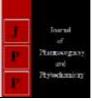


Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com



E-ISSN: 2278-4136 P-ISSN: 2349-8234 www.phytojournal.com JPP 2021; 10(2): 1113-1122

Received: 13-01-2021 Accepted: 17-02-2021

Nguyen Viet Thanh

Department of Crop Physiology, Faculty of Agriculture, Assam Agricultural University, Jorhat, Assam, India

Bhagawan Bharali

Department of Crop Physiology, Faculty of Agriculture, Assam Agricultural University, Jorhat, Assam, India

Corresponding Author: Nguyen Viet Thanh Department of Crop Physiology, Faculty of Agriculture, Assam Agricultural University, Jorhat, Assam, India

Physiological performance of some rice (*Oryza* sativa L.) genotypes under salinity stress condition

Nguyen Viet Thanh and Bhagawan Bharali

Abstract

In Agriculture, among the abiotic factors, salinity damages more than 45 million hectares of irrigated land, and 1.5 million hectares becomes unfit for cultivation due to high salinity worldwide. Physiological aberrations are caused by high salinity which are yield limiting in crops. To assess the differential salt sensitivity, a pot experiment was conducted on ten upland rice genotypes (*viz.*, Bahadur, Joymati, Ashoni Bara, Mulagachuru, Gitesh, Monoharsale and Moniram from Assam, and OM 5451, OM 6976, OM 4900 from Vietnam) under salinity condition (@ 0-30 mM \approx EC 0, 40dSm⁻¹) imposed at vegetative and reproductive stages. In the study, the salinity stress reduced RLWC (1.6-9.2%), Pⁿ rate (25.6-46.6%), SLW (0.35-14.4%), root biomass (1.64-19.5%), shoot biomass (3.03-27.5%), Chl-a (14.53-81.89%), Chl-b (8.4-88.1%), total Chl (5.25-84.90%), NR activity (2.44-27.59%), grain carbohydrate contents (19.12-49.41%), but increased Proline content (2.954-88.49%) and lipid Peroxidase activity (10.87-93.94%). Based on the overall performance, the tolerance range of the rice varieties to salt stress condition as compared to control was Joymati>Bahadur>Gitesh>Monoharsali&Moniram>OM6976>Aghonibora=Mulagabhoru>OM5451=OM4900. Hence, in the experiment, varieties Joymati, the cultivar from India, and OM 6976, the cultivar from Vietnam emerged suitable under salt stress condition.

Keywords: Rice, salinity, chlorophyll, photosynthesis, nitrate reductase, proline, lipid peroxidase, carbohydrate, and biomass

Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world, being almost exclusively consumed as grain by human population. Salinity is one of the major impediments to production in rice growing areas worldwide (Flowers and Yeo, 1995)^[22]. The physiological lethal effects of salt stress include reduction of leaf expansion, photosynthetic leaf area and dry matter production (Ashrof, 2010)^[10]. The threshold of salt stress in rice is $3dSm^{-1}$; beyond it, yield is reduced by 12% per dS per meter. This makes rice as a salt sensitive crop (Maas and Hoffman, 1977)^[43]. Salt stress affects rice crop differentially at growth stages (Lutts *et al.*, 1995)^[42]; Shannon *et al.*, 1998)^[68]. The rice crop is sensitive to salt stress at seedling and reproductive growth phases remarkably rather than vegetative phase (Zeng and Shannon, 2000)^[84].

In rice, the economic yield and yield attributes are reduced substantially by salt stress (Thanh and Bharali, 2019) ^[74]. Pollen viability and poorer percentage of seed set cause these consequences to rice. (Khatun *et al.*, 1995) ^[39]. High salinity (>50mM NaCl) lessens temporary stomatal conductance and gaseous exchange for carbon assimilation (Moradi and Ismail, 2007) ^[50]. Abscissic acid accumulation in guard cells (Moons *et al.*, 1995) ^[49] also regulates stomatal movement (Zhang *et al.*, 2006) ^[85]. Proline plays a key role on osmoprotection against salt stress in plants (Ashrof and Foolad, 2007)⁸. Salt stress generates reactive oxygen species (ROS) or its intermediates i.e. (O₂⁻, H₂O₂, OH⁻) (Mittler, 2002) ^[47]. Antioxidant activities e.g. Super oxide dismutase, Catalases, peroxidase, Ascorbate and reduced Glutathion scavenge different types of ROS in salt tolerant plants (Foyer and Noctor, 2005) ^[23].

In the past, efforts have been made on rice improvement for salinity stress through physiological and biochemical interventions (Shannon, 1998^[68]; Ashrof, 1994^[9], Munns *et al.*, 2006^[52] & Munns and Tester, 2008^[51]). Despite, availability of lakhs of salt tolerant rice genotypes around the tropics and subtropics (Negrao, *et al.*, 2011^[56]), information on physiological sensitivity of land races of Assam to induced salt stress is lacking so that it could be cultivated in some salty areas in a different place. Such a salt prone area is the Mekong Delta in southern Vietnam. It's a hot spot for production of nearly half of the country's rice.

However, this area is relatively more vulnerable to global sea level rise in the context of global climate change. As a ramification, submergence of rice fields with saline water for a long time becomes a great hindrance to three-seasoned rice farming in this densely populated, low-lying region. Thereby, this type of situation could be threat to livelihood of millions of people elsewhere in future decades. Hence, the present work was undertaken to study the physiological performance of seven rice genotypes from Assam in conjunction with three varieties from Vietnam under saline condition.

Materials and Methods

A pot experiment was laid in Randomised Block Design (RBD) with three replications. Seven rice genotypes (Bahadur, Joymati, Aghoni Bara, Mulagabharu, Gitesh, Monoharsale and Moniram) from Assam, and there varieties (OM 5451, OM 6976, OM 4900) from Vietnam were included in the study. Thus, the rice germplasm were indifferent in salt habitats. Salinity stress was imposed by application of NaCl @ 0, 30 mM (\approx EC 0, 30dSm⁻¹), 1000 cm³ to the soil at vegetative and flowering stages. The condition was retained for maximum 7 days, and flushed the rice crop with tap water afterwards. The initial soil pH values were determined using a digital pH meter (Model 510 Bench Meter). Electrical conductance (EC) was measured in dilute (<0.1 mol⁻kg⁻¹) aqueous NaCl solutions using Conductivity Meter, Model 304.

