

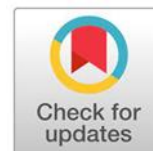
Physiological Adaptive Capabilities of Fifteen Different Local Rice Cultivars Under Salinity Condition

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Abstract

Rice is a major cereal contributing to the world's calories consumption and staple food crop over for one-third of the world's population. At present salinity is the second most widespread soil problem after drought and is considered as a serious constraint to increase rice production. Soil salinity affects plants through osmotic effects, ion-specific effects and oxidative stress. The effect of salinity stress in plants is mediated at least in part by an enhanced generation of active oxygen species, especially in chloroplast and in mitochondria which cause lipid peroxidation and membrane injury, protein degradation and enzyme inactivation. Plants have developed a complex anti-oxidant complex which mitigates and repairs the damage initiated by reactive oxygen species, toward enzyme synthesis to protect the cellular and subcellular system degradation. The seedling stage is one of the most sensitive stages to salt stress in rice and studies on salt tolerance during this stage could probably provide insights for enhancing tolerance throughout the plant life cycle. The present investigation was undertaken to examine the influence of NaCl on metabolic status of chlorophyll, protein, starch, soluble sugar and salt-tolerant capabilities among different rice cultivars.



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Introduction

Salinity causes reduction in percentage of germination, seedling vigor and other growth attributes (Farsaraei et. al. 2021). The main problem exerted by the salinity stress in rice plant is the accumulation of sodium and chloride ions at toxic level which in turn produces reactive oxygen species (ROS), free radicals and subsequently damages various photosynthetic pigments viz. chlorophyll, carotenoid, xanthophyll. Saline soil also restricts the absorption of potassium ions and thereby imbalances the normal Na^+/K^+ ratio within the plant body (Khare et. al. 2015). There are 3 basic stages of seed germination; water imbibition, activation of metabolic process and final germination phase. It has been reported that salinity adversely affected the first stage of germination i.e. water absorption phase by establishing osmotic pressure and contriving the

symptoms related to drought like insufficient water availability, wilting and reduced growth (Ucarli et. a. 2020). Disruption of ionic balance as a result of salinity also negatively influences the production of vital hormones and enzymes involved during seedling establishment (Kumar et. al. 2021). One of these enzymes is α -amylase, functions in the hydrolysis of stored form of carbohydrate i.e., starch into the less complex utilizable form (e.g., glucose, maltose etc.) during germination (Apar et. al. 2004). Salinity stress strongly affected the activity of hydrolytic enzymes (Liu et. al 2018). It has been observed that the immediate response of plant against high salt concentration is to adjust osmotic pressure by reducing the expansion and division of cell. The reduction in turgor pressure is also mediated by closing of stomata and minimization of leaf surface area to prevent transpiration. This results in declined photosynthesis and stunted plant growth (Sarker et. al. 2020). As glycophytic plant, rice is

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much more sensitive to saline soil as compared to other cereal crops. Several reports have been made that rice have developed a number of salinity-tolerant mechanisms up to a certain degree (Horie et. al. 2012). One of the physiological indicators to this salinity-adaptability is the accumulation of compatible osmolyte or solutes. The compatible osmolytes or solutes are a diverse group of chemical compounds which are polar, uncharged and soluble in nature. The main function of accumulated osmolyte is to maintain osmotic balance through continuous water inflow and thereby protecting the cell's structural integrity (Chen et. al. 2021). The major advantage of osmolyte accumulation is that, these compounds are unreactive to any cellular metabolic process even if present at higher concentration. Proline is such an amino acid group of organic osmolyte present in diverse taxonomic class including rice. There are two possible ways of maintaining the concentration of compatible osmolytes within the plant body either by continual synthesis of compounds or by combined synthesis with degradation (Gupta et. al. 2014). The accumulated intracellular proline provides tolerance-capability under salinity by supplying organic nitrogen reserve for stress recovery (Kaur et. al. 2015). Endosperm utilization during early seedling growth is greatly hampered under salinity stress (Blum et. al. 1994). It is largely depended on the capabilities of specific seed hydrolytic enzymes to produce soluble sugar and support seedling growth. Relative water content (RWC) is another important physiological parameter related to salt tolerance. Relative water content is the measurement of maximum amount of water that plants leaf can take up under full turgid condition and hence it is considered as an appropriate determinant of plant water status under stress. Relative water content is efficient over leaf water potential which estimates the plant water status only during conduction between soil-water-atmosphere interface, but it does not take osmotic adjustment (OA) under consideration. OA is an influential mechanism for conservation of cellular hydration status under water scarcity. Hence RWC is most crucial in estimating water status as it accounts both OA and leaf water potential experienced by plants at early seedling stage (Suriya-arunroj et. al. 2004). So, the salt-tolerant cultivars could be selected on the basis of their ability to maintain the higher RWC during initial stages of salt stress.

