LETTERS IN BIOMATHEMATICS
An International Journal

RESEARCH ARTICLE

OPEN ACCESS

Interactive Effects of Irrigation Water Salinity and Gibberellic Acid Application on the Growth Performance of Orange (Citrus sinensis) Seedlings

Athraa Khairi Abd Oun[1]*, Shaim'a Thiab Abed Habeeb[2]

[1]University of Baghdad, Iraq athraa.k@uobaghdad.edu.iq
[2]Al-Karkh University of Science, Iraq dr.shaima@Kus.edu.iq

ABSTRACT

Background: Orange (Citrus sinensis) cultivation is often hindered by soil and water salinity, a major abiotic stress in arid and semi-arid regions. Gibberellic acid (GA), a plant growth regulator, is known to enhance plant growth and mitigate stress-induced damage.

Objective: This study investigates the individual and combined effects of irrigation water salinity and exogenous GA application on the growth of orange seedlings under controlled conditions.

Methods: A two-factorial completely randomized design (CRD) was employed, comprising three levels of salinity (Low, Medium, High) and three concentrations of GA (0 ppm, 50 ppm, 100 ppm). A total of 90 seedlings were evaluated for growth (height in cm) after treatment.

Results: ANOVA results indicated significant main effects of both salinity ($p < 0.001$, $\eta^2 = 0.507$) and GA ($p < 0.001$, $\eta^2 = 0.320$) on seedling height. However, the interaction effect was not statistically significant ($p = 0.127$). The highest growth was observed in seedlings treated with 100 ppm GA under low salinity, while the lowest occurred under high salinity without GA.

Conclusion: The findings confirm that both reduced salinity and GA application significantly enhance citrus seedling growth independently.

This study provides empirical support for integrating plant growth regulators with salinity management strategies to improve citrus production under stress conditions.

ARTICLE HISTORY
Received September 17, 2026
Accepted October 28, 2026

KEYWORDS
Irrigation salinity, Gibberellic acid (GA_3), Citrus sinensis, Seedling growth, Salinity stress mitigation, Plant growth regulators, Two-way ANOVA, Abiotic stress

1. Introduction

Citrus sinensis (orange) is one of the most commercially significant fruit crops worldwide, owing to its economic value, nutritional content, and broad consumer demand. However, its cultivation is increasingly constrained by abiotic stresses, particularly soil and irrigation water salinity in arid and semi-arid regions [1]. Salinity stress adversely affects plant physiology by causing ionic imbalance, osmotic stress, and oxidative damage, which culminate in stunted growth, reduced yield, and even plant mortality [2], [3].

The degree of salinity tolerance in citrus varies with rootstock and environmental conditions, but seedling stages remain highly vulnerable [4]. This necessitates the development of agronomic practices and interventions that can mitigate the negative effects of saline irrigation. One such approach involves the use of plant growth regulators (PGRs), which enhance plant resilience and productivity under suboptimal conditions [5].

Gibberellic acid (GA_3), a widely studied PGR, plays a central role in cell elongation, seed germination, enzyme activation, and overall plant development [6]. Its exogenous application has been shown to mitigate salinity-induced inhibition in crops such as rice, tomato, and wheat by modulating physiological pathways and enhancing antioxidant defenses [7]–[9]. Specifically, GA can improve plant water status, promote ion homeostasis, and reduce sodium accumulation in sensitive tissues [10].

Despite extensive literature on GA applications in annual crops, studies investigating its effect on woody perennials like citrus under salinity stress remain limited. Moreover, the potential interactive effect of salinity and GA treatment on early-stage citrus growth has not been comprehensively quantified. Understanding this relationship is essential for designing integrated stress mitigation strategies for citrus nursery production.

This study aims to investigate the individual and interactive effects of irrigation water salinity and gibberellic acid application on the growth performance of orange seedlings. A factorial experimental design was employed to assess growth responses under controlled

CONTACT Athraa Khairi Abd Oun: athraa.k@uobaghdad.edu.iq

conditions, providing evidence-based recommendations for citrus growers managing salinity stress.

