IJPSR (2023), Volume 14, Issue 12 **(Review Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 May 2023; received in revised form, 20 July 2023; accepted 28 July 2023; published 01 December 2023

COMPARATIVE PHYSIOLOGICAL AND MOLECULAR RESPONSES OF A COMMON AROMATIC INDICA RICE CULTIVAR TO HIGH SALINITY WITH NON-AROMATIC INDICA RICE CULTIVAR

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Keywords:

Salinity, Anti-oxidant, Aromatic, Chlorophyll, Peroxide

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ABSTRACT: With a threshold of 3 DSM^{-1}, rice is one of the crops that are most sensitive to salt. Salinity in agricultural areas is primarily brought on by the overuse of irrigation water that is overly salty. The early vegetative and seedling stages of rice are the most vulnerable to salt stress, followed by the reproductive phases. When osmotic stress inhibits root water uptake, too much salt in the soil hurts plant growth, development, and productivity. Direct salt buildup impairs metabolic functions and all key morphological-physiological and yield-related features, drastically reducing yield. To investigate the cellular basis of the response to salt stress, extensive analysis involving physiological or biochemical assays and gene expression studies under high salt regimes is reviewed and contrasted with an aromatic salt-sensitive (Chinigura) and non-aromatic salt tolerant (IET 4786) rice variety. The detrimental effects of salinity stress were most evident in Chinigura, where the root-to-shoot ratio was higher, chlorophyll degeneration was more severe, foliar concentrations of Na^+ ions were higher, and peroxide content increased most after salt treatment. Following salt stress, catalase activity occurred in all types. In contrast, antioxidants were most abundant in salt-tolerant plants, which suggests that salt-tolerant genes are less expressed in aromatic rice.

INTRODUCTION: Among all the abiotic stress (such as high salinity, drought, flood, and high and low temperature). Salt stress is one of the most harmful environmental factors impacting crop development and productivity. According to Hagemann and Erdmann (1997), Na^+ is the main harmful ion that causes ionic and osmotic toxicity in salinity soils. Maintaining low levels of Na^+ in the cytoplasm is crucial for survival during saline stress. The low plant survival rate has been associated with too much Na^+ in the apoplast [1].

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.14(12).5587-94

This article can be accessed online on
www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.14(12).5587-94

Reactive oxygen species like superoxide, hydrogen peroxide and hydroxyl radicals, which are scavenged by both enzymatic and non-enzymatic processes, are produced at higher levels, causing increased lipid peroxidation, membrane damage, ion imbalances brought on by the accumulation of Na^+ and Cl^-, and ion imbalances caused by the buildup of Na^+ and Cl^-, salt stress severely inhibits the growth and development of plants.

Soil salinity is a complicated physiological and phenotypic phenomenon in plants [2]. Additionally, salt negatively affects plant metabolism and gene expression, which can result in the accumulation or depletion of specific metabolites and cellular proteins [3]. F_v/F_m (maximum quantum yield of PSII) was, however, almost not impacted by salt stress rice cultivars comparable in salinity tolerance, but qN (non-photochemical coefficient) rose in sensitive cultivars as salt stress increased. Salinity significantly reduces SOD (Superoxide) activity in rice seedlings with variation between cultivars[4].In general, rice can tolerate a small amount of salt water without compromising its growth and yield depending on the type and species of rice and their growth stage. Because of their antagonistic effect, more Na^+ in the zone of root limits the uptake of K^+ which reduces plant development. Chlorosis and necrosis in plants are caused by ionic stressors, which either speed up senescence or hinder growth and development [5]. The stages of seedling and reproductive growth are often the most vulnerable to salt stress [6]. The reproductive stage affects yield and quality the most [7]. Panicle sterility is regarded as a significant risk during the rice reproductive stage when there is salt stress [8]. Salt stress decreased the relative water content of leaves. Crop productivity is significantly hampered staged by abiotic salinity stress. Typically, a saline soil is a reservoir of various soluble salts, including Na^+, Ca^{2+}, $Mg^{2+,}$ and anions Cl^-, HCO^{3-}, SO_4^{2-} with particularly high levels of CO_3^{2-}, K^+, and NO^{3-}, When the soil's EC (electrical conductivity) is 4dS/m or greater (about 40mM NaCl), together with an osmotic pressure of 0.2 MPa, the soil is considered to be saline [9].

