

Salt Tolerance in Rice: Glasshouse Screening, Field Experiment and Salt-induced Polypeptides

การทนเค็มในข้าว: การทดสอบในเรือนทดลอง, ภาคสนามและโปรตีนที่ถูกชักนำด้วยความเค็ม

Kanlaya Kong-ghen (กัญญา กองเงิน)* Dr. Piyada Treerakulpisut (ดร. ปิยะดา ธีระกุลพิศุทธิ์)**
Dr. Sumonthip Bunnag (ดร. สุมนทิพย์ บุนนาค)** Dr. Manit Kositrakun (ดร. มานิตย์ โฆษิตตระกุล)**

ABSTRACT

Salt tolerance of 98 Thai rice varieties was determined at seedling stage based on the visual symptoms of salt injury and Na-K ratio in shoots. The rice varieties test were classified into 3 groups: moderately tolerant, susceptible and highly susceptible. In a field experiment conducted in saline soil, eight varieties were selected for evaluation of salt tolerance based on growth, yield and yield components. Salt tolerance in rice showed positive correlation with number of productive tillers per plant, followed by straw yield per plant, panicle length and number of spikelets per panicle. Protein changes induced by NaCl stress were investigated in roots of rice seedlings using SDS-PAGE. Intensity of a 90 kilodalton polypeptide increased in cvs. Leuang Yai, Leuang Anan, Khao Med Lek and Pokkali but not in KDML 105.

บทคัดย่อ

การทนเค็มของข้าวไทย 98 สายพันธุ์ในระยะต้นกล้า วัดได้จากอาการผิดปกติที่เกิดจากความเค็มของเกลือและค่าอัตราส่วนของโซเดียมต่อโพแทสเซียมในส่วนยอดต้น จำแนกการทนเค็มของข้าวไทยได้ 3 กลุ่มคือ ทนเค็มปานกลาง, ไม่ทนเค็มและไม่ทนเค็มมาก การศึกษาการทนเค็มข้าว 8 สายพันธุ์ในพื้นที่ดินเค็มวัดจากค่าการเจริญเติบโต ผลผลิตและองค์ประกอบของผลผลิต พบว่า การทนเค็มของข้าวสัมพันธ์กับจำนวนหน่อต่อต้น ตามด้วยน้ำหนักฟางแห้งต่อต้น ความยาวรวง และจำนวนเมล็ดต่อรวง การศึกษาการเปลี่ยนแปลงของโปรตีนที่ถูกชักนำด้วยเกลือโซเดียมคลอไรด์ในเนื้อเยื่อของรากข้าวโดยใช้เทคนิค SDS-PAGE พบว่า โปรตีนเปปไทด์ขนาด 90 กิโลดาลตันถูกชักนำให้สร้างมากขึ้นในรากเมื่อได้รับความเค็มในข้าวพันธุ์ เหลืองใหญ่, เหลืองอนันต์, ขาวเม็ดเล็ก และพอลคาลี แต่ไม่พบในข้าวพันธุ์ขาวดอกมะลิ 105

Keywords : Rice, Salt tolerance, Salt-induced Polypeptides

คำสำคัญ : ข้าว การทนเค็ม โปรตีนที่ถูกชักนำด้วยความเค็ม

*Student, Doctor of Philosophy Program in Biology, Khon Kaen University.

**Associate Professor, Department of Biology, Faculty of Science Khon Kaen University.

Introduction

Salinity is one of the most important abiotic stress factors adversely affecting rice production. Research in salt tolerance of crop plants represents an important area contributing to practical goal of breeding for salt-tolerant varieties and to our understanding of basic plant biology questions such as signal transduction, gene regulation, ion regulation and metabolic responses. Numerous work has been published on physiology and molecular biology of salt tolerance in rice, however, most of the work has been concentrated on only a few varieties such as the well-known salt tolerant cvs. Pokkali and Nona-bokra. The aim of this project is to try to obtain more information on responses of Thai rice varieties to salinity stress. This report presents some results on the screening 99 Thai rice for salinity tolerance at seedling stage, the effects of salinity on yield and yield components of eight rice varieties and the NaCl effects on the pattern of polypeptides. We plan to identify genes encoding the salt-induced polypeptides from the amino acid sequence information.

Materials and Methods

Seeds of 99 varieties of *Oryza sativa* L. were kindly provided by Pathumthani Rice Research Institute, Thailand.

