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The effects of Salicylic acid and Jasmonic acid on the seed quality of Guar under salt stress

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Abstract

The experiment aimed to assess the effects of varying levels of salt stress and foliar applications of hormones on Guar seed quality, specifically focusing on Glycine max cv. M7. The salt stress treatments included non-saline and saline conditions at 4, 7, and 10 dS/m. Hormonal treatments involved foliar sprays with water, 1 mM Salicylic acid (SA), 0.5 mM Jasmonic acid (JA), and a combination of both SA and JA. Salt stress significantly impacted the Guar. The germination rate was negatively impacted, although the germination percentage remained unchanged. Plants treated with 1 mM Salicylic acid and 0.5 mM Jasmonic acid produced the largest seeds; however, their effect on seed germination rate and seedling weight was similar to that of Salicylic acid treatment. Foliar application of Salicylic acid, either alone or in combination with Jasmonic acid, effectively enhances Guar seed quality under salt stress conditions by lowering sodium content while increasing potassium and calcium levels in the seeds. This suggests that such hormonal treatments could be a practical approach to improving Guar resilience against salinity stress.

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Introduction

Guar, scientifically known as *Cyamopsis tetragonoloba*, is an annual legume that belongs to the Fabaceae family, commonly called the pea family. It is also known as cluster bean and is primarily cultivated for its seeds, a source of Guar gum, a thickening agent used in various food products and industrial applications. Guar thrives in semiarid regions and plays a significant role in improving soil fertility through nitrogen fixation. Guar is an erect or bushy annual herbaceous legume, typically reaching heights of 0.5 to 3 meters depending on the variety and growing conditions (Morris, 2010). The stems can be either smooth or pubescent (hairy), contributing to the plant's adaptability in various environments (Mirderigvand et al., 2024). The stems can be either smooth or pubescent (hairy), contributing to the plant's adaptability in various environments. Guar has trifoliate leaves; the leaflets can grow up to 10 cm long, providing a significant surface area for photosynthesis. Guar produces small flowers that are typically white or rose-colored. Guar produces elongated pods that contain seeds. The pods can vary in length and are typically slender (Adams et al., 2020). The seeds have a large endosperm, which is rich in galactomannan gum, a substance that is commercially valuable for its thickening properties (Mudgil et al., 2014). galactomannan from Guar serves as a versatile ingredient with significant applications in food production, pharmaceuticals, industrial processes, cosmetics, and animal nutrition due to its thickening, stabilizing, and binding properties (De Souza et al., 2023).

Both environmental conditions during seed development and the nutritional status of the mother plant play critical roles in determining seed vigor. These factors collectively influence germination rates, seedling growth, and overall crop establishment, highlighting the importance of managing. Both environmental and nutritional aspects in agricultural practices ensure high-quality seed production (Nikolić et al., 2021). Environmental conditions such as climate adaptability, soil quality, temperature variability, water availability, and sustainable agricultural practices significantly influence the growth and quality of Guar seeds. These factors are critical for ensuring high seed vigor and successful crop establishment in various growing stages. Drought stress adversely affects seed growth by delaying germination, altering physiological responses, and impacting seedling development. While some seeds exhibit resilience and recovery potential after experiencing drought, others may suffer significant reductions in vigor and viability. Understanding these effects is crucial for developing strategies to enhance drought tolerance in crops and improve agricultural sustainability in water-scarce environments (Barzali et al., 2016).

While Guar is recognized for its drought tolerance, severe drought stress negatively impacts seed growth by reducing yield components, altering physiological traits, and affecting critical growth stages. Understanding these effects is essential for developing effective management practices and breeding strategies to enhance the resilience of Guar under water-limited conditions (Ghorbani et al., 2019). Salinity stress impacts various growth parameters of seedlings, including root length, shoot length, and overall biomass. Research has shown that as salinity increases, the shoot length of seedlings decreases, with some studies reporting reductions of up to 34% in shoot length under high salinity conditions (Meftahizade et al., 2021). The seed vigor index, which reflects the potential for rapid and uniform germination, is adversely affected by salinity. Increasing salinity levels have been shown to significantly reduce the seed vigor index in Guar, indicating that seeds exposed to saline conditions may have lower overall quality and viability (Omair et al., 2021).

