

Osmotic Adjustment: A Defence Mechanism in Four High-Yielding *Oryza Sativa* L. Genotypes to Salt Stress

Foluso Ologundudu¹, Otitolaju Kekere²

^{1,2} Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria

Abstract: Osmotic adjustment is an important and effective defence mechanism of salinity resistance in crop plants. The compatible osmolytes in leaf and root of IR20, POKKALI, IR29 and NERICA1 rice varieties were examined under NaCl and Na₂SO₄ solutions at 0 (control), 5, 10 and 15 ds/m. Leaf proline and lipid peroxidase as well as root protein contents increased with increasing salinity in all the cultivars with the highest values recorded at 15 ds/m except in NERICA1 where there was a significant reduction in leaf proline and lipid peroxidase under Na₂SO₄ treatment, and IR29 with a significant decrease compared to the control and other treatments under both salts. The highest increase in the parameters obtained at 15 ds/m was generally more under NaCl than Na₂SO₄ relative to their respective control treatments in all the cultivars. The highest percentage increase in leaf proline was best in IR20 and IR29 under NaCl and Na₂SO₄ respectively while lipid peroxidase was best in IR20 under both salts. Salinity increased root protein content significantly in plants treated with saline solution except in IR29. The percentage increase was also higher under NaCl than Na₂SO₄ in IR20 and NERICA1 while the reverse was the case in POKKALI. Root total phenol was significantly reduced by salinity in the plants except in IR20 under NaCl as well as in IR20 and POKKALI under Na₂SO₄ treatment. The reduction was highest at 15 ds/m, which was more under NaCl than Na₂SO₄. Root reducing and non-reducing sugars declined under salt treatment. The decrease in reducing sugar was more in POKKALI and IR29 under NaCl as well as in IR20 and NERICA1 under Na₂SO₄. The non-reducing sugar decrease was however more under Na₂SO₄ in the plants except IR20. IR20 and NERICA1 showed the highest reduction under NaCl and Na₂SO₄ respectively. The rice cultivars showed considerable changes in compatible osmolytes in response to salinity stress with variations depending on the cultivar and salt type.

Keywords: adaptation, compatible osmolyte, proline, rice cultivars, salinity.

1. INTRODUCTION

All plants are subjected to multitude of environmental stress factors throughout their life cycle, and the major one reducing plant productivity is salinity [1]. Soil salinization is a serious problem in the entire world and it has grown substantially causing loss in crop productivity [2]. It is a major constraint limiting agricultural productivity on nearly 20% of the cultivated and irrigated areas worldwide [3]. Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. High exogenous salt concentrations affect seed germination, water deficit, ion imbalance of the cellular ions resulting in ion toxicity and osmotic stress [4]. Salt stress has been reported to cause an inhibition of growth and development, reduction in photosynthesis, respiration and protein synthesis in sensitive species [5], [6]. The most important process that is affected in plants growing under saline conditions is photosynthesis. Reduced photosynthesis under salinity is not only attributed to stomata closure leading to a reduction in intercellular CO₂

concentration, but also to non-stomata factors. There is strong evidence that salt affects photosynthetic enzymes, chlorophylls and carotenoids [7]. Salt also affects photosynthetic parameters, including osmotic and leaf water potential, transpiration rate, leaf temperature, and relative leaf water content [8].

Accumulation of carbohydrates enhances the plant stress tolerance [9], and might be a useful character to select salt-tolerance genotypes. The accumulation of soluble carbohydrates depends on the type of plant and genotype. Some researchers reported that tolerant genotypes have the ability to accumulate more soluble carbohydrates [9], [10], whereas some others reported the different response of soluble carbohydrate accumulation in different tomato cultivars [11].

Proline is known to play an important role in osmotic adjustment in salt stressed plants [12]. Osmotic adjustment gained considerable attention as a significant and effective mechanism of salinity resistance in crop plants. For example, studying the mechanism of salt tolerance in salt tolerant grass species, a positive relationship of growth with osmotic adjustment was found, which occurred mainly due to the accumulation of potassium, glycinebetaine and reducing sugars under saline conditions [12]. In wheat, a salt tolerant cultivar Giza 164, was found to have higher osmotic adjustment as compared to a salt-sensitive cultivar Sakha 69 [13]. In the same crop, similar result was recorded while comparing two cultivars differing in salt tolerance [14]. In contrast to all these reports, there are some reports in which there is little or no correlation between osmotic adjustment and salt tolerance. For example, while comparing different classes of plants with regard to their degree of salt tolerance, any correlation between the amount of osmotic adjustment, leaf area, and yield reduction in plants sensitive to salinity could not be found [15]. In view of these contrasting reports on the relationship of osmotic adjustment and plant growth under saline stress, it is not yet clear that osmotic adjustment is a useful physiological mechanism of salt tolerance. Tolerance to abiotic stresses is very complex at the whole plant and cellular levels [16]. This is due to the complexity of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development [17].

