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Original Article

Silver nanoparticles enhance the mitigation of osmotic stress in *Chenopodium quinoa* microshoots grown under *in vitro* osmo-stressing conditions

Nanopartículas de prata aumentam a mitigação do estresse osmótico em microbrotos de *Chenopodium quinoa* cultivados sob condições de estresse osmótico *in vitro*

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Abstract

Osmotic stress is one of the main destructive abiotic factors that hinder plant growth and development. In this research, the role of silver nanoparticles (Ag NPs) in mitigating the negative impact of osmotic stress on *in vitro* grown *Chenopodium quinoa* (Quinoa 6 Line; Q6) was investigated to determine whether Ag NPs were able to reduce the negative effects on the *in vitro* grown cultures of the Q6 line. The explants were subcultured onto a special osmostressing media containing sucrose, sorbitol, or mannitol at different levels (0.1, 0.2, 0.3, and 0.4 mol/L) to mimic the osmotic stressing environment for four weeks. Then, stress physiological responses of *in vitro* grown Q6 under the induced osmotic stress were investigated to determine the highest stress level that the microshoots could tolerate. Next, Ag NPs; 25, 50, and 75 mg/L were added to the medium that contained the highest stress level of the induced osmotic stress to determine if their addition improved the physiological performance of the Q6 microshoots under the most severe osmotic agent levels. The results revealed that 0.4 mol/L sucrose, 0.3 mol/L sorbitol, and 0.3 mol/L mannitol were the highest stress levels that the microshoots could tolerate. The addition of 75 mg/L Ag NPs to the previous highest stress levels resulted in a significant increase in the following: stem length (SL), leaves number (LN), fresh weight (FW), dry weight (DW), total chlorophyll, protein, calcium (Ca), and phosphorus (P) contents, while it caused a reduction in proline, sodium (Na) ions, and potassium (K) ions. These results indicate that the negative consequences of osmotic stress on Q6 quinoa microshoots could be mitigated by adding specific concentrations of Ag NPs to the culture medium.

Keywords: *quinoa*, *in vitro* culture, microshoots, osmotic stress, nanoparticles.

resumo

O estresse osmótico é um dos principais fatores abióticos destrutivos que impedem o crescimento e o desenvolvimento das plantas. Nesta pesquisa, o papel das nanopartículas de prata (Ag NPs) na mitigação do impacto negativo do estresse osmótico em *Chenopodium quinoa* cultivado *in vitro* (Quinoa 6 Line; Q6) foi investigado para determinar se Ag NPs foram capazes de reduzir os efeitos negativos nas culturas cultivadas *in vitro* da linha Q6. Os explantes foram subcultivados em um meio especial de osmosestressante contendo sacarose, sorbitol ou manitol em diferentes níveis (0,1, 0,2, 0,3 e 0,4 mol/L) para simular o ambiente de estresse osmótico por quatro semanas. Em seguida, as respostas fisiológicas ao estresse de Q6 cultivado *in vitro* sob o estresse osmótico induzido foram investigadas para determinar o nível de estresse mais alto que os microbrotos poderiam tolerar. Em seguida, Ag NPs; 25, 50 e 75 mg/L foram adicionados ao meio que continha o maior nível de estresse do estresse osmótico induzido para determinar se sua adição melhorou o desempenho fisiológico dos microbrotos Q6 sob os níveis mais severos de agente osmótico. Os resultados revelaram que 0,4 mol/L de sacarose, 0,3 mol/L de sorbitol e 0,3 mol/L de manitol foram os maiores níveis de estresse que os microbrotos puderam tolerar. A adição de 75 mg/L de Ag NPs aos maiores níveis de estresse anteriores resultou em um aumento significativo no seguinte: comprimento do caule (SL), número de folhas (LN), peso fresco (FW), peso seco (DW), clorofila total, proteína, cálcio (Ca) e teores de fósforo (P), enquanto causou uma redução nos íons prolina, sódio (Na) e potássio (K). Esses resultados indicam que as consequências negativas do estresse osmótico nos microbrotos de quinoa Q6 poderiam ser mitigadas pela adição de concentrações específicas de Ag NPs ao meio de cultura.