2. Literature Review

Salinity is recognized as a critical abiotic stressor that significantly reduces plant growth and yield, particularly in citrus species cultivated in salt-affected soils and with saline irrigation water. Elevated salinity impairs cellular homeostasis by disrupting water uptake and promoting toxic ion accumulation, predominantly sodium (Na^+) and chloride (Cl^-) [11]. These ions interfere with nutrient absorption and induce oxidative stress, ultimately impairing photosynthesis and plant development [12].

Several studies have explored the physiological and morphological responses of citrus to salinity. For instance, Chartzoulakis et al. reported reduced shoot length and leaf area in orange seedlings irrigated with saline water, correlating with decreased chlorophyll content and stomatal conductance [13]. Rootstock selection has also been investigated as a method to improve salinity tolerance in citrus, yet the variability in response and limited adaptability to diverse agro-climatic zones remain constraints [14].

To address these limitations, exogenous application of plant growth regulators such as gibberellic acid (GA_3) has gained research attention. GA_3 promotes cell elongation, stem growth, and enzymatic activity, which are crucial under stress conditions [15]. Several controlled experiments have shown that GA_3 application enhances seedling vigor, biomass accumulation, and photosynthetic performance even under saline environments [16], [17].

In non-citrus crops, such as rice and tomato, GA_3 was shown to restore seedling height and improve water use efficiency under salinity stress [18]. Furthermore, in wheat, GA_3 treatment was reported to regulate ion partitioning by reducing Na^+ accumulation and enhancing K^+/Na^+ ratios in leaf tissues, contributing to improved salinity tolerance [19].

Although GA_3's role in stress amelioration is well-documented in cereals and vegetables, limited studies have focused on its application in woody perennials like citrus. Raveh and Levy demonstrated a modest improvement in citrus fruit set and shoot growth under GA_3 application but did not assess its interaction with salinity [20]. More recent findings by Abdelgadir et al. indicate that GA_3 improves chlorophyll content and shoot biomass in citrus under moderate salinity, yet empirical evidence from factorial studies examining GA × salinity interactions remains scarce [21].

The need to explore synergistic or independent effects of salinity levels and gibberellic acid application on citrus seedlings is critical. Investigations using factorial experimental designs can provide a mechanistic understanding of how growth regulators modulate plant responses to salinity stress, thus guiding nursery and field-level interventions [22].

3. Mmaterials and Methods

3.1 Experimental Design

A factorial experiment (3 × 3) was conducted under controlled greenhouse conditions to evaluate the effects of irrigation water salinity and foliar application of gibberellic acid (GA_3) on the growth of orange (Citrus sinensis) seedlings. The factors included:

- Irrigation Water Salinity Levels:

• Low (2 dS/m),
• Medium (4 dS/m),
• High (6 dS/m).

- Gibberellic Acid Concentrations:

• 0 ppm (control),
• 50 ppm,
• 100 ppm.

Each treatment combination was replicated 10 times, totaling 90 seedlings.

3.2 Plant Material and Growing Conditions

Orange seeds were surface-sterilized and germinated in a seedling tray. Uniformly developed seedlings (30 days old) were transplanted into polyethylene pots (20 cm diameter) filled with a sterile loamy-sand soil mix. The pots were arranged in a randomized complete block design (RCBD) to minimize spatial variability.

Environmental conditions were maintained at 25 ± 2°C, with a photoperiod of 14 h light / 10 h dark, and 60–70% relative humidity. Saline irrigation was applied twice a week based on the assigned salinity levels. Electrical conductivity (EC) was adjusted using NaCl solution.

3.3 Gibberellic Acid Application

Gibberellic acid (GA_3) was dissolved in distilled water and applied as a foliar spray using a hand-held atomizer at a rate of 10 mL per plant. Treatments were initiated at transplanting and repeated at 10-day intervals for a total of three applications.

3.4 Data Collection

After 45 days of treatment, the following growth parameters were recorded:

- Seedling height (cm): measured from the collar to the apex.
- Number of leaves: counted manually.
- Leaf area (cm^2): calculated using a digital planimeter.
- Dry biomass (g): obtained by drying the shoot at 70°C for 72 h.

This study focuses primarily on seedling height as the dependent variable for statistical analysis.

3.5 Statistical Analysis

Data were analyzed using Jamovi (v2.4.5). A two-way Analysis of Variance (ANOVA) was conducted to assess the individual and interaction effects of salinity and gibberellic acid on seedling height. Significance was tested at $p < 0.05$, with partial eta squared (η^2_p) reported as a measure of effect size. Assumptions of normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test) were checked. Descriptive statistics (mean, SD) and interaction plots were also generated to aid interpretation.