Three plants in a Hill were selected randomly. For root biomass at harvest, plants were uprooted after loosening the soil with sufficient water. Roots were cleaned in tap water gently without any loss. The cleaned roots were dried in shade prior to putting in hot air oven at 80°C to measure its dry mass to a constant weight, and it was expressed in gram per plant. Shoot biomass at harvest was dried also in hot air oven at 80°C to a constant weight, and dry weight was estimated in gram per plant. Specific Leaf weight (SLW) was measured at maximum tillering stage of the crop following method suggested by Singh, (1988) ^[67]. Flag leaf (fully expanded 2nd leaf from the top) was collected; area of the leaf was measured using scale, and dried at 80°C in oven. Fresh weight, dry weight and turgid weights of green leaves were used to calculate relative leaf water content (RLWC) at maximum tillering and flowering stages of the crop following the method of Boyer (1968)^[16].

The rate of Photosynthesis (μ molm⁻² s⁻¹) at maximum tillering was determined using Infra Red Gas Analyzer (IRGA: Model LI-COR 6400). Fully expanded upper most leaves were used for measuring photosynthesis rate. Free proline content (μ g g

¹) in leaves at panicle initiation (PI) and Flowering stages was estimated using method described by Bates et al. (1973)^[13]. Chlorophyll 'a', Chlorophyll 'b' and total Chlorophyll contents (mgg⁻¹f.w) in leaves were estimated by colorimetric method suggested by Arnon (1949)^[5]. In vivo NR activity (µmoles NO₂ formed g⁻¹fresh tissue wt hr⁻¹) was estimated following the method of Keeper et al. (1971)^[36]. Lipid peroxidation was measured in terms of Malondialdehyde content (nmol MDA per g fresh weight) following the procedure of Heath and Packer (1968) ^[28]. Total carbohydrate contents in leaf tissues were estimated at PI and Flowering stages of the crop following Anthrone method (Hedge and Hofreiter, 1962)^[29]. Carbohydrates were first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose was dehydrated to hydroxymethyl furfural. These compounds react with Anthrone reagent and form a green coloured product with an absorption maximum at 630 nm.

Fisher's method of analysis of variance (Panse and Sukhatme, 1978)^[57] was used for analysis of data in case of easch character. The level of significance or non-significance of variance due to the treatment or varietal effects was determined by calculating the respective 'F' values. The Critical difference (CD) worked out from the standard error of the means (S.Ed.) was used to judge the level of significance between a pair of treatments or two genotypes at P(0.05).

Results & Discussion

The crop was subjected to salinity stress (≈EC 30dSm⁻¹) by exposing to NaCl (30mM) in soil both at maximum tillering and flowering stages. As the soil was strongly acidic in nature (pH 5.64), and the initial soil N, P, K contents were in the medium ranges, fertilizers @40:20:20 in terms of Urea, Single Super Phosphate (SSP) and Muriate of Potash (MoP) were applied to avoid any deficiency of the nutrients during the growth of the crop. However, imposition of salt stress altered the soil physicochemical properties. Soil pH increased to a maximum of 7.97, Soil EC increased by 66.61-83.07%, but the soil N (20.09%), P (25.32%) and K (29.25%) decreased significantly during the crop growth following application of salt solutions as compared to the controlled plots. Therefore, these values indicate that soil salinity was induced properly, which was further examined by the physiological changes of the rice crop varieties during growth stages.

There were significant effects of salinity on RLWC at maximum tillering and flowering stages of the crop (Table 1). All the varieties had lesser RLWC at the panicle initiation and

Parameter→	RLWC (%) at maximum tillering stage			RLWC (%) at maximum flowering stage		
Treatments→ Varieties↓	Control	NaCl	Mean	Control	NaCl	Mean
Bahadur	81.25	78.89	80.07	82.90	79.83	81.37
Joymati	78.24	73.24	75.74	84.75	81.82	83.29
Aghoni Bora	83.94	76.21	80.08	86.64	85.30	85.97
Mulagabharu	80.71	79.87	80.29	76.96	73.35	75.16
Gitesh	79.49	78.69	79.09	80.06	72.69	76.38
Monoharsale	83.54	82.20	82.87	86.36	84.85	85.61
Moniram	81.58	75.13	78.36	89.04	82.08	85.56
OM 5451	73.41	70.36	71.89	87.82	84.70	86.26
OM 6976	84.21	80.63	82.42	80.99	67.94	74.47
OM 4900	71.05	69.17	70.11	82.53	77.41	79.97
Mean	79.74	76.44		83.81	79.00	
	S.Ed (±)	CD (0.05)		S.Ed (±)	CD (0.05)	

Table 1: Effects of salinity (30mM NaCl) on Relative leaf water content (RLWC) in rice crop

Journal of Pharmacognosy and Phytochemistry

Treatment (T)	3.85772	12.34151	7.624107	24.39084	
Variety (V)	3.900449	70.08828	7.402567	12.76325	
T imes V	3.852402	12.56338	8.082698	26.35915	

Flowering stages of crop under salinity condition as compared to the non saline condition. As such, at maxium tillering stage, the highest reduction in RLWC was in Aghoni Bara (9.20%), and OM 4900(4.06%) experienced the lowest reduction of it at the same stage due to salinity as compared to control. At flowering stage, the highest reduction in RLWC was in Gitesh (9.21%), and the lowest reduction was in Aghonibora (1.55%). Abdelkrim *et al.*, (2014) ^[2] also reported

RLWC reduction of about 50% on exposure to salinity stress. Fresh weight of treated and non treated plants reduced to 95% and 75% respectively as compared to the control. Salinity stress also caused reduction in RLWC in rice seedling (Mohammad *et al.*, 2011 ^[48]; Kaur *et al.*, 2014 ^[36]). A decrease in RLWC indicates loss of turgor, which occurs due to disturbances in the growth of individual leave, in other words in leaf expansion (Katerji *et al.* 1997 ^[34]).

