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Research Article

Exploring the Role of Bioactive Polyphenolic Antioxidants in Salinity Tolerance of Two Rice Landraces from Coastal Areas of Bangladesh

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Abstract

The bioactive polyphenolic compounds (BPC), play an important role in the restoration of cellular redox homeostasis but are seldom studied in context of salinity stress tolerance in rice. RP-HPLC based comparative analysis of some important bioactive polyphenolic compounds (gallic acid, protocatechuic acid, para-hydroxy benzoic acid, catechin, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid, salicylic acid, naringin, rutin, ellagic acid, myricetin, quercetin, naringenin, apigenin and kaempferol) from seedling of two rice landraces (*Oryza sativa* L. landraces Kutepatnai and Charobalam) subjected to post imbibitional salinity stress (PISS) not only exhibited differential landrace specific accumulation but also exhibited strong correlation with salinity tolerance. The landrace Kutepatnai exhibiting the ability to maintain redox homeostasis under PISS (assessed in terms of biomarkers of oxidative stress like the relative reactive oxygen species accumulation, relative total antioxidant competence and relative oxidative membrane damage) showed significantly greater up-regulation of majority of the polyphenolic compounds derived from chalcone synthase, and cinnamic acid dependent pathway as compared to the salt susceptible landrace Charobalam. The positive correlation between bioaccumulation of BPC (protocatechuic acid, ellagic acid, caffeic acid, syringic acid, rutin, catechin, myricetin, quercetin and apigenin) with redox parameters suggests their role in regulation of redox homeostasis necessary for salt tolerance in experimental land races of coastal areas of Bangladesh.

Keywords: Rice Landraces; Salinity Tolerance; Bioactive Polyphenolic Compounds; Antioxidants; Redox Homeostasis

Abbreviations

ROS: Reactive Oxygen Species; DNA: Deoxyribonucleic Acid; BPC: Bioactive Polyphenolic Compounds; $HgCl_2$: Mercuric Chloride; PISS: Post Imbibitional Salinity Stress; DPPH: 2, 2/-diphenyl-1-pycryl hydrazyl; DCFDA: 2', 7'-dichlorofluorescindiacetate; TRIS-HCl: Tris (hydroxymethyl) Aminomethane (THAM) Hydrochloric Acid; RRA: Relative ROS Accumulation; TAC: Total Antioxidant Capacity; TBARS: Thiobarbituric Acid Reactive Compounds; TCA: Trichloroacetic Acid; TBA: Thiobarbituric Acid; RLP: Relative Lipid Peroxidation; T_{50}: Time (in hour) of 50% Germination of Seeds Sown; RP-HPLC: Reverse Phase High Performance Liquid Chromatography; DAD: Diode Array Detector; NaCl: Sodium Chloride

Introduction

Salinity is one of the prime abiotic stress factors that affects various metabolic and physiological pathways in plants such as reducing growth, development, and ultimately impacting global crop yield, slowing down growth and development, and ultimately

impacting crop yields globally [1,2]. Plants are basically harmed by ion toxicity, nutritional imbalance, secondary osmotic and oxidative stresses when there is a high salt concentration in the soil and irrigation water [3-6]. Different plant species and genotypes within a species react to salt stress in different ways. In saline environment, certain moderately or highly salt-tolerant plants can survive. In presence of high salt concentrations, these species are able to avoid ion toxicity and maintain water uptake [7]. Furthermore, salt-tolerant varieties have a stronger antioxidant defense system to combat oxidative damages induced by reactive oxygen species (ROS) induced oxidative damages [8]. Rice landraces tolerant to salinity thus play a vital part in local food security in saline coastal areas and agriculture's long-term sustainability, in addition to their usefulness as a genetic resource for rice genetic improvement [9,10].

