



Effectiveness of Plant Growth Regulators on Growth Attributes of Different Malaysian Rice Cultivars Primed with NaCl on Germination

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ARTICLE INFO

Keywords:

PGR
 Phytohormone
 Priming
 Rice
 Salinity

Article History:

Received: March 6, 2024

Accepted: May 8, 2025

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ABSTRACT

Plants have a hard time in its growth and development under salt stress. Salinity stress increase the activity of antioxidants and lessen the damage that salt does, phytohormones might be a good answer. Phytohormones application has emerged as a promising strategy in modern stress management as it defends plants against various abiotic stresses. The study was planned to define whether salt primed seeds treated with plant growth regulators (PGRs) can germinate normally and which level of PGRs can better assist rice germination and seedling growth. Seeds of Malaysian rice cultivars MR263 and MR284 were presoaked with 100 mM NaCl. Salt primed seeds were then treated with 0.25, 0.5, 0.75 and 1 mM Gibberellic acid (GA_3), Salicylic acid (SA) and 10, 20, 30, 40 mM Thiourea (TU) in petridishes in controlled lab conditions. The experiment was designed in factorial CRD (completely randomized design) with two factors and four replicates. The interaction between rice cultivars and treatments (PGRs) significantly affected water uptake potential, germination percentage, germination index, seedling fresh and dry weight and seed vigor index. The application of 0.25 mM GA_3 , 0.25 mM SA and 10 mM TU reduced the inhibitory effect of NaCl priming at germination level.

INTRODUCTION

Agricultural lands are prone to artificial salinity due to excessive use of fertilizers for boosting crop growth and yield (Krasilnikov et al., 2022). This is the one way to fulfill the high demand of food by utilizing the limited land for excessive crop production. The other way is to increase the agricultural land which is again limited due to different soil type, its condition and weather. The naturally saline saline-sodic or sodic soils are poor soils that hinder the high crop production (Daba & Qureshi, 2021). Plants have the capability to cope this artificial or natural salinity naturally, but selection of those salinity resisted crops required

study and selection process. Scientists have devised various techniques and studies to cope with stresses which plants have to face for their survival and life cycle completion.

Seed priming is a pre-sowing and physiological method to trigger germination activities of the seed embryo (Salih et al., 2022). The presoaking of seeds regulates the hormones that induce cytological and gene processes of a dormant juvenile plant. In other words, triggering of cytological and genes machinery produce hormones require. for embryo cells to divide, multiply, growth and development (Hussain et al., 2012).

ISSN: 0126-0537

Cite this as: Zahra, S. M., Ibrahim, M. H., Nulit, R., Yaapar, M. N., & Maqbool, N. (2025). Effectiveness of plant growth regulators on growth attributes of different Malaysian rice cultivars primed with NaCl on germination. *AGRIVITA Journal of Agricultural Science*, 47(2), 326-337. <https://doi.org/10.17503/agrivita.v47i2.4503>

Plants produce multiple ranges of hormones to regulate their cytological, genetical, physiological phenomena of life (Yamaguchi et al., 2010). The widely studied Plant growth regulators (PGRs) includes gibberellins, salicylic acid (SA), and indole acetic acid make it easier for plants to adjust to salt stress. Plants grow, anti-oxidants work better, and they maintain the body's balance. Protecting plants from the harmful effects of salt with PGRs is an inexpensive method that helps them keep developing and creating even when things are tough.

The widely studied plant growth regulators (PGRs) include gibberellin or gibberellic acid (GA_3), Salicylic acid (SA) and Indole acetic acid (IAA). PGR is an easy low-cost technique to alleviate damaging effects of salinity (Quamruzzaman et al., 2021). SA mitigates the impact of salinity in various crops such as rice (Jini & Joseph, 2017), wheat (Fardus et al., 2018), maize (Aslam et al., 2021), marigold (Afzal et al., 2017), faba beans (Anaya et al., 2018). GA_3 induced salt tolerance in summer squash (Al-harathi et al., 2021), leaf lettuce and rocket (Vetrano et al., 2020), papaya (Álvarez-Méndez et al., 2022), hagel (Salih et al., 2022) and rice (Farooq et al., 2022). The negative consequences of abiotic stress can be mitigated to some extent with the supplementation of exogenous natural or synthetic PGRs (Sabagh et al., 2021). GA_3 , abscisic acid (ABA) and Cytokines (CK) applied solely or in combination to improve plant stress tolerance (Raza et al., 2022). The foliar application of GA_3 , ABA and CK showed higher grain yield, increased number of filled grains per panicle, improved harvest index in dry seeded rice (Pal et al., 2020). GA_3 supplementation delayed senescence at the grain filling stage (Luo et al., 2021). Thiourea (TU) is a synthetic bioregulator for growth and development of crops. Its exogenous application boosts plants growth and productivity under normal and stressful conditions (Granaz et al., 2022).

