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RESEARCH ARTICLE

Enhancement of drought tolerance in potato employing nanoparticles of different biostimulants

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ABSTRACT

Improving drought tolerance of potato (*Solanum tuberosum* L.) is integral, particularly under current climate fluctuations. The objective of this study was to assess the impact of different concentrations of nanoparticles of ascorbic acid (AS), benzoic acid (BA), and salicylic acid (SA) individually and in combined treatments on potato under drought-induced stress. The assessed biostimulants with different concentrations (0.50 and 0.75 mM) were applied to two potato cultivars (Spunta and Lady-Rosetta). Nodal cutting of each cultivar was exposed to drought stress via 30% polyethylene glycol (PEG) in MS media. Five growth characters (plantlet length, number of leaves plant^{-1}, number of roots, number of lateral branches plantlet^{-1}, and root length) were measured after 7, 14, and 21 d. The results indicated that the evaluated cultivars exhibited highly significant differences (< 0.001) in all characters. Moreover, all evaluated nano biostimulants recorded highly significant differences (< 0.001) compared to the untreated control. The co-application of two nano biostimulants was stronger than the sole use of one material. The co-application of AS+BA was the most effective, and its impact was more considerable compared to the other treatments. The assessed cultivars displayed significant interaction with the application of nano biostimulants. 'Lady-Rosetta' responded more to the applied nano biostimulants in all studied characters. In conclusion, applying AS, SA, and BA improved the growth of 'Lady-Rosetta' under drought stress conditions. Furthermore, the combined treatment AS+BA is more powerful in modulating drought stress's adverse impacts on potato plants.

Key words: Drought-induced stress, heatmap, and hierarchical clustering, polyethylene glycol, principal component analysis, *Solanum tuberosum*.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important food crops worldwide (Gikundi et al., 2023). It ranks as the first non-cereal highest-produced food crop (FAOSTAT, 2023). Global potato production in 2021 was 376.12 million tons, produced from 181.32 million hectares. However, the yield average was down 6% from the previous year (FAOSTAT, 2023). Potato production is vulnerable due to environmental stresses, which are aggravated by current climate fluctuations. Potato is severely sensitive to drought stress (Wishart et al., 2014). Most commercial potato cultivars are sensitive to drought stress, and substantial improvement in their tolerance level is difficult owing to the narrow genetic base (Sallam et al., 2022). Accordingly, finding innovative approaches to enhance tolerance to abiotic stresses to guarantee competitive production under suboptimal environments is decisive.

Climate change is an unavoidable, inevitable phenomenon worldwide that impacts all aspects of life, including food security (Balasundram et al., 2023). Climate change causes long-term heating, severe drought, particularly in arid regions, increased salinity, and unpredictably heavy rainfall that devastatingly impacts potato production (Nasir and Toth, 2022). Water deficit is one of the most threatening environmental stresses that restrict plant development and growth, causing considerable losses in crop productivity (Essa et al., 2023). It causes detrimental impacts on biochemical, molecular, physiological, and morphological aspects of the plant that deleteriously impact crop productivity and quality (ALshamrani et al., 2022). Numerous biochemical, molecular, physiological, and morphological processes in the plants are extremely impaired under drought stress due to oxidative stress, ion toxicity, and nutrient deficiency, resulting in poor plant performance (Hill et al., 2021). Accordingly, water shortage may lead to a dangerous food lack that will worsen due to climate change and the growing global population (Kah et al., 2019). Therefore, there is a dire need to enhance drought tolerance in plants using efficient, economical, and safe approaches.

Nanotechnology provides great applicable strategies in environmental research, food industry, and agriculture (Kandhol et al., 2022). Nanoparticles (NPs) are characterized by powerful interaction with plants due to nanoscale size, physicochemical structural properties, and elevated ratio of surface to volume (Kavitha et al., 2023). Numerous products, including NPs are widely utilized in the agricultural sector, such as nano fertilizers, nano pesticides, and nano-sensors (Alabdallah and Hasan, 2021). The application of NPs is highly effective in enhancing plant tolerance against various environmental stresses (Linh et al., 2020). The positive impact of NPs was previously demonstrated in ameliorating plant tolerance and adaptation under several environmental stresses, including water scarcity, through various biochemical and physiological processes (Desoky et al., 2021a; Kandhol et al., 2022). Thus, the objective of the present study was to explore the impact of nanoparticles of salicylic acid (SA), ascorbic acid (AS), and benzoic acid (BA), in attenuating adverse effects of drought stress in potato.

MATERIALS AND METHODS

Synthesis of nanoparticles for applied biostimulants

Synthesis of nanoparticles for the applied biostimulants was performed utilizing ball mill method. In a typical synthesis, 1 g dry ascorbic acid (AS), 1.1 g dry benzoic acid (BA), or 0.8 g dry salicylic acid (SA) were put in the stainless-steel gear of the ball mill machine (MM 500 nano, Retsch GmbH, Haan, Germany). Then, 4 mL deionized water was added to wet powder for AS while 2 mL for BA or 2.5 mL for SA to void oxidation or decomposition results from milling heat. Wet biostimulants were milled using several steps with different ball diameters and times of milling with a constant mass of powder to a mass of balls 1:6 for AS, 1:4 for BA, or 1:7 for SA. To obtain AS nanoparticles, in the first step, a ceramic ball of 0.4 cm diameter was used for 1 h, then 0.05 cm for 3 h, and finally 0.04 cm for 3 h. Moreover, to obtain BA nanoparticles in the first step, a ceramic ball of 0.2 cm diameter was used for 3 h, then 0.02 cm for 1.5 h, and finally 0.01 cm for 3 h. Besides, to obtain SA nanoparticles in the first step, a stainless-steel ball with a diameter of 0.25 cm was used for 4 h, then 0.03cm for 2 h, and finally 0.01 cm for 2 h.

Nanoparticle characterization

The texture and shape of nanoparticles of used biostimulants were scanned employing a high-resolution transmission electron microscope (HRTEM-JEOL-TEM-2100-Japan Akishima, Tokyo, Japan) with a 20X magnification and 250 kV accelerating voltage. Fifty microns were added to scanning electron microscopy (SEM) grade with air drying for 5 h. The angular intensity distribution was utilized to detect the size of particles using Stokes-Einstein equation. Dynamic light scattering (DLS) was performed using a particle size analyzer (NanoSight-NS500; Malvern Panalytical, Malvern, Worcestershire, UK). Zeta potential was also performed using zeta sizer analyzer (NanoSight-NS500).

