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Effects of Salinity Stress on Growth and Phenolics of Rice (*Oryza sativa* L.)

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Abstract. This study was conducted to determine the correlation between of salinity stress on growth and phenolic compounds in rice. It was observed that salinity stress caused a significant decrease in shoot lengths, fresh and dry weights of all rice varieties. Under salinity stress, changes of chemical contents also differed among phenolic compounds and rice cultivars. Total phenolics and flavonoids, and contents of vanillin and protocatechuic acid in tolerant varieties were strongly increased, whereas in contrast, they were markedly reduced in the susceptible cultivar. Ferulic acid and *p*-coumaric acid were detected only in tolerance rice. Vanillin and protocatechuic acid may play a role, but ferulic acid and *p*-coumaric acid may be much involved in the tolerant mechanism against salinity stress. Ferulic acid and *p*-coumaric acid and their derivatives are potent to be exploited as promising agents to reduce detrimental effects of salinity stress on rice production.

Introduction

Salt stress is one of the most brutal abiotic stresses that limit profitable rice production worldwide [1]. Munns and Tester [2] noted that the areas of land in the world are seriously affected by high salinity increasing over 800 million hectares. According to Wahhab [3], the salinity sensitive level in rice crop ranges from 3.6 dS m^{-1} to 18 dS m^{-1}, and the seedling stage in rice is the most vulnerable stage to salinity [4,5]. This makes a problem for rice farmers because all transplanted seedlings may die, and the establishment of a sufficient crop stand can become very difficult. The influence factors of salinity on the growth of rice include salt concentrations, and types of salt, duration of exposure to salt stress, soil pH, water regime, temperature, humidity and solar radiation [6].

Salinity causes complex interactions among different morphological, physiological and biochemical processes. Salinity may cause oxidative stress due to highly producing of reactive oxygen species (ROS) leading to alteration plant metabolism. As a result, DNA, proteins, lipids, carbohydrates, and membranes are damaged [7,8]. A comparatively high injury always belongs to cell membranes in salt susceptible rice varieties under salt stress [9]. Besides, salt tolerant varieties can uphold better antioxidant defense system to counteract the ROS [10].

Plants may vary widely in their phenolic contents and compositions, with both genetic and environment affecting the type and level of these compounds [11,12]. Phenolic compounds, a group of secondary metabolites, have different biological activities, and the most important capability is antioxidant activities [13,14]. Additionally, these compounds are accumulated to response in the increases of ROS under salt stress [15,16,17] by exhibiting antioxidant activity in tissues to inactivate lipid free radicals or prevent decomposition of hydroperoxides into free radicals [18,19,20]. Higher buildup of phenolics and flavonoids in the plant under salt stress may assist the plant to lighten the salinity-induced oxidative stress [21]. For example, the phenolic content and antioxidant activity of leaves of the halophyte *Cakile maritime* were increased by salinity [22]. Leaf phenolic content of Artichoke was significantly increased at 25-50 mM NaCl [23]. The phenolics in matured fruits increased in salinity conditions in red matured paper fruits [24]. Parida et al. [25] reported the accumulation of the phenolic content in moderate salinity in the mangrove. The effects

of salt stress on secondary metabolites in rice should be determined. The use of effective secondary metabolites is promising in development bioactive reagents to protect rice production under salinity stress. Therefore, the study aimed to clarify the correlation between effects of salinity stress on rice growth and changes in chemical components including phenolics in rice.

Materials and Methods

Phenolic standards and reagents

The reagents and standards included Folin-Ciocalteu's reagent, benzoic acid, caffeic acid, catechol, cinnamic acid, chlorogenic acid, ellagic acid, ferulic acid, gallic acid, protocatechuic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, sinapic acid, syringic acid, vanillic acid, rutin, and vanillin. All solvents were used of analytical grade purchased from KANTO chemical, Tokyo Japan.

Plant materials and screening method

Six rice varieties (OM4900, X7KD, OM8108, BC15TB, BT, and Q5) were obtained from the Cuu Long Delta Rice Research Institute, Vietnam. In laboratory, seeds were surface-sterilized by soaking in 0.1 % NaOCl for 30 min followed through washed in distilled water and then germinated in Petri dishes for 5 days. The germinated seeds were placed in screening trays in a greenhouse for one week. After that, NaCl was added into these trays to obtain desired electrical conductivity (EC) at 5 dS m^{-1} and 10 dS m^{-1}. Trays without salt considered as the control (0 dS m^{-1}). The EC of the solution were checked using an EC meter (Hanna HI 4321, USA). The modified standard evaluation system was used to assess the visual symptoms of salt injury by a scoring system in salinized condition (Table 1) [26]. The survival plants were selected randomly in all treatments to record shoot height, root length, fresh and dry weight after 14 days of treatment. All experiments were conducted from May to September 2014 in Hiroshima University, Japan.

Table 1. Modified standard evaluation score (SES) of visual salt injury at seedling stage

Score	Observation	Response category
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips of 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 plants dead or dying	Highly susceptible

Extraction procedure

An amount of 0.5 g dried ground plant sample was extracted in 100 ml polystyrene bottle by adding 50 ml of ethanol 99.5 % and shaken for 12 h and then filtered through filter papers. The residue was re-extracted twice under the same conditions. The solvent was then removed in a rotary evaporator at 30 ºC. The precipitate was weighed, and dissolved in methanol and kept in the dark at 4 ºC. Extracts were used to determine total phenolic and flavonoid contents.

