Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 5, Issue, 9, pp.1602-1611,September, 2014

International Journal of Recent Scientific Research

ISSN: 0976-3031

RESEARCH ARTICLE

REDUCTION OF MAJOR PHOTOSYNTHETIC PIGMENTS UNDER SALINITY STRESS IN SOME NATIVE RICE CULTIVARS OF NORTH KERALA, INDIA

[1]Abhilash Joseph, E, [2]Radhakrishnan, VV, [2]Chandramohanan, KT and [1&2]*Mohanan, KV

[1]Interuniversity Centre for Plant Biotechnology, Department of Botany, University of Calicut, Kerala India
[2]Genetics and Plant Breeding Division, Department of Botany, University of Calicut, Kerala India

ARTICLE INFO

Article History:

Received 12th, August, 2014
Received in revised form 20th, August, 2014
Accepted 15th, September, 2014
Published online 28th, September, 2014

Key words:

Abiotic stress; carotenoids; leaf chlorophyll; Oryza sativa; salt tolerance.

ABSTRACT

Salt stress as a major adverse factor can lower leaf water potential, interrupting the metabolic processes of plants, leading to reduced turgor and some other responses, and ultimately lower crop productivity. Leaf chlorophyll content, a good indicator of photosynthetic activity is of special significance to precision agriculture. Chlorophyll is an essential element of photosynthesis and its content in plant leaves indicates their photosynthetic capacity as well as the presence of stress or diseases. The purpose of this work was to estimate chlorophyll and total carotenoid contents in different rice cultivars collected from the rice tracts of North Kerala, India. Results showed that Chlorophyll a, Chlorophyll b, total chlorophyll and total carotenoid contents got significantly reduced among the rice cultivars under different salinity conditions (0, 10, 30, 50, 70, 100 and 200mM NaCl). Five rice cultivars viz; Orthadian, Chovvarian, Kuttusan, Kuthiru and Orkazhama collected from a saline rice tract and two cultivars Kunhutty and Veliyan collected from a traditional non saline rice tract were used for the experiment. Both the groups exhibited significant reduction in chlorophyll content and carotenoid content under salt stress and the reduction was in proportion with the increase in salt content in the growth medium. Chlorophyll b showed higher percentage of reduction when compared to chlorophyll a content and total chlorophyll content.

INTRODUCTION

Rice (*Oryza sativa* L.) is a main staple crop around the world, feeding millions of people and providing the necessary daily calories to more than half the world's population (Khush, 1997; Khush, 2005). It is one of the most widely grown crops in coastal areas inundated with sea water during high tidal period; although it is usually considered moderately susceptible to salinity (Akbar and Yabuno, 1972; Korbe and Abdel-Aal, 1974; Mori and Kinoshita, 1987; Hong *et al*, 2007), it is salt sensitive (Maas and Hoffman, 1977). Salinity of soil and water resources is the most basic and oldest environmental problems that it can limit crop production in many parts of the world and is considered as serious danger for agriculture (Abrol*et al*, 1988; Munns, 2002, Netondo *et al*, 2004; Haq *et al*, 2010). It has been predicted that the demand for rice in the world will increase to 780 million tons by the year 2020 (Shabbir *et al*, 2001). Major environmental limitations on rice production are salinity and drought (Toenissen, 1995). According to reports, about 900 million hectares of lands are affected by salinity in the world that is nearly 20% of the world's cultivated area and about half of the world's total irrigated lands (Munns, 2002; FAO, 2007). Soil salinity is a complex effect causing disturbance to membrane integrity, nutrient imbalance and disturbances on general metabolic activities. Accumulation of salts in the soils of arid and semi-arid regions is a continuing threat to crop production. A possible alternative is the introduction of crop species/cultivars capable of tolerating higher soil salinities with moderate economic yield (Yeo and Flowers, 1986). Two types of plant responses to salinity have been distinguished: pre-existing resistance mechanisms and adaptation or acquired tolerance (Amzallag *et al*, 1990). Response of plants to any stress agent is particularly of adaptive nature when the stress is sublethal. On the other hand, response shown may be biased towards cell death if the stress is lethal (Grover *et al*, 2001). Salinity can limit plant growth and yield by reducing osmotic potential, ion toxicity creation, uptake disarrangement and ion imbalance and can cause disorders in enzyme activities and membrane and metabolic activities in plants (Marschner, 1986; Gorham, 1993; Hasegawa *et al*, 2000; Basu *et al*, 2002; Murphy *et al*, 2003; Islam *et al*, 2008). These processes could affect morphological parameters and plant growth and will reduce vegetative growth (Linghe and Shannon, 2000; Sairam and Tyagi, 2004; Rogers *et al*, 2009), active leaf area, chlorophyll content (Netondo *et al*, 2004, Cha-um *et al*, 2007, Saleh and Maftoun, 2008) and chlorosis is a common morphological and physiological characteristic in response to salt stress (Harinasut *et al*, 2000). Chlorophyll content of salt stressed rice can be described as a function of the leaf sodium content (Yeo and Flowers, 1983). The response of plants to excess NaCl is complex and involves changes in their morphology, physiology and metabolism (Hilal *et al*, 1998;Djanaguiraman *et al*, 2003;Rahman*et al*, 2008) and consequently reducing plant dry weight (Zeng and Shannon, 2000;Pesqueira*et al*, 2003; Razzaque*et al*, 2009; Rogers *et al*, 2009) and dry matter production (Mansour and Salama, 2007) and ultimately crop yield (Shannon *et al*, 1998; Zeng and

* Corresponding author: **Mohanan, KV**
Inter university Centre for Plant Biotechnology, Department of Botany, University of Calicut, Kerala India
Genetics and Plant Breeding Division, Department of Botany, University of Calicut, Kerala India

Shannon, 2000; Sairam and Tyagi, 2004; Jamil *et al*, 2010; Osakabe *et al*, 2011).

Salinization is rapidly increasing on a global scale causing decline in average yield for most of the major crop plants (Bray *et al*, 2000). Due to a number of environmental factors the coastal soils are slightly to moderately saline on the surface and highly saline in sub-surface layers and substrata. Saline soil contains an excess of soluble salts, especially sodium chloride. In other words, soil salinity develops under the influence of the electrolytes of sodium salts, with a nearly neutral reaction. Salinity imposes both ionic and osmotic stresses on plants (Munns *et al*, 2006) and salt exclusion from photosynthetic tissues is considered an important mechanism associated with salt tolerance in monocots (Yeo *et al*, 1990;Moradi *et al*, 2003; Davenport *et al*, 2005). Soil salinity is one of the major constraints of arid and semiarid regions, where soluble salts are frequently high in the soil or in irrigation water. It adversely affects the growth of most agricultural crops through its influence on certain aspects of plant metabolism such as osmotic adjustment (Bernstein, 1963; Yokoi *et al*, 2002; Zeng*et al*, 2003), the uptake of certain essential nutrients (Greenway *et al*, 1996), photosynthesis (Downton, 1977) and enzyme activity (Weimberg, 1970) as well as causes hormonal imbalance (Shah and Loomis, 1965). Chlorophyll fluorescence is a rapid and non-intrusive tool used to screen varieties for salinity tolerance (Maxwell and Johnson, 2000, Ashraf 2010). Some toxic effects of salt stress include decreased germination and seedling growth (Heenan *et al*, 1988; Zeng and Shannon, 2000).

