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## Variation in Accumulation of Phenolics in Some Native Rice Cultivars of North Kerala, India in Response to Salt Stress

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### ABSTRACT

Rice plants respond to salinity stress in different ways and accumulation of phenolic compounds is one of them. An experiment was carried out presently to analyze the variation of accumulation of phenolics in some native rice cultivars of North Kerala, India in response to salt stress. Five cultivars namely *Orthadian*, *Orkazhama*, *Kuthiru*, *Kuttusan* and *Chovvarian* collected from one of the saline rice tracts of Kerala and two cultivars namely *Kunhutty* and *Veliyan* collected from one of the non-saline rice tracts of Kerala were used for the study. The plants were treated with gradually increasing concentrations of NaCl varying from 0-200 mM starting from the 45th day of growth onwards mimicking the gradually increasing salt content of the salinity prone rice farms of the study area and the quantity of total phenolics was investigated in each case. The cultivars collected from both the saline and non-saline rice tracts showed gradual increase in the accumulation of total phenolics in response to increase in salt stress. The cultivars showed differential variation in the accumulation of total phenolics in relation to salt stress showing that the character was cultivar specific. Among the cultivars studied, *Kuthiru* showed the highest quantity of phenolic compounds and *Orkazhama* showed the highest percentage of increase of phenolics over the control plants in relation to increase in salt stress. Salt stress tolerant cultivars can be further screened for the production of higher quantity of phenolics in response to salt stress with an objective of improving the antioxidant production potential of such cultivars.

**Key words:** Native rice cultivars, *Oryza sativa*, phenolics, salt stress

### INTRODUCTION

Rice (*Oryza sativa* L.) is the most important staple cereal food of nearly 50-80% of the world's population either directly as human food or indirectly as animal feed (Osawa *et al.*, 1992; Zhai *et al.*, 2001; Zhou *et al.*, 2002; Conforti and Sarris, 2011). Asia represents about 90% of global rice production and consumption (Osawa *et al.*, 1992; Salekdeh *et al.*, 2002). It is a semi-aquatic tropical C<sub>3</sub> crop and hence it requires high level of soil moisture (McKersie and Lesham, 1994). Nutritional quality of rice has received more attention in the developing countries, where monotonous consumption of rice may lead to deficiencies of essential minerals, vitamins and other nutritional components (Asamarai *et al.*, 1996; Bouis *et al.*, 2003). Salinity is a prevalent abiotic stress that limits the productivity and geographical distribution of plants worldwide and induces a wide range of adverse metabolic responses in higher plants. High concentrations of salts disrupt

homeostasis in water potential and ion distribution in plants (Dash and Panda, 2001). Soil salinity is becoming a serious problem in many coastal, arid and irrigated rice production systems. Increasing salinity of agricultural irrigation water together with progressive salinization of agricultural land is of increasing importance to agriculture because it limits the distribution of plants in certain natural habitats. The impact of salinity is the most serious in countries where all or most of the agricultural production is based on irrigation and when agriculture is a substantial part of the national economy (Mc Williams, 1986; Ghassemi *et al.*, 1995).

Crucial changes in ion and water homeostasis lead to molecular damage, growth arrest and even death of the plant. Specific effects of salt stress on plant metabolism, especially on leaf senescence, have been related to the accumulation of toxic ions ( $\text{Na}^+$  and  $\text{Cl}^-$ ) or to  $\text{K}^+$  and  $\text{Ca}^{2+}$  depletion (Chutipaijit *et al.*, 2011; Rengel, 1992). As a consequence of ion imbalance and hyperosmotic stresses which are primary effects of salt stress, secondary stresses such as oxidative damage may occur. Limited  $\text{CO}_2$  fixation due to stress conditions leads to a decrease in carbon reduction by the Calvin cycle and to a decrease in oxidized  $\text{NADP}^+$  to serve as an electron acceptor in photosynthesis. When ferredoxin is over reduced during photosynthetic electron transfer, electrons may be transferred from PS-I to oxygen to form superoxide radicals ( $\text{O}_2^-$ ) by the process called Mehler reaction which triggers chain reactions that generate more aggressive oxygen radicals (Heldt, 1997; Hsu and Kao, 2003). It is already known that these cytotoxic Active Oxygen Species (AOS) which are also generated during metabolic processes in the mitochondria, chloroplast and peroxisomes, can destroy normal metabolism through oxidative damage of lipids, proteins and nucleic acids. Lipid peroxidation, induced by free radicals, is also important in membrane deterioration (Halliwell, 1987; McCord, 2000).

