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Ameliorative effects of foliar polyamines on leaf traits of guava (*Psidium guajava* L.) cv. Hisar Safeda under saline water irrigation

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Abstract

Salinity stress poses a significant constraint to guava production, particularly in arid and semi-arid regions where saline irrigation is prevalent. This study investigated the mitigating effects of exogenous polyamine application on guava leaves parameters under saline water irrigation conditions. The experiment was conducted at the Experimental Orchard, Department of Horticulture, CCS Haryana Agricultural University, Hisar during 2023-2025 using a Factorial Completely Randomized Design with three replications. Treatments included four irrigation water salinity levels (Canal water, 6.0, 7.0 and 8.0 dSm⁻¹) and seven polyamine treatments (Putrescine at 0.5 and 1.0 mM, Spermidine at 0.5 and 1.0 mM, Spermine at 0.5 and 1.0 mM, and Control). Results demonstrated that increasing salinity levels significantly reduced most of leaf traits. However, exogenous application of polyamines, particularly Putrescine at 0.5 mM, significantly ameliorated salt stress effects. The highest values for number of leaves (51.56), leaf area (359.79 cm²), fresh leaf weight (7.39 g) and dry leaf weight (1.66 g) were recorded with Putrescine 0.5 mM treatment. The study demonstrates that foliar application of polyamines, especially Putrescine at 0.5 mM, can serve as an effective strategy to enhance guava tolerance to salinity stress and maintain productive growth under challenging irrigation conditions.

Keywords: Guava, salinity stress, polyamines, putrescine, growth parameters

Introduction

Guava (*Psidium guajava* L.), belonging to the Myrtaceae family with a diploid chromosome number of 2n = 22, is a tropical and subtropical fruit crop that has gained significant economic importance globally (Nakasone and Paull, 1998) [14]. Originating from tropical America, spanning from Mexico to Peru, guava cultivation has spread worldwide and has become particularly significant in India, where it ranks as the fourth most important subtropical fruit crop after mango, banana, and citrus (Meena *et al.*, 2013) [11]. Historical records indicate that Portuguese explorers introduced guava to India in the 17th century, where it has since earned the moniker "Apple of the Tropics" due to its nutritional value and adaptability.

Soil salinity represents one of the most pressing agricultural challenges globally, affecting approximately 20% of irrigated land worldwide (FAO, 2024) [4]. In India, approximately 200,000 square kilometers are affected by saline water with electrical conductivity exceeding 4,000 µS/cm according to the Central Board of Ground Water. In Haryana specifically, about 65% of the area features brackish or saline groundwater (Anonymous, 2024) [2], making salinity management crucial for sustainable agriculture. This widespread occurrence of saline irrigation water threatens guava cultivation, especially in arid and semi-arid regions where alternative water sources are limited.

Salt stress adversely affects plant growth through multiple mechanisms including osmotic stress, ionic toxicity, and oxidative damage (Munns and Tester, 2008) [13]. While guava demonstrates moderate tolerance to salinity compared to other fruit crops, prolonged exposure to saline conditions significantly impairs its physiological processes and reduces yield potential (Hussain *et al.*, 2021) [7]. Traditional approaches to salinity management such as leaching, gypsum application, and salt-tolerant rootstock development are often costly, time-consuming, or have limited effectiveness under severe stress conditions.

Polyamines, including putrescine, spermidine and spermine, are low molecular weight aliphatic nitrogen compounds that play crucial roles in plant growth, development, and stress responses (Kusano *et al.*, 2008) [10]. These naturally occurring compounds are involved in various physiological processes including cell division, membrane stabilization, protein synthesis, and stress tolerance mechanisms (Minocha *et al.*, 2014) [12]. Recent research has demonstrated that exogenous application of polyamines can enhance plant tolerance to various abiotic stresses, including salinity, drought, and temperature extremes (Hasanuzzaman *et al.*, 2014) [6]. The protective mechanisms of polyamines under salt stress include stabilization of cellular membranes, scavenging of reactive oxygen species, maintenance of ionic homeostasis, and modulation of gene expression related to stress responses (Ahmad *et al.*, 2018) [1]. Polyamines also contribute to osmotic adjustment and help maintain cell turgor pressure under saline conditions (Todorova *et al.*, 2007) [18]. Furthermore, these compounds can enhance photosynthetic efficiency and chlorophyll stability, thereby supporting continued growth under stress conditions.