In general, salinity leads to the accumulation of antioxidant molecules, which are important in counterbalancing oxidative stress [11-13]. Overproduction of ROS is exacerbated by salinity, which causes oxidative damage by oxidizing proteins, lipids, DNA, and other biological macromolecules [14,15]. Plants have evolved an antioxidative system that includes antioxidative enzymes as well as non-enzymatic low-molecular-mass secondary metabolites and bioactive metabolites like phenolic compounds and flavonoids to minimize ROS-induced damage [15-17]. The dynamics of ROS-antioxidant interactions at metabolic interface are crucial not just for decreasing oxidative damage, but also for triggering ROS signaling during saline environment stressful situations [18,19]. Role of polyphenolic compounds in restoration of redox homeostasis, otherwise disturbed by salinity can be vouched by the inhibition of lipid peroxidation and protection of photosynthetic system from photo damage [20,21]. Many studies are being carried out to determine the involvement of different antioxidant metabolites in plant stress tolerance. Because of increased H^+ donating ability and radical stabilization property of phenolic compounds, among other metabolites, have been researched extensively for their antioxidant characteristics [22-26]. The stronger activity of enzymes related to the metabolization of phenolics and the accumulation of phenolic compounds have been linked with the tolerance of cereals to abiotic stress [27]. For example, phenolics are electron donors and hence serve as ideal substrates for antioxidant enzymes such as peroxidases, which may help to reduce the effects of oxidative stress [17].

Plants raised under saline environment, thus exhibit diverse metabolic imprints of non-enzymatic antioxidant based redox regulatory mechanisms and oxidative deterioration. In this context, standardization of parameters of non–enzymatic redox metabolic fingerprints especially BPC and correlating them with redox status of salinity-induced plants are of utmost significance for understanding mechanistic aspect and screening salinity resistant cultivars of rice and hence is explored in present study. Thus the study is aimed to explore and standardize the accumulation of BPC for the assessment of redox health of two contrasting landraces of rice of coastal areas of Sundarban, Bangladesh.

Materials and Methods

Plant growth and treatment of NaCl to induce post-imbibitional salinity stress (PISS)

Two landraces of two rice (*Oryza sativa* L.) germplasm (landraces Kutepatnai and Charobalam) that are commonly cultivated in different coastal areas of Bangladesh were selected and their seeds were collected from Bangladesh Resource Center for Indigenous Knowledge (BARCIK). The seeds of two rice germplasm were multiplied and maintained at the Crop Research and Seed Multiplication Farm (CRSMF), University of Burdwan, Burdwan, West Bengal, India.

The seeds were surface sterilized (with 0.2% $HgCl_2$) for 5 min and imbibed in distilled water for 48 h in the dark at 25 ± 2°C, after which seeds were added in petri plate lined with moist filter paper and placed in standardized conditions of a thermostat-controlled seed germinator cum stability chamber maintained at 25 ± 2°C. Water imbibed seed lots were treated with 200 mM NaCl for 7 days to induce post-imbibitional salinity stress (PISS). Water imbibed seeds were added directly in petri plates for the untreated control set. All seed lots were grown at a temperature of 25 ± 2°C, with a 14 h photoperiod (light intensity of 270 µmol m^{-2} s^{-1}) and relative humidity of 78 ± 2%. For all biochemical analyses, 7 days old seedlings raised from aforesaid conditions were used.

Determination of reactive oxygen species (ROS) and total antioxidant capacity (DPPH radical scavenging property)

Total ROS generation

For extraction and estimation of total ROS, methodology of [28] was adopted. 30 mg of seedling tissue was incubated for 60 min at 30°C in 100 µM solution of 2', 7'-dichlorofluorescindiacetate (DCF-

DA, Sigma) dissolved in TRIS-HCl (p^H-7.0). Excitation at 504 nm and emission at 525 nm were used to detect the fluorescence in the supernatant using spectrofluorometry. To distinguish ROS from other long-lived compounds capable of reacting with DCFDA, the same technique was used without DCFDA in the control set.