Experiment 1. Screening Thai rice varieties for salinity tolerance at seedling stage

Methods for growing plants, salinization of nutrient solution and evaluation of salt stress symptoms were modified from Gregorio et al. (1997). Rice seeds were surfaced sterilized and sown on plastic grids placed above 4-liter black plastic pots containing nutrient solution (Yoshida et al., 1976). Three replicate pots each with 30 seedlings were used for each variety. Seedlings were grown in glasshouse for 14 days before NaCl was introduced initially at the level of 6 dS m⁻¹ for 3 days then to 12 dS m⁻¹ for 13 days. The solution was renewed every 7 days and the pH maintained at 5.0 daily. The screening was performed between July to November 2000. Due to limitation of greenhouse space, rice varieties were divided into three

groups. The first group comprising 25 varieties were tested between July 20 - August 16, 2000; the second group of 31 varieties between August 21 - September 14, 2000 and the third group of 41 varieties between November 2-29, 2000. Two varieties were used as check varieties:- Pokkali (an Indian variety), tolerant; and KDML 105, susceptible. Sixteen days after initial salinization, seedlings were scored for salt tolerance using the modified standard evaluating score in rating the visual symptoms of salt toxicity (Gregorio et al., 1997 see Table 1). The number of plants survived were recorded, plants able to maintain three or more greenish and elongating leaves are considered surviving. The concentration of Na and K in shoots was analyzed from three replicates of oven-dried shoot samples of each variety using atomic absorption spectrophotometry and Na-K ratio was determined. The varieties which were scored moderately tolerant were rescreened during March 2001.

Experiment 2. Salinity effects on yield and yield components of rice

Field experiment was performed between August 25 - December 16, 2001. A total of eight varieties were tested including Leuang Yai, Leuang Anan, Khao Med Lek, KDML 105, RD6, Supanburi 2 and two check varieties: Pokkali and Pathumthani 60. Rice seeds were grown in the salt-affected field at Tumbum Muang Pai, Amphur Ban Pai, Khon Kaen Province. The experiment was arranged in CRD with 3 replications. Data collected included number of productive tillers per plant, panicle length, number of filled-unfilled spikelets per panicle, 100 grain weight, straw yield per plant and grain yield per plant.

Experiment 3. The NaCl effects on the pattern of polypeptides

The 21 day old seedlings of rice cvs.; Leuang Yai, Leuang Anan, Khao Med Lek, Pokkali, Pathumthani 60 and KDML 105 were exposed to 12dS m⁻¹ NaCl for 10 days after salinization. Total proteins were extracted from leaf and root tissue and solubilized completely in loading buffer (0.2 M Tris-HCl pH 6.8 buffer containing 2% w/v SDS, 5% v/v, 2-mercaptoethanol and 10% v/v glycerol). The

homogenate was centrifuged at 15,000 rpm for 30 min at 4°C and the supernatant was stored at -70°C. The amount of protein in the extracts was determined following Biorad's standard assay procedure, and analyzed by SDS-PAGE stained with Coomassie blue or silver nitrate.

Results and discussion

Experiment 1. Screening rice for salinity tolerance at seedling stage

In the first screening, among the 99 varieties tested, Pokkali was the most tolerant and the only variety rated as tolerant (score - 3). Among the remaining 98 Thai rice varieties, 31 were scored moderately tolerant (score - 5), 61 susceptible (score - 7) and 6 highly susceptible (score - 9) (data not shown). Concentration of Na, K and Na-K ratio of shoots tested during the period of high average daily temperature between July 20 - August 16, 2000 ranged from 6.9-15.7%, 0.4-2.2% and 3.3-22.8 respectively. During the period of higher humidity and lower daily temperature between August 21-September 14, 2000, concentration of Na decreased to 3.7-8.7%, K content increased to 6.0-7.0% and Na-K ratio decreased to 0.6-2.9. Similar values were obtained during November 2-29, 2000; 3.4-9.9% for Na content, 1.9-5.8% for K content and 0.8-2.8 for Na-K ratio. Pokkali which was included in all three screenings as a check variety for tolerance reproducibly showed the salinity score of 3 and the lowest Na-K ratio and KDML 105, the check variety for susceptibility showed the salinity score of 7.