Determination of total phenolic content (TPC)

TPC was assayed by Folin–Ciocalteu's reagent method [27]. Briefly, an aliquot of 0.125 ml extract was placed into test tubes and then with 0.5 ml of distilled water and 0.125 ml of 10 % Folin–Ciocalteu's reagent. After 6 min, 1.25 ml of sodium carbonate solution (7.5 %) was added. Then, 1 ml of distilled water was added to bring the total volume to 3 ml. The mixture was vigorously shaken and allowed to stand for 90 min at room temperature. The absorbance of the reaction was recorded at 760 nm by using a spectrophotometer (HACH DR/4000U-Japan). Gallic acid was used as standard and TPC was expressed as mg gallic acid equivalents (GAE) per gram dry weight.

Determination of total flavonoid content (TFC)

TFC of extracts was estimated as mg rutin equivalents (RE) per gram dry weight, from the rutin calibration curve. The reaction was prepared by mixing 1 ml of extract with 1 ml of 2 % aluminum

chloride ($AlCl_3$) in methanolic solution. The mixture was vigorously shaken and allowed to stand for 30 min at room temperature. The absorbance of reaction was read at 430 nm by using a spectrophotometer [28].

Identification and quantification of phenolic acids

Five-microliter samples were analyzed by using an HPLC system (LC-Net II/ ADC, UV-2075 Plus and PU-2089 Plus), the column Jasco RPC18 (250 mm x 4.6 mm x 5 μm). The mobile phase was composed of solvent A (99.8 % methanol) and solvent B (0.1 % acetic acid). The flow rate was 1 ml/ min and integrated at 254 nm. The program was performed as follows 0-5 min (5 % A), 5-10 min (20 % A), 10-20 min (50 % A), 20-30 min (80 % A), 30-40 min (100 % A), 40-50 (100 % A) min, 50-60 min (5 % A). Fifteen standard phenolic acids including benzoic acid, caffeic acid, catechol, cinnamic acid, chlorogenic acid, ellagic acid, ferulic acid, gallic acid, protocatechuic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, sinapic acid, syringic acid, vanillic acid, and vanillin were used. Phenolic acids in the samples were identified by comparing retention times and peak area with those of pure standards. All trials were replicated in thrice.

Statistical analysis

The results of all assays were showed as the means ± standard errors (SE). Data were analyzed by the software Minitab 16. Analysis of variance (ANOVA of one factor) was used to determine if significant differences existed at a level of confidence of $p < 0.05$.

Results

Effects of salinity stress on rice emergence

The salinity tolerance scores for six rice varieties ranged from 1 to 7 (Table 2). At the dose 5 dS m^{-1}, the highly tolerant (score 1) varieties were recorded in OM4900, OM8108, BC15TB and Q5. But when the EC was increased to 10 dS m^{-1}, only two varieties OM4900 and BC15TB showed a tolerant response (score 3). The susceptible variety was X7KD with the salinity tolerance scores of 7 at both 5 and 10 dS m^{-1}.

Table 2. Tolerance level of different rice varieties in salinity conditions

Varieties	Standard evaluation score	
	Salinized 5 dS m^{-1}	Salinized 10 dS m^{-1}
OM4900	1	3
X7KD	7	7
OM8108	1	5
BC15TB	1	3
BT	3	5
Q5	1	5

Salinity caused a decrease in growth performance of seedlings in all rice varieties, as shown in Table 3. In term of shoot height, at 5 dS m^{-1}, the varieties OM4900 and BC15TB showed a minimum decrease (3.57% and 6.65%, respectively), followed by Q5 (7.06%), X7KD (7.07%), and BT (8.53%), while the maximum reduction in shoot height (14.21 %) was noted in OM8108. Similarly, slight decreases (8.00% and 15.26%) at 10 dS m^{-1} were recorded in BC15TB and OM4900, respectively. Most of the rice varieties indicated dramatically increases in root length at 5 dS m^{-1}. At 10 dS m^{-1}, the varieties BC15TB and OM4900 showed the highest elongation (40.50% and 30.27%, respectively) whereas the X7KD exhibited a dramatic reduction (59.04%) in root length, indicating that this cultivar is the most susceptible.

Table 3. Effect of salinity on shoot and root lengths of rice varieties

Shoot height (cm plant^{-1})					
Varieties	Control 0 dS m^{-1}	Salinized 5 dS m^{-1}	Decrease over control (%)	Salinized 10 dS m^{-1}	Decrease over control (%)
OM4900	12.34 ± 0.40a	11.90 ± 0.68ab	3.57	10.46 ± 0.55b	15.26
X7KD	12.59 ± 0.52a	11.70 ± 1.73ab	7.07	9.80 ± 0.50b	22.16
OM8108	14.85 ± 0.34a	12.74 ± 0.41b	14.21	9.23 ± 0.67c	37.88
BC15TB	12.53 ± 0.25a	11.70 ± 0.59a	6.65	11.53 ± 0.70a	8.00
BT	10.10 ± 0.34a	9.24 ± 0.56a	8.53	8.27 ± 0.15b	18.15
Q5	10.63 ± 0.47a	9.88 ± 0.31a	7.06	8.00 ± 0.26b	24.74
Root length (cm plant^{-1})					
Varieties	Control 0 dS m^{-1}	Salinized 5 dS m^{-1}	Increase over control (%)	Salinized 10 dS m^{-1}	Increase over control (%)
OM4900	5.11 ± 0.38b	6.10 ± 0.45a	19.37	6.66 ± 0.53a	30.27
X7KD	6.71 ± 0.65a	6.30 ± 1.10a	-6.17	2.75 ± 0.25b	-59.04
OM8108	5.22 ± 0.33ab	5.60 ± 0.31a	7.28	4.53 ± 0.54b	-13.31
BC15TB	9.82 ± 0.65b	10.28 ± 1.17b	4.66	13.8 ± 0.59a	40.50
BT	5.00 ± 0.46a	5.28 ± 0.45a	5.66	3.80 ± 0.17b	-24.00
Q5	7.40 ± 0.37b	8.93 ± 0.39a	20.68	7.83 ± 0.11b	5.85

Salinity substantially reduced the fresh and dry weights of seedlings in all varieties. Table 4 showed that at 5 dS m^{-1}, the varieties OM4900 and BC15TB indicated slight decreases (8.08% and 16.05%, respectively) in fresh weight while the variety X7KD showed the maximum reduction (34.56%). However, at 10 dS m^{-1}, the decrease of fresh weights was not significantly different compared with 5 dS m^{-1}. Likewise, at both 5 dS m^{-1} and 10 dS m^{-1} in saline conditions the variety OM4900 observed the least reduction (2.71% and 4.07%, respectively), followed by BC15TB (3.57% and 4.28%, respectively) in dry weight.