 Table 2: Effects of salinity (30mM NaCl) on Net photosynthesis rate (Pn) of rice crop

Parameter→	Rate of P ⁿ (µmolm ⁻² s ⁻¹)	at Maximum tillering stage			
Treatments→ Varieties↓	Control	NaCl	Mean		
Bahadur	13.76	8.42	11.09		
Joymati	10.61	5.82	8.22		
Aghoni Bora	11.60	6.20	8.90		
Mulagabharu	11.02	6.07	8.55		
Gitesh	15.99	10.66	13.33		
Monoharsale	13.34	9.72	11.53		
Moniram	13.20	9.82	11.51		
OM 5451	13.09	9.68	11.39		
OM 6976	12.65	8.28	10.47		
OM 4900	11.51	8.21	9.86		
Mean	12.68	8.29			
	S.Ed (±)	CD (0.05)			
Treatment (T)	3.85772	12.34151			
Variety (V)	3.900449	70.08828			
$T \times V$	3.852402	12.56338			

Salinity affected Net Pⁿ rate of rice crop at maximum tillering stage significantly (Table 2). On an average total treatment mean, Gitesh (13.33 µmolm⁻² s⁻¹) had the highest Pⁿ rate, and the lowest P^n was in Joymati (8.22 μ molm⁻² s⁻¹). The variety Gitesh maintained the highest Pn rate under both salinity $(15.99 \ \mu molm^{-2} \ s^{-1})$ and non saline $(10.66 \ \mu molm^{-2} \ s^{-1})$ conditions. There was significant reduction of Pⁿ rate in all the varieties under salinity condition as compared to the non saline control one. The process of photosynthesis is a primary target of many forms of environmental stress, including salinity (Michael et al., 1996^[44]; Stepien and Kłbus, 2006 ^[72]). Salinity reduced photosynthetic rate of rice crop (Strasser and Strasser 1995 [73]; Ji et al., 2012 [32]; Lauteri, 2014 [41]; Yang et al., 2014 [81]). In the current study, in general, there was reduction in Pⁿ rates in all the genotypes. At the vegetative (maximum tillering) stage, the highest reduction in Pⁿ rate was caused in Aghoni Bara (46.55%). The lowest reduction in Pⁿ was in Moniram (25.60%) under salinity stress as compared to normal condition. Centritto et al. (2009) ^[19] reported that the major components limiting photosynthesis are the slow diffusion of CO₂ due to early stomatal closure, reduced activity of photosynthetic enzymes, the biochemical components related to triose-phosphate formation and decreased photochemical efficiency of PSII. Change in any of these components alters the final photosynthesis rate (Strasser and, Strasser 1995^[73]). Munns et al,. (2006)^[52] also reported that photosynthesis and plant growth are among the primary processes affected directly by salinity, and indirectly salinity induced drought condition. Water stress and salinity can affect photosynthesis by decreasing CO_2 availability caused by diffusion limitations.

Salinity affected SLW at maximum tillering stage of rice crop significantly (Table 3). On an average, Monoharsale (137.07 mg cm⁻²) exhibited the highest SLW > Aghoni Bora (131.51) mg cm⁻²). Overall, the varieties under saline condition had significantly lower SLW than in the non saline condition. The highest reduction of SLW was found in variety Bahudur (14.44 %), and OM 4900 had the lowest per cent decrease (0.35%) of it under salinity as compared to non saline condition. SLW is a characteristic feature for tolerance to stress condition e.g. drought (Balasimha 1987 [11]; Balasimha et.al. 1985 ^[12]), low light, (Sahu 1984 ^[61]; Bormudoi and Bharali, 2016^[14]). The decrease in SLW could be due to variation in mesophyll tissue density or leaf thickness, as suggested by Araus et al., (1986)^[4]. Many plants develop mechanisms either to exclude salt from their cells or to tolerate its presence within the cells (Greenway and Munns, 1980) [25].

Biomass accumulated in root and shoot was affected significantly by salinity stress (Table 4). Overall, salinity reduced root biomass of the varieties significantly than in control.

Table 3: Effects of salinity (30mM NaCl) on Specific Leaf Weight (SLW) of rice crop

Parameter→	SLW (mg cm ⁻²) at ma			
Treatments→ Varieties↓	NaCl	Control	Mean	
Bahadur	113.04	132.13	122.59	
Joymati	121.48	128.73	125.11	
Aghoni Bora	130.88	132.14	131.51	
Mulagabharu	124.65	130.50	127.58	
Gitesh	106.34	114.70	110.52	
Monoharsale	132.88	141.26	137.07	
Moniram	124.94	131.77	128.36	
OM 5451	121.57	125.92	123.75	
OM 6976	126.57	133.25	129.91	
OM 4900	130.48	130.94	130.71	
Mean	123.28	130.13		
	S.Ed (±)	CD (0.05)		
Treatment (T)	0.533385	26.4567		
Variety (V)	3.465689	27.54788		
$T \times V$	3.786687	26.40289		

The highest decline in root biomass was found in OM 5451 (19.48%) under salinity condition as compared to non saline condition. The varieties had significant reductions of root biomass and shoot biomass under salinity condition as compared to non saline condition. The highest reduction in shoot biomass was found in OM 4900 (27.5%) followed by

Monoharsale (27.27 %) under salinity condition as compared to non saline one. Shoot growth is more sensitive than root growth to salt- induced osmotic stress probably, because a reduction in the leaf area development relative to root growth would decrease the water use by the plant, thus allowing it to conserve

 Table 4: Effect of Salinity (30mM NaCl) on Shoot biomass and Root biomass at harvest of rice crop

Parameters→	(a)	Shoot biomass (g)		(b) Root biomass (g)		
Treatments→ Varieties↓	Control	NaCl	Mean	Control	NaCl	Mean
Bahadur	0.27	0.31	0.29	0.59	0.57	0.58
Joymati	0.32	0.33	0.33	0.49	0.45	0.47
Aghoni Bora	0.29	0.38	0.34	0.72	0.66	0.69
Mulagabharu	0.27	0.31	0.29	0.74	0.69	0.72
Gitesh	0.32	0.33	0.33	0.66	0.64	0.65
Monoharsale	0.32	0.44	0.38	0.61	0.60	0.61
Moniram	0.35	0.38	0.37	0.47	0.45	0.46
OM 5451	0.29	0.39	0.34	0.77	0.62	0.70
OM 6976	0.29	0.37	0.33	0.57	0.53	0.55
OM 4900	0.29	0.40	0.35	0.51	0.49	0.50
Mean	0.30	0.36		0.61	0.57	
	S.Ed (±)	CD (0.05)		S.Ed (±)	CD (0.05)	
Treatment (T)	0.031623	NS		0.124499	0.398294	
Variety (V)	0.03873	NS		0.180278	3.239459	
$T \times V$	0.031623	NS		0.11619	0.378915	

soil moisture and prevent salt concentration in the soil (Munns and Tester, 2008)^[49]. Reduction in shoot growth due

to salinity is commonly expressed by a reduced leaf area and stunted shoots (Läuchli and Epstein, 1990)^[40].