In order to select for the most tolerant test entries, 25 varieties which were scored moderately tolerant in the first screening were rescreened using the same salinization regime and evaluation method. Pokkali was included as a check variety for tolerance and KDML 105 and Pathumthani 60 for susceptible. The second screening was performed between March 4-21, 2001. Under the condition of high temperature, even Pokkali showed injury symptoms of drying and rolling of lower mature leaves. However, most Pokkali plants survived and showed significantly higher level of greenness than

the other varieties. Thirteen varieties which were scored moderately tolerant during the mild growing conditions of the first screening were rated "susceptible" or "highly susceptible" (Table 2). Most varieties accumulated more Na but less K, hence much higher Na-K ratio during harsh growing conditions than in mild conditions. The Na-K ratio in shoots correlates well with salinity scores and the amount of greenness. Among Thai local varieties, Leuang Yai and Leuang Anan showed the Na-K ratios comparable to that of Pokkali.

It is well established that the typical mechanism of salinity tolerance in rice is the Na exclusion and increased absorption of K to maintain low Na-K ratio. High correlation between Na-K ratio and salinity tolerance has also been noted by several workers (Yeo and Flowers, 1986; Heenan et al., 1988; Gregorio and Senadhira, 1993). The salinity tolerance rating in this screening technique has been shown to relate well with grain yield and yield reduction under saline field condition (Gregorio et al., 1997).

Experiment 2. Salinity effects on yield and yield components of rice

Results from the experiment in salt-affected field, are presented in Table 3, 4 and 5. Leuang Anan showed the highest grain yield per plant (20.05) and the highest number of productive tillers per plant (8). Khao Dawk Mali 105 showed the lowest grain yield per plant (6.43) and the lowest number of productive tillers per plant (2). Grain yield per plant showed a strong significant correlation with number of productive tillers per plant (0.723**), straw yield per plant (0.707**), panicle length (0.655**) and number of spikelets per panicle (0.568**). Plant height showed less correlation with grain yield per plant (0.508*) than other characters. The fact that the grain yield per plant was strongly correlated with the number of productive tillers per plant, followed by straw yield per plant, panicle length and number of spikelets per panicle suggested that these components can be used for the prediction of the grain yield per plant. Rao et al. (1997) reported that number of productive tillers per plant, followed by plant height, panicle length and flag leaf area, had the highest direct effect on grain yield.

In general, more tolerant varieties in the glasshouse experiment tend to produce higher number of productive tillers per plant and other yield component characters leading to higher grain yield in the field experiment. More tolerant cvs. Leuang Anan, Leuang Yai, , Khao Med Lek produced higher number of productive tillers per plant than less tolerant Pathum Thani 60, RD 6, Supanburi 2, and Khao Dawk Mali 105. Number of productive tillers per plant can be used as an early indication of salt tolerance in rice.

At reproductive stage, the processes of panicle initiation and spikelet formation of the more tolerant varieties Leuang Yai, Leuang Anan, and Khao Med Lek were less sensitive to salinity than those of the less tolerant varieties Pathum Thani 60, RD 6, Supanburi 2, and Khao Dawk Mali 105 resulting in higher number of spikelets per panicle. However, salinity had more adverse effect on pollination, fertilization and/or grain development in Leuang Yai, Leuang Anan, and Khao Med Lek leading to higher percentage of unfilled spikelets compared to Pathum Thani 60, RD 6, and Supanburi 2.

The relationship between the greenhouse screening technique based on seedling growth (Exp.1) and the field test based on grain yield (Exp.2) was demonstrated in Table 5. The order of ranking of salt tolerance among the most tolerant Thai varieties Leuang Yai, Leuang Anan, and Khao Med Lek in the field experiment is consistent with that obtained from the greenhouse experiment. However, the order of ranking of salt tolerance among the medium and less tolerant varieties (Pathum Thani 60, RD 6, Supanburi 2, and Khao Dawk Mali 105) differed between seedling and reproductive stage.

