



CONSEQUENCES OF SALINITY STRESS ON RICE GENOTYPES INFLUENCED BY SALICYLIC ACID

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ABSTRACT

Rice (*Oryza sativa*), one of the most important cereal crops is sensitive to salinity stress at early growth stage. Soil salinity is a major abiotic environmental threat that adversely affected the productivity of rice crop in all over the world. Salinity stress badly effects on the germination, root length, shoot length, total dry weight and seedling vigor index at early seedling growth stage of rice plant. In the present research work, salinity was induced in soil by the application of NaCl at different concentrations under study. Much significant reduction in germination percentage were recorded @ 200 mM NaCl in both Sahbhagi Dhan and MTU 1010, *i.e.*, 22.3%, 27.2% respectively as compared to control. Other growth parameters such as root length, shoot length, total dry weight and seedling vigor index (SVI) also significantly decrease at higher concentration of NaCl (@ 200 mM NaCl) in both genotypes. However, maximum reduction in all growth parameters @ 200 mM NaCl was noticed in MTU 1010 genotype. Excessive sodium and chloride ions can interfere with water absorption and metabolic processes within the seed by creating osmotic stress and nutrient imbalance. As detected, in general, SA play vital role in mitigation the harmful effect of salinity in both rice genotype. SA induced to have more germination%, root length, shoot length, total dry weigh and seedling vigor index in both rice genotypes at threshold combination of SA and NaCl (*i.e.*, 1mM SA + 100 mM NaCl) as compared to two other combinations of NaCl with SA. Thus, it is summarized that Sahbhagi Dhan exhibited more tolerance than MTU 1010, which showed relatively susceptible reaction under salinity stress.

Key words : Salinity, Salicylic acid, Germination, Seedling vigor index.

Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food crops grown in diverse ecology in India but it is highly sensitive to salinity. Soil salinity poses a significant threat for the growth and development process in rice by affecting and modulating key parameters. Salinity affects all aspects of rice growth from germination to maturity (Heenan *et al.*, 1988 and Lutts *et al.*, 1995). The major inhibitory effect of salinity on plant growth and development has been attributed to osmotic inhibition of water availability as well as the toxic effect of salt ions responsible for salinization. Nutritional imbalance

caused by such ions leads to reduction in photosynthetic efficiency and other physiological disorders (Yeo *et al.*, 1990).

Salicylic acid (SA) is a very important endogenous growth regulator (Sakhabutdinova *et al.*, 2003) and play a vital role in the regulation of various physiological processes (Hayat *et al.*, 2010). SA enhanced salinity tolerance of various crops by reducing Na⁺ and increasing K⁺ content which may influence the transport of K⁺ within the plant cells. SA application improves plant growth and germination rates in both salt- and stress-free conditions resulted stomatal regulation, chlorophyll

content, and photosynthesis (Jangra *et al.*, 2022). This seems important to identify or develop tolerant cultivars or to make the plants tolerant for the stress through the technique of chemical hardening of seeds by SA before sowing.

Keeping these facts in view, the present investigation was carried out to observed the effect of induced salinity and mitigate the salinity stress by using salicylic acid through seed hardening for better germination and seedling establishment.

Materials and Methods

Plant material and salinity stress applications

Rice genotypes (Sahbhagi Dhan and MTU1010) seeds were surface sterilized with 0.2% HgCl₂ solution. Salicylic acid (SA) was dissolved in absolute ethanol then added drop wise to water (ethanol/water: 1/1000 v/v) (Williams *et al.*, 2003). Twenty seeds of each genotype for each treatment were soaked for overnight in water. Ten seeds of each genotype for each treatment were soaked in water (0 mM SA) and hardened with salicylic acid (1.0 mM) by repeatedly soaking and drying four times for overnight before sowing in pots. Thereafter, salinity stress was created in soil in single shock by the application of NaCl (@ 100, 150 and 200 mM) to achieve electrical conductivity (EC) 8, 12 and 16 dSm⁻¹, respectively. Carefully 10 seeds of both rice genotypes were sown in plastic pots (size 20 cm × 20 cm) containing farm soil having 12-14% moisture at the time of sowing. The individual and combined treatments were taken as T₀ (control: without NaCl and SA), T₁ (1.0 mM SA), T₂ (100 mM NaCl), T₃ (150 mM NaCl), T₄ (200 mM NaCl), T₅ (100 mM NaCl + 1.0 mM SA), T₆ (150 mM NaCl + 1.0 mM SA), T₇ (200 mM NaCl + 1.0 mM SA). The treatments were replicated three times. Germination percentage, root length, shoot length, root dry weight, shoot dry weight and seedling vigour index were recorded at 10 days after germination.

