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Screening of Rice Genotypes for Salinity Tolerance Using Salinity Induction Response (SIR) Technique

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ABSTRACT

Rice (*Oryza sativa* L.) is among the cereal crops most severely affected by soil salinity, which greatly hinders its cultivation in regions with high salt levels. The present study tested the tolerance of eighteen different rice genotypes under controlled *in-vitro* conditions using the Salinity Induction Response (SIR) method to investigate how rice adapts to adverse conditions. The effects of salinity on root and shoot growth, as well as the plants' recovery from the stress, were closely examined. Significant genotypic responses were observed under both gradual and sudden lethal exposure to salt. Dhaksha and Gen_254 exhibited moderate growth reductions but the highest recovery capacities (root: 75% and 44%; shoot: 74% and 46%, respectively), classifying them as tolerant. Genotypes 214, 204, and 628 showed intermediate reductions and moderate recovery, while IR64, Gen_85, 618, and 578 displayed severe reductions with recovery below 25%, indicating high susceptibility. These findings suggest that rice plants may be primed for enhanced tolerance through the activation of adaptive physiological mechanisms through gradual salt exposure. Overall, the results show that the SIR method is a useful strategy for identifying salt-resilient rice genotypes, offering insightful information for breeding initiatives meant to improve crop performance in saline conditions.

Keywords : Rice, Salinity Induction Response, Salinity Tolerance, Root & Shoot Growth

RICE (*Oryza sativa* L.) is a staple food grain crop around the world. It provides the majority of the calories and diet for more than half of the world's population. Improving rice yield is crucial to preserving global food security in light of the world's constantly expanding population. Despite its significance, rice is extremely sensitive to salinity, which affects its overall productivity, growth and physiological processes. Soil salinization, intensified by climate change and unsustainable irrigation practices, poses a major abiotic constraint to rice cultivation, particularly in salt-affected regions such as India, where approximately 6.73 million hectares about 2.1 per cent of the total land area are impacted, including significant areas in Karnataka (CSSRI, 2020). Salt stress is one of the most significant abiotic threats to sustainable rice production due to rice's high sensitivity to salinity. It is caused by the accumulation of excessive soluble salts, primarily sodium (Na^+) and chloride (Cl^-) ions, in the soil, which disrupts water uptake and ionic balance in plants. It imposes osmotic stress by reducing soil water potential, limiting the plant's ability to absorb water and ionic stress by causing toxic ion accumulation that interferes with nutrient uptake and damages cellular structures. In rice (*Oryza sativa* L.), salt stress leads to stunted growth, chlorosis, reduced tillering and ultimately a decline in yield and grain quality. Studies shows that under moderate to severe salinity stress (6-8 dS/m), rice yields can decline by 40-60%, depending on the cultivar and the level of stress (Xu *et al*., 2024). Zheng *et al*. (2023) revealed that rice yield decreased by up to 65% under salinity levels above 8 dS/m, with a significant reduction in grain filling and quality observed across different rice varieties.

Salinity stress continues to be one of the major challenges facing rice production globally, particularly in coastal regions and irrigated farming systems where soil salt accumulation is frequent. According to Rodríguez *et al*. (2023), excessive salinity disrupts the regular physiological functions of plants, leading to decreased photosynthetic efficiency, stunted growth, leaf yellowing, and ultimately substantial yield reductions or crop loss. By preventing water uptake and disrupting the ionic balance within plant cells, excess salts impair seedling establishment and lower productivity (Ketehouli *et al*., 2019).

Rising sea levels and increasing soil salinisation are expected to exacerbate the problem by expanding the area of salt-affected agricultural land. Therefore, improving rice's ability to withstand saline environments has emerged as a top research priority in order to ensure food production in the future.

Significant progress has been made by virtue of conventional breeding, molecular genetics and modern biotechnological methods. These efforts have enabled the identification and incorporation of genes that govern critical adaptive traits such as ion regulation, osmotic balance and antioxidant defence (Afzal *et al*., 2020). Thus, the creation and spread of salt-tolerant rice cultivars can contribute to long-term food security, stabilise yields and enhance livelihoods in areas that are at risk.

In addition to breeding-based advances, several integrated solutions have been investigated to enhance rice's tolerance to salt. These include gene-assisted breeding, effective irrigation methods, genetic engineering and soil management strategies that reduce salt accumulation. A promising way for assessing tolerance is the salinity-induction response (SIR) method, which gradually exposes seedlings to increasing salt concentrations. The physiological distinction between lethal and gradual salt stress must be understood. Gradual exposure to salt enables plants to start defence responses, such as changing root architecture to access deeper water reserves, whereas exposure to extremely high salt concentrations overwhelms these defence mechanisms and results in irreparable damage. The advantage of SIR over traditional methods lies in its ability to differentiate between tolerant and sensitive genotypes based on their capacity to acclimate progressively to salinity stress, providing a more realistic evaluation of plant performance under field conditions and enabling the identification of mechanisms involved in acquired tolerance. This method thereby offers enhanced sensitivity and relevance for breeding and physiological studies compared to single-dose salt stress assays. The patterns are similar for other crops. For instance, moderate salinity in wheat maintains water balance by promoting osmolyte production and antioxidant activity, while excessive salinity significantly inhibits root growth and biomass accumulation (Fu *et al*., 2023). Similar adaptive responses have been observed in crops such as *Moringa oleifera* and alfalfa, where physiological deterioration occurs as a result of lethal stress and partial recovery is promoted by gradually increasing salinity (Azeem *et al*., 2023).

These findings highlight why gradual stress studies are so valuable-they mimic real-world conditions and reveal how plants build tolerance over time. By combining physiological studies with breeding and modern molecular tools, scientists aim to develop rice varieties that can thrive even in saline soils, ensuring stable production for the growing global population.

Thus, in the present study, an effort was made to standardise the technique of salinity induction response for rice seedlings, which is necessary for evaluating salinity tolerance in rice genotypes at the seedling level.

MATERIAL AND METHODS

Plant Material, Growth Conditions and Stress Treatment

Seeds of sixteen rice germplasm lines, including GEN_RIC_712, GEN_RIC_319, GEN_RIC_498, GEN_RIC_238, GEN_RIC_214, GEN_RIC_254, GEN_RIC_790, GEN_RIC_604, GEN_RIC_584, GEN_RIC_769, GEN_RIC_204, GEN_RIC_628, GEN_RIC_618, GEN_RIC_85, GEN_RIC_81 and GEN_RIC_578 (Table 1) and two varieties (Dhaksha and IR-64) were obtained from the Department of Crop Physiology, University of Agricultural Sciences, Bangalore. The study was conducted at the Department of Plant Biotechnology under laboratory conditions (28 ± 2°C). Different levels (100 mM, 200 mM, 300 mM, and 350 mM) of sodium chloride (NaCl) solution were prepared to induce gradual salinity stress (Fig.1). Healthy seeds of eighteen genotypes were soaked in distilled water for six hours. subsequently, 25 seeds were arranged in Petri plates (90 mm×14 mm) containing filter paper and further kept incubated for germination in the incubator (28 ºC and 60 % RH) for 72 h. The 3-day-old seedlings were then subjected to different treatments, *viz*, control, gradual and lethal (Fig. 1), to evaluate their performance during stress conditions. Three replications for each salt treatment, *i.e.*, control, gradual stress and lethal stress, were maintained.