Palavras-chave: quinoa, cultura *in vitro*, microbrotos, estresse osmótico, nanopartículas.

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1. Introduction

Nanotechnology has gained the interest of scientists in plant tissue culture as it has been reported to boost growth in tissue-cultured plants. Several research-based studies have explored the utilization of nanoparticles (NPs) on *in vitro*-grown plants. For example, the addition of silver nanoparticles (Ag NPs) to the medium where *Tecomella undulata* was grown enhanced shoot number, shoot induction percentage, and callus formation (Aghdaei et al., 2012).

Plant tissue culture is routinely used as a tool for studying stress physiology because it can focus on the targeted stress factor while neutralizing others, providing a better understanding of plant reactions to stress. Osmotic stress can be induced in the culture medium by adding osmotic agents such as sugars (Dubois and Inzé, 2020). This can be achieved by increasing the sugar level in the growth medium (Cui et al., 2010; Molassiotis et al., 2006; Lee and Huang, 2014; Snyman et al., 2019). Sugars such as sucrose, sorbitol, and mannitol have been found to decrease the water potential and availability to plants grown under laboratory conditions (Traversari et al., 2020). Many morphological and physiological characteristics of *in vitro*-grown plants are negatively influenced by induced water stress and osmotic stress (Aliche et al., 2020). The extent of these alterations depends on several factors, such as the type of osmotic substances, their concentration, induction times, and the specific *in vitro*-grown plant species (Kiełkowska, 2017).

A few studies have confirmed better growth performance in tissue-cultured plants grown under abiotic stress conditions due to the addition of NPs. For instance, zinc nanoparticles were used to mitigate the adverse effects of salinity in cotton plants (Hussein and Abou-Baker, 2018). Zinc NPs lowered the phosphorus to zinc ratio in plant leaves, thereby providing protection from salinity (Hussein and Abou-Baker, 2018). Additionally, adding 2-10 mg/L of zinc oxide NPs to the culture medium enhanced quinoa plant growth (Al Gethami and El Sayed, 2020).

These studies demonstrate a significant link between plant tissue culture and nanotechnology (AL-Mohusaien et al., 2022; Shibli et al., 2022). Therefore, integrating biotechnology via *in vitro* growing of the Q6 quinoa line under induced osmotic stress conditions, and nanotechnology via Ag NPs, could be considered an attractive research scope. In this research, we studied the growth features and physiological responses of osmotically stressed quinoa (Q6 line) microshoots grown *in vitro*. Additionally, we examined whether adding different levels of silver NPs to the stressing mediums would improve the performance of the Q6 cultures under stress.

2. Material and Methods

2.1. Plant material

2.1.1. Plant micropropagation

The establishment and multiplication of plant material, as well as the synthesis and characterization of silver nanoparticles (Ag NPs), were performed according to Shibli et al. (2022).

2.1.2. Osmotic agents and culture medium for Q6 quinoa microshoots grown in vitro

Microshoots were sub-cultured in Murashige and Skoog (MS) medium (without hormones) (Murashige and Skoog, 1962) that was supplemented with elevated levels of sucrose (0.1, 0.2, 0.3, and 0.4 mol/L), sorbitol (0, 0.1, 0.2, and 0.3 mol/L), and mannitol (0, 0.1, 0.2, and 0.3 mol/L). The media were poured into 250 mL Erlenmeyer flasks (100 mL per flask). Then, 5 µm microshoots were transferred to the culture medium. Replications were performed five times, with each flask considered one replicate and containing 3 shoots per replicate. Data on microshoot length, fresh weight (FW), dry weight (DW), and number of leaves were collected after 4 weeks of culturing to determine the most tolerant stress level for each osmotic agent.