The effects of salt stress on rice are highly dependent on plant phenology: young seedlings and plants at the flowering stage appear to be the most sensitive while tillering plants are less sensitive (Sahu and Mishra, 1987;Lutts *et al*, 1995). Salinity applied at the seedling stage frequently induces premature senescence of leaves (Kura-Hotta *et al*, 1987; Yeo *et al*, 1991). Leaf senescence is most often quantified by decrease in protein or chlorophyll concentration (Dhindsa *et al*, 1981; Hashimoto *et al*, 1989; Chen and Kao, 1991; Chen *et al*, 1991) and by increase in membrane permeability (Björkman, 1987). Chlorophyll fluorescence kinetics, and more especially the ratio of the maximal variable fluorescence to the maximum level of chlorophyll fluorescence (Fv/Fm), which is directly related to PS II photochemical efficiency, may also be modified during ageing although the relationship between chlorophyll fluorescence and naturally occurring senescence processes is rarely considered (Bohra and Dörffling, 1993). In rice, it is found that NaCl salinity lowered the Fv/Fm chlorophyll fluorescence ratio after 47 day of stress and also at the flowering stage (Bohra *et al*, 1995; Pu and Gong, 2000).
Chlorophyll, the green pigment common to all photosynthetic cells, absorbs all wave lengths of visible light except green. All photosynthetic organisms have chlorophyll *a*. Chlorophyll *a* absorbs its energy from the violet-blue and reddish orange-red wavelengths, and little from the intermediate (green-yellow-orange) wavelengths. Due to chlorophyll absorption, the visible region of green plants shows a maximum reflectance at approximately 550 nm and lower reflectance in blue (450 nm) and red (680 nm) (Bohnert and Jensen, 1996).

Sugar may play key role in salt defence mechanisms, including membrane stability, *via* interaction with phospholipid head groups and reactive oxygen species detoxification (Bentsink*et al*, 2000; Smeekens, 2000; Roy *et al*, 2005). Moreover, soluble sugar, produced by photosynthesis in higher plants and a major energy source, also plays a critical role in signal transduction in primary and secondary metabolites, including the building blocks of macromolecules in the developmental processes of plants (Price *et al*, 2004; Yamane *et al*, 2008). However, photosynthetic pigment degradation, chloroplast destruction, chlorophyll fluorescence diminution and net photosynthetic rate reduction in salt stressed plants have been reported (Asch *et al*, 2000; Cha-um *et al*, 2004; Cha-um *et al*, 2009).

The effects of salinity on chlorophyll synthesis and integrity seem to vary with the level of salt stress (Santo, 2004; Rout *et al*, 1997). However, significant differences between genotypes were sometimes observed regarding the effects of salt stress on chlorophyll concentration in leaves (Sies and Stahl, 1995; Datta *et al*, 2009). Chlorophyll concentration usually is a good indicator of plant nutrient stress, photosynthesis and growing periods, the content of chlorophyll in the plant leaves indicates the growth status of the crops, also it is the important condition for exchange of mass and energy from the outside world and therefore real-time monitoring of the content of chlorophyll is a key step to complete crop monitoring and yield estimation (Canfield *et al*, 1993; Rao *et al*, 2007; Costache *et al*, 2012). The presence of pigments in plant tissues gives colour to leaf, vegetables and fruit, which is different depending on variety and species. Pigments are substances with very different chemical structure; they are present in the form of porphyrin pigments, carotenoids, anthocyanin and flavones. The main porphyrin pigments found in vegetables are chlorophyll *a*, *b* and *c*. Chlorophyll *a*, the main pigment in plants, converts light energy into chemical energy through photosynthetic process. The content of chlorophyll pigments varies by species (Richardson *et al*, 2002). Carotenoid pigments can be located in chromoplasts, contributing to the colour of vegetables and fruits, or in chloroplasts together with chlorophylls. Among carotenoid pigments in vegetables and fruits, *β*-carotene is the most popular and widespread.

In photosynthesis, antenna pigments in leaf chloroplasts absorb solar radiation, and through resonance transfer the resulting excitation is channelled to the reaction centre pigments, which release electrons and set in motion the photochemical process. The chlorophylls, Chl *a* and Chl *b*, are the most important of these pigments, and are thus virtually essential for the oxygenic conversion of light energy to the stored chemical energy that powers the biosphere (Groff *et al*, 1995). Carotenoids constitute a family of pigmented lipophilic compounds that are widely distributed in biological systems. They are synthesized by plants and microorganisms but not animals. They are yellow to red pigments and include non-polar hydrocarbons, carotenes (*α*- carotene, *β*-carotene, lycopene) and their oxygenated derivatives, xanthophylls (Peto*et al*, 1981). They are highly physiologically important and protect plants and microorganisms against excessive irradiation. Also, some carotenoids possess provitamin A activity. Carotenoids interact with reactive oxygen species and thus act as free radical quenchers, singlet oxygen scavengers and lipid antioxidants.

The present experiment was carried out so as to study the effect of salt stress on the variation in chlorophyll content and total carotenoid content in native rice cultivars collected from

traditional saline and non saline rice tracts of Kerala State of India. Progressive salinity stress starting from 45th day of planting was applied in this case so as to mimic the situation in the saline fields of the geographical area where the fields are almost free from salinity in the early stages of plant development thanks to high rainfall from south-west monsoon and salinity increases as the monsoon recedes.

MATERIALS AND METHODS

Germination of seeds and transfer to the experiment site

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 (*Oryzasativa*), including five cultivars namely *Orthadian, Orkazhama, Kuthiru, Kuttusan* and *Chovvarian* collected from one of the saline rice tracts of Kerala and two native rice cultivars namely *Kunhutty* and *Veliyan* collected from one of the non-saline rice tracts of Kerala were used for the study. Enough numbers of healthy mature caryopses from a single plant were used. The seeds were washed in running tap water to remove infected, unfilled grains and dust particles, soaked in distilled water and allowed to germinate in 10cm diameter Petri dishes (Borosil) covered with lid under room temperature. The water was changed every day. The seeds started to germinate from the third day.

Plant materials and treatments

On the 10th day, required numbers of the germinated seedlings were transferred to coloured plastic pots of 25cm diameter filled with paddy soil mixed with enriched compost in 3:1 ratio. Two seedlings were initially planted per pot and after 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 3cm of water above the soil level. The soil was fertilized with 1g of 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 and observations

The experimental treatment was started from the 45th day onwards starting from 10mM (0.91dSm^{-1}) to 200mM (18.26 dSm^{-1}) aqueous solution of sodium chloride as detailed in Table 1.

Estimation of pigment composition of leaf

Estimation of the pigments was done according to the protocol advocated by Arnon (1949). Fresh leaves of control as well as experimental plants were collected for analysis on 90th day, washed with water and blotted between sheets of filter paper. To estimate chlorophyll and carotenoids, chilled 80% acetone was used as the extraction medium. Enough precautions were taken to avoid any exposure of the extract to light. 0.1g of fresh leaf sample was weighed in an electronic balance (Sartorius). It was then powdered using liquid nitrogen, crushed with the help of mortar and pestle in 20ml of 80% acetone (v/v) (Merck, India). Then the homogenate was centrifuged at 5,000 rpm for 10 minutes in a cooling centrifuge at 4°C (Sigma, Germany) and the supernatant was collected in a polypropylene tube (Tarsons, India). The residue was again washed with 80% acetone and centrifuged again. The process was repeated till the pellet became colour less.