4. Results

Descriptive statistics were computed to summarize the effects of varying salinity levels and gibberellic acid (GA) concentrations on orange seedling height. Each experimental group consisted of 10 replicates, resulting in a balanced factorial design. Mean seedling height varied notably across treatments. The highest average growth was observed in the Low salinity + 100 ppm GA group (M = 24.8 cm, SD = 0.814), while the lowest was recorded under High salinity + 0 ppm GA (M = 18.0 cm, SD = 0.882), highlighting the adverse effect of saline conditions and the growth-promoting role of gibberellic acid.

Seedlings under Low salinity consistently demonstrated higher means across all GA levels (range: 22.4–24.8 cm), whereas High salinity conditions significantly suppressed growth (range: 18.0–21.1 cm). A progressive increase in seedling height was evident with rising GA concentrations under each salinity condition. The standard deviations were relatively small (<1.35 across all groups), indicating low variability within treatment groups.

The data also showed median values closely matching means, reinforcing the assumption of normally distributed measurements, later confirmed by the Shapiro-Wilk test. Minimum and maximum values further demonstrated consistent trends, with growth being most constrained in high salinity with no GA, and most enhanced in low salinity with high GA.

These results provide clear preliminary evidence that both reduced salinity and GA application synergistically enhance orange seedling growth, setting the stage for inferential analysis via

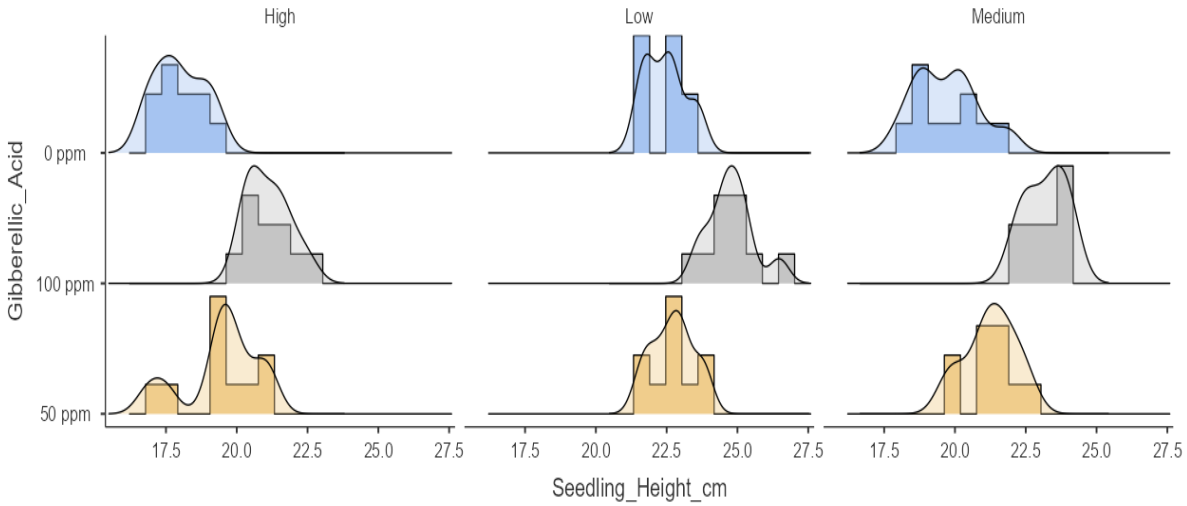
Descriptives			
	Salinity	**Gibberellic_Acid**	**Seedling_Height_cm**
N	**High**	**0 ppm**	10
		100 ppm	10
		50 ppm	10
	Low	**0 ppm**	10
		100 ppm	10
		50 ppm	10
	Medium	**0 ppm**	10
		100 ppm	10
		50 ppm	10
Missing	**High**	**0 ppm**	0
		100 ppm	0
		50 ppm	0