Assessment of Relative ROS accumulation (RRA)

Relative ROS accumulation (RRA) was calculated from the result of Total ROS, calculated by the following formula:

$$\text{Relative ROS accumulation (RRA)} = \frac{T(TBARS)}{C(TBARS)} \times 100\ \%$$

Where, T (ROS) = Total ROS accumulation during the treated condition, C (ROS) = Total ROS accumulation during the untreated condition.

Total antioxidant capacity in terms of 2, 2/-diphenyl-1-pycryl hydrazyl (DPPH) radical scavenging activity

The extraction of dry seedling tissue was performed by incubation in 80% methanol at 28°C for 24 h in a shaking incubator for the measurement of DPPH free radical scavenging activity, as described by [29]. Extracts were centrifuged at 3500 rpm for 20 min at 4°C. To determine the radical scavenging activity, 1 mL of sample was combined with 3 mL DPPH (0.04 mg mL-1 ethanol) and incubated in the dark for 30 min before measuring the absorbance at 517 nm.

Finally total antioxidant capacity (TAC) was determined by the following formula:

$$\text{TAC (\%)} = {}^{=\frac{T(TBARS)}{C(TBARS)} \times 100\ \%} \times 100\ \%$$

[A_i= 1 mL sample + 3 mL DPPH; A_j = 1mL sample + 3 mL ethanol; A_c= 1 mL ethanol + 3 mL DPPH].

Assessment of Relative total antioxidant capacity

The result of total antioxidant capacity was used to compute relative total antioxidant capacity (DPPH radical scavenging activity), calculated by the following formula:

$$\text{Relative total antioxidant capacity (RTAC)} = \frac{T(TBARS)}{C(TBARS)} \times 100\ \%$$

Where, T (TAC) = Total antioxidant capacity during the treated condition, C (TAC) = Total antioxidant capacity during the untreated condition.

Assessment of membrane lipid peroxidation

Heath and Packer's [30] technique was used to test for thiobarbituric acid reactive compounds (TBARS) to determine membrane lipid peroxidation. 200 mg of sample was homogenized in 5 mL 0.1% trichloroacetic acid (TCA) and then centrifuged at 10,000 rpm for 15 min and finally supernatant was taken. To 1 mL of supernatant, 3 mL of 5% TCA containing 1% thiobarbituric acid (TBA) was added and heated in a hot water bath for 30 min and cooled quickly in cold water bath. It was finally centrifuged at 10,000 rpm for 10 min. At 530 nm, the absorbance of the supernatant was measured. The extinction coefficient of TBARS, which is 155 µM cm^{-1}, was used to calculate its concentration. By subtracting A_{600} from A_{530}, the non-specific turbidity was adjusted. The formula employed as:

$$\text{Conc. of unknown} = \frac{T(TBARS)}{C(TBARS)} \times 100\ \%$$

The TBARS content was finally expressed in n mol g^{-1} dry mass of tissue.

Assessment of relative lipid peroxidation

Relative lipid peroxidation was calculated from the result of TBARS content, calculated by following formula:

$$\text{Relative Relative lipid peroxidation (RLP)} = \frac{T(TBARS)}{C(TBARS)} \times 100\ \%$$

Where, T (TBARS) = TBARS content during treated condition, C (TBARS) = TBARS content during untreated condition.

Determination of early growth performances

- T_{50} values were obtained according to [31,32] for analyzing early growth performances.
- T_{50} value of germination = Time (in hour) of 50% germination of seeds sown.

RP-HPLC based quantitative assessment of redox-sensitive phenolic acids and flavonoids

Sample preparation

Samples were prepared by repeating two cycles of soxlet-mediated hydroethanolic extraction of dry powdered tissue (150 mL 80% ethanol for 15 g powder) at 60°C temperature for each sample, followed by rotary vacuum evaporation for concentrating the samples. 20 µL of solution was taken for HPLC study [33].