Materials and methods

Seed sample collection

Matured seeds (caryopses) of fifteen local rice cultivars viz., Amalmona (AMA), Chinigura (CHI), Dudheshwar (DUD), Gitanjali (GIT), Gobindobhog (GOB), Harinakhuri (HAR), Kalojira (KAL), Kanakchur (KAN), Kartick khas (KAR), Khas dhan (KHA), MTU-1153 (MTU), Radhunipagal (RAD), Sabita (SAB), Satabdi IET-4786 (SAT), Sukumar C (SUK) were collected from two research stations i.e., Bidhan Chandra Krishi Vishwavidyalaya, Mohanpur, Nadia, West Bengal, and Indian Council of Agricultural Research (ICAR), Dighirpar, Canning, West Bengal. The collected mature seeds were categorized into two subtypes, viz., aromatic and high yielding cultivars.

Growth condition for seedling

For biochemical assessment at seedling stage, seeds were surface sterilized with 1% sodium hypochlorite solution and imbibed in distilled water for 24 h. Then germinated seedlings were raised in soil up to 14 days. Seedlings were maintained in natural conditions (Rice experimental field, University of North Bengal, attitude 26°84' North, longitude 88°44' East) at $28 \pm 1^\circ\text{C}$, 70-80% relative humidity and 12h day/night photoperiod in the month of July 2022. For salinity treatment, aqueous solution (200 mM) of NaCl was prepared. Soil was saturated with NaCl solution at 7 days' stage. Control sets were treated with distilled water.

Salinity stress indices

Shoot and root phyto-toxicity was calculated according to the formula described by Asmare 2013. Relative water content was measured using procedure described by Suriya-arunroj et. al. 2004. Endosperm utilization was calculated by method used by Blum et. al. 1994.

Biochemical procedures

For assessment of chlorophyll content, the method described by Arnon was followed. Protein content was estimated as described by Lowry et. al. For starch and total soluble sugar, the method by Hedge et. al. (1962) was followed. Proline content was estimated as described by Bates et. al. (1973). Endosperm utilization efficiency (EUE) was calculated according to Blum et. al. (1994). Shoot and root Phytotoxicity was calculated as described by Asmare et. al. (2013).