Despite the recognized potential of polyamines as stress protectants, empirical evidence regarding their specific application in guava under saline irrigation conditions remains limited. This knowledge gap highlights the need for comprehensive research to evaluate the effectiveness of different polyamines and their optimal concentrations for mitigating salt stress in guava production systems. Given the increasing prevalence of salinity problems in guava-growing regions and the need for sustainable, cost-effective solutions, this study was undertaken to evaluate the mitigating effects of exogenous polyamine application on guava growth and physiological parameters under saline water irrigation.

Materials and Methods

Experimental Details

The present investigation entitled "Effect of exogenous application of polyamines on the growth and physio-biochemical characteristics of guava under saline water irrigation" was conducted at the Experimental Orchard, Department of Horticulture, CCS Haryana Agricultural University, Hisar during 2023-2025. The experiment was conducted using a Factorial Completely Randomized Design (FCRD) with three replications. The study comprised 28 treatment combinations derived from a 4×7 factorial arrangement. Each treatment combination was represented by three pots, with each pot measuring 12 inches in size. The experimental material consisted of grafted plants of guava cultivar Hisar Safeda established on L-49 rootstock, which served as the test subjects for evaluating the interactive effects of the applied treatments under the specified experimental conditions.

Treatment Details

A₁-Canal Water; A₂- 6.0 dSm⁻¹; A₃-7.0 dSm⁻¹; A₄-8.0 dSm⁻¹; C₁-Putrescine (0.5mM); C₂-Putrescine (1.0mM); C₃-Spermidine (0.5mM); C₄-Spermidine (1.0mM); C₅-Spermine (0.5mM); C₆-Spermine (1.0mM); C₇-Control

Note: Polyamines were applied as foliar sprays at 60 and 75 days after transplanting using appropriate concentrations dissolved in distilled water with 0.01% as surfactant.

Data Collection and Observations

For data collection, three plants per replication were selected randomly. The following growth parameters were recorded: Number of leaves per plant were recorded by counting leaves fortnightly after polyamine application for three representative plants per replication and averaged. Leaf area per plant (cm²) was measured using a leaf area meter fortnightly after polyamine application for three representative plants per replication and averaged. Fresh leaf weight (g/plant) was calculated by taking ten leaves from representative plants were selected per replication, cleaned and weighed using a digital electronic balance. Average weight was calculated and expressed as gram per plant. For calculation of dry leaf weight (g/plant), the same leaves used for fresh weight determination were dried in a hot air oven at 65±2°C for 72 hours or until constant weight was achieved. Dry weight was determined using a digital electronic balance and expressed as gram per plant. Statistical analysis was conducted using Completely Randomized Design for analysis of variance (ANOVA) as recommended by Sheoran (2004) [17]. Data were analyzed using OPSTAT software developed by CCS Haryana Agricultural University, Hisar. Critical Difference (CD) was calculated using the formula:

C.D. = S.E.(d) × "t" at 5% or 1% for error degrees of freedom

Where S.E.(d) = $\sqrt{(2EMS/r)}$ EMS = Error mean squares "t" = Tabulated value at 5% level of significance r = Number of replications

Results and Discussion

Number of Leaves

The data revealed significant effects of both salinity levels and polyamine treatments on leaf number across all observation periods (60, 75 and 90 DAT). The number of leaves showed an interesting pattern where a progressive increase was observed with increasing salinity levels at all observation periods. At 60 DAT, leaf count increased from 35.72 leaves under canal water to 37.24 leaves under 8.0 dS m⁻¹ in 2023-24. Among polyamine treatments, Putrescine at 1.0 mM (C₂) and 0.5 mM (C₁) consistently recorded the highest leaf numbers across all time points. The maximum number of leaves (53.62) was observed with C₁A₄ combination at 90 DAT in 2024-25.

