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RESEARCH ARTICLE

Evaluation of nanoparticle biostimulants in alleviating salinity-induced stress: A study on morphological and molecular responses in potato cultivars

Amirah S. Alahmari[1], Mahmoud Magdy[2, 3], Ahmed B. El-Mansy[4], Nabil S. Awad[5, 6], Mohammad E. Eldenary[7], Rahma Alshamrani[8], Makhdora Almuziny[8], Amani Omar Abuzaid[8], Nora M. Al Aboud[9], and Diaa Abd El Moneim[10*]

[1]Princess Nourah bint Abdulrahman University, College of Science, Department of Biology, Riyadh 11671, Saudi Arabia.
[2]Murcia University, Faculty of Biology, Department of Plant Biology, Murcia, 30100, Spain.
[3]Ain Shams University, Faculty of Agriculture, Department of Genetics, Cairo, 11241, Egypt.
[4]Arish University, Faculty of Environmental Agricultural Sciences, Department of Plant Production (Vegetable Breeding and Biotechnology Branch), El-Arish 45511, Egypt.
[5]Misr University for Science and Technology, College of Biotechnology, Giza, 12563, Egypt.
[6]Aswan University, Faculty of Agriculture and Natural Resources, Department of Genetics, Aswan, 81528, Egypt.
[7]Horticulture Research Institute, Potato and Vegetatively Propagated Vegetables Research Department (PVPV), Giza, Egypt.
[8]King Abdulaziz University, Faculty of Science, Department of Biological Sciences, Jeddah, Saudi Arabia.
[9]Umm Al-Qura University, Faculty of Science, Department of Biology, Makkah, Saudi Arabia.
[10]Arish University, Faculty of Environmental Agricultural Sciences, Department of Plant Production (Genetic Branch), El-Arish 45511, Egypt.
*Corresponding author (dabdelmoniem@aru.edu.eg).
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ABSTRACT

Potato (*Solanum tuberosum* L.), a critical food crop, faces productivity challenges due to salinity stress, which is exacerbated by climate change. This study evaluates the potential of nanoparticle-based biostimulants—salicylic acid (SA), ascorbic acid (AS), and benzoic acid (BA)—to alleviate salinity-induced stress in 'Spunta' and 'Lady Rosetta' potatoes. The biostimulants were applied at 0.5 mM (T1) and 0.75 mM (T2) concentrations under salinity stress levels of 50 and 100 mg L^{-1} NaCl. Morphological assessments revealed that T2 treatment significantly improved root length (up to 12.17 cm) and root number (up to 4.17) in both cultivars. Gene expression analysis showed that the *MYB1* gene, a key regulator of stress responses, was upregulated under salinity stress in control plants but was significantly downregulated by nanoparticle treatments. This reduction suggests that nanoparticles modulate stress signaling pathways, reducing the need for MYB1 activation. The *P5CS* gene, involved in proline biosynthesis, was also downregulated by nanoparticle treatments, indicating enhanced osmotic stress tolerance and reduced reliance on proline accumulation. Furthermore, expression of the *P450* gene, associated with stress responses, was reduced by nanoparticles, highlighting their role in modulating stress-responsive pathways. The *SOS1* gene, crucial for salt tolerance, was significantly reduced under high salt concentrations with nanoparticle treatments, suggesting improved ion homeostasis. Our study confirms that nanoparticle-based biostimulants effectively enhance potato plant resilience to salinity stress by improving growth parameters and modulating stress-related gene expression. These findings underscore the potential of nanotechnology in advancing crop management strategies under challenging environmental conditions.

Key words: Biostimulants, gene expression, nanoparticles, MYB1 gene, P5CS gene, potato cultivars, salinity stress, *Solanum tuberosum*, stress response.

INTRODUCTION

Potatoes (*Solanum tuberosum* L.) are a significant food crop globally, ranking as the top non-cereal food crop in terms of production (Sánchez et al., 2007). However, potato cultivation faces challenges, particularly concerning environmental stresses (Dahal et al., 2019). The susceptibility of potatoes to environmental stresses is further exacerbated by climate change, which brings about prolonged heat, severe droughts, increased soil salinity, and unpredictable heavy rainfall, all of which negatively impact potato production (Chourasia et al., 2021). Salinity stress poses a significant threat to potato cultivation, affecting plant growth, productivity, and overall crop quality (Carreño-Quintero et al., 2012). Understanding the physiological, biochemical, and molecular responses of potatoes to salinity stress is crucial for developing effective strategies to enhance stress tolerance and crop performance (Magdy et al., 2023; Abd El Moneim et al., 2023).