TABLE 1: CLASSES OF SALINITY AND EC (DS/M) TAKEN FROM NRCS SOIL HANDBOOK

Sl. no.	EC(Electrical Conductivity)	Salinity Class	Crop response
1	0<2	Non-saline	The impact of salinity is almost nonexistent.
2	2<4	Very slightly saline	Reduction in the yield of very sensitive crops.
3	4<8	Slightly saline	Most crops' yields have decreased.
4	8<16	Moderately saline	Only tolerant plants produce sufficient yields.
5	≥16	Strongly saline	Yields that are satisfactory for very low-tolerant crops.

The osmolytes, which are low molecular weight, are osmotically active molecules that build up in the cytosol to lower the osmotic potential inside the cell through a process known as an osmotic correction, are an essential component of maintaining homeostasis in a stressful scenario (OA). Simple compounds among them are inositol, polyols, sugar alcohols, complex sugars, quaternary amino substances such as glycine-proline, betaine the triamine, and higher polyamines (PAs) tetramine spermine (Spm4+) and spermidine (Spd3+).

In a research performed by Hasegawa *et al* in 2000 it is noticed that when there is a water shortage then osmoprotectants serve by preserving membrane structure, play an antioxidant role, and act as free radical scavengers acting as inhibitors of lipid peroxidation or as controllers of K+ receptors in the stomata. The salt-challenged plant's stress produces the plant hormone abscisic acid as well. (ABA) as a corrective action to lessen transpiration by stomatal closure [10]. Rice's sensitivity to salt stress varies greatly genetically. The higher endogenous ABA levels in Indica varieties like IR-48 and IR-29 during osmotic shock are classified as highly salt tolerant. By the research of Moons *et al.* in 1995, applying ABA to plants, an important phytohormone, the effects of stress are lessened (s). It has long been known that an increase in this hormone is brought on by a shortage of soil moisture around the root. The production of ABA in shoots and roots is increased by osmotic stress and water shortage brought on by salt stress. The production of ABA can lessen the negative effects of salinity on photosynthesis, growth, and assimilate translocation. The buildup of K+, Ca+2, and compatible solutes in root vacuoles, which is counterbalanced by the absorption of Na+ and Cl-, is thought to be at least partially responsible for the positive association between ABA accumulation and salinity tolerance.

Several salt and water-responsive genes have their expression altered by the important cellular signal ABA [11]. Rice is a traditional crop in most parts of the countries even it has a significant role in medicinal properties due to its anti-oxidation, anti-tonic, and anti-inflammatory activity.

Aromatic rice has a priceless diversity of genetic varieties in the state of West Bengal. Due to their superior quality attributes and lovely aroma, Gobindobhog, Tulaipanji, Kataribhog, *etc.* are among them and well-liked in domestic markets. There are very few documents about the salt-tolerant biochemical parameters of aromatic rice.

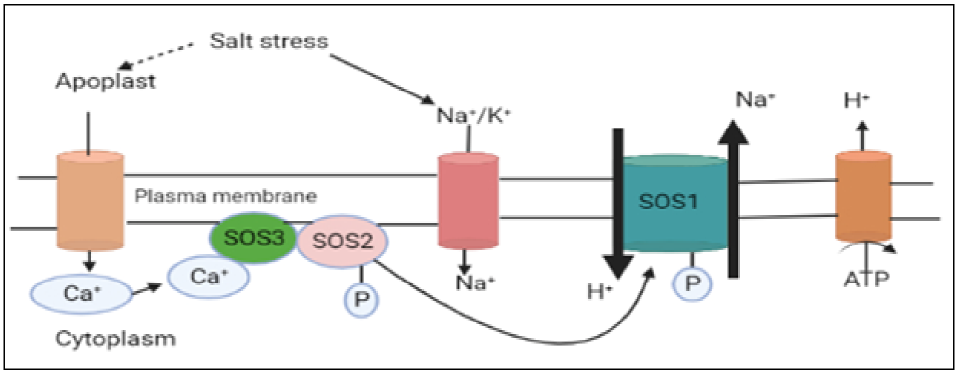

FIG. 1: SOS PATHWAY MODEL FOR SALINITY STRESS RESPONSE

Effect of Salinity-stress on Rice Cultivar: Rice farming has long faced challenges from salt in the soil. Rice has often been farmed on salty soils along the shore. Rivers, canals, streams, and other surrounding water bodies are frequently contaminated by salt. The bulk of rice farming has relied on irrigation water since the start of the green revolution, and salt intrusion in coastal regions has lately become a common occurrence. In general, rice can tolerate a little amount of saltwater without experiencing negative effects on growth and production. However, a lot relies on the varieties and species of rice as well as their stage of development [12].