Micropropagation and salinity drought

Plantlets of two potato (*Solanum tuberosum* L.) cultivars (Spunta and Lady-Rosetta) were utilized in the present study. The explants were sterilized using 70% ethanol for 1 min then sodium hypochlorite (2%) for 10 min, and washed thrice using sterile distilled water. The prepared explants were cultured on semi-solid media, including 30 g sucrose and 8 g agar (pH 5.8). Drought stress was applied by 30% polyethylene glycol (PEG). The PEG nutrient solution was prepared utilizing PEG 8000 (C000Q01EA, AMRESCO, Solon, Ohio, USA). Treatments with nanoparticles of SA, BA, and AS were carried out using two concentrations of 0.5 and 0.75 mM separately or in combination with each other, as illustrated in Table 1. Five quantitative characters were measured after 7, 14, and 21 d. The measured characters were number of leaves per plant, plantlets length (cm), number of lateral branches per plantlets, number of roots, and root length (cm).

Table 1. The applied nanoparticles of different biostimulants salicylic acid (SA), benzoic acid (BA), and ascorbic acid (AS) at two concentrations (0.50 and 0.75 mM) under drought stress (30% polyethylene glycol).

Nr	Nano treatment	Code
1	Unstressed untreated control	Unstressed untreated
2	Salicylic acid (SA) at 0.50 mM	SA_0.50
3	Benzoic acid (BA) at 0.50 mM	BA_0.50
4	Ascorbic acid (AS) at 0.50 mM	AS_0.50
5	SA at 0.50 mM + BA at 0.50 mM	SA_0.50+BA_0.50
6	SA at 0.50 mM + AS at 0.50 mM	SA_0.50+AS_0.50
7	BA at 0.50 mM + AS at 0.50 mM	BA_0.50+AS_0.50
8	SA at 0.50 mM + BA at 0.50 mM + AS at 0.50 mM	SA_0.50+BA_0.50+AS_0.50
9	SA at 0.75 mM	SA_0.75
10	BA at 0.75 mM	BA_0.75
11	AS at 0.75 mM	AS_0.75
12	SA at (0.75 mM + BA at 0.75 mM	SA_0.75+BA_0.75
13	SA at 0.75 mM + AS at 0.75 mM	SA_0.75+AS_0.75
14	BA at 0.75 mM + AS at 0.75 mM	BA_0.75+AS_0.75
15	SA at 0.75 mM + BA at 0.75 mM + AS at 0.75 mM	SA_0.75+BA_0.75+AS_0.75

Statistical analysis

The obtained data were analyzed using a completely randomized design with factorial arrangements (three factors) in three replicates. The least significant differences (LSD) test was employed to compare means at the statistical probability of 0.01. Principal component analysis, heatmap, and hierarchical clustering were performed using R statistical software version 4.1.1.

RESULTS

Nanoparticle characterization

The nanoparticle size of the three applied biostimulants SA, BA, and AS was determined employing transmission electron microscopy (TEM) as presented in Figures 1A, 1D, and 1G, respectively. In addition, the surface charge was explored employing zeta potential, as shown in Figures 1B, 1E, and 1H for SA, BA, and AS in the same order. Moreover, the size distribution of nanoparticles was detected by dynamic light scattering (DLS) as displayed in Figures 1C, 1F, and 1I for SA, BA, and AS, respectively.

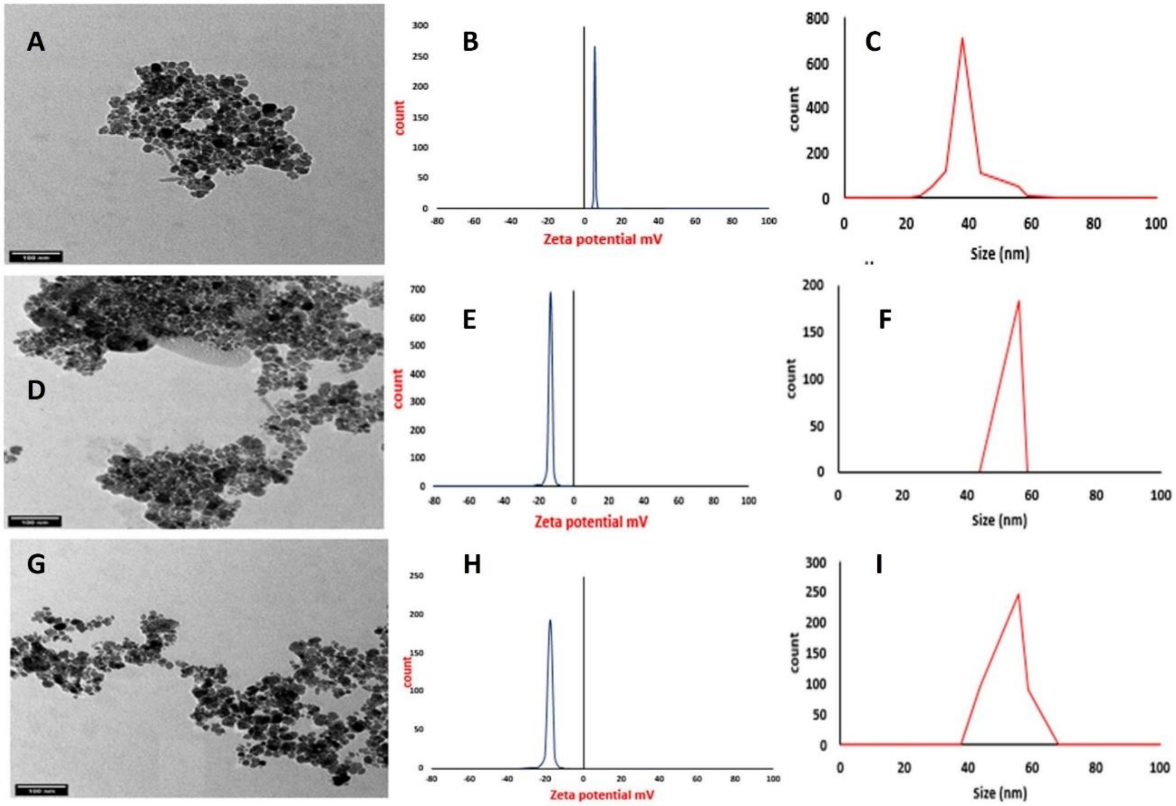


Figure 1. Transmission electron microscopy (TEM) image, zeta potential, and hydrodynamic size of salicylic acid (A, B, and C respectively), benzoic acid (D, E, and F respectively) and ascorbic acid nanoparticles (G, H, and I respectively).

