



## EFFECT OF EXOGENOUS APPLICATION OF POLYAMINES ON SHOOT GROWTH OF KINNOW MANDARIN UNDER SALINE WATER IRRIGATION

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Salinity stress is a major constraint limiting growth and productivity of citrus crops, particularly Kinnow mandarin grown under semi-arid conditions. The present investigation was conducted to evaluate the effect of exogenous application of polyamines on shoot growth parameters of Kinnow mandarin under saline water irrigation. One-year-old grafted Kinnow plants were subjected to four irrigation water salinity levels [canal water ( $0.4 \text{ dS m}^{-1}$ ), 3.0, 4.0 and  $5.0 \text{ dS m}^{-1}$ ] and seven polyamine treatments comprising putrescine, spermidine and spermine at two concentrations each, along with an untreated control. The experiment was laid out in a Completely Randomized Design with three replications and observations on number of shoots, shoot diameter and shoot length were recorded at 90 days after transplanting. Increasing salinity levels significantly reduced all shoot growth parameters during both years of study. However, exogenous application of polyamines significantly improved shoot growth under saline conditions. Among the treatments, putrescine at  $0.5 \text{ mM}$  consistently recorded higher number of shoots, greater shoot diameter and increased shoot length, followed by spermine at  $0.5 \text{ mM}$ , whereas untreated control plants exhibited the lowest values. Although polyamines partially mitigated the adverse effects of higher salinity, their effectiveness was more pronounced under canal water and moderate salinity ( $3.0 \text{ dS m}^{-1}$ ).

### ABSTRACT

**Keywords:** Kinnow mandarin, polyamines, salinity stress, shoot growth, putrescine.

### Introduction

Citrus is a globally significant fruit crop, renowned for its nutritional content and beneficial health properties (Zou *et al.*, 2016). Citrus species are categorized as glycophytes and exhibit significant sensitivity to salinity stress, which markedly impedes development and productivity, especially in dry and semi-arid environments where saline irrigation water is commonly utilized (Simpson *et al.*, 2015). Citrus is the third most significant fruit crop in India and is essential for horticultural sustainability, particularly in the north-western regions of Punjab, Rajasthan, and Haryana (Kumar *et al.*, 2023).

*Citrus nobilis* L.  $\times$  *Citrus deliciosa* L., often known as the Kinnow mandarin, is grown extensively because of its high potential production, excellent juice quality, and capacity to adapt to semi-arid regions

(Saini *et al.*, 2022). Kinnow productivity is significantly hindered by salinity, which negatively impacts vegetative growth, shoot development, and overall plant vitality. Salinity stress disturbs ionic and osmotic equilibrium, diminishes nutrient absorption, and triggers oxidative stress via the overproduction of reactive oxygen species (ROS), resulting in growth suppression (Hasanuzzaman *et al.*, 2021).

Polyamines (PAs), including putrescine, spermidine, and spermine, are low-molecular-weight aliphatic amines that are essential for regulating plant growth and enhancing stress tolerance. These chemicals facilitate membrane stability, regulate ion homeostasis, and augment antioxidant defence mechanisms under saline circumstances (Kolesnikov *et al.*, 2024). The Exogenous application of polyamines has been shown to mitigate growth inhibition caused by salt in many crops by enhancing cellular integrity

and fostering vegetative growth (Blázquez, 2024). Nonetheless, data about the influence of polyamines on the regulation of shoot growth parameters in Kinnow mandarin under saline irrigation is scarce. This study aimed to assess the impact of exogenous polyamine treatment on shoot length, shoot diameter, and shoot quantity in Kinnow mandarin plants grown under saline water irrigation.

### Materials and Methods

The experiment was conducted during 2023–2025 at the Experimental Orchard, Department of Horticulture, Chaudhary Charan Singh Haryana Agricultural University (CCS HAU), Hisar, India (29.10° N, 75.46° E; 215 m above mean sea level). The region experiences a semi-arid climate characterized by hot summers, cold winters and an average annual rainfall of about 450 mm.

Uniform, disease-free, one-year-old grafted plants of Kinnow mandarin (*Citrus nobilis* L. × *Citrus deliciosa* L.) on rough lemon (*Citrus jambhiri* Lush.) rootstock were used. Plants were grown in 12-inch pots filled with sandy soil and maintained under pot culture conditions.

