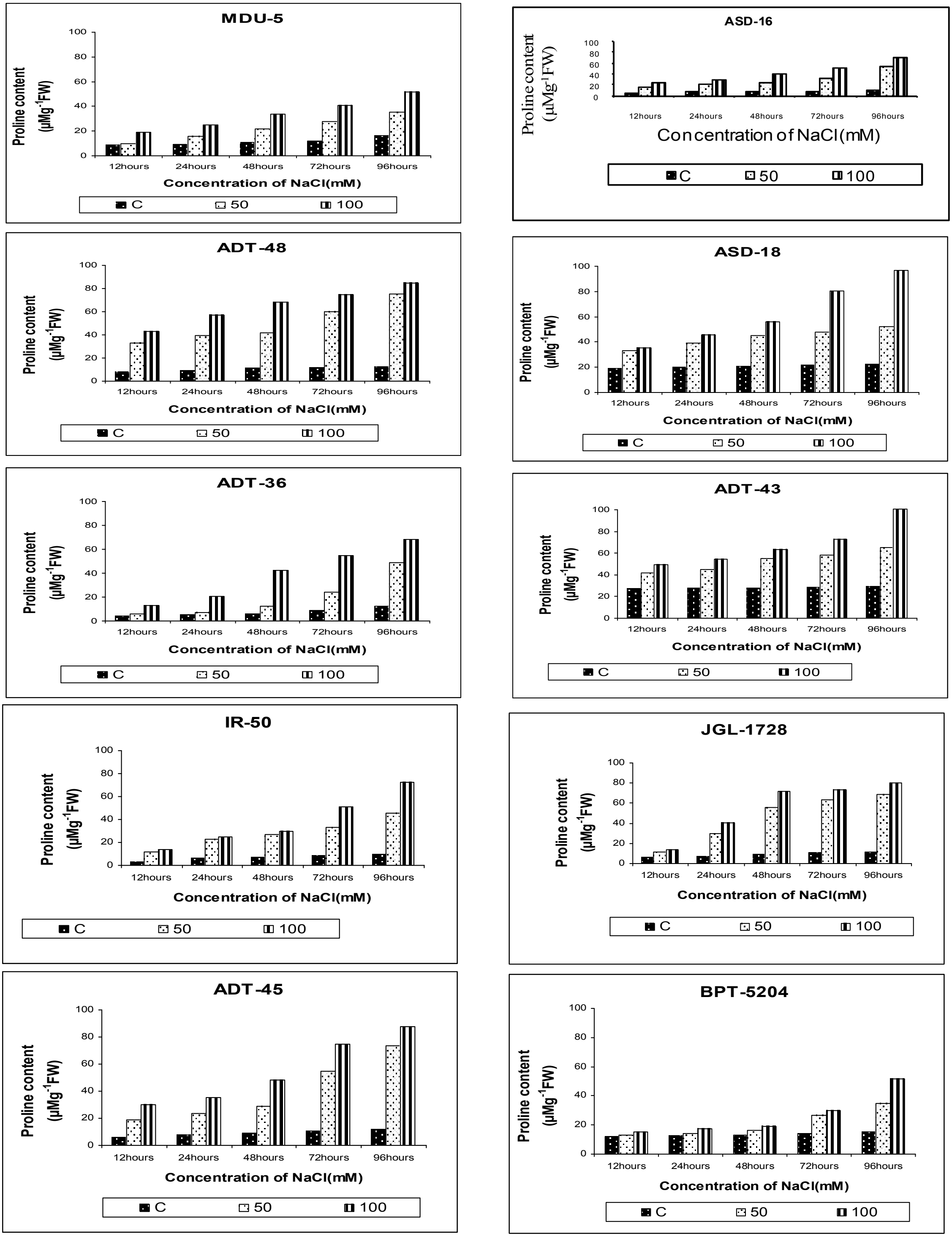

Fig. 5. Impact of salinity on the accumulation of proline in stressed seedlings of rice genotypes

increase in apoplastic pH and a decrease in acidification rate (Boyer 1982; Blum 1999). Impact of salt stress is not only by osmotic effects on water uptake but also by variable effects on plant cell metabolism to alter the plasma membrane, cell wall and cytoskeleton and or induce nutritional disorder and simultaneous reduction in leaf area and root length, affecting photosynthesis, water and mineral uptake.

Morphologically the most typical symptoms of saline injury to a plant is, retarded growth resulting in a stunted appearance. Higher plants produces osmolytes such as proline, glycine betaine and soluble carbohydrates, among others. These solutes, which accumulate mainly in the cytoplasm, contribute the osmotic adjustment, protecting cell structures and functions and also constitute as a source metabolic energy (Kemble and Macpherson 1954; Ludlow and Muchow 1988; Ludlow and Muchow 1990; Morgan 1983; Morgan 1984; Morgan 1986; Rodriguez-Maribona 1992).

Proline acts as a major osmolyte or osmoprotectant. Proline synthesis and degradation have special significance in which proline accumulate to very high levels in response to various kinds of stresses in plants; proline can be synthesized either from glutamate or ornithine. During stress conditions proline is synthesised from glutamate. Proline might act as a safety valve to adjust cellular redox state during stress. The inhibitory effect of NaCl on the growth and germination of seeds of higher plants has been reported by several researchers. In this study also NaCl inhibited germination of seeds of different genotypes of rice. Genotypes such as IR-50, ADT-48, JGL-1728 and MDU-5, showed less reduction in seed germination and also more growth under salinity compared to other genotypes (Table 1-7 and Fig. 1–4). Many eubacteria, algae and higher plants accumulate free proline in response to osmotic stress (Dealauney and Verma, 1993; Anoop and Gupta 2003; Kavi Kishore at al 2005). Significant increase in the level of proline in seedlings stressed with different concentrations of NaCl compared with unstressed was observed during the course of this study. The increase in proline levels was found to be directly proportional to the concentration of NaCl. Genotypes such as IR-50, ADT-48, JGL-1728 and MDU-5, showed higher rate of proline accumulation when compared to other genotypes (Figure 5). Based on the growth performance and the ability to accumulate free proline the genotypes IR-50, ADT-48, JGL-1728 ADT-43 are believed to be tolerant ones against osmotic stress.

Conclusion

It has been observed in the present study that NaCl have adverse impact on the growth and development of rice seedlings as evidenced by their impact on seed germination, shoot length and root length. Therefore, attempts were made to screen different genotypes of rice in their ability to withstand salinity by quantifying the proline content in seedlings subjected to stress assuming that proline could serve as a biochemical marker to screen genotypes in their ability tolerate osmotic stress.

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