The hypothesis of this study is that the NaCl priming effect on Malaysian rice could be strengthened by the treatment of PGRs including GA_3 , SA and TU and selection of suitable dose of these PGRs. The objective of this study was to assess the impact of NaCl priming on and to alleviate this effect by different doses of PGRs on germination and seedling growth of Malaysian rice cultivars.

MATERIALS AND METHODS

Plant Materials

The two rice cultivars MR263 and MR284 were obtained from MARD Parit, Perak, Malaysia. The healthy seeds of two Malaysian rice cultivars were surface sterilized with 70% ethanol for 30 seconds, then washed with 5% NaOCl solution containing one drop of Tween 20 for another 20 minutes. Seeds were washed with autoclaved distilled water five times following air dried on tissue paper for 24 hours (Goswami et al., 2013).

Experimental Design

The study was conducted in controlled environment at Tissue Culture Laboratory Biology Department of Faculty of Science in Universiti Putra Malaysia, on January 2023. The research was designed in a factorial experiment (variety x PGR) arranged in a completely randomized design (CRD) with four replications. The surface sterilized seeds of MR263 and MR284 were primed with 100 mM NaCl (Sodium Chloride) for 16 hours at swirled incubator at room temperature to ensure proper aeration. After 16 hours, seeds were rinsed with distilled water and surface dried on filter paper for ± 3 hours to retrieve original moisture (Budiastuti et al., 2020). The exogenous plant growth regulators levels were 0mM (control) 0.25, 0.5, 0.75, 1.0 mM GA_3 and SA; 10, 20, 30, 40 mM Thiourea (TU). Salinity primed seeds were soaked in various concentration of PGR (5 ml) for 12 hours in glass jars at room temperature in jar. Then seven seeds of each treatment were placed in a 9 cm petri dish with two layers of filter paper moistened with distilled water. All the petri dishes were covered with lids to reduce evaporation, incubated at $\pm 26^\circ\text{C}$ 16/8 hours (light/dark) cycled Philips Fluorescent light (Salih et al., 2022). The data of germination parameters were recorded.

Germination Parameters

Hypocotyl and radical length were measured manually and their dry and fresh weights were weighed. Water uptake potential, germination percentage, seed vigor index, seedling height reduction and mean germination time are measured using following formulas:

Water uptake potential (WUP) =

$$\frac{\text{Seed fresh weight} - \text{Seed dry weight}}{\text{Seed fresh weight}} \times 100 \dots\dots\dots 1)$$

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Germination Percentage (GP) =

$$\frac{\text{Number of germinated seeds}}{\text{Total number of seed sown}} \times 100 \dots\dots\dots 2)$$

Seed vigor index (SVI) =

$$\frac{\text{Length of Hypocotyl} + \text{length of radical}}{100} \times \text{GP} \dots\dots\dots 3)$$

Seedling height reduction (SHR) =

$$\frac{\text{Seedling height at control} - \text{Seedling height at treated condition}}{\text{Seedling height at control}} \dots\dots\dots 4)$$

$$\text{Mean germination time (MGT)} = \frac{\sum Dn}{\sum n} \dots\dots\dots 5)$$

Where: n is number of germinated seeds which are germinated on day D and D is the number of days counted from beginning of germination.

Inferential Analysis

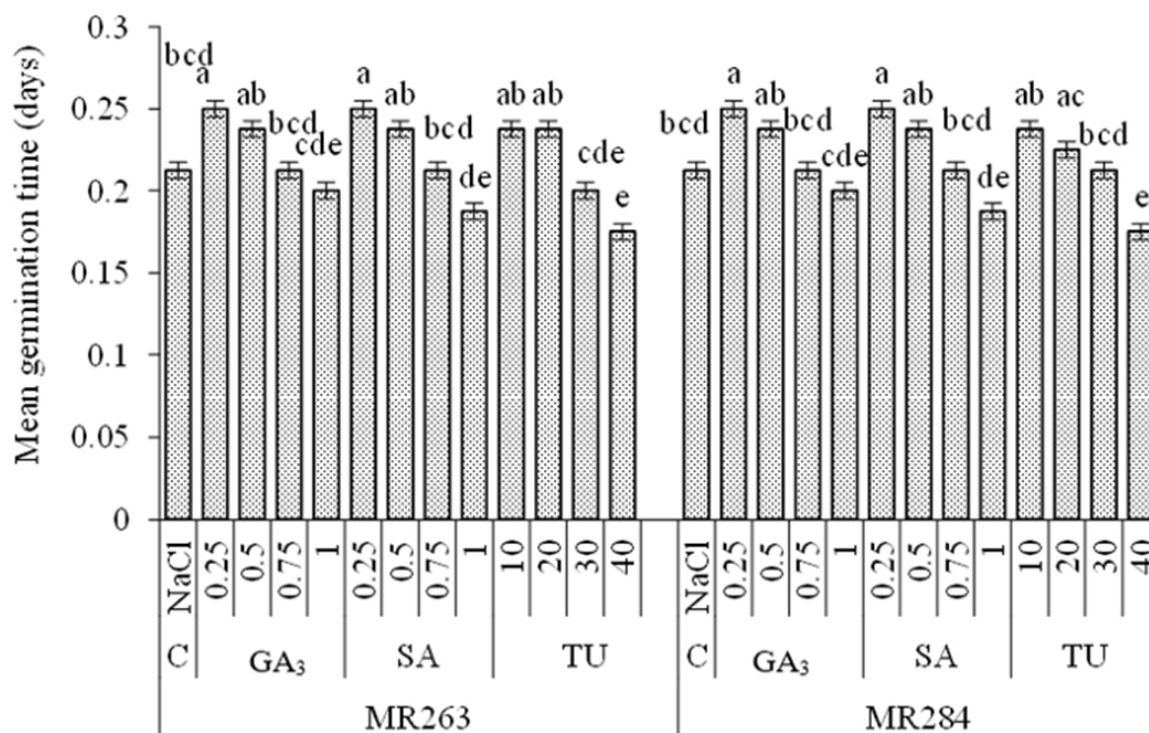
Data was computed for two factors factorial ANOVA with probability level $P \leq 0.05$ using SPSS 22 version for Windows. The means values were compared with DMRT Test.