The exploration of biotechnological approaches and nanomaterial applications underscores their potential in enhancing abiotic stress tolerance in crops (Saad et al., 2019). Nanotechnology offers innovative solutions in agriculture, environmental research, and the food industry, with nanoparticles interacting effectively with plants due to their unique properties (Wang et al., 2023). Various nanoparticle products, such as nano fertilizers, pesticides, and sensors, are widely used in agriculture to improve plant tolerance to environmental stresses, including salinity (Liang et al., 2023). Research has shown the potential of nanoparticle-based biostimulants in alleviating salinity-induced stress in crops, including potatoes (Gao et al., 2015). Nanoparticles have demonstrated promise in enhancing plant tolerance to various environmental stresses through complex biochemical and physiological mechanisms (Murtaza et al., 2023). Studies on the physiological responses of potato plants to salinity stress have highlighted the role of osmoprotectants and antioxidant enzymes in plant adaptation (Ahmed et al., 2020). Studies on the application of polyamine precursors and antioxidants under salt stress offer valuable insights into enhancing plant stress tolerance (Girija et al., 2021).

Metabolomic and biochemical analyses of potato cultivars under osmotic and salt stresses provide insights into plant stress physiology and the importance of understanding these responses for crop improvement (Ma et al., 2022). The use of osmoregulators and antioxidants, such as glycine betaine, proline, and salicylic acid, has been shown to mitigate the adverse impacts of salinity stress in potato genotypes, enhancing stress tolerance (Kalsoom et al., 2023). Investigations into the synergistic effects of nanoparticles like Zn, B, Si, and zeolite have shown potential in improving salinity stress tolerance in potatoes (Mahmoud et al., 2019). Research on a nanoparticle mixture containing salicylic acid, ascorbic acid, and benzoic acid showed promise in enhancing stress tolerance in potatoes (Azeem et al., 2023; Raees et al., 2023). These studies collectively emphasize the importance of innovative strategies, including nanotechnology, in improving crop productivity and resilience to environmental stresses for sustainable potato production under changing climatic conditions.

The present study aimed to investigate the potential of a nanoparticle mixture containing salicylic acid (SA), ascorbic acid (AS), and benzoic acid (BA) in mitigating the detrimental impacts of salinity stress on 'Spunta' and 'Lady Rosetta' potatoes.

MATERIALS AND METHODS

Synthesis of nanoparticles for applied biostimulants

The synthesis of nanoparticles for the applied biostimulants was performed using a ball mill method (Alowaiesh et al., 2024). Specifically, 1 g dry ascorbic acid (AS), 1.1 g dry benzoic acid (BA), or 0.8 g dry salicylic acid (SA) were placed in the stainless-steel gear of a ball mill machine (MM 500 nano, Retsch GmbH, Haan, Germany). For the AS nanoparticle synthesis, 4 mL deionized water was added to the dry powder, while 2 mL was added for BA and 2.5 mL for SA to prevent oxidation or decomposition caused by milling heat. The wet biostimulants were milled using various steps with different ball diameters and milling times, maintaining a constant mass ratio of powder to balls: 1:6 for AS, 1:4 for BA, and 1:7 for SA (Alowaiesh et al., 2024).

Micropropagation and salinity stress treatment

Plantlets of 'Spunta' (SN) and 'Lady Rosetta' (CV) potatoes (*Solanum tuberosum* L.) were used in this study. The experiment followed a completely randomized design (CRD) with five replicates (pentaplicate) for each

treatment. The explants were sterilized using 70% ethanol for 1 min, followed by 2% sodium hypochlorite for 10 min, and then rinsed three times with sterile distilled water. The sterilized explants were cultured on semi-solid media containing 30 g sucrose and 8 g agar, with a pH adjusted to 5.8. The nanoparticle treatments, a mixture composed of SA, BA, and AS, was applied at concentrations of 0.5 (T1) and 0.75 mg L^{-1} (T2). Plantlets were morphologically assessed at 7, 14 and 21 d. At 22 d, salinity stress was applied using sodium chloride (NaCl) at concentrations of 50 and 100 mg L^{-1} in media.

Morphological assessment under biostimulants effect

Five quantitative traits were measured at 7, 14, and 21 d: Number of leaves per plant, plantlet length (cm), number of lateral branches per plant, number of roots, and root length (cm). Observed measurements were transformed (z-score) on Microsoft Excel. All traits were compared visually using heatmap and linear plots showing means and ranges of all traits under different treatments over the three ages using Orange 3.37 (Demšar et al., 2013). For each trait, a univariate ANOVA was estimated using SPSS V29 (IBM, Armonk, New York, USA) to determine the effect of plantlet age (in days), different concentration of biostimulants mixtures (treatments), and cultivar type.