TABLE 1

List of 16 germplasm lines used for validation of genetic variability

Id	Name: Accession	Gen_Ric No.	Iris Id	Box Code	Sub Population	Country
936	LOCAL:: IRGC 53300-1	GEN_RIC 204	IRIS 313-11478	AN59	ind2	India
1030	AMAKOYALI:: IRGC 60878-1	GEN_RIC 214	IRIS 313-11595	AO83	aus	India
336	ASU::IRGC 62154-1	GEN_RIC 790	IRIS 313-9572	AF24	indx	Bhutan
419	DA NUO (ZHAN):: IRGC 72025-1	GEN_RIC 85	IRIS 313-10189	AG19	ind1A	China
1515	SOM::IRGC 92221-1	GEN_RIC 618	IRIS 313-12228	AV63	subtrop	Lao People's Democratic Republic
1124	GORA DHAN 2:: IRGC 66269-1	GEN_RIC 238	IRIS 313-11712	AQ14	aus	India
1029	CHAN THANH HOA:: IRGC 60647-1	GEN_RIC 769	IRIS 313-11594	AO82	indx	Vietnam
1378	CHAO LAO SOUNG:: IRGC 78799-1	GEN_RIC 604	IRIS 313-11992	AT14	ind3	Lao People's Democratic Republic
1643	HR 22::IRGC 6394-1	GEN_RIC 254	IRIS 313-10531	AY12	admix	India
685	YAHNG LEU::IRGC 78279-1	GEN_RIC 584	IRIS 313-8565	AK49	Subtrop	Subtrop

Continued....

TABLE 1 Continued....

Id	Name: Accession	Gen_Ric No.	Iris Id	Box Code	Sub Population	Country
1980	ARC 13373:: IRGC 22691-1	GEN_RIC 319	IRIS 313-10897	BC4	ind3	India
1563	NGAM KON DAM:: IRGC 98486-1	GEN_RIC 628	IRIS 313-12300	AW42	indx	Lao People's Democratic Republic
290	WP 65::IRGC 36526-1	GEN_RIC 578	IRIS 313-9227	AE64	ind1B	Thailand
335	NX 3533::IRGC 63796-1	GEN_RIC 81	IRIS 313-9570	AF23	indx	China
240	KURULUTUDU:: IRGC 36304-1	GEN_RIC 712	IRIS 313-8925	AE04	ind2	Sri Lanka
1206	MHARAKA:: IRGC 70612-1	GEN_RIC 498	IRIS 313-11809	AR18	aus	Kenya

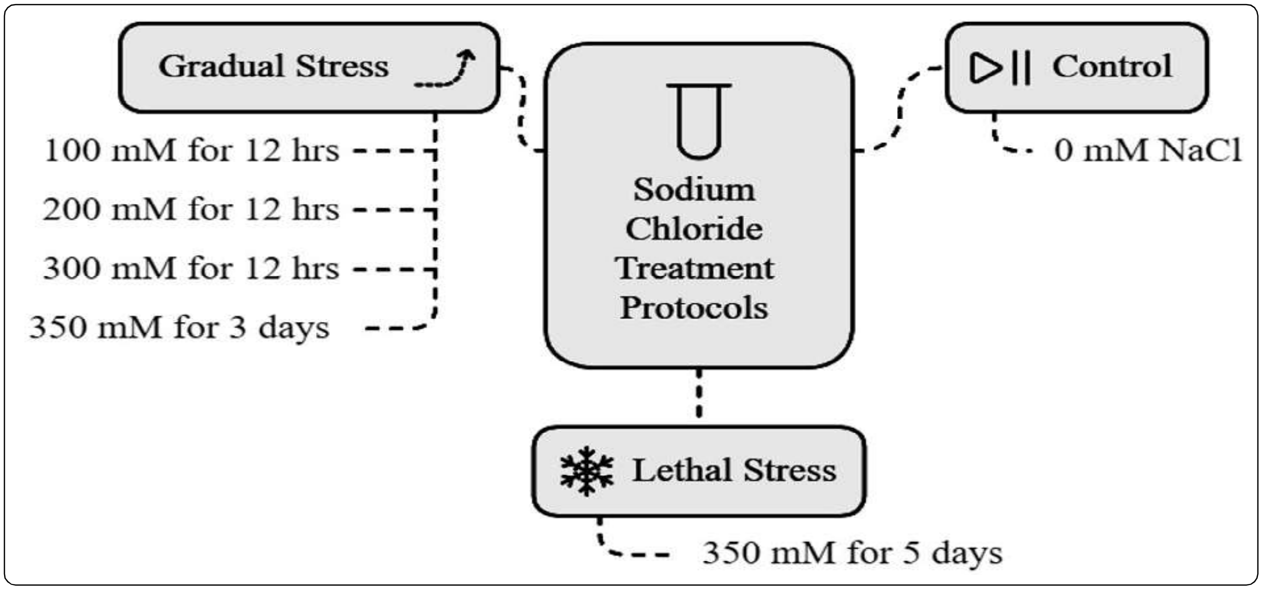

Fig. 1 : Treatment details involved in the salinity induction response experiment

Standardising Salinity Induction Response (SIR) Protocol in Rice

Standardisation of lethal NaCl concentration was done using different concentrations of NaCl, *viz.*, 100 mM, 200 mM, 300 mM, 350 mM, 400 mM, 500 mM, 550 mM and 600 mM, to check the 50% mortality of seedlings.

Screening for Cellular-level Tolerance at Early Seedling Stage

Using the SIR technique, sixteen rice lines were phenotyped for tolerance. 72 hours seedlings were selected for screening under control (0), gradual (seedlings were initially exposed to a mild NaCl concentration (sub-lethal or induction stress) *i.e.*, 100 mM, 200 mM, and 300 mM each for 12 hours each, and subsequently, these seedlings were exposed to lethal NaCl concentration *i.e.*, 350 mM for three days) and non-induced Lethal stress of 350 mM for 5 days and then allowed to recover by transferring them back to distilled water and *percent* recovery growth of seedlings after 72 hours of recovery was determined as a measure of tolerance (Fig. 2).