Experiment 3. The NaCl effects on the pattern of polypeptides

In an attempt to study the relationship between salt tolerance and salt-induced changes in polypeptide pattern, certain local Thai rice varieties with varying levels of salt tolerance were grown and subjected to the same salinity treatment used for the screening. A set of controlled plants was grown in the same culture solution, without added NaCl, under the same conditions as the salinized plants. Patterns of polypeptides extracted from leaves of salt-treated plants

were not different from those of controlled plants, except that the intensity of some polypeptide bands decreased in the salt-treated leaves (results not shown). An observation of polypeptide profiles from salt-treated roots, however, revealed a profound increase in intensity of a 90-kD polypeptide band, almost undetectable in controlled roots. This salt-induced polypeptide was clearly observed in tolerant, Pokkali; moderately tolerant, Leuang Yai, Leuang Anan and Khao Med Lek but almost undetectable in both controlled and treated roots of susceptible KDML 105 (Fig. 1)

Comparison of polypeptide pattern was performed as a preliminary attempt to study differential gene function. The 90-kD polypeptide which was specifically induced in roots of tolerant Pokkali and moderately tolerant Leuang Yai, Leuang Anan and Khao Med Lek but not in susceptible KDML 105 could be compared to the 90-kD polypeptide found in salt tolerant cultivar CSR 19 studied by Ashwani et al. (1998). The biochemical and molecular aspects of this polypeptide will be further investigated. Several workers have detected a number of polypeptides induced by salt reflecting the complexity of biochemical and physiological responses, many polypeptides were found to be cross-induced by other types of stress. We plan to obtain amino acid sequences of the salt-induced polypeptides. The sequence information will be used to obtain the genes encoding the induced polypeptides.

Conclusion

A total of ninety-eight Thai rice varieties were screened at seedling stage for salt tolerance and classified into 3 groups: moderately tolerant, susceptible and highly susceptible. Based on the visual symptoms of salt injury and Na-K ratio in shoots. In general, more tolerant varieties accumulated less Na⁺ but uptake more K⁺ resulting in lower Na-K ratio than less tolerant varieties. Eight varieties were selected for evaluation of salt tolerance in salt affected- experiment based on yield and yield components. The degree of salt tolerance obtained from the greenhouse screening technique condition at seedling stage can be reliably used as a selection criterion for rapid screening of large amount of breeding materials.

Acknowledgements

We wish to thank Pathumthani Rice Research Center for the generous supply of rice seeds. This research was funded by the scholarship from the University Staff Development Program.

References

- Ashwani, P; Singla, SL; Anil, G. 1998. Protein alterations associated with salinity, desiccation, high and low temperature stresses and abscisic acid application in seedling of Pusa 169, a high yielding rice (*Oryza sativa* L.) cultivar. *Current Science*. 75 (10): 1023-1035.
- Gregorio, GB; Senadhira, D. 1993. Genetic. analysis of salinity tolerance in rice (*Oryza sativa* L.). *Theo Appl Gen*. 86: 333-338.
- Gregorio, GB; Dharmawansa, S; Mendoza, RD. 1997. *Screening rice for salinity tolerance*. IRRI. Discussion Paper Series No. 22. International Rice Research Institute, Manila, Philippines.
- Heenan, DP; Lewin, LG; McCaffery, DW. 1988. Salinity tolerance in rice varieties at different growth stages. *Aust J Exp Agric*. 28: 343-349.
- Rao, SA; Khan, MA; McNeilly, T; Khan, AA. 1997. Cause and effect relations of yield and yield components in rice (*Oryza sativa* L.). *J.Genet.& Breed*. 51: 1-5.
- Yeo, AR ; Flowers, TJ. 1986. Salinity resistance in rice (*Oryza sativa* L.) and a pyramiding approach to breeding varieties on saline soils. *Aust J Plant Physiol*. 13: 161-173.
- Yoshida, S; Forno, DA; Cock, JH; Gomez, KA. 1976. *Laboratory Manual for Physiological studies of Rice*. The International Rice Research Institute, Manila, Philippines

Table 1 Modified standard evaluation score (SES) of visual salt injury at seedling stage (Gregorio et al., 1997)

Score	Observation	Tolerance
1	Normal growth, No leaf symptoms	Highly tolerant
3	Nearly normal growth, But leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded; Most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete cessation of growth; Most leaves dry; some plants dying	Susceptible
9	Almost all plant dead or dying	Highly susceptible

Table 2 Salinity score, Na and K content, Na-K ratio in shoots and % survival of 28 varieties of rice seedlings grown in salinized culture solution during hot summer condition between March 4-31, 2001. Values in parentheses were obtained from plants growing in mild conditions between July - November, 2000.