Gene expression quantification under salinity stress

Gene expression analysis was performed using quantitative PCR (qPCR) to quantify the expression levels of *MYB1*, *P5CS*, *P450*, and *SOS1* genes, with *ACTB* serving as the housekeeping gene. Total RNA was extracted from the treated plantlets at 25 d of age using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The concentration and purity of the RNA were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). For cDNA synthesis, 1 µg total RNA was reverse transcribed using the QuantiTect Reverse Transcription Kit (Qiagen) in a final reaction volume of 20 µL. The reaction conditions were as follows: 42 °C for 15 min, followed by 95 °C for 3 min to inactivate the reverse transcriptase.

The qPCR reactions were conducted in a 20 µL volume containing 10 µL SYBR Green Master Mix (Applied Biosystems, Foster City, California, USA), 1 µL cDNA, 0.5 µL each forward and reverse primer (10 µM), and 8 µL nuclease-free water. The primer sequences for each target gene and the housekeeping gene *ACTB* are provided in Table 1. The qPCR program was run on an Agilent Mx3000P QPCR System (Agilent, Santa Clara, California, USA) with the following cycling conditions: Initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. A melting curve analysis was performed to ensure the specificity of the amplified products, with a gradual increase in temperature from 60 to 95 °C.

Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), normalizing the expression of target genes to the *ACTB* gene and comparing the expression levels in treated samples to the control.

Table 1. Primer sequences for quantitative PCR analysis of target genes in potato, including *MYB1*, *P450*, *P5CS*, *SOS1*, and *ACTB*. Forward and reverse sequences are provided for each gene.

Gene	5'-Forward Primer Sequence-3'	5'-Reverse Primer Sequence-3'
MYB1	CCAGCTGAGGACGACACCAT	CCAGGTAGCAGTCGGGCAAT
P450	TCGGATGACGTCAACAAGTGTCA	TGCTGTCAATAAACCTATCCGGCAA
P5CS	TGCAAGGAGAGCGTATCGGT	CCTGAAGTCGTCTGGAACTCTCC
SOS1	CCCGCACCCTCAACATGGAT	CCCTGCGAACAACCGGAAGA
ACTB	TGGCATCACACTTTCTACAA	GATCTTGAGAGCTTAGGCAA

RESULTS

Morphological assessment under salinity stress

The morphological assessment of the 'Spunta' (SV) and 'Lady Rosetta' (CV) potatoes under various nanoparticle treatments (Ctrl, T1, T2) over 7, 14, and 21 d highlighted the impact of plantlet age on growth traits. Analysis using z-score transformations and heatmap visualization revealed that plant age predominantly influenced the number of branches (NB), number of leaves (NL), and plantlet length (PL). Notably, the T2 treatment (0.75 mg L^{-1} nanoparticle mixture) significantly enhanced the number of roots (NR) and root length (RL) in the 'Spunta', with similar improvements observed for RL in 'Lady Rosetta' (Figure 1a). Furthermore, T2-treated plants exhibited higher mean values and greater ranges across all traits, underscoring the potential of nanoparticle biostimulants to improve growth performance (Figure 1b). 'Spunta' consistently outperformed 'Lady Rosetta', particularly in response to the T2 treatment (Figure 1c).