Genotypic differences and applied nano-treatments and their interactions

Statistically high significant differences (< 0.001) were observed among the evaluated cultivars, biostimulant nanoparticle treatments, time of measurement, and their interaction in all studied characters (Table 2).

The two assessed potato cultivars (Spunta and Lady-Rosetta) exhibited highly significant differences in all studied characters (Table 2). 'Lady-Rosetta' recorded the highest performance of all characters. Moreover, the evaluated three biostimulants in two concentrations displayed a highly significant impact on all characters.

The co-application of BA+AS at concentrations of 0.50 mM possessed superior performance for most studied characters following the unstressed plants. Furthermore, sole use of AS at 0.75 and 0.50 mM, 0.75 and 0.50 mM, and co-application of BA+AS at 0.75 mM recorded high performance for most evaluated characters. The measurement time showed significant differences with significant gradient increments in all studied characters over time 7, 14, and 21 d.

Table 2. Influence of sole use or co-application of nanoparticle salicylic acid (SA), ascorbic acid (AS), benzoic acid (BA), at two concentrations (0.50 and 0.75 mM) on growth characters of two potato cultivars (Spunta and Lady-Rosetta) at three times 7, 14 and 21 d after treatment under drought stress (30% polyethylene glycol). The averages represent the main effect of studied factors; potato cultivars and nano-applications. Means followed by different letters within the same factor differ significantly by least significant differences (LSD) test at $P \leq 0.01$.

Studied factor		Leaves per plant	Plantlets length	Lateral branches per plantlets	Number of roots	Root length
		nr	cm	nr	nr	cm
Cultivar						
Spunta		7.904^{b}	6.823^{b}	0.970^{b}	1.445^{b}	5.408^{b}
Lady-Rosetta		9.253^{a}	7.088^{a}	1.620^{a}	1.756^{a}	5.862^{a}
Nano-application						
Unstressed untreated		10.722^{a}	8.177^{b}	1.915^{c}	2.587^{b}	7.965^{a}
SA_0.50		7.755^{i}	6.685^{g}	1.342^{f}	1.965^{d}	5.083^{j}
BA_0.50		8.555^{g}	6.515^{h}	0.618^{g}	1.397^{h}	6.007^{f}
AS_0.50		7.793^{i}	7.092^{e}	2.297^{a}	3.277^{a}	5.982^{f}
SA_0.50+BA_0.50		9.512^{d}	8.233^{ab}	0.632^{g}	1.140^{i}	6.205^{e}
SA_0.50+AS_0.50		7.005^{j}	6.885^{f}	1.657^{de}	1.477^{gh}	4.887^{k}
BA_0.50+AS_0.50		8.910^{e}	8.290^{a}	1.303^{f}	1.790^{e}	6.927^{b}
SA_0.50+BA_0.50+AS_0.50		6.987^{j}	6.202^{i}	0.578^{g}	0.970^{j}	4.678^{l}
SA_0.75		9.422^{d}	7.412^{c}	1.947^{c}	1.630^{f}	5.457^{h}
BA_0.75		8.745^{f}	7.263^{d}	1.617^{e}	1.695^{f}	6.617^{c}
AS_0.75		10.190^{b}	7.500^{c}	2.042^{b}	2.307^{c}	5.293^{i}
SA_0.75+BA_0.75		8.292^{h}	6.160^{i}	0.598^{g}	0.638^{k}	5.685^{g}
SA_0.75+AS_0.75		8.498^{g}	6.430^{h}	0.600^{g}	0.985^{i}	4.635^{l}
BA_0.75+AS_0.75		9.937^{c}	6.722^{g}	1.717^{d}	1.512^{g}	6.347^{d}
SA_0.75+BA_0.75+AS_0.75		6.357^{k}	4.768^{j}	0.567^{g}	0.637^{k}	2.762^{m}
Days						
7		3.194^{c}	2.561^{c}	0.476^{c}	0.593^{c}	2.121^{c}
14		6.399^{b}	5.242^{b}	1.017^{b}	1.281^{b}	4.187^{b}
21		16.14^{a}	13.06^{a}	2.393^{a}	2.927^{a}	10.59^{a}
$LSD_{0.01}$						
ANOVA	df			*P*-value		
Cultivar (C)	1	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Application (P)	14	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Time (T)	2	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
C×P	14	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
C×T	2	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
P×T	28	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
C×P×T	28	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

The interaction between potato cultivars and applied nano-treatments was highly significant for all evaluated characters (Table 2). The applied nano-treatments significantly impacted the number of leaves per plant (Table 3). The most potent treatment was the co-application of BA+AS at both concentrations, recording the highest values, particularly with 'Lady-Rosetta' (Table 3). The uppermost number of leaves per plant in 'Spunta' was assigned for SA+BA using the concentration of 50 mM and the sole use of AS at 0.75 mM (Table 3). The interaction effect of nano-treatment and cultivars on plantlet length under drought conditions is illustrated in Table 4. The results indicated that 'Lady-Rosetta' was the most responding cultivar in both concentrations. The superior plantlet length was produced by 'Lady-Rosetta' utilizing BA+AS at both concentrations. Meanwhile, in 'Spunta', the highest value was recorded for SA+BA at 0.50 mM. Otherwise, the co-application SA+AS at 75 mM exhibited the highest value in 'Spunta'.