Measurement of germination and various growth parameters

Germination (%) : Ten days after sowing, germination was calculated in percentage by the following formula:

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds grown}} \times 100$$

Root length (cm) and Shoot length (cm) : The distance between the tip of the root and the portion of the plant just touching the surface soil was measured as the root length. Plant height was measured from the base

of the plant to the top of the main shoot. The average height of five plants was considered.

Total dry weight plant⁻¹ (g) : The plants were harvested carefully and oven dried at 105°C for 1 h followed by keeping the plant materials at 65-70°C up to 72 h; weighed with the help of electronic balance (Model; KEA 200) at every 24 h till the constant weight was obtained.

Seedling vigour index (SVI) : Seedling vigour index (SVI) was calculated by using the formula:

$$\text{Seedling vigour index} = (\text{Root length} + \text{Shoot length}) \times \text{Germination \%}$$

Vigour index value was computed using the formula suggested by Abdul-Baki and Anderson (1973).

Statistical analysis

Experimental design was conducted in FCRD. Data was analyzed for analysis of variance (ANOVA) and means were compared by the least significant difference (LSD) test and those at P < 0.05. Standard error of mean was also calculated (Gomez and Gomez, 1984).

Results and Discussion

Present research was carried out to deal with effect of salinity stress on germination and seedling growth of rice genotypes (Sahbhagi Dhan, MTU 1010), their tolerance level and advantageous effect of salicylic acid for mitigating the deleterious effect of salinity stress on germination, root length, shoot length, total dry weight and seedling vigor index.

Germination (%)

Seed germination is one of the most important vigorous phases in the life cycle of plants that play significant role in plant establishment and the yield of the crops. Salinity is an important factor that may limit crop productivity by inhibiting seed germination (Yu *et al.*, 2016). Salinity stress significantly reduced germination in both the rice genotypes (Sahbhagi Dhan, MTU1010). Maximum inhibition in germination percentage was recorded in genotype MTU1010 than genotype Shahbhagi Dhan at different concentrations of NaCl (Table 1). Significant reduction in germination percentage were recorded @ 200 mM NaCl in both Shahbhagi Dhan and MTU 1010, *i.e.*, 22.3%, 27.2% respectively as compared to control. Salicylic acid (SA) hardened seeds @ 1.0 mM increased the germination percentage in combination with different levels of NaCl, however, significantly increased germination percentage were recorded in combination of SA (@ 1.0 mM) and NaCl (@ 100 mM) in Sahbhagi Dhan than two other combinations of NaCl, which were 90.0% in Sahbhagi Dhan and 82.7% in MTU 1010

Table 1 : Consequences of Salinity Stress on Germination (%) of Rice Genotypes influenced by Salicylic acid.

Year 2021		
Treatment	Sahbhagi Dhan	MTU1010
T ₀ (Control: 0.0 mM NaCl & SA)	100.0	100.0
T ₁ (1.0 mM SA)	90.3	93.3
T ₂ (100 mM NaCl)	92.0	80.0
T ₃ (150 mM NaCl)	83.3	70.0
T ₄ (200 mM NaCl)	70.3	63.3
T ₅ (100 mM NaCl + 1.0 mM SA)	66.7	83.3
T ₆ (150 mM NaCl + 1.0 mM SA)	90.0	80.0
T ₇ (200 mM NaCl + 1.0 mM SA)	72.7	70.0
	SEm±	CD 5%
Genotypes (G)	7.12	20.55
Treatments (T)	3.56	10.28
G×T	2.52	7.27

Table 2 : Consequences of Salinity stress on Root Length (cm) of Rice Genotypes influenced by Salicylic acid.