The experiment was laid out in a Completely Randomized Design (CRD) with three replications, comprising four irrigation water salinity levels [canal water (0.4 dS m<sup>-1</sup>), 3.0, 4.0 and 5.0 dS m<sup>-1</sup>] and seven polyamine treatments: putrescine (0.5 and 1.0 mM), spermidine (0.5 and 1.0 mM), spermine (0.5 and 1.0 mM) and an untreated control. Polyamines were applied as foliar sprays at 60 and 75 days after transplanting, while irrigation was scheduled as per treatment to maintain uniform soil moisture.

All plants were maintained under uniform cultural practices throughout the experimental period. Nutrient management was carried out through foliar application of a water-soluble NPK fertilizer (19:19:19) at 15-day intervals. The crop remained free from major pest and disease incidence.

Growth observations were recorded at 90 days after transplanting. The number of primary shoots per plant was counted manually. Shoot length was measured from the point of emergence to the apical tip using a meter scale and expressed in centimeters. Shoot diameter was recorded at the mid-portion of the primary shoot using Vernier calipers and expressed in millimeters. The recorded data were subjected to analysis of variance (ANOVA) using OPSTAT software. Treatment means were compared using the critical difference (CD) test at the 5% level of significance.

## Results and Discussion

### Number of shoots

The number of shoots per plant was significantly influenced by irrigation water salinity (Factor A) and polyamine treatments (Factor B) during both years of study. A marked decline in shoot number was observed with increasing salinity levels. Mean number of shoots decreased from 6.1 under canal water (A<sub>1</sub>) to 2.8 at 5.0 dS m<sup>-1</sup> (A<sub>4</sub>) during 2023–2024 and from 5.9 to 2.6 during 2024–2025, clearly indicating the suppressive effect of saline water irrigation on shoot proliferation (Table 1).

Exogenous application of polyamines significantly increased the number of shoots under saline conditions. Among the treatments, putrescine at 0.5 mM (C<sub>1</sub>) recorded the maximum mean number of shoots (5.5 and 5.2), whereas the untreated control (C<sub>7</sub>) consistently recorded the minimum values (3.4 and 3.3) during both years. Polyamine-treated plants maintained higher shoot numbers even under moderate salinity; however, the response declined under higher salinity levels (5.0 dS m<sup>-1</sup>), suggesting partial alleviation of severe salt stress.

The increase in number of shoots due to polyamine application may be attributed to improved bud activity, maintenance of meristematic growth and enhanced physiological stability under saline conditions. Polyamines are known to promote cell division and protect developing tissues from salt-induced injury, thereby supporting shoot initiation and development. Similar enhancement in shoot proliferation under salinity stress following polyamine application has been reported in citrus and other crops (Sharma *et al.*, 2011; Khoshbakht *et al.*, 2017).

### Shoot Diameter

Shoot diameter of Kinnow plants was significantly affected by irrigation water salinity (Factor A), polyamine treatments (Factor B) and their interaction (A × B) during both the years of study (Table 2). A progressive and significant reduction in shoot diameter was observed with increasing salinity levels. Mean shoot diameter declined from canal water (A<sub>1</sub>) to 5.0 dS m<sup>-1</sup> (A<sub>4</sub>), recording values of 4.95 to 4.18 mm during 2023–2024 and 4.98 to 3.89 mm during 2024–2025, indicating the adverse effect of saline water irrigation on shoot growth.

Exogenous application of polyamines significantly improved shoot diameter under saline conditions. Among the treatments, putrescine at 0.5 mM (C<sub>1</sub>) recorded the maximum mean shoot diameter

during both years (4.91 and 4.86 mm), followed by spermine at 0.5 mM (C<sub>5</sub>). The minimum shoot diameter was consistently recorded in the untreated control (C<sub>7</sub>), particularly under higher salinity levels. These results indicate the effectiveness of polyamines in alleviating salinity-induced growth suppression.

The interaction between salinity and polyamines revealed that polyamine application was more effective under canal water and moderate salinity (3.0 dS m<sup>-1</sup>) compared to higher salinity levels. Although polyamines improved shoot diameter even at 5.0 dS m<sup>-1</sup>, the magnitude of response was comparatively lower, suggesting partial mitigation of severe salinity stress.

The improvement in shoot diameter due to polyamine application may be attributed to their role in maintaining membrane stability, enhancing cell expansion and regulating cambial activity under salt stress. Similar findings have been reported in citrus and other crops where exogenous polyamines improved stem or shoot thickness under saline conditions (Khoshbakht *et al.*, 2017; Rathinapriya *et al.*, 2020).