2. MATERIALS AND METHODS

2.1 Experimental plants:

Seeds of four rice varieties; IR20, IR29, NERICA1 and POKKALLI were utilized in the experiment. They were collected from the African Rice Centre, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

2.2 Source of soil:

The soil used was river sand and it was collected behind the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The soil has a high porosity and leaching rate, thus, excess salt accumulation could be avoided by flooding with water and allowed to drain. Soil samples were dried, passed through a 2-mm sieve, and analyzed for the physico-chemical parameters. Particle distribution was determined using the rapid method, pH was measured in 1:1 soil: water suspension, Nitrogen was determined by the modified Kjeldahl method while phosphorus was assayed by Bray's P1 solution and read on a spectrophotometer. Cations were extracted with 1.0 M ammonium acetate solution at pH 7.0; sodium and potassium contents in the extract were determined by flame photometry while calcium and magnesium were measured by atomic absorption spectrophotometry. Organic carbon was determined by the wet oxidation method while cation exchange capacity (CEC) was by ammonium distillation. All the procedures followed the standard method of the Association of Official Analytical Chemists [18] at the Department of Soil Science, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria.

2.3 Preparation of nutrient and salt solutions:

Nutrient solution was prepared according to the method of [19]. Stock solution was prepared by dissolving equimolar amounts of salts of nutrients in 1L distilled water. About 150ml of each stock solution was mixed and made up to 120 litres, which was used to irrigate the plants to provide adequate nutrient for growth. Pure sodium chloride (NaCl) and sodium sulphate (Na_2SO_4) salts obtained from the laboratory of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria were used to prepare solutions of NaCl and Na_2SO_4 salts each at the concentrations of 5, 10 and 15 ds/m by diluting the salt with water gradually until the desired concentrations were achieved using conductivity meter.

2.4 Experimental set up:

Five kilograms (5 kg) of air-dried soil was filled into perforated plastic pots (21 cm diameter and 17 cm depth) and arranged in the screen house of the Department of Botany, Obafemi Awolowo University, Ile Ife, Nigeria (Latitude-7°28'N, Longitude 4° 33'E and 272 m above sea level). Five seeds of rice were sown in each pot and watered regularly for germination to take place in March 2015. Plants were thinned to two seedlings per pot at 3 weeks after sowing. Seedlings were nurtured with the nutrient solution with each pot supplied with 200 ml every other day up to 14 days to allow for sufficient growth and adequate nutrient availability before salinization. Salinity treatments commenced at 5 weeks after sowing and each pot received 200 ml of saline solution every 3 days till the end of the experiment. The saline solutions were stored in plastic kegs and kept inside a refrigerator in which no change in concentration was noticed throughout the experimental period. Meanwhile, each pot was flooded with water and allowed to drain once per week to prevent salt accumulation beyond the desired salinity level in the soil. This was immediately followed by application of 200 ml nutrient solution to prevent nutrient deficiency. The treatments lasted for 3 months and plants were harvested in July 2015.

2.5 Experimental design:

It was a 4 x 4 x 2 factorial experiment with factors of the experiment including rice varieties (IR20, IR29, POKKALI and NERICA 1), salinity concentrations (0, 5, 10 and 15 ds/m) and salt types (NaCl and Na₂SO₄). There were 8 treatments for each variety (4 treatments each for NaCl and Na₂SO₄) with 5 replicates per treatment, which amounted to 160 pots laid out in a randomized complete block design (RCBD). The average temperature, relative humidity and light intensity in the screen house during the experimental period were 30°C, 40°F and 14000 lux respectively. There was no rain intrusion throughout the experimental period, only the salt solutions were used for irrigation.

2.6 Biochemical contents:

2.6.1 Sugar contents:

Sugar contents in rice roots were extracted by the modified method of [20]. Fifty-milligrams of rice roots were ground in liquid nitrogen with a pestle in a pre-cooled eppendorf tube. One milliliter nanopure water was added and then sonicated for 15 minutes. The aliquot was centrifuged at 12,000 rpm for 15 minutes. Supernatant was collected and filtered through a 0.45 µm millipore filter (VertiClean™; NYLON Syringe, Vertical Chromatography Co., Ltd., Thailand) and stored at -20°C prior to sugar content determinations.

2.6.2 Determination of Starch (non-reducing sugar):

Starch was estimated using anthrone reagent by following the method given by [21]. Fresh roots samples (250 mg each) were separately homogenized in hot 80% ethanol (v/v) to remove sugars. Residue was retained after centrifugation at 5000 x g for 15 min at room temperature. The starch was extracted by 52% perchloric acid at 0°C for 20 min. Starch was estimated by using anthrone reagent spectrophotometrically at 630 nm wavelength on UV-visible spectrophotometer (Chemito, UV-2600) and calculated from graph plotted using glucose as a standard.

2.6.3 Determination of Total Protein Content:

Proteins were estimated using the method of [22]. Fresh samples (250 mg) were homogenized in 2.5 ml of phosphate buffer (pH 7.0). The extract was centrifuged at 5000 g for 15 minutes at 4°C and the supernatant was transferred to a tube containing a mixture of 20 ml acetone and 14 ml β- Mercaptoethanol for precipitation of protein. The sample tubes were stored at 0°C for 5 hours and then centrifuged at 10000 rpm 20 minutes. The supernatant was discarded and the pellet was dissolved in 2.5 ml 1 N sodium hydroxide solution. Aliquot of 0.2 ml from this sample was used to prepare the reaction mixture. The intensity of blue color developed was recorded at 660 nm and protein concentration was measured using bovine serum albumin as standard.

2.6.4 Determination of Total Phenols:

Total phenol content was estimated following Malik and Singh [24] method. Total phenols were extracted from 500 mg of fresh roots and shoot tissues separately in 80% (v/v) ethanol and estimated by Folin-Ciocalteu reagent. The absorbance

of the reaction was measured at 650 nm wavelength on UV-visible spectrophotometer (Chemito, UV-2600). Total phenols were calculated by using standard graph of catechol.