Year 2021		
Treatment	Sahbhagi Dhan	MTU1010
T ₀ (Control: 0.0 mM NaCl & SA)	24.54	22.61
T ₁ (1.0 mM SA)	28.11	24.86
T ₂ (100 mM NaCl)	22.72	18.61
T ₃ (150 mM NaCl)	20.32	18.08
T ₄ (200 mM NaCl)	19.12	13.96
T ₅ (100 mM NaCl + 1.0 mM SA)	26.04	21.38
T ₆ (150 mM NaCl + 1.0 mM SA)	24.22	18.98
T ₇ (200 mM NaCl + 1.0 mM SA)	20.32	13.03
	SEm±	CD 5%
Genotypes (G)	1.47	4.24
Treatments (T)	0.73	2.12
G×T	0.52	1.50

genotypes as compared to salt treated ones. In this respect, Xu *et al.* (2011) reported that salinity stress reduced the germination in rice crop. Simultaneously, Anuradha and Rao (2001), also reported that salinity inhibits seed germination as well as seedling growth of rice. Present findings are in accordance with the results recorded by Khan and Weber (2008), who stated that germination process influenced by salinity, it alters the imbibition of water by seeds due to lower osmotic potential of germination media. This may be explained critically by Liu *et al.*, (2022), who reported that SA can alleviate ion toxicity by reducing Na⁺ content, thereby helping to maintain reactive oxygen species, hormone homeostasis,

promote starch hydrolysis and deliver sufficient energy for seed germination, as a result improves rice seed germination under salinity stress.

Root and Shoot length (cm)

Salinity stress adversely affects growth of rice genotypes at early seedling stage. Salinity induces Na⁺ accumulation and K⁺ efflux, destroy ion homeostasis, causes ion toxicity, inhibits plant growth that leads to decrease cell turgidity may cause drastic growth reductions (Yang *et al.*, 2018). Data reveals in Table 2 that Maximum reduction percentage in root length was noted in the Sahbhagi Dhan than MTU 1010 at three different concentrations of NaCl. Root length decreased significantly @ 200 mM of NaCl in both genotypes showed 39.9% in Sahbhagi Dhan, and 30.6% in MTU 1010 as compared to control. Present findings are supported with the observations recorded by Dolatabadian *et al.* (2011), who observed that salinity stress significantly decreased shoot and root length. Salicylic acid (SA) @ 1.0 mM increase much root length in both genotypes with the combination NaCl @ 100 mM; as compared to two other combination of SA and NaCl. The decreased reduction percentage in root length was 23.3% in Sahbhagi Dhan and 8.8% in MTU in combination with SA (@ 1.0 mM) and NaCl (@ 100 mM) as compared to salt treated ones.

Shoot length decreased with increasing NaCl concentrations @ 100, 150, 200 mM in both the genotypes, *i.e.*, Sahbhagi Dhan and MTU 1010. Significant reduction percentage in shoot length was recorded @ 200 mM NaCl in both the genotypes, *i.e.*, 22.1% in Sahbhagi Dhan and 38.3% in MTU 1010, respectively as compared to control (Table 3). Salicylic acid (SA) @ 1.0 mM, increased shoot length at all the levels of NaCl in Sahbhagi Dhan. However, reduction percentage in shoot length decreased significantly in both the genotypes in combination with SA (@ 1.0 mM) and NaCl (@ 100 mM) as compared to two other combinations, *i.e.*, 14.6% in Sahbhagi Dhan and 14.9% in the MTU 1010. The results were similar to the findings reported by Jini and Joseph (2017), which indicates that the shoot and root length of rice varieties were reduced by salinity that were significantly enhanced by the application of SA under salt stress. From the result, it was proved that SA application to the salinity stress medium helps to mitigate the salinity stress to the rice plants during early seedling growth.