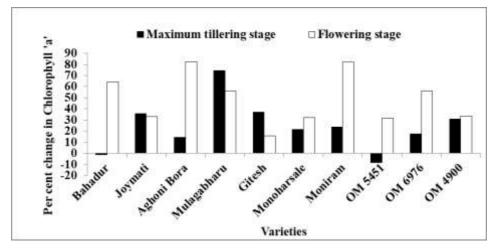


Fig 1(a): +ve values indicate decreaes in Chlorophyll'a' under salinity condition as compared to normal ~ 1116 ~

Chlorophyll 'a', Chlorophyll 'b' and total Chlorophyll contents were affected significantly by salinity stress irrespective of growth stages in rice crop (Fig.1a, b & c). Overall.Chlorophyll 'a', Chlorophyll 'b' and total chlorophyll contents were higher in natural condition as compared to saline condition at both maximum tillering and flowering stages. At maximum tillering stage, the highest reduction Chl 'a' was found in Mulagabharu (74.58%). However, there were increases in Chl.'a' in Bahadur (by1.14%) and OM5451 (by 8.25%) at this stage. Similarly, at flowering stage, the highest reduction of Chl 'a' was recorded in Aghonibora (82.52%), and the minimum was in Gitesh (15.83%). In case of Chl'b' at maximum tillering stage, the highest reduction was in Jaymati (81.40%). In contrast, Monoharsale increased Chl'b' by 65.85% under salinity condition as compared to control. At flowering stage, Aghonibora showed 88.12% decrease

whereas Monoharsali increased Chlo'b' by 28.67%. Likewise, maximum reduction in total chlorophyll contents was found in Mulagabharu (68.3%) and Aghonibora (84.9%) at maximum tillering and flowering stages respectively. Overall, Chl'a' (27.07%, 49.06%), Chl'b' (25%, 52.58%) and Total Chl. (26.93%, 50.05%) were reduced by salinity stress as compared to the control at the two stages respectively. Chlorophyll is the main pigment responsible for

photosynthesis. Under adverse circumstances, the chlorophyll level is a good indicator of the photosynthesis function (Xu, *et al.*, 2008^[79]). Chlorophyll degradation is induced by many stresses, leading to changes of certain enzyme activities, photosynthetic electron transport, carbon metabolism and photophosphorylation in the process of photosynthesis. During salt stress, salt-

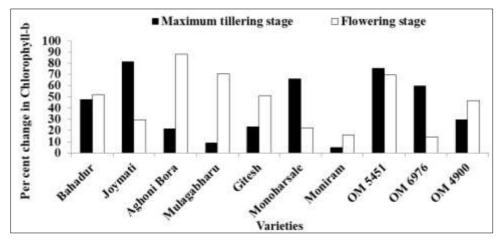


Fig 1(b): +ve values indicate decreaes in Chlorophyll 'b' under salinity condition as compared to normal

sensitive plants clearly showed chlorophyll degradation and growth reduction. Salinity significantly reduced chlorophyll 'a' content in leaf. It also significantly reduced chlorophyll 'b', total Chl., and Carotenoids contents after ten days of salinity treatment (Amira and Abdul, 2011) ^[3]. The loss of chlorophyll under salt stress could be related to photo inhibition or ROS formation (Kato and Shimizu, 1985) ^[35]. The reduction in photosynthesis under salinity can also be attributed to a decrease in chlorophyll content. Salinity reduces the chlorophyll content in salt susceptible plants, and increases it in salt tolerant plants. Reduced growth in radish (*Raphanus sativus* L.) at high salinity level could be attributed

to a reduction in leaf area expansion, and hence to a lower light interception (Marcelis and Hooijdonk, 1999)^[44]. Reduction of chlorophyll content due to salinity stress is very common in salt-sensitive plant species because of salt toxicity which mostly causes burning of leaves or other succulent parts and degradation of other pigments too. But those are salinity tolerant species that can protect themselves from such deterioration of salinity stress. Chlorophyll content of salt stressed rice can be described as a function of the leaf's sodium content (Yeo, and Flowers., 1983)^[82]. Sodium chloride accumulation in the leaf laminate reduces net

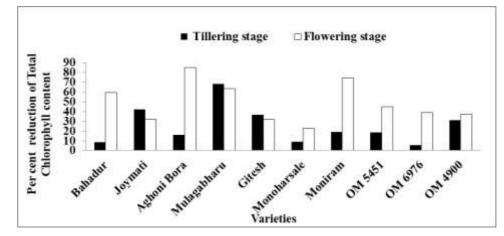


Fig 1(c): +ve values indicate decrease in total chlorophyll under salinity condition as compared to normal

There were significant effects of salinity stress on proline content at both maximum tillering and flowering stages. On an average, variety OM 4900 (361.59 µgmol⁻g⁻¹ f.w.) had the highest proline content on leaf, and variety Monoharsale (243.63 µgmol⁻g⁻¹ f.w..) had the lowest proline content in leaf at maximum tillering stage. The proline accumulation was the highest in OM 4900 at flowering stages (414.02 µgmol⁻g⁻¹ f.w.). The lowest was in Monoharsale (243.63 µgmol⁻g⁻¹ f.w.) at maximum tillering stage, and Bahadur (261.09 µgmol⁻g⁻¹ f.w.) had the lowest at flowering stage. The proline content was higher in saline condition as compared to natural condition at both maximum tillering and flowering stages. Overall, salinity increased proline contents in leaf significantly in the varieties as compared to the control irrespective of the stages of the crop. Proline accumulation is an important mechanism for osmotic regulation under salt stress (Huang et al, 2013)^[31]. It is apparent from the Fig.2 that there were significant increases in proline contents both at maximum tillering (upto 88.49 % in OM 6976) and flowering stages (39.56% in OM 6976) of all the varieties under salinity stress condition. Proline accumulation is a wellknown measure adopted for alleviation of salinity stress (Matysik, 2002) ^[45]. Among the best known compatible solutes, proline and glycine betaine (GB) have been reported to increase greatly under salt and drought stresses (Munns, 2002 ^[54]), and constitute the major metabolites found in durum wheat under salt stress, as in other poaceae (Ashraf and Foolad, 2007 ^[8]; Sairam and Tyagi, 2004 ^[63]). Proline can play an important role

in enhancing plant stress tolerance. This role can be in the form of either osmoprotection (Wyn Jones and Gorham 1983 ^[78]; Handa *et al.* 1986 ^[27], Hamdia, 1987 ^[26]) or cryoprotection (Snngstad *et al.* 1990 ^[71], Santarius 1992 ^[65]). For example, in various plant species growing under saline conditions, exogenously-supplied proline provided osmoprotection and facilitated growth (Csonka and Hanson 1991 ^[20], Yancey, 1994 ^[84]). Results showed that proline concentration increased in all the cultivars studied in relation to increase in salt stress, and it was progressive along with increase in stress (Joseph *et al.*, 2015) ^[33].