2.6.5 Proline Content:

Proline content was determined according to the conventional method [15]. Leaf tissue (5 g) was homogenized in 3% sulfosalysilic acid and filtered. To 2 ml of filtrate, 2 ml of acid ninhydrin and 2 ml of glacial acetic acid were added and incubated for 1 hour in a boiling water bath followed by an ice bath. To this, 4 ml of toluene was added and mixed vigorously and the chromophore containing toluene was aspirated from the aqueous phase and the absorbance was measured at 520 nm. A standard curve was obtained using a known concentration of authentic proline.

2.6.6 Lipid Peroxidation:

Lipid peroxidation in rice leaf samples was determined in terms of malondialdehyde (MDA) content according to the standard method [25]. One (1) g of leaf tissue was homogenized by adding 0.5 ml, 1% (w/v) TCA. The homogenate was centrifuged for 10 minutes (15000 x g, 40°C) and 0.5 ml of the supernatant was later mixed with 1.5 ml, 0.5% TBA and was diluted in 20% TCA. The supernatant was incubated in water bath at 95°C for 25 minutes. The solution was later incubated on ice. Where there were cases of unclear solutions, it was centrifuged further for 5 minutes (15000 x g, 40 °C). The absorbance was measured at 532 and 600 nm. OD600 values were subtracted from the MDA-TBA complex values at 532 nm and MDA concentration was calculated using the Lambert-Beer law.

2.6.7 Statistical Analysis:

Data were subjected to single factor analysis of variance (One-Way ANOVA) and means were separated with Duncan Multiple Range (DMR) at 95% significant level using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).

3. RESULTS

The soil used for planting was a river sand with pH 5.9, %clay 6.8, %silt 4.0, %sand 89.2, 3.30g/kg C, 0.14% N, 10.4mg/kg AP, 272.5 mg/kg K, -260.5mg/kg H and 243.5mg/kg CEC, which has earlier been described [23]. The osmolyte concentrations as affected by salinity in the four rice cultivars are shown in Tables 1 and 2. Leaf proline and lipid peroxidase contents increased with increasing salinity in all the cultivars with the highest values recorded at 15 ds/m except in NERICA1 where there was a significant reduction under Na₂SO₄ treatment (Table 1). The highest increase in proline content obtained at 15 ds/m was generally higher under NaCl than in Na₂SO₄ relative to their respective control treatments (Fig. 1). The highest percentage increase in leaf proline content recorded at 15 ds/m NaCl and Na₂SO₄ was best in IR20 and IR29 respectively while it was lowest in NERICA1 and IR20 respectively (Fig. 1). Increase in leaf peroxidase was also generally higher under NaCl than in Na₂SO₄ except in IR29. However, the highest percentage increase values obtained at 15 ds/m was best in IR20 under both salts but lowest in IR29 and POKKALI under NaCl and Na₂SO₄ salts respectively (Fig. 2).

Salinity increased root protein content significantly in plants treated with saline solution except in IR29 where there was a significant decrease compared to the control and other treatments (Table 1). Relative to the control, the best percentage increase at 15 ds/m was higher under NaCl than Na₂SO₄ in IR20 and NERICA1 while the reverse was the case in POKKALI (Fig. 3). The highest percentage increase was obtained under NaCl and Na₂SO₄ in NERICA1 and IR29 respectively while the least values were recorded in POKKALI and IR20 under NaCl and Na₂SO₄ respectively (Figure 3). Root total phenol was significantly reduced by salinity in the plant except in IR20 under NaCl as well as in IR20 and POKKALI under Na₂SO₄ where there was a significant increase (Table 2). The highest percentage decrease relative to the control recorded at 15 ds/m was more under NaCl salt in the cultivars than under Na₂SO₄ (Fig. 4). Salinity reduced the concentrations of both root reducing sugars and non-reducing sugars with increasing concentration of salt (Table 2). Relative to the control, the highest reduction values recorded at 15 ds/m in root reducing sugar was more in POKKALI and IR29 under NaCl as well as in IR20 and NERICA1 under Na₂SO₄ (Fig. 5). In the case of non-reducing sugar however, the highest reduction as obtained at 15 ds/m was more under Na₂SO₄ in the cultivars except IR20. Meanwhile, IR20 and NERICA1 showed the highest reduction under NaCl and Na₂SO₄ respectively while the least decrease was obtained in NERICA1 and IR20 under NaCl and Na₂SO₄ respectively (Fig. 6).