Next, the threshold levels of each osmotic agent were used to evaluate the influence of different Ag NP concentrations (25, 50, and 75 mg/L) on *in vitro* grown Q6 quinoa. These concentrations were added to the medium containing the highest tolerated stress level of each osmotic agent. After 4 weeks, 5 mm Q6 microshoots were transferred to MS medium supplemented with the highest tolerance levels of osmotic agents, which were sucrose (0.4 mol/L), sorbitol (0.3 mol/L), and mannitol (0.3 mol/L), separately. Ag NP concentrations were then added to the media (Table 1) following media sterilization. The media were aseptically filtered into each media bottle under continuous stirring before being poured into sterile 250 mL Erlenmeyer flasks (100 mL per flask). Replications were performed five times for each treatment, with each replicate containing 3 microshoots. Finally, data were collected after 4 weeks of culturing. Data on microshoot length, fresh weight (FW), dry weight (DW), and number of leaves were organized after 4 weeks of culturing.

Table 1. The treatments that were used in the experiment.

Treatments	Medium Description
0	Sucrose (0.4 mol/L) + 0.0 mg/L Ag NPs
1	Sucrose (0.4 mol/L) + 25 mg/L Ag NPs
2	Sucrose (0.4 mol/L) + 50 mg/L Ag NPs
3	Sucrose (0.4 mol/L) + 75 mg/L Ag NPs
4	Sorbitol (0.3 mol/L) + 0.0 mg/L Ag NPs
5	Sorbitol (0.3 mol/L) + 25 mg/L Ag NPs
6	Sorbitol (0.3 mol/L) + 50 mg/L Ag NPs
7	Sorbitol (0.3 mol/L) + 75 mg/L Ag NPs
8	Mannitol (0.3 mol/L) + 0.0 mg/L Ag NPs
9	Mannitol (0.3 mol/L) + 25 mg/L Ag NPs
10	Mannitol (0.3 mol/L) + 50 mg/L Ag NPs
11	Mannitol (0.3 mol/L) + 75 mg/L Ag NPs

2.2. Silver NPs formation and characterization

2.2.1. Silver NPs formation and characterization

The synthesis and characterization of silver nanoparticles (Ag NPs) were performed according to Shibli et al. (2022).

2.3. Assessments of plant physiological responses

2.3.1. Chlorophyll pigment content

The chlorophyll pigment content, including chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (Total Chl), was evaluated spectrophotometrically as described by Arnon (1949).

2.3.2. Proline determination

Proline content was determined on a fresh weight basis with modifications. The absorbance was measured at 520 nm using a BIO-RAD UV/Visible Spectrophotometer (Bates et al., 1973).

2.3.3. Protein content

The protein content was determined spectrophotometrically at a wavelength of 660 nm according to Lowry et al. (1951), with some modifications.

2.3.4. Determination of calcium, phosphorus, sodium, and potassium

The dried plant material from *in vitro*-cultured plants was weighed for the determination of calcium (Ca) and phosphorus (P) through acid digestion (Jones Junior, 1984). Mineral content was analyzed using an inductively coupled plasma optical emission spectrometer. Sodium (Na) and potassium (K) ions were measured using a flame photometer (Jenway Flame Photometer PFP7, 230V, 50/60Hz). Results were expressed on a dry weight basis.

2.4. Statistical design

A completely randomized design (CRD) was used for all treatments in each experiment. There were five replicates for the growth parameters and three replicates for the determination of plant physiological responses. Data were analyzed using SAS software for Windows Version 9.2 (SAS Institute Inc., 2004). Analysis of variance (ANOVA) and standard error were used for result analysis. Mean separation was performed using Tukey's Honestly Significant Difference (HSD) test with a probability level of 0.05.