Results and Discussion

Table 1. Morphological indices of different cultivars under salinity stress

Cultivar	Treatment	Germination (%)	Shoot length (cm)	Root length (cm)
Kalojira	Control	86.67 ^f ± 3.53	8.08 ^{kn} ± 0.12	8.9 ^{eg} ± 0.03
	NaCl	61.33 ⁿ ± 1.33	4.58 ^p ± 0.18	3.31 ^{mn} ± 0.48
Kanakchur	Control	93.33 ^e ± 1.33	12.26 ^{eg} ± 0.12	12.58 ^b ± 0.15
	NaCl	50.67 ^p ± 1.33	6.18 ^{mp} ± 0.30	3.5 ^{mn} ± 0.18
Harinakhuri	Control	88.00 ^{ef} ± 2.31	12.28 ^{eg} ± 0.82	7.74 ^{tj} ± 0.12
	NaCl	54.67 ^o ± 1.33	7.16 ^o ± 0.17	1.94 ⁿ ± 0.20
Gobindobhog	Control	81.33 ^{gh} ± 3.53	10.26 ^h ± 0.63	9.56 ^{df} ± 0.44
	NaCl	40.00 ⁿ ± 2.31	4.38 ^p ± 0.51	6.26 ^l ± 0.33
Kartick khas	Control	82.67 ^g ± 3.53	9.24 ^k ± 0.64	9.4 ^{ef} ± 0.20
	NaCl	76.00 ^j ± 2.31	6.82 ^o ± 0.60	9.02 ^{eg} ± 0.86
Khas dhan	Control	90.67 ^d ± 3.53	9.6 ^k ± 0.31	9.88 ^{dc} ± 0.25
	NaCl	76.00 ^j ± 2.31	5.6 ^{op} ± 0.20	4.72 ^{lm} ± 0.09
Sabita	Control	96.00 ^b ± 4.00	21.64 ^a ± 0.73	12.98 ^b ± 0.25
	NaCl	65.33 ^m ± 1.33	13.22 ^{df} ± 0.49	10.38 ^{ec} ± 0.37
Gitanjali	Control	94.67 ^{bc} ± 1.33	13.52 ^{df} ± 0.63	11.96 ^a ± 0.19
	NaCl	64.00 ^m ± 2.31	6.62 ^{mo} ± 0.36	10.16 ^{bd} ± 0.28
Amalmona	Control	89.33 ^{de} ± 1.33	19.44 ^b ± 0.54	18.84 ^b ± 0.61
	NaCl	76.00 ^j ± 2.31	13.96 ^{dc} ± 0.50	11.32 ^f ± 1.47
Dudheshwar	Control	98.67 ^a ± 1.33	16.94 ^c ± 0.54	12.76 ^b ± 0.61
	NaCl	80.00 ^h ± 2.31	14.5 ^d ± 0.21	7.8 ^{il} ± 0.15
Chinigura	Control	86.67 ⁱ ± 3.53	11.72 ^{fh} ± 0.24	12.48 ^{ef} ± 0.33
	NaCl	69.33 ^l ± 1.33	3.42 ^p ± 0.48	5.82 ^{gk} ± 0.11
Radhunipagal	Control	82.67 ^g ± 1.33	11.08 ^g ± 0.56	9.3 ^l ± 0.53
	NaCl	72.00 ^k ± 2.31	5.38 ^{op} ± 0.38	7.32 ^{ef} ± 0.23
MTU-1153	Control	78.67 ^h ± 3.53	13.9 ^{dc} ± 0.42	5.88 ^{gk} ± 0.19
	NaCl	49.33 ^p ± 3.53	9.56 ^k ± 0.58	6.9 ^l ± 0.79
Satabdi IET-4786	Control	86.67 ⁱ ± 5.33	7.7 ^{kp} ± 0.23	7.7 ^{hk} ± 0.15
	NaCl	54.67 ^o ± 5.81	5.4 ^{op} ± 0.17	5.4 ^{tj} ± 0.21
Sukumar C	Control	76.00 ^j ± 2.31	8.68 ^{il} ± 0.73	8.68 ^{kl} ± 0.52
	NaCl	64.00 ^m ± 2.31	5.82 ^{op} ± 0.59	5.82 ^{il} ± 0.24