Table 3. Impact of sole use or co-application of nanoparticle salicylic acid (SA), ascorbic acid (AS), benzoic acid (BA), at two concentrations (0.50 and 0.75 mM) on number of leaves per plant of two potato cultivars (Spunta and Lady-Rosetta) at three times 7, 14 and 21 d after treatment under drought stress (30% polyethylene glycol). C: Potato cultivars; P: nano-applications; T: time of measurement. LSD C×P×T $_{0.01}$: least significant difference (LSD) value to detect significant differences among the averages of the interaction among studied three factors; potato cultivars, nano-applications, and time of measurement at $P \leq 0.01$.

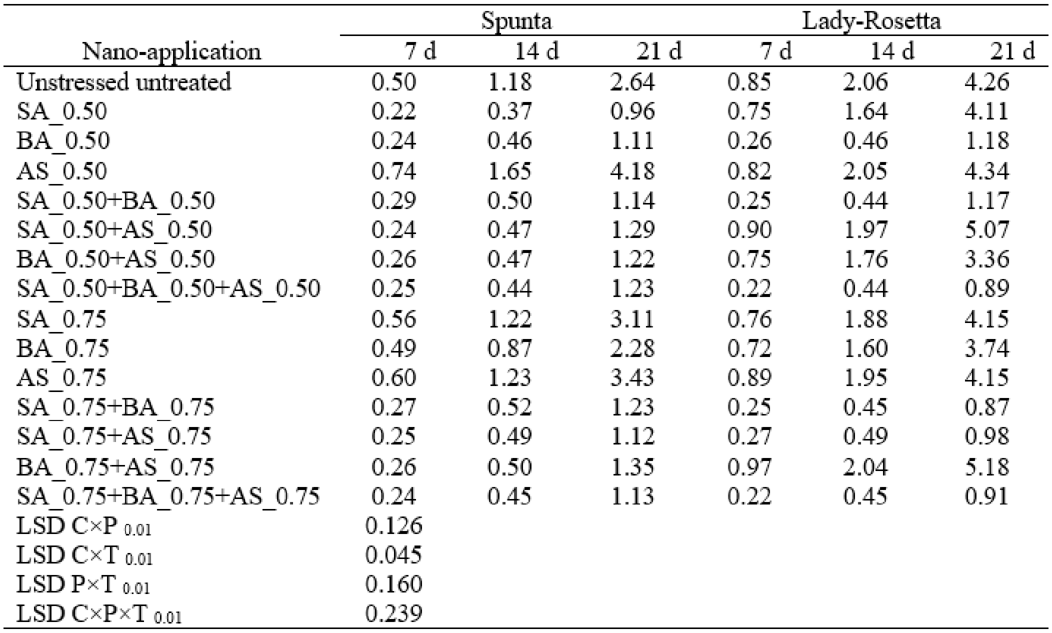
	Spunta			Lady-Rosetta		
Nano-application	7 d	14 d	21 d	7 d	14 d	21 d
Unstressed untreated	3.260	7.330	18.230	4.420	8.820	22.270
SA_0.50	2.240	4.710	12.110	3.430	6.600	17.440
BA_0.50	2.950	5.650	15.190	3.330	6.650	17.560
AS_0.50	3.010	6.050	15.110	2.750	5.550	14.290
SA_0.50+BA_0.50	3.660	7.160	16.640	4.000	7.370	18.240
SA_0.50+AS_0.50	2.750	5.630	13.160	2.550	4.950	12.990
BA_0.50+AS_0.50	2.940	5.160	13.370	4.020	8.140	19.830
SA_0.50+BA_0.50+AS_0.50	2.780	5.590	14.470	2.350	4.340	12.390
SA_0.75	3.020	6.580	15.800	3.860	7.520	19.750
BA_0.75	2.460	4.810	12.350	4.150	8.360	20.340
AS_0.75	3.340	6.580	16.740	4.460	8.750	21.270
SA_0.75+BA_0.75	3.100	6.450	16.530	2.960	5.940	14.770
SA_0.75+AS_0.75	3.040	6.360	15.630	3.240	6.330	16.390
BA_0.75+AS_0.75	3.030	6.240	15.140	4.350	8.820	22.040
SA_0.75+BA_0.75+AS_0.75	2.320	4.760	12.270	2.060	4.770	11.960
LSD C×P $_{0.01}$	0.182					
LSD C×T $_{0.01}$	0.065					
LSD P×T $_{0.01}$	0.231					
LSD C×P×T $_{0.01}$	0.347					

Table 4. Impact of sole use or co-application of nanoparticle salicylic acid (SA), ascorbic acid (AS), benzoic acid (BA), at two concentrations (0.50 and 0.75 mM) on plantlets length of two potato cultivars (Spunta and Lady-Rosetta) at three times 7, 14 and 21 d after treatment under drought stress (30% polyethylene glycol). C: Potato cultivars; P: nano-applications; T: time of measurement. LSD C×P×T $_{0.01}$: least significant difference (LSD) value to detect significant differences among the averages of the interaction among studied three factors; potato cultivars, nano-applications, and time of measurement at $P \leq 0.01$.

	Spunta			Lady-Rosetta		
Nano-application	7 d	14 d	21 d	7 d	14 d	21 d
Unstressed untreated	2.740	5.730	13.650	3.540	7.210	16.190
SA_0.50	2.140	4.050	10.200	2.860	5.970	14.890
BA_0.50	2.340	4.970	12.080	2.410	4.890	12.400
AS_0.50	3.040	6.050	15.270	2.330	4.420	11.440
SA_0.50+BA_0.50	3.120	6.560	15.760	2.960	5.950	15.050
SA_0.50+AS_0.50	2.820	5.900	14.820	2.140	4.410	11.220
BA_0.50+AS_0.50	2.940	5.940	15.040	3.240	6.340	16.240
SA_0.50+BA_0.50+AS_0.50	2.540	4.980	12.250	2.040	4.340	11.060
SA_0.75	2.740	5.610	13.440	2.740	5.820	14.120
BA_0.75	2.020	4.350	11.280	3.110	6.460	16.360
AS_0.75	2.410	4.810	12.170	3.060	6.340	16.210
SA_0.75+BA_0.75	2.290	4.840	12.550	2.050	4.100	11.130
SA_0.75+AS_0.75	2.960	5.980	15.010	1.750	3.650	9.230
BA_0.75+AS_0.75	2.000	3.960	9.460	3.090	6.360	15.460
SA_0.75+BA_0.75+AS_0.75	1.550	3.820	8.860	1.860	3.460	9.060
LSD C×P $_{0.01}$	0.163					
LSD C×T $_{0.01}$	0.059					
LSD P×T $_{0.01}$	0.207					
LSD C×P×T $_{0.01}$	0.310					