On the basis of standard evaluation system score and phenotypic performance, two varieties OM4900 and BC15TB were identified as salt tolerant, whilst OM8108, BT, and Q5 were moderately tolerant, and X7KD was susceptible at seedling stage (Table 2). Therefore, the two cultivars BC15TB and X7KD were selected for analyzing changes in chemical components under salinity stress.

Table 4. Effect of salinity on seedling fresh and dry weights of different rice varieties

Fresh weight (g plant^{-1})					
Varieties	Control 0 dS m^{-1}	Salinized 5 dS m^{-1}	Decrease over control (%)	Salinized 10 dS m^{-1}	Decrease over control (%)
OM4900	0.094 ± 0.002a	0.081 ± 0.000b	8.08	0.068 ± 0.002b	19.84
X7KD	0.085 ± 0.003a	0.055 ± 0.001b	34.56	0.065 ± 0.002b	23.19
OM8108	0.095 ± 0.004a	0.088 ± 0.005a	6.90	0.060 ± 0.006b	36.30
BC15TB	0.081 ± 0.003a	0.068 ± 0.006b	16.05	0.063 ± 0.005b	22.22
BT	0.073 ± 0.002a	0.053 ± 0.001b	27.40	0.048 ± 0.006b	34.25
Q5	0.093 ±0.005a	0.088 ± 0.004a	5.03	0.073 ± 0.002b	21.04
Dry weight (g plant^{-1})					
Varieties	Control 0 dS m^{-1}	Salinized 5 dS m^{-1}	Decrease over control (%)	Salinized 10 dS m^{-1}	Decrease over control (%)
OM4900	0.019 ± 0.001a	0.018 ± 0.000a	2.71	0.018 ± 0.001a	4.07
X7KD	0.013 ± 0.000a	0.013 ± 0.000a	1.78	0.012 ± 0.000a	5.26
OM8108	0.017 ± 0.000a	0.016 ± 0.000b	7.50	0.016 ± 0.001b	9.14
BC15TB	0.019 ± 0.000a	0.018 ± 0.001a	3.57	0.018 ± 0.001a	4.28
BT	0.015 ± 0.000a	0.014 ± 0.001ab	7.68	0.013 ± 0.001b	12.23
Q5	0.017 ± 0.001a	0.016 ± 0.000a	0.34	0.014 ± 0.000b	13.98

Influence of salinity stress on total phenolic and flavonoid contents

Salinity resulted in changes of TPC and TFC of rice varieties, as shown in Table 5. Most of the rice varieties presented an increase in TPC at 5 dS m^{-1} compare with the control. Especially, at 10 dS m^{-1}, only two tolerant varieties OM4900 and BC15TB had an increased TPC (65.74% and 32.93%, respectively) while TPC was reduced in the others. The susceptible variety X7KD had the highest reduction of the phenolics (50.17%). Moreover, the varieties OM4900 and BC15TB presented the maximum increase of TFC at both 5 dS m^{-1} (17.15% and 10.81%, respectively) and 10 dS m^{-1} (55.58% and 16.87%, respectively) over the controls, whereas other varieties showed a decrease in total flavonoid production. The most reduction of flavonoid contents was noted in X7KD under salt stress.

Table 5. Effect of salinity on seedling total phenolic and flavonoid contents of rice varieties

Total phenolic content (mg GAE g^{-1} dry weight)					
Varieties	Control 0 dS m^{-1}	Salinized 5 dS m^{-1}	Increase over control (%)	Salinized 10 dS m^{-1}	Increase over control (%)
OM4900	0.60 ± 0.05gh	0.89 ± 0.02fg	47.69	1.00 ± 0.03f	65.74
X7KD	0.82 ± 0.01fg	0.69 ± 0.03fgh	-15.55	0.41 ± 0.01h	-50.17
OM8108	0.68 ± 0.01fgh	0.77 ± 0.01fg	12.81	0.59 ± 0.00gh	-13.77
BC15TB	1.85 ± 0.07bc	2.08 ± 0.05b	12.71	2.45 ± 0.08a	32.93
BT	1.45 ± 0.02de	1.46 ± 0.13de	1.66	1.43 ± 0.02e	-1.79
Q5	1.84 ± 0.07bc	1.94 ± 0.06bc	5.19	1.73 ± 0.04cd	-6.31
Total flavonoid content (mg RE g^{-1} dry weight)					
Varieties	Control 0 dS m^{-1}	Salinized 5 dS m^{-1}	Increase over control (%)	Salinized 10 dS m^{-1}	Increase over control (%)
OM4900	0.05 ± 0.00k	0.06 ± 0.00k	17.15	0.08 ± 0.00j	55.58
X7KD	0.37 ± 0.01a	0.11 ± 0.00i	-70.41	0.11 ± 0.00i	-70.93
OM8108	0.27 ± 0.01cd	0.21 ± 0.00f	-23.10	0.12 ± 0.00i	-56.72
BC15TB	0.24 ± 0.00e	0.27 ± 0.00d	10.81	0.29 ± 0.00c	16.87
BT	0.30 ± 0.01b	0.20 ± 0.00fg	-33.51	0.19 ± 0.01g	-37.02
Q5	0.30 ± 0.00b	0.19 ± 0.00fg	-34.52	0.14 ± 0.00h	-53.89