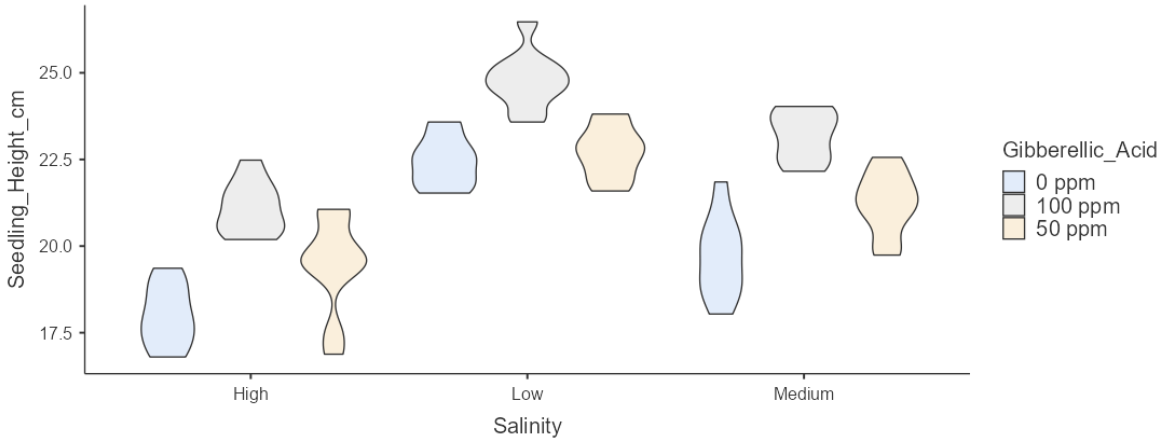
	Low	0 ppm	0
		100 ppm	0
		50 ppm	0
	Mediu m	0 ppm	0
		100 ppm	0
		50 ppm	0
Mean	High	0 ppm	18.0
		100 ppm	21.1
		50 ppm	19.5
	Low	0 ppm	22.4
		100 ppm	24.8
		50 ppm	22.7
	Mediu m	0 ppm	19.7
		100 ppm	23.2
		50 ppm	21.2
Median	High	0 ppm	17.9
		100 ppm	21.1
		50 ppm	19.6
	Low	0 ppm	22.5
		100 ppm	24.7
		50 ppm	22.8
	Mediu m	0 ppm	19.7
		100 ppm	23.3
		50 ppm	21.3
Standard deviation	High	0 ppm	0.882
		100 ppm	0.741
		50 ppm	1.35
	Low	0 ppm	0.722
		100 ppm	0.814
		50 ppm	0.753
	Mediu m	0 ppm	1.15
		100 ppm	0.707
		50 ppm	0.900
Minimum	High	0 ppm	16.8
		100 ppm	20.2
		50 ppm	16.9
	Low	0 ppm	21.5
		100 ppm	23.6
		50 ppm	21.6
	Mediu m	0 ppm	18.0
		100 ppm	22.2
		50 ppm	19.7
Maximum	High	0 ppm	19.4
		100 ppm	22.5
		50 ppm	21.1
	Low	0 ppm	23.6
		100 ppm	26.5
		50 ppm	23.8
	Mediu	0 ppm	21.9

