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ROLE OF VARIOUS TREATMENTS IN SEED INVIGORATION AND SALINITY TOLERANCE IN SPINACH

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ABSTRACT

Slow and erratic emergence restricts spinach cultivation in saline soils. Seed priming, a shotgun approach, has been used in several crops for seed enhancement that enables better seed germination under optimal and sub-optimal conditions. Different seed priming treatments, viz., hydropriming, IAA 100ppm, Kinetin 25ppm, Moring leaf extract (MLE), and GA_3 10ppm, were tested in comparison with non-primed seeds in this study. Primed and non-primed seeds were sown in non-saline (0mM NaCl) or saline (200mM NaCl) conditions according to factorial arrangements. Data were recorded for various emergence traits (final emergence percentage, time to 50% emergence, mean emergence time), seedling traits (radicle, plumule and total seedling length, seedling fresh and dry weight), and indices (vigour index, emergence index, Timson's index and emergence energy). Results revealed that seed priming in GA_3 10ppm increased final emergence percentage, seedling fresh and dry weight, and vigour index under both saline and non-saline conditions. Priming in IAA 100ppm also improved seedling traits and vigour index, but was second to GA_3 in overall performance. Results depicted that priming treatments were performed in the following order: GA_3>IAA>hydropriming>Kinetin≥MLE. Notably, GA_3 performed the best among all treatments and can be utilized by farmers under both saline and non-saline conditions.

Keywords: *Spinacea oleracea*, Emergence, Priming, Salt stress, Vigour index.

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1. INTRODUCTION

Soil and water salinity are major hurdles in obtaining a good crop stand of direct-seeded vegetable crops, such as spinach, coriander, and fenugreek (Romero-Aranda et al., 2001). This reduction in growth is associated with the accumulation of salts in plant tissues, leading to premature aging due to higher production of reactive oxygen species (ROS). Meanwhile, limited nutrient uptake further reduces plant growth (Niu et al., 2018). Salt stress tolerance is a function of multiple traits involving genes controlling osmotic, ionic, and biochemical processes in plants (Hossain et al., 2022). Because of its complex nature, the development of salt-tolerant varieties of vegetable crops with high-yielding capacity is not an easy job. Therefore, scientists try short, medium, and long-term approaches to tackle not only salt stress but also other abiotic stresses (Forni & Borromeo, 2023).

Seed priming has been recognized as an efficient, cheap, and simple technique to induce abiotic stress tolerance in several agronomic and horticultural crops (Forni & Borromeo, 2023; Hameed et al., 2025). At the molecular level, it induces a series of transcriptional changes that enable the seed to germinate and improve seedling growth, e.g., suppression of dormancy-causing and stress-sensitive genes while increasing transcript level of germination-inducing and stress-responsive genes (Elkholy & Almutairi, 2018; Nie et al., 2022). However, these changes are mainly dependent on method of priming used, type of seed (crop), priming duration, priming agent, etc. (Forni & Borromeo, 2023; Hameed et al., 2025). Common priming techniques are hydropriming, hormonal/hormo-priming, halopriming, osmopriming, nutripriming, biopriming, magneto-priming and solid matrix priming (Nisha & Rahul, 2022; Forni & Borromeo, 2023; Razzaq et al., 2023; Hameed et al., 2025).

Exogenous application of Plant Growth Regulators (PGRs), as foliar and seed priming treatment has gained much popularity. Improved germination and stress alleviation has been reported in several studies using auxins, gibberellins, cytokinins, polyamines, salicylic acid, melatonin, brassinosteroids, etc. (Basit et al., 2021; Forni and Borromeo, 2023). Besides PGRs, natural growth stimulants have also gained popularity for inducing stress tolerance. One such example is Moring Leaf Extract (MLE) that has been used for foliar as well as seed priming treatment in agricultural

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crops (Khan et al., 2022). Allah has bestowed MLE with several qualities due to the presence of hormones (IAA, GA_4, zeatin, jasmonic acid and salicylic acid) (Brockman et al., 2020), as well as antioxidants, minerals, and vitamins (Khan et al., 2022). Moringa extract has been used for foliar sprays and also for seed treatments in agronomic crops (Brockman et al., 2020; Khan et al., 2022) and only a few vegetable crops, including pepper (Yuniati et al., 2023) and fenugreek (Al-Khazanm, 2020).