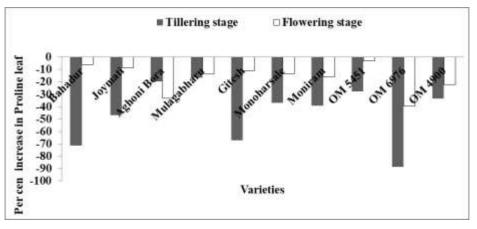


Fig 2: -ve values indicate increase Proline under salinity condition as compared to normal

Salinity stress affected nitrate reductase activity (NR) activity in leaf tissues of rice crop significantly. The NR activity was lesser at flowering stage as compared to the maximum tillering stage. On an average, the highest NR activity in leaf tissue was in Monoharsale (2.41 NO₂⁻µmole g⁻¹ f.w.hr⁻¹), and the lowest was in Aghoni Bora (1.55 µmole NO₂⁻ g⁻¹ f.w.hr⁻¹). Overall, salinity reduced NR activity significantly in the varieties as compared to the control irrespective of the stages of the crop (Fig. 3). Among the varieties, OM 5451 had the highest NR reduction (21.92%) followed by OM 4900 (20.77%) >Mulagabharu (19.7%) under salinity as compared to normal. The lowest reduction was observed in Gitesh (2.44%) under salinity as compared to normal.

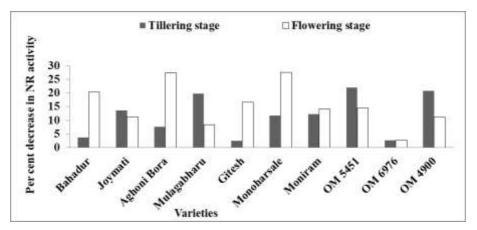


Fig 3: +ve values indicate decrease in Nitrate reductase (NR) under salinity condition as compared to normal ~ 1118 ~

At flowering stage, the highest NR reduction was obtained in Monoharsale (27.59%) followed by Ashoni Bara (27.37%) while the lowest reduction was found in OM 6976 (2.70%). There were significant declines of NR at maximum tillering (2.44-20.77%) and flowering (2.70-27.59%) stages in the varieties. Silveira *et al.* (2001) presented a possible homeostasis between nitrate assimilation and plant growth for cowpea, algarroba plants subjected to NaCl stress. Even though the plants showed lower growth rates, they were compatible with the general processes of nitrate reduction. A similar coordinate balance between C and N assimilation was proposed for maize plants subjected to drought stress (Foyer *et al.*, 1998 ^[24]). The fast NR decrease in the leaves by the external supply of NaCl may be related to osmotic changes following NaCl addition to the medium. In fact, NR is inhibited by osmotic effects of NaCl treatment in cashew (Viegas *et al.*, 1999)^[76]. The reduction of the maximum extractable NR in the leaves could be due to a lower NR protein content (Férrario-Méry *et al.*, 1998^[21]). The decrease in NO₃⁻ concentrations by NaCl treatment may result from a disruption of root membrane integrity (Carvajal *et al.*, 1999^[18]), an inhibition of NO₃⁻ uptake (Bourgeais-Chaillou *et al.*, 1992^[15]; Parida et al., 2002^[59]) and low NO₃⁻ loading into root xylem (Abd-El Baki *et al.*, 2000^[1]).

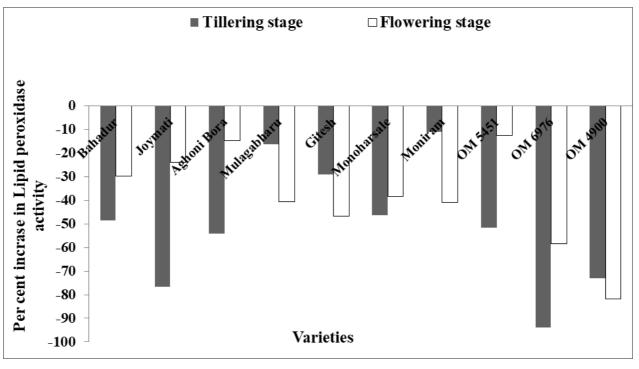


Fig 4: -ve values indicates increase in Lipid peroxidase activity under salinity condition as compared to normal

There were significant effects of salinity on Lipid peroxidase activity in leaf at maximum tillering and flowering stages. The Lipid peroxidase activity (nmol MDA g⁻¹f.w⁻¹) in leaf was lesser at flowering stage as compared to the maximum tillering stage. On an average, the Lipid peroxidase activity in leaf tissues was the highest in Aghoni Bora (0.77 nmol g⁻¹f.w⁻¹ ¹), and the lowest was recorded in OM 5451 (0.23 nmol g⁻ ¹f.w⁻¹). At flowering stage, overall, salinity increased lipid peroxidase activity significantly in the varieties as compared to the control irrespective of the stages of the crop (Fig. 4). It was interesting to note that all the varieties experienced increase in lipid peroxidase activity under saline condition. OM 6976 had the highest increase in lipid peroxidase activity (93.94%) followed by Joymati (76.79%) > OM 4900 (72.97%) under salinity as compared to normal. The lowest increase in lipid peroxidase activity was observed in Moniram (10.87%) under salinity compared to normal. At flowering

stage, the highest increases in lipid peroxidase activity was found in OM 4900 (81.82%) followed by OM 6976 (58.33%), while the lowest increase was in OM 5451 (12.50%). Overall, there were significant increases of lipid peroxidase activity at maximum tillering (10.87-9.94%) and flowering (12.50-81.82%) stages in the varieties. As lipid peroxidation is the mostly ascribed symptom to oxidative damage, it is often used as a marker of oxidative stress (Hernandez et al., 2000 [30]; Khan and Panda, 2008 [38]). The elevated antioxidant activity led to the lower lipid peroxidation under salinity as it was reported in high-yielding (Shalata and Tal, 1998 [67]). In increasing level of salinity stress, the MDA content increased in the sensitive varieties thus indicating an increase in lipid peroxidation (Satoshi, et al 1998 [66]). High salinity induces oxidative stress by decreasing the concentrations of Lipid in plants (Vaidyanathan et al. 2003^[75]).