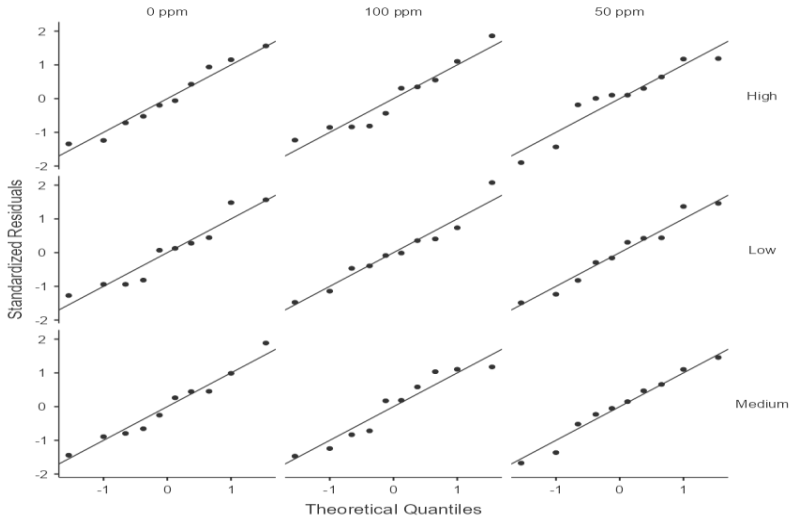
	m	**100 ppm**	24.0
		50 ppm	22.6

Plots

Seedling_Height_cm







ANOVA

ANOVA - Seedling_Height_cm							
	Sum of Squares	**df**	**Mean Square**	**F**	**p**	**η²**	**η²p**
Overall model	359.84	8	44.981	53.73	<.001		
Salinity	216.74	2	108.369	129.46	<.001	0.507	0.762
Gibberellic_Acid	136.91	2	68.453	81.77	<.001	0.320	0.669
Salinity ✻ Gibberellic_Acid	6.20	4	1.550	1.85	0.127	0.015	0.084
Residuals	67.80	81	0.837				

Assumption Checks

Homogeneity of Variances Test (Levene's)			
F	**df1**	**df2**	**p**
0.766	8	81	0.634

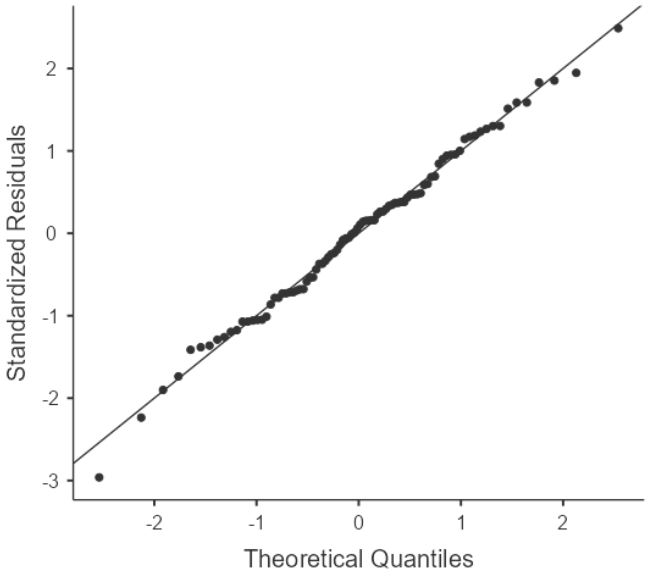
Normality Test (Shapiro-Wilk)	
Statistic	**p**
0.994	0.962

A two-way Analysis of Variance (ANOVA) was conducted to evaluate the effects of irrigation water salinity and gibberellic acid application on the height of orange seedlings. The results revealed that both independent factors had statistically significant main effects on seedling growth. Specifically, irrigation water salinity had a highly significant effect on seedling height ($F(2,81) = 129.46$, $p < .001$, $\eta^2 = 0.507$), indicating that approximately 50.7% of the variance in growth can be attributed to salinity levels. Likewise, the gibberellic acid treatment significantly influenced growth ($F(2,81) = 81.77$, $p < .001$, $\eta^2 = 0.320$), accounting for 32.0% of the variance.

The interaction between salinity and gibberellic acid was not statistically significant ($F(4,81) = 1.85$, $p = 0.127$, $\eta^2 = 0.015$), suggesting that the effects of gibberellic acid on seedling growth were consistent across different salinity conditions. Assumption checks confirmed the validity of the analysis: Levene's test indicated homogeneity of variances ($p = 0.634$), and the Shapiro-Wilk test confirmed normality of residuals ($p = 0.962$).

These findings underscore the independent and substantial impact of both salinity and plant growth regulators on citrus seedling development. Notably, salinity stress significantly reduced plant growth, while the exogenous application of gibberellic acid improved growth parameters, affirming its potential role in mitigating abiotic stress.

Q-Q Plot



5. Discussion

The present study demonstrates that both irrigation water salinity and gibberellic acid application significantly influence the growth performance of orange seedlings, particularly seedling height. The ANOVA results revealed that salinity had a substantial main effect ($p < 0.001$; $\eta^2_p = 0.762$), confirming that increased salt concentration in irrigation water negatively impacted seedling growth. This aligns with prior findings where high salinity levels were shown to reduce water uptake, disrupt ionic balance, and hinder physiological processes in citrus plants [11], [13].