Overall, increasing salinity significantly reduced shoot diameter of Kinnow plants, while exogenous application of polyamines, particularly putrescine at lower concentration, effectively alleviated the adverse effects of salinity by sustaining shoot growth.

### Shoot length

Shoot length of Kinnow plants was significantly affected by irrigation water salinity (Factor A),

polyamine treatments (Factor B) and their interaction (A × B) during both years of study. A consistent decline in shoot length was observed with increasing salinity levels. Mean shoot length decreased from 13.85 cm under canal water (A<sub>1</sub>) to 11.50 cm at 5.0 dS m<sup>-1</sup> (A<sub>4</sub>) during 2023–2024 and from 14.57 cm to 11.91 cm during 2024–2025, indicating the inhibitory effect of saline water irrigation on shoot elongation (Table 3).

Exogenous application of polyamines significantly improved shoot length under saline conditions. Among the treatments, putrescine at 0.5 mM (C<sub>1</sub>) recorded comparatively higher shoot length, followed by spermine at 0.5 mM (C<sub>5</sub>), whereas the untreated control (C<sub>7</sub>) consistently recorded the lowest values during both years. Although polyamines enhanced shoot length at all salinity levels, their effectiveness declined under higher salinity (5.0 dS m<sup>-1</sup>), suggesting partial mitigation of severe salt stress.

The improvement in shoot length due to polyamine application may be attributed to enhanced cell elongation, stabilization of cellular membranes and regulation of ionic balance under saline conditions. Polyamines are known to protect meristematic tissues and maintain metabolic activity, thereby sustaining shoot growth under stress environments. Similar enhancement in shoot elongation following exogenous polyamine application under salinity has been reported in citrus and other crops (Sharma *et al.*, 2011; Khoshbakht *et al.*, 2017)

**Table 1:** Effect of exogenous application of polyamines on number of shoots of Kinnow plants under saline water irrigation

Different concentration of Polyamines	Quality of Irrigation Water											
	2023-2024					2024-2025						
	A1	A2	A3	A4	Mean C		A1	A2	A3	A4	Mean C	
C1	7.0	6.0	5.0	4.0	5.5	C1	6.7	5.7	4.7	3.7	5.2	
C2	6.7	5.7	4.3	3.3	5.0	C2	6.3	5.3	4.3	3.3	4.8	
C3	6.3	5.3	4.0	3.0	4.7	C3	6.0	5.0	4.0	3.0	4.5	
C4	6.0	5.0	3.7	2.7	4.3	C4	6.0	5.0	3.3	2.3	4.2	
C5	6.0	5.0	3.7	2.7	4.3	C5	5.7	4.7	3.3	2.3	4.0	
C6	5.7	4.3	3.0	2.0	3.8	C6	5.3	4.3	3.0	2.0	3.7	
C7	5.3	4.0	2.7	1.7	3.4	C7	5.3	4.0	2.3	1.7	3.3	
Mean A	6.1	5.0	3.8	2.8		Mean A	5.9	4.9	3.6	2.6		
C.D.	Factor A= 0.26 Factor C= 0.35 Factor AxC=N/A					C.D.	Factor A= 0.3 Factor C= 0.45 Factor AxC=N/A					

A<sub>1</sub>-Canal Water; A<sub>2</sub>-3.0 dSm<sup>-1</sup>; A<sub>3</sub>-4.0 dSm<sup>-1</sup>; A<sub>4</sub>-5.0 dSm<sup>-1</sup>; C<sub>1</sub>-Putrescine (0.5mM); C<sub>2</sub>-Putrescine (1.0mM); C<sub>3</sub>-Spermidine (0.5mM); C<sub>4</sub>-Spermidine (1.0mM); C<sub>5</sub>-Spermine (0.5mM); C<sub>6</sub>-Spermine (1.0mM); C<sub>7</sub>-Control

**Table 2:** Effect of exogenous application of polyamines on shoot diameter (mm) of Kinnow plants under saline water irrigation