Table 1 Salinity treatment details

Treatment No.	Treatment
T1	Control
T2	10mM (0.91dSm^{-1}) on 45th day
T3	10mM (0.91dSm^{-1}) on 45th day & 30mM (2.74 dSm^{-1}) on 53rd day
T4	10mM (0.91dSm^{-1}) on 45th day, 30mM (2.74 dSm^{-1}) on 53rd day & 50mM (4.57 dSm^{-1}) on 61st day
T5	10mM (0.91dSm^{-1}) on 45th day, 30mM (2.74 dSm^{-1}) on 53rd day, 50mM (4.57 dSm^{-1}) on 61st day & 70mM (6.39 dSm^{-1}) on 69th day
T6	10mM (0.91dSm^{-1}) on 45th day, 30mM (2.74 dSm^{-1}) on 53rd day, 50mM (4.57 dSm^{-1}) on 61st day, 70mM (6.39 dSm^{-1}) on 69th day & 100mM (9.13 dSm^{-1}) on 77th day
T7	10mM (0.91dSm^{-1}) on 45th day, 30mM (2.74 dSm^{-1}) on 53rd day, 50mM (4.57 dSm^{-1}) on 61st day, 70mM (6.39 dSm^{-1}) on 69th day, 100mM (9.13 dSm^{-1}) on 77th day & 200mM (18.26 dSm-1) on 85th day

The final volume of the pooled supernatant was noted. The absorbance was read at 663nm, 646nm, 750nm and 470nm against the solvent blank (80% acetone) using a UV visible spectrophotometer (Systronics, India). Then the amount of chlorophyll and carotenoids present in the extract was calculated using the following formulae adopted from Arnon (1949), Manuela *et al*, (2005), Molazem *et al*, (2010) and Khaleghi *et al*, (2012). The concentration of chlorophyll and carotenoids are expressed in mg/g fresh weight of the leaf tissue.

Chlorophyll a (mg/g) =

$$\frac{[12.7 \times (A663) - 2.69 \times (A645)] \times V}{(1000 \times W)}$$

Chlorophyll b (mg/g) =

$$\frac{[22.9 \times (A645) - 4.68 \times (A663)] \times V}{(1000 \times W)}$$

Total Chlorophyll (mg/g) =

$$\frac{[20.2 \times (A645) + 8.02 \times (A663)] \times V}{(1000 \times W)}$$

Carotenoids total (mg/g) =

$$\frac{[1000 \times (A470) + 3.27 \times (chl\ a - chl\ b)] \times V}{W \times (229) \times 1000}$$

Where, W is the fresh mass of the leaf sample taken and V is the total volume of the sample solution.

Statistical analysis

Differences between the values for control and salt stressed plants were analysed by one-way *ANOVA* taking $P< 0.05$ as significance level. Data are shown as mean ± standard error (*SE).*

RESULTS

The photosynthetic pigments Chl *a*, Chl *b* and carotenoids showed significant reduction in rice plants when subjected to incremental dozes of salinity treatments (Table 2, Figs. 1& 2).

Table 2 Details of variation in Chlorophyll a, Chlorophyll b, Total Chlorophyll, Chlorophyll a/ Chlorophyll b ratio and carotenoids as affected by salt stress in the rice cultivars studied

Treatments	Chlorophyll a			Chlorophyll b			Total Chlorophyll			a/b ratio			Carotenoids		
	Mean± SE (mg/g)	CD @5%	% of reduction over control	Mean± SE (mg/g)	CD @5%	% of reduction over control	Mean± SE (mg/g)	CD @5%	% of reduction over control	Mean± SE	CD @5%	% of increase over control	Mean± SE (mg/g)	CD @5%	% of reduction over control
							Orthadian								
Control	1.724±0.001		-	0.497±0.003		-	2.220±0.002		-	3.470±0.02		-	0.143±0.001		-
10mM	1.691±0.003*		1.95	0.469±0.004*		5.63	2.160±0.001*		2.7	3.606±0.03		3.92	0.130±0.000*		9.09
30mM	1.605±0.002*	0.016	6.90	0.407±0.004*	0.026	18.11	2.012±0.003*	0.019	9.37	3.953±0.05*	0.35	13.92	0.101±0.001*	0.005	29.37
50mM	1.602±0.002*		7.08	0.405±0.002*		18.51	2.007±0.003*		9.59	3.953±0.01*		13.92	0.087±0.001*		39.16
70mM	1.343±0.002*		22.10	0.336±0.004*		32.39	1.678±0.001*		24.41	4.003±0.05*		14.41	0.071±0.001*		50.35
100mM	1.290±0.003*		25.17	0.273±0.004*		45.07	1.563±0.002*		29.59	4.740±0.08*		36.60	0.062±0.001*		56.64
200mM	1.254±0.001*		27.26	0.263±0.002*		47.08	1.517±0.003*		31.67	4.763±0.03*		37.26	0.041±0.000*		71.33
							Chovvarian								
Control	2.036±0.002		-	0.437±0.005		-	2.457±0.003		-	4.663±0.05		-	0.170±0.000		-
10mM	2.034±0.003		0.10	0.429±0.006		1.83	2.447±0.003		0.41	4.763±0.07		2.14	0.156±0.001*		8.24
30mM	1.787±0.002*	0.015	12.23	0.418±0.004	0.030	4.35	2.181±0.006*	0.026	11.23	4.286±0.04	0.90	8.08	0.138±0.001*	0.005	18.82
50mM	1.769±0.001*		13.11	0.407±0.002*		6.86	2.181±0.001*		11.23	4.347±0.02		6.78	0.116±0.000*		31.76
70mM	1.672±0.002*		17.88	0.377±0.002*		13.73	2.050±0.000*		16.56	4.333±0.03		7.08	0.112±0.000*		34.12
100mM	1.553±0.001*		23.72	0.171±0.003*		60.87	1.723±0.003*		29.87	9.147±0.17*		96.16	0.083±0.000*		51.18
200mM	1.542±0.002*		24.26	0.159±0.003*		63.62	1.717±0.003*		30.12	9.757±0.22*		109.24	0.073±0.001*		57.06
							Kuttusan								
Control	1.959±0.003		-	0.706±0.004		-	2.665±0.002		-	2.777±0.02		-	0.112±0.000		-
10mM	1.797±0.002*		8.27	0.638±0.001*		9.63	2.434±0.003*		8.67	2.817±0.01		1.44	0.094±0.000*		16.07
30mM	1.782±0.003*		9.04	0.582±0.003*		17.56	2.363±0.004*		11.33	3.063±0.01		10.30	0.084±0.000*		25.00
50mM	1.775±0.006*	0.026	9.39	0.561±0.006*	0.036	20.54	2.335±0.006*	0.030	12.38	3.170±0.04	0.97	14.15	0.074±0.001*	0.004	33.93
70mM	1.664±0.001*		15.06	0.493±0.004*		30.17	2.157±0.005*		19.06	3.383±0.03		21.82	0.065±0.000*		41.96
100mM	1.496±0.003*		23.63	0.261±0.004*		63.03	1.756±0.001*		34.11	5.757±0.10*		107.31	0.054±0.001*		51.79
200mM	1.437±0.004*		26.65	0.190±0.007*		73.09	1.627±0.004*		38.95	7.707±0.30*		177.53	0.041±0.001*		63.39
							Kuthiru								
Control	2.221±0.004		-	0.471±0.005		-	2.691±0.001		-	4.730±0.06		-	0.110±0.001		-
10mM	2.066±0.001*		6.98	0.414±0.003*		12.10	2.480±0.004*		7.84	4.993±0.04		5.56	0.097±0.001*		11.82
30mM	2.059±0.007*		7.29	0.394±0.007*		16.35	2.452±0.004*		8.88	5.250±0.12*		10.99	0.084±0.001*		23.64
50mM	1.921±0.003*	0.028	13.51	0.377±0.003*	0.035	19.96	2.297±0.005*	0.031	14.64	5.107±0.04	0.52	7.97	0.082±0.000*	0.005	25.45
70mM	1.918±0.001*		13.64	0.371±0.004*		21.23	2.289±0.005*		14.94	5.180±0.06		9.51	0.074±0.000*		32.73
100mM	1.888±0.002*		14.99	0.355±0.003*		24.63	2.243±0.002*		16.65	5.327±0.05*		12.62	0.066±0.001*		40.00
200mM	1.718±0.002*		22.65	0.280±0.002*		40.55	1.998±0.003*		25.75	6.140±0.05*		29.81	0.062±0.001*		43.64
							Orkazhama								
Control	1.724±0.001		-	0.497±0.003		-	2.220±0.002		-	3.470±0.02		-	0.143±0.001		-
10mM	1.691±0.003*		1.91	0.469±0.004*		5.63	2.160±0.001*		2.70	3.607±0.03		3.95	0.126±0.001*		11.88
30mM	1.605±0.002*	0.016	6.90	0.407±0.004*	0.026	18.11	2.012±0.003*	0.019	9.37	3.953±0.05*	0.35	13.92	0.106±0.000*	0.004	25.87
50mM	1.602±0.002*		7.08	0.405±0.002*		18.51	2.007±0.003*		9.59	3.953±0.01*		13.92	0.088±0.000*		38.46
70mM	1.343±0.002*		22.10	0.336±0.004*		32.39	1.678±0.001*		24.41	4.003±0.05*		15.36	0.075±0.000*		47.55
100mM	1.290±0.003*		25.17	0.273±0.004*		45.07	1.563±0.002*		29.59	4.740±0.08*		36.60	0.071±0.000*		50.35
200mM	1.254±0.001*		27.26	0.263±0.002*		47.08	1.517±0.003*		31.67	4.763±0.03*		37.26	0.063±0.000*		55.94
							Kunhutty								
Control	2.184±0.010		-	0.751±0.007		-	2.935±0.006		-	2.910±0.04		-	0.117±0.000		-
10mM	2.025±0.002*		7.28	0.670±0.003*		10.79	2.695±0.001*		8.18	3.023±0.02		3.88	0.099±0.001*		15.38
30mM	1.891±0.002*		13.48	0.594±0.003*		20.91	2.485±0.003*		15.33	3.180±0.01*		9.28	0.081±0.000*		25.17
50mM	1.773±0.001*	0.033	18.82	0.533±0.001*	0.032	29.03	2.305±0.001*	0.028	21.47	3.327±0.01*	0.25	14.33	0.070±0.001*	0.004	40.17
70mM	1.664±0.001*		23.81	0.507±0.002*		32.49	2.170±0.001*		26.06	3.283±0.02*		12.82	0.064±0.001*		45.30
100mM	1.633±0.002*		25.23	0.411±0.003*		45.27	2.043±0.002*		30.39	3.980±0.03*		36.77	0.053±0.001*		54.70
200mM	1.562±0.002*		28.48	0.351±0.004*		53.26	1.912±0.006*		34.86	4.467±0.06*		53.51	0.042±0.000*		64.10
							Veliyan								
Control	1.940±0.002		-	0.724±0.007		-	2.664±0.005		-	2.683±0.03		-	0.087±0.000		-
10mM	1.782±0.003*		8.14	0.641±0.002*		11.46	2.422±0.005*		9.08	2.780±0.00		3.62	0.073±0.001*		16.09
30mM	1.748±0.002*		9.90	0.584±0.002*		19.34	2.332±0.001*		12.46	2.993±0.01		11.55	0.057±0.001*		34.48
50mM	1.745±0.002*	0.012	10.05	0.545±0.001*	0.03	24.72	2.289±0.004*	0.078	14.08	3.200±0.00*	0.51	19.27	0.034±0.001*	0.005	60.62
70mM	1.637±0.002*		15.62	0.444±0.005*		38.67	2.080±0.007*		21.92	3.693±0.03*		37.64	0.030±0.000*		65.52
100mM	1.516±0.003*		21.86	0.237±0.005*		67.27	1.752±0.003*		34.23	6.443±0.15*		140.14	0.019±0.001*		78.16
200mM	1.475±0.040*		23.97	0.197±0.004*		79.97	1.620±0.023*		39.19	7.413±0.06*		176.30	0.014±0.000*		83.91