RESULTS AND DISCUSSION

Germination Attributes

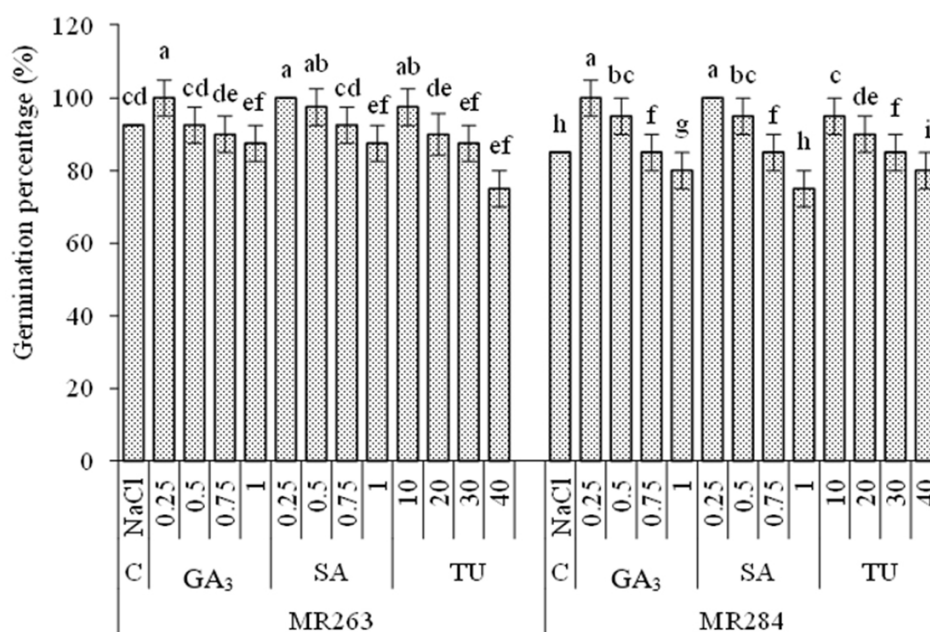
Mean Germination Time (MGT)

Seed priming with 100 mM NaCl (control) have significantly affected the mean germination time (Fig. 1). The treatment with high levels of PGRs reduced germination time compared with low concentrations. The highest mean germination time for MR263 and MR284 was recorded at a dose of 0.25 mM GA_3 (0.25), followed by the dose of 0.5 mM GA_3 (0.2375). While dose of 0.25 mM SA and dose of 10 mM TU increased mean germination time by 0.25 and 0.2375 respectively, compared to 100 mM NaCl without PGRs.



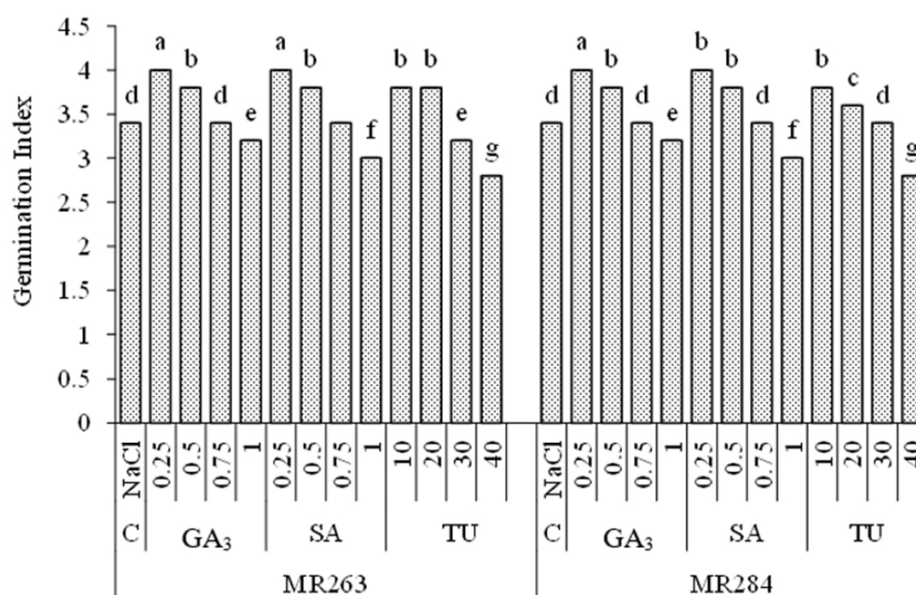
Remarks: Graphical bars represent means values $\pm$ errors bars represent SD and alphabetical labels above bars represent the statistical significance among cultivars and treatments