Table 1: Effect of exogenous application of polyamines on the number of leaves per plant of guava under saline water irrigation at 60 DAT

Factor B-Doses of Polyamines	Factor A- Quality of Irrigation Water									
	2023-24					2024-25				
	A ₁	A ₂	A ₃	A ₄	Mean	A ₁	A ₂	A ₃	A ₄	Mean
C ₁	36.48	37.26	37.46	38.47	37.42	38.20	38.97	39.19	40.20	39.14
C ₂	36.94	36.96	37.35	38.67	37.48	38.67	38.68	39.09	40.40	39.21
C ₃	35.71	36.37	36.79	37.85	36.68	37.43	38.10	38.54	39.59	38.42
C ₄	36.53	36.77	36.92	37.86	37.02	38.25	38.48	38.66	39.59	38.75
C ₅	35.17	35.24	34.86	36.40	35.42	36.91	36.98	36.63	38.15	37.17
C ₆	34.82	34.81	36.07	36.29	35.50	36.56	36.55	37.82	38.04	37.24
C ₇	34.40	34.57	34.37	35.17	34.63	36.15	36.31	36.13	36.93	36.38
Mean	35.72	36.00	36.26	37.24		37.45	37.72	38.01	38.99	
C.D. at 5%	A = 0.56 B = 0.74 A×B = NS					A = 0.59 B = 0.78 A×B = NS				

Table 2: Effect of exogenous application of polyamines on the number of leaves per plant of guava under saline water irrigation at 75 DAT

Factor B-Doses of Polyamines	Factor A- Quality of Irrigation Water									
	2023-24					2024-25				
	A ₁	A ₂	A ₃	A ₄	Mean	A ₁	A ₂	A ₃	A ₄	Mean
C ₁	42.06	41.86	42.08	42.85	42.21	42.29	42.13	42.09	42.51	42.26
C ₂	41.22	41.47	42.28	41.63	41.65	41.62	41.82	42.4	41.48	41.83
C ₃	41.67	41.2	41.51	42.17	41.64	41.86	41.41	41.46	41.83	41.64
C ₄	41.65	41.24	41.72	42.27	41.72	41.87	41.51	41.79	41.97	41.79
C ₅	39.59	40.61	40.75	39.87	40.21	39.78	40.47	40.65	40.65	40.14
C ₆	39.37	39.75	40.16	40.87	40.04	39.61	39.96	40.23	40.58	40.10
C ₇	39.80	40.14	40.67	41.34	40.49	39.77	40.16	40.45	40.66	40.26
Mean	40.77	40.90	41.31	41.57		40.97	41.07	41.30	41.34	
C.D. at 5%	A = 0.25 B = 0.34 A×B = 0.67					A = 0.13 B = 0.17 A×B = 0.34				

Table 3: Effect of exogenous application of polyamines on the number of leaves per plant of guava under saline water irrigation at 90 DAT

Factor B-Doses of Polyamines	Factor A- Quality of Irrigation Water									
	2023-24					2024-25				
	A ₁	A ₂	A ₃	A ₄	Mean	A ₁	A ₂	A ₃	A ₄	Mean
C ₁	48.17	50.56	51.09	51.39	50.30	49.47	51.32	51.83	53.62	51.56
C ₂	47.18	48.61	48.57	48.71	48.27	48.87	49.03	49.58	52.09	49.89
C ₃	47.57	49.71	50.34	50.66	49.57	49.3	50.67	51.25	53.17	51.10
C ₄	46.76	48.72	49.79	49.13	48.60	48.58	50.2	50.85	51.53	50.29
C ₅	45.87	47.26	47.45	48.71	47.32	47.68	48.52	48.78	51.2	49.05
C ₆	45.8	47.54	47.77	47.47	47.15	47.51	47.92	48.88	50.85	48.79
C ₇	45.74	47.89	48.8	49.67	48.03	47.73	50.42	50.24	51.93	50.08
Mean	46.73	48.61	49.12	49.39		48.45	49.73	50.20	52.06	
C.D. at 5%	A = 0.15 B = 0.20 A×B = 0.40					A = 0.22 B = 0.29 A×B = 0.57				