GAE, gallic acid equivalents; RE, rutin equivalents

Correlation efficient between salt tolerant with phenotype traits, TPC, and TFC

Correlation coefficients among salt tolerance indexes were showed in Table 6. At the seedling stage, highly significant and negative correlations were found between salt tolerant with shoot height, fresh weight and TFC under salt stress. Moreover, at salinized condition correlation between salt tolerant and TPC was inverse and significant. Whereas, there were no significant correlations between salt tolerant with root length and dry weight. These results implied that salt tolerant genotypes (having lower salt tolerance score) exhibited higher shoot height, fresh weight, total phenolic and flavonoid contents. Regression analysis amongst the phenotype traits (i.e. shoot height, root length, fresh and dry weights) against the TPC and TFC did not yield any meaningful relationships.

Table 6. Correlation coefficients between salt tolerant with phenotype traits, total phenolic and flavonoid contents

	Salt tolerant	Shoot height	Root length	Fresh weight	Dry weight
Salt tolerant					
Shoot height	-0.468**				
Root length	-0.150	0.004			
Fresh weight	-0.589**	0.472**	0.171		
Dry weight	-0.276	0.336*	0.183	0.508**	
TPC	-0.250*				
TFC	-0.509**				

**, ** Correlation is significant at the 0.05 and 0.01 levels, respectively*

Influence of salinity stress on phenolic acids

Interestingly, among fifteen standard phenolic acids, only five phenolic acids (vanillin, cinnamic acid, protocatechuic acid, ferulic acid and *p*-coumaric acid) were determined in tolerant variety (BC15TB), while only three (vanillin, cinnamic acid, and protocatechuic acid) were found in susceptible variety (X7KD) (Table 7).

Table 7. Effect of salinity on seedling phenolic components (µg g-1 dry weight) of strong and weak tolerant varieties

Phenolic acids	BC15TB (tolerant)		
	Control 0 dS m^{-1}	Salinized 5 dS m^{-1}	Salinized 10 dS m^{-1}
Vanillin	26.33 ± 0.21j	33.88 ± 0.55i	41.54 ± 1.19gh
Cinnamic acid	15.74 ± 0.85l	0.70 ± 0.15m	2.90 ± 0.39m
Protocatechuic acid	43.62 ± 0.24g	52.25 ± 0.07f	64.13 ± 0.05e
Ferrulic acid	61.30 ± 0.71e	67.97 ± 0.25d	81.13 ± 0.16c
p-Coumaric acid	82.14 ± 1.92c	94.81 ± 0.47b	115.78 ± 0.31a
Phenolic acids	X7KD (susceptible)		
	Control 0 dS m^{-1}	Salinized 5 dS m^{-1}	Salinized 10 dS m^{-1}
Vanillin	39.52 ± 0.28h	21.95 ± 0.05k	13.87 ± 0.03l
Cinnamic acid	3.12 ± 0.21m	3.67 ± 0.14m	1.63 ± 0.30m
Protocatechuic acid	80.51 ± 0.18c	52.86 ± 0.013f	33.73 ± 0.46i
Ferulic acid	nd	nd	nd
p-Coumaric acid	nd	nd	nd

nd: not detected

It is found that ferulic acid and *p*-coumaric acid were detected only in tolerant rice, whereas no trace of the two phenolic acids was found in the susceptible rice. The contents of the constituents were also strongly increased at 5 and 10 dS m^{-1} doses. In the control condition, the content of cinnamic acid in tolerant rice was higher than in the susceptible rice, but in contrast, quantities of vanillin and protocatechuic acid were greater in susceptible rice than in the tolerant rice. However, under salinity stress, the concentrations of vanillin, protocatechuic acid, ferulic acid and *p*-coumaric acid increased in tolerant variety. Quantities of vanillin and protocatechuic acid in susceptible variety were reduced.

Discussion

Salinity stress and seedling growth

Rice seedlings in salinized conditions expressed different visual symptoms of physical injury. The symptoms of salt effects on rice were envisioned by leaf rolling, new leaf formation, the color of leaf tips, drying of leaves and also decrease in root growth, stunted shoot growth and thickened stem caused a complete reduction of growth and dying of seedlings (Table 1). Salt causes osmotic stress [29], alter metabolism, the inability of apoplastic acidification and lack of turgor lead to a decrease in rice growth [2]. In the study of Suplick-Ploense, Qian, and Read [30], the less reduction of growth was always noted in tolerant varieties in salinized conditions. Kumar et al. [31] also reported that salt resistant rice varieties had larger biomass than susceptible under salt stress. Tolerant varieties may have mechanisms for maintenance of growth and protection of the metabolic process in elongation cell against salinity. Therefore, in this study, minimum decrease in shoot lengths, seedling fresh and dry weights was observed in tolerant varieties OM4900 and BC15TB (Tables 3 & 4).