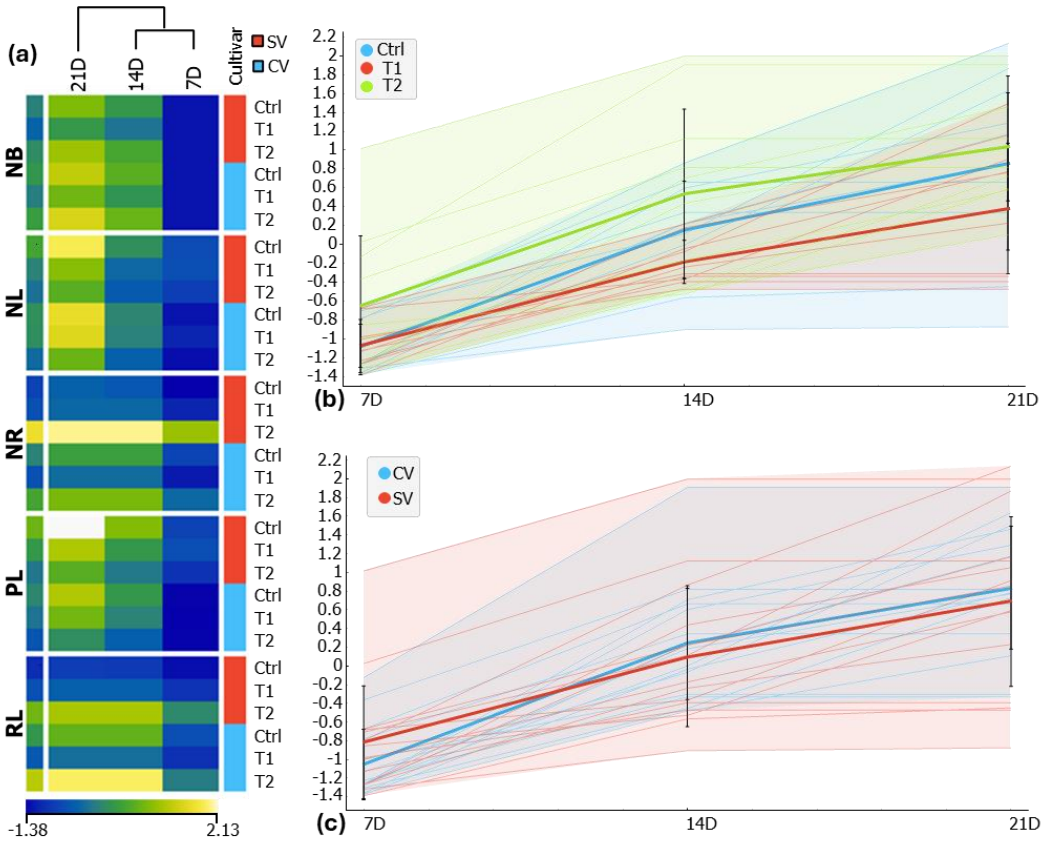

Figure 1. Effect of nanoparticle treatments on morphological traits of potato cultivars under salinity stress. (a) Heatmap of z-score transformed morphological trait data for 'Spunta' (SV) and 'Lady Rosetta' (CV) under control (Ctrl), T1, and T2 treatments across 7, 14, and 21 d, with a gradient from -1.38 (dark blue) to 2.13 (yellow). (b) Linear plot showing means (horizontal bold colored lines) and ranges (shaded colors: Blue for control, red for T1, green for T2) of morphological traits across 7, 14, and 21 d. Vertical means and error bars are shown in black lines. (c) Linear plot comparing means (horizontal bold lines) and ranges (shaded colors: Blue for CV, red for SV) between the cultivars. NB: Number of branches; NL: number of leaves; NR: number of roots; PL: plantlet length; RL: root length.

In detail, the morphological analysis of SV and CV potatoes under different nanoparticle treatments (Ctrl, T1, T2) over 7, 14, and 21 d revealed significant differences in plant growth parameters. The number of leaves (NL) in SV showed a substantial increase over time, with control plants reaching an average of 12.50 by day 21. Treatments T1 and T2 resulted in lower leaf counts, indicating a potential inhibitory effect of the nanoparticles on leaf development. In the CV cultivar, the number of leaves also increased over time, but the effect of treatments was less pronounced compared to SV. The control plants had an average of 11.67 leaves by day 21, while T1 and T2 treatments resulted in averages of 11.19 and 8.43, respectively. The PL in SV was significantly affected by nanoparticle treatments, with control plants reaching 14.50 cm by day 21. T1 and T2 treatments showed reduced lengths of 10.81 and 8.57 cm, respectively.

For CV, control plants reached 10.83 cm, while T1 and T2 treatments resulted in 9.33 and 6.76 cm, respectively. The NB exhibited minor changes under treatment. In SV, control plants had an average of 1.14 branches by day 21, while T1 and T2 treatments had 0.83 and 1.24 branches, respectively. For CV, the control had 1.35 branches, T1 had 1.10, and T2 had 1.44 branches. The NR in SV control plants was 2.10 by day 21, while T1 and T2 treatments resulted in 2.14 and 4.17 roots, respectively. The CV control plants had 2.76 roots, T1 had 2.19, and T2 had 3.17 roots. The RL in SV was significantly affected by treatments, with control plants having 3.28 cm roots by day 21. The T1 and T2 treatments resulted in increased lengths of 4.57 and 9.67 cm, respectively. For CV, control plants had 8.19 cm roots, T1 had 5.10 cm, and T2 had 12.17 cm (Table 2).

Table 2. Morphological quantitative traits of 'Spunta' (SV) and 'Lady Rosetta' (CV) potatoes under nanoparticle treatments over 7, 14, and 21 d. The average and standard errors for number of leaves per plant (NL), plantlet length (PL), number of lateral branches per plantlet (NB), number of roots (NR), and root length (RL) for the two potato cultivars are shown. Ctrl: Control; T1: 0.5 mg L^{-1}; T2: 0.75 mg L^{-1}.