The increase in leaf number with salinity may represent a compensatory mechanism where plants produce more leaves to offset reduced photosynthetic efficiency per unit leaf area under stress conditions (Ran *et al.*, 2021) ^[16]. However, the superior performance of polyamine-treated plants indicates enhanced vegetative proliferation and stress tolerance. These findings align with Ahmad *et al.* (2018) ^[1], who reported increased leaf production in salt-stressed crops following polyamine application. The comprehensive evaluation of growth parameters clearly demonstrated that salinity stress significantly impaired guava growth, while exogenous polyamine application, particularly Putrescine at 0.5 mM, effectively mitigated these adverse effects. The decline in most growth parameters with increasing salinity can be attributed to multiple stress mechanisms including osmotic stress, ionic toxicity and oxidative damage (Nawaz *et al.*, 2010; Baranova and Gulevich, 2021) ^[15, 3]. the increase in leaf number with salinity, despite reduced leaf area and biomass, suggests a compensatory mechanism where plants attempt to maintain photosynthetic capacity through increased leaf production (Ran *et al.*, 2021) ^[16].

However, the quality of these leaves, as indicated by reduced area and biomass, remains compromised under stress conditions.

Leaf Area per Plant

Leaf area exhibited contrasting responses to salinity and polyamine treatments across the experimental period. Unlike leaf number, leaf area showed a clear declining trend with increasing salinity levels. At 60 DAT, mean leaf area decreased from 305.21 cm² under canal water to 231.03 cm² under 8.0 dS m⁻¹ in 2023-24. This decline continued at subsequent observations, indicating the adverse effect of salinity on leaf expansion and development. Among treatments, Putrescine at 0.5 mM (C₁) consistently produced the highest leaf area across all observation periods and salinity levels. The maximum leaf area (379.08 cm²) was recorded with C₁A₁ combination at 90 DAT. The significant interaction effects (A×B) across all time points demonstrate that polyamine effectiveness varies with salinity levels, with greater benefits observed under moderate stress conditions.

Table 4: Effect of exogenous application of polyamines on leaf area per plant (cm²/plant) of guava under saline water irrigation at 60 DAT

Factor B-Doses of Polyamines	Factor A- Quality of Irrigation Water									
	2023-24					2024-25				
	A ₁	A ₂	A ₃	A ₄	Mean	A ₁	A ₂	A ₃	A ₄	Mean
C ₁	362.75	330.50	320.25	282.25	323.94	360.33	345.59	340.86	323.37	342.54
C ₂	357.00	310.25	287.00	270.75	306.25	357.69	336.13	325.57	318.09	334.37
C ₃	352.25	287.00	291.00	264.50	298.69	355.49	325.57	327.44	315.23	330.93
C ₄	345.25	279.50	264.50	255.75	286.25	352.30	322.05	315.23	311.16	325.19
C ₅	275.00	260.50	259.00	216.50	252.75	320.07	313.36	312.70	293.23	309.84
C ₆	256.00	253.00	223.75	197.50	232.56	311.38	309.95	296.53	284.54	300.60
C ₇	245.75	215.00	209.25	174.00	211.00	306.65	292.57	289.82	273.76	290.70
Mean	305.21	267.54	255.75	231.03		333.93	316.61	311.22	300.54	
C.D. at 5%	A = 4.29 B = 5.67 A×B = 11.34					A = 3.96 B = 5.23 A×B = 10.47				

Table 5: Effect of exogenous application of polyamines on leaf area per plant (cm²/plant) of guava under saline water irrigation at 75 DAT