*: Shows significant variation from the control

Among the cultivars collected from the saline rice tract, *Orthadian* and *Orkazhama* showed the highest reduction in Chl *a* (27.26%) followed by *Kuttusan* (26.65%) and *Kuthiru* showed the minimum reduction (22.65%) under the highest salt concentration when compared to the control plants. While considering the cultivars collected from the non saline rice tract, *Kunhutty* showed the highest reduction (28.48%) in Chl *a* content at the highest salinity level. In the case of Chl *b* content, *Kuttusan* showed the maximum percentage of reduction (73.09%) followed by *Chovvarian* (63.62%) among the cultivars collected from the saline tract. Minimum reduction was shown by *Kuthiru* (40.55%) at the highest salinity level.Among the cultivars collected from the non saline tract, *Veliyan* showed the maximum reduction in the case of Chl *b* (79.97%). In the case of Total Chl content, *Kuttusan* showed the maximum reduction (38.95%) among the cultivars collected from the saline tract and *Kuthiru* showed the minimum reduction (25.75%) at the highest salinity level. *Veliyan* showed the maximum reduction among the cultivars collected from the non saline rice tract (39.19%). Reduction in total carotenoid content among the cultivars collected from the saline rice tract was the highest in *Orthadian* (71.33%) followed by *Kuttusan* (63.39%) and *Chovvarian* (57.06%) and the minimum in *Kuthiru* (43.64%) at the highest salinity level. Among the cultivars collected from the non saline area, *Veliyan* showed the maximum reduction percentage (83.91%) of total carotenoids over control plants. Increase in Chl *a*/Chl *b* ratio has also been observed under salt stress in the study and this variation is also cultivar specific. This variation takes

place due to the differential variation in Chl *a* andChl *b* content.

collected from the saline tract and the non saline tract, progressive increase in salinity resulted in reduction of