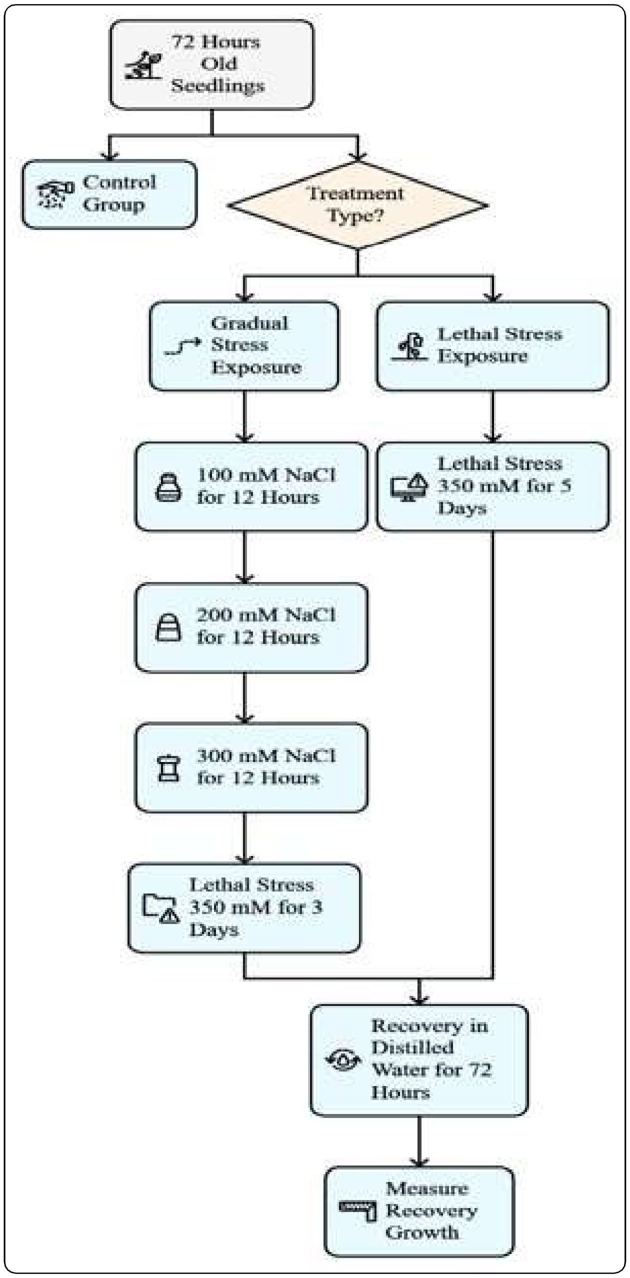

Fig. 2 : A schematic representation of the Salinity Induction Response protocol for acquired tolerance

Observations recorded

During the stress period

On the fifth day of the incubation, *i.e.*, at the end of the stress period, different observations were recorded.

Seedling attributes

Five seedlings from each replication were taken to record the following parameters.

Root and Shoot Length (cm)

The measurement of shoot and root lengths of control, gradual, and lethal stress seedlings was recorded, with values expressed in centimetres. The measurements included the distance from the seed base to the leaf apex and the root tip for both shoot and root length.

Per cent* reduction in growth *(%* RG*): Reduction in root and shoot growth was measured in both gradual and lethal treatments of salt (Manasa *et al.*, 2017).

$$\begin{array}{l} \textit{Percent}\ \text{reduction} \\ \text{in growth (\% RG)} \end{array} = \frac{\text{Lc} - \text{Lt}}{\text{Lc}}\ x\ 100$$

Where Lc is the length of the absolute control seedlings,

Lt is the length of treated seedlings.

During the Recovery Period

Seedling Attributes

Similarly, after 72 hrs of the recovery period, five seedlings from each replication were taken to record the following parameters.

Root and Shoot Length (cm)

The measurement of shoot and root lengths of control, gradual, and lethal seedlings during the recovery period was done according to the previous section.

***Per cent* Recovery Growth (% RrG)**

Per cent recovery growth was estimated based on relative growth during salt recovery (Chen *et al.*, 2016).

$$\%\ \text{RrG} = \frac{\text{RGr} - \text{RGs}}{\text{RGrc} - \text{RGsc}}\ x100$$

Where,

RGr : recovery growth of treatment during recovery

RGs : recovery growth of treatment during salinity stress

RGrc : recovery growth of control during recovery

RGsc : recovery growth of control during salinity stress

RESULTS AND DISCUSSION

Standardisation of Lethal NaCl Concentration

The lethal NaCl concentration was standardised by exposing 3-day-old seedlings of the Dhaksha and IR64 varieties to varying NaCl concentrations, namely 50, 100, 150, 200, 250, 300, 350, 400 and 500 mM, for varying durations (Table 2). The seedlings were then allowed to recover for 72 hours after the treatment and were closely observed for their recovery. The concentration at which nearly 50 *per cent* of seedling mortality occurred was considered a lethal concentration.

TABLE 2

Standardization of lethal NaCl concentration for evaluating salinity tolerance in rice

NaCl Conc. (mM)	Mortality after 3 Days	Mortality after 5 Days	Mortality after 7 Days
50	10%	20%	30%
100	20%	30%	40%
200	30%	40%	60%
300	40%	50%	80%
350	50%	60%	90%
400	80%	90%	100%
500	90%	100%	100%

When rice seedlings were exposed to varying NaCl concentrations, pronounced results were observed on the 5-day-long treatment as compared to the 3-day and 7-day treatments. Mortality rates were found to increase with increasing salinity, especially after extended exposure. Remarkably, after 7 days, even mild salt stress (50 mM NaCl) can result in considerable mortality. Complete mortality occurs within a week due to rapid and widespread cell death caused by higher concentrations, such as 400 mM and above. About 60% mortality was noted at 350 mM NaCl for five days, which was deemed a lethal concentration of NaCl for further research.

The seedlings were first subjected to salinity stress at concentrations of 100 mM, 200 mM and 300 mM for 12 hours each, depending on the lethal stress concentration. These were then subjected to 350 mM of NaCl, a lethal concentration, for 5 days.

Genetic Variability for Salinity Tolerance in Rice

Significant variations in the adaptive mechanisms of rice genotypes under lethal and gradual salt stress were revealed by genetic variation. Similarly, genotypes subjected to gradual stress were able to maintain root and shoot growth and exhibited higher recovery growth (RrG), thus demonstrating better adaptive capacity. In contrast, these traits were reduced significantly under lethal stress, with only a few genotypes showing resilience. Parameters such as root length, *per cent* reduction in growth (RG) and RrG highlight significant variability, making them essential markers for selecting salt-tolerant genotypes. This variability underscored the potential for targeted breeding programs to enhance salinity resilience in rice.

Screening of Rice Genotypes for Salinity Tolerance using the Salinity Induction Response (SIR) Technique

The evaluation of acquired salinity tolerance was conducted on eighteen varied genotypes of *Oryza sativa* L. The genotypes of rice under investigation exhibited significant genetic variability in all recorded traits, including root length, shoot length and the percentage decrease in these parameters after treatments with salinity stress.

Root Length and Shoot Length

Salinity had a significant effect on root and shoot growth, with varying degrees of reduction depending on the genetic background of the genotype and the severity of the stress (Sandesh *et al.*, 2022). Root length analysis of the three treatments (control, gradual salt stress and lethal stress) showed significant genotypic differences (Table 3). The average root length was 2.45 cm under control conditions, 1.36 cm under gradual stress and 1.19 cm under lethal stress. This showed a steady decline with increasing salinity intensity. Even in the presence of higher salt concentrations, some genotypes strangely showed greater resilience, maintaining relatively longer roots and shoots, suggesting the presence of innate or learned tolerance mechanisms. Unlike genotypes 'Dhaksha' and '769', which retained relatively longer roots even under lethal saline conditions, sensitive lines such as genotypes '618' and '85' showed a significant decline, with roots barely expanding under lethal stress. Notably, under lethal stress, in contrast to gradual stress, genotype '214' showed an abnormal response, with roots growing longer. This implies that in response to sudden and extreme stress, some plants could activate compensatory growth mechanisms. The idea that gradual stress allowed plants to adapt physiologically by regulating water uptake, ion transport and growth distribution, while lethal stress acted as a shock that severely limited growth, is supported by this model. Salinity