Salinity stress and total phenolic and flavonoid content

Salinity limits the photosynthesis in the plant due to carbon dioxide reduction [32]. Additionally, at high salt concentration, uptake of phosphor and potassium, main substances of secondary

metabolites (such as polyphenols), are declined [33]. Moreover, the disturbance of enzymatic activities under salt stress leads to decline the photosynthesis process in the plant [34]. In this study, the reductions of total phenolic and flavonoid contents of X7KD, OM8108, BT and Q5 in saline conditions were observed. Salt tolerant plant varieties regulate the movement of ion and water and maintain better antioxidant defense system against the ROS due to salinity [35]. High accumulation of phenolics in plant plays an imperative physiological role in overcoming the salinity-induced oxidative stress [8]. Recently, Danai-Tambhale, Kumar, and Shriram [36] also quoted that a higher buildup of total polyphenols in tolerant rice variety than sensitive one under salt stress. Similarly, tolerant varieties OM4900 and BC15TB increased total phenolic and flavonoid compounds. The enhancement in the synthesis of flavonoids and phenolics of strong tolerant varieties might be the adaptive mechanism of rice under salt stress (Table 5).

Salinity stress and phenolic components

Secondary metabolites play an important role as antioxidants and antiradicals supporting plants to deal with oxidative stress [37]. Phenolic acids are secondary metabolites extensively spread throughout the plant kingdom [38]. Phenolic compounds are crucial for plant growth and reproduction, and are produced as a response to unfavorable environmental factors (light, chilling, salinity etc.) and to defend injured plants [39]. The results obtained from this research showed that the concentrations of vanillin, protocatechuic acid, ferulic acid, and *p*-coumaric acid increased in tolerant variety BC15TB compared to the control under salt stress while there was a decrease of vanillin and protocatechuic acid in susceptible variety X7KD at the saline condition. Ferulic acid and *p*-coumaric acid might play a certain role in salinity tolerance mechanism when they increased in tolerant variety and absent in susceptible variety (Table 7). A significant increase in the accumulation of *p*-coumaric acid assists to decrease oxidative pressure because *p*-coumaric acid expresses high radical scavenging activity due to their hydroxyl nature [40]. The presence of ferulic acid under osmotic stress may be related to the strengthening of the plant cell wall and the overall cell elongation [41]. Besides, ferulic acid copes with dehydration stress by decreasing of lipid peroxidation due to activation of antioxidant enzymes and increasing of proline and soluble sugar content in cucumber leaves [42].

In this study, vanillin, cinnamic acid, protocatechuic acid, ferulic acid, and *p*-coumaric acid were detected as free phenolic acids. Generally, free phenolic acids can be obtained by extraction by aqueous solutions of alcohol or acetone. After centrifugation, the combined supernatants were analyzed for free phenolic acids and soluble phenolic acid esters, using HPLC or GC [43]. The bounded phenolic acids should be hydrolyzed with 4 NaOH high temperature of 45-50^{o}C, adjusted pH to 1-2 and extracted with ethyl acetate [44]. The other phenolic acids other than vanillin, cinnamic acid, protocatechuic acid, ferulic acid, and *p*-coumaric acid may exist in these rice, but possible are in bounded forms with sugar or glycoside and need the mentioned above procedure to separate. The five phenolic acids may be available in rice and play a direct role in salinity resistance in rice, therefore, the present study aimed at detecting only free phenolic acids in rice.

Conclusions

To increase the tolerance of rice against salinity is an important task for rice researchers to reduce the effect of climate change against rice production. In this study, ferulic acid and *p*-coumaric acid were found only in the tolerant rice and their contents were strongly increased under salinity stress. Vanillin and protocatechuic acid may play a role, but ferulic acid and *p*-coumaric acid may be much involved in the salinity tolerant mechanism of rice. However, it should elaborate how much ferulic acid and *p*-coumaric acid can promote salinity tolerance of rice in saline soil. This evidence may help to develop bioactive agents from the two constituents and their derivatives to reduce detrimental effects of salinity stress on rice production.

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References

[1] T. J. Flowers, Improving crop salt tolerance, J. Exp. Bot. 55 (2004) 307-319.

[2] R. Munns, M. Tester, Mechanisms of salinity tolerance, Annu. Rev. Plant Biol. 59 (2008) 651–681.

[3] A. Wahhab, Salt tolerance of various varieties of agricultural crops at the germination stage, in Salinity Problems in the Arid Zone. Proc. Teheran Symposium on Arid Zone Research, 14, UNESCO, 1961, 185-192.

[4] R. Munns, Comparative physiology of salt and water stress, Plant Cell Environ. 25 (2002) 239-250.

[5] J. Cuartero, M.C. Bolarin, M.J. Asins, V. Moreno, Increasing salt tolerance in the tomato, J. Exp. Bot. 57 (2006) 1045-1058.

[6] M. Akbar, Breeding for salinity resistance in rice, in Prospects for bio-saline research; Ahmed, R., Pietro, A.S., Eds.; Department of Botany, University of Karachi, Pakistan, 1986, 37-55.

[7] L. Menezes-Benavente, S.P. Kernodle, M. Margis-Pinheiro, J.G. Scandalios, Salt-induced antioxidant metabolism defenses in maize (*Zea mays* L.) seedlings, Redox. Rep. 9 (2004) 29–36.

[8] H. Hichem, D. Mounir, E.A. Naceur, Differential responses of two maize (*Zea mays* L.) varieties to salt stress: Changes on polyphenols composition of foliage and oxidative damages, Ind. Crops Prod. 30 (2009) 144–151.

[9] D.A. Meloni, C.A. Oliva, J. Cambraia, Photosynthesis and activity of superoxide dismiotase, peroxidase and glutathione reductase in cotton under salt stress, Braz. J. Plant Physiol. 15 (2003) 12–21.

[10] N.P. Rout, B.P. Shaw, Salt tolerance in aquatic macrophytes: possible involvement of the antioxidative enzymes, Plant Sci. 160 (2001) 415–423.

[11] J.M. Awika, L.M. Rooney, Sorghum phytochemicals and their potential impact on human health, Phytochemistry. 65 (2004) 1199-1221.