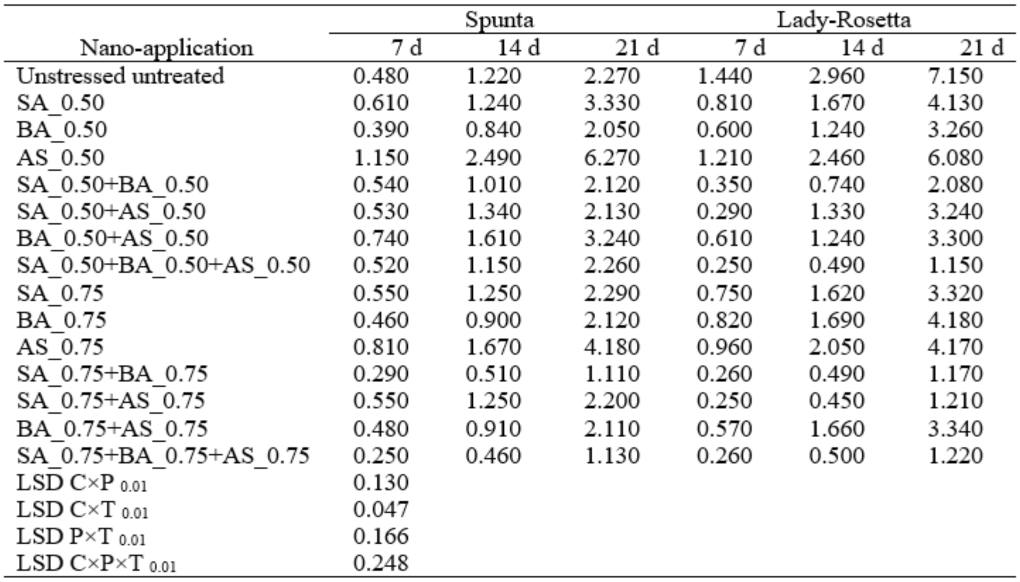
Furthermore, 'Lady-Rosetta' was the most affected cultivar by utilized treatments and concentrations and exhibited the highest number of lateral branches per plantlet (Table 5). The co-application SA+AS at 0.50 mM recorded the highest number of lateral branches and the co-application BA+AS at 0.75 mM (Table 5). The interaction between studied cultivars and nano-treatments significantly affected the number of roots. The application of AS at both applied concentrations clearly recorded great rooting performance in both cultivars over measurement time (Table 6). Furthermore, the sole application of SA or BA at both concentrations recorded a good number of roots compared to the other treatments in both cultivars over measurement times. The applied nano-treatments in both concentrations substantially impacted root length in both evaluated cultivars at different measurement times (Table 7). The co-application BA+AS with both concentrations recorded the greatest root length in both cultivars. However, the impact of co-application BA+AS was superior in 'Lady-Rosetta'. Furthermore, the application of AS at 0.75 mM showed a high root length in 'Lady-Rosetta'. Otherwise, 'Spunta' displayed high values with the co-application BA+AS at 0.75 mM (Table 7).

Table 5. Impact of sole use or co-application of nanoparticle salicylic acid (SA), ascorbic acid (AS), benzoic acid (BA), at two concentrations (0.50 and 0.75 mM) on number of lateral branches per plantlets of two potato cultivars (Spunta and Lady-Rosetta) at three times 7, 14 and 21 d after treatment under drought stress (30% polyethylene glycol). C: Potato cultivars; P: nano-applications; T: time of measurement. LSD C×P×T $_{0.01}$: least significant difference (LSD) value to detect significant differences among the averages of the interaction among studied three factors; potato cultivars, nano-applications, and time of measurement at $P \leq 0.01$.



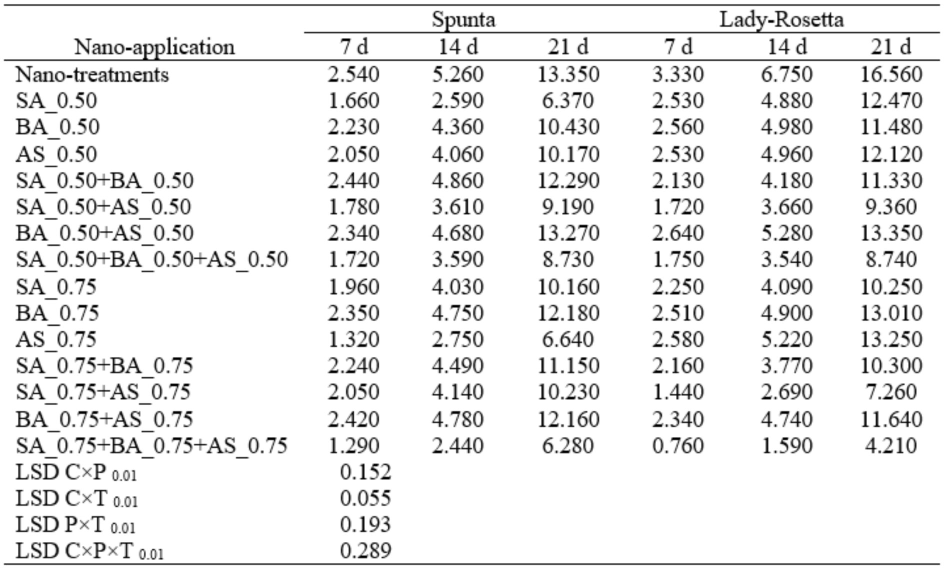
	Spunta			Lady-Rosetta		
Nano-application	7 d	14 d	21 d	7 d	14 d	21 d
Unstressed untreated	0.50	1.18	2.64	0.85	2.06	4.26
SA_0.50	0.22	0.37	0.96	0.75	1.64	4.11
BA_0.50	0.24	0.46	1.11	0.26	0.46	1.18
AS_0.50	0.74	1.65	4.18	0.82	2.05	4.34
SA_0.50+BA_0.50	0.29	0.50	1.14	0.25	0.44	1.17
SA_0.50+AS_0.50	0.24	0.47	1.29	0.90	1.97	5.07
BA_0.50+AS_0.50	0.26	0.47	1.22	0.75	1.76	3.36
SA_0.50+BA_0.50+AS_0.50	0.25	0.44	1.23	0.22	0.44	0.89
SA_0.75	0.56	1.22	3.11	0.76	1.88	4.15
BA_0.75	0.49	0.87	2.28	0.72	1.60	3.74
AS_0.75	0.60	1.23	3.43	0.89	1.95	4.15
SA_0.75+BA_0.75	0.27	0.52	1.23	0.25	0.45	0.87
SA_0.75+AS_0.75	0.25	0.49	1.12	0.27	0.49	0.98
BA_0.75+AS_0.75	0.26	0.50	1.35	0.97	2.04	5.18
SA_0.75+BA_0.75+AS_0.75	0.24	0.45	1.13	0.22	0.45	0.91
LSD C×P $_{0.01}$	0.126					
LSD C×T $_{0.01}$	0.045					
LSD P×T $_{0.01}$	0.160					
LSD C×P×T $_{0.01}$	0.239					