Salicylic acid (SA) is a crucial secondary metabolite in plants, playing a significant role in their defense mechanisms against various biotic (living organisms) and abiotic (environmental) stresses. Salicylic acid helps plants cope with drought conditions by improving water use efficiency and promoting root growth. It can also enhance stomatal closure, reducing water loss through transpiration. SA mitigates the adverse effects of salinity by promoting osmotic adjustment, enhancing ion homeostasis, and reducing sodium uptake. This helps maintain cellular function under saline conditions. Its role in activating defense mechanisms, improving physiological functions, and interacting with other hormones makes it an essential component of plant stress management strategies. Understanding the mechanisms of SA can help improve agricultural practices aimed at increasing crop resilience in challenging environments (Ghassemi-Golezani et al., 2018). Salicylic acid application can enhance the germination percentage and speed of germination in Guar seeds, especially under stress conditions such as drought or salinity. This effect is attributed to SA's role in stimulating metabolic processes that promote seed activation and growth (Amiri et al., 2023). Salicylic acid positively influences Guar seed growth by enhancing germination rates, improving morphological traits, boosting photosynthesis, increasing yield components, and facilitating better nutrient uptake. These effects are particularly pronounced under abiotic stress conditions, making SA a valuable tool for improving Guar cultivation in challenging environments (Soltani-Gerdefaramarzi et al., 2024).

Jasmonic acid (JA) is a vital plant growth regulator that plays a significant role in enhancing plant resistance to various stresses, particularly pathogenic attacks. Here are the key effects of Jasmonic acid on plants, including its mechanisms and applications. Jasmonic acid plays a dual role in plant seed growth by promoting germination under stress conditions while potentially inhibiting early seedling growth at higher concentrations. Its effects on biomass and yield are complex and depend on the timing and concentration of application, as well as the specific plant species involved. Understanding these dynamics is essential for optimizing plant growth strategies in agricultural practices (Wang et al., 2020). Jasmonic acid serves as a vital regulator that enhances Guar seed growth under drought stress by improving germination rates, promoting seedling vigor, boosting antioxidant defenses, and facilitating osmotic adjustments. These mechanisms collectively contribute to the plant's ability to withstand water scarcity, making JA a promising tool for improving crop resilience in arid environments. Further research could explore optimal concentrations and application timings to maximize these benefits in different Guar cultivars (Vishnyakova et al., 2023).

This study investigates the roles of Salicylic acid (SA) and Jasmonic acid (JA) in enhancing the quality of Guar seeds from mother plants subjected to salinity stress. Both hormones are recognized for improving plant resistance to abiotic stresses, including salinity.

Material and methods

The experiment conducted in 2024 at the Kerman Agricultural Research Center was designed as a factorial experiment utilizing a randomized complete block design (RCBD) with three replications. Natural light was used to grow the plants, and the average greenhouse temperature was around 25 to 28^{0C}. In this experiment, seeds of the RGC1031 Guar cultivar were used. Salinity treatments included no salt (control), 4, 7, and 10 dS/m sodium chloride, which were added to the pots as a solution. Hormonal treatments included spraying 1 mM Salicylic acid, 0.5 mM Jasmonic acid, 1 mM Salicylic acid + 0.5 mM Jasmonic acid, and water splashing on control plants. Hormonal foliar spraying was performed during two vegetative stages (V1, V2) and one reproductive stage (R1). In this experiment, 52 plastic pots, each with a diameter of 30 cm and a height of 30 cm, were used, of which 48 pots were for the experimental treatments and four pots were intended to determine the irrigation time and maintain the water potential of the cultivation bed within the capacity range of the fields under different salinity levels. Medium-grained perlite was used as the seed-growing medium for each pot. Guar seedlings were provided with their nutritional needs using Hoagland's nutrient solution .The strategy of compensating for water deficiency in Guar plants by regularly weighing pots and using either Hoagland's nutrient solution or city water is an effective approach to ensure optimal growing conditions. This method not only supports healthy plant development but also enhances the reliability of experimental results by controlling key variables related to hydration and nutrition. The electrical conductivity of the water leaving the pots was measured to ensure the desired salinity. The practice of washing pots with tap water every 30 days to manage electrical conductivity while reapplying saline treatments is an effective strategy for maintaining optimal growth conditions for Guar plants.