Note: Values are mean ± SE of three independent determinant

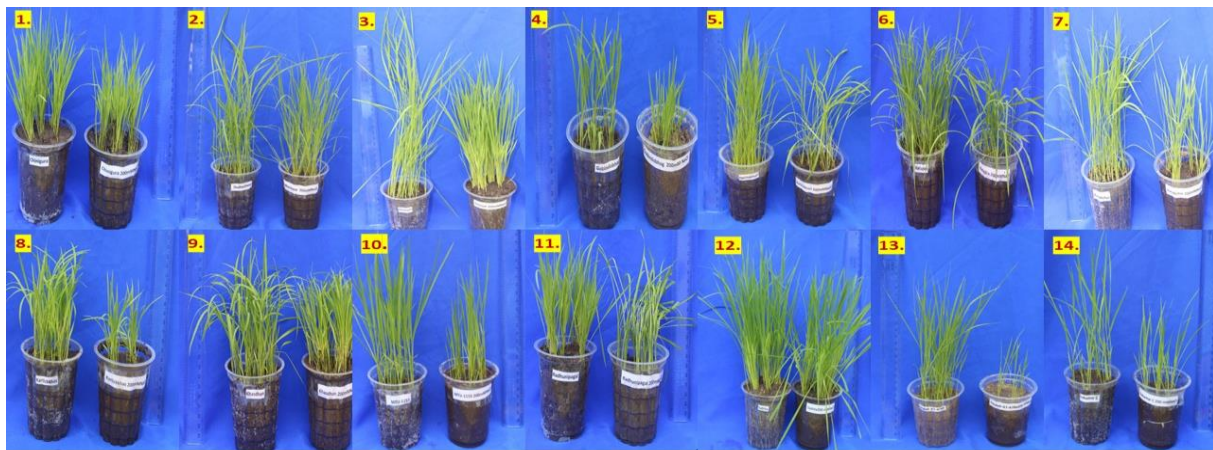


Fig 1: Salinity tolerance of different rice cultivars (1) Chinigura (2) Dudheshwar (3) Gitanjali (4) Gobindobhog (5) Harinakhuri (6) Kalojira (7) Kanakchur (8) Kartickkhas (9) Khasdhan (10) MTU-1153 (11) Radhunipagal (12) Sabita (13) Satabdi (14) Sukumar-C

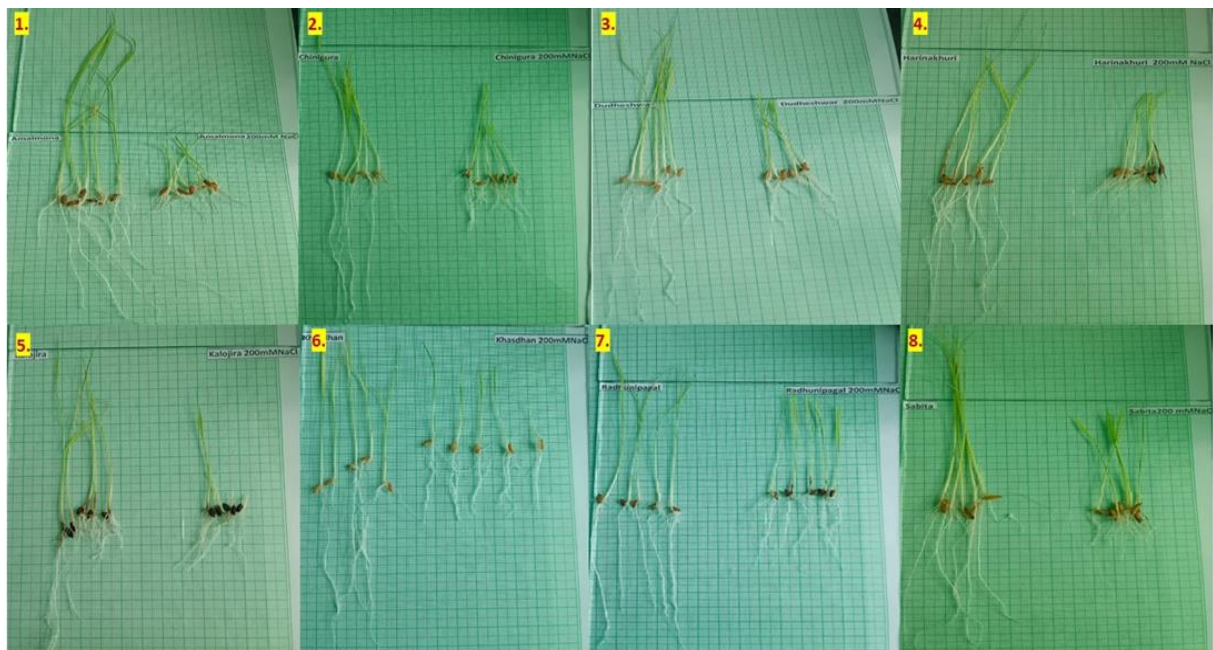


Fig 2 : Salinity treatment in 14 days seedling stage-(1) Amalmona (2) Chinigura (3) Dudheshwar (4) Harinakhuri (5) Kalojira (6) Khasdhan (7) Radhunipagal (8) Sabita