TABLE 3

Effect of salinity stress on root and shoot length in rice seedlings

Genotype	Root length (cm)				Shoot length (cm)			
	Control	Gradual	Lethal	Mean	Control	Gradual	Lethal	Mean
Dhaksha	4.86	3.44	2.59	3.63	6.15	1.78	1.25	3.06
IR64	1.51	0.88	0.83	1.07	4.07	2.05	1.42	2.52
712	1.82	1.51	0.98	1.44	2.47	1.77	1.71	1.98
319	2.69	0.90	1.13	1.57	3.68	1.36	0.81	1.95
498	1.20	0.99	0.95	1.05	6.17	2.05	1.43	3.22
238	2.69	2.21	2.14	2.35	5.16	1.74	1.19	2.70
214	3.57	2.07	4.23	3.29	5.44	1.85	0.64	2.64
254	0.85	1.36	0.49	0.90	4.52	1.61	1.33	2.49
790	1.99	0.88	0.91	1.26	2.94	1.04	0.53	1.50
604	1.57	0.81	0.49	0.96	4.27	1.75	1.24	2.42
584	1.97	0.45	0.48	0.97	3.35	1.17	1.51	2.01
769	3.12	2.01	1.87	2.33	5.69	2.13	4.21	4.01
204	1.69	3.05	1.71	2.15	4.92	1.27	4.07	3.42
628	2.30	1.21	0.41	1.31	4.20	1.13	2.89	2.74
618	2.77	0.25	0.53	1.18	2.27	0.81	2.14	1.74
85	4.53	0.76	0.35	1.88	5.51	0.77	0.78	2.35
81	3.28	1.97	2.45	2.57	5.99	1.02	0.99	2.67
578	2.29	1.11	0.39	1.26	2.07	1.00	1.03	1.36
Mean	2.45	1.36	1.19		4.38	1.46	1.62	
	SEM	CD	CV		SEM	CD	CV	
Genotype	0.11	0.31	6.61		0.14	0.38	5.46	
Treatment	0.05	0.13			0.06	0.16		
G*T	0.19	0.54			0.24	0.66		

inhibited root growth, as evidenced by a significant decrease in root length in the lethal, gradual and control treatments. The idea of acquired tolerance is supported by the fact that plants perform better when exposed to gradual stress, suggesting that roots can adapt when exposed to gradual stress.

These results are consistent with previous *in vitro* studies. Ashraf *et al.* (2004) demonstrated that salinity causes significant reductions in root elongation but that the degree of impact varies among genotypes. Similarly, Naser *et al.* (2010) identified genotypic differences in root length as a key determinant of salt tolerance, reinforcing the value of root traits in screening programs.

Shoot length is a sensitive indicator of salt stress as it varies more than roots. Under gradual stress conditions, the average shoot length decreased from 4.38 cm in control plants to 1.46 cm. Unexpectedly, the average was slightly higher, at 1.62 cm, when exposed to lethal stress. While many genotypes were retarded under lethal conditions, some genotypes performed better than under gradual stress, suggesting genotype-specific responses in this unexpected fashion. For example: under lethal conditions, genotypes '769' and '204' produced longer shoots (4.21 cm and 4.07 cm, respectively) and genotypes '628' and '618' showed a similar pattern. In contrast, under lethal stress, genotypes '214', '85' and '81' showed virtually no shoot growth, indicating that they were extremely sensitive.

One of the most striking observations was that some genotypes did not follow the expected progressive decline from control to gradual to lethal stress. Instead, certain lines seemed to 'activate' tolerance mechanisms only under extreme conditions, producing stronger roots or shoots when suddenly exposed to lethal stress. For example, '214' displayed longer roots under lethal stress, possibly as a compensatory strategy to seek less saline zones, while '769' and '204' showed enhanced shoot growth, hinting at rapid stress response activation.

The intricacy of salinity tolerance is highlighted by this non-linear pattern. While some genotypes have tolerance pathways that are only activated by acute stress, others may rely on slow acclimation mechanisms and thrive under gradual stress. In order to capture the entire range of adaptive responses and prevent the exclusion of significant tolerance traits, genotypes must be evaluated under both gradual and lethal stress.

These findings are consistent with previous research like Hakim *et al*. (2014) reported that salinity severely reduces shoot elongation but also highlighted genotype-specific responses. Afzal *et al*. (2019) demonstrated that *in vitro* screening is an effective approach to identify salt-tolerant rice genotypes, particularly by examining shoot growth under stress. Roy *et al*. (2014), further emphasised the importance of shoot traits as reliable indicators of salt tolerance, as genotypes with higher shoot elongation potential tend to be more resilient. Likewise, Ashraf *et al*. (2004) noted that salt stress impairs shoot length through ionic imbalance and osmotic stress, but tolerant genotypes can maintain growth through better ion regulation and stress signaling.

Overall, this study reinforces the importance of both root and shoot traits in assessing salt tolerance. While gradual stress allows for acclimation and reveals adaptive capacity, lethal stress exposes the limits of tolerance. Identifying genotypes like 'Dhaksha', '214' and '769' that show resilience under stress provides valuable candidates for breeding programs aimed at developing salt-tolerant rice varieties.

***Per cent* Reduction in Root Length and Shoot Length**

Recording the per cent reduction in root length (RL) and shoot length (SL) in seedlings is a critical parameter for assessing plant tolerance to abiotic stresses such as salinity (Plate 1). Root and shoot growth are fundamental indicators of a plant's ability to maintain physiological and metabolic processes under stress conditions. Roots play a vital role in water and nutrient uptake, while shoots are essential for photosynthesis and energy production. Measuring the reduction in their lengths provides a quantitative means to evaluate how effectively a genotype can

Plate 1 : Comparative seedling growth response of rice genotype (Dhaksha, GEN_RIC_712 and GEN_RIC_85) under different treatments *viz.,* Control, Gradual salt stress and Lethal salt stress conditions

withstand adverse conditions. Seedlings are particularly vulnerable to salt stress, which disrupts ion balance, induces osmotic stress, and hinders cell division and elongation. A greater reduction in RL and SL reflects higher sensitivity to stress, while minimal reductions suggest tolerance. These measurements are essential for identifying resilient genotypes that can maintain growth under stress, aiding in breeding programs for developing stress-resistant crops. Furthermore, *percentage* reduction values serve as a standardised metric for comparing genotypes and treatments, offering insights into physiological and genetic mechanisms driving stress adaptation.

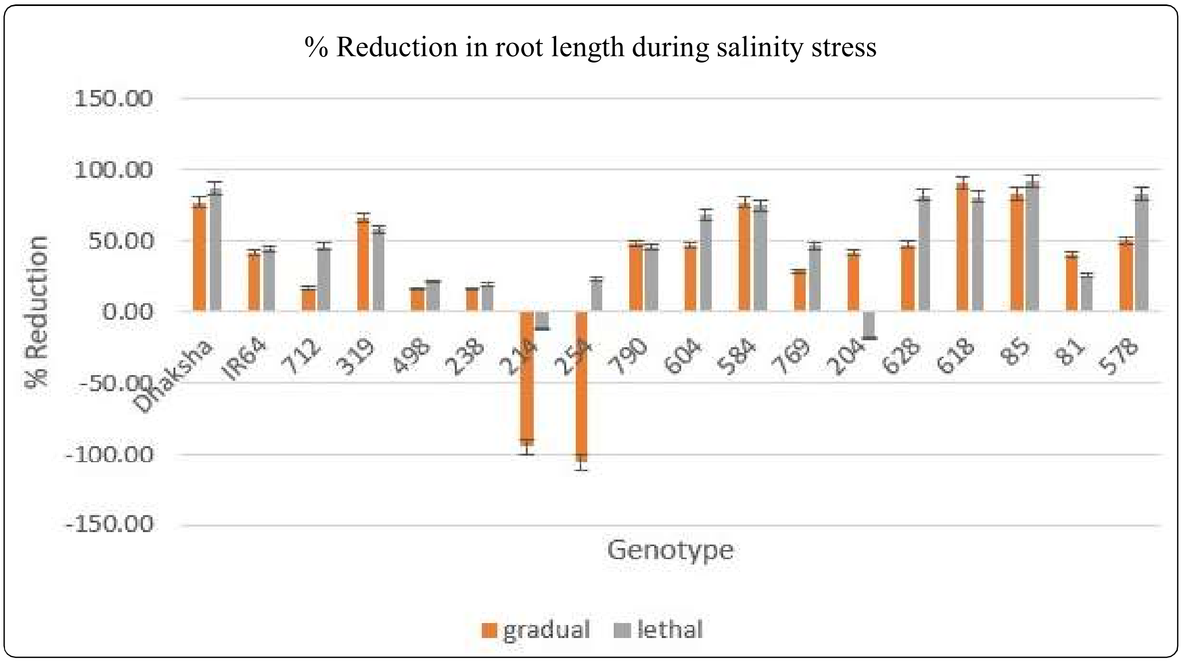

Fig. 3 : Per cent reduction in root length in gradual and length stress across genotypes