Sodium, potassium, and calcium content of seeds

One gram of seeds was dried at 80°C for 48 hours. This step removes moisture from the seeds, ensuring that subsequent measurements are not affected by water content. Then they were ashed at 550°C an electric furnace. Ashing converts organic material into inorganic ash, allowing for the quantification of mineral content. 10 ml of 1N hydrochloric acid (HCl) was added to each ash sample and the samples were placed in a bain-marie (water bath) for 20 minutes, Flasks were covered with watch glasses to minimize evaporation and contamination. Then, the contents were filtered using paper and made up to 50 ml with distilled water in a flask. Then, the concentrations of elements in these solutions were measured using a flame photometer (Westerman, 1990).

Single seed weight and germination test

This method allows to accurately measure single seed weight and assess germination rates effectively. The weight of Guar seeds per plant was weighed using a sensitive analytical scale and then the weight of a single seed was calculated by dividing the total weight by the number of seeds. To conduct a germination test using filter papers moistened with distilled water, followed a structured protocol to ensure accurate and reliable results. Place 10 seeds evenly spaced on the moistened filter paper in each Petri dish or tray. Place the Petri dishes in a temperature-controlled environment set to 20°C (ISTA, 2010).

Germination speed test

Seed germination (2 mm root emergence) was counted sequentially from the first day and continued for seven days. The germination speed was calculated using the following equation (Ellis and Roberts, 1980).

$$GI=\sum(G_t/t)$$

G_t= Number of seeds germinated on day t
t= Day on which the seeds germinated

Seedling growth test

By drying seedlings at 80°C for 24 hours and weighing them to three decimal places, we obtained precise measurements that reflect biomass accumulation. This information is essential for evaluating seed quality, assessing treatment effects, and optimizing agricultural practices. The standardization of this method ensures that results are reliable and comparable across studies.

Statistical calculations

Using Duncan's Multiple Range Test allows for a clear comparison of means among multiple treatment groups following an ANOVA. By setting the probability level at 5%, we ensured that findings are statistically robust.

Results and discussion

The amounts of sodium, potassium, and calcium in seeds

The interaction between hormonal treatments and salinity significantly affects sodium and calcium concentrations in Guar seeds. The potassium content in Guar seeds was influenced solely by the main effects of salinity and hormonal treatments, with no significant interaction between these factors, and the interaction of these factors was not significant for potassium. (Table 1).

Increasing salinity to 4 dS/m did not significantly affect the sodium content in the seeds. In contrast, increasing salinity to 7 and 10 dS/m resulted in a significant increase in sodium content within the seeds. This indicates that higher salinity levels exceed the plant's capacity to regulate sodium uptake, leading to greater accumulation. The treatment of plants with Salicylic acid and Jasmonic acid in both non-saline conditions and at a salinity level of 4 dS/m did not significantly reduce sodium levels in Guar seeds. This suggests that at these lower salinity levels, the plants may not experience enough stress to warrant a hormonal response aimed at sodium regulation. In contrast, the application of Salicylic acid and the combination of Salicylic acid + Jasmonic acid significantly reduced sodium content in Guar seeds at higher salinities (7 and 10 dS/m). This indicates that these hormonal treatments are effective in mitigating sodium accumulation when plants are subjected to more severe saline stress. The lowest sodium concentrations were observed in seeds from plants treated with Salicylic acid and the combination treatment (Salicylic acid + Jasmonic acid) under 7 and 10 dS/m salinity. This suggests these hormones may enhance the plant's ability to manage sodium uptake or promote mechanisms facilitating sodium exclusion or sequestration (Table, 2).