			NL		PL		NB		NR		RL	
Cultivar	Days	Nano treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
					cm						cm	
SV	7	Ctrl	3.34	0.11	3.34	0.11	0.10	0.05	1.30	0.10	1.87	0.11
		T1	3.43	0.12	3.81	0.10	0.10	0.04	1.52	0.09	2.95	0.10
		T2	2.81	0.10	2.57	0.09	0.10	0.03	3.34	0.08	6.17	0.09
	14	Ctrl	6.25	0.21	9.67	0.25	0.83	0.09	2.00	0.14	3.19	0.13
		T1	4.55	0.20	7.20	0.21	0.60	0.08	2.14	0.13	4.57	0.12
		T2	3.97	0.18	5.71	0.18	0.94	0.07	4.17	0.12	9.67	0.11
	21	Ctrl	12.50	0.33	14.50	0.35	1.14	0.12	2.10	0.18	3.28	0.15
		T1	9.09	0.30	10.81	0.32	0.83	0.10	2.14	0.17	4.57	0.14
		T2	7.95	0.25	8.57	0.28	1.24	0.09	4.17	0.15	9.67	0.13
CV	7	Ctrl	1.34	0.09	1.17	0.08	0.10	0.05	1.81	0.09	3.91	0.08
		T1	1.81	0.08	1.10	0.07	0.10	0.04	1.43	0.08	2.86	0.07
		T2	1.19	0.07	1.14	0.06	0.10	0.03	2.17	0.07	5.67	0.06
	14	Ctrl	5.84	0.21	7.22	0.20	1.01	0.08	2.76	0.12	8.19	0.10
		T1	5.59	0.20	6.21	0.19	0.81	0.07	2.19	0.11	5.10	0.09
		T2	4.21	0.19	4.51	0.18	1.07	0.08	3.17	0.10	12.17	0.08
	21	Ctrl	11.67	0.31	10.83	0.30	1.35	0.11	2.76	0.14	8.19	0.12
		T1	11.19	0.32	9.33	0.27	1.10	0.10	2.19	0.13	5.10	0.11
		T2	8.43	0.27	6.76	0.26	1.44	0.11	3.17	0.12	12.17	0.10

The ANOVA results revealed the statistical significance of the effects of three factors (cultivar, days, and nanoparticle treatments) on the morphological traits. The factor 'days' had a highly significant effect on NL ($F = 106.949$, $p < 0.001$), PL ($F = 41.417$, $p < 0.001$), NB ($F = 134.487$, $p < 0.001$), NR ($F = 5.180$, $p = 0.032$), and RL ($F = 5.713$, $p = 0.025$). Nanoparticle treatments also significantly influenced NL ($F = 7.288$, $p = 0.013$), PL ($F = 5.527$, $p = 0.027$), NB ($F = 5.855$, $p = 0.024$), NR ($F = 14.019$, $p = 0.002$), and RL ($F = 12.446$, $p = 0.003$).

The interaction between cultivar and days did not show significant effects on most traits, except for NR ($F = 4.827$, $p = 0.029$), indicating some interaction effects on root number. For the factor 'days', comparisons between 7, 14, and 21 d were highly significant for NL, PL, and NB ($p < 0.001$). The differences between control and 0.75 mM treatments were significant for NL ($p = 0.004$), PL ($p = 0.009$), NR ($p = 0.002$), and RL ($p = 0.003$). Significant differences were also observed between 0.5 and 0.75 mM treatments for NB ($p = 0.009$), NR ($p < 0.001$), and RL ($p = 0.001$) (Table 3).

Table 3. ANOVA summary for morphological traits of potato cultivars treated with biostimulants nanoparticles. The F-values and p-values from the ANOVA test the effects of cultivar type, days, and nanoparticle treatments on the number of leaves (NL), plantlet length (PL), number of branches (NB), number of roots (NR), and root length (RL). LSD post-hoc comparisons are shown between specific time points and treatment concentrations. Significant p-values are written in bold.