Total dry weight plant⁻¹ (g)

It is obvious from the data that Salinity stress reduced the total dry weight plant⁻¹ in both the rice genotypes. The maximum reduction percentage was recorded in genotype MTU 1010 than Sahbhagi Dhan as compared

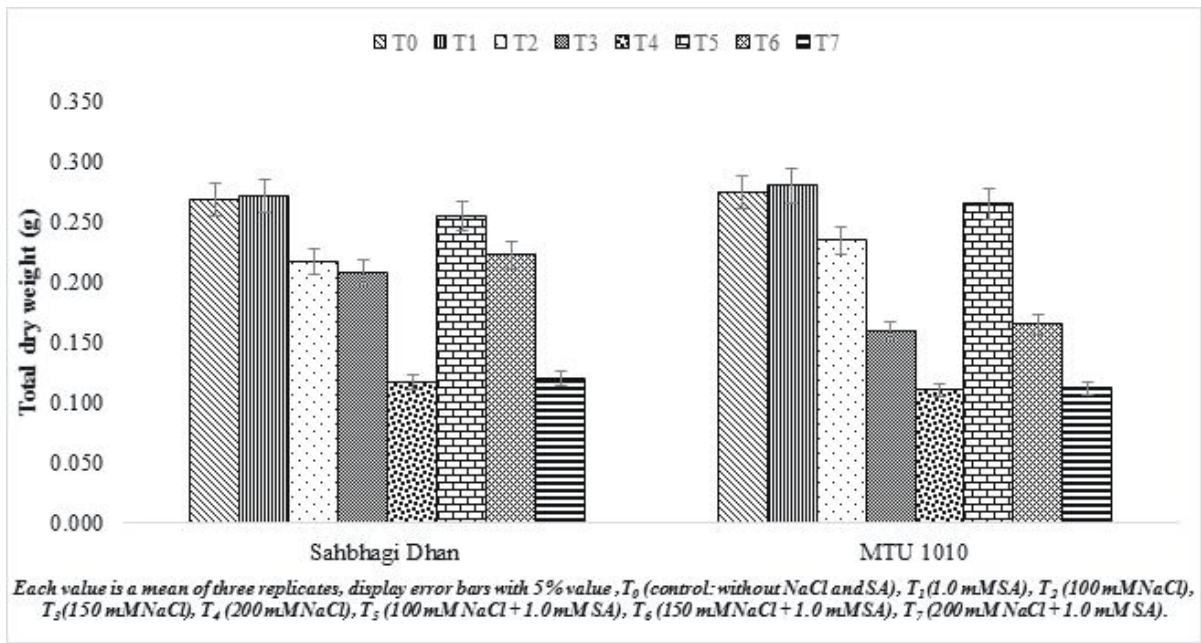


Fig. 1 : Consequences of Salinity Stress on Total dry weight (g) of Rice Genotypes Influenced by Salicylic acid.