Spinach (*Spinacia oleracea*) is a leafy green vegetable, popular in many countries worldwide. It was grown on 7861 hectares with total production of 102047 tonnes in Pakistan during 2022-23, with maximum production in Punjab (44964 tonnes from 2279 ha), followed by Balochistan (29186 tonnes from 1709 ha), Khyber Pakhtunkhwa (16029 tonnes from 1534 ha) and Sindh (11868 tonnes from 2339 ha) (Government of Pakistan, 2024). Crop is mainly grown in the suburbs of cities because of its delicate nature and short postharvest life. Agricultural land in these areas is usually irrigated by using a canal, underground, sewage water, or a combination, depending upon availability. Therefore, the salinity/EC level of these soils is high (774µScm) (Bibi et al., 2025), rendering these soils unfit for most of the direct-seeded vegetable crops. Although, spinach is moderately salt tolerant but germination, growth and yield are negatively affected due to salinity (Keshavarzi et al., 2011; Mwazi, 2012).

Previously, it has been investigated that spinach seeds primed in IAA (200ppm) performed better at higher salinity levels (200mM) than control by exhibiting higher germination percentage and vigour index followed by priming in distilled water and kinetin (25ppm) (Ziaf et al., 2017). Here we report the comparative effect of IAA, kinetin, MLE and GA_3 on seed emergence and seedling traits of spinach at two salinity levels. Aim of this investigation was to compare the effect of seed priming, using distilled water, IAA (100ppm), kinetin (25ppm), GA_3 (10ppm) with MLE, in enhancing salt tolerance of spinach. It was hypothesized that GA_3, which is involved in conferring stress tolerance to various crops, can also improve spinach crop establishment. Moreover, MLE that is rich in gibberellins, IAA, and kinetin, can be used as an alternative to these PGRs.

2. MATERIALS AND METHODS

Seeds of spinach cv. Desi Palak were obtained from Ayub Agricultural Research Institute, Faisalabad, Pakistan. Seeds were first washed with tap water, followed by distilled water. Seeds were placed in beakers containing distilled water (hydropriming; HP), 100ppm IAA (IAA 100), 25ppm kinetin (Kin 25), 10ppm GA_3 (GA_3 10), or 3% moringa leaf extract (MLE) and primed for 30 hours using an aquarium pump at 24±1°C. After priming treatment, primed and un-primed (NP) seeds were washed with distilled water and sown in planters filled with sandy loam soil having pH 7.8 and electrical conductivity 1.93 dS m^{-1}. Two different salinity levels (0 and 200mM) were developed in these planters using NaCl solution, essentially as described by Raza et al. (2016). Twenty-five seeds of each treatment were sown in each planter containing one salinity (0 or 200mM NaCl). Trays were irrigated with tap (drinking) water. Treatments were laid out according to a Completely Randomized Design (CRD) under factorial arrangement with four replications.

2.1. Data Collection

Data were recorded for the following attributes employing standard procedures as described previously (Ziaf et al., 2017). Briefly, emergence was recorded daily till 14 days after sowing. On 15th day, seedlings were taken out, washed with tap water, surface dried and used for data collection. Ten seedlings from each replication were used to record radicle, plumule and total seedling length (cm), seedling fresh and dry weight (mg). Daily emergence data were used to calculate final emergence (%), mean emergence time (Ellis & Roberts, 1981), time taken to 50% emergence (Farooq et al., 2005), emergence energy (Ruan et al., 2002), emergence index (AOSA, 1983), seedling vigour index (Abdul-Baki & Andereson, 1973) and Timson index (Timson, 1965).

2.2. Statistical Analysis

The collected data were statistically analyzed using the ANOVA technique, and differences among treatments were compared by using the LSD test at 5% probability level through the statistical software "Statistica" (version 5.5).