The data represented the percentage reduction in root length (RL) of various rice genotypes under salt stress conditions, "gradual," and "lethal" salt stress treatments (Fig. 3, 5). In most cases, lethal stress resulted in a higher reduction, indicating more severe damage to root growth. For instance, Dhaksha showed a reduction of 85% (gradual) and 92% (lethal), while IR64 experienced 46% (gradual) and 51% (lethal). Genotype *712* has a reduction of 43% (gradual) and 49% (lethal). In contrast, genotypes like 319 (27%,

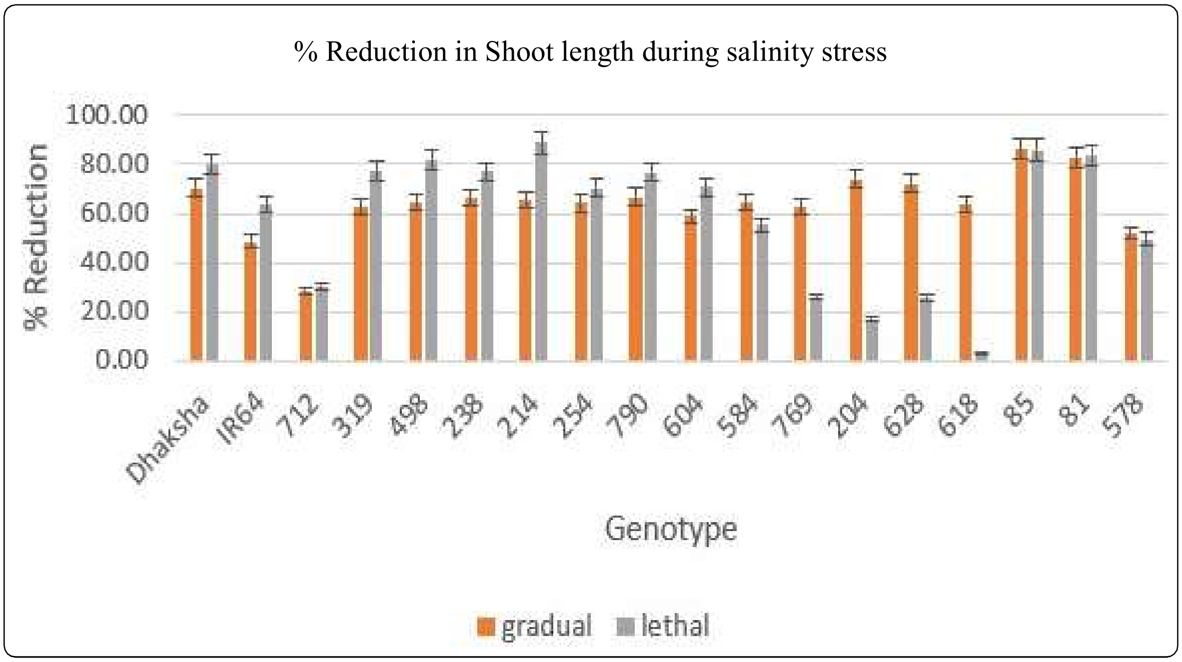

Fig. 4 : *Per cent* reduction in shoot length in gradual and lethal stress across genotypes

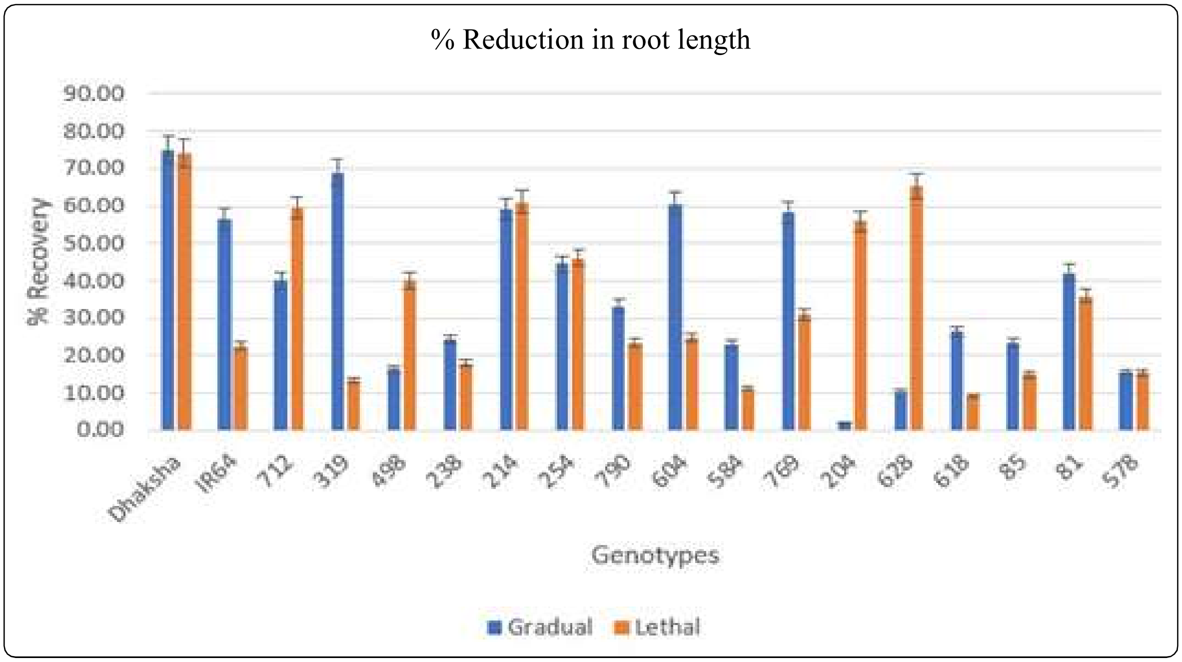

Fig. 5 : *Per cent* recovery in root length during salinity stress recovery

33%) and 498 (21%, 28%) exhibit relatively lower reductions, suggesting better tolerance. 238 shows nearly equal reduction under both stresses (16% gradual, 18% lethal), highlighting a stable response. Genotypes such as 790 (49%, 54%), 604 (70%, 72%) and 584 (71%, 74%) show moderately high reductions under both conditions. On the more sensitive side, genotypes like 618 (91% gradual, 94% lethal), 85 (96%, 98%) and 81 (90%, 92%) suffer some of the highest reductions. The findings align with *in vitro* studies on salt stress in rice, such as research by Mishra *et al*. (2020), which demonstrated that salt-sensitive genotypes experience greater root damage due to oxidative stress and reduced antioxidant enzyme activities, supporting the observed trends in this data. Similarly, Prakash *et al*. (2021) emphasised that tolerant genotypes maintain better root growth under salt stress through improved Na+/K+ regulation.