RP HPLC analysis of phenolic acids and flavonoids

For HPLC analyses, a Dionex Ultimate 3000 liquid chromatograph with a diode array detector (DAD) and a 5 cm flow cell was employed, along with a Chromeleon system manager as a data processor. Separation was achieved by a reversed-phase Acclaim C18 column (5 micron particle size, 250 x 4.6 mm). All extracted solutions were filtered using an HPLC filter [0.45 mm membrane filter (Millipore)]. The quantification of phenolic acids and flavonoids in the sample extracts was carried out by measuring the integrated peak area. The contents were determined by plotting peak area versus concentration of the relevant standard sample using the calibration curve. Standard stock solutions of twenty-one phenolic acids and flavonoids (protocatechuic acid, gallic acids, naringenin, para hydroxyl benzoic acid, chlorogenic acid, gentisic acid, vanillic acid, ellagic acid, ferulic acid, caffeic acid, synaptic acid, syringic acid, para caumaric acid, salicylic acid, quercetin, rutin, myricetin, apigein, kaempferol and catechin) were prepared in methanol at a concentration 10 µg mL^{-1}.

Statistical analysis

Each treatment had three replicates, and each experiment was repeated twice at different times. Results are mean of three replicates ± standard error. The data were statistically analyzed using Microsoft Excel 2010, which revealed substantial differences between untreated control and post-imbibitional salinity stress-raised seedlings.

Results and Discussion

In order to ascertain the metabolic role of polyphenolic compounds particularly phenolic acids and flavonoids in ROS-antioxidant interaction necessary for redox-regulation and their reliability as standard metabolic fingerprints in adjudging salinity tolerance, RP-HPLC based identification and quantification of some important redox-sensitive phenolic acids and flavonoids were done in PISS-raised seedlings of two experimental landraces Kutepatnai and Charobalam vis-a-vis their untreated control (Table 1, 2). Here, we have identified and quantified important redox-sensitive polyphenolic compounds through RP-HPLC, like gallic acid (R_t 7.69), protocatechuic acid (R_t 17.56), para-hydroxy benzoic acid (R_t 36.76), catechin (R_t 40.50), chlorogenic acid (R_t 43.37), vanillic acid (R_t 45.58), caffeic acid (R_t 47.05), syringic acid (R_t 49.17), p-coumaric acid (R_t 55.27), ferulic acid (R_t 57.88), sinapic acid (R_t 62.66), salicylic acid (R_t 69.53), naringin (R_t 70.78), rutin (R_t 72.40), ellagic acid (R_t 75.41), myricetin (R_t 77.90), quercetin (R_t 88.14), naringenin (R_t 92.76), apigenin (R_t 93.60) and kaempferol (R_t 99.42) (Figure 1).

When compared between the untreated control and PISS-raised seedlings, the landrace Kutepatnai exhibited the highest individual level up-regulation of phenolic compounds tested for PISS-raised seedlings over their untreated control. The landrace Kutepatnai showed a significantly greater accumulation of phenolic acids like Proto-catechuic acid (204.282% increment), Ellagic acid (151.129% increment), Chlorogenic acid (30.23% increment), Caffeic acid (67.379% increment), Syringic acid (195.035% increment), p-Coumaric acid (35.016% increment) and Sinapic acid (139.8% increment) over untreated control along with *de-novo* synthesis of the phenolic acid p-Hydroxy benzoic acid and Ferulic acid which were all together absent in untreated control seedlings. Gallic acid on the other hand suffered an only marginal loss (28.823%) in PISS-raised seedlings over their untreated control (Table 1).

The flavonoids like rutin, catechin, myricetin, quercetin, and apigenin, similarly showed an up-regulation by 408.927%, 17.22%, 28.30%, 129.17% and 56.69% respectively for PISS-raised seedlings of landrace Kutepatnai over their untreated control. Kutepatnai showed significant down-regulation in the accumulation of Naringin, Naringenin and Kaempferol by 14%, 92.29%, and 92.61% respectively in PISS-raised seedlings over their untreated control (Table 2).