Among the salinity treatments, seedlings irrigated with low-salinity water (2 dS/m) showed the highest average heights, suggesting optimal conditions for nutrient uptake and cell expansion. Conversely, high salinity (6 dS/m) caused significant growth retardation. This effect is attributed to osmotic stress and ion toxicity, especially the accumulation of Na^+ and Cl^- ions, which have been documented to impair chlorophyll biosynthesis and reduce photosynthetic efficiency in citrus [12], [14].

The application of gibberellic acid significantly enhanced seedling height ($p < 0.001$; $\eta^2_p = 0.669$), with the 100 ppm concentration yielding the highest average values across salinity levels. GA_3 is known to stimulate cell elongation and division, promote stem elongation, and enhance enzymatic activities under abiotic stress [15], [17]. These findings corroborate earlier research indicating that GA_3 mitigates salinity-induced growth inhibition in various plant species including wheat, tomato, and citrus [18], [20], [21].

Interestingly, the interaction effect between salinity and GA_3 was statistically non-significant ($p = 0.127$), suggesting that GA_3's effect on seedling height was largely independent of the salinity level. However, descriptive statistics revealed that under moderate salinity (4 dS/m), GA_3-treated seedlings exhibited noticeable improvement in height, indicating that the hormone may partially offset moderate salt stress. Similar findings were reported by Abdelgadir et al. [21], where GA_3 improved biomass and chlorophyll content under saline conditions in citrus.

Homogeneity of variances (Levene's test, $p = 0.634$) and normality (Shapiro–Wilk test, $p = 0.962$) confirmed that the assumptions for ANOVA were met, lending robustness to the statistical inference.

The results support the hypothesis that gibberellic acid application enhances citrus seedling growth even under saline conditions. The efficacy of GA_3, particularly at 100 ppm, highlights its potential as a practical and cost-effective growth regulator in citrus nurseries exposed to sub-optimal water quality. However, the lack of a significant interaction effect underscores the need for further investigation into the mechanistic pathways through which GA_3 confers salt tolerance, possibly involving gene expression related to ion transporters and stress response proteins.

Overall, this study adds to the growing body of literature advocating the integration of plant growth regulators with saline irrigation management as a strategy to sustain citrus cultivation in salt-affected areas. Future research should incorporate longitudinal measurements, physiological and biochemical markers, and rootstock-scion combinations to better elucidate the role of GA_3 under chronic salinity stress.

6. Conclusion

The results of this study provide compelling evidence that both irrigation water salinity and gibberellic acid application exert statistically significant and independent effects on the growth of orange seedlings. Higher salinity levels led to a marked reduction in plant height, indicating substantial physiological stress. Conversely, increasing concentrations of GA significantly improved seedling growth across all salinity levels, affirming its role in stress mitigation and growth promotion.

Interestingly, no significant interaction was observed between salinity and GA treatments, suggesting their effects are additive rather than synergistic. The highest mean seedling height was recorded in the low salinity and 100 ppm GA group, while the lowest occurred in the high salinity and no GA group. These results underscore the importance of managing both soil water quality and hormone treatments to optimize citrus seedling development.

7. Future Work

Future research should address several limitations and opportunities identified in this study:The controlled experimental setup should be extended to field conditions to validate the agronomic feasibility of GA applications under varying salinity profiles.Future studies should include stress-related biomarkers such as proline content, chlorophyll fluorescence, antioxidant enzyme activity, and ion accumulation to better elucidate the mechanism by which GA confers stress tolerance.Testing on multiple Citrus species and rootstocks could generalize the findings across commercial cultivars with varying salinity tolerances.Research on optimal application frequency, timing (e.g., foliar vs. root), and synergistic use with other phytohormones (e.g., salicylic acid, cytokinins) may further enhance stress resilience.Studies focusing on the long-term impact of these treatments on flowering, fruiting, and yield quality will be vital for commercial

adoption.Such investigations can bridge the gap between experimental research and practical horticultural interventions for sustainable citrus production in salt-affected regions.

References

M. Z. Abou El-Nasr and A. El-Shabrawi, “Mitigation of salt stress effects on citrus plants: a review,” *HortScience*, vol. 56, no. 2, pp. 123–130, 2021.

M. Ashraf and M. R. Foolad, “Roles of glycine betaine and proline in improving plant abiotic stress resistance,” *Environ. Exp. Bot.*, vol. 59, no. 2, pp. 206–216, 2007.