Rice is categorized as a cereal that is sensitive to salinity in its early stages of growth, which reduces its ability to produce when it reaches maturity [13]. For rice, soil salinity above EC 4 dS m^{-1} is regarded as moderate salinity, while salinity above EC 8 dS m^{-1} is high, according to IRRI. Ionic toxicity and osmotic stress were both brought on by too much salt in rice plants. When exposed to high salinity, rice plants exhibit a variety of morphological, physiological, or biochemical changes and symptoms, and in extreme cases, they may even die. Plants are directly damaged by sodium ions, and a larger concentration of sodium ions in the root zone restricts the uptake of potassium ions due to their antagonistic effects. Because K$^+$ is essential for maintaining membrane potential, enzyme activity and cell turgor, a lack of K$^+$ inside the cell inevitably results in a reduction in plant development. Some cations, including Cl$^-$, exhibit toxicity in rice in addition to Na$^+$ or other anions. Chlorosis and necrosis, which are brought on by ionic stress, can speed up senescence or hinder growth and development. During NaCl salinity, cellular metabolisms such as protein synthesis and enzyme activity are hindered, which affects source-sink interactions and photosynthesis. Because multiple studies have shown that Na$^+$ buildup in shoots is closely related to rice plant survival under salinity stress, maintaining a lower cytosolic Na$^+$ concentration is regarded to be one of the key methods for salt tolerance in glycophytes [14].

Response at Morpho- Physiological and Biochemical Level: The weights and lengths of roots and shoots, both fresh and dried, were drastically reduced by high NaCl concentrations. In water culture as opposed to soil culture, NaCl had a quicker effect.

Root growth was more severely reduced in wet cultures than in shoot growth. According to a study using electron microscopy, the impacts of NaCl included the growth of thylakoids, the accumulation of starch grains and lipid droplets, the distortion of grana stacking, the increase in the size and number of plastoglobuli, and the vesiculation of cellular membrane. The mitochondrial cristae in salt-treated plants developed defects and the matrix turned pale in comparison to control plants [15]. Therefore, it is possible to understand how salt stress affects photosynthetic machinery by looking at changes in chlorophyll concentration, the fluorescence of chlorophyll and membrane permeability [16]. When salt accumulation reaches a dangerous level, ionic stress sets in, which can be classified as the second stage of the salinity effect on crop plants. The initial osmotic impact in plants is defined as the lowered water potential to the increased salt concentration with increased osmotic potential. One of the main plant defenses is the osmotic adjustment that plants make by collecting large concentrations of inorganic ions or low molecular weight organic solutes [17].

Proline, which is widely distributed in higher plants, assimilates more readily in salt-stressed plants, according to studies [18]. Rice has been found to accumulate proline, which perhaps contributes to osmotic correction, protecting membranes and enzymes, and providing energy and nitrogen for use when exposed to salinity [19]. According to the buildup of glycine betaine in rice masks the harmful effects of salt. These substances are said to have a crucial role in osmotic regulation, cellular macromolecule fortification, and nitrogen storage. They are necessary to detoxify cells, scavenge ROS species, and preserve cellular pH homeostasis [20]. Altering or accumulating protein levels is another strategy for combating salt. De -novo protein synthesis or an up-regulation of the process in response to salt may cause the concentration of certain proteins that are already present in the plant to increase [21]. In plants cultivated in saline circumstances, proteins build up and serve as a sort of nitrogen store that is used again when stress is not present [22]. The osmotic correction will also be actively aided by protein synthesis in the future. In comparison to sensitive rice seedlings, tolerant rice seedlings have been found to have a considerable increase in soluble protein content and a favorable connection [23]. The ability of plants to feel the initial osmotic stress or osmo-sensing triggers their reaction to counteract salt stress. The control of the Na^+/K^+ ratio at the genetic level and the regulatory system alone provided the basis for understanding the triggered signaling pathway in response to this stress, even though the Na^+/K^+ ratio may be quantified phenotypically to measure changes occurring at the cellular or tissue level [23]. Previous research on the mechanisms of salinity tolerance showed the intricacy of salinity as abiotic stress and that plants' responses to this stress vary not only between species but also between varieties, particularly when it comes to rice as a crop [24].