Fig. 1. Mean germination time (MGT) of two Malaysian rice cultivars (MR263 and MR284) seeds primed with NaCl (100 mM) and treated with different doses of PGRs (GA_3 , SA and TU)



Remarks: Graphical bars represent means values $\pm$ errors bars represent SD and alphabetical labels above bars represent the statistical significance among cultivars and treatments

Fig. 2. Germination percentage (GP) of two Malaysian rice cultivars (MR263 and MR284) seeds primed with NaCl (100 mM) and treated with different doses of PGRs (GA₃, SA and TU)



Remarks: Graphical bars represent means values $\pm$ errors bars represent SD and alphabetical labels above bars represent the statistical significance among cultivars and treatments

Fig. 3. Germination index (GI) of two Malaysian rice cultivars (MR263 and MR284) seeds primed with NaCl (100 mM) and treated with different doses of PGRs (GA₃, SA and TU)

Germination Percentage (GP)

Seed priming of Malaysian rice cultivars with NaCl (100 mM) showed decrease in germination percentage (Fig. 2). Among all PGR treatments the 0.25 mM GA₃ and SA enhanced 100% germination percentage of NaCl primed seeds of both cultivars compared to all other doses of PGRs. Among all doses of TU, 10 mM TU increased germination percentage of MR263 (97.5) and MR284 (95) compared to 20, 30 and 40 mM of TU.

Germination Index (GI)

Seed priming with 100 mM NaCl (control) have significantly affected the germination index (Fig. 3). The treatment with high levels of PGRs reduced germination index compared with low concentrations. The highest germination index for MR263 and MR284 was recorded at a dose of 0.25 mM GA₃ (4), followed by the dose of 0.5 mM GA₃ (3.8). While 0.25 mM SA and 10 mM TU enhanced germination index by 4 and 3.8 respectively compared to 100 mM NaCl without PGRs.

Water Uptake Potential (WUP)

Cultivars and PGRs interactions exhibited significant ($P \leq 0.05$) effect at various levels of PGRs (Table 1). WUP of seeds of MR263 was 83.2 and MR284 was 87.9 when primed with 100 mM NaCl (control). However, when seeds were treated with 0.25 mM GA₃ and SA, WUP was increased by

89.6% for both cultivars while treatments of TU decreased the WUP of both cultivars as compared to control (Fig. 4).

**Growth Attributes
Seedling Height (SDH)**

Treatment with different levels of PGRs has significantly affected the salt primed rice cultivars seedling height (Table 1). Among PGRs, seedling height of both Malaysian rice cultivars was significantly enhanced by GA₃ as compared to different doses of SA and TU respectively. Among different levels of GA₃, the highest values of SDH of MR263 and MR284 were 34.5 cm/plant and 54.7 cm/plant recorded at 0.25 mM GA₃ respectively (Fig. 5).

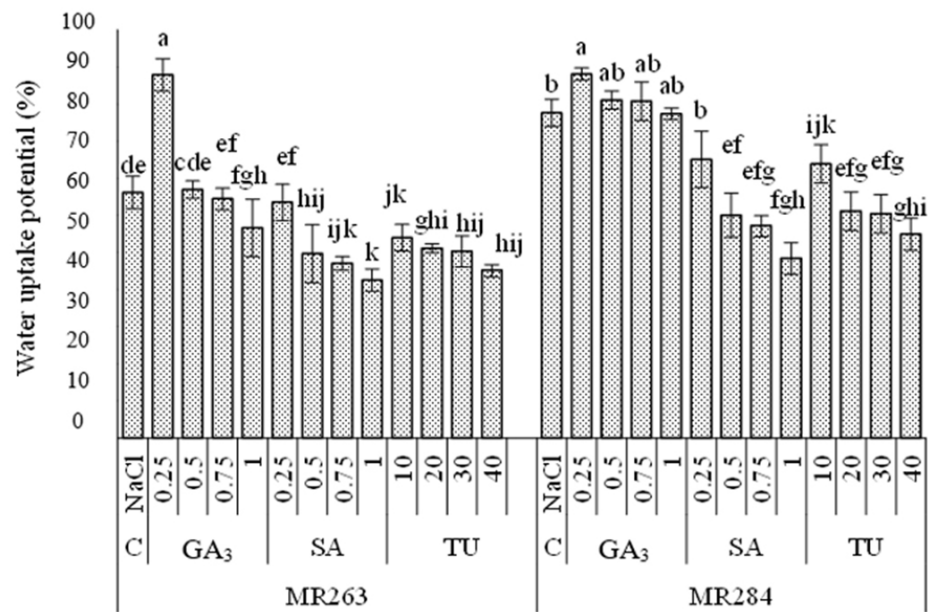
Plant Fresh Weight (PFW)