A remarkable trend was observed in genotypes 204 and 214. Unlike most other genotypes, these lines exhibited negative reduction values not only under gradual stress but also under lethal stress. During gradual stress, genotype 204 showed approximately -10% reduction while genotype 214 showed about -20%, indicating enhanced root elongation relative to the control as an adaptive growth response to moderate salinity. Under lethal stress, genotype 204 maintained around -2% reduction and genotype 214 had about -15% reduction, demonstrating that root elongation was not inhibited but stimulated even by high salt concentrations, a response that is atypical among the tested genotypes. Similarly, genotype 254 also followed this trend, showing negative reduction values, with approximately -50% and -60% under gradual and lethal stress, respectively, reflecting strong root growth stimulation under salt stress conditions compared to the control. Under gradual stress, both genotypes showed enhanced root elongation relative to the control, reflecting an adaptive growth response triggered by moderate salinity. Under lethal stress, instead of showing severe inhibition, genotypes 204 and 214 maintained enhanced root growth, again recording negative reduction values. This indicates that root elongation was not suppressed but in fact stimulated by high salt concentrations, an atypical response that distinguishes them from all other tested genotypes.

Conversely, genotypes such as 712 (19.55%), 618 (22.36%) and 769 (29.53%) displayed lower mean reductions, highlighting their potential salt tolerance. Studies by Ali *et al*. (2019) demonstrated that tolerant genotypes sustain shoot growth under salinity by maintaining ion homeostasis and reducing oxidative damage, which supports these findings. Similarly, Zhu *et al*. (2021) reported that salt stress impacts shoot growth less in tolerant varieties due to the regulation of stress-responsive genes.

When root and shoot responses are considered together, it becomes evident that salinity tolerance is organ-specific and genotype-dependent. Genotype 204 demonstrated consistent tolerance across both roots and shoots, whereas genotypes 618 and 214 displayed differential responses between the two organs. Such phenotypic diversity highlighted the intricate nature of salinity tolerance mechanisms and indicates that comprehensive selection should integrate both root- and shoot-related traits. A Similar result was observed in a previous study where salinity stress mainly affected shoot length more significantly than root length in finger millet, with roots sometimes showing increased growth under moderate salinity (Veena *et al*., 2025).

Recovery Growth (cm)

Assessing the recovery growth of *in vitro* seedlings following salinity stress provides a reliable approach to assess how plants withstand and recover from adverse environmental conditions. Post-stress growth measurements provide valuable information about the physiological resilience of plants and help identify genotypes with superior adaptability-essential information for breeding rice varieties with increased salt tolerance. A distinct trend was observed among the tested genotypes: seedlings exposed to gradually increasing salinity showed significantly stronger resilience than those exposed to sudden lethal salt

TABLE 4

Root and shoot length during recovery from salinity stress in rice genotypes

Genotype	Root length (cm)				Shoot length (cm)			
	Control	Gradual	Lethal	Mean	Control	Gradual	Lethal	Mean
Dhaksha	4.90	3.47	2.61	3.66	6.42	1.98	1.31	3.24
IR64	1.90	1.10	0.98	1.33	4.78	2.21	1.54	2.84
712	2.32	1.71	1.01	1.68	3.26	2.24	1.90	2.47
319	3.14	1.21	1.41	1.92	6.30	1.71	1.11	3.04
498	1.69	1.07	0.99	1.25	6.22	2.07	1.44	3.24
238	4.41	2.63	2.34	3.13	7.17	2.10	1.46	3.58
214	4.01	2.33	4.54	3.63	7.65	3.20	1.29	4.05
254	1.21	1.52	0.58	1.10	5.91	2.25	1.92	3.36
790	2.08	0.91	0.92	1.30	7.83	2.19	1.56	3.86
604	1.90	1.01	0.53	1.15	6.36	2.42	1.51	3.43
584	3.87	0.89	0.76	1.84	7.21	2.32	1.93	3.82
769	5.71	3.52	2.64	3.96	7.05	2.55	4.80	4.80
204	5.66	3.13	4.39	4.39	6.71	2.27	5.11	4.70
628	6.31	1.63	0.73	2.89	5.98	2.40	3.37	3.92
618	4.55	0.72	0.95	2.07	6.39	1.21	2.42	3.34
85	5.64	1.02	0.45	2.37	6.18	0.89	0.81	2.63
81	4.42	2.45	2.71	3.19	7.41	1.30	1.21	3.31
578	3.25	1.26	0.46	1.66	5.51	1.58	1.41	2.83
Mean	3.72	1.75	1.61		6.35	2.05	2.01	
	SEM	CD	CV		SEM	CD	CV	
Genotype	0.135	0.38	6.07		0.203	0.569	5.75	
Treatment	0.055	0.155			0.083	0.232		
G*T	0.235	0.658			0.351	0.986		

concentrations (Table 4). Rapid exposure to extreme lethal salinity often overwhelms the plant's defences, resulting in significant inhibition of root and shoot growth. Such inhibition is often associated with ion toxicity, oxidative stress and osmotic disorders, which impair essential cellular and metabolic functions, thereby reducing recovery capacity (Ashraf *et al.*, 2004 and Hakim *et al.*, 2014).

Conversely, progressive salt exposure enables plants to gradually adjust through physiological acclimation, activating crucial defence systems such as ion regulation, osmotic adjustment and antioxidant protection. These adaptive mechanisms help preserve cellular homeostasis and facilitate renewed growth once the stress is lifted (Roy *et al.*, 2014 and Afzal *et al.*, 2019).

In general, both root and shoot lengths decreased as stress severity increased. The mean root length declined from 2.45 cm under control conditions to 1.36 cm under gradual stress and 1.19 cm under lethal stress, indicating that stress strongly limited root growth.

Among the genotypes, Dhaksha (3.63 cm), 214 (3.29 cm), and 81 (2.56 cm) recorded relatively higher mean root lengths, suggesting better tolerance under stress conditions. In contrast, 254 (0.90 cm), 604 (0.95 cm), and 578 (1.26 cm) showed the lowest mean root lengths, reflecting greater sensitivity to increasing stress levels.

Shoot length followed a similar declining trend. The average shoot length decreased from 6.35 cm in the control to 2.05 cm under gradual stress and 2.01 cm under lethal stress. However, a few genotypes maintained higher shoot growth despite stress. For instance, 769 (4.80 cm) and 204 (4.70 cm) showed the highest mean shoot lengths, indicating stronger resilience, while 85 (2.63 cm) and 578 (2.83 cm) recorded the lowest, showing that they were more adversely affected. Overall, stress conditions significantly reduced both root and shoot growth in all genotypes, with lethal stress showing the strongest inhibitory effect. Nevertheless, some genotypes, particularly Dhaksha, 214, 204 and 769, performed comparatively better under stress, suggesting they possess traits that confer improved stress tolerance. These genotypes could serve as promising material for breeding programs aimed at developing stress-tolerant rice varieties.