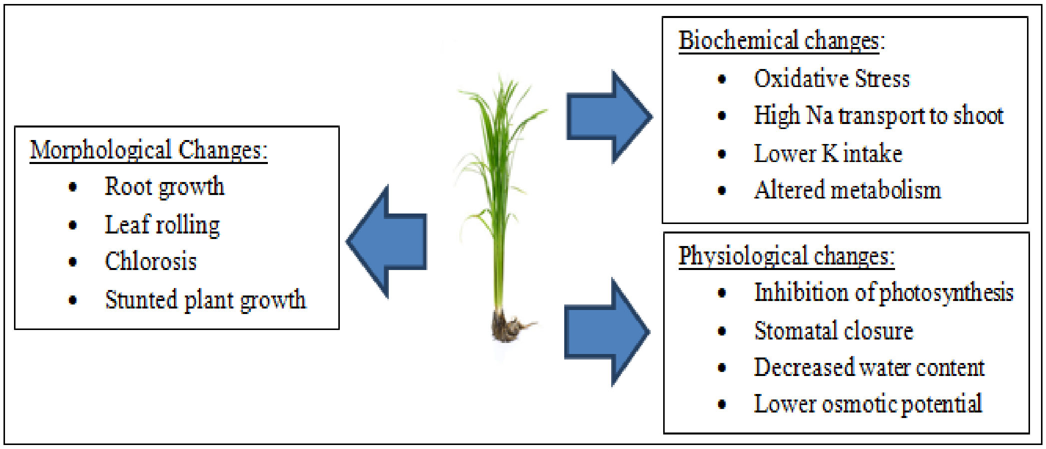

FIG. 2: THE ADVERSE EFFECT OF SALT STRESS ON PLANT GROWTH

The Defense Mechanism of Rice against Saline Stress: The three processes that make up a plant's defense mechanisms against the toxic effect of salt stress are a) tolerance to osmotic stress through osmotic adjustment; b) Na^+ exclusion from leaf blades by selective ion uptake and also the regulation of sodium ion uptake at the molecular level; and c) tissue tolerance, or tolerance of tissue to accumulated Na^+, or in some species, to Cl^- *via* compartmentalization of absorbed or accumulated salt [25, 26]. Plants are also prone to oxidative stress, which is primarily brought on by salinity's inhibition of photosynthesis. Along with ionic stress and osmotic stress, this stress exists. The heat-induced breakdown of the xanthophyll pigments and the production of reactive oxygen species (ROS) from electron transfer to oxygen acceptors rather than water are two examples of how plants modify their metabolic pathways in preparation for the approaching photo-inhibitory impacts (reactive oxygen species). However, the latter reaction is lessened by the activation of multiple regulatory enzymes, including catalase, ascorbate peroxidase, superoxide dismutase, and other peroxidases [27–29]. The degree of oxidative damage is determined by how long it takes for ROS to develop and then be removed by the body's antioxidative scavenging mechanism. Additionally, the functional antioxidant system has been seen in the roots of rice cultivars with varying salt tolerance [30, 31].