F-statistics	ANOVA	NL	PL	NB	NR	RL
Cultivars	F-value	0.023	1.052	0.248	0.367	3.686
	p-value	0.883	0.325	0.627	0.556	0.079
Days	F-value	106.949	41.417	134.487	5.180	5.713
	p-value	**< 0.001**	**< 0.001**	**< 0.001**	**0.032**	**0.025**
Nano treatments	F-value	7.288	5.527	5.855	14.019	12.446
	p-value	**0.013**	**0.027**	**0.024**	**0.002**	**0.003**
Cultivar × Days	F-value	1.311	0.074	1.008	0.019	0.258
	p-value	0.305	0.930	0.394	0.981	0.777
Cultivar × Nano treatments	F-value	0.056	0.041	0.003	4.827	1.145
	p-value	0.945	0.960	0.997	**0.029**	0.351
Days × Nano treatments	F-value	1.845	1.200	1.523	0.036	0.449
	p-value	0.204	0.375	0.275	0.997	0.771
Post-Hoc	Case	NL	PL	NB	NR	RL
Days	7D × 14D	**< 0.001**	**< 0.001**	**< 0.001**	**0.022**	**0.017**
	7D × 21D	**< 0.001**	**< 0.001**	**< 0.001**	**0.020**	**0.017**
	14D × 21D	**< 0.001**	**< 0.001**	**0.001**	0.956	0.990
Nano treatments	Control × 0.5 mM	0.139	0.150	**0.038**	0.541	0.614
	Control × 0.75 mM	**0.004**	**0.009**	0.401	**0.002**	**0.003**
	0.5 mM × 0.75 mM	0.057	0.114	**0.009**	**< 0.001**	**0.001**

Gene expression analysis under salinity stress

The *MYB1* gene, a crucial transcription factor involved in stress responses, exhibited significant upregulation in 'Spunta' under salinity stress without biostimulants treatment. Specifically, *MYB1* expression increased by approximately 15-fold at both 50 and 100 mg L^{-1} salt concentrations in control plants, indicating a robust stress response. However, the application of nanoparticle biostimulants led to a dramatic reduction in *MYB1* expression. At 50 mg L^{-1} salt concentration, T1 and T2 treatments decreased *MYB1* expression by about 98% and 80%, respectively. In 'Lady Rosetta', *MYB1* expression under control conditions and 50 mg L^{-1} salt stress remained relatively low. The T1 treatment led to a slight increase in *MYB1* expression at 50 mg L^{-1} salt, whereas T2 treatment resulted in a decrease. At 100 mg L^{-1} salt concentration, both T1 and T2 treatments significantly reduced *MYB1* expression compared to the control, demonstrating the biostimulants' role in moderating the stress response (Figure 2).

The *P5CS* gene, crucial for proline biosynthesis and osmotic stress tolerance, showed increased expression in 'Spunta' under salinity stress. Control plants exhibited significant upregulation of *P5CS*, with a 12-fold increase at 50 mg L^{-1} and a 20-fold increase at 100 mg L^{-1} salt concentrations. Nanoparticle biostimulants treatments, particularly T1, significantly lowered *P5CS* expression by approximately 90% compared to control, indicating enhanced osmotic stress tolerance and reduced proline accumulation needs as a stress response. In 'Lady Rosetta', *P5CS* expression was low across all conditions, with minor fluctuations observed upon nanoparticle biostimulants treatment (Figure 2).