Among the genotypes studied, genotype 214 demonstrated remarkable recovery ability, showing a 2-fold increase in root length under lethal stress (4.23 cm) compared to the gradual stress condition (2.07 cm). This fold change indicates a strong acclimation response and suggests that genotype 214 can effectively restore and even enhance root growth under severe stress conditions.

These findings are consistent with earlier research demonstrating that plants exposed to incremental salinity levels maintain better growth and recovery compared to those exposed directly to high salt concentrations. Similar responses have been documented in rice and various other crops, where stepwise salt exposure strengthens tolerance mechanisms, while abrupt stress often inflicts irreversible physiological injury (Naser *et al*., 2010 and Roy *et al*., 2014).

In conclusion, assessing recovery growth provides a more holistic measure of salinity tolerance than evaluating stress-phase performance alone. It reflects not only a genotype's capacity to endure stress but also its potential to resume normal growth once favourable conditions return. This emphasises the significance of adaptive priming mechanisms in enhancing salinity resilience and highlights the importance of identifying genotypes with strong recovery abilities for breeding programs aimed at stabilising yields in salt-affected regions.

Similarly, Dhaksha showed improvement from 2.59 cm under lethal stress to 3.44 cm under gradual stress, indicating partial recovery of root growth when the stress was applied more gradually. This trend was consistent in other relatively tolerant lines, such as 238 and 712, which maintained more stable root lengths of 2.21 cm and 1.51 cm, respectively, under gradual stress. In contrast, under lethal stress, root elongation was severely restricted in most genotypes, likely due to rapid cellular damage and impaired meristem activity. Genotype 85, which displayed a strong baseline under control conditions (4.53 cm), sharply declined to 0.35 cm under lethal stress, highlighting its pronounced sensitivity to abrupt salinity exposure.

In terms of shoot length, which reflects above-ground biomass and photosynthetic recovery potential, gradual stress again proved more favourable in most genotypes. Genotype 214 recorded a shoot length of 3.20 cm under gradual stress compared to 1.29 cm under lethal stress, while Dhaksha maintained 1.98 cm under gradual conditions and 1.31 cm under lethal stress, demonstrating better shoot sustainability. Interestingly, a few genotypes, such as 204 and 769, exhibited higher shoot lengths under lethal conditions (204: 5.11 cm and 769: 4.80 cm) compared to their gradual treatments (2.27 cm and 2.55 cm, respectively). These responses may represent genotype-specific adaptive mechanisms, possibly linked to stress-induced elongation or escape strategies rather than true physiological resilience.

These findings are in agreement with Acosta-Motos *et al*. (2017) that the gradual imposition of salt stress allows plants to activate adaptive mechanisms, providing a physiological buffer period that enhances root and shoot growth during recovery. Guo *et al.* (2015) found that the gradual imposition of salt stress allowed cotton plants to activate adaptive mechanisms, leading to improved recovery of root and shoot growth after stress relief.

The study strongly supports that the gradual imposition of salt stress results in more favourable root and shoot growth during recovery, as it provides a physiological buffer period that enables plants to activate adaptive mechanisms. This study highlights the importance of gradual salinity stress screening for identifying truly resilient rice genotypes. Gradual exposure more accurately mimics field conditions and allows plants to physiologically adjust, leading to improved root and shoot growth compared with sudden, lethal stress. Genotypes such as 214, 204 and 769 exhibited strong acclimation and recovery under salinity stress, indicating the effective activation of adaptive mechanisms that support sustained root and shoot growth. These responses reflect acquired tolerance, where prior or mild stress enhances the plant's ability to withstand subsequent severe stress. Such genotypes, capable of dynamic physiological adjustment, represent valuable material for breeding programs targeting salinity-prone environments.

From a breeding perspective, the identification of genotypes that exhibited stable tolerance under both gradual and lethal salinity stress (e.g., genotypes 204, 214, 769, 238, 712, Dhaksha and 81) provided valuable genetic resources. In particular, genotypes with acquired tolerance traits represented ideal candidates for developing new cultivars capable of thriving in saline-prone environments, where salinity levels typically build up gradually during the growing season. Integrating these traits into breeding pipelines could contribute significantly to the development of resilient rice varieties suitable for salt-affected ecosystems.

Per cent Recovery in Root and Shoot Length

The *percentage* recovery of root and shoot length during salinity stress is a critical measure for evaluating a plant's ability to cope with and recover from adverse environmental conditions. Salinity stress severely impacts plant growth, leading to reduced root and shoot elongation due to osmotic stress, ion toxicity, and nutrient imbalances. *Per cent* recovery provides a quantitative measure to assess a genotype's resilience, reflecting its capacity to regain growth after stress is alleviated. This parameter is particularly important in identifying salt-tolerant genotypes, as it captures both immediate stress response and post-stress recovery efficiency. Root recovery is vital for water and nutrient uptake, while shoot recovery is essential for photosynthesis and biomass production.

The percentage root recovery during salt stress recovery across 18 genotypes under gradual and lethal stress was calculated (Fig. 5). In general, gradual salinity stress resulted in noticeably higher root length recovery across most genotypes compared to the lethal treatment. For example, Dhaksha showed the highest recovery, with approximately 75-80% recovery under gradual stress and around 25% under lethal stress, indicating a strong ability to restore root growth when stress intensity increased progressively. Similarly, genotypes such as 319, 214 and 769 maintained relatively high recovery levels under gradual stress (around 60-65%) compared to their corresponding lethal treatments (around 40-45%), reflecting effective acclimation and adaptive root responses.

In contrast, genotypes like 85 and 204 showed very limited recovery potential under both treatments, with recovery percentages below 20%, suggesting high sensitivity to salinity. A few lines, including 238, 498, and 604, exhibited only modest improvement, maintaining recovery levels between 20-30%, indicating partial tolerance. Notably, IR64, 712, and 628 displayed intermediate recovery under gradual stress but sharp reductions under lethal conditions, suggesting that abrupt stress disrupts root function more severely.

Overall, the mean percentage recovery was higher under gradual stress (approximately 60-65%) than under lethal stress (around 30-35%), confirming that gradual salinity exposure allows plants to activate compensatory mechanisms that support root system recovery. The high variability in the genotype × treatment interaction (G × T) further highlights the genotype-specific nature of root recovery, demonstrating that both genetic background and stress intensity play crucial roles in determining salinity tolerance.

These findings align with studies like Munns and Tester (2008) and Zhu (2002), which emphasise that root recovery under salt stress is a critical trait for overall plant resilience. These findings align with *in vitro* studies, such as those by Negrao *et al.* (2017) and Ashraf *et al.* (2010), which have highlighted the utility of *in-vitro* screening for salt tolerance traits, including root elongation and recovery. *In-vitro* conditions allow precise control over salinity levels, facilitating the identification of genotypes with superior stress mitigation traits.

Similarly, the percentage shoot recovery during salt stress recovery for 18 genotypes under gradual stress and lethal stress was calculated (Fig. 6). Shoot length recovery varied considerably among the rice genotypes under both gradual and lethal salinity stress. Overall, recovery was higher under gradual stress, suggesting that a progressive increase in salinity allows for better physiological adjustment compared to abrupt lethal exposure. Dhaksha showed the highest recovery, with 74.07% under gradual stress and 22.22% under lethal stress, indicating strong resilience and effective stress adaptation mechanisms. In contrast, IR64 recorded one of the lowest recoveries (22.54% and 16.90%, respectively), confirming its high sensitivity to salt. Genotypes such as 214 (61.09% vs. 29.41%), 254 (46.04% vs. 42.45%) and 628 (71.35% vs. 26.97%) demonstrated moderate to high resilience, maintaining better recovery potential under gradual stress and reflecting partial tolerance.