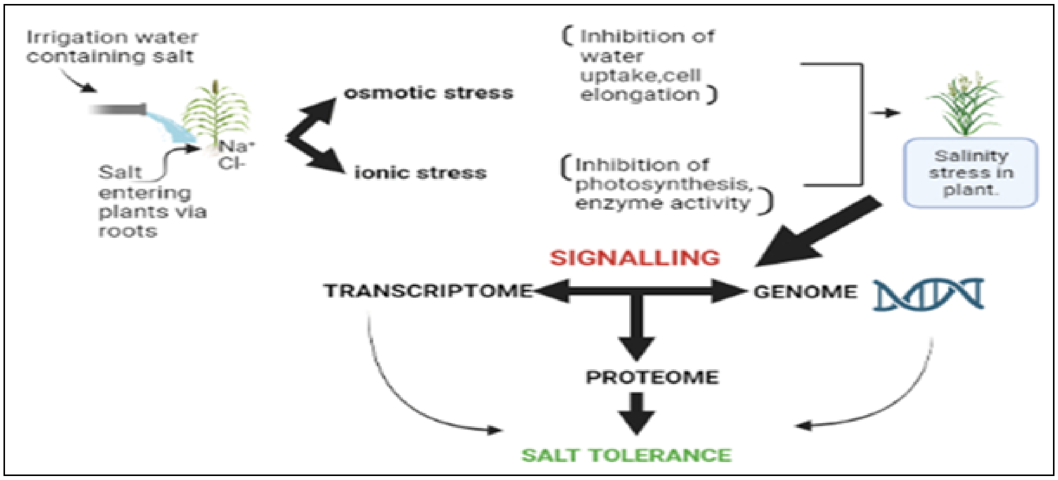

FIG. 3: PATHWAYS OF SALT STRESS TOXICITY AND DIFFERENT PLANT TOLERANCE TECHNIQUES [32]

Screening to Prevent Salinity Stress: One of the elements influencing salt tolerance phenotypes is differential salinity sensitivity at different growth stages. Rice plants are often particularly susceptible to salinity while they are in young seedling stages and less sensitive when they are in the reproductive stages [33]. A suitable screening technique is needed to evaluate how germplasms react to salinity. When paddy is in either of its two salt-sensitive phases, screening can be done. Screening at the seedling stage is a quick procedure that uses straightforward criteria. Screening might be carried out in a lab setting or on the ground. The former is challenging because of the soil's heterogeneity, as well as climatic and other external conditions that may interfere with physiological processes. Three levels of the plant's development namely, the growth, development, and pleiotropic effect are altered in response to salt stress and this needs to be properly investigated. The measures referred to as effective salinity indices, such as root length, shoot length, plant biomass, and shoot Na^+/K^+ ratio, can be used for morphological screening [34]. However, because agronomic characteristics are so strongly impacted by the environment, it may not be possible or fruitful to screen for salinity tolerance using these criteria alone. The understanding of the processes underlying salt tolerance as well as the evaluation of salt tolerance is projected to become much more precisely defined through genotype screening at the biochemical level, as many scientists have done. It gave a general understanding of the potential markers of plant, tissue, or cellular salt tolerance. It is also known how to identify the metabolic processes that are impacted by salinity stress and, conversely, how plants escape salinity stress. The most modern methods for comprehending how rice reacts are based on molecular screening for salt tolerance [35].

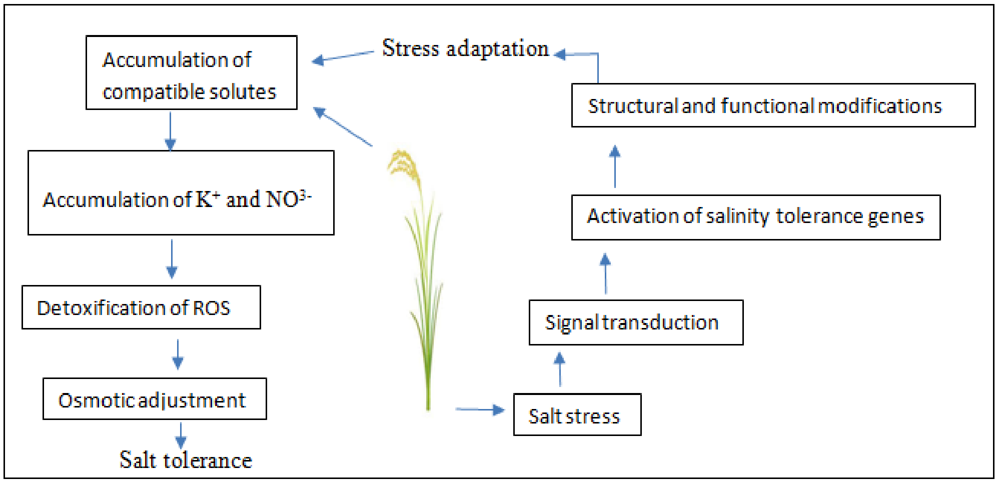

FIG. 4: SALT STRESS SIGNALS CONNECT TO THEIR PARTICULAR RECEPTORS AND START THE PHYSIOLOGICAL AND MOLECULAR PROCESSES THAT ALLOW A PLANT TO SURVIVE UNDER STRESSFUL CIRCUMSTANCES [32]