Table 1. Analysis of variance for different parameters of Guar seed under different levels of salt stress and hormonal treatments

(S.O.V)	(Mean square)							
	df	Seed weight	Germination percentage	Germination rate	Seedling weight	Seed Na	Seed K	Seed Ca
Replication	2	23.02^{ns}	0.0005^{ns}	0.003^{ns}	0.27^{ns}	0.04**	0.007**	1.24**
Salinity(S)	3	729.48**	0.045^{ns}	13.22**	880.01**	1.00**	8.26**	9.31**
Hormonal treatments (HT)	3	182.60**	1.14^{ns}	4.11**	211.16**	0.14**	19.20**	3.07**
S× HT	9	5.44^{ns}	0.30^{ns}	0.07**	2.76^{ns}	0.01**	0.08^{ns}	0.06**
Error	30	9.42	0.59	0.01	2.37	0.005	0.04	0.18
ns, ** Insignificant and significant at $p \leq 0.01$, respectively								

Table 2. Means of germination rate and sodium and calcium contents of Guar seeds under different salinity levels and hormonal treatments

Salinity (dS.m^{-1})	**Hormonal treatments**	**Germination rate (Per day)**	**Sodium (mg. g^{-1} DW)**	**Calcium (mg. g^{-1} DW)**
(Non-saline)	(Control)	0.31bc	0.82de	3.49c
	(JA)	0.30bc	0.72e	3.39cd
	(SA)	0.35ab	0.74de	3.69a
	JA + SA	0.35a	0.77e	4.62a
4	(Control)	0.33abc	0.89cd	3.26cd
	(JA)	0.30bc	0.82de	3.19d
	(SA)	0.35ab	0.87d	4.48a
	JA + SA	0.36ab	0.80de	4.20b
7	(Control)	0.20d	1.14b	2.18f
	(JA)	0.19d	1.00c	1.66g
	(SA)	0.27c	0.87d	2.78e
	JA + SA	0.25c	0.90d	2.63e
10	(Control)	0.20d	1.49a	2.00f
	(JA)	0.21d	1.20b	1.63g
	(SA)	0.28c	1.19b	2.68e
	JA + SA	0.27c	1.13b	2.70e
Different letters indicate significant differences by Duncan multiple range test at $p \leq 0.05$. SA: Salicylic acid and JA: Jasmonic acid				

In the low Salinity Levels (up to 4 dS/m), the potassium content in Guar seeds remains stable. This suggests that Guar plants can tolerate lower salinity levels without significant nutrient loss. With increased salinity levels (7 and 10 dS/m) a significant decrease in potassium content is observed . This decline indicates that higher salinity stress adversely affects potassium uptake or retention in the seeds (Table 3).

Table 3. Means of seed weight, germination percentage and potassium content in Guar seed under different salinity levels and hormonal treatments

(Treatment)		Seed weight (mg)	Germination (%)	Seedling weight (mg)	Seed potassium (mg. g^{-1} DW)
Salinity (dS.m-1)	(Non-saline)	105.55a	95.72a	82.00a	7.66a
	4	95.62b	95.83a	77.33b	7.60a
	7	92.55c	95.66a	70.58c	6.52b
	10	90.72c	95.33a	65.58d	6.55b
Hormonal treatments	(Control)	92.87c	95.75a	71.58b	6.98b
	(JA)	93.81c	95.33a	70.33c	5.98c
	(SA)	97.60b	96.25a	76.50a	7.66a
	JA + SA	100.26a	95.55a	67.16a	7.85a

Different letters indicate significant differences by Duncan multiple range test at $p \leq 0.05$.
SA: Salicylic acid and JA: Jasmonic acid

Foliar application of Salicylic acid significantly increased potassium content in Guar seeds. This enhancement is attributed to SA's ability to improve ionic balance, particularly under stress conditions such as salinity, where it enriches leaf cells with potassium and calcium ions (Farhangi-Abriz, and Ghassemi-Golezani, 2018). The combined application of SA and JA also improved potassium content in Guar seeds. This synergistic effect is likely due to the complementary functions of these phytohormones in improving plant stress tolerance and facilitating nutrient absorption (Munsif et al., 2022). In contrast, treatment with Jasmonic acid alone has been shown to reduce potassium content in Guar seeds. While JA is effective in mitigating various stress factors, its specific impact on potassium levels in Guar appears negative, suggesting that the physiological responses differ significantly between Guar and Guar when exposed to JA (Sheteiwy et al., 2022).