Table 6. Impact of sole use or co-application of nanoparticle salicylic acid (SA), ascorbic acid (AS), benzoic acid (BA), at two concentrations (0.50 and 0.75 mM) on number of roots of two potato cultivars (Spunta and Lady-Rosetta) at three times 7, 14 and 21 d after treatment under drought stress (30% polyethylene glycol). C: Potato cultivars; P: nano-applications; T: time of measurement. LSD C×P×T $_{0.01}$: least significant difference (LSD) value to detect significant differences among the averages of the interaction among studied three factors; potato cultivars, nano-applications, and time of measurement at $P \leq 0.01$.



Nano-application	Spunta			Lady-Rosetta		
	7 d	14 d	21 d	7 d	14 d	21 d
Unstressed untreated	0.480	1.220	2.270	1.440	2.960	7.150
SA_0.50	0.610	1.240	3.330	0.810	1.670	4.130
BA_0.50	0.390	0.840	2.050	0.600	1.240	3.260
AS_0.50	1.150	2.490	6.270	1.210	2.460	6.080
SA_0.50+BA_0.50	0.540	1.010	2.120	0.350	0.740	2.080
SA_0.50+AS_0.50	0.530	1.340	2.130	0.290	1.330	3.240
BA_0.50+AS_0.50	0.740	1.610	3.240	0.610	1.240	3.300
SA_0.50+BA_0.50+AS_0.50	0.520	1.150	2.260	0.250	0.490	1.150
SA_0.75	0.550	1.250	2.290	0.750	1.620	3.320
BA_0.75	0.460	0.900	2.120	0.820	1.690	4.180
AS_0.75	0.810	1.670	4.180	0.960	2.050	4.170
SA_0.75+BA_0.75	0.290	0.510	1.110	0.260	0.490	1.170
SA_0.75+AS_0.75	0.550	1.250	2.200	0.250	0.450	1.210
BA_0.75+AS_0.75	0.480	0.910	2.110	0.570	1.660	3.340
SA_0.75+BA_0.75+AS_0.75	0.250	0.460	1.130	0.260	0.500	1.220
LSD C×P $_{0.01}$	0.130					
LSD C×T $_{0.01}$	0.047					
LSD P×T $_{0.01}$	0.166					
LSD C×P×T $_{0.01}$	0.248					

Table 7. Impact of sole use or co-application of nanoparticle salicylic acid (SA), ascorbic acid (AS), benzoic acid (BA), at two concentrations (0.50 and 0.75 mM) on root length (cm) of two potato cultivars (Spunta and Lady-Rosetta) at three times 7, 14 and 21 d after treatment under drought stress (30% polyethylene glycol). C: Potato cultivars; P: nano-applications; T: time of measurement. LSD C×P×T $_{0.01}$: least significant difference (LSD) value to detect significant differences among the averages of the interaction among studied three factors; potato cultivars, nano-applications, and time of measurement at $P \leq 0.01$.



Nano-application	Spunta			Lady-Rosetta		
	7 d	14 d	21 d	7 d	14 d	21 d
Nano-treatments	2.540	5.260	13.350	3.330	6.750	16.560
SA_0.50	1.660	2.590	6.370	2.530	4.880	12.470
BA_0.50	2.230	4.360	10.430	2.560	4.980	11.480
AS_0.50	2.050	4.060	10.170	2.530	4.960	12.120
SA_0.50+BA_0.50	2.440	4.860	12.290	2.130	4.180	11.330
SA_0.50+AS_0.50	1.780	3.610	9.190	1.720	3.660	9.360
BA_0.50+AS_0.50	2.340	4.680	13.270	2.640	5.280	13.350
SA_0.50+BA_0.50+AS_0.50	1.720	3.590	8.730	1.750	3.540	8.740
SA_0.75	1.960	4.030	10.160	2.250	4.090	10.250
BA_0.75	2.350	4.750	12.180	2.510	4.900	13.010
AS_0.75	1.320	2.750	6.640	2.580	5.220	13.250
SA_0.75+BA_0.75	2.240	4.490	11.150	2.160	3.770	10.300
SA_0.75+AS_0.75	2.050	4.140	10.230	1.440	2.690	7.260
BA_0.75+AS_0.75	2.420	4.780	12.160	2.340	4.740	11.640
SA_0.75+BA_0.75+AS_0.75	1.290	2.440	6.280	0.760	1.590	4.210
LSD C×P $_{0.01}$	0.152					
LSD C×T $_{0.01}$	0.055					
LSD P×T $_{0.01}$	0.193					
LSD C×P×T $_{0.01}$	0.289					

Interrelationship among evaluated treatments and studied characters.