The landrace Charobalam on the other hand, also showed significant up-regulation in the accumulation of gallic acid, protocatechuic acid, para-hydroxy benzoic acid, chlorogenic acid, caffeic acid, p-coumaric acid and sinapic acid by 39.996%, 105.462%, 373.112%, 51.279%, 7.377%, 61.51% and 175.25% respectively in PISS-raised seedlings over their untreated control. The landrace Charobalam particularly suffers significant loss in the accumulation of ellagic acid, syringic acid and Ferulic acid over untreated control by 27.87%, 52.696% and 59.209% respectively. Vanillic acid, which was present in untreated control of landrace Charobalam in an appreciable amount (29.031 µg/100g dm) got completely disappeared under PISS. Salicylic acid, which was absent in control seedlings showed *de-novo* expression under PISS in an

appreciable amount (165.054 µg/100g dm) in the landrace Charobalam.

The landrace Charobalam showed a significantly greater accumulation of flavonoids like Naringin, rutin, quercetin, Naringenin, apigenin and Kaempferol over untreated control by 487.528%, 12.554%, 101.721%, 677.38%, 40.735% and 54.851% respectively. Charobalam particularly suffers significant loss in the accumulation of catechin and myricetin, over untreated control by 43.702% and 47.607% respectively.

The correlative evaluation of Post imbibitional salinity stress-induced changes in the endogenous titer of redox-sensitive phenolic acids and flavonoids with redox health was done in terms of relative ROS accumulation, relative lipid peroxidation, relative total antioxidant capacity and germination performance (T_{50} value of germination) of the two rice landraces differing in sensitivity towards NaCl salinity stress. The result exhibited significant accumulation of some up-regulated important redox–sensitive individual phenolic acids like protocatechuic acid, ellagic acid, caffeic acid and syringic acid in PISS raised seedlings of Kutepatnai than its counterpart Charbalam (Table 3). Whereas some individual important up-regulated redox-sensitive flavonoids like rutin, catechin, myricetin, quercetin and apigenin have greater elicitation in PISS raised seedlings of Kutepatnai than its counterpart Charobalam (Table 4).

Relative ROS accumulation and relative lipid peroxidation is significantly reduced in Kutepatnai than its counterpart Charobalam. Thus these results indicate the correlation between the capacity for elicitation of phenolic acids and flavonoids with PISS-induced oxidative damages to the germinating tissue. T_{50} value is significantly reduced in Kutepatnai than its counterpart Charobalam but it is noticeable that the relative total antioxidant capacity is increased in Kutepatnai than Charobalam. Now, when we compared the salinity induced changes in redox health of the PISS – raised seedlings of two experimental land races of rice, we find strong correlation between the ability of the accumulation of BPC tested with the redox health of both the cultivars. The cultivar Kutepatnai, being able to elicit the overall production of flavonoids and phenolic was found to be capable of restoring the redox homeostasis more efficiently than its counterpart i.e. the landraces Charobalam. The redox metabolic shift towards prooxidants was found to be more for the landrace Charobalam which shows significantly lesser ability in elicitation of polyphenolic compounds tested by RP–HPLC. Several previous works like [19,34,35] strongly vouch the metabolic role of polyphenolic compounds as potent radical scavenger and inhibitor of lipid peroxidation and fenton reaction, necessary for maintenance of redox homeostasis of crops under stress. Lee., *et al.* [36] and Fiorani., *et al.* [34] through their studies also exhibited significant up regulation of the enzymes of flavonoid and phenolic acid biosynthesis, under oxidative threat as potent mechanism of redox regulation. Moreover, other studies also exhibited significant impact of BPC in stabilizing enzymatic redox hub necessary for redox regulation of crops [19,35].