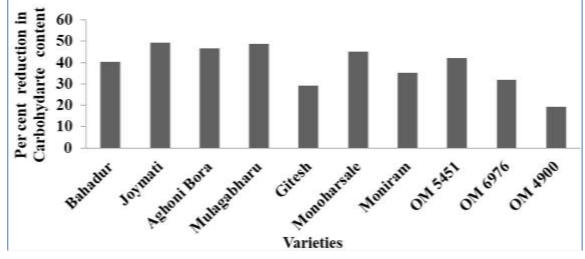


Fig 5: +ve values indicate decrease in grain carbohydrate content under salinity condition as compared to normal

There were significant differences of total carbohydrate contents among the varieties under treatments at harvest stage of rice crop. On an average, the highest carbohydrate content in grains was in Mulagabharu (2.11%), and the lowest was in Gitesh (1.26%). In general, salinity reduced grain carbohydrate contents in all the varieties as compared to the control condition. There were significant reductions (19.12-49.41%) of grain carbohydrate contents among the varieties under salinity condition (Fig.5). Among the varieties, Joymati (49.41%) had the highest reduction in carbohydrate followed by Mulagabharu (48.74%) > Monoharsale (45.00%) under salinity as compared to normal. The lowest reduction was observed in OM 4900 (19.12%) under salinity compared to normal. There was a significant decline of carbohydrate at harvest stage in the varieties. The accumulation of soluble carbohydrates in plants had been widely reported as a response to salinity or drought, despite a significant decrease in net CO₂ assimilation rate (Murakeozy *et al.* 2003 ^[55]). Carbohydrates such as sugars (glucose, fructose, sucrose, and fructans) and starch accumulate under salt stress. A decrease in starch content, an increase in both reducing, non-reducing sugars and polyphenol levels have been reported (Parida et al. 2002 ^[59]). Wattana and Maysaya (2008) ^[77]. The results showed that when salinity stress increases, starch decreases in plants. Moreover, it was investigated whether a relatively higher salt-tolerance was related with the ability to retain higher concentrations of Na⁺ in the roots, and whether this capacity interfered with macronutrient uptake by roots. Because NaCl salinity impairs leaf metabolism in sensitive species, photosynthesis is reduced and carbohydrate production is limited (Rahimi et al., 2011)^[60].

It could be inferred that Joymati, the cultivar from India and OM 6976, the cultivar from Vietnam were found physiologically efficient among the ten varieties tested against salt stress condition. In earlier report (Thanh and Bharali, 2019^[74]) also, we found that these two varieties had the adaptive physiological traits, higher yield and yield attributes under salinity condition. So, in this paper, the mechanisms of physiological tolerance of the rice genotypes to salt stress are illustrated especially in terms of higher proline accumulation and lower peroxidase activity in association with other physiological attributes e.g. higher RLWC, SLW; root & shoot biomasses; biochemical traits like higher Chlorophyll contents, NR activity, Pⁿ rate and grain carbohydrate content.

Acknowledgements

All kinds of support received from Assam Agricultural University for conducting the experiment are duly accredited. The pecuniary support given by the ICCR, Govt. of India, to pursue the Masters research by the first author successfully in AAU, Jorhat, Assam, is acceptable, and the authors always remain grateful for it. Special thanks are due to Dr. Ranjan Das, Professor, Crop Physiology, Assam Agricultural University, for rendering equipments for measuring photosynthesis in the experimental plants.

References

- 1. Abd-El Bak J, Naseer S, Geldner N. A developmental framework for endodermal differentiation and polarity. Proc Natl Acad Sci USA 2000;107:5214-5219.
- Abdelkrim BN El, Fazaa S El, Ati J. Time-motion analysis and physiological data of elite under-19-year-old basketball players during competition. Br J Sports Med 2014;41:69-75.
- 3. Amira MS, Abdul Qados. Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.), Journal of the Saudi Society of Agricultural Sciences 2011;10(1):7-15.
- 4. Araus G, Alscher RG, Hess JL. Salinity, oxidative stress and antioxidant responses in shoot cultures of rice. J Exp Bot 1986;48:325-331.
- 5. Arnon DI. Copper enzymes in isolated chloroplast. Polyphenol oxidase in Beta vulgaris. Plant Physiol 1949;24:1-15.
- Asch F, Dingkuhn M, Dörffling K. Physiological stresses of irrigated rice caused by soil salinity in the Sahel, in In Irrigated Rice in the Sahel: Prospects for sustainable development, K.M. Miezan, *et al.*, Editors.: Côte d'Ivoire 1997,247-273p.
- Asch F, Dingkuhn M, Dorffling K. Salinity increases CO₂ assimilation but reduces growth in fieldgrown, irrigated rice", Plant and Soil 2000;218:1-10.
- 8. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany 2007;59:206-216.
- 9. Ashrof M. Breeding for salinity tolerance in plants. Crit. Rev. Plant Sci 1994;13:17-42.
- 10. Ashraf M. Inducing drought tolerance in plants: some recent advances. Biotechnol. Adv 2010;28:169-183.