TABLE.1: THE ROOT CONTENT OF SOME COMPATIBLE OSMOLYTES IN FOUR RICE CULTIVARS GROWN UNDER DIFFERENT CONCENTRATIONS OF NaCl AND Na₂SO₄ SALTS

NaCl (ds/m)	Leaf proline (mg/g fresh weight)				Leaf lipid peroxidation (mg/g fresh weight)				Root total protein content (mg/g fresh weight)			
	IR20	POK KALI	IR29	NERI CA1	IR20	POK KALI	IR29	NERI CA1	IR20	POK KALI	IR29	NERI CA1
0	65.06 ^c	71.72 ^c	80.2 ^c	87.8 ^c	23.4 ^c	51.2 ^b	78.0 ^c	100.0 ^d	483.3 ^a	403.6 ^a	438.1 ^a	419.0 ^a
5	74.36 ^b	75.0 ^c	87.3 ^b	95.9 ^b	28.4 ^c	51.2 ^b	82.3 ^b	145.0 ^c	463 ^a	364 ^b	422 ^a	418 ^a
10	80.95 ^b	89.9 ^b	99.1 ^a	106.6 ^a	37.9 ^b	60.7 ^{ab}	83.3 ^b	147.0 ^b	400 ^b	311 ^c	408 ^a	408 ^a
15	94.08 ^a	98.3 ^a	104.5 ^a	107.3 ^a	45.9 ^a	66.1 ^a	88.8 ^a	152.0 ^a	403 ^b	304 ^c	323 ^b	390 ^b
Mean	78.3	83.3	92.5	98.7	33.4	57.4	82.75	136.0	437.3	345.5	397.75	408.5
LSD	6.74	5.38	5.72	6.36	6.20	10.67	2.67	1.88	55.03	20.08	36.3	12.57
0	98.63 ^d	87.52 ^b	87.0 ^b	110.7 ^a	49.87 ^c	88.9 ^d	93.0 ^c	151.7 ^a	502 ^a	489.6 ^a	370 ^a	435 ^a
5	104.4 ^c	90.21 ^b	88.1 ^b	92.7 ^c	66.0 ^b	97.7 ^c	121.0 ^b	135.6 ^{bc}	465 ^b	439 ^b	339 ^b	414 ^a
10	108.5 ^b	98.3 ^a	101.1 ^a	96.0 ^b	71.0 ^b	102.1 ^b	129.0 ^b	142.3 ^b	424 ^c	422 ^c	328 ^b	391 ^b
15	112.3 ^a	100.5 ^a	105.1 ^a	103.1 ^b	78.4 ^a	110.2 ^a	138.0 ^a	128.6 ^c	364 ^d	380 ^d	318 ^c	353 ^c
Mean	105.98	94.15	95.7	100.7	66.31	99.75	120.64	139.65	438.91	433.0	339.2	398.5
LSD	1.19	3.75	8.89	5.53	7.24	3.58	8.60	8.87	22.09	13.28	20.17	37.45

Means with the same letter in the columns do not differ significantly ($P>0.05$). values in parenthesis indicate percent reduction of respective cultivars

TABLE.2: THE LEAF CONTENT OF SOME COMPATIBLE OSMOLYTES IN FOUR RICE CULTIVARS GROWN UNDER DIFFERENT CONCENTRATIONS OF NaCl AND Na₂SO₄ SALTS

NaCl (ds/m)	Root total phenols (mg/g fresh weight)				Relative Root reducing sugar (mg/g fresh weight)				Root non-reducing sugar (mg/g fresh weight)			
	IR20	POK KALI	IR29	NERI CA1	IR20	POK KALI	IR29	NERI CA1	IR20	POK KALI	IR29	NERI CA1
0	15.38 ^c	20.6 ^c	24.4 ^a	14.76 ^d	144. ^d	258.0 ^a	323.0 ^a	116.0 ^a	101 ^a	84.0 ^a	127 ^a	435 ^a
5	19.46 ^b	21.7 ^c	22.4 ^b	17.20 ^c	203. ^c	222.0 ^b	301.0 ^b	107.0 ^b	87 ^b	74 ^b	119 ^a	414 ^a
10	20.46 ^b	25.6 ^b	19.6 ^c	23.06 ^b	247. ^b	192.0 ^c	274.0 ^c	92.0 ^c	61 ^c	65 ^c	106 ^b	391 ^b
15	24.73 ^a	29.5 ^a	22.0 ^b	26.7 ^a	393. ^a	156.0 ^d	254.0 ^d	74.0 ^d	55 ^c	51 ^d	92 ^d	353 ^c
Mean	20.05	24.35	21.75	20.24	246.7	207	287.75	97.25	76.4	68.5	111.0	398.5
LSD	1.97	1.92	1.33	0.99	24.95	6.55	8.08	7.15	7.88	3.63	8.08	37.45
Na ₂ SO ₄ (ds/m)												
0	29.4 ^c	31.43 ^d	24.36 ^c	21.7 ^c	121.6 ^b	340.1 ^a	195.0 ^a	190.1 ^a	125.0 ^a	94.1 ^a	132.1 ^a	72.7 ^a
5	31.6 ^{bc}	36.2 ^c	30.3 ^b	24.7 ^b	125.0 ^b	306.1 ^b	179.1 ^{ab}	179.0 ^{ab}	124 ^a	63 ^b	111 ^b	57 ^b
10	34.3 ^b	41.17 ^b	31.5 ^b	29.0 ^a	152.0 ^a	275.0 ^c	167.1 ^{bc}	156.0 ^b	108 ^b	54 ^c	96 ^c	50 ^b
15	40.5 ^a	45.20 ^a	38.1 ^a	30.0 ^a	168.0 ^a	243.2 ^d	155.2 ^c	125.0 ^c	80 ^c	53 ^d	78 ^d	40 ^c
Mean	33.98	38.50	31.20	26.59	141.92	291.17	174.5	162.7	109.4	61.7	104.7	55.2
LSD	4.04	1.69	2.17	1.65	25.5	10.59	22.15	30.10	9.05	5.85	8.20	9.69

Values are mean of 5 replicates. Means with the same letter in the columns do not differ significantly ($P>0.05$) from each other at 95% level of significance (DMR test).