Similar to other cereal grains, rice is rich in many bioactive non-nutrient compounds known as antioxidants, including phenolic compounds (Frei and Becker, 2004). There are different types of rice that contain colour pigments, such as purple rice, black rice and red rice. They contain phytochemicals that are responsible for their colours. Generally, these coloured compounds or pigments fall into a number of large groups such as chlorophylls, riboflavin, carotenoids, flavonoids and quinones. Most of these pigments are reported to form in plant for vital functions (Chiang *et al.*, 2006). Phenolic compounds are a large group of naturally occurring and chemically diverse substances, universally distributed in the plant kingdom as secondary metabolic products with a large range of structures and functions (Harbone, 1980). They are generally characterized by an aromatic ring bearing one or more hydroxyl substituents (Martinez-Valverde *et al.*, 2000; Liu, 2007) and include alkaloids, flavonoids, terpenoids and glycosides (Appel, 1993) and are primarily synthesized through the shikimate/chorismate or succinyl benzoate metabolic pathway. Evidence indicates that phenolic compounds have potent antioxidant properties and free radical scavenging capabilities (Shahidi *et al.*, 1992). They are widely distributed in medicinal plants, spices, vegetables, fruits, grains, pulses and other seeds (Stratil *et al.*, 2007). They also exhibit a wide range of physiological properties such as anti-allergenic, anti-atherogenic, anti-microbial, antioxidant, antithrombotic, cardioprotective and vasodilatory effects (Puupponen-Pimia *et al.*, 2001). The most important groups of phenolics are flavonoids, phenolic acids and polyphenols (King and Young, 1999). Phenolic acids contribute to allelopathic expression in numerous crops, including sorghum, wheat, oat and rice.

Flavonoids are one group of phenolics which consists of two aromatic rings linked by 3 carbons that are usually in an oxygenated heterocycle ring with one or more hydroxyl groups that may be variously elaborated with methyl, methoxyl, amino or glycosyl groups (Liu, 2004, 2007).

Anthocyanins are a group of reddish to purple water soluble flavonoids that are the primary pigments in the red and black grains and have been widely identified and characterized in cereal grains (Abdel-Aal *et al.*, 2006). There have been a few reports on the characterization of other flavonoids such as flavonols, flavones, flavanols and flavanones and these phenolic compounds are also known as antioxidants (Adom and Liu, 2002; Hu *et al.*, 2003; Abdel-Aal *et al.*, 2006) and have long been recognized to have protective functions against oxidative damage and are associated with reduced risk of chronic diseases (Adom and Liu, 2002; Liu, 2007). Other phytochemicals such as carotenoids, tocopherols and oryzanol are also antioxidants (Xu *et al.*, 2001; Aguilar-Garcia *et al.*, 2007; Choi *et al.*, 2007).

Plants have the ability to synthesize a large number of aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Cowan, 1999). Most are secondary metabolites which may be in the form of simple phenols and phenolic acids, quinones, flavones, flavonoids, flavonols, tannins, terpenoids, essential oils and alkaloids. Some such as terpenoids give plants their odours and flavour; many compounds like tannins and quinones are responsible for plant pigments and others such as some alkaloids may be toxic to the consumer. In many instances, these substances serve as plant defence mechanisms against predation by insects, herbivores and microorganisms (Williams and Harborne, 1989; Cowan, 1999; Beckman, 2000). Different studies show that there are often large increases in phenolic synthesis in plants after attack by plant pathogens (De Ascensao and Dubrey, 2003). In resistant plants, phenolic based defence responses are characterized by the early and rapid accumulation of phenolics at the infection site resulting in the effective isolation of the pathogen. These physical responses may include the elaboration of cell wall thickenings and appositions such as papillae, as well as the occlusion of plant vessels.