- Balasimha D. Cocoa In: Tree crop physiology. Sethuraj, M.R.; Raghavendra, A.S. (eds) Elsevier Sci. Publ. Amsterdam 1987,263-285p.
- Balasimha D, Subramonian N, Subbhaiah CC. Leaf characteristic in cocoa (*Theobroma cacao* L.) accessions. Cafe Cacao The 1985;29:95-98.
- 13. Bates HJ, Nelson DE, Jensen RG. Adaptation to environmental stresses. Plant Cell 1973;7:1099-1111.
- Bormudoi B, Bharali B. Effects of light intensity and quality on physiological changes in winter rice (*Oryza* sativa L.). International Journal of Environmental and Agricultural Research (IJOEAR) ISSN [2454-1850] 2016;2(3).
- 15. Bourgeais-Chaillou P, Perez-Alfocea F, Guerrier G. Comparatives effects of N-sources on growth and physiological responses of soybean exposed to NaCl-stress. Journal of Experimental Botany 1992;43:1225-1233.
- 16. Boyer JS. Measurement of the water status of plants. Annual Review of Plant Physiol 1968;9:351-363.
- 17. Carillo P, Mastrolonardo G, Nacca F, Fuggi A. Nitrate reductase in durum wheat seedlings as affected by nitrate nutrition and salinity. Functional Plant Biology 2005;32(3):209-219.
- Carvajal M, Martnez V, Alcaraz FC. Physiological function of water channels as affected by salinity in roots of paprika pepper. Physiological Plantarum 1999;105:95-101.
- Centritto M, Lauteri M, Monteverdi MC, Serraj R. Leaf gas exchange, carbon isotope discrimination, and grain yield in contrasting rice genotypes subjected to water deficits during the reproductive stage. J. Exp. Bot 2009;60:2325-2339.
- Csonka LN, Hanson AD. Prokaryotic osmoregulation: genetics and physiology. Annual Review of Microbiology 1991;45:569-606.
- 21. Férrario-Méry S, Valadier MH, Foyer CH. Over expression of nitrate reductase in tobacco delays droughtinduced decreases in nitrate reductase activity and mRNA. Plant Physiology 1998;117:293-302.
- 22. Flowers JJ, Yeo AR. Breeding for salinity resistance in crop plants where next/ Aust. J. Plant Physiol 1995;22:875-884.
- 23. Foyer CH, Noctor G. Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ 2005;28:1056-71.
- 24. Foyer CH, Valadier M, Migge A, Beeker TW. Droughtinduced effects on nitrate reductase activity and mRNA and on coordination of nitrogen and carbon in maize plants. Physiol. Plant 1998;117:283-292.
- 25. Greenway H, Munns R. Mechanisms of Salt Tolerance in Nonhalophytes, Annual review of Plant Physiology 1980;31:149-190.
- Hamdia MA. Physiological studies of some plants in relation to salinity injury. Ph. D. thesis. Fac. Sci., Miniauniv., Egypt 1987,1-242.
- 27. Handa S, Handa AK, Hasegawa PM, Bressan RA. Proline accumulation and the adaptation of cultured plant cells to water stress. Plant Physiol 1986;80:938-945.
- Heath LR, Packer L. Photo-oxidation in isolated chloroplast. I kinetics and stochiomentry of fatty acid peroxidation. Arch. Biochem. Biophysic 1968;125:189-198.
- 29. Hedge JE, Hofreiter BT. In carbohydrate chemistry 17 (Eds whistler RL and Be Millee, J.N) Academic press, New York 1962.

31. Huang H, Hernandez JA, Jimenez A, Mullineaux P, Sevilla F. Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defences. Plant Cell Environ 2013;23:853-86.

2000;23:853-862.

- 32. Ji Y, Zuo L, Wang F, Li D, Lai C. Nutritional value of 15 corn gluten meals for growing pigs: chemical composition, energy content and amino acid digestibility. Arch. Anim. Nutr 2012;66(4):283-302.
- 33. Joseph A, Ford K, Kretschmer J, Tester M. Rice plants expressing the moss sodium pumping Atpase PpENA1 maintain greater biomass production under salt stress. Plant Biotech J 2015;9:838-847.
- 34. Katerji R, Megdiche W, Debez A, Falleh H, Grignon C, Abdelly C. Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*. Plant Physiol Biochem 1997;45:244-24.
- 35. Kato M, Shimizu S. Chlorophyll metabolism in higher plant. VI. Involvement of peroxidase in chlorophyll degradation. Plant Cell Physiol 1985;26:1291-1301.
- 36. Kaur H, Ozdemir F, Turkan I. Effect of salt stress on lipid peroxidation and superoxide dismutase and peroxidase activities of *Lycopersicon esculentum* and *L. pennellii*. Biol Plant 2014;50:745-748.
- Keeper C, Tuna AL, Ashraf M, Altunlu H. Improved salt tolerance of melon (*Cucumis melo* L.) by the addition of proline and potassium nitrate. Environ Exp Bot 1971;60:397-403.
- Khan MA, Panda SK. Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. Acta Physyol Plant 2008;30:91-89.
- Khatun S, Rizzo CA, Flowers TJ. Genotypic variation in the effect of salinity on fertility on rice.. affected by salinity. In: Skaggs RW, van Schilfgaarde J (eds.). Agricultural Drainage. Agron Monogr 38. Plant Soil 1995;173:239-50.
- 40. Lauchli A, Epstein E. Plant Growth And Development Under Salinity Stress, Department of Land, Air and Water Resources, University of California, One Shields Ave., Davis, CA 95616, USA 1990.
- 41. Lauteri A. Correction; Photosynthetic Diffusional Constraints Affect Yield in Drought Stress Rice Cultivars during Flowering. Plos one 2014;10(2):e0117631.
- 42. Lutts S, Kinet JM, Bouharmont J. Changes in plant response to NaCl during development of rice (*Oryza sativus* L.) varieties differing in salinity resistance. J Exp. Bot 1995;46:1843-1852.
- 43. Maas EV, Hoffman GJ. Crop salt tolerance current assessment. J. Irrig. and Drainage Div., ASCE 1977;103:115-134.
- 44. Marcelis MV, Hooijdonk VR. Field performance evaluation of vertical conveyer paddy reaper. Karnataka J. Agric. Sci 1999;22:140-142.
- 45. Matysik J, Alia A, Bhalu B, Mohanty P. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants, Current Science 2002;82(5):525-532.
- 46. Michael D, Mess EV, Hoffman GK. Crop salt tolerance: Current assessment ASCEJ Irrig Drain Div 1996;103:115-134.
- 47. Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 2002;7:405-410.