The salinity-induced decline in growth may be due to the creation of osmotic stress that inhibits transport and absorption of water as shown by salt-sensitive cultivar chinigura (CHI), kanakchur (KAN), harinakhuri (HAR) (Table 1 & Fig. 1). Considering the proline content, it is shown to be highest in kanakchur (KAN). As proline is a stress marker, most of the energy get utilized in survival mechanism and less energy is allocated for growth purpose. As a result of which reduced root and shoot length can be seen as represented in Fig. 2. It has been reported that proline not always make reduction in growth but also provides nitrogen source to overcome the saline toxicity and support growth as shown by cultivars amalmona (AMA), sabita (SAB) and kalojira (KAL) supported by result of Cha-Um (2009). The remaining cultivars do not show any

significant changes in proline content. If we consider the chlorophyll content, it is severely affected during salinity imposition. Salinity stress generates reaction species that cause oxidative damage to chlorophyll pigment, thereby reduce the photosynthetic potentiality according to the report of Gharsallah (2016). To cope up with this deadly photo-oxidative damage, plants possess a diverse phenolic group of non-enzymatic anti-oxidant molecules, that function in neutralizing free radicals and detoxifying its effect up to a certain level supported by results of Yan (2022). Cultivars MTU-1153, satabdi (SAT), amalmona (AMA) were found to have quite higher phenol content, could scavenge the reactive oxygen species (ROS) molecules and maintain the pigment component to a static level. As we know that starch, the ultimate storage product of vital metabolic

Table 2. Stress tolerance indices of different rice cultivars

Cultivar	Treatment	Relative water content (%)	Endosperm utilization efficiency (EUE)	Shoot-phytotoxicity	Root-phytotoxicity
Kalojira	Control	69.7	73.2	43.3	62.8
	NaCl	62.5	56.1		
Kanakchur	Control	84.4	88.9	49.6	72.2
	NaCl	80.9	60.5		
Harinakhuri	Control	80.9	45.7	41.7	74.9
	NaCl	71.4	60.9		
Gobindobhog	Control	73.9	79.2	57.3	34.5
	NaCl	43.8	58.9		
Kartickkhas	Control	83.9	85.7	26.2	4
	NaCl	63.6	56.3		
Khasdhan	Control	80.6	87.9	41.7	52.2
	NaCl	90.9	58.2		
Sabita	Control	81.8	88.0	38.9	20
	NaCl	78.9	69.6		
Gitanjali	Control	87.3	93.2	51	15.1
	NaCl	66.3	81.2		
Amalmona	Control	88.7	86.3	28.2	39.9
	NaCl	79.2	76.1		
Dudheshwar	Control	73.7	81.7	14.4	38.9
	NaCl	70.0	70.9		
Chinigura	Control	83.8	65.7	70.8	53.4
	NaCl	48.6	43.8		
Radhunipagal	Control	71.4	77.8	51.4	21.3
	NaCl	50.0	54.2		
MTU-1153	Control	64.5	84.2	31.2	17.3
	NaCl	59.3	69.3		
Satabdi-IET 4786	Control	78.2	71.5	29.9	6.8
	NaCl	74.6	60.2		
Sukumar C	Control	67.5	70.5	32.9	2.7
	NaCl	63.5	59.6		