Salinity induced alteration in redox homeostasis due to obligation of secondary oxidative stress is a well-known event that directly influence growth, development and yield performance of the crops [37,38]. Present experiment entitled a strong correlation between PISS-induced changes in biomarkers of redox status (assessed in terms of relative oxidative membrane damage through lipid peroxidation, relative changes in ROS accumulation and antioxidative capacity) and early growth performances of germinating rice seeds. Oxidative membrane lipid peroxidation is one of the major metabolic incident under salinity that possess several adverse and physiological consequences [39,40]. This course of event disturbs not only membrane architecture but also caters production of ROS and other toxic secondary products oxidative stress, further exasperating the oxidative membrane injury causing loss of cellular redox and metabolic homeostasis [41].

Further, for understanding whether PISS-induced oxidative MLP and altered redox status has any impact on germination, sensitive germination phenotype T50 value were assessed and compared between PISS-raised seedlings of two experimental landraces Kutepatnai and Charobalam vis-a-vis their untreated control. The results in general showed significant inverse relationship between relative ROS accumulation and oxidative membrane lipid peroxidation with T50 values (Figure). The greater the magnitude of oxidative lipid peroxidation and relative changes in redox status of germinating tissue under PISS, more was the inhibitory impact on germination (T50 values). So, in the current study, the assessment of redox biomarkers of PISS exhibited strong correlation with the sensitive germination phenotype of the experimental rice cultivars. This result also corroborated by other studies, showing the impact of accumulation of BPCs in restoration of redox homeostasis and improvement of germination phenotypes and stress tolerance [42,43].

Rice (*Oryza sativa* L.) Landraces				Treatment	Accumulation of redox sensitive phenolic acids ($\mu g\text{-}^{100g}$ dm)
Charobalam		Kutepatnai			
PISS (200 mM NaCl)	Untreated Control	PISS (200 mM NaCl)	Untreated Control		
652.587 (+39.996%)	466.146	555.169 (-28.823%)	779.98	**Gallic acid**	
199.871 (+105.462%)	97.279	321.909 (+204.282%)	105.793	**Proto-catechuic acid**	
273.227 (+373.112%)	57.751	218.811	-	**p-Hydroxy benzoic acid**	
157.655 (-27.87%)	218.569	454.132 (+151.129%)	180.836	**Ellagic acid**	
106.936 (+51.279%)	70.688	149.158 (+30.23%)	114.534	**Chlorogenic acid**	
-	29.031	37.820 (+0.484%)	37.638	**Vanillic acid**	
515.688 (+7.377%)	480.258	225.039 (+67.379%)	134.449	**Caffeic acid**	
100.877 (-52.696%)	213.253	234.624 (+195.035%)	79.524	**Syringic acid**	
1817.932 (+ 61.51%)	1125.602	2366.106 (+35.016%)	1752.464	**p-Coumaric acid**	
141.987 (- 59.209%)	348.085	249.899	-	**Ferulic acid**	
481.292 (+175.25%)	174.857	519.091 (+139.8%)	216.468	**Sinapic acid**	
165.054	-	-	-	**Salicylic acid**	

Table 1: Post imbibitional salinity stress (PISS) induced changes in endogenous titer of some important redox sensitive phenolic acids in two rice landraces (*Oryza sativa* L. landraces Kutepatnai and Charobalam) differing in sensitivity towards NaCl salinity stress (200 mM NaCl/ EC 0.18S cm^{-1}). Values within parenthesis represent % increment or reduction under PISS over control.