The principal component was analyzed to explore the association among the applied nano-treatments in different concentrations and studied characters in ‘Spunta’ (Figure 2A) and ‘Lady-Rosetta’ potatoes (Figure 2B). The first two principal components presented 84.20% (55.21 + 28.99) of the variability in ‘Spunta’ and 89.27% (74.98 + 14.29) in ‘Lady-Rosetta’. The first principal component displayed 55.21% and 74.98% of the variation in ‘Spunta’ and ‘Lady-Rosetta’, respectively, and was associated with the impact of evaluated nano-treatments on studied characters. The first principal component divided the evaluated nano-treatments based on their impacts on growth characters. The nano-treatments with high performance were on the positive side, while the lowest performance was on the negative side (Figures 2A and 2B). Unstressed control, AS at both concentrations, SA at both concentrations, the co-applications BA+AS at both concentrations were situated on the positive side of the first principal component and positively related with studied growth characters in both cultivars (Figures 2A and 2B). Moreover, the heatmap and hierarchical clustering analysis based on the studied growth characters distinguished the evaluated nano-applications into different clusters in assessed ‘Spunta’ (Figure 3A) and ‘Lady-Rosetta’ potatoes (Figure 3B). The application of AS at both concentrations, SA at both concentrations and the co-applications BA+AS at both concentrations exhibited superior values for studied growth characters.

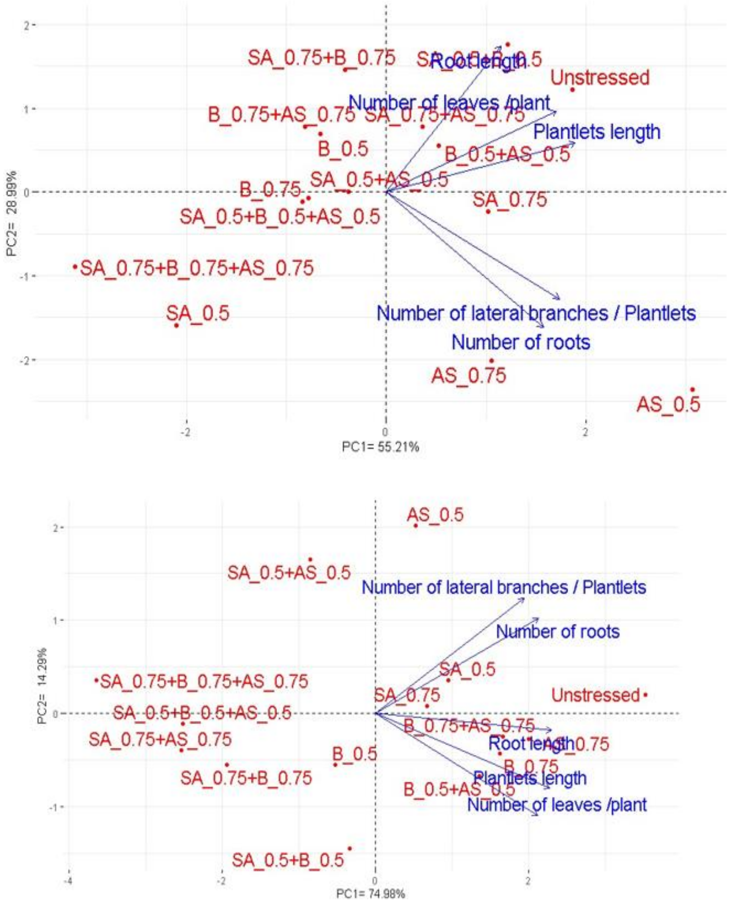


Figure 2. Principal components biplot for the evaluated nano-treatments in ‘Spunta’ (A) and ‘Lady-Rosetta’ potatoes (B) based on studied growth characters. SA_0.5: Salicylic acid (SA) in 0.5 mM; BA_0.5: benzoic acid (BA) in 0.5 mM; AS_0.5: ascorbic acid (AS) in 0.5 mM; SA_0.75: SA in 0.75 mM; BA_0.75: BA in 0.75 mM; AS_0.75: AS in 0.75 mM; Unstressed: unstressed untreated control.

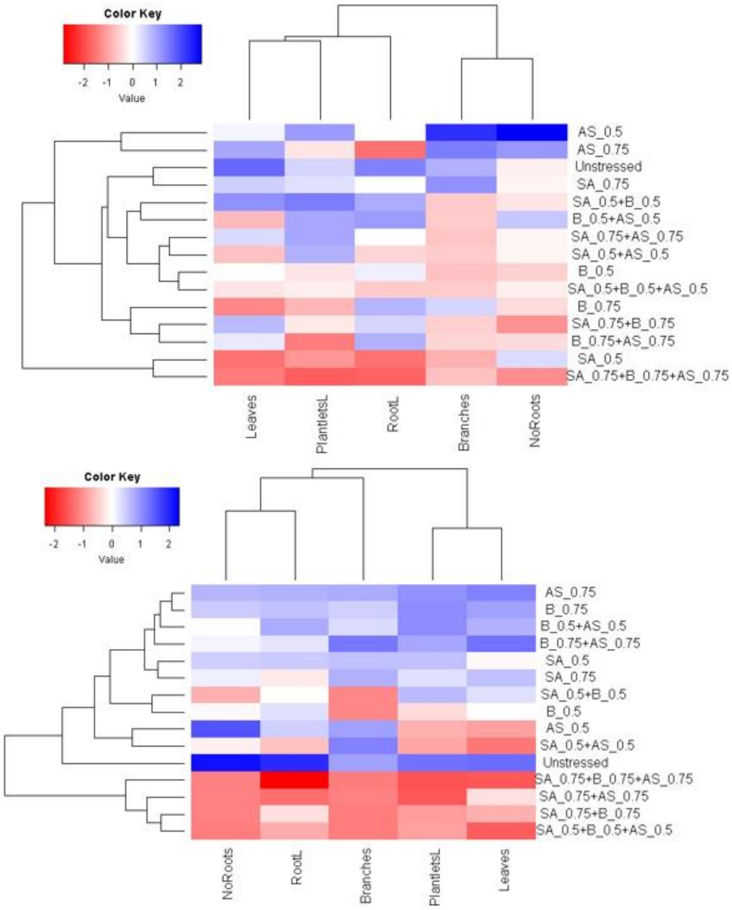


Figure 3. Heatmap and hierarchical clustering for the evaluated nano-treatments in ‘Spunta’ (A) and ‘Lady-Rosetta’ potatoes (B) based on studied growth characters. Red and blue colors indicate high and low values for the corresponding trait, respectively. SA_0.5: Salicylic acid (SA) in 0.5 mM; BA_0.5: benzoic acid (BA) in 0.5 mM; AS_0.5: ascorbic acid (AS) in 0.5 mM; SA_0.75: SA in 0.75 mM; BA_0.75: BA in 0.75 mM; AS_0.75: AS in 0.75 mM; Unstressed: unstressed untreated control; NoRoots: number of roots; RootL: root length; Leaves: number of leaves per plant; PlantletsL: plantlets length; Branches: number of lateral branches per plantlets.