3. RESULTS

3.1. Priming Effects on Emergence and Related Traits

Final emergence percentage was highest at 0mM (92.0%) and 200mM (82.6%) salinity levels in GA_3 10ppm treatment (Fig. 1A). The least value of final emergence percentage was recorded in hydroprimed seeds at 200mM (48.0%). However, it was statistically similar to non-primed seeds (48.0%). There was no significant difference among various treatments for emergence and related traits at 0mM salinity level, except GA_3 10ppm. However, at 200mM salinity level, GA_3 10ppm superseded all other treatments, followed by IAA 100ppm (68.0%) and Kinetin 25ppm (70.6%); both (IAA and Kin) were statistically similar. MLE (61.3%) followed IAA and Kinetin. At the same time, non-primed and hydroprimed treatments were statistically similar, i.e., 48.0% in both treatments (Fig. 1A).

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Mean emergence time (MET) was the least in GA_3 10ppm (9.3 days) at 0mM salinity level. In contrast, all other treatments including control were statistically similar (Fig. 1B). Highest MET value was noticed in non-primed seeds followed by GA_3 10ppm (10.5 days) at 200mM salinity level; however, it was statistically similar to other treatments, indicating that none of the treatments reduced the number of days to emergence at 200mM salinity level. Similar trend was observed for time taken to 50% emergence (E_{50}) that was lowest in hydroprimed seeds (5.8 days) followed by IAA 100ppm (5.9 days), Kinetin 25ppm (6.0 days), GA_3 10ppm (6.0 days) and MLE (6.6 days), while highest in control (7.9 days) at 0mM salinity level. At 200mM salinity level, non-primed seeds (10.4 days) took maximum time for E_{50} followed by GA_3 treated seeds (9.4 days) and hydroprimed seeds (9.4 days). Priming in IAA, Kinetin and MLE significantly reduced E_{50} with values 6.7, 7.0 and 7.1 days, respectively, indicating significant positive impact of these treatments (Fig. 1B).