Factor B-Doses of Polyamines	Factor A- Quality of Irrigation Water									
	2023-24					2024-25				
	A ₁	A ₂	A ₃	A ₄	Mean	A ₁	A ₂	A ₃	A ₄	Mean
C ₁	371.69	356.57	351.74	333.82	353.46	373.94	360.08	355.60	339.22	357.21
C ₂	368.96	346.91	335.99	328.29	345.04	371.42	351.19	341.18	334.11	349.48
C ₃	366.72	335.99	337.95	325.42	341.52	369.39	341.18	343.00	331.45	346.26
C ₄	363.43	332.42	325.42	321.29	335.64	366.38	337.89	331.45	327.67	340.85
C ₅	330.39	323.46	322.76	302.81	319.86	336.00	329.70	329.07	310.73	326.38
C ₆	321.43	320.03	306.24	293.85	310.39	327.81	326.55	313.88	302.54	317.70
C ₇	316.60	302.11	299.38	282.86	300.24	323.40	310.10	307.58	292.39	308.37
Mean	344.59	326.82	321.29	310.29		349.07	332.77	327.69	317.68	
C.D. at 5%	A = 4.08 B = 5.40 A×B = 10.80					A = 3.74 B = 4.94 A×B = 9.89				

Table 6: Effect of exogenous application of polyamines on leaf area per plant (cm²/plant) of guava under saline water irrigation at 90 DAT

Factor B-Doses of Polyamines	Factor A- Quality of Irrigation Water									
	2023-24					2024-25				
	A ₁	A ₂	A ₃	A ₄	Mean	A ₁	A ₂	A ₃	A ₄	Mean
C ₁	377.49	360.97	355.72	336.26	357.61	379.08	363.06	357.96	339.06	359.79
C ₂	374.48	350.47	338.64	330.24	348.46	376.20	352.86	341.40	333.24	350.93
C ₃	372.10	338.64	340.74	327.09	344.64	373.86	341.40	343.44	330.18	347.22
C ₄	368.53	334.72	327.09	322.61	338.24	370.38	337.62	330.18	325.80	341.00
C ₅	332.48	324.99	324.22	302.45	321.04	335.40	328.14	327.42	306.30	324.32
C ₆	322.75	321.21	306.23	292.72	310.73	325.98	324.48	309.96	296.82	314.31
C ₇	317.50	301.75	298.74	280.75	299.69	320.88	305.58	302.70	285.18	303.59
Mean	347.97	328.63	322.61	310.51		350.45	331.68	325.85	314.12	
C.D. at 5%	A = 4.11 B = 5.44 A×B = 10.87					A = 4.15 B = 5.48 A×B = 10.97				

The reduction in leaf area under salinity is attributed to impaired cell expansion, reduced chloroplast development, and disrupted water relations (Zhao *et al.*, 2021) ^[19]. However, polyamines enhance leaf development through membrane stabilization, osmotic adjustment, and protection of photosynthetic apparatus (Hussain *et al.*, 2024) ^[8]. The superior performance of Putrescine at 0.5 mM across most parameters suggests its optimal concentration for stress mitigation in guava. Putrescine's effectiveness may be attributed to its role in membrane stabilization, enhanced nutrient uptake, and modulation of

stress-related gene expression (Jangra *et al.*, 2023) ^[9].

Fresh and Dry Leaf Weight

Both fresh and dry leaf weights demonstrated similar patterns of response to salinity and polyamine treatments. Fresh weight decreased progressively from 7.26 g under canal water to 6.07 g under 8.0 dS m⁻¹ in 2023-24, indicating salt-induced reduction in leaf biomass. Putrescine at 0.5 mM (C₁) consistently recorded the highest values for both parameters.