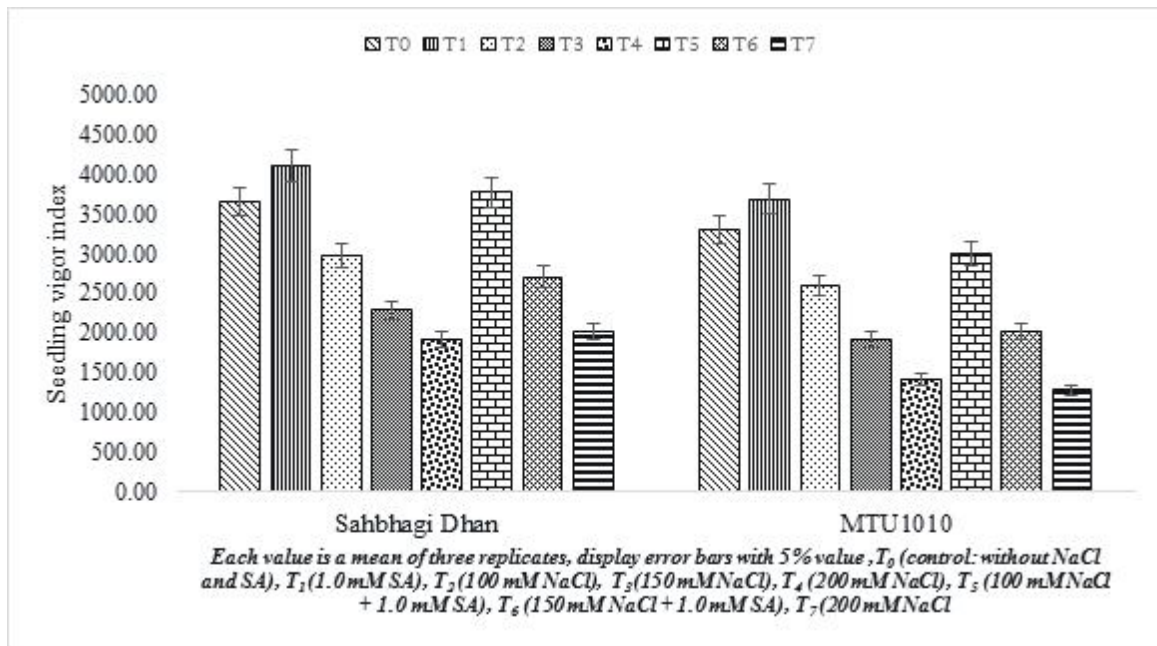


Fig. 2 : Consequences of Salinity stress on Seedling Vigor Index (SVI) of Rice Genotypes influenced by Salicylic acid.

to control (Fig. 1). In both the rice genotypes, total dry weight plant⁻¹ significantly decreased @ 200 mM NaCl. The reduction percentages of total dry weight plant⁻¹ @ 200 mM NaCl were 56.43% in Sahbhagi Dhan and 56.38% in MTU 1010. Salicylic acid (SA) hardened seeds (@ 1.0 mM) decreased the reduction percentage in total dry weight at all the three levels of NaCl, but significant decrease in reduction percentage was noted in combination with SA (@ 1.0 mM) and NaCl (@ 100 mM), *i.e.*, 17.4% in Sahbhagi Dhan and 12.9% in MTU 1010. Naheed *et al.* (2007) reported significant reduction in dry weight of rice at higher concentration of NaCl and

confirmed the findings. Identical results were also noticed in several other crops such as maize (Nawaz and Ashraf, 2010), wheat (Khan *et al.*, 2006).

Seedling Vigor Index

The seedling vigor index decreased significantly with increased salinity stress levels. Seedling vigor decreased with increasing NaCl concentrations *i.e.*, @ 100, 150 and 200 mM in both the genotypes, (Sahbhagi Dhan and MTU 1010). Significant reductions percentage in seedling vigour index were recorded @ 200 mM NaCl in both the genotypes, *i.e.*, 47.6% in Sahbhagi Dhan and 57.4% in

Table 3 : Consequences of Salinity Stress on Shoot Length (cm) of Rice Genotypes influenced by Salicylic acid.

Year 2021		
Treatment	Sahbhagi Dhan	MTU1010
T ₀ (Control:0.0 mM NaCl & SA)	15.9	14.4
T ₁ (1.0 mM SA)	16.7	15.4
T ₂ (100 mM NaCl)	12.9	13.9
T ₃ (150 mM NaCl)	12.2	11.5
T ₄ (200 mM NaCl)	9.6	10.0
T ₅ (100 mM NaCl + 1.0 mM SA)	15.9	15.1
T ₆ (150 mM NaCl + 1.0 mM SA)	13.0	11.9
T ₇ (200 mM NaCl + 1.0 mM SA)	9.7	9.8
	SEm ±	CD 5%
Genotypes (G)	1.32	3.82
Treatments (T)	0.66	1.91
G × T	0.47	1.35