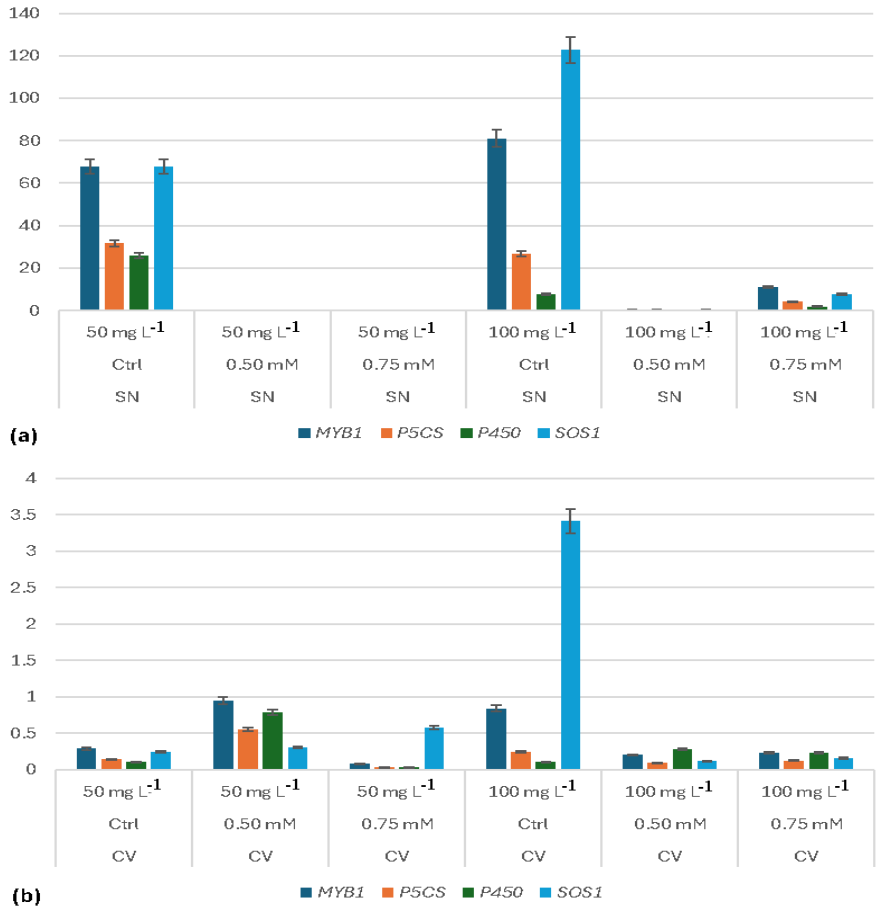

Figure 2. Histogram plots showing the relative expression levels of MYB1, P5CS, P450, and SOS1 genes in potato cvs. Spunta (SN) (a) and Lady Rosetta (CV) (b). Gene expression was quantified using the ΔΔCt method, with *ACTB* as the housekeeping gene. Each bar represents the mean fold change in gene expression under different treatment conditions, including control (Ctrl), and nanoparticle treatments at concentrations of 0.5 and 0.75 mM under salinity stress levels of 50 and 100 mg L^{-1}.

The *P450* gene, associated with various stress responses, demonstrated highly elevated expression in 'Spunta' under 50 mg L^{-1} salt stress without biostimulants treatment, with an approximately 18-fold increase. This expression was significantly reduced by both T1 and T2 treatments, indicating that the biostimulants modulate stress-responsive pathways involving *P450*. At 100 mg L^{-1} salt, *P450* expression was initially low in the control but increased to about 5-fold under T2 treatment, suggesting a dose-dependent response to biostimulants. In 'Lady Rosetta', *P450* expression remained low under control and salt stress conditions, with slight increases upon nanoparticle treatment (Figure 2).

The *SOS1* gene, critical for salt tolerance, was significantly upregulated in 'Spunta' under salinity stress in control plants, showing a 25-fold increase at 50 mg L^{-1} salt. The application of nanoparticle biostimulants markedly reduced *SOS1* expression, particularly under T1 treatment at 50 mg L^{-1} salt, by over 99%. This dramatic reduction suggests that the biostimulants enhance ion homeostasis and salt tolerance, thereby reducing the necessity for *SOS1*-mediated stress responses. In 'Lady Rosetta', *SOS1* expression exhibited variable responses. At 100 mg L^{-1} salt, control plants showed increased *SOS1* expression, which was significantly reduced by T1 and T2 treatments, demonstrating the biostimulants' effectiveness in modulating salt tolerance mechanisms across different cultivars (Figure 2).

DISCUSSION

The impact of nanoparticle biostimulant treatments on potato cultivars under salinity stress has garnered considerable attention due to its significant effects on plant growth and development. This study focused on the morphological and genetic responses of 'Spunta' and 'Lady Rosetta' potatoes under salinity stress conditions, revealing that a 0.75 mg L^{-1} nanoparticle mixture notably enhanced root development, particularly by increasing the number and length of roots. The statistical analysis utilizing ANOVA indicated that both plant age and nanoparticle treatments significantly influenced various morphological traits and root characteristics. The interaction between cultivar and nano treatments also showed significance, suggesting cultivar-specific responses. Post-hoc comparisons further emphasized the influence of time and nanoparticle concentrations on growth traits, underscoring the effectiveness of higher nanoparticle concentrations in improving plant performance under salinity stress (Zhang et al., 2023). The root length and number enhancement are the key findings as it underscores the role of nanoparticle biostimulants in promoting root system architecture, which is crucial for improved water and nutrient uptake under stress conditions.

The genotype-specific responses observed between the two cultivars are particularly noteworthy. 'Spunta' exhibited superior performance compared to 'Lady Rosetta', especially under the T2 treatment, highlighting the importance of tailoring biostimulant strategies to specific genotypes. This finding aligns with emerging research emphasizing the necessity of considering genetic factors when developing interventions for salinity stress management (Djaman et al., 2021). The differential responses underscore the complexity of plant stress responses and the potential for genotype-specific biostimulant applications to maximize crop resilience and productivity (Zaki and Radwan, 2022).

Furthermore, the study's findings suggest that the observed enhancement in root growth under nanoparticle treatments may represent an adaptive mechanism whereby plants prioritize root development as a strategy to cope with salinity stress. This response is vital for improving plant water and nutrient uptake, which are essential for sustaining growth and enhancing stress tolerance. By bolstering root systems, plants are better equipped to withstand adverse environmental conditions, ultimately contributing to improved crop resilience and productivity (Khalid et al., 2023).