Table 7: Effect of exogenous application of polyamines on fresh weight (g/plant) of guava leaves under saline water irrigation

Factor B-Doses of Polyamines	Factor A- Quality of Irrigation Water									
	2023-24					2024-25				
	A ₁	A ₂	A ₃	A ₄	Mean	A ₁	A ₂	A ₃	A ₄	Mean
C ₁	8.03	7.53	7.37	6.77	7.43	8.12	7.61	7.45	6.38	7.39
C ₂	7.94	7.21	6.85	6.59	7.15	8.02	7.28	6.92	6.21	7.11
C ₃	7.87	6.85	6.91	6.49	7.03	7.95	6.69	6.98	6.12	6.94
C ₄	7.76	6.73	6.49	6.36	6.84	7.84	6.80	6.56	5.98	6.80
C ₅	6.66	6.43	6.41	5.74	6.31	6.73	6.50	6.48	5.39	6.28
C ₆	6.36	6.31	5.86	5.44	5.99	6.43	6.38	5.92	5.10	5.96
C ₇	6.20	5.72	5.63	5.08	5.66	6.27	5.78	5.69	5.14	5.72
Mean	7.26	6.68	6.50	6.07		7.34	6.72	6.57	5.76	
C.D. at 5%	A = 0.10 B = 0.14 A×B = 0.27					A = 0.10 B = 0.14 A×B = 0.27				

Table 8: Effect of exogenous application of polyamines on dry weight (g/plant) of guava leaves under saline water irrigation

Factor B-Doses of Polyamines	Factor A- Quality of Irrigation Water									
	2023-24					2024-25				
	A ₁	A ₂	A ₃	A ₄	Mean	A ₁	A ₂	A ₃	A ₄	Mean
C ₁	1.50	1.44	1.42	1.35	1.43	1.73	1.67	1.65	1.57	1.66
C ₂	1.49	1.40	1.36	1.32	1.39	1.72	1.63	1.58	1.55	1.62
C ₃	1.48	1.36	1.36	1.31	1.38	1.71	1.58	1.59	1.53	1.60
C ₄	1.47	1.34	1.31	1.29	1.35	1.70	1.56	1.53	1.52	1.58
C ₅	1.33	1.30	1.30	1.22	1.29	1.56	1.53	1.52	1.44	1.51
C ₆	1.30	1.29	1.23	1.18	1.25	1.52	1.51	1.45	1.41	1.47
C ₇	1.28	1.22	1.20	1.13	1.21	1.5	1.44	1.42	1.35	1.43
Mean	1.41	1.34	1.31	1.26		1.63	1.56	1.53	1.48	
C.D. at 5%	A = 0.02 B = 0.03 A×B = NS					A = 0.02 B = 0.03 A×B = NS				

The reduction in leaf biomass under salinity stress can be attributed to osmotic stress, ion toxicity, and disrupted photosynthetic processes (Hussain *et al.*, 2021) ^[7]. The significant interaction effects for fresh weight but not dry weight suggest that polyamines primarily influence water retention capacity more than structural dry matter accumulation. This aligns with the known role of polyamines in osmotic adjustment and membrane stability (Hameed *et al.*, 2021) ^[5]. The protective mechanisms of polyamines under salt stress includes stabilization of cellular membranes through interaction with phospholipids, scavenging of reactive oxygen species to prevent oxidative damage, maintenance of ionic homeostasis by regulating Na⁺/K⁺ transport, enhancement of photosynthetic efficiency through chloroplast protection, and modulation of stress-responsive gene expression (Ahmad *et al.*, 2018; Todorova *et al.*, 2007) ^[1, 18]. The differential responses of various polyamines (Putrescine > Spermidine > Spermine) may be related to their molecular structures, cellular uptake mechanisms and metabolic pathways. Putrescine, being the simplest polyamine, may have better cellular penetration and faster metabolic conversion to other beneficial compounds (Minocha *et al.*, 2014) ^[12].

Conclusion

Salinity stress significantly reduced leaves in guava. Exogenous application of polyamines, especially putrescine at 0.5 mM, consistently mitigated these effects by enhancing growth in term of leaves number and leaf physiology. These findings suggest that integrating polyamines and PGRs into guava cultivation practices could help sustain productivity in saline-prone regions.

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