MTU 1010 respectively as compared to control. Treatment of Salicylic acid (SA) @ 1.0 mM, increased seedling vigor index at all the levels of NaCl in Sahbhagi Dhan as compared to salinity treated ones. While, reduction percentage in seedling vigor index decreased significantly in both the genotypes in combination with SA (@ 1.0 mM) and NaCl (@ 100 mM) as compared to two other combinations, *i.e.*, 27.1% in Sahbhagi Dhan and 15.9% in the MTU 1010 (Fig. 2). Salicylic acid @ 1.0 mM and higher concentration of NaCl @200 mM treatment showed that the genotypes differed in their ability to tolerate effect of salinity stress at the same salinity levels. The findings are fully in accordance with Kandil *et al.* (2019) and Karlidag *et al.* (2009) research, suggested that salinity affects the seedling vigour index by suppressing the growth of root length and shoot length because Salicylic acid treatment able to mitigate the deleterious effect of salinity stress on seedling growth.

Conclusion

Present findings indicate that germination and early seedling growth stage of both rice genotypes were inhibited by increasing salt concentration. From the result, it was proved that SA hardened rice seeds sowing them in different levels of salty medium alleviates the deleterious effect of salinity stress on rice plants during germination and seedling growth stage. Seed hardening or priming is an important technique for enhancing germination performance and seedling growth under saline conditions (Benidire *et al.*, 2015) by controlling osmoregulation processes (Rhaman *et al.*, 2021). In the present study, the effects of Salicylic acid (SA) on germination and seedling growth of rice were studied.

Salinity stress causes water stress, ion toxicity and nutritional disorders. Higher concentration salt causes the osmotic changes in outside the root, which reduces the ability of the plant to absorb water and minerals like K⁺ and Ca²⁺. Sahbhagi Dhan has the more tolerance than genotype MTU 1010 at different levels of NaCl as compared to control. Salicylic acid (SA) treatment could not alleviate the 100 per cent deleterious effects of salinity stress on rice genotypes, but decreased the reduction per cent in germination and growth parameters significantly at optimum level of NaCl than higher levels of NaCl. This study strongly suggests that salicylic acid treatment in seeds before sowing is beneficial at optimum levels of salts for attaining optimum size, shape and appearance of morphological features by each and every plant indispensable for efficient performance of a number of physiological processes with a view to accomplish needed plant growth and development.

Future scope

It is the need of hour and future strategies to have a multidisciplinary approach that integrates the exact mechanism of Salicylic acid in biochemical and molecular investigations for enhancing the survival and plant vigor under salinity stress. This kind of investigation may be crucial for expanding the actual concentration and use of Salicylic acid in various crops for sustainable and resilient farming system in adverse environmental situations.

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Conflict of interest : None.

References

- Abdul-Baki, A.A. and Anderson J.D. (1973) Vigour determination in soybean seed by multiple criteria. *Crop Sci.*, **13**, 630-633.
- Benidire, L., Daoui K., Fatemi Z.A., Achouak W., Bouarab L. and Oufdou K. (2015). Effect of salt stress on germination and seedling of *Vicia faba* L. *J. Mater. Environ. Sci.*, **6**, 840-851.
- Dolatabadian, A., Modarressanavy S.A.M. and Ghanati F. (2011). Effect of salinity on growth, xylem structure and anatomical characteristics of soybean. *Notulae Sci. Biolog.*, **3**, 41-45.
- Gomez, K.A. and Gomez A.A. (1984). *Statistical procedures for agricultural research*. A. Willey-Interscience Publication, New York.