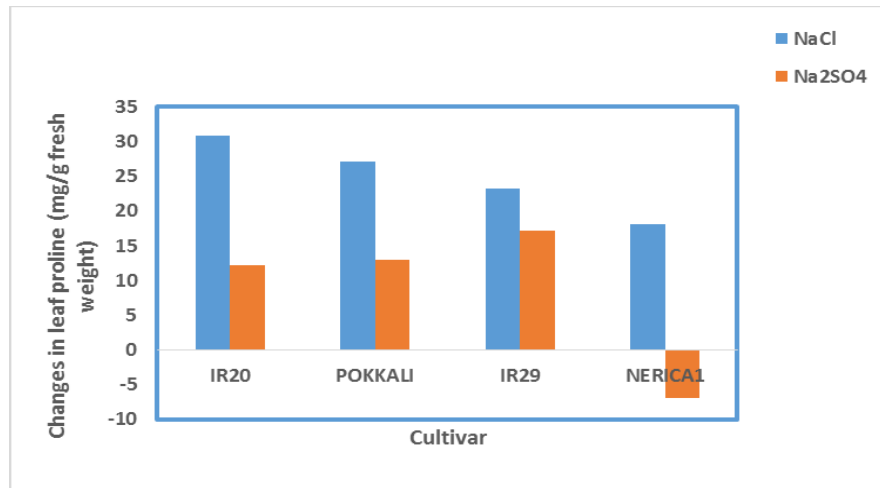


Fig.1: Changes in leaf proline content of four rice cultivars at 15 ds/m salinity (concentration at which the effect was highest) relative to the control

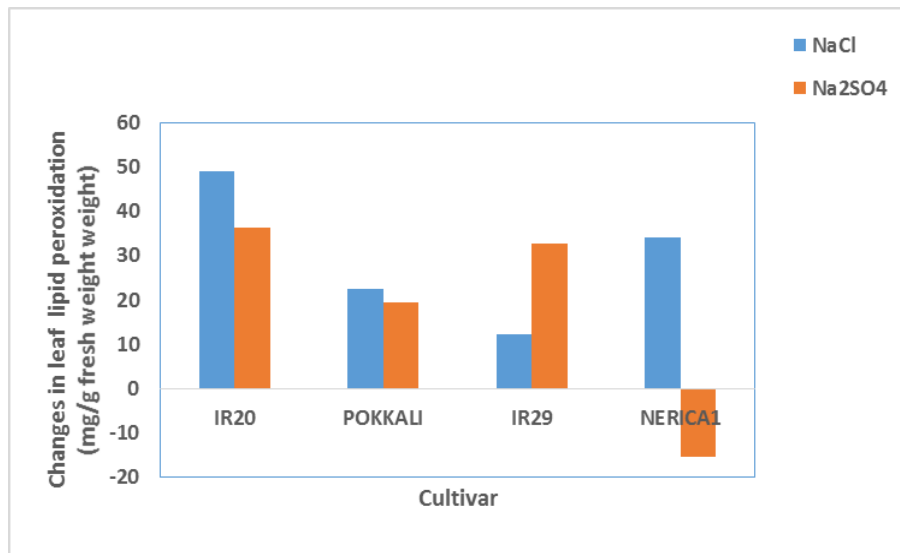


Fig.2: Changes in leaf lipid peroxidation of four rice cultivars at 15 ds/m salinity (concentration at which the effect was highest) relative to the control

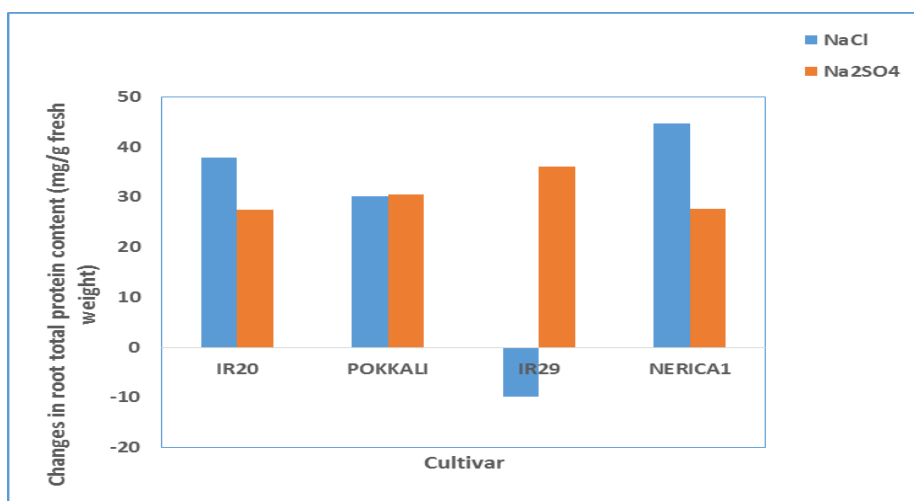


Fig. 3: Changes in root protein content of four rice cultivars at 15 ds/m salinity (concentration at which the effect was highest) relative to the control