PFW was significantly affected by PGRs and cultivars interactions (Table 1). Increased levels of PGRs significantly decreased the PFW of NaCl (100 mM) seed primed MR263 and MR284. At the control without PGRs, the NaCl priming decreased PFW by (0.3 g and 0.4 g) of both rice cultivars. At 0.25 mM GA₃, the values of PFW were 0.5 g/plant for MR263 and MR284 respectively. The PFW was lowered at all levels of SA and TU as compared to control (Fig. 6).

Table 1. ANOVA studies of two Malaysian rice cultivars (MR263 and MR284) seeds primed with NaCl (100 mM) and treated with different doses of PGRs (GA₃, SA and TU)

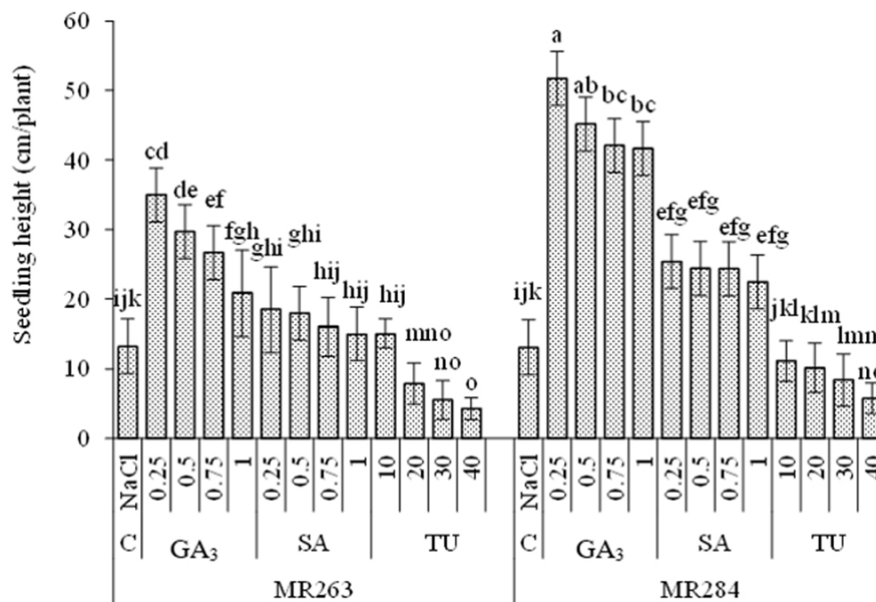
Attributes	Cultivars (C)	Treatments (T)	C×T
Mean Germination Time	5.99e ⁻³⁵ ns	0.004***	5.2e ⁻⁵ ns
Germination percentage	808.65***	396.15***	87.82***
Germination index	0.003ns	1.09***	0.01***
Water uptake potential	224.79***	70.41***	8.36***
Seedling height	1541.29***	1184.77***	110.56ns
Plant fresh weight	0.15***	0.06***	0.009***
Plant dry weight	5.64e ⁻⁴ ***	4.66e ⁻⁴ ***	2.24e ⁻⁴ ***
Seed vigor index	298.21***	583.91***	28.89***
Seedling height reduction	16.4ns	87.11***	0.84ns

Remarks: Values represents means square values and * represents significance level ($P \leq 0.05$); *** very highly significant; ** highly significant; * significant; ns – non-significant