[12] I.N. De Abreu, P. Mazzafera, Effect of water and temperature stress on the content of active constituents of Hypericum brasilienne Choisy, Plant Physiol. Biochem. 43 (2005) 241-248.

[13] S. Kuntz, U. Wenzel, H. Daniel, Comparative analysis of the effects of flavonoids on proliferation, cytotoxity, and apoptosis in human colon cancer cell lines, Eur. J. Nutr. 38 (1999) 133-142.

[14] H. Czeczot, Biological activities of flavonoids: A review, Pol. J. Food Nutr. 950 (2000) 3-13.

[15] R.A. Dixon, N. Paiva, Stress-induced phenylpropanoid metabolism, The Plant Cell. 7 (1995) 1085-1097.

[16] M.R. Roberts, N.D. Paul, Seduced by the dark side: integrating molecular and ecological perspectives on the influence of light on plant defense against pests and pathogens, New Phytol. 170 (2006) 677-699.

[17] R. Julkunen-Tiito, N. Nenadis, S. Neugart, M. Robson, G. Agati, J. Vepsa¨la¨inen, G. Zipoli, L. Nybakken, B. Winkler, M. Jansen, Assessing the response of plant flavonoids to UV radiation: anoverview of appropriate techniques, Phyto. Rev. 14 (2015) 273-279.

[18] D. Krishnaiah, R. Sarbatly, R. Nithyanandam, A review on the antioxidant potential of medicinal plant species, Food Bioprod. Process. 89 (2011) 217-233.

[19] G. Agati, E. Azzarello, S. Pollastri, M. Tattini, Flavonoids as antioxidants in plants: location and functional significance, Plant Sci. 196 (2012) 67-76.

[20] C. Brunetti, M. Di Ferdinando, A. Fini, S. Pollastri, M. Tattini, Flavonoids as antioxidants and development regulators: relative significance in plants and humans, Int. J. Mol. Sci. 14 (2013) 3540-3555.

[21] A. Wahid, A. Ghazanfar, Possible involvement of some secondary metabolites in salt tolerance of sugarcane, J. Plant Physiol. 163 (2006) 723–730.

[22] R. Ksouri, W. Megdiche, A. Debez, H. Falleh, C. Grignon, C. Abdelly, Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*, Plant Physiol. Biochem. 45 (2007) 244-249.

[23] F. Hanen, R. Ksouri, W. Megdiche, N. Trabelsi, M. Boulaaba, C. Abdelly, Effect of salinity on growth, leaf phenolic content and antioxidant scavenging activity in *Cynara cardunculus* L., in Biosaline Agriculture and High Salinity Tolerance; Abdelli, C., Ozturk, M., Ashraf, M., Grignon, Y.C., Eds.; Birkhauser Verlag, Switzerland, 2008, 335-343.

[24] J.M. Navarro, P. Flores, C. Garrido, V. Martinez, Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity, Food Chem. 96 (2006) 66-73.

[25] A.K. Parida, A.B. Das, Y. Sanada, P. Mohanty, Effects of salinity on biochemical components of the mangrove, *Aegiceras corniculatum*, Aqua. Bot. 80 (2004) 77-87.

[26] G.B. Gregorio, D. Senadhira, R.D. Mendoza, Screening rice for salinity tolerance, in IRRI Discussion Paper Series no. 22, International Rice Research Institute, Manila, Philippines, 1997, 1-30.

[27] H.H. Ti, Q. Li, R.F. Zhang, M.W. Zhang, Y.Y. Deng, Z.C. Wei, Free and bound phenolic profiles and antioxidant activity of milled fractions of different indica rice varieties cultivated in southern China, Food Chem. 159 (2014) 166-174.

[28] A. Djeridane, M. Yousfi, B. Nadjemi, D. Boutassouna, P. Stocker, N. Vidal, Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds, Food Chem. 97 (2006) 654-660.

[29] E. Bandeoglu, F. Eyidogan, M. Yuceland, H.A. Oktem, Antioxidant responses of shoots and roots of lentil to NaCl-salinity stress, Plant Growth Regul. 42 (2004) 69-77.

[30] M.R. Suplick-Ploense, Y.L. Qian, J.C. Read, Salinity tolerance of Texas bluegrass, Kentucky bluegrass, and their hybrids, Crop Sci. 42 (2002) 2025-2030.

[31] V. Kumar, V. Shriram, T.D. Nikam, N. Jawali, M.G. Shitole, Antioxidant enzyme activities and protein profiling under salt stress in indica rice genotypes differing in salt tolerance, Arch Agron Soil Sci. 55 (2009) 379-394.

[32] M.M. Chaves, J. Flexas, C. Pinheiro, Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell, Annal. Bot. 103 (2009) 551-560.

[33] A. Rezazadeh, A. Ghasemzadeh, M. Brani, T. Telmadarrehei, Effect of salinity on phenolic composition and antioxidant activity of Artichoke (*Cynara scolymus* L.) leaves, J. Med. Plant Res. 6 (2012) 245-252.

[34] J. Miljuš-Djukić, N. Stanisavljević, S. Radović, Ž. Jovanović, A. Mikić, V. Maksimović, Differential response of three contrasting pea (*Pisum arvense*, *P. sativum* and *P. fulvum*) species to salt stress: assessment of variation in antioxidative defence and miRNA expression, Aust. J. Crop. Sci. 7 (2013) 2145-2153.

[35] M. Hussain, M. Farooq, M. Shehzad, M.B. Khan, A. Wahid, G. Shabir, Evaluating the performance of elite sunflower hybrids under saline conditions, Int. J. Agric. Biol. 14 (2012) 131-135.

[36] S. Danai-Tambhale, V. Kumar, V. Shriram, Differential response of two scented indica rice (Oryza sativa) cultivars under salt stress, J. Stress Physiol. Biochem. 7 (2011) 387-397.