Comparative Analysis: Due to its high aroma and flavor, aromatic rice has increased demand among the population. The majority of these high-quality aromatic rice genotypes have limited yield potential because of their susceptibility to stresses both biotic and abiotic. There are very few examples of high-quality aromatic rice being connected to salt tolerance [36]. The body's reaction to salt stress is complicated, starting with instantaneous osmotic stress that is shown by decreased water intake, decreased growth retardation, and cell expansion. Tolerant cultivars probably adjust their stomatal apertures and produce compatible solutes in response to this initial stage of salt stress [37].

Maintaining a reduced shoot Na^+ level is a typical strategy for salt tolerance in rice. This can be accomplished through sodium exclusion, effective harmful salt sequestration in older leaves and roots, compartmentalization, and Na^+ extrusion into vacuoles and out of cells. Despite these shared physiological patterns in tolerant rice cultivars in response to salt stress, damage scores and the level of Na^+ in the seedling vary widely. There is a case to be made for the independent study of several tolerant genotypes because they could have cutting-edge ways of avoiding salt stress [38].

The addition of sorbitol and Tre enhanced the tolerance of the salt-stressed rice plant (*Oryza sativa* L.) to oxidative stress by lowering H_2O_2 generation, lipid peroxidation, and membrane electrolyte leakage [39]. According to Li *et al.*, pretreatment of rice seedlings with ABA increased their ability to withstand salt stress because it provided an ample supply of energy and promoted the active anabolism of nitrogen, nucleotides, and carbohydrates. By lowering Na^+ and Cl^- concentrations, the Na^+/K^+ ratio, and raising K^+ and Ca^{+2} contents, seed treatment with ABA may have helped to mitigate the effects of salt stress damage [40]. According to Uchida *et al.*, pretreatment with NO increased the activity of antioxidant enzymes such SOD, CAT, and APX, and as a result, NO reduced salt stress-related damage to rice seedlings [41]. Exogenous Spd and Spm supplementation were found to reduce growth inhibition, and cellular damage, decrease H_2O_2, MDA, LOX activity, protein oxidation, and protease activity, maintain K^+/Na^+ balance, modulate osmolytes, and enhance the activity of antioxidant enzymes in various rice cultivars by conferring salt stress tolerance (GPX, APX, and CAT). However, salt-sensitive rice cultivars such as M-1-48 (sensitive to salt), Nonabokra (tolerant to salt) and Gobindobhog (extremely sensitive) reacted better to exogenous PAs treatment under salt stress than salt-tolerant cultivars [42]. In a study, Konakci *et al.* investigated the impact of exogenously administered GA on the susceptibility to salt (NaCl) stress of two rice cultivars, the sensitive IR-28, and the tolerant Pokkali. Salt stress decreased the real quantum yield (PSII), photochemical quenching coefficient (qP) and maximal photochemical efficiency (Fv/Fm) ratio of two rice cultivars; however, the effect was more pronounced in IR-28. Additionally, lipid peroxidation and a considerable rise in H_2O_2 concentration were seen after NaCl treatment. Compared to plants in Pokkali, IR-28 plants were more susceptible to salt stress. In contrast to salt stress alone, adding GA to NaCl-stressed Pokkali rice resulted in a significant decrease in H_2O_2 and thiobarbituric acid reactive substances (TBARS) levels, an improvement in the activities of SOD, CAT, APX, and POX, as well as an increase in relative growth rate (RGR), osmotic potential, Fv/Fm ratio, and Pro level. Therefore, it can be claimed that GA (gallic acid) can increase antioxidant activity and photosynthetic efficiency, which can lessen the toxicity of NaCl to rice plants [43]. So, these are a few approaches tried by different authors to combat salt stress in distinct types and overcome this difficult abiotic stress for good yield and plant development.

ACKNOWLEDGEMENTS: Nil

CONFLICTS OF INTEREST: Nil

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How to cite this article:
Jha V and Choudhury B: Comparative physiological and molecular responses of a common aromatic Indica rice cultivar to high salinity with non-aromatic Indica rice cultivar. Int J Pharm Sci & Res 2023; 14(12): 5587-94. doi: 10.13040/IJPSR.0975-8232.14(12).5587-94.