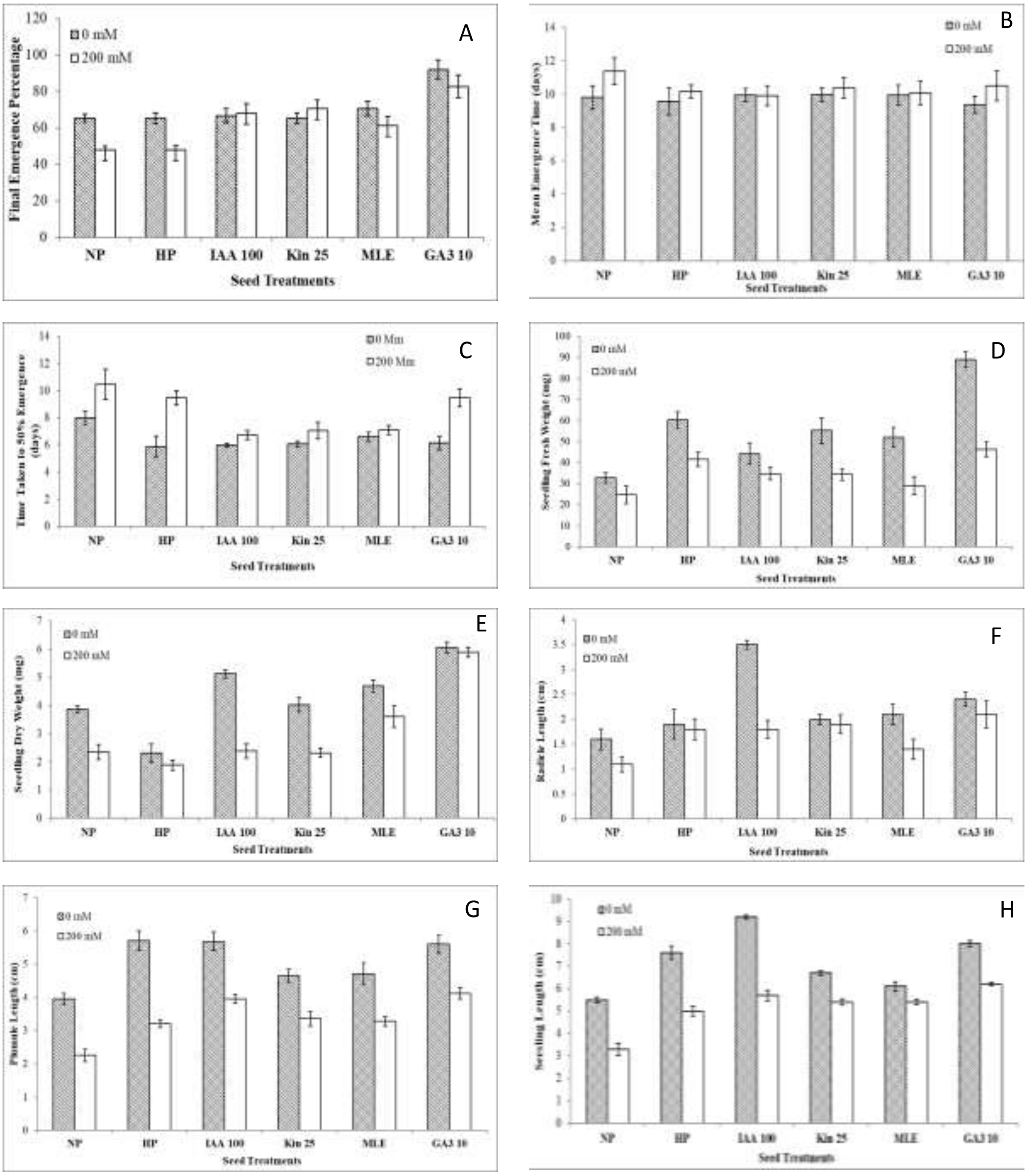

Fig. 1: Seed priming effects on final emergence percentage (A), mean emergence time (B), time to 50% emergence (C), seedling fresh weight (D), seedling dry weight (E), radicle length (F), plumule length (G) and total seedling length (H) of spinach.

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3.2. Priming Effects on Seedling Traits

Salt stress reduced fresh and dry weight of seedlings in all treatments but there was significant variation among treatments (Fig. 1D). Seedling fresh weight was minimum in non-primed seeds (32.6 and 24.6mg) at both salinity (0 and 200mM) levels. Hydropriming and Kinetin 25ppm treatments were statistically similar. GA_3 treatment showed highest seedling fresh weight at both (0 and 200mM) salinity levels, i.e. 89.1 and 46.3mg, respectively. However, GA_3 treatment was statistically similar to hydroprimed seeds at 200mM salinity level. Seedling dry weight was minimum in response to hydropriming treatment while highest for GA_3 10ppm treatment at both salinity levels (Fig. 1E). After GA_3, seedling dry weight was higher in MLE (3.6mg) treatment at 200mM salinity level, while IAA 100ppm (2.4mg), Kinetin 25ppm (2.3mg) and non-primed (2.3mg) treatments were statistically similar.

Priming treatments significantly affected radicle, plumule and total seedling length. IAA 100ppm resulted in the highest radicle length (3.5cm) while minimum in response to seed priming in non-primed seeds (1.6cm) at 0mM salinity level (Fig. 1F). Seeds primed in GA_3 10ppm produced highest radicle length (2.1cm) at 200mM salinity level. Hydropriming (1.8cm), IAA 100ppm (1.9cm) and Kinetin 25ppm (1.9cm) treatments were statistically similar. Plumule length was maximum in hydropriming (5.7cm), IAA 100ppm (5.7cm), and GA_3 (5.6cm) treatments at 0mM salinity level (Fig. 1G). At 200mM salinity level, GA_3 10ppm (4.1cm) treatment showed the best results for plumule length followed by IAA 100ppm (4.0cm) treatment. MLE (3.3cm), Kinetin (3.4cm) and hydropriming (3.2cm) treatments were statistically similar. A similar trend was observed for total seedling length, which was highest in the IAA 100ppm treatment at 0mM, while in response to GA_3 10ppm (6.2cm) at 200mM salinity level (Fig. 1H). At 200mM salinity level, IAA 100ppm (5.7cm), Kinetin 25ppm (5.4cm) and MLE (5.4cm) were statistically similar.