[37] K.S. Gould, J. McKelvie, K.R. Markham, Do anthocyanins function as antioxidants in leaves? Imaging of H2O2 in red and green leaves after mechanical injury, Plant Cell Environ. 25 (2002) 1261-1269.

[38] F. Tomas-Barberan, J.C. Espin, Phenolic compounds and related enzymes as determinants of quality of fruits and vegetables, J. Sci. Food. Agric. 81 (2001) 853-876.

[39] I.K. Valentine, V.K. Maria, B. Bruno, Phenolic cycle in plants and environment, J. Mol. Cell Biol. 2 (2003) 13-18.

[40] S. Jamalian, M. Gholami, M. Esna-Ashari, Abscisic acid-mediated leaf phenolic compounds, plant growth and yield is strawberry under different salt stress regimes, Theor. Exp. Plant Physiol. 25 (2013) 291-299.

[41] K. Wakabayashi, T. Hoson, S. Kamisaka, Osmotic stress suppresses cell wall stiffening and the increase in cell wall bound ferulic and diferulic acids in wheat coleoptiles, Plant Physiol. 113 (1997) 967-973.

[42] D.M. Li, Y.X. Nie, J. Zhang, J.S. Yin, Q. Li, X.J. Wang, J.G. Bai, Ferulic acid pretreatment enhances dehydration-stress tolerance of cucumber seedlings, Biol. Plant. 57 (2013) 711-717.

[43] K. Krygier, F. Sosulski, L. Hogge, Free, esterified, and insoluble-bound phenolic acids. 1. Extraction and purification procedure, J. Agric. Food. Chem. 30 (1982) 330-334.

[44] T.D. Xuan, E. Tsuzuki, H. Terao, T.D. Khanh, Correlation between growth inhibitory exhibition and suspected allelochemicals (phenolic compounds) in the extract of alfalfa (*Medicago sativa* L.), Plant Prod. Sci. 6 (2003) 165-171.

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Effects of Salinity Stress on Growth and Phenolics of Rice (*Oryza sativa* L.)
10.18052/www.scipress.com/ILNS.57.1

DOI References
[1] T. J. Flowers, Improving crop salt tolerance, J. Exp. Bot. 55 (2004) 307-319.
10.1093/jxb/erh003
[2] R. Munns, M. Tester, Mechanisms of salinity tolerance, Annu. Rev. Plant Biol. 59 (2008) 651- 681.
10.1146/annurev.arplant.59.032607.092911
[4] R. Munns, Comparative physiology of salt and water stress, Plant Cell Environ. 25 (2002) 239- 250.
10.1046/j.0016-8025.2001.00808.x
[5] J. Cuartero, M.C. Bolarin, M.J. Asins, V. Moreno, Increasing salt tolerance in the tomato, J. Exp. Bot. 57 (2006) 1045-1058.
10.1093/jxb/erj102
[7] L. Menezes-Benavente, S.P. Kernodle, M. Margis-Pinheiro, J.G. Scandalios, Salt-induced antioxidant metabolism defenses in maize (Zea mays L.) seedlings, Redox. Rep. 9 (2004) 29- 36.
10.1179/135100004225003888
[8] H. Hichem, D. Mounir, E.A. Naceur, Differential responses of two maize (Zea mays L.) varieties to salt stress: Changes on polyphenols composition of foliage and oxidative damages, Ind. Crops Prod. 30 (2009) 144-151.
10.1016/j.indcrop.2009.03.003
[9] D.A. Meloni, C.A. Oliva, J. Cambraia, Photosynthesis and activity of superoxide dismiotase, peroxidase and glutathione reductase in cotton under salt stress, Braz. J. Plant Physiol. 15 (2003) 12-21.
10.1016/s0098-8472(02)00058-8
[10] N.P. Rout, B.P. Shaw, Salt tolerance in aquatic macrophytes: possible involvement of the antioxidative enzymes, Plant Sci. 160 (2001) 415-423.
10.1016/s0168-9452(00)00406-4
[11] J.M. Awika, L.M. Rooney, Sorghum phytochemicals and their potential impact on human health, Phytochemistry. 65 (2004) 1199-1221.
10.1016/j.phytochem.2004.04.001
[12] I.N. De Abreu, P. Mazzafera, Effect of water and temperature stress on the content of active constituents of Hypericum brasilienne Choisy, Plant Physiol. Biochem. 43 (2005) 241-248.
10.1016/j.plaphy.2005.01.020
[13] S. Kuntz, U. Wenzel, H. Daniel, Comparative analysis of the effects of flavonoids on proliferation, cytotoxity, and apoptosis in human colon cancer cell lines, Eur. J. Nutr. 38 (1999) 133-142.
10.1007/s003940050054
[15] R.A. Dixon, N. Paiva, Stress-induced phenylpropanoid metabolism, The Plant Cell. 7 (1995) 1085-1097.
10.1105/tpc.7.7.1085
[16] M.R. Roberts, N.D. Paul, Seduced by the dark side: integrating molecular and ecological perspectives on the influence of light on plant defense against pests and pathogens, New Phytol. 170 (2006) 677-699.
10.1111/j.1469-8137.2006.01707.x
[17] R. Julkunen-Tiito, N. Nenadis, S. Neugart, M. Robson, G. Agati, J. Vepsa ·la · inen, G. Zipoli, L. Nybakken, B. Winkler, M. Jansen, Assessing the response of plant flavonoids to UV radiation: anoverview of