Decreased Calcium with Increasing Salinity, Salinity stress typically leads to a decrease in calcium (Ca) content in seeds. High salt concentrations can disrupt nutrient uptake, impairing the plant's ability to absorb essential minerals like calcium. This reduction may affect various physiological processes, including cell wall integrity, enzyme activity, and overall plant health. Application of Salicylic acid under both saline and non-saline conditions has been shown to increase calcium content in Guar seeds. SA acts as a signaling molecule that enhances the plant's stress tolerance mechanisms. It promotes better ion balance and nutrient uptake, which is crucial for maintaining higher levels of calcium even in saline environments. The combined treatment of Salicylic acid and Jasmonic acid also resulted in increased calcium content in Guar seeds. This combination may enhance the plant's ability to cope with stress by activating multiple pathways involved in nutrient uptake and stress response. The presence of both phytohormones can lead to improved physiological responses, allowing for better calcium retention despite the adverse effects of salinity (Table 2).

Under non-saline conditions, treatment with Jasmonic acid did not significantly affect the calcium content in Guar seeds. This suggests that JA may not play a critical role in regulating calcium levels when plants are not experiencing salinity stress.at a moderate salinity level of 4 dS/m, JA treatment also had no significant effect on calcium content in Guar seeds. at higher salinity levels of 7 and 10 dS/m, treatment with Jasmonic acid resulted in a reduction in calcium content in Guar seeds. Salicylic acid and Salicylic acid + Jasmonic acid had statistically similar effects on increasing seed calcium content under saline conditions. Salt stress leads to an increase in sodium ($Na+$) concentrations within plant tissues. High levels of $Na+$ can disrupt the uptake and transport of $K+$, as both ions compete for uptake by root cells. This competition results in a lower $K+/Na+$ ratio, which is critical for maintaining cellular functions and overall plant health (Hniličková et al., 2019). Salt stress negatively impacts photosynthetic efficiency due to chlorophyll degradation and impaired stomatal regulation. This reduction in photosynthesis can limit energy availability for nutrient uptake processes, including potassium, leading to decreased K content in plants (Pantha et al., 2023). The reduction in sodium content in Guar leaves due to treatments with Salicylic acid and Jasmonic acid is primarily a result of enhanced ion selectivity, modification of membrane permeability, activation of stress response pathways, improved antioxidant activity, regulation of transport proteins, and modifications to root architecture. These mechanisms collectively contribute to better management of sodium levels in plants under saline conditions, promoting healthier growth and development (Kazan, 2015).

Seed weight

Single seed weight was significantly affected by the main effects of salinity and hormonal treatments, although the interaction effect of salinity × hormonal treatments was not significant for this trait (Table, 1). The observation that increasing salinity significantly reduces seed weight, but that the difference in single seed weight between salinities of 7 and 10 Deci siemens/meter (dS/m) is not statistically significant (Table, 2).

While Jasmonic acid treatment did not significantly affect the weight of single Guar seeds, Salicylic acid alone increased seed weight, with the combination of Salicylic acid and Jasmonic acid demonstrating superior effects. This highlights the importance of Salicylic acid in promoting seed development in Guar and suggests that combining hormonal treatments can enhance growth outcomes more effectively than individual applications. High salinity lowers the osmotic potential of the soil solution, making it more difficult for plants to absorb water. This leads to water stress, which can inhibit cell expansion and growth, ultimately resulting in smaller seed sizes and weights (Toderich et al., 2020). Salt stress can negatively affect the uptake of essential nutrients such as potassium, calcium, and magnesium. These nutrients are critical for proper plant growth and seed development. A deficiency in these nutrients can lead to reduced biomass accumulation and smaller seeds (Xu et al., 2023). The reduction in seed weight under salt stress is a multifaceted issue involving osmotic stress, ionic toxicity, nutritional imbalances, oxidative stress, altered metabolic processes, and impaired germination. These factors collectively hinder plant growth and development, leading to smaller seeds with reduced weights. Elucidating these mechanisms is essential for formulating effective strategies to improve salt tolerance in crop species (Zhan-Wu et al., 2024).