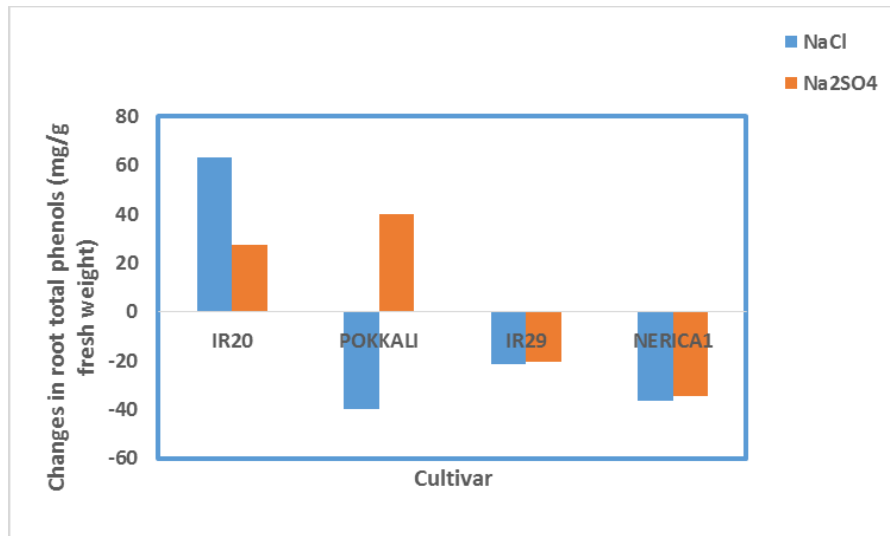


Fig.4: Changes in root total phenol content of four rice cultivars at 15 ds/m salinity (concentration at which the effect was highest) relative to the control

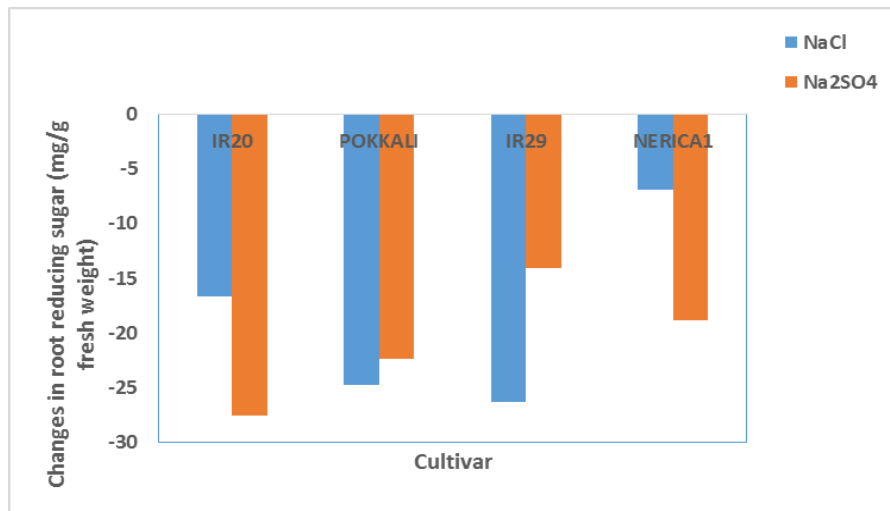


Fig.5: Changes in root reducing sugar content of four rice cultivars at 15 ds/m salinity (concentration at which the effect was highest) relative to the control

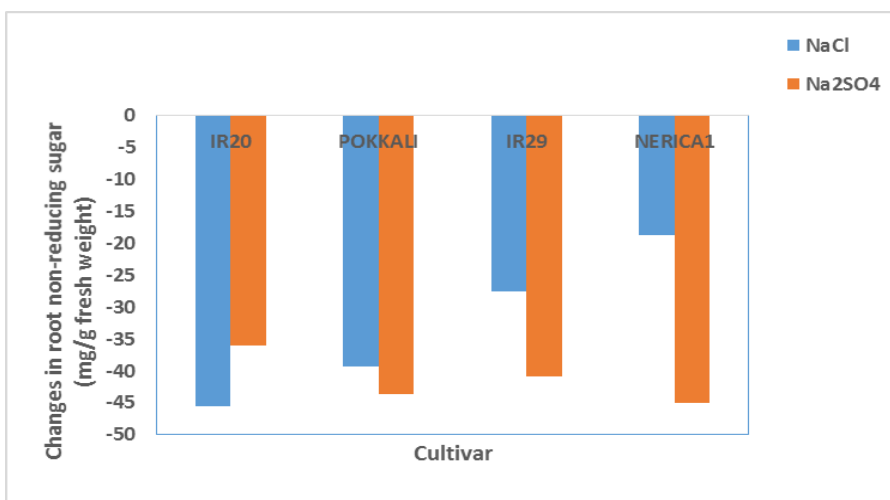


Fig.6: Changes in root non-reducing sugar content of four rice cultivars at 15 ds/m salinity (concentration at which the effect was highest) relative to the control