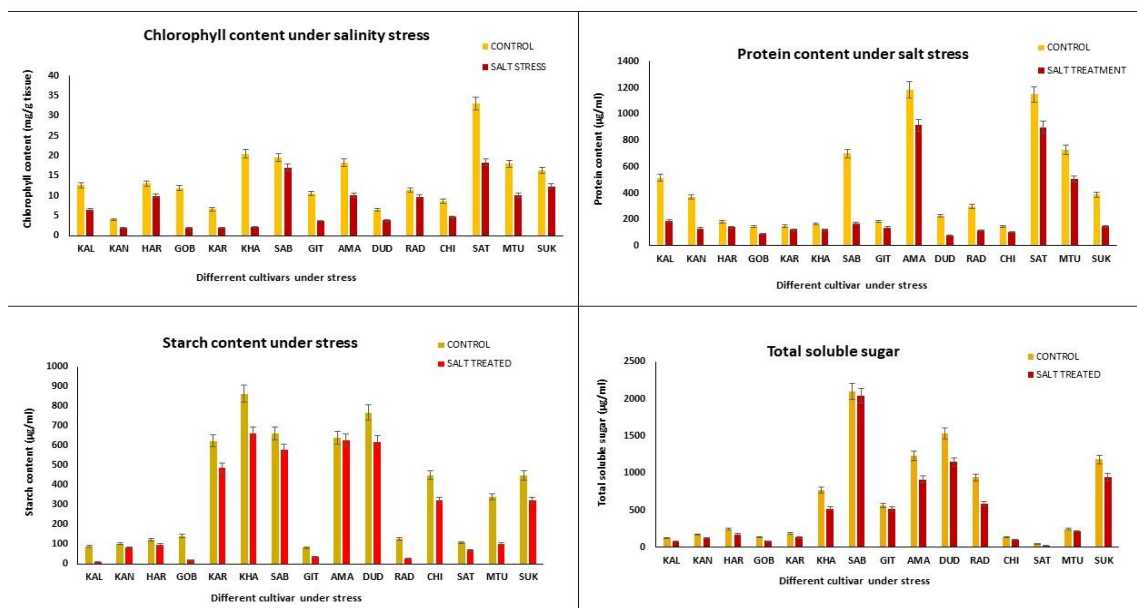
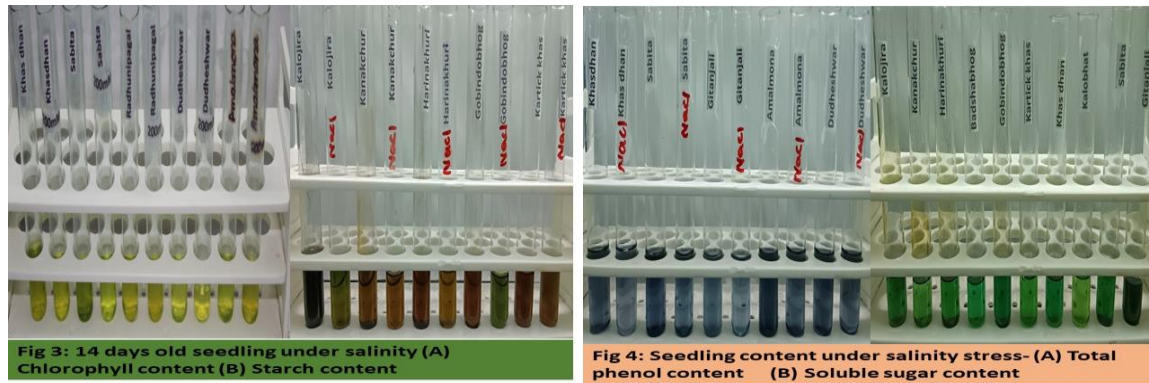


Fig 5: Biochemical attributes of seedling of different cultivars under salinity stress

process photosynthesis, the accumulation of starch is directly related to the principal pigment chlorophylls (chl-a and chl-b) as represented in Fig. 3 & 5. Direct proportionality between chlorophyll pigment and starch product can be seen in cultivars khasdhan (KHA), amalmona (AMA), MTU-1153, satabdi (SAT); The remaining cultivars represent moderate changes under saline condition. Upon endo-hydrolytic enzyme activity, stored starch gets hydrolyzed into soluble form and mobilized into the germinating seeds to support its seedling growth. Such higher level of soluble sugar can be observed in case of cultivars sabita (SAB), dudheshwar (DUD), amalmona (AMA) and khasdhan (KHA) (Fig. 4 & 5). But this carbohydrate mobilization can be severely affected by salinity stress and negatively influence the seedling growth as shown by cultivars kalojira (KAL), gobindobhog (GOB), chinigura (CHI). Endosperm content utilization efficiency was shown to be reduced in salt sensitive cultivars as documented in Table 2.

Conclusion

In the present study, salt treatment resulted in the reduced level of chlorophyll, protein and soluble sugar content. This happens perhaps due to the generation of excess ROS, which may lead to the oxidative degradation of chlorophyll pigment and ultimate accumulation of less photo-assimilates during photosynthesis. In our study proline content is significantly increased under salt-treatment, a key indicator representing salt-tolerance capabilities among different rice cultivars. Furthermore, our finding demonstrated a positive interdependent correlation between chlorophyll content and dry matter accumulation.

Conflicts of interest

There are no actual or potential conflicts of interest to declare.

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