DISCUSSION

Drought stress has a devastating impact on agricultural production, and its frequency and severity are predicted to increase due to global climate fluctuations (Bibi and Rahman, 2023). In the present study, results indicated that drought stress significantly disrupted the potato-studied growth parameters. These decreases might be attributed to the overproduction of reactive oxygen species which caused oxidative damage to the membranes lipids and elevated the content of malondialdehyde (Abd El Moneim 2020). Accordingly, it is decisive to enhance tolerance to drought stress of different crops to ensure sustainable agriculture and food security (Safhi et al., 2022). In this regard, Desoky et al. (2021b) treated potato plants externally with stimulating adjuvants such as clove fruit extract (CFE) and/or salicylic acid (SA) to enable them to overcome the water deficit stress.

The present study assessed the impact of nanomaterials of ascorbic acid (AS), SA, and benzoic acid (BA) for ameliorating the tolerance of potato plants to water shortage. The effect of applied nanomaterials was assessed either solely or in combination on two potato cultivars. The results showed that all nano treatments substantially impacted all studied growth characters. The co-application of assessed nanomaterials in combinations was stronger than the sole use of only one material. Particularly, the co-application of AS+BA and SA+BA was the most effective, and its results were more significant compared to other treatments. These effects can be ascribed to the positive consequence of AS, SA, and BA on plants' biochemical and physiological processes, particularly under abiotic stresses (Zhou et al., 2016). The plants have an advanced defense system against abiotic stresses that contains enzymatic antioxidants such as catalase, peroxidase, and superoxide dismutase as well as nonenzymatic antioxidant compounds such as SA, AS, tocopherols, glutathione, for eliminating reactive oxygen species (Mohammadi et al., 2020; Chen et al., 2022; Osei et al., 2022). In the same context, Seleiman et al. (2023) found that the foliar application of ZnO nanoparticles (NPs) and SiO_2-NPs positively enhanced the mineral uptake and the morphological, growth, and physiological traits of potato plants grown under water shortage conditions. However, the application of ZnO-NPs resulted in the highest values for most of the potato plant traits that were grown under different water deficit stress treatments in comparison with other treatments.

Ascorbic acid is involved in numerous biochemical and physiological processes from germination into plant senescence, such as cell division and enlargement, synthesis of ethylene, resistance against pathogens, maintaining water relations, and increasing productivity and stress tolerance (Latif et al., 2016). Ascorbic acid exists as a regular antioxidant under favorable conditions and would increase under abiotic stress conditions. It has an integral role in preserving the activity of enzymatic antioxidants such as catalase, superoxide dismutase, and peroxidase under environmental stresses (Liu et al., 2014). Furthermore, AS elevates the accumulation of osmoprotectant as proline and accordingly enhances cell membrane stability and osmotic adjustment under water shortage (Sun et al., 2013). Moreover, BA ameliorates gas exchange and tolerates different abiotic stresses (Senaratna et al., 2003). Besides, BA promotes stomatal conductance, intercellular CO_2, net photosynthetic rate, and transpiration rate under abiotic stresses (Zhu et al., 2017). These positive impacts are reflected in sustaining plant development and growth under environmental stress (Farghaly et al., 2022). Besides, SA is a nonenzymatic antioxidant compound that directly reduces the destructive reactive oxygen species and reduces damage markers' contents (Li et al., 2019). Salicylic acid substantially boosts chlorophylls, anthocyanin contents, carotenoids, upregulation of antioxidant enzymes, and regulation of nutrient uptake under abiotic stresses (Naz et al., 2021; Azeem et al., 2023). Our results in line with results of Desoky et al. (2021b), who reported that foliar spraying of SA noticeably enhanced growth parameters, yield traits, and physio-biochemical attributes of water deficit-stressed potato plants. Moreover, Ghazi (2017) showed that the exogenous of SA treatment increased growth and yield parameters under drought stress as compared with the untreated plants with SA in maize.

These alterations are accounted for as adaptabilities, which enhance the tolerance of plants against abiotic factors (Yazdanpanah et al., 2011). This confirms the considerable role of applied nanomaterials in enhancing potato tolerance to drought stress. This is in consonance with the previous results of Mahmoud et al. (2019), Awad et al. (2021), and Al-Selwey et al. (2023) manifested the positive impacts of nanomaterials on potato growth and productivity under different abiotic stresses. Furthermore, Raees et al. (2022) elucidated that seed priming using growth regulators such as ascorbic acid, tocopherol, and salicylic acid is considered a beneficial approach for ameliorating the development and growth of *Brassica napus* under water deficit and salinity stresses.

CONCLUSIONS

The assessed potato cultivars and nanobiostimulants displayed substantial variations in studied growth characteristics: Plantlet length, number of leaves per plant, number of roots, number of lateral branches per plantlet, and root length. The co-application of nano biostimulants was more effective than the sole use, particularly ascorbic acid + benzoic acid (AS+BA), which exhibited considerably enhancing growth under drought stress compared to the other treatments. The interaction showed that 'Lady-Rosetta' was more responsive to the applied nano biostimulants AS, salicylic acid (SA), and BA in all studied characters than 'Spunta' under drought stress conditions.

Author contributions

Conceptualization: B.F.A., D.A-E. Methodology: B.F.A., N.S.A., M.E.E. Software: M.E.E., D.A-E. Validation: B.F.A., N.S.A., M.E.E., D.A.-E. Formal analysis: B.F.A., M.E.E., D.A.-E. Investigation: B.F.A., N.S.A., D.A.-E. Resources: B.F.A., D.A.-E. Data curation: B.F.A., N.S.A., M.E.E., D.A.-E. Writing-original draft preparation: B.F.A., D.A-E. Visualization: B.F.A., N.S.A., M.E.E., D.A.-E. Funding acquisition: B.F.A., D.A.-E. All authors have read and agreed to the published version of the manuscript.

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