4. DISCUSSION

A positive correlation between magnitude of free proline accumulation and salt tolerance has been suggested as an index for determining salt tolerance potentials among cultivars [26]. The magnitude of increase in free proline accumulation was higher in IR20 and NERICA 1. However some researchers reported that proline accumulation cannot be used as a sole criterion for salt tolerance [27]. Rapid accumulation of Lipid peroxidation content and proline are typical response to salt stress [28]. However, these biochemical compounds alone may not determine the salt tolerance potentials in some plants. Rice cultivar, IR20 rapidly produced increased free proline and lipid peroxidation when subjected to salinity stress of 5 to 15 ds/m. In addition, it is the only rice cultivar that accumulated increased reducing sugar as salt stress increased under NaCl and Na₂SO₄ salts. These biochemical compounds including reducing sugar might have improved the osmotic adjustment and hence improved water efficiency, nutrient uptake and metabolic processes. When exposed to high salt content in soil, many plants have been observed to accumulate high amounts of lipid peroxidation and soluble sugars [29]. The above is in agreement with the result of these findings as the cultivars POKKALI and NERICA1 were observed to have considerably high accumulation of Lipid peroxidation and sugar content respectively at high salt concentration. Proline is a known osmo-protectant and plays an important role in osmotic balancing, protection of sub-cellular structures and increasing cellular osmolarity (turgor pressure) that provide the turgor necessary for cell expansion under stress conditions [30], [31]. The result of these findings demonstrates that sulphate stress (Na₂SO₄) can cause higher accumulation of proline than the chloride type. This could possibly indicate that induction of proline synthesis is related not only to variations in salinity concentration but also resulted from interruptions in metabolic activities by high stress intensity from specific salt or from an adaptive response with special physiological function. The higher accumulation of proline under salt stress is favourable to plants as proline participates in the osmotic potential of leaf and thus in the osmotic adjustment [32]. The apparent reduction in amylose content with increased salinity in the cultivars and the positive correlation with protein content is not in agreement with the report that a negative correlation was established between amylose and protein content in rice, and the amylose content was considered to be one of the most important traits related to the taste and cooking quality which determine the viscosity of cooked rice [33]. Protein accumulation under salt stress conditions depends on the genotypic nature of plants and cultivars. The degradation of protein content with salinity increase in IR20 and POKKALI might be the result of breakdown of protein molecules, which are used as substrate for biosynthesis of proline [34]. It was suggested that there is the possibility of using proteins as molecular marker to improve the salt stress [35]. Similar results were reported in tomato cultivars [36] and cucumber [7]. The content of soluble protein in leaves of *Lens culinaris* was decreased with salinity increase. A difference in phenol content of potato cultivars under salt stress was discovered [37]. Some cultivars showed decrease in phenol content, whereas other cultivars showed positive correlation with salt stressed conditions. The above findings are in conformity with the result of this study. Protein content decreased in sweet sorghum species in response to salinity stress [36]. A different response was reported in tomato cultivars in accumulation of soluble protein in Isfahani cv. but adversely decreasing in Shirazy cv. A decrease in protein content was also recognized in barley varieties under salt stress [38].

5. CONCLUSION

The study revealed that the rice cultivars showed considerable variations in the adjustment of compatible osmolytes in response to salinity. The decrease in carbohydrates by salt stress was due to a reduction in photosynthetic rate which disrupts the carbohydrate metabolism in leaves and might have led to the reduction in assimilate transported to the sink organs. The accumulation of proline and lipid peroxidation in the plant leaves as well as protein content in the root was for osmotic adjustment to salt stress and protection of sub-cellular structures. As such, the qualities and desirable traits of such cultivars can be used as templates for the development of other genotypes in future breeding programmes.

REFERENCES

- [1] P. B. Majeed and S. Inanaga, "Physiological response of common bean (*Phaseolus vulgaris* L.) seedlings to salinity stress". Afr. J Biotechnol. vol 6. pp. 079-088, 2010.
- [2] FAO, "World food and agriculture". IN: FAO Statistical Year book, pp.307, 2013.
- [3] L. Zheng, M. C. Shannon and S. M. Lesch. "Timing of salinity stress affecting rice growth and yield components". Agriculture and Water Management, vol.48, pp.191-206, 2001.