No	variety	G.S.No.	salinity score	%Na	%K	Na-K ratio	% survival
1	Pokkali	-	3 (3)	6.6 (4.4)	1.1 (5.0)	6.2 (0.8)	93 (86)
2	Leuang Anan	7224	5 (5)	4.3 (3.6)	0.8 (2.2)	5.2 (1.6)	53 (93)
3	Leuang Yai	5557	5 (5)	5.4 (6.1)	0.8 (3.5)	6.4 (1.7)	53 (80)
4	Jao Daeng	-	5 (5)	5.9 (5.4)	0.9 (3.4)	6.7 (1.6)	73 (100)
5	Khao Nahng Mon	9373	5 (5)	6.3 (5.3)	0.9 (3.7)	6.8 (1.5)	47 (60)
6	Jek Gra-Dod	3580	5 (5)	5.7 (5.0)	0.9 (3.5)	6.1 (1.4)	33 (67)
7	Khao Setti	6783	5 (5)	7.9 (5.3)	1.1 (3.9)	7.4 (1.4)	33 (67)
8	Nam Sa-Gui	3023	5 (5)	7.1 (6.5)	0.9 (6.9)	7.5 (0.9)	33 (73)
9	Khao Med Lek	9423	5 (5)	5.6 (5.9)	0.7 (3.3)	7.6 (1.8)	27 (87)
10	Supanburi 2	13746	5 (5)	7.0 (8.7)	0.9 (6.0)	7.9 (1.5)	33 (47)
11	RD7	2600	5 (5)	7.0 (4.8)	0.9 (3.5)	7.9 (1.4)	33 (80)
12	Khao Ah-Gahd	5491	5 (5)	6.0 (5.1)	0.7 (3.4)	8.2 (1.5)	53 (87)
13	Khao Luang	5530	5 (5)	7.3 (3.5)	0.9 (2.2)	8.4 (1.6)	60 (80)
14	Khao Yai Sai	9389	7 (5)	6.4 (5.2)	0.8 (3.3)	7.9 (1.6)	33 (80)
15	Khao Mahk Kaek	9387	7 (5)	8.7 (7.5)	1.0 (5.0)	9.1 (1.5)	27 (42)
16	RD 6	3062	7 (5)	9.4 (7.6)	0.9 (4.8)	10.1 (1.6)	20 (54)
17	Nahng Nuan	2995	7 (5)	9.2 (5.8)	0.9 (3.8)	10.4 (1.5)	47 (93)
18	Puang Nahk 16	256	7 (5)	9.9 (4.5)	0.9 (3.4)	10.9 (1.3)	40 (87)
19	Leuang Bahng Kem	8179	7 (5)	8.0 (5.2)	0.7 (3.3)	10.9 (1.6)	13 (60)
20	Daeng Dawk Kok	20849	7 (5)	10.7 (5.0)	0.8 (2.9)	12.7 (1.7)	27 (67)
21	Pathum Thani 1	23898	7 (5)	12.0 (5.6)	0.8 (3.4)	14.4 (1.7)	47 (87)
22	Man Poo	7629	9 (5)	n.a. (4.5)	n.a. (3.3)	n.a. (1.4)	0 (67)
23	Bun Mah	91119	9 (5)	n.a. (4.6)	n.a. (3.4)	n.a. (1.4)	0 (55)
24	Krasaen	85018	9 (5)	n.a. (8.3)	n.a. 0.9	n.a. (9.6)	0 (87)
25	Khao Daeng	86070	9 (5)	n.a. (10.1)	n.a. (1.0)	n.a. (10.4)	0 (55)
26	Leuang Tah Mo	8195	9 (5)	n.a. (6.6)	n.a. (7.0)	n.a. (0.9)	0 (83)
27	Khao Dawk Mali 105	-	9 (7)	n.a. (6.4)	n.a. (2.3)	n.a. (2.8)	0 (13)
28	Pathum Thani 60	17770	9 (7)	n.a. (7.2)	n.a. (2.5)	n.a. (2.8)	0 (33)

n.a. :- all plants died, no living tissues were available for analysis.

Table 3 Growth, yield components and grain yield per plant of eight varieties of rice grown in salt-affected field at Tumbum Muang Pai, Amphur Ban Pai, Khon Kaen Province between August 25 - December 16, 2001.