3.3. Priming Effects on Various Indices

Priming improved vigour index under control (non-saline) as well as saline conditions. The highest vigour index was recorded for the GA_3 10ppm treatment, both at 0mM (736.9) and 200mM (515.0) salinity level (Fig. 2A). At 200mM salinity level, IAA 100ppm and Kinetin 25ppm showed statistically similar results, i.e., 392.6 and 386.3, respectively. Timson's index was also highest for GA_3 10ppm treatment at 0mM salinity level (Fig. 2B). However, IAA 100ppm (745.6) showed the highest Timson's index value at 200mM salinity but was statistically similar to GA_3 10ppm treatment (741.3). MLE (653) and Kinetin 25ppm (672.5) were statistically alike at 200mM salinity level. Similarly, emergence index was the highest for GA_3 10ppm treatment (19.7) at 0mM salinity level while lowest for Kinetin 25ppm treatment (Fig. 2C). At 200mM salinity level, IAA 100ppm showed the highest value (12.5) but was statistically similar to MLE. GA_3 10ppm and Kinetin 25ppm were statistically similar. Emergence energy, i.e. emergence percentage on 10th day of test, was highest for GA_3 10ppm (32.0) treatment followed by hydropriming (26.6) at 0mM salinity level (Fig. 2D). Emergence energy at 200mM was highest for MLE (8) but was statistically similar to IAA 100ppm (6.6). Non-primed seeds exhibited the least value of emergence energy (0) at 200mM salinity level.

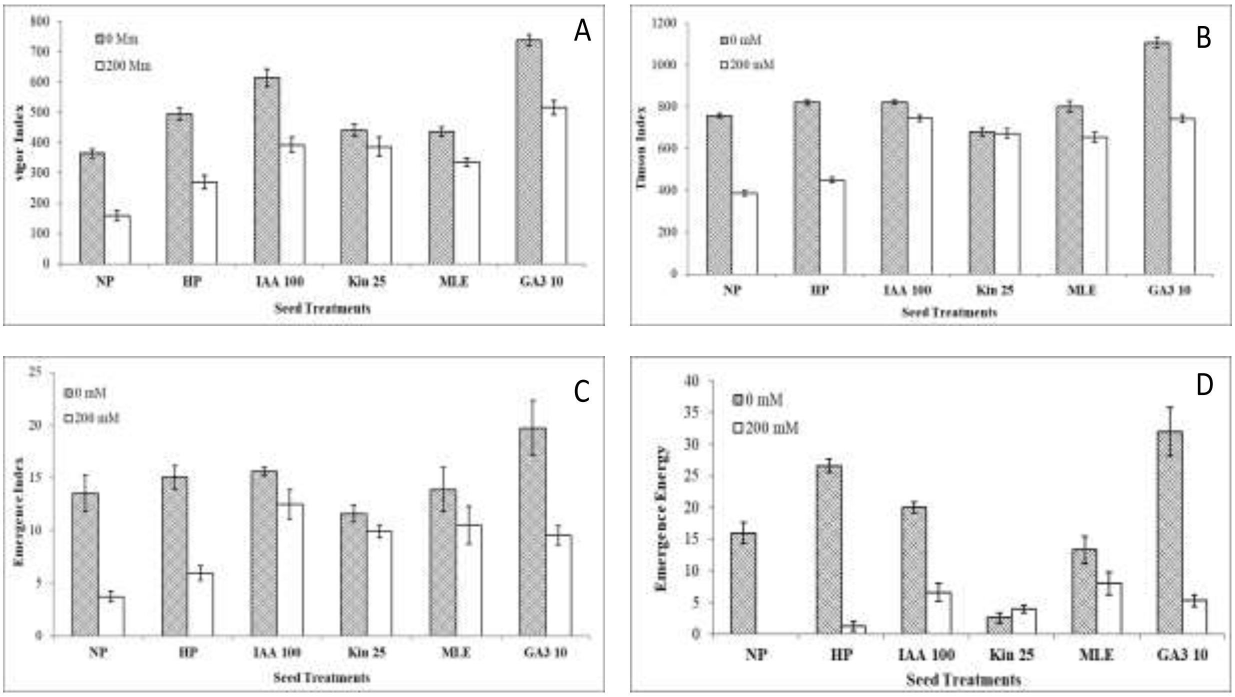

Fig. 2: Seed priming effects on vigour index, Timson's index, emergence index and emergence energy of spinach.