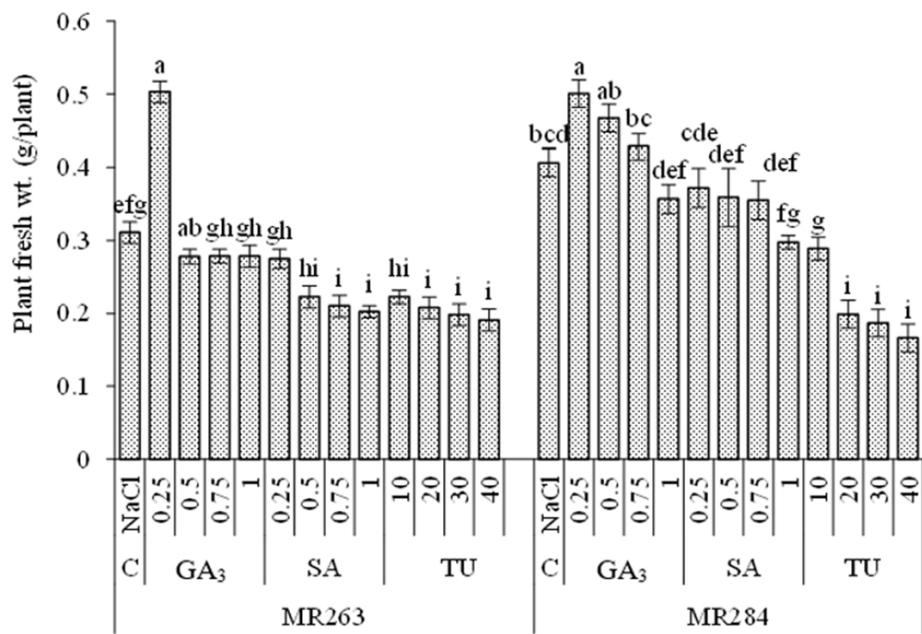
Remarks: Graphical bars represent means values $\pm$ errors bars represent SD and alphabetical labels above bars represent the statistical significance among cultivars and treatments

Fig. 4. Water uptake potential (WUP) of two Malaysian rice cultivars (MR263 and MR284) seeds primed with NaCl (100 mM) and treated with different doses of PGRs (GA₃, SA and TU)



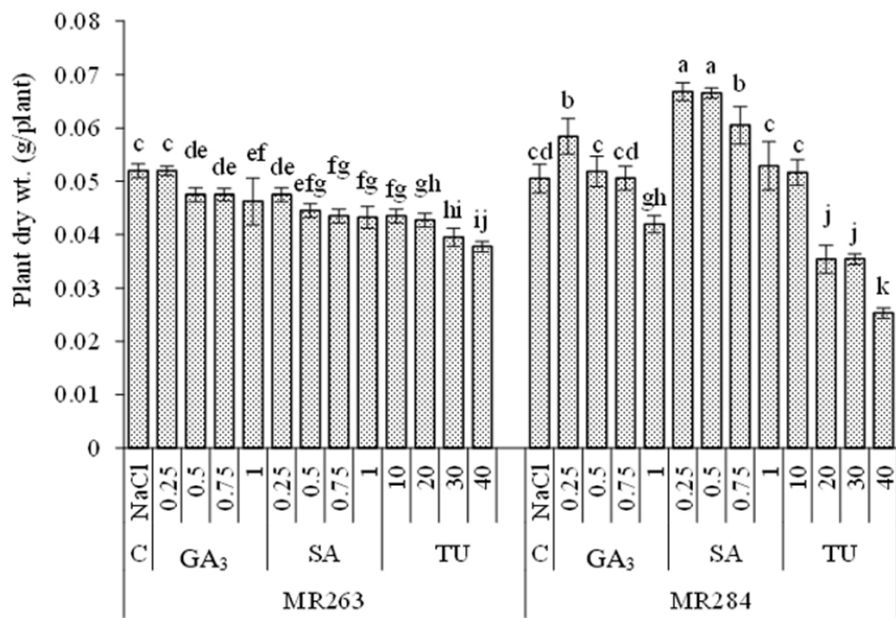
Remarks: Graphical bars represent means values $\pm$ errors bars represent SD and alphabetical labels above bars represent the statistical significance among cultivars and treatments

Fig. 5. Seedling height (SH) of two Malaysian rice cultivars (MR263 and MR284) seeds primed with NaCl (100 mM) and treated with different doses of PGRs (GA₃, SA and TU)



Remarks: Graphical bars represent means values ± errors bars represent SD and alphabetical labels above bars represent the statistical significance among cultivars and treatments

Fig. 6. Plant fresh weight (PFW) of two Malaysian rice cultivars (MR263 and MR284) seeds primed with NaCl (100 mM) and treated with different doses of PGRs (GA₃, SA and TU)



Remarks: Graphical bars represent means values ± errors bars represent SD and alphabetical labels above bars represent the statistical significance among cultivars and treatments

Fig. 7. Plant dry weight (PDW) of two Malaysian rice cultivars (MR263 and MR284) seeds primed with NaCl (100 mM) and treated with different doses of PGRs (GA₃, SA and TU)

Plant Dry Weight (PDW)

PDW was significantly affected by PGRs and cultivars interactions (Table 1). Elevated levels of PGRs significantly reduced the PDW of NaCl (100 mM) seed primed MR263 and MR284. At the control without PGRs, the NaCl priming declined the PDW by (0.05 g/plant) of both rice cultivars. The values of PDW at 0.25 mM GA₃ were 0.058 g/plant and 0.25 mM SA (0.048 and 0.067g/plant) for MR263 and MR284 respectively. The PDW was lowered at all levels of TU as compared to control (Fig. 7).