The concentration of a particular phenolic compound within a plant tissue is dependent on season and may also vary at different stages of growth and development (Lynn and Chang, 1990; Thomas and Ravindra, 1999; Ozyigit *et al.*, 2007). Several internal and external factors, including trauma, wounding, drought and pathogen attack affect the synthesis and accumulation of phenolics. Furthermore, the biosynthesis of phenolics in chloroplasts and their accumulation in vacuoles are enhanced on exposure to light (Kefeli *et al.*, 2003). Photoinhibition and nutrient stresses such as deficiencies in nitrogen, phosphate, potassium, sulphur, magnesium, boron and iron, also trigger the synthesis of phenyl propanoid compounds in some plant species (Dixon and Paiva, 1995; Hollman and Katan, 1999; Balasundram *et al.*, 2006). Studies have also shown that in rice, induction of salt stress resulted in increased synthesis of phenolics and other antioxidants. The potential of induction of antioxidant production through induction of salt stress in rice has also been reported (Daiponmak *et al.*, 2010; Umnajkitikorn *et al.*, 2013). Under the above circumstances, the present study has been carried out to assess the variation in the accumulation of phenolics in some native rice cultivars of North Kerala, India in response to induction of salinity stress. Incremental salinity stress has been applied mimicking the pattern of variation in the salinity levels of the salinity prone rice habitats of the study area.

## **MATERIALS AND METHODS**

**Plant materials and planting method:** The experiment was conducted in the experimental rainout poly house of Department of Botany, University of Calicut, Kerala, India located at 11°35' N latitude and 75°48'E longitude in the first crop season of 2013. Seven native cultivars of rice including five cultivars collected from one of the saline rice habitats of Kerala namely *Orthadian*, *Orkazhama*, *Kuthiru*, *Kuttusan* and *Chovvarian* and two native rice cultivars collected

Table 1: Salinity treatment details

Sl. No.	Treatments
T1	Control
T2	10 mM (0.91 dS m <sup>-1</sup> ) on 45th day
T3	10 mM (0.91 dS m <sup>-1</sup> ) on 45th day and 30 mM (2.74 dS m <sup>-1</sup> ) on 53rd day
T4	10 mM (0.91 dS m <sup>-1</sup> ) on 45th day, 30 mM (2.74 dS m <sup>-1</sup> ) on 53rd day and 50 mM (4.57 dS m <sup>-1</sup> ) on 61st day
T5	10 mM (0.91 dS m <sup>-1</sup> ) on 45th day, 30 mM (2.74 dS m <sup>-1</sup> ) on 53rd day, 50 mM (4.57 dS m <sup>-1</sup> ) on 61st day and 70 mM (6.39 dS m <sup>-1</sup> ) on 69th day
T6	10 mM (0.91 dS m <sup>-1</sup> ) on 45th day, 30 mM (2.74 dS m <sup>-1</sup> ) on 53rd day, 50 mM (4.57 dS m <sup>-1</sup> ) on 61st day and 70 mM (6.39 dS m <sup>-1</sup> ) on 69th day and 100 mM (9.13 dS m <sup>-1</sup> ) on 77th day
T7	10 mM (0.91 dS m <sup>-1</sup> ) on 45th day, 30 mM (2.74 dS m <sup>-1</sup> ) on 53rd day, 50 mM (4.57 dS m <sup>-1</sup> ) on 61st day and 70 mM (6.39 dS m <sup>-1</sup> ) on 69th day, 100 mM (9.13 dS m <sup>-1</sup> ) on 77th day and 200 mM (18.26 dS m <sup>-1</sup> ) on 85th day

from one of the non-saline rice habitats of Kerala namely *Kunhutti* and *Veliyan* were used for the study. Healthy mature seeds from single plant each were collected and washed in running tap water to remove infected and unfilled grains and dust particles. The seeds were soaked in distilled water and allowed to germinate in 10 cm diameter Petri dishes covered with lid under room temperature. The water was changed every day. The seeds started to germinate from the third day.