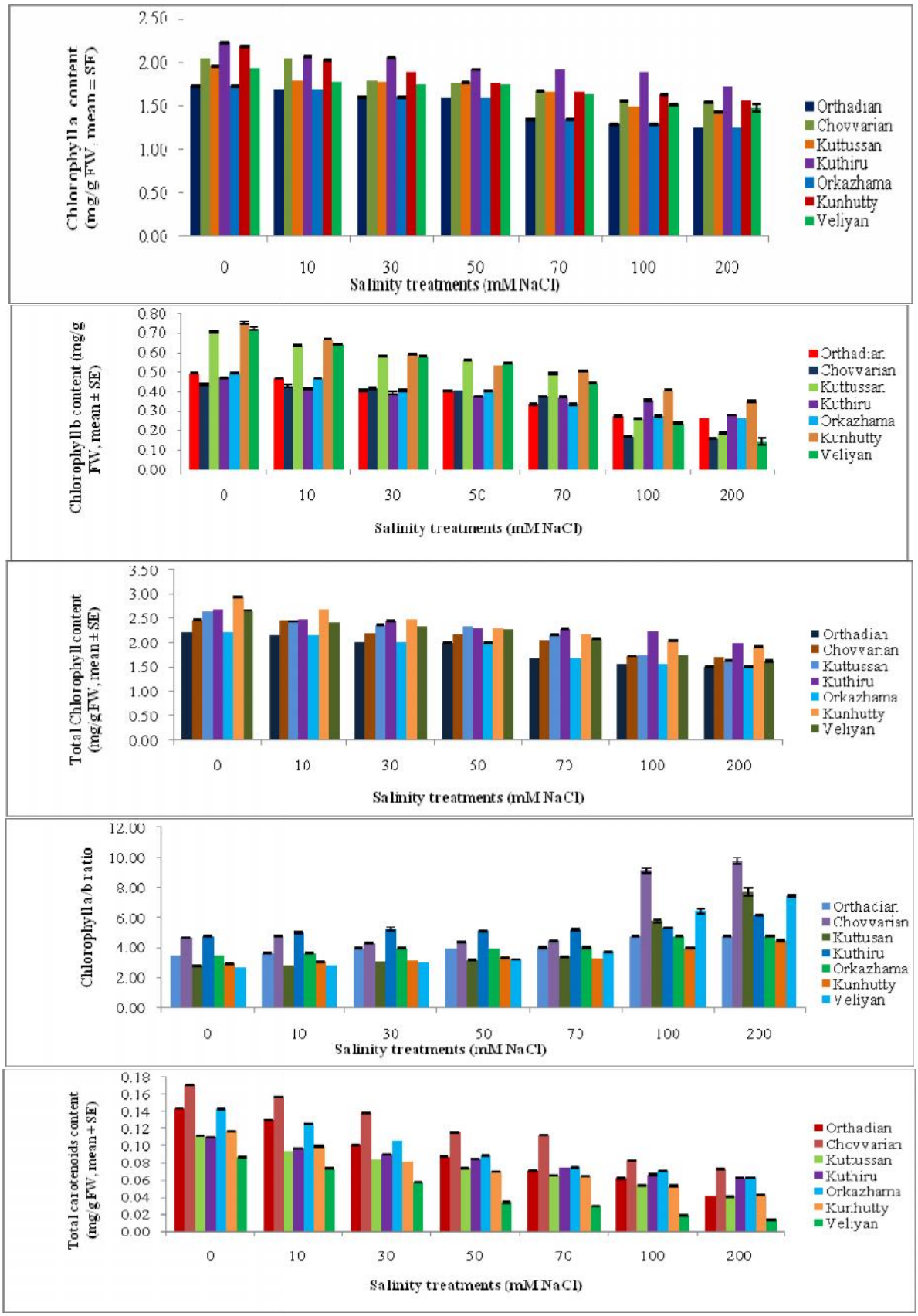

Fig. 1 Variation in Chlorophyll a, Chlorophyll b, Total chlorophyll, Chlorophyll a/ Chlorophyll b ratio and carotenoids as affected by salt stress in the rice cultivars studied

DISCUSSION

The present study shows that in the case of the rice cultivars

chlorophyll production. Among the cultivars studied, *Kuthiru* showed the minimum reduction in Chl *a* content (22.65%), Chl *b* content (40.55%) and Total Chl content (25.75%)

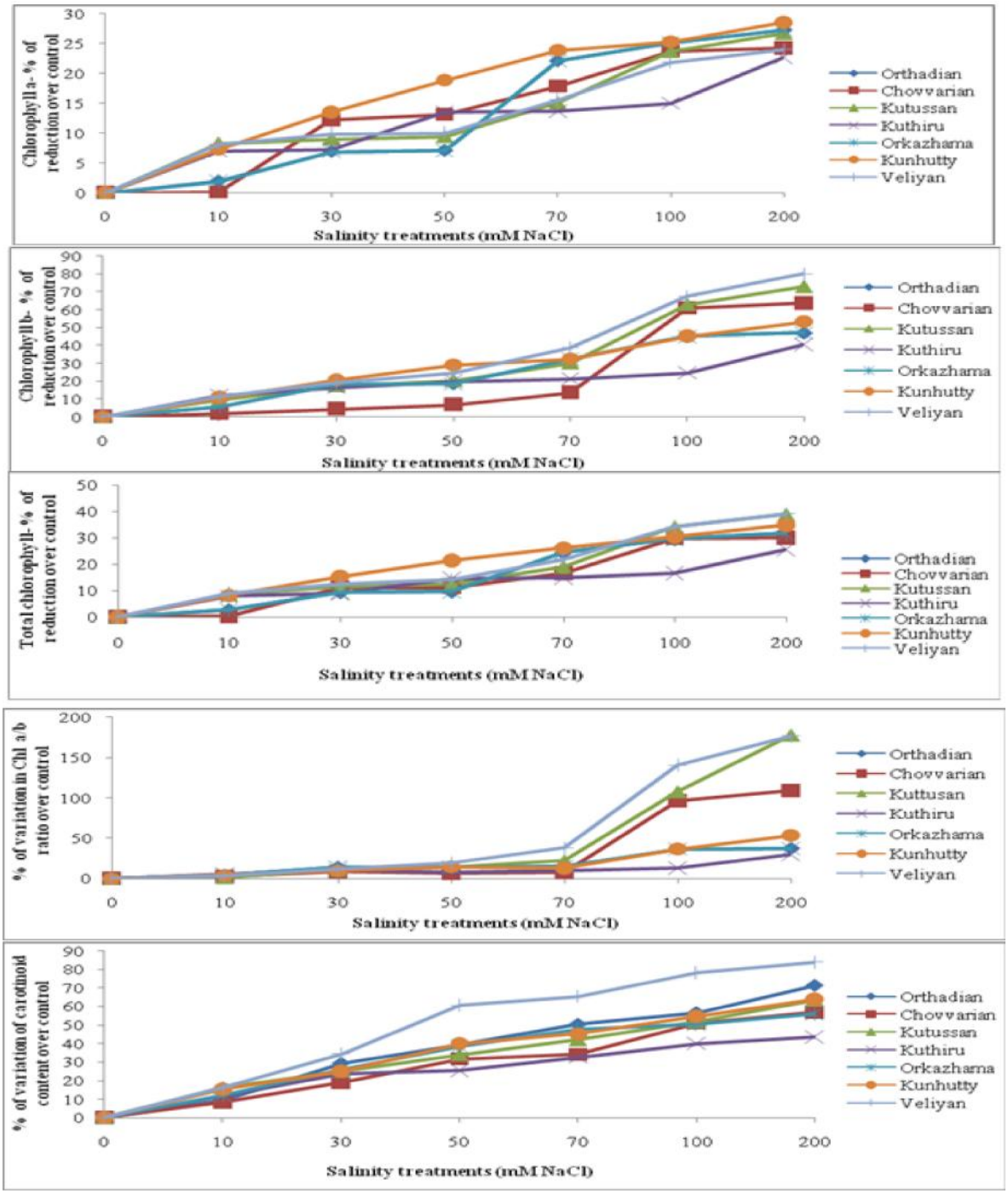

Fig. 2 Percentage of variation in Chlorophyll a, Chlorophyll b, Total chlorophyll, Chlorophyll a/ Chlorophyll b ratio and carotenoids as affected by salt stress in the rice cultivars studied

under salinity stress followed by *Chovvarian* and *Orkazhama*. The trend shown by Chl *a*/Chl *b* ratio indicated that Chl *b* content exhibited higher reduction due to salt stress when compared to reduction in Chl *a* content and Total Chl content. Moreover, the percentage of reduction was in proportion to relative increase in salinity.

Relative percentage of reduction was the highest in the case of Chl*b* content when compared to Chl *a* content and Total Chl content. However, the reduction in Total Chl content was the maximum among the cultivars collected from the non saline tract. Cultivars collected from the saline tract showed comparatively lesser reduction in chlorophyll content when compared to others. More over, the results showed that reduction in chlorophyll content and carotenoid content were cultivar specific. This difference in the variation in chlorophyll content manifests one major adaptation of salinity tolerant rice cultivars making them capable of growing under saline conditions. Earlier workers have reported that rice cultivars such as *Kuthiru*and *Orkazhama* performed comparatively well under conditions of moderate salt stress (Chandramohanan and Mohanan, 2012). Reports have established that salt stress causes reduction in leaf surface expansion ratio, leading to cessation of expansion as salt concentrations increase (Wang and Nil, 2000). Srivastava *et al*, (1988) have reported chlorophyll content as one of the parameters of salt tolerance in crop plants. In the present study, the salt treatments significantly decreased chlorophyll and carotenoid contents in the salt treated plants when

compared to control plants both in the case of the rice cultivars collected from the saline tract and the non saline tract. However, the reduction was higher in the case of the plants collected from the non saline tract.

High soil salinity is one of the important environmental factors that limit distribution and productivity of major crops. NaCl stress decreases chlorophyll content even at the lowest concentration (Santo, 2004). Rice is a major grain crop and carbohydrate source, supplying the necessary daily calories for more than half the world's population (Dubey and Singh, 1999; Khush, 2005). It has been predicted that the demand for rice in the world will increase to 780 million tons by the year 2020 (Shabbir *et al*, 2001). However, environmental stress is a serious issue confronting rice production, especially the problem of salinity (Yokoi *et al*, 2002; Zeng *et al*, 2003).