Different concentration of Polyamines	Quality of Irrigation Water										
	2023-2024					2024-2025					
	A1	A2	A3	A4	Mean C	A1	A2	A3	A4	Mean C	
C1	5.08	4.97	4.85	4.73	4.91	C1	5.24	5.01	4.74	4.45	4.86
C2	5.02	4.84	4.66	4.47	4.75	C2	5.04	4.71	4.35	3.96	4.52
C3	4.98	4.74	4.51	4.25	4.62	C3	4.91	4.54	4.12	3.67	4.31
C4	4.93	4.62	4.33	4.00	4.47	C4	5.05	4.67	4.37	4.07	4.54
C5	5.06	4.92	4.79	4.66	4.86	C5	5.15	4.87	4.56	4.25	4.71
C6	4.84	4.46	4.12	3.71	4.28	C6	4.83	4.37	4.01	3.60	4.20
C7	4.73	4.29	3.88	3.41	4.08	C7	4.64	4.09	3.69	3.22	3.91
Mean A	4.95	4.69	4.45	4.18		Mean A	4.98	4.61	4.26	3.89	
C.D.	Factor A= 0.11 Factor C= 0.14 Factor AxC =0.28				C.D.	Factor A= 0.12 Factor C=0.15 Factor AxC =0.31					

A<sub>1</sub>-Canal Water; A<sub>2</sub>- 3.0 dSm<sup>-1</sup>; A<sub>3</sub>-4.0 dSm<sup>-1</sup>; A<sub>4</sub>-5.0 dSm<sup>-1</sup>; C<sub>1</sub>-Putrescine (0.5mM); C<sub>2</sub>-Putrescine (1.0mM); C<sub>3</sub>-Spermidine (0.5mM); C<sub>4</sub>-Spermidine (1.0mM); C<sub>5</sub>-Spermine (0.5mM); C<sub>6</sub>-Spermine (1.0mM); C<sub>7</sub>-Control

**Table 3:** Effect of exogenous application of polyamines on shoot length (cm) of Kinnow plants under saline water irrigation

Different concentration of Polyamines	Quality of Irrigation Water										
	2023-2024					2024-2025					
	A1	A2	A3	A4	Mean C	A1	A2	A3	A4	Mean C	
C1	14.13	13.36	13.00	11.45	12.99	C1	14.86	13.72	13.16	11.92	13.42
C2	13.94	13.28	13.10	11.73	13.01	C2	14.61	13.64	13.34	12.04	13.41
C3	13.92	13.23	13.21	11.68	13.01	C3	14.66	13.54	13.42	12.16	13.45
C4	13.65	13.22	12.98	11.58	12.86	C4	14.31	13.61	13.16	11.86	13.23
C5	13.80	13.46	13.08	11.38	12.93	C5	14.55	13.91	13.34	12.00	13.45
C6	13.99	13.21	12.86	11.45	12.88	C6	14.81	13.55	13.08	11.84	13.32
C7	13.52	12.92	12.64	11.20	12.57	C7	14.22	13.31	12.86	11.54	12.98
Mean A	13.85	13.24	12.98	11.50		Mean A	14.57	13.61	13.19	11.91	
C.D.	Factor A= 0.05 Factor C= 0.07 Factor AxC =0.14				C.D.	Factor A= 0.04 Factor B=0.05 Factor AxB =0.10					

A<sub>1</sub>-Canal Water; A<sub>2</sub>- 3.0 dSm<sup>-1</sup>; A<sub>3</sub>-4.0 dSm<sup>-1</sup>; A<sub>4</sub>-5.0 dSm<sup>-1</sup>; C<sub>1</sub>-Putrescine (0.5mM); C<sub>2</sub>-Putrescine (1.0mM); C<sub>3</sub>-Spermidine (0.5mM); C<sub>4</sub>-Spermidine (1.0mM); C<sub>5</sub>-Spermine (0.5mM); C<sub>6</sub>-Spermine (1.0mM); C<sub>7</sub>-Control

### Conclusion

Salinity stress markedly diminished shoot growth of Kinnow mandarin, as indicated by a reduction in number of shoots, shoot diameter and shoot length with increasing irrigation water salinity. Exogenous application of polyamines significantly mitigated the adverse effects of salinity and improved shoot growth parameters during both years of study. Among the evaluated polyamines, putrescine at 0.5 mM exhibited the highest effectiveness in enhancing shoot proliferation and development, followed by spermine at 0.5 mM, whereas untreated plants recorded the greatest growth inhibition. Although polyamine application partially alleviated severe salinity stress (5.0 dS m<sup>-1</sup>), the magnitude of response was comparatively greater under canal water and moderate salinity levels.

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