appropriate techniques, Phyto. Rev. 14 (2015).
10.1007/s11101-014-9362-4
[18] D. Krishnaiah, R. Sarbatly, R. Nithyanandam, A review on the antioxidant potential of medicinal plant species, Food Bioprod. Process. 89 (2011) 217-233.
10.1016/j.fbp.2010.04.008
[19] G. Agati, E. Azzarello, S. Pollastri, M. Tattini, Flavonoids as antioxidants in plants: location and functional significance, Plant Sci. 196 (2012) 67-76.
10.1016/j.plantsci.2012.07.014
[20] C. Brunetti, M. Di Ferdinando, A. Fini, S. Pollastri, M. Tattini, Flavonoids as antioxidants and development regulators: relative significance in plants and humans, Int. J. Mol. Sci. 14 (2013) 3540-3555.
10.3390/ijms14023540
[21] A. Wahid, A. Ghazanfar, Possible involvement of some secondary metabolites in salt tolerance of sugarcane, J. Plant Physiol. 163 (2006) 723-730.
10.1016/j.jplph.2005.07.007
[22] R. Ksouri, W. Megdiche, A. Debez, H. Falleh, C. Grignon, C. Abdelly, Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte Cakile maritima, Plant Physiol. Biochem. 45 (2007) 244-249.
10.1016/j.plaphy.2007.02.001
[23] F. Hanen, R. Ksouri, W. Megdiche, N. Trabelsi, M. Boulaaba, C. Abdelly, Effect of salinity on growth, leaf phenolic content and antioxidant scavenging activity in Cynara cardunculus L., in Biosaline Agriculture and High Salinity Tolerance; Abdelli, C., Ozturk, M., Ashraf, M., Grignon, Y.C., Eds.; Birkhauser Verlag, Switzerland, 2008, 335-343.
10.1007/978-3-7643-8554-5_31
[24] J.M. Navarro, P. Flores, C. Garrido, V. Martinez, Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity, Food Chem. 96 (2006) 66-73.
10.1016/j.foodchem.2005.01.057
[25] A.K. Parida, A.B. Das, Y. Sanada, P. Mohanty, Effects of salinity on biochemical components of the mangrove, Aegiceras corniculatum, Aqua. Bot. 80 (2004) 77-87.
10.1016/j.aquabot.2004.07.005
[27] H.H. Ti, Q. Li, R.F. Zhang, M.W. Zhang, Y.Y. Deng, Z.C. Wei, Free and bound phenolic profiles and antioxidant activity of milled fractions of different indica rice varieties cultivated in southern China, Food Chem. 159 (2014) 166-174.
10.1016/j.foodchem.2014.03.029
[28] A. Djeridane, M. Yousfi, B. Nadjemi, D. Boutassouna, P. Stocker, N. Vidal, Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds, Food Chem. 97 (2006) 654-660.
10.1016/j.foodchem.2005.04.028
[29] E. Bandeoglu, F. Eyidogan, M. Yuceland, H.A. Oktem, Antioxidant responses of shoots and roots of lentil to NaCl-salinity stress, Plant Growth Regul. 42 (2004) 69-77.
10.1023/b:grow.0000014891.35427.7b
[30] M.R. Suplick-Ploense, Y.L. Qian, J.C. Read, Salinity tolerance of Texas bluegrass, Kentucky bluegrass, and their hybrids, Crop Sci. 42 (2002) 2025-(2030).
10.2135/cropsci2002.2025
[31] V. Kumar, V. Shriram, T.D. Nikam, N. Jawali, M.G. Shitole, Antioxidant enzyme activities and protein profiling under salt stress in indica rice genotypes differing in salt tolerance, Arch Agron Soil Sci. 55 (2009) 379-394.
10.1080/03650340802595543

[32] M.M. Chaves, J. Flexas, C. Pinheiro, Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell, Annal. Bot. 103 (2009) 551-560.
10.1093/aob/mcn125
[33] A. Rezazadeh, A. Ghasemzadeh, M. Brani, T. Telmadarrehei, Effect of salinity on phenolic composition and antioxidant activity of Artichoke (Cynara scolymus L.) leaves, J. Med. Plant Res. 6 (2012) 245-252.
10.3923/rjmp.2012.245.252
[37] K.S. Gould, J. McKelvie, K.R. Markham, Do anthocyanins function as antioxidants in leaves? Imaging of H2O2 in red and green leaves after mechanical injury, Plant Cell Environ. 25 (2002) 1261-1269.
10.1046/j.1365-3040.2002.00905.x
[38] F. Tomas-Barberan, J.C. Espin, Phenolic compounds and related enzymes as determinants of quality of fruits and vegetables, J. Sci. Food. Agric. 81 (2001) 853-876.
10.1002/jsfa.885
[41] K. Wakabayashi, T. Hoson, S. Kamisaka, Osmotic stress suppresses cell wall stiffening and the increase in cell wall bound ferulic and diferulic acids in wheat coleoptiles, Plant Physiol. 113 (1997) 967-973.
10.1093/oxfordjournals.pcp.a029239
[42] D.M. Li, Y.X. Nie, J. Zhang, J.S. Yin, Q. Li, X.J. Wang, J.G. Bai, Ferulic acid pretreatment enhances dehydration-stress tolerance of cucumber seedlings, Biol. Plant. 57 (2013) 711-717.
10.1007/s10535-013-0326-0
[43] K. Krygier, F. Sosulski, L. Hogge, Free, esterified, and insoluble-bound phenolic acids. 1. Extraction and purification procedure, J. Agric. Food. Chem. 30 (1982) 330-334.
10.1021/jf00110a028
[44] T.D. Xuan, E. Tsuzuki, H. Terao, T.D. Khanh, Correlation between growth inhibitory exhibition and suspected allelochemicals (phenolic compounds) in the extract of alfalfa (Medicago sativa L.), Plant Prod. Sci. 6 (2003) 165-171.
10.1626/pps.6.165