Rice (*Oryza sativa* L.) Landraces		Treatment	Accumulation of redox sensitive flavonoids ($\mu g \cdot {}^{100g}$ dm)							
			Naringin	Rutin	Catechin	Myricetin	Quercetin	Naringenin	Apigenin	Kaempferol
Kutepatnai		Untreated Control	16.650	230.618	129.772	102.534	353.852	183.306	295.208	216.246
		PISS (200 mM NaCl)	14.175 (-14%)	1173.677 (+408.927%)	152.119 (+17.22%)	131.555 (+28.30%)	810.937 (+129.17%)	14.136 (-92.29%)	462.548 (+56.69%)	15.984 (-92.61%)
Charobalam		Untreated Control	19.090	299.448	104.077	126.365	256.362	75.487	324.889	8.122
		PISS (200 mM NaCl)	112.159 (+487.528%)	337.040 (+12.554%)	58.593 (-43.702%)	66.207 (-47.607%)	517.135 (+101.721%)	586.821 (+677.38%)	457.233 (+40.735%)	12.577 (+54.851%)

Table 2: Post imbibitional salinity stress (PISS) induced changes in endogenous titer of some important redox sensitive flavonoids in two rice landraces (*Oryza sativa* L. landraces Kutepatnai and Charobalam) differing in sensitivity towards NaCl salinity stress (200 mM NaCl/EC 0.18S cm^{-1}). Values within parenthesis represent % increment or reduction under PISS over control.

Rice (*Oryza sativa* L.) Landraces		Treatment	Redox status			Major phenolic acids enhanced								Germination Performance
Charobalam	Kutepatnai		Relative ROS accumulation (%)	Relative total antioxidant capacity (%)	Relative lipid peroxidation (%)	Gallic acid	Proto-catechuic acid	p-Hydroxy benzoic acid	Ellagic acid	Chlorogenic acid	Caffeic acid	Syringic acid	p-Coumaric acid	Sinapic acid	T_{50} (hrs)
PISS (200 mM NaCl)	Untreated Control	PISS (200mM NaCl)													
184.439 ± 0.607	100	130.522 ± 0.510	100	100	100	-	105.793	-	180.836	114.534	134.449	79.524	1752.464	216.468	20.5 ± 0.136
100.788 ± 0.158	100	101.972 ± 0.208	100	100	100	-	321.909 (+204.28%)	218.811	454.132 (+151.13%)	149.158 (+30.23%)	225.039 (+67.38%)	234.624 (+195.04%)	2366.106 (+35.02%)	519.091 (+139.80%)	24.67 ± 0.208
288.235 ± 0.587	100	120.898 ± 0.553	100	100	100	466.146	97.279	57.751	-	70.688	480.258	-	1125.602	174.857	20.83 ± 0.157
652.587 (+40.00%)	466.146	-													
199.871 (+105.46%)	97.279	321.909 (+204.28%)													
273.227 (+373.11%)	57.751	218.811													
-	-	454.132 (+151.13%)													
106.936 (+51.28%)	70.688	149.158 (+30.23%)													
515.688 (+7.38%)	480.258	225.039 (+67.38%)													
-	-	234.624 (+195.04%)													
1817.932 (+61.51%)	1125.602	2366.106 (+35.02%)													
481.292 (+175.25%)	174.857	519.091 (+139.80%)													
60.83 ± 0.079	20.83 ± 0.157	24.67 ± 0.208													

Table 3: Corelative evaluation of up-regulated major polyphenolic acids with redox status and germination performance of two rice landraces (*Oryza sativa* L. landraces Kutepatnai and Charobalam) of Sundarban, Bangladesh raised from PISS (200 mM NaCl/ EC 0.18S cm^{-1}). Values are mean of three independent replicates ± standard error and values with in parenthesis represent % increment or reduction under PISS over control.