On the 10th day, required numbers of the germinated seedlings were transferred to coloured plastic pots of 25 cm diameter filled with paddy soil mixed with enriched compost in 3:1 ratio. Two seedlings were initially planted per pot and after the establishment of the seedlings the smaller among the two were removed. The plants were maintained in the experimental poly house under wetland conditions, always maintaining 3 cm of water above the soil level. The soil was fertilized with 1 g N: P: K = 18: 18: 18 per pot at fortnightly intervals starting from the 30th day. Weeding was done manually whenever required. Plants were grown in Randomized Block Design with three replications.

**Experimental treatments:** The experimental treatment was started from the 45th day of germination onwards starting from 10 mM (0.91 dS m<sup>-1</sup>) to 200 mM (18.26 dS m<sup>-1</sup>) aqueous solution of sodium chloride as detailed in Table 1.

**Determination of total phenolic content:** The total phenolic content of leaf was determined using the Folin-Ciocalteu reagent (Singleton and Rossi Jr., 1965; Osawa and Namiki, 1981; Gaxiola *et al.*, 2001). Leaf samples (0.1 g) were weighed separately (Sartorius, Germany), homogenized in 80% aqueous ethanol (Merck, India) at room temperature using a clean mortar and pestle, centrifuged in refrigerated centrifuge (Sigma, Germany) at 4°C at a speed of 10,000×g for 20 min and the supernatant was saved. The residue was re-extracted twice with 80% ethanol and supernatants were pooled, put into evaporating dishes and evaporated to dryness at room temperature. Residue was re-dissolved in 5 mL of distilled water. Hundred microliter of this extract was diluted to 3 mL with water and 0.5 mL of freshly prepared Folin-Ciocalteu reagent (Merck, India) was added. After 3 min 2 mL of 20% of sodium carbonate (w/v) (Himedia, India) was added and the contents were mixed thoroughly. The colour was developed and absorbance measured at 650 nm in a spectrometer (Thermo Scientific, USA) after 60 min using catechol as standard. The results were expressed as mg catechol/g of fresh weight of the material. The reaction was conducted in triplicate and results were averaged.

## RESULTS AND DISCUSSION

From the results obtained, it is clear that total phenol content got gradually increased with progressing salt stress conditions. The data revealed that *Orkazhama*, a salinity tolerant rice

Table 2: Variation in total phenolic content in the different rice cultivars studied (mg catechol g<sup>-1</sup> fresh weight)