Chlorophyll is an important part of chlorophyll protein complexes on the thylakoid membranes. It is the key photosynthetic pigment and its content directly reflects the photosynthetic efficiency and assimilation capacity. As a result, chlorophyll content is an important index in determining salt stress level (Munns, 1993). Considering that Chl*a* is the main photosynthetic pigment (Daiz*et al*, 2002; Santo, 2004) reduction in its quantity could probably be one of the main reasons for reduced photosynthesis under salt stress (Moradi and Ismail, 2007). Significant differences in chlorophyll concentrations under salt stress have been observed between genotypes, with the tolerant genotypes having higher Chl *a*, but lower Chl *b*, resulting in substantially higher chlorophyll a/b ratio than the moderately tolerant genotypes. Ability of the tolerant genotypes to maintain higher concentration of Chl*a* is probably one of the important mechanisms contributing to salinity tolerance in this genotype, which could consequently result in higher photosynthetic capacity and carbohydrate formation (Moradi and Ismail, 2007; Rout *et al*, 1997, Datta *et al*, 2009). The structural integrity of chloroplasts is also affected by salt stress (Yang *et al*, 2008). Decrease in total chlorophyll content may occur due to ion accumulation and functional disorders observed during stoma opening and closing under salinity stress (Seemann and Critchley, 1985;Aranda and Syvertsen, 1996; Khalehi *et al*, 2012; Nawaz *et al*, 2010). Another reason for the decrease of chlorophyll content under salt conditions is stated to be the rapid maturing of leaves (Yeo *et al*, 1991). Decrease in chlorophyll content under salinity stress is observed more in salt sensitive genotypes in comparison to cultivars with low tolerance (Khan *et al*, 2009). The observed reduction of chlorophyll in water stressed plants may be due to a reduction in the lamellar content of the light harvesting chlorophyll a/b protein (Randall *et al*, 1977). The efficiency of light captured to drive photosynthesis is directly correlated to the chlorophyll concentration in the leaf (Netondo *et al*, 2004).

CONCLUSION

Significant reduction in the concentration of Chl *a*, Chl *b* Total Chl and total carotenoids has been caused by salt stress in the case of all the rice cultivars studied presently. Reduction in Chl *b* content is higher when compared to reduction in Chl *a* content and Total Chl content. Chl *a/b* ratio has also been altered. The rate of reduction is proportionate to the rate of increase in salt stress. Cultivars collected from saline rice tracts show comparatively lesser reduction in pigment content when compared to the cultivars collected from non saline rice tracts. This reduction presently observed may be probably due to the inhibitory effect of the accumulated ions on the biosynthesis of the different chlorophyll fractions. As the chloroplast is membrane bound its stability is dependent on membrane stability which under high salinity condition seldom remains intact. Salt tolerance is not a function of single organ or plant attribute, but it is the product of all the plant attributes. Therefore a genotype exhibiting relative salt tolerance for all the plant attributes may be the ideal one.

References

Abrol I.P., Yadav J.S.P. and Massoud I. 1988. Salt affected soils and their management. FAO Soils Bull. 39. FAO., Rome, p.131.

Akbar M. and Yabuno Y. 1972. Breeding for saline resistant varieties of rice- Variability for salt-tolerance among some rice varieties. Jpn J Breed, 22: 277-284.

Amzallag G.N., Lerner H.R. and Poljakoff Mayber A. 1990. Induction of increased salt tolerance in *Sorghum bicolor* by NaCl pre-treatment. J Exp Bot, 41: 29-34.

Aranda R.R. and Syvertsen J.P. 1996. The influence of foliar applied urea nitrogen and saline solutions on net gas exchange of citrus leaves. J Amer Soc Hort Sci, 121: 501-506.

Arnon D.I. 1949. Copper enzymes in isolated chloroplasts-Polyphenol oxidase in *Beta vulgaris*. Plant Physiol, 24(1): 1-15.

Asch F., Dingkuhn M. and Dorffling K. 2000. Salinity increases CO_2 assimilation but reduces growth in field grown, irrigated rice. Plant Soil, 218: 1-10.

Ashraf M. 2010. Inducing drought tolerance in plants: some recent advances. Biotechnol Adv, 28: 169-183.

Basu S., Gangopadhyay G. and Mukherjee B. 2002. Salt tolerance in rice in vitro: Implication of accumulation of Na^+, K^+ and proline. Plant Cell Tiss Org Cult, 69: 55-64.

Bentsink L., Alonso-Blanco C., Vreugdenhil D., Tesnier K.J.Y. and Groot S.P.C. 2000. Genetic analysis of seed soluble oligosaccharides in relation to seed storability of Arabidopsis. Plant Physiol, 124: 1595-1604.

Bernstein L. 1963. Osmotic adjustment of plant to saline media- II. Dynamic phase. Am J Bot, 48: 909-918.

Björkman O. 1987. Low temperature chlorophyll fluorescence in leaves and its relationship to photon yield of photosynthesis in photoinhibition. In: D.J. Kyle, C.B. Osmond and C.J. Arntzen (ed.): Photoinhibition. Elsevier Science Publishers, Amsterdam. pp.123-144.

Bohra J.S. and Dörffling K. 1993. Potassium nutrition of rice (*Oryza sativa* L.) varieties under NaCl salinity. Plant Soil, 152: 299-303.

Bohra J.S., Dörffling H. and Dörffling K. 1995. Salinity tolerance of rice (*Oryza sativa* L.) with reference to endogenous and exogenous abscisic acid. J Agron Crop Sci, 174: 79-86.

Bohnert H.J. and Jensen R.G., 1996. Strategies for engineering water stress tolerance in plants. Trends Plant Sci, 14: 89-97.

Bray E.A., Bailey-Serres J. and Weretilnyk E. 2000. Responses to abiotic stresses. In: W. Gruissem, B. Buchnnan and R. Jones (ed.), Biochemistry and Molecular

Biology of Plants. American Society of Plant Physiologists, Rockville, M.D, pp. 1158-1249.

Canfield L.M., Krinsky N.I. and Olson J.A. 1993. Carotenoids in human health. - In: Annals of New York Academy of Sciences, 691. The New York Academy of Sciences, New York, NY, USA.

Chandramohanan K.T. and Mohanan K.V. 2012. Kaipad rice farming in North Kerala- An indigenous saline resistant organic farming system. Indian J Tradi Know, 11(1): 185-189.

Cha-um S., Supaibulwatana K. and Kirdmanee C. 2007. Glycinebetaine accumulation, physiological characterization and growth efficiency in salt-tolerant and salt-sensitive lines of indica rice (*Oryza sativa* L. ssp. *indica*) in response to salt stress. J Agron Crop Sci, 193: 157-166.

Cha-um S., Charoenpanich A., Roytrakul S. and Kirdmanee C. 2009. Sugar accumulation, photosynthesis and growth of two indica rice varieties in response to salt stress. Acta Physiol Plant, 31: 477-486.

Cha-um S., Trakulyingcharoen T., Smitamana P. and Kirdmanee C. 2004. Salt tolerance in two rice cultivars differing in salt tolerant abilities in response to iso-osmotic stress. Aust J Crop Sci, 3: 221-230.

Chen C.T. and Kao C.H. 1991. Senescence of rice leaves- XXIX. Ethylene production, polyamine level and polyamine biosynthetic enzyme activity during senescence. Plant Science, 78: 193-198.

Chen C.T., Li C.C. and Kao C.H. 1991. Senescence of rice leaves- XXXI. Changes of chlorophyll, protein and polyamine contents and ethylene production during senescence of a chlorophyll deficient mutant. J Plant Growth Regul, 10: 201-205.

Costache M.A., Campeanu G. and Neata G. 2012. Studies concerning the extraction of chlorophyll and total carotenoids from vegetables. Rom Biotech Lett, 17(5): 7702-7708.