- Hayat, Q., Hayat S., Irfan M. and Ahmad A. (2010). Effect of exogenous salicylic acid under changing environment: A review. *Environ Exp Bot.*, **68**(1), 14–25.
- Heenan, D.P., Lewin L.G. and McCaffery D.W. (1988). Salinity tolerance in rice varieties at different growth stages. *Aust. J. Exp. Agric.*, **28**, 343–349.
- Jangra, M., Devi S., Satpal K.N., Goyal V. and Mehrotra S. (2022). Amelioration effect of salicylic acid under salt stress in *Sorghum bicolor* L. *Appl Biochem Biotechnol.*, **194**(10), 4400–4423.
- Jini, D. and Joseph B. (2017). Physiological Mechanism of salicylic acid for alleviation of salt stress in rice. *Sci. Direct Rice Sci.*, **24**(2), 97–108.
- Kandil, A.A., Sharief A.E., Fatma M. and Abd EL-Fatah (2019). Influence of antioxidants and salinity stress on seedling parameters of some rice cultivars. *J. Plant Stress Physiol.*, **5**, 15–21.
- Karlidag, H., Yildirim E. and Turan M. (2009). Salicylic Acid Ameliorates The adverse effect of salt stress on strawberry. *Sci. Agric. (Piracicaba, Braz.)*, v.66, n.2, p
- Khan, A., Ahmad M.S.A., Athar H.R. and Ashraf M. (2006). Interactive effect of foliar applied ascorbic acid and salt stress on wheat (*Triticum aestivum* L.) at seedling stage. *Pak J. Bot.*, **39**(5), 1407–1414.
- Khan, M.A. and Weber D.J. (2008). *Ecophysiology of high salinity tolerant plants* (tasks for vegetation science), 1st edn. Springer, Amsterdam.
- Liu, Z., Ma C., Hou L., Wu X., Wang D., Zhang L. and Liu P. (2022). Exogenous SA Affects Rice Seed Germination under Salt Stress by Regulating Na⁺/K⁺ Balance and Endogenous GAs and ABA Homeostasis. *Int J Mol Sci.*, **18**;23(6), 3293.
- Lutts, S., Kinet J.M. and Bouharmont J. (1995). Changes in plant response to NaCl during development of rice (*Oryza sativa*) varieties differing in salinity resistance. *J. Exp. Bot.*, **46**, 1843–1852.
- Naheed, G., Shahbaz M., Latif A. and Rha E.S. (2007). Alleviation of the adverse effects of salt stress on rice (*Oryza sativa* L.) by phosphorus applied through rooting medium: Growth and gas exchange characteristics. *Pak. J. Bot.*, **39**(3), 729–737.
- Nawaz, K. and Ashraf M. (2010). Exogenous application of glycinebetaine modulates activities of antioxidants in maize plants subjected to salt stress. *J. Agron. Crop Sci.*, **196**(1), 28–37.
- Rhaman, M.S., Imran S., Rauf F., Khatun M., Baskin C.C., Murata Y. and Hasanuzzaman M. (2021). Seed priming with phytohormones: An effective approach for the mitigation of abiotic stress. *Plants*, **10**, 37 [CrossRef] [PubMed].
- Sakhabutdinova, A.R., Fatkhutdinova D.R., Bezrukova M.V. and Shakirova F.M. (2003). Salicylic acid prevents the damaging action of stress factors on wheat plants. *Bulg J Plant Physiol.*, **29**, 314–319.
- Williams, M., Senaratna T., Dixon K. and Sivasithamparam K. (2003). Benzoic acid induces tolerance to biotic stress caused by *Phytophthora cinnamomi* in Banksai attenuate. *Plant Growth Regulation*, **41**, 89–91.
- Yang, Y. and Guo Y. (2018). Elucidating the molecular mechanisms mediating plant salt-stress responses. *New Phytol.* **217**, 523–539. [Google Scholar] [CrossRef] [PubMed] [Green Version].
- Yeo, A.R., Yeo M.E., Flowers S.A. and Flowers T.J. (1990). Screening of rice (*Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance and their relationship to overall performance. *Theor. Appl. Genet.*, **79**, 377–384.
- Yu, Y., Wang J., Shi H., Gu J., Dong J., Deng X. and Huang R. (2016). Salt stress and ethylene antagonistically regulate nucleocytoplasmic partitioning of COP1 to control seed germination. *Plant Physiol.*, **170**, 2340–2350.