Treatments (mM)	Mean±SE	CD at 5%	Percentage of increase
<b>Orthadian</b>			
0 (control)	0.240±0.08	0.059	0.00
10	0.297±0.10		23.75
30	0.300±0.03*		25.00
50	0.412±0.10*		71.67
70	0.498±0.07*		107.50
100	0.578±0.04*		140.83
200	0.648±0.05*		170.00
<b>Chovvarian</b>			
0 (control)	0.242±0.07	0.053	0.00
10	0.262±0.05		8.26
30	0.297±0.10*		22.73
50	0.328±0.07*		35.54
70	0.408±0.06*		68.60
100	0.592±0.07*		144.63
200	0.662±0.04*		173.55
<b>Kuttusan</b>			
0 (control)	0.255±0.04	0.043	0.00
10	0.322±0.07*		26.27
30	0.335±0.05*		31.37
50	0.353±0.04*		38.43
70	0.400±0.02*		56.86
100	0.525±0.09*		105.88
200	0.567±0.04*		122.35
<b>Kuthiru</b>			
0 (control)	0.360±0.08	0.054	0.00
10	0.385±0.06		6.94
30	0.442±0.08*		22.78
50	0.453±0.02*		25.83
70	0.475±0.03*		31.94
100	0.582±0.10*		61.67
200	0.668±0.06*		85.56
<b>Orkazhama</b>			
0 (control)	0.208±0.07	0.042	0.00
10	0.240±0.08		15.38
30	0.312±0.06*		50.00
50	0.398±0.02*		91.35
70	0.457±0.03*		119.71
100	0.548±0.03*		163.46
200	0.615±0.04*		195.67
<b>Kunhutty</b>			
0 (control)	0.197±0.04	0.047	0.00
10	0.235±0.06		19.29
30	0.313±0.06*		58.88
50	0.348±0.04*		76.65
70	0.405±0.04*		105.58
100	0.493±0.04*		150.25
200	0.533±0.09*		170.56
<b>Veliyan</b>			
0 (control)	0.225±0.04	0.051	0.00
10	0.243±0.04		8.00
30	0.287±0.07*		27.56
50	0.333±0.08*		48.00
70	0.430±0.07*		91.11
100	0.512±0.07*		127.56
200	0.550±0.07*		144.44

\*Significant variation from the control at 5%

cultivar from the saline rice tracts of North Kerala showed the highest percentage of increase in total phenolic content (195.67%) (Table 2 and Fig. 1-2). *Kuthiru* showed the lowest percentage of

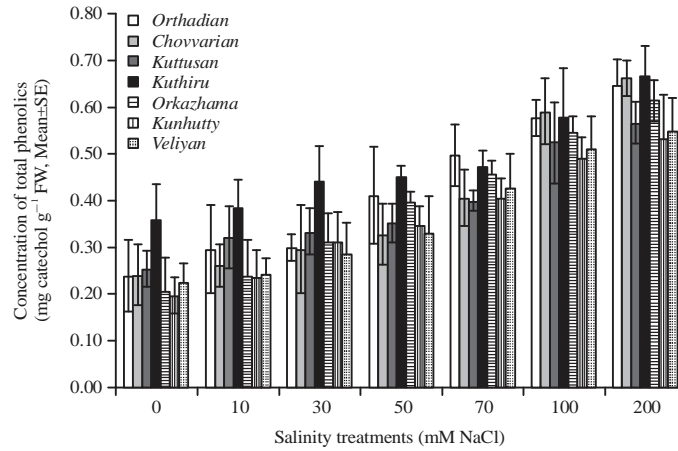


Fig. 1: Graph showing the variation in total phenolics in different rice cultivars studied under different levels of salinity treatments

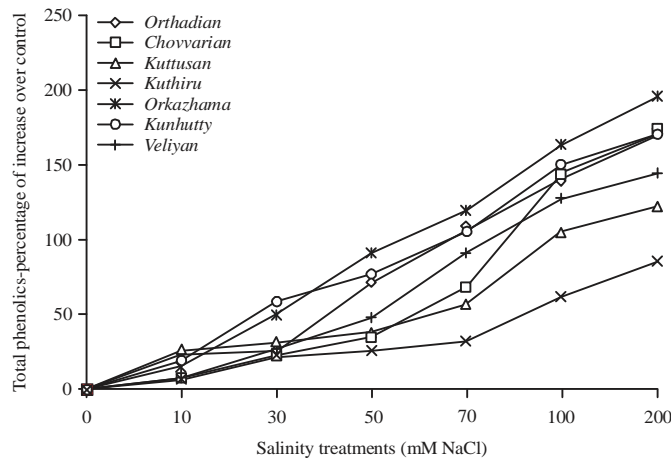


Fig. 2: Graph showing the percentage of increase in the total phenolic content in different rice cultivars under salinity stress