Daiz M.G.Q., Bebing N.N., Villegas M.J. and Manuel M.C. 2002. Cell Biology, Laboratory Manual 5thEdn, Genetics and Molecular Biology Division, The University of the Philippines, Philippines, p. 47.

Datta J.K., Nag S., Banerjee A. and Mondal N.K. 2009. Impact of salt stress on five varieties of wheat (*Triticum aestivum* L.) cultivars under laboratory condition. J Appl Sci Environ Manage, 13(3): 93-97.

Davenport R., James R.A., Plogander Z.A., Tester M. and Munns R. 2005. Control of sodium transport in durum wheat. Plant Physiol, 137: 807-818.

Dhindsa R.S., Plumb-Dhindsa P. and Thorpe T.A. 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. J Exp Bot, 32: 93-101.

Djanaguiraman M., Senthil A. and Ramadass R. 2003. Assessment of rice genotypes for salinity tolerance at germination and seedling stage. Madras Agric J, 90(7-9): 506-510.

Downton W.J.S. 1977. Photosynthesis in salt stressed grape vines. Aus J Plant Physiol, 4: 183-192.

Dubey R.S. and Singh A.K. 1999. Salinity induces accumulation of soluble sugars and alters the activity of sugar metabolising enzymes in rice plants. Biol Plant, 42: 233-239.

FAO, 2007. FAO Land and Plant Nutrition Management Service. http://www.fao.org/ag/agl/agll/spush

Groff J.L., Gropper S.S. and Hunt S.M. 1995. The fat soluble vitamins. In: Advanced Nutrition and Human Metabolism. West Publishing Co., New York, NY, USA, pp 284-312.

Gorham J. 1993. Genetics and physiology of enhanced K/Na discrimination. - In: P.J. Randall, E. Delhaize and R.A. Richards. (eds.): Genetic Aspects of Plant Mineral Nutrition. Kaluwer Academic Publishers, Netherlands. pp. 151-159.

Greenway H., Gunn A. and Thomas D.A. 1966. Plant response to saline substrate. Regulation of ion concentration in salt sensitive and halophytic species. Aust J Biol Sci, 19: 741-756.

Grover A., Kapoor A., SatyaLakshmi O., Agarwal S., Sahi C., Katiyar-Agarwal S., Agarwal M. and Dubey H. 2001. Understanding molecular alphabets of the plant abiotic stress response. Curr Sci, 80: 206-216.

Haq I., Dahri A.M., Dahot M.U., Parveen N., Ghaffar A. and Laghari A.L. 2010. Growth responses of NaCl stressed rice (*Oryza sativa* L.) plants germinated from seed in aseptic nutrient cultures supplemented with proline. African J Biotech, 9(39): 6534- 6538.

Harinasut P., Srisunak S., Pitukchaisopol S. and Charoensataporn R. 2000. Mechanisms of adaptation to increasing salinity of mulberry: proline content and ascorbate peroxidase activity in leaves of multiple shoots. Sci Asia, 26: 207-211.

Hashimoto H., Kura-Hotta M. and Katoh S. 1989. Changes in protein content and in the structure and number of chloroplasts during leaf senescence in rice seedlings. Plant Cell Physiol, 30: 707-715.

Hasegawa P.M., Bressan R.A., Zhu J.K. and Bohnert H.J. 2000. Plant cellular and molecular responses to high salinity. Annual Rev Plant Phys, 51: 463-499.

Heenan D.P., Lewin L.G. and McCaffery D.W. 1988. Salinity tolerance in rice varieties at different growth stages. Aust J Exp Agr, 28: 343-349.

Hilal M., Zenoff A.M., Ponessa G., Moreno H. and Massa E.M. 1998. Saline stress alters the temporal patterns of xylem differentiation and alternative oxidative expression in developing soybean roots. Plant Physiol., 11: 695-701, 1998.

Hong C.Y., Hsu Y.T., TsaiY.C. and Kao, C.H. 2007. Expression of ascorbate peroxidase in roots of rice (*Oryza sativa* L.) seedlings in response to NaCl. J Exp Bot, 58: 3273-3283.

Islam M.S., Hur J.H. and Wang M.H. 2008.The influence of abiotic stresses on expression of zinc finger protein gene in rice. Russ J Pl P, 56: 695-701.

Jamil M., Iqbal W., Bangash A., Rehman S.U., Imran Q.M. and Rha E.S. 2010. Constitutive expression of OSC3H33, OSC3H50 and OSC3H37 genes in rice under salt stress. Pak J Bot, 42: 4003-4009.

Khalehi E, Arzani K., Moallemi N. and Barzegar M. 2012. Evaluation of chlorophyll content and chlorophyll fluorescence parameters and relationships between chlorophyll a, b and chlorophyll content index under water stress in *Olea europaea* cv. Dezful. World Academy of Science, Engineering and Technology, 68**:** 1154-1157.

Khan M.A., Shıraz M.U., Muhammad Al Khan. Mujtaba S.M., Islam E., Mumtaz S., Shereen A., Ansarı R.U. and Yasın Ashraf M. 2009. Role of proline, K/Na ratio and

chlorophyll content in salt tolerance of wheat (*Trıtıcum aestıvum* L.). Pak J Bot, 41(2): 633-638.

Khush G.S. 1997. Origin, dispersal, cultivation and variation of rice. Plant Mol Biol, 35: 25-34.

Khush G.S. 2005. What it will take to feed 5.0 billion rice consumers in 2030. Plant Mol Biol, 59: 1-6.

Korbe S.A. and Abdel-Aal R.M. 1974. Effect of total salinity and type of salts on rice crop. Agric. Res. Rev., 52: 73-78.

Kura-Hotta M., Satoh K. and Katoh S. 1987. Relationship between photosynthesis and chlorophyll content during leaf senescence of rice seedlings. Plant Cell Physiol, 28: 1321-1329.

Linghe Z. and Shannon C.M. 2000. Salinity effect on seedling growth and yield components of rice. Crop Sci, 40: 996-1003.

Lutts S., Kinet J.M. and Bouharmont J. 1995.Changes in plant response to NaCl during development of rice (*Oryzasativa* L.) varieties differing in salinity resistance. J Exp Bot, 46: 1843-1852.

Maas E.V. and Hoffman G.J. 1977. Crop salt tolerance: Current assessment. J Irrig Drain Div Amer Soc Civ Eng,103: 115-132.

Mansour M.M. and Salama K.H. 2004. Cellular basis of salinity tolerance in plants. Environ Exp Bot, 52: 113-122.

Manuela A.C., Gheorghe C. and Gabriela N. 2012. Studies concerning the extraction of chlorophyll and total carotenoids from vegetables. Rom Biotech Lett, 17(5): 7702-7708.

Marschner H. 1986. Mineral Nutrition of Higher Plants, II Edition. Academic Press, London. p. 674.

Maxwell K. and Johnson G.N. 2000. Chlorophyll fluorescence: a practical guide. J Exp Bot, 51: 659–668.

Molazem D., Qurbanov E.M. and Dunyamaliyev S.A. 2010. Role of proline, Na and chlorophyll content in salt tolerance of corn (*Zea mays* L.). American-Eurasian J Agric & Environ Sci, 9(3): 319-324.

Moradi F. and Ismail A.M. 2007. Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. Ann Bot, 99: 1161-1173.

Moradi F., Ismail A.M., Gregorio G.B. and Egdane J.A. 2003. Salinity tolerance of rice during reproductive development and association with tolerance at the seedling stage. Indian J Plant Physi, 8: 276-287.

Mori I.K. and Kinoshita T. 1987. Salt tolerance of rice callus clones. Rice Genet Newsl, 4: 112-113.

Munns R. 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant Cell Environ, 16: 15-24.

Munns R. 2002. Comparative physiology of salt and water stress. Plant Cell Environ., 25: 239-250.

Munns R., James R.A. and Läuchli, A. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. J Exp Bot, 57: 1025–1043.