Seed Vigor Index (SVI)

Seed priming with 100 mM NaCl (control) have significantly affected the seed vigor index (Fig. 8). The treatment with high levels of PGRs reduced SVI compared with low concentrations. The highest SVI for MR263 and MR284 was recorded at a dose of 0.25 mM GA₃ (27.1 and 38.4), followed by the dose of 0.5 mM GA₃ (22.6 and 29.5). While 0.25 mM SA enhanced SVI of MR263 by 15.8 and MR284 by 17.4 respectively compared to 100 mM NaCl without PGRs. All the treatments of TU decreased the seed vigor index of both the cultivars as compared to control.

Seedling Height Reduction (SHR)

NaCl seed priming of both the rice cultivars and treatment with different levels of PGRs had significantly affected SHR (Table 1). The highest value of SHR was 11.5 cm/plant, 12 cm/plant and 12.9 cm/plant recorded at the dose of 1 mM GA₃, 1 mM SA and 40 mM TU respectively for MR263 (Fig. 9). The highest value of SHR was 9.6 cm/plant, 11.2 cm/plant and 12.6 cm/plant recorded at the dose of 1 mM GA₃, 1 mM SA and 40 mM TU respectively for MR284 (Fig. 9). The low levels of PGRs, 0.25 mM GA₃ and SA, 10 mM TU showed lowest values of SHR in both rice cultivars compared to high levels of PGRs (Fig. 9).

Discussion

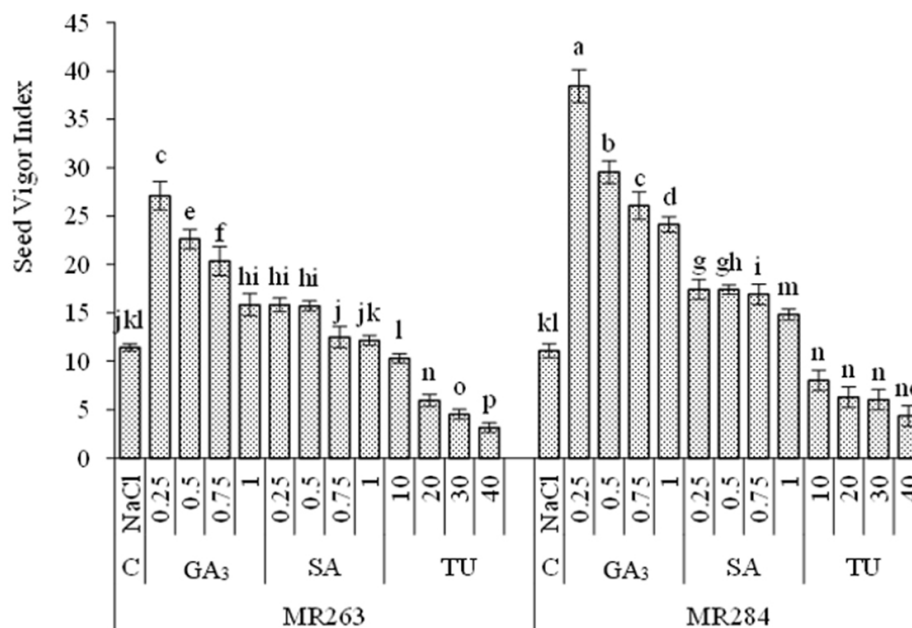
Germination constitutes the most important stage in the life cycle of any plant as it determines the survival of the seedlings in its native soil (Guo et al., 2020). Successful germination of a seed depends on appropriate water absorption and ionic balance outside and inside of a seed. Salinity or high concentration of salts in the vicinity of seed holds the movement of water molecules from high water

potential to low water potential (De La Reguera et al., 2020).

This xerophytic osmotic stress prone the seed embryo desiccation and uphold its germination (Peng et al., 2022). Salinity influences the amount of seed germination stimulants such as reduction in amount of GA₃ and ABA, membrane permeability and seed water behavior (Uçarlı, 2021). The final germination percentage, germination energy of all rice varieties was arrested at 20 dS/m salinity along with reduction in their root and shoot length and dry weight respectively (Farooq et al., 2021). Germination percentage, germination index, mean germination time, seedling vigor index, ion leakage, radicle and plumule length, root and shoot fresh and dry weights were significantly affected by increasing salinity in pumpkin (Irik & Bikmaz, 2024).