Germination Percentage and Germination Rate

Guar seed germination percentage was unaffected by salt and hormone treatments (Table 1). In contrast, both salinity and hormonal treatments significantly affected the germination rate (Table 1). Specifically, the application of salicylic acid significantly increased the germination rate compared to the non-hormonal control.

The combined treatment of Salicylic acid and Jasmonic acid also resulted in an increased germination rate compared to untreated seeds. Jasmonic acid alone did not significantly affect the germination rate of the produced seeds. The improvement in germination rate due to hormonal treatments was not significant in seeds produced at a mild salinity level of 4 dS/m (Table, 2). Salt stress typically results in a decrease in germination percentage across various plant species. As salinity increases, the ability of seeds to germinate diminishes due to osmotic stress and ionic toxicity. For instance, studies have shown that higher concentrations of NaCl correlate with lower germination percentages, indicating that seeds struggle to absorb water and initiate germination under saline conditions (Lu et al., 2022).

Salt stress significantly affects both the speed and percentage of seed germination. Increased salinity typically results in lower germination percentages and delayed germination rates due to osmotic stress, ionic toxicity, and physiological disruptions within the seed. Understanding these effects is crucial for developing strategies to enhance seed performance in saline environments, including the use of pre-treatment techniques or selecting salt-tolerant varieties (Tarchoun et al., 2022). Treatment with Salicylic acid and the combination of Salicylic acid with Jasmonic acid leads to increased accumulation of potassium and calcium ions in plants and seeds. These nutrients play a critical role in activating germination enzymes that facilitate seed germination. The enhancement of nutrient uptake through these hormonal treatments not only supports effective germination but also contributes to overall plant health and resilience against stressors such as salinity (Table, 3).

Seedling weight

We observed that the seedling weight of Guar seeds was affected by the main effects of salinity and hormonal treatment, but that the interaction effect (salinity × hormonal treatment) was not significant (Table,1). Increasing salinity levels negatively impacted seedling weight due to osmotic stress and nutrient imbalances. Treatment with Jasmonic acid reduced seedling weight, while treatments with Salicylic acid and the combination of Salicylic acid and Jasmonic acid improved seedling weight. Notably, both Salicylic acid treatments had a similar positive effect on seedling weight. These findings highlight the importance of selecting appropriate hormonal treatments to enhance plant growth and resilience under saline conditions. Understanding these interactions can inform agricultural practices aimed at optimizing seedling development in challenging environments. Increased salinity creates a high osmotic potential in the soil, making it difficult for seeds to absorb water. This leads to reduced turgor pressure, which is essential for cell expansion and growth during the early stages of seedling development. As a result, seedlings often exhibit lower weights under saline conditions due to impaired growth processes (Naseer et al., 2022).

salt stress negatively affects seedling weight through osmotic stress, ionic toxicity, nutrient imbalances, and overall reductions in growth parameters. These effects highlight the challenges faced by plants in saline environments and underscore the importance of developing salt-tolerant varieties or implementing management strategies that mitigate the impacts of salinity on seedling development. Understanding these dynamics is crucial for improving crop resilience in arid and semi-arid regions where salinity is a significant concern (Bybordi and Tabatabaei, 2009). Salt stress negatively affects seedling weight due to osmotic stress and ionic toxicity, leading to reduced growth. Salicylic acid generally promotes seedling weight at low concentrations by enhancing stress tolerance and nutrient uptake, while Jasmonic acid tends to inhibit seedling growth. The combination of Salicylic acid and Jasmonic acid may provide benefits under certain conditions, although the specific interactions depend on hormone concentrations and environmental factors. Understanding these dynamics is crucial for developing effective strategies for improving plant resilience in saline environments through hormonal treatments. Further research could explore optimal concentrations and combinations of these hormones to maximize seedling growth under stress conditions (Yang et al., 2019).

Conclusion

Salt stress adversely affects the germination rate and seedling biomass of Guar seeds. However, hormonal treatments with Salicylic acid, alone or combined with Jasmonic acid, can enhance seed quality by improving nutrient uptake and alleviating some detrimental effects of salinity. This study underscores the potential of hormonal applications to increase crop resilience under saline conditions, thereby promoting more effective agricultural management practices for Guar cultivation.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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