No	Variety	Plant height (cm)	No. of prod. tillers /plant	Panicle Length (cm)	No. of Spikelets /panicle	No. of filled spikelets /panicle	No. of Unfilled spikelets /panicle	%of unfilled spikelets	100 grain weight (g)	Straw yield/ plant (g)	Grain yield per plant (g)
1	Leuang Anan	131	8	24.96	151	119	32	22	2.46	21	20.05
2	Leuang Yai	104	7	25.60	164	110	54	32	2.52	21	16.08
3	Khao Med Lek	137	6	25.27	154	117	37	24	2.43	19	14.67
4	Pokkali	139	4	24.70	100	90	10	10	2.72	9	11.76
5	Pathum Thani 60	121	4	24.79	122	99	23	18	2.47	9	11.55
6	RD 6	84	4	21.67	99	81	18	18	2.16	5	7.79
7	Supanburi 2	101	4	21.99	88	75	13	14	2.20	7.5	7.26
8	Khao Dawk Mali 105	115	2	24.04	128	98	30	23	2.43	8	6.43

Table 4 Correlation coefficient of seven yield related characters and total grain yield per plant in rice.

	No. of prod. tillers/plant	Panicle length	No. of spikelets/panicle	% of unfilled spikelets	100 grain weight	Straw yield/plant	Grain yield Per plant
Plant height	.918**	.212	.250	.045	.157	.919**	.508*
No. of prod. Tillers/plant		.331	.321	.146	.114	.960**	.723**
Panicle length			.728**	.252	.713**	.433*	.655**
No. of spikelets/panicle				.587**	.416*	.482*	.568**
% of unfilled spikelets					.028	.242	.204
100 grain weight						.239	.353
Straw yield/plant							.707*

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

Table 5 Ranking of rice varieties in order of salinity tolerance at seedling stage and reproductive stage.

Degree of salt tolerance	seedling stage (Exp.1)	reproductive stage (Exp.2)
More tolerant ↑ ↓ Less tolerant	Pokkali Leuang Anan Leuang Yai Khao Med Lek Supanburi 2 RD 6 Khao Dawk Mali 105 Pathum Thani 60	Leuang Anan Leuang Yai Khao Med Lek Pokkali Pathum Thani 60 RD 6 Supanburi 2 Khao Dawk Mali 105

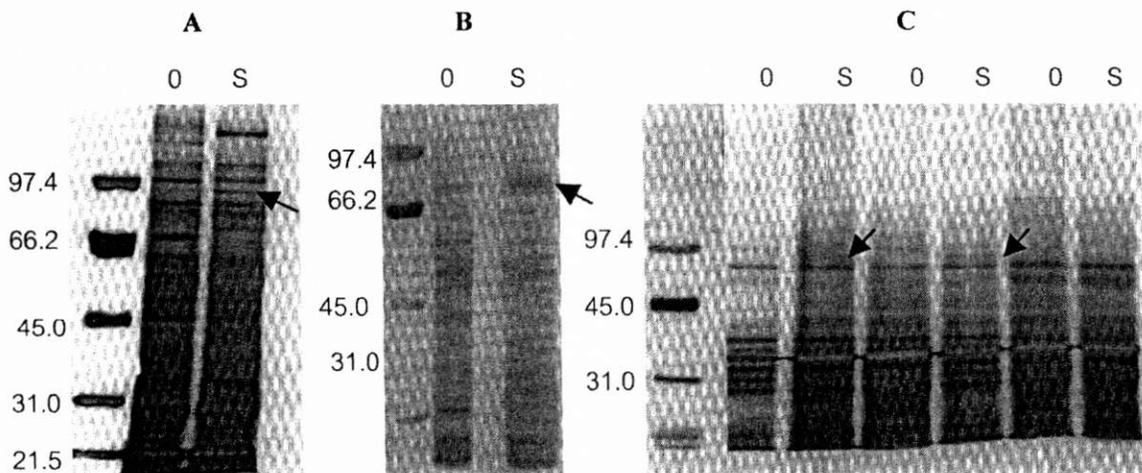


Fig. 1 Profiles of polypeptides extracted from controlled (0) and salt-treated roots (S) of rice var. Luang Yai (A); Khao Med Lek (B); Luang Anan (C, lane 2,3); Pokkali (C, lane 4,5); KDML 105 (C, lane 6, 7).