Murphy L.R., Kinsey S.T. and Durako M.J. 2003.Physiological effects of short-term salinity changes on *Ruppia maritima*. Aquat Bot, 75: 293- 309.

Nawaz K., Talat A.I., Hussain K. and Majeed A. 2010. Induction of salt tolerance in two cultivars of sorghum (*Sorghum bicolor* L.) by exogenous application of proline at seedling stage. World Applied Sciences Journal, 10: 93-99.

Netondo G.W., Onyango J.C. and Beck, E. 2004. Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. Crop Sci Society Am, 44: 806-811.

Osakabe Y., Kajita S. and Osakabe K. 2011. Genetic engineering of woody plants: current and future targets in a stressful environment. Physiol Plant, 142: 105-117.

Pesqueira J., Garcia M.D. and Molina M.C. 2003. NaCl tolerance in maize (*Zea mays* ssp. Mays) x *Tripsacum dactyloides* L. hybrid calli and regenerated plants. Span J Agric Res, 1(2): 59-63.

Peto R., Doll R., Buckley J.D. and Sporn M.B. 1981. Can dietary beta-carotene materially reduce human cancer rates? Nature, 290: 201-208.

Price J., Laxmi A., Martin S.K. and Jang J.C. 2004. Global transcription profiling reveals multiple sugar signal transduction mechanisms in Arabidopsis. Plant Cell, 16: 2128-2150.

Pu R.L. and Gong P. 2000. Hyperspectral remote sensing and its applications. Higher Education Press, Beijing. p. 254.

Rahman M., Soomro U.A., Haq M.Z. and Gul, S. 2008. Effects of NaCl salinity on wheat (*Triticum aestivum* L.) cultivars. World J Agric Sci, 4(3): 398-403.

Randall S.A., Thornber P. and Fiscus E. 1977. Water stress effects on the content and organization of chlorophyll in mesophyll and bundle sheath chloroplasts of maize. Plant Physiol, 59: 351-353.

Rao L.G., Mackinnon E.S., Josse R.G., Murray T.M., Strauss A. and Rao A.V. 2007. Lycopene consumption decreases oxidative stress and bone resorption markers in postmenopausal women. Osteoporosis Int. 18(1): 109-15.

Razzaque M.A., Talukder N.M., Islam M.S., Bhadra A.K. and Dutta R.K. 2009. The effect of salinity on morphological characteristics of seven rice (*Oryza sativa* L) genotypes differing in salt tolerance. Pakistan J Biological Sci, 12(5): 406-412.

Richardson A.D., Duigan P.S. and Berlyn G.P. 2002. An evaluation of non-invasive methods to estimate foliar chlorophyll content. New Phytol, 153: 185-194.

Rogers M.E., Colmer T.D., Frost K., Henry D., Cornwall D., Hulm E., Hughes S., Nichols P.G.H. and Craig A.D. 2009. The influence of NaCl salinity and hypoxia on aspects of growth in *Trifolium* species. Crop Pasture Sci., 60: 71-82, 2009.

Rout N.P., Tripathi S.B. and Shaw B.P. 1997. Effect of salinity on chlorophyll and proline contents in three aquatic macrophytes. Biol Plant, 40: 453-458.

Roy P., Niyogi K., Sengupta D.N. and Ghosh B. 2005. Spermidine treatment to rice seedlings recovers salinity stress-induced damage of plasma membrane and PM-bound H^+-ATPase in salt-tolerant and salt-sensitive rice cultivars. Plant Sci, 168: 583-591.

Sahu A.C. and Mishra D. 1987. Changes in some enzyme activities during excised rice leaf senescence under NaCl stress. Biochemie und Physiologie der P¯anzen, 182: 501-505.

Sairam R.K. and Tyagi A. 2004. Physiology and molecular biology of salinity stress tolerance in plants. Curr Sci, 86: 407-421.

Saleh J. and Maftounm M. 2008. Interactive effect of NaCl levels and Zinc sources and levels on the growth and mineral composition of rice. J Agric Sci Techno, 10: 325-336.

Santo C.V. 2004. Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. Sci Hortic, 103: 93-99.

Seemann J.R. and Critchley C. 1985. Effects of salt stress on growth, ion content, stomatal behaviour and photosynthetic capacity of a salt sensitive species, *Phaseolus vulgaris* L. Planta, 164: 15-16.

Shabbir G., Hussain N., Bhatti M.K., Ahmad A., Javed M.A. and Shakir, M.A. 2001. Salt-tolerance potential of some selected fine rice cultivars. J Biol Sci, 1: 1175-1177.

Shah C.B. and Loomis R.S. 1965. Ribonucleic acid and protein metabolism in sugar beet during drought. Physiol Plant, 18: 240-254.

Shannon M.C., Rhodes J.D., Draper J.H., Scardaci S.C. and Spyres M.D. 1998. Assessment of salt tolerance in response to salinity problems in California. Crop Sci., 38: 394-398.

Sies H. and Stahl W. 1995. Vitamins E and C, β-carotene, and other carotenoids as antioxidants. Am J Clin Nutr, 62: 1315-1321.

Smeekens S. 2000. Sugar induced signal transduction in plants. Ann Rev Plant Physiol Plant Mol Biol,51: 49-81.

Srivastava T.P., Gupta S.C., Lal P., Muralia P.N. and Kumar A. 1988. Effect of salt stress on physiological and biochemical parameters of wheat. Ann Arid Zone, 27: 197-204.

Toenissen G.H. 1995. The Rockefeller Foundation's International Program on Rice Biotechnology. In: D.W. Altman, and K.N. Watanabe (ed.): Plant Biotechnology in Developing Countries. R.G. Landes Company, Austin. pp. 193-212.

Wang Y. and Nil N. 2000. Changes in chlorophyll, ribulosebiphosphate carboxylase- oxygenase, glycine betaine content photosynthesis and transpiration in *Amaranthus tricolour* leaves during salt stress. J Hortic Sci Biotech, 75: 623-627.

Weimberg R. 1970. Enzyme levels in pea seedlings grown on highly salinized media. Plant Physiol, 46: 466-470.

Yamane K., Kawasaki M., Taniguchi M. and Miyake H. 2008. Correlation between chloroplast ultrastructure and chlorophyll fluorescence characteristics in the leaves of rice (*Oryza sativa* L.) grown under salinity. Plant Prod Sci, 11: 139-145.

Yang C.W., Li C.Y., Yin H.J., Ju M. and Shi, D.C. 2008. pH and ion balance in wheat-wheatgrass under salt-or alkali stress. Chinese J Applied Ecol, 19(5): 1000-1005.

Yeo A.R. and Flowers T.J. 1983. Varietal differences of sodium ions in rice leaves. Physiol. Plant., 59: 189-195.

Yeo A.R. and Flowers, T.J. 1986. Salinity resistance in rice (*Oryza sativa* L.) and a pyramiding approach to breeding varieties for saline soils. Aust J Plant Physiol, 13: 161-173.

Yeo A.R., Lee K.S., Izard P., Boursier P.J. and Flowers T.J. 1991. Short and long-term effects of salinity on leaf growth in rice (*Oryza sativa* L.). J Exp Bot, 42: 881-889.

Yeo A.R., Yeo M.E., Flowers S.A. and Flowers T.J. 1990.Screening of rice (*Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance and their relationship to overall performance. Theor. Appl. Genet., 79: 377-384.

Yokoi S., Bressan R.A. and Hasegawa P.M. 2002. Salt stress tolerance of plants, JIRCAS Working Report. pp 25-33.

Zeng L. and Shannon M.C. 2000. Effects of salinity on grain and yield components of rice at different seeding densities. Agron J, 92: 418-423.

Zeng L., Lesch S.M. and Grieve C.M. 2003. Rice growth and yield respond to changes in water depth and salinity stress. Agric. Water Manage., 59: 67-75.

****