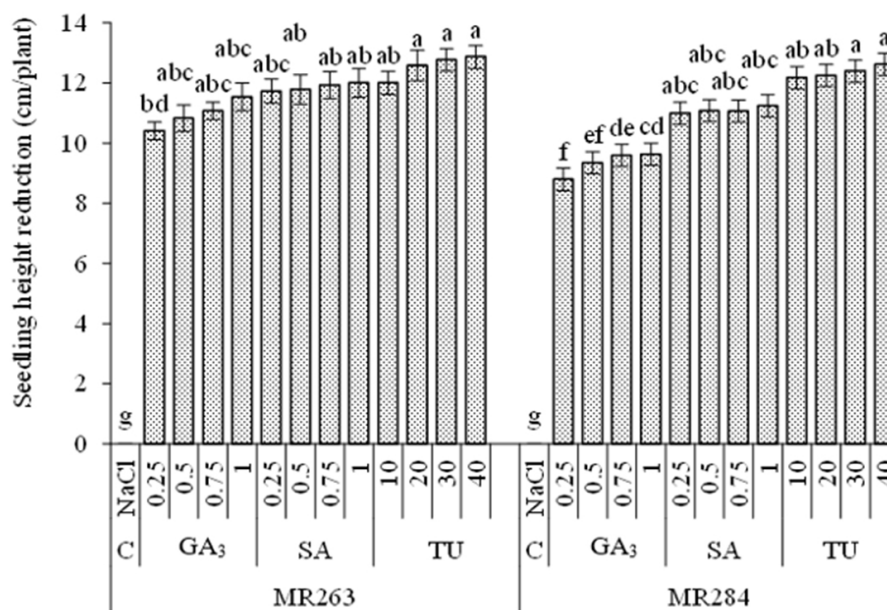
The SA treatment reduced mean germination time, enhanced germination, germination index and plant height of Indian squash under heat stress (Qamar et al., 2022). Eight varieties of rice had a large distribution of salinity tolerances at 120 mM NaCl (Farid et al., 2021). Chauhan et al. (2019) reported the reduction in germination and growth of three oat cultivars under salinity. Application of 150 ppm GA₃ maximized the germination and seed vigor but inhibited the root growth of sensitive oat cultivars. Seeds of *Zea mays* L., *Pisum sativum* and *Lathyrus sativus* L. primed with 0.2 g/l GA₃ had improved germination percentage, reduced mean germination time, increased shoot and root length, weight under 8 dS/m salinity (Tsegay & Andargie, 2018).

The treatment of GA₃ alleviates the impact of 200 and 300 mM NaCl on *Leymus chinensis* by increasing the germination percentages in light and dark conditions (Wu et al., 2016). The 400 ppm GA₃ resulted in maximum plant height, number of leaves, fresh and dry weight of root and shoot of Jatti Khatti when treated for 12 hours (Yadav et al., 2022). The 50 ppm GA₃ induced significant effect on germination, vigor index, shoot root leaf length and weight of Asparagus (Dhoran & Gudadhe, 2012). *Limonium bicolor* germination was significantly suppressed at 200 mM NaCl and treatment of Salicylic acid upregulated the key genes that enhances GA₃ biosynthesis and inhibited the expression of ABA biosynthesis thereby enhanced seed germination (Li et al., 2019).



Remarks: Graphical bars represent means values $\pm$ errors bars represent SD and alphabetical labels above bars represent the statistical significance among cultivars and treatments

Fig. 8. Seed vigor index (SVI) of two Malaysian rice cultivars (MR263 and MR284) seeds primed with NaCl (100 mM) and treated with different doses of PGRs (GA₃, SA and TU)



Remarks: Graphical bars represent means values $\pm$ errors bars represent SD and alphabetical labels above bars represent the statistical significance among cultivars and treatments

Fig. 9. Seedling height reduction (SHR) of two Malaysian rice cultivars (MR263 and MR284) seeds primed with NaCl (100 mM) and treated with different doses of PGRs (GA₃, SA and TU)

Thiourea primed late sown wheat showed uniform germination, good stand establishment and greater productive tillers (Chattha et al., 2007). The combined application of KCl, 2 mM thiourea and 0.06 mM GA₃ improved germination and seedling growth of rice under drought in hydroponic conditions (Mahadi et al., 2020). Thiourea seed priming of Camelina varieties improved root and shoot length, biomass, water relations and seed yield under heat stress (Waraich et al., 2021). Thiourea capped nanoparticles amplify osmotic stress tolerance in *Zea mays* L. by conserving photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids), catalase, antioxidant biosystems (APX, SOD, POD) and osmolytes biosynthesis (sugar, protein, proline) (Faryal et al., 2022).

CONCLUSION

Seed priming with NaCl decreased germination percentage, germination index, average of germination time, seed vigor index and seedling height, fresh and dry weight of Malaysian rice cultivars. However, the NaCl primed seeds when treated with GA₃, SA and Thiourea fostered germination and salt tolerance of both MR284 and MR263. The application of 0.25 mM GA₃, 0.25 mM SA and 10 mM Thiourea reduced the inhibitory effect of NaCl priming at germination level. The selected levels of PGRs are studied at control laboratory conditions and needs further investigation at field environment.

ACKNOWLEDGEMENT

The author would like to thank IPS Putra Grant for the financial support throughout this project and Department of Biology, Faculty of Science, Universiti Putra Malaysia Serdang and Malaysian Research and Development Institute (MARDI) for providing all the seed materials and facilities to carry out this study.

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