Rice (*Oryza sativa* L.) Landraces				Redox status			Major Flavonoids enhanced							Germination Performance
Charobalam		Kutepatnai		Treatment										
PISS (200 mM NaCl)	Untreated Control	PISS (200 mM NaCl)	Untreated Control	Relative ROS accumulation (%)	Relative total antioxidant capacity (%)	Relative lipid peroxidation (%)	Naringin	Rutin	Catechin	Myricetin	Quercetin	Naringenin	Apigenin	Kaempferol	T_{50} (hrs)
184.439 ± 0.607	100	130.522 ± 0.510	100	100	100	100	-	230.618	129.772	102.534	353.852	-	295.208	-	20.5 ± 0.136
100.788 ± 0.158	100	101.972 ± 0.208													
188.235 ± 0.587	0	20.898 ± 0.553													
112.159 (+487.53%)	19.09	-													
337.04 (+12.55%)	299.448	1173.677 (+408.93%)													
-	-	152.119 (+17.22%)													
-	-	131.555 (+28.30%)													
517.135 (+101.72%)	256.362	810.937 (+129.17%)													
586.821 (+677.38%)	75.487	-													
457.233 (+40.74%)	324.889	462.548 (+56.69%)													
12.577 (+54.85%)	8.122	-													
60.83 ± 0.079	20.83 ± 0.157	24.67 ± 0.208													

Table 4: Corelative evaluation of up-regulated major flavonoid compounds with redox status and germination performance of two rice landraces (*Oryza sativa* L. landraces Kutepatnai and Charobalam) of Sundarban, Bangladesh raised from PISS (200 mM NaCl/EC 0.18S cm^{-1}). Values are mean of three independent replicates ± standard error and values with in parenthesis represents % increment or reduction under PISS over control.

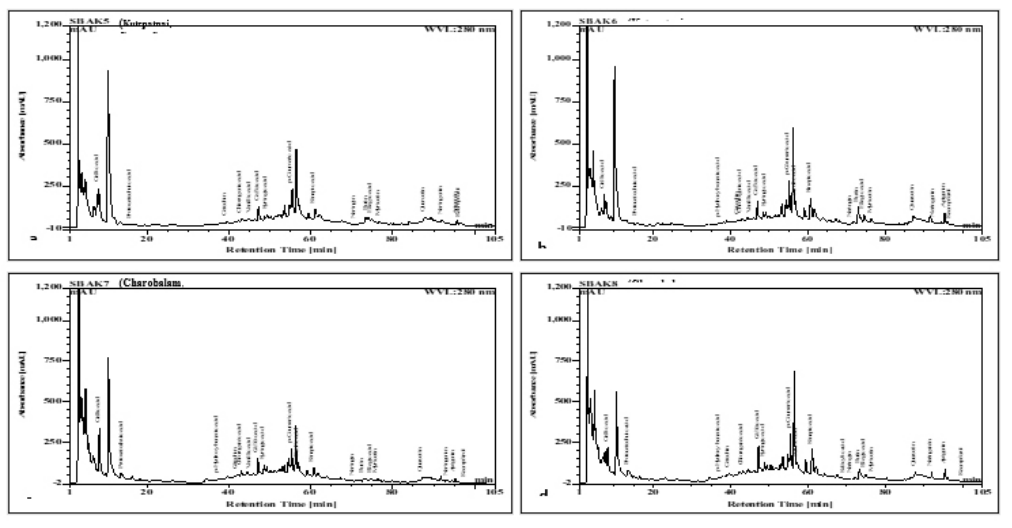

Figure 1: RP-HPLC based Chromatograms of polyphenolic compounds extracted from PISS (200 mM NaCl/EC 0.18S cm^{-1}) raised seedlings of two landraces of Rice
(*Oryza sativa* L. landraces Kutepatnai and Charobalam).

Conclusion

Taken as a whole, the present investigation suggests the significance of bioactive polyphenolic compounds derived from Phenylpropanoid pathway in restoration of redox homeostasis under salinity in rice landraces of Sundarbans, Bangladesh. Further, the vulnerability of newly assembled membrane system towards oxidative deterioration through peroxidation and alteration of redox homeostasis at metabolic interface under PISS in experimental rice cultivars and elicitation of redox-sensitive BPCs to combat such deteriorative event found to regulate the germination performance under salinity.

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Conflict of Interest

The authors have no conflict of interest in preparing of this research article.

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