- 48. Mohammad MM, Narayanan SL, Ibrahim SM. Chlorophyll stability index (CSI): its impact on salt tolerance in rice. IRRN 2255 2011;22(24).
- 49. Moons A, Bauw G, Prinsen E, Van-Montagu M, Vander-Rhodes-Straeten D. Molecular and physiological responses to abscisic acid and salts in roots of saltsensitive and salt-sensitive and salt-tolerant indica rice varieties. Plant Physiol 1995;107:177-186.
- 50. Moradi F, Ismail AM. Responses of photosynthesis, chlorophyll fluorescence and ROS scavenging system to salt stressduring seedling and reproductive stages in rice. Ann. Bot 2007;99:1161-1173.
- 51. Munns R, Tester M. Mechanisms of salinity tolerance, Annual Review of Plant Biology 2008;59:651-681.
- 52. Munns R, James RA, La^{*}uchli A. Approaches to increasing the salt tolerance of wheat and other cereals. J Exp. Bot 2006;57:1025-1043.
- 53. Munns R, Tester M. Mechanisms of Salinity Tolerance. The Annual Review of Plant Biology 2008;59:651.
- 54. Munns R. Comparative physiology of salt and water stress. Plant, Cell & Environment 2002;25(2):239-250.
- 55. Murakeozy EP, Nagy Z, Duhaze C, Bouchereau A, Tuba Z. Seasonal changes in the levels of compatible osmolytes in three halophytic species of inland saline vegetation in Hungary. J. Plant Physiol 2003;160:395-401.
- 56. Negrao S, Courtois B, Ahmadi N, Abreu I, Saibo N, Oliveira MM. Recent updates on salinity stress in rice: From physiological to molecular responses. Crit. Rev. Plant Sci 2011;30(4):329-377.
- 57. Panse VG, Sukhatme PV. Statistical methods for Agricultural workers, ICAR, New Delhi 1978.
- 58. Parida AK, Das AB. Salt tolerance and salinity effects con plants: A review. Ecotoxicology and Environmental Safety 2005;60(3):324-49.
- 59. Parida AK, Das AB, Das P. NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora*, in hydroponic cultures. J Plant Biol 2002;45:28-36.
- 60. Rahimi KP, Reed RH, Richardson DL, Stewart WDP. Na⁺ uptake and extrusionin the cyanobacterium Synechocystis PCC 6714 in response to hypersaline treatment. Evidence for transient changes in plasmalemma Na+ permeability. *Biochim. Biophysic*. Acta 2011;814:347-355.
- 61. Sahu G. Screening for shade tolerance in rice. Intl. Rice Res. Newl 1984;9(3):26-27.
- 62. Janardhan KV, Murty KS, Das NB. Effect of low light during ripening period on grain yield and translocation of assimilates in rice varieties. Indian J Plant Physiol 1980;23:163-168.
- 63. Sairam RK, Tyagi A. Physiology and molecular biology of salinity stress tolerance in plants. Current Science 2004;86(3):407-421.
- 64. Sakamoto A, Murata N. The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. Plant Cell and Environment 2002;25(2):163-171.
- 65. Santarius V. Salt-induced changes in the vegetative anatomy of Prosopis strombulifera (Leguminosae), Canadian Journal of Botany 1992;82(5):618–628,
- Satoshi Tobita, Maribel L, Dionisio-Sese. Antioxidant responses of rice seedlings to salinity stress, Journal of the Saudi Society of Agricultural Sciences 1998;135 I(1).
- 67. Shalata A, Tal M. The effects of salt stress on lipid peroxidationand antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*. Physiol Plant 1998;104:169-174.

- 68. Shannon MC, Rhoades JD, Draper JH. Assessment of salt tolerance in rice cultivars in response to salinity problems in California. Crop Sci 1998;38:394-8.
- 69. Silveira JAG, Melo ARB, Viégas RA, Oliveira JTA. Salinity-induced effects on nitrogen assimilation related to growth in cowpea plants. Environmental and Experimental Botany 2001;46:171-179.
- 70. Singh KN. Proline: a multifunctional amino acid. Trends in Plant Science 1988;15(2):89-97.
- 71. Snngstad CC, Sreenivasulu N, Sopory SK, Kishor PBK. Deciphering the regulatory mechanisms of abiotic stress tolerance in plants by genomic approaches. Gene 1990;388:1-13.
- 72. Stępień P, Kłbus G. Water relations and photosynthesis in *Cucumis sativus* L. leaves under salt stress, Biologia Plantarum 2006;50(4):610-616.
- 73. Strasser BJ, Strasser RJ. Measuring fast fluorescence transients to address environmental questions: The JIP-test. In: Mathis P (ed), Photosynthesis: From light to biosphere. Kluwer Academic Publishers, Dordrecht, The Netherlands 1995;5:977-980.
- 74. Thanh NV, Bharali B. Salinity stress on rice (*Oryza sativa* L.) crop and its amelioration. Journal of Pharmacognosy and Phytochemistry 2019;8(6):1435-1441.
- 75. Vaidyanathan H, Sivakumar P, Chakrabarsty R, Thomas G. Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.) differential response in salt-tolerant and sensitive varieties, Plant Science 2003;165:1411-1418.
- 76. Viegas FH, Van Kooten O, Snel JFH. The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynthesis Res 1999;25:147-150.
- 77. Wattana P, Maysaya T. Effect of salinity stress on growth and carbonhydrate metabolism in three rice (*Oryza sativa*. L) cultivas differing in salinity tolerance. India and early seedling growth of Atriplex triangularis under saline conditions. Physiol. Plant 2008;63:109-113.
- Wyn Jones RG, Gorham J. Osmoregulation. In: O.L. Lange Editor Encyclopedia of Plant Physiology, Springer-Verlag Berlin 1983, 35-58.
- 79. Xu X, Xu H, Wang Y, Wang X, Qiu Y, Xu B. The effect of salt stress on the chlorophyll level of the main sandbinding plants in the shelterbelt along the Tarim Desert Highway, Chinese Science Bulletin 2008;53(2):109-111.
- Yancey PH. Compatible and counteracting colutes. In Cellular and Molecular Physiology of Cell Volume Regulation (ed. K. Strange), CRC Press, Boca Raton, FL, USA 1994, 81-109p.
- Yang E, Yamauchi N, Watada AE. Regulated chlorophyll degradation in spinach leaves during storage. J Amer. Soc. Hort. Sci 2014;116:58-62.
- 82. Yeo AR, Flowers TJ. The effect of salinity on photosynthesis in rice Exp. Bot 1983;36:1240-1248.
- Yeo AR, Flowers TJ, Caporn JM. Varietal differences of sodium ions in wheat leaves, Physiol. Plant 1985;59:180-190.
- 84. Zeng L, Shannon MC. Effects of salinity on grain yield and yield components of rice at different seedling densities. Iranian J. of Agric. Sci 2000;23:173-185.
- 85. Zhang Y, Zurita JL, Roncel M, Aguilar M, Ortega JM. A The rmoluminescence study of photosystem II back electron transfer reactions in rice leaves 2006.