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4. DISCUSSION

Salinity is a major issue that affects germination and crop stand establishment in direct seeded crops such as spinach (Leskovar et al., 1999). Seed priming has been reported to increase germination and seedling traits under normal and saline conditions in spinach (Ziaf et al., 2017). Previously, it was noticed that IAA 100ppm and hydropriming significantly improved germination and seedling traits under normal and saline (200mM NaCl) conditions, particularly IAA outperformed all treatments under saline conditions (Ziaf et al., 2017). The best treatments (hydropriming, IAA 100ppm, Kinetin 25ppm) from that previous study (Ziaf et al., 2017) were compared with GA_3 10ppm and MLE in this study. It was observed that spinach seeds primed in GA_3 10ppm showed best results for most of the studied traits, particularly emergence traits. This improvement in emergence and related traits can be due to endosperm (perisperm) weakening and seed invigoration due to GA_3 (Chandrasekaran et al., 2022). Priming reduced lipid peroxidation, particularly in IAA and hydropriming treatments (Ziaf et al., 2017), which can be the reason for enhanced seedling traits in priming treatments, particularly the GA_3 treatment. Moreover, strengthening of the seedling antioxidant system might have improved the seedling traits as stated earlier by Chen and Arora (2011). GA_3 has been reported to increase salinity tolerance when applied as a foliar treatment by reducing MDA levels, improving membrane integrity as evidenced by reduced electrolyte leakage and increased activities of antioxidant enzymes (Janah et al., 2024). Improved seedling vigour was evidenced by higher vigour index due to seed priming with GA_3 followed by IAA. The emergence index, which reflects the rapid emergence of seedlings from the growing media (Demir et al., 2019), was highest for GA_3 under non-saline conditions; other seed priming treatments were better than non-primed seeds (control). However, under salt (200mM NaCl) stress, IAA, Kinetin, and MLE performed better than GA_3, although the emergence index of GA_3 treatment was twice as high as that of non-primed and hydroprimed seeds. Timson's index indicates the sum of emergence percentage noticed daily for a specified time and depicts a true picture of seed response under stress conditions (Al-Ansari & Ksiki, 2016). Timson's index was also highest for GA_3 treatment under non-saline conditions. While under saline conditions, it was statistically at par with IAA seed priming treatment, indicating that both GA_3 and IAA can be used to improve seedling emergence under saline conditions in spinach. Emergence energy also indicates speed and uniformity of seedling emergence (Domin et al., 2020); it was also highest for GA_3 under non-saline (0mM NaCl) conditions. While under 200mM NaCl stress conditions, MLE showed the highest value, followed by IAA and GA_3. Emergence energy for non-primed seeds was almost zero. These results clearly indicate the role of seed priming with GA_3 and IAA in improving the emergence of spinach seedlings under both saline and non-saline conditions.

5. CONCLUSION

It can be concluded on the basis of results that spinach seed priming, using solutions of different growth regulators, distilled water (hydropriming) and MLE, can be an effective strategy to improve spinach seedling emergence under saline (200mM NaCl) and non-saline (0mM NaCl) conditions. Ranking of treatments was: GA_3>IAA>hydropriming>Kinetin≥MLE. Farmers can easily use GA_3 for spinach seed priming, which will help them to establish a good crop stand even under saline conditions.

Declarations

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Author's Contribution: Conceptualization, K.Z.; methodology, Z.S.; software, M.I.; validation, W.A.; Y.M.; writing-original draft preparation, M.H.; writing—review and editing, W.A.; supervision, K.Z.; project administration and funding acquisition, K.Z.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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