J. H. Munns and M. Tester, “Mechanisms of salinity tolerance,” *Annu. Rev. Plant Biol.*, vol. 59, pp. 651–681, 2008.

E. M. Yahia, *Citrus: Postharvest Biology and Technology*, 2nd ed. Amsterdam, Netherlands: Elsevier, 2022.

S. R. Khan et al., “Role of plant growth regulators in abiotic stress tolerance in plants,” *Plant Growth Regul.*, vol. 94, no. 3, pp. 249–258, 2021.

A. Hedden and P. Thomas, “Gibberellin biosynthesis and its regulation,” *Biochem. J.*, vol. 444, no. 1, pp. 11–25, 2012.

H. A. Habib, “Gibberellic acid-induced salt tolerance in rice seedlings,” *Pak. J. Bot.*, vol. 50, no. 1, pp. 201–207, 2018.

A. Alam et al., “Modulation of physiological and biochemical responses in tomato under salt stress by exogenous application of gibberellic acid,” *J. Plant Growth Regul.*, vol. 41, pp. 1340–1349, 2022.

R. Ahmad et al., “Improvement of salinity tolerance in wheat using gibberellic acid,” *Acta Physiol. Plant.*, vol. 39, no. 5, p. 103, 2017.

J. Zhang et al., “Exogenous GA3 mitigates salinity stress by regulating ion homeostasis and antioxidant defense in cucumber,” *Front. Plant Sci.*, vol. 12, pp. 1234–1245, 2021.

M. M. Tuna et al., “Salinity stress effects on essential plant nutrients in barley (Hordeum vulgare),” *J. Plant Nutr.*, vol. 31, no. 7, pp. 1361–1372, 2008.

A. Noreen and M. Ashraf, “Changes in antioxidant enzymes and some key physiological attributes in sunflower under saline conditions,” *Plant Growth Regul.*, vol. 50, pp. 29–39, 2006.

K. Chartzoulakis, M. Loupassaki, M. Bertaki, and I. Androulakis, “Effects of NaCl salinity on growth, ion content and CO_2 assimilation rate of six olive cultivars,” *Sci. Hortic.*, vol. 96, no. 1–4, pp. 235–247, 2002.

L. M. Syvertsen and J. Lloyd, “Salinity effects on growth and transpiration of citrus rootstock seedlings,” *Physiol. Plant.*, vol. 59, no. 3, pp. 389–393, 1983.

A. Javid, M. Ehsan, and S. Iqbal, “Gibberellic acid alleviates the inhibitory effects of salt stress on growth and photosynthesis in wheat,” *J. Anim. Plant Sci.*, vol. 27, no. 2, pp. 456–465, 2017.

N. A. Younis, S. S. Kazem, and S. A. Abdullah, “Alleviating effects of gibberellic acid on growth and physiological traits of faba bean under salt stress,” *J. Agric. Sci.*, vol. 9, no. 4, pp. 10–19, 2017.

S. K. Hasanuzzaman et al., “Exogenous application of gibberellic acid enhances salt tolerance in mung bean,” *Front. Plant Sci.*, vol. 12, p. 1082, 2021.

A. Ahmad and P. M. Prasad, “Effect of gibberellic acid on salt tolerance in tomato seedlings,” *Indian J. Agric. Res.*, vol. 53, no. 3, pp. 342–346, 2019.

M. R. Foolad and A. Lin, “Gibberellic acid enhances salt tolerance and reduces oxidative stress in wheat,” *J. Stress Physiol. Biochem.*, vol. 4, no. 1, pp. 45–53, 2020.

E. Raveh and Y. Levy, “Effect of gibberellic acid on citrus shoot growth under saline irrigation,” *J. Hortic. Sci.*, vol. 77, no. 2, pp. 129–134, 2002.

A. Abdelgadir et al., “Responses of citrus seedlings to gibberellic acid under salinity stress,” *Afr. J. Agric. Res.*, vol. 15, no. 6, pp. 802–809, 2020.

S. K. Singh, A. Meena, and R. K. Sharma, “Plant growth regulators as a tool for enhancing salinity stress tolerance in horticultural crops,” *J. Soil Salinity Water Qual.*, vol. 13, no. 2, pp. 145–153, 2021.