increase in total phenolic content over the control plants (85.56%). But the highest phenolic content was observed in *Kuthiru* followed by *Chovvarian* and *Orthadian*, the cultivars collected from the traditional saline rice tract. Phenolic content of all the cultivars increased progressively in relation to increase in salt stress and in all the cultivars the highest phenolic content was produced under the highest salt stress induced. All the varieties showed the highest phenolic content under the highest salt stress applied (200 mM NaCl). Among the cultivars studied, *Kuttusan* showed significant increase in phenolic content over the control starting from 10 mM salt treatment itself, while all the others showed significant variation from 30 mM salt concentration onwards.

A wide array of environmental stresses seriously affect plant growth and crop yield and among that salt stress is one of the major abiotic factor that limit rice productivity. High salt content increases osmotic stress (Mohanty *et al.*, 2002) and leads to the accumulation of excess sodium in plants (Niu *et al.*, 1995; Zhu, 2001). In response to environmental stresses, plants accumulate more soluble osmotic molecules such as proline, betaine and soluble sugar as osmoprotectants

(Zhang *et al.*, 2004). They accumulate phenolics and other osmoprotectants also (Daiponmak *et al.*, 2010; Umnajkitikorn *et al.*, 2013). Here, we observed greater accumulation of phenolic compounds in the leaf tissue of all the rice cultivars studied under salt stress. This study provides a clear idea on the accumulation of phenolic compounds due to salinity stress in the rice cultivars that are cultivated in the saline as well as non-saline rice tracts of North Kerala. The cultivars collected from both the saline and non-saline rice tracts showed gradual increase in the accumulation of total phenolics in response to increase in salt stress. The cultivars showed differential variation in the accumulation of total phenolics in relation to salt stress showing that the character was cultivar specific. Among the cultivars studied, *Kuthiru* showed the highest quantity of phenolic compounds and *Orkazhama* showed the highest percentage of increase of phenolics over the control plants in relation to increase in salt stress.

Besides ionic and osmotic effects which are the primary stresses of salinity, high salt concentrations additionally induce secondary stresses such as oxidative stress due to the excessive generation of ROS in plants (Zhu, 2001; Vaidyanathan *et al.*, 2003; Kaniuga, 2008; Xu *et al.*, 2011). Under various stresses, ROS production increased quickly in chloroplasts, resulting in damage to the structure of plant cells (Gechev *et al.*, 2006). The ROS are very small molecules and they are highly reactive due to the presence of unpaired valence shell electrons. When plants are exposed to abiotic stresses, their ROS levels can increase dramatically, leading to lipid peroxidation, DNA damage, denaturation of proteins and metabolic disorders. The ROS degrade polyunsaturated lipids and subsequently generate MDA which causes toxic stress in cells by forming covalent protein adducts. Furthermore, ROS triggered by various stresses also affect membrane integrity and cell compartmentation in plants. To prevent oxidative damage, plants process both enzymatic mechanisms such as APX and CAT and non-enzymatic mechanisms such as phenolic compounds for ROS scavenging (Mittler, 2002; Vaidyanathan *et al.*, 2003; Gill and Tuteja, 2010). The present result is consistent with Chutipaijit *et al.* (2009), Daiponmak *et al.* (2010) and Ghosh *et al.* (2011), who found that total phenolic content increased in rice seedlings under salinity stress.

The genetic variations among crop plants are useful in providing a valuable resource in the selection of cultivars with desirable traits (Misra and Dwivedi, 2004). Anil *et al.* (2005) emphasized the importance of identifying the traits that impart salt tolerance to rice lines and advocated that it would have significant agronomic consequences. The results presented make it clear that total phenol content was gradually increased with progressing salt stress in all the native rice cultivars studied with much higher magnitude of increase in phenol contents in them. Moreover, salt stress tolerant cultivars can be further screened for the production of higher quantity of phenolics in response to salt stress with an objective of improving the antioxidant production potential of such cultivars.

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