Optimizing the concentration and formulation of nanoparticle biostimulants is critical for maximizing their beneficial effects on both above-ground and below-ground plant traits. The current findings highlight the need for further research to fine-tune these parameters, aiming to unlock the full potential of nanoparticle biostimulants in improving crop performance under salinity stress (Zarzecka et al., 2022). The study's results provide a foundation for future investigations into the optimal use of biostimulants in enhancing plant resilience and productivity in challenging environments.

In addition to morphological assessments, this study delved into the molecular mechanisms underlying the observed stress responses by examining gene expression patterns in potato cultivars under salinity stress. The upregulation of the *MYB1* gene in 'Spunta' under salinity stress indicates an active defense response, which was significantly mitigated by nanoparticle biostimulant applications. This modulation of *MYB1* expression demonstrates the effectiveness of biostimulants in attenuating stress responses, likely by enhancing the plant's intrinsic defense mechanisms. Similarly, the regulation of the *P5CS* gene, associated with osmotic stress tolerance, revealed that nanoparticle treatments reduced the gene's expression in 'Spunta', suggesting improved osmotic stress management through biostimulant intervention. The altered expression of the *P450* gene further underscores the role of biostimulants in modulating stress-responsive pathways, highlighting their impact on overall plant stress physiology (Zuzunaga-Rosas et al., 2022; Zuzunaga-Rosas, 2024).

The study also highlighted the modulation of the *SOS1* gene, a crucial player in salt tolerance, under salinity stress. The upregulation of *SOS1* in response to salinity stress was markedly reduced by nanoparticle treatments, indicating that biostimulants can enhance ion homeostasis and salt tolerance mechanisms. This finding is particularly important as it demonstrates how biostimulants can modulate critical molecular pathways to improve crop resilience in salt-affected soils. The gene expression analysis conducted in this study offers valuable insights into the molecular underpinnings of biostimulant action, providing a mechanistic understanding of how these treatments enhance plant stress tolerance and resilience (Rajput et al., 2021).

The broader implications of this research are supported by parallel studies on the use of biostimulants in mitigating abiotic stress in plants. For instance, research on biostimulants based on polyphenols and glycine betaine has shown similar benefits in improving salt tolerance and overall plant performance under high salinity conditions (Zuzunaga-Rosas et al., 2022). Moreover, microbial biostimulants have also been demonstrated to enhance plant responses to salt stress (Kałużewicz et al., 2017). Additionally, studies on the physiological impacts of biostimulants, such as improved gas exchange and transpiration rates under drought stress, highlight the multifaceted benefits of these treatments in enhancing plant resilience to various abiotic stressors (Jesus et al., 2023). Previous research on a nanoparticle mixture containing salicylic acid, ascorbic acid, and benzoic acid shows promise in enhancing drought tolerance in potatoes (Alowaiesh et al., 2024).

CONCLUSIONS

In conclusion, the research on nanoparticle biostimulants and their impact on potato cultivars under salinity stress reveals significant potential for these treatments in improving plant growth and development. The study underscores the importance of understanding genotype-specific responses, optimizing treatment concentrations, and exploring the synergistic effects of biostimulants to enhance crop resilience and productivity. The interaction between nanoparticle biostimulants and gene expression in potato cultivars under salinity stress uncovers a complex network of molecular responses that contribute to plant adaptation and resilience. By elucidating these genetic mechanisms, researchers can tailor interventions to enhance stress tolerance in crops, paving the way for more sustainable and productive agricultural practices in the face of environmental challenges. Continued research in this area is essential for fully harnessing the potential of biostimulants in enhancing crop performance under adverse conditions.

Author contribution

Conceptualization: A.S.A., M.M. Methodology: A.S.A., N.M.A., D.A.E.M. Software: M.M., A.B.E.M., D.A.E.M. Validation: N.M.A., D.A.E.M. Formal analysis: A.S.A., N.S.A., M.E.E., R.A., M.A., A.O.A., N.M.A. Investigation: M.M., A.B.E.M., N.S.A., M.E.E., R.A., D.A.E.M. Resources: A.S.A., N.S.A., M.E.E., M.A., A.O.A., D.A.E.M. Data curation: A.S.A., M.M., A.B.E.M., N.S.A., M.E.E., R.A., M.A. Writing-original draft: A.S.A., M.M., A.B.E.M., N.S.A., M.E.E., R.A., M.A., A.O.A., N.M.A., D.A.E.M. Writing-review & editing: A.S.A., M.M., A.B.E.M., N.S.A., M.E.E., R.A., M.A., A.O.A., N.M.A., D.A.E.M.

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