- [4] M. H. Khan and S. K. Panda, "Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl salinity stress". *Acta Physiology of Plant*, vol. 30, pp. 81-89, 2008.
- [5] A. Meloni, M.P. Reddy and R.J. Joly, R. "Leaf gas exchange and solute accumulation in the halophyte, *Salvadora persica* grown at moderate salinity". *Journal of Experimental Botany*, vol.44, pp.31-38, 2003.
- [6] H. Pal, K. Lu. "Salt stress magnitude can be quantified by integrating salinity with respect to duration". *Proceedings of 4th International Crop Sci Congress. Brisbane, Aust. 26th Sept-1st Oct 2004*, pp 1-5, 2004.
- [7] P. Stepien and G. Klobus, "Water relations and photosynthesis in *Cucumis sativus* L. leaves under salt stress". *Biologia Plantarum*. vol. 50, pp. 610-616, 2006.
- [8] M. Ashraf and P. J. Foolad, "Potential biochemical indicators of salinity tolerance in plants". *Plant Science*, vol. 166. pp. 3-16, 2007.
- [9] Z. Kerepesi and C. M. Shannon, "Salinity effects on seedling growth and yield components of rice (*Oryza sativa* L.)". *Australian Journal of Agricultural Research*, vol. 35, pp. 239-252, 2000.
- [10] G. S. Almodares, 2008. "Physiological basis of differential response to salinity in rice cultivars. *Plant Soil*". vol. 123. pp. 277-294, 2008.
- [11] M. Amini and R. D. Jini. "Biological and physiological perspectives of specificity in abiotic salt stress response from various rice plants". *Asian Journal of Agricultural Science*, vol. 2. pp. 99-105, 2005.
- [12] S. F. Alraham and W. K. Silk. "Effects of salinity on xylem structure and water use in growing leaves of sorghum. *New Phytologist*". vol. 146. pp. 119-127, 2005.
- [13] R. T. Tobita. "Salinity effects on seedling growth and yield components of rice". *Crop Science*. vol. 40: pp. 996-1003, 2000.
- [14] M. Tari and K. Postini, "Evaluation of thirty wheat cultivars in respect of response to salinity", *Iranian Journal of Agricultural Science*. Vol.33. pp. 57-64, 2002.
- [15] C. A. Gulmar and K. Fares. "Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five beet cultivars." *Environment and Experimental Botany*, vol. 47, pp. 39-50, 2000.
- [16] Sotiropoulou, "Leaf growth and K⁺/Na⁺ ratio as an indication of the salt tolerance of three sorghum cultivars grown under salinity stress", *Acta Agron Hungarica*, vol. 52, pp. 291-297, 2007.
- [17] J. K. Zhu. "Salt and drought stress signal transduction in plants". *Annual Review of Plant Biology*, vol.53,
- [18] Association of official analytical chemist (AOAC), Rockville, Maryland, USA, vol. 19, 2012
- [19] S. Yoshida, D. A. Forno and K. A. Gomez. *Laboratory manual for physiological studies of rice*, Philippines; IRRI; vol.83, 1976
- [20] H. J. Gong, D. P. Randall and T. J. Flowers. "Silicon deposition in the root reduces sodium uptake in rice (*Oryza sativa* L.) seedlings by reducing bypass flow". *Plant Cell Environment* vol. 29, pp.1970-1979, 2006.
- [21] B. Joseph, D. Jini and S. Sujatha. "Biological and physiological perspectives of specificity in abiotic salt stress response from various rice plants". *Asian Journal of Agric. Science*. vol. 2, pp. 99-105, 2010
- [22] R. A. James, A. R. Rivelli, and R. Munns. Factors affecting high apoplastic solute concentrations in leaves alter water relations of the halophytic shrub, *Sarcobatus vermiculatus*. *Journal of Experimental Botany*, vol. 57, pp.139-147, 2002.
- [23] F. Ologundudu and O. Kekere. "Leaf nutrient content and yield of rice cultivars under different concentrations of chloride and sulphate salts of sodium." *International Journal of Life Sciences Research*, vol. 4 (2), pp. 178-186, 2016.
- [24] J. J. Grant and J. G. Loake. "Role of reactive oxygen intermediate and cognate green gram". *Plant Science*. vol. 169, pp. 331-339, 2000.
- [25] M. Hodges. *Oxidative stress and post-harvest produce*. In: Hodges, M. (Ed.), *Post-Harvest Oxidative Stress in Horticultural Crops*. Food Products Press, New York, pp. 1-12, 1999.
- [26] N. Misra and A. K. Gupta. "Effect of salt stress metabolism in two high yielding genotypes of green gram". *Plant Science*. vol. 169, pp. 331-339, 2005.
- [27] F. A. Moradi, M. Ismail, G. B. Gregorio and J. A. Egdane. "Salinity tolerance of rice during reproductive development and association with tolerance at the seedling stage". *Indian Journal of Plant Physiology*. vol. 8, pp. 276-287, 2007.
- [28] A. K. Parida and A. B. Das. "Salt tolerance and salinity effects on plants". Relationship with the salt tolerance of two genetically diverse lines of canola (*Brassica napus* L.). *Pakistan Journal of Botany*, vol.38, pp.663-672.

- [29] M. Kumer and M. A. Rashid. "Effect of salinity on growth of some modern rice cultivar". *Journal of Agronomy*. vol. 3. Pp.1-10, 2009.
- [30] A. Matysik and H. R. Moussa. 2006. "Influence of Exogenous Application of Silicon on physiological Response of Salt-stressed Maize" (*Zea mays L.*). *International Journal of Biological Sciences*. vol. 8, pp. 293-297, 2002.
- [31] R. K. Sairan and R. C. Tyagi. "Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes". *Plant Biology*. vol. 49. pp. 85-91, 2004.
- [32] K. J. Kayona and D. Stocva: "Effect of salinity stress on dry matter production and ion accumulation in hybrid maize varieties". *Turkish Journal of Agriculture*. vol. 30. pp. 365-373, 2006.
- [33] F.T. Lacerda, H. R. Rhoades and J. D. Keren, "Effects of salinity and varying boron concentrations on boron uptake and growth of wheat". *Plant and Soil*, vol. 97, pp. 345-351, 2003.
- [34] B. T. Stewart, "Inhibitory effect of water deficit on maize leaf elongation". *Plant Physiol*. vol. 77, pp. 190–194, 1985.
- [35] J. C. Pareek, "A comparative study of the effects of NaCl salinity on respiration, photosynthesis and leaf extension growth in *Beta vulgaris*". *Plant Cell Environment*. vol. 6. pp. 675–677, 1997.
- [36] I. Shibli, and T. Demiral, "Recent developments in understanding salinity tolerance". *Environment and Experimental Botany*. vol. 67, pp. 2–9, 2007.
- [37] M. G. Potluri and A. Zhu. 1994. Understanding and improving salt tolerance in plants. *Crop Science*. vol. 45, pp. 437-448, 1994.
- [38] R. T. Khosraunejad, "Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress". *Acta Physiology of Plant*. vol. 30. pp. 81-89, 2009.