Interestingly, genotype 204 exhibited nearly equal recovery under both conditions (55.87% under gradual and 58.10% under lethal stress), suggesting stable performance and potential acquired tolerance. A similar trend was observed in 769, which showed slightly higher recovery under lethal stress (43.38%) than under gradual stress (30.88%), implying a genotype-specific compensatory response. In contrast, genotypes such as 618, 85 and 578 exhibited the lowest recovery values under

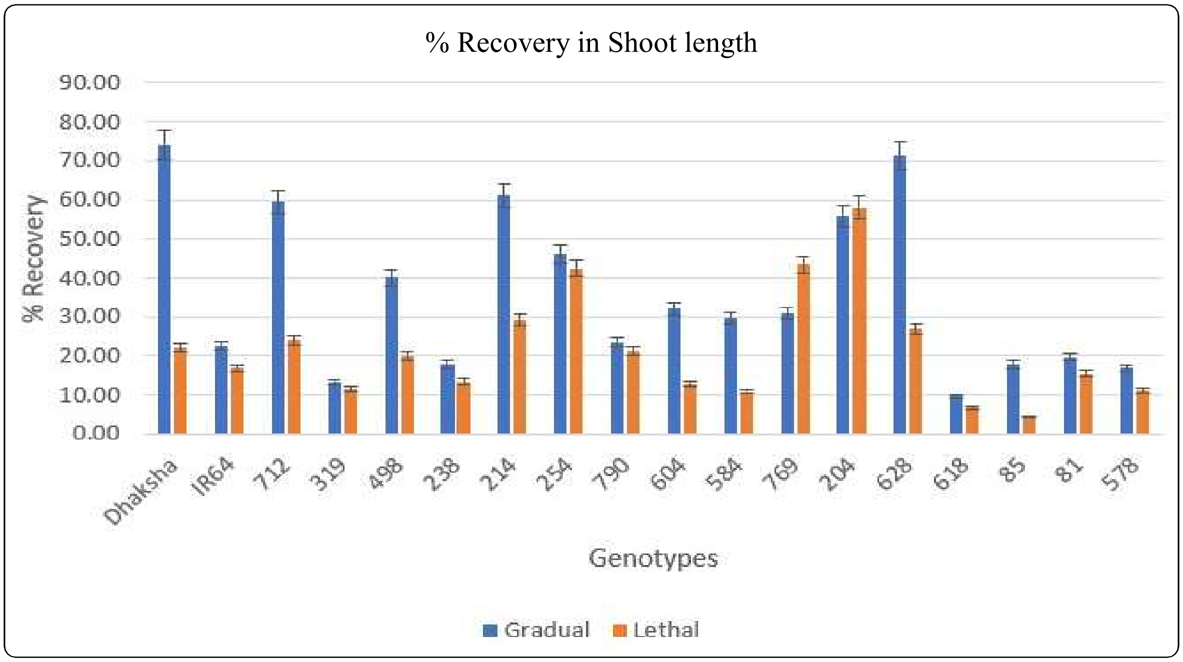

Fig. 6 : *Per cent* recovery in shoot length during salinity stress recovery

both conditions, indicating heightened sensitivity and limited adaptive capacity.

On average, mean shoot length recovery was higher under gradual stress (approximately 38-40%) than under lethal stress (around 25-30%), highlighting the advantage of gradual salinity exposure in supporting partial growth restoration (Fig. 6). The significant genotype × treatment (G × T) interaction underscores the strong genotype-specific responses to salinity stress, emphasising the importance of identifying and selecting genotypes with better adaptive recovery potential. Dhaksha's superior shoot recovery likely reflects efficient hormonal balance, osmotic regulation and ion compartmentalisation-traits commonly associated with salt-tolerant genotypes reported in previous studies. These findings align with *in vitro* research, such as Ashraf *et al.* (2010), which demonstrated that shoot recovery is influenced by osmotic adjustment and improved photosynthetic efficiency under salt stress. Moreover, studies by Munns and Tester (2008) and Negrao *et al.* (2017) emphasise that genotypic differences in shoot recovery often correlate with enhanced stress signalling pathways, ion regulation and antioxidant activity.

Acquired tolerance refers to a plant's ability to cope better with extreme stress after first being exposed to a milder form of that stress. In many ways, it functioned as a form of 'training', where gradual exposure allowed plants to activate protective mechanisms that later supported recovery and survival (Munns & Tester, 2008). Under salinity conditions, such preconditioning was known to trigger physiological and molecular responses, including osmolyte accumulation and stress-responsive gene expression, which enhanced resilience to harsher environments (Chinnusamy *et al*., 2005).

The results of this study showed clear evidence of acquired tolerance in several rice genotypes. In terms of root recovery, genotypes such as Dhaksha, 214 and 769 exhibited strong recovery under gradual salinity conditions compared to lethal stress, with Dhaksha recording the highest overall recovery (around 75-80%) under gradual treatment. Genotypes 319 and 254 also maintained relatively good recovery under gradual stress, reflecting their capacity to partially restore root growth when salinity developed progressively.

For shoot recovery, Dhaksha, 214, 712 and 628 demonstrated the best responses, each showing notably higher recovery percentages under gradual stress than under lethal conditions. Dhaksha again stood out with 74.07% shoot recovery under gradual stress, confirming its consistent performance across both below and above-ground traits. Genotypes 214 (61.09%) and 628 (71.35%) also maintained strong shoot recovery, indicating efficient physiological adjustment and sustained growth despite salinity exposure. Overall, the results highlighted that acquired tolerance was strongly genotype-dependent rather than uniform across all rice lines. The superior performance of Daksha, 214, 712, and 628 suggested that these genotypes carried adaptive traits that could be harnessed in breeding programs to improve salt tolerance. Their ability to recover more effectively under gradual stress pointed to their potential value as donor parents for the development of resilient rice varieties suitable for saline environments.

The comparative evaluation of rice genotypes under salinity stress revealed that gradual treatment enhanced tolerance and recovery compared to lethal exposure. Significant genotypic variation was observed in both stress response and post-stress regeneration. Dhaksha exhibited moderate reductions in root (H-80%) and shoot (H-70%) lengths but demonstrated the highest recovery (75% root, 74% shoot), indicating strong tolerance. Genotype 254 also showed stable performance with moderate reductions and substantial recovery (44% root, 46% shoot), classifying it as tolerant. Genotypes 214, 204 and 628 displayed intermediate reductions and moderate recovery, suggesting partial tolerance, while IR64, 85, 618 and 578 experienced severe growth inhibition and poor recovery (<25%), confirming susceptibility. Overall, Dhaksha and 254 were identified as tolerant, 214, 204 and 628 as moderately tolerant, and IR64, 85, 618 and 578 as susceptible. These results support that gradual salinity stress promotes acclimation and adaptive responses, whereas lethal stress causes irreversible damage, emphasising the role of stress progression rate in salinity tolerance and recovery.

Salt induction treatments have an assimilative impact on plant adaptive processes. For example, previous studies reported that mung bean seeds treated with NaCl solution exhibited positive root growth compared to untreated seeds (Saha *et al.,* 2010) and similarly, induced eggplant seedlings demonstrated increased root growth and higher biomass following salinity stress, showing that moderate salt exposure could prime plants for improved adaptation to subsequent stress (David *et al.,* 2022).

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