3. Results

3.1. The impact of osmoticum on quinoa microshoot growth

Quinoa growth parameters were affected by different types and levels of osmoticum. Our results indicated a significant decrease in microshoot length, fresh weight (FW), dry weight (DW), and number of leaves at elevated levels of sucrose, sorbitol, and mannitol (Table 2). For example, shoot length decreased significantly with increasing levels of sucrose, sorbitol, and mannitol compared to the control treatment (3.20 cm). A significant adverse effect of osmotic stress on the number of leaves was observed ($P \leq 0.05$) (Table 2). Additionally, the highest levels of 0.4 mol/L sucrose, 0.3 mol/L sorbitol, and 0.3 mol/L mannitol resulted in reductions in fresh and dry weights. Specifically, the highest levels of sucrose (0.4 mol/L), sorbitol (0.3 mol/L), and mannitol (0.3 mol/L) significantly reduced FW to 1.10 g, 1.10 g, and 1.08 g, respectively, compared to the control treatment (0.1 mol/L sucrose) which had a FW of 1.75 g (Table 2).

Table 2. The Impact of Osmotic Agent Levels on Q6 Microshoot Length, FW, DW, and Leaf Number.

Osmotic levels mol/L	length(cm)	Leaf number	FW (mg)	DW (mg)
0.1 sucrose	3.03a*	7.40a	297.6a	176.8a
0.2 sucrose	2.13bc	7.20ab	186.6abc	13.22ab
0.3 sucrose	1.75cd	6.50ab	143.3bc	9.408bcd
0.4 sucrose	1.22de	5.90abc	151.0bc	9.57bcd
0.1 sorbitol	2.33b	6.60ab	201.5ab	11.98abc
0.2 sorbitol	1.78c	5.70abc	132.0bc	7.732bcd
0.3 sorbitol	1.16e	4.70bc	83.8c	5.452d
0.1 mannitol	1.91bc	6.10abc	136.4bc	9.376bcd
0.2 mannitol	1.80bc	5.70abc	103.9bc	6.795bcd
0.3 mannitol	1.06e	3.90c	98.7bc	5.80cd
P- values				
TRT	<.0001	0.1874	<.0001	<.0001
Sugar	<.0001	<.0001	<.0001	<.0001
TRT*Sugar	0.0026	0.1535	0.5542	0.6011

*Means within the columns characterized by the same letter were not different significantly at the $P \leq 0.05$ (according to the Tukeys HSD).

3.1.1. The addition of Ag NPs to the highest level of osmotic agents on microshoot growth

The growth parameters of microshoots were influenced by different concentrations of silver nanoparticles (Ag NPs) when they were incorporated with the highest osmotic level in the nutrient medium. The results revealed a significant impact of various concentrations of silver nanoparticles added to the highest osmotic level for each 0.4 M sucrose, 0.3 M sorbitol, and 0.3 M mannitol. For instance, the addition of 75 mg/L Ag NPs to 0.4 M sucrose, 0.3 M sorbitol, and 0.3 M mannitol significantly increased shoot length compared with the highest osmotic levels alone (Table 2). Furthermore, there was an increase in the number of leaves in microshoots subjected to various levels of Ag NPs compared to the control treatment. The highest leaf numbers, 8.8 and 8.6, were recorded with 0.3 mol/L sorbitol + 50 mg/L Ag NPs and 0.3 mol/L sorbitol + 75 mg/L Ag NPs, respectively (Table 3). Regarding plant fresh weight (FW) and dry weight (DW), both increased significantly with the increasing levels of Ag NPs. Adding 75 mg/L of Ag NPs to 0.4 mol/L sucrose, 0.3 mol/L sorbitol, and 0.3 mol/L mannitol caused an increase in FW and DW compared with the highest osmotic levels alone (Table 3).

3.2. Effect of osmoticum on microshoot physiological responses before and after adding Ag NPs

3.2.1. Effect on chlorophyll content

The physiological parameters of the quinoa plant were impacted by various levels of osmotic stress. For instance, the amounts of chlorophyll a decreased to 0.12743 mg/g, 0.14831 mg/g, and 0.13658 mg/g in 0.4 mol/L sucrose and 0.3 mol/L mannitol, respectively, compared to 0.39385 mg/g obtained under the control treatment (0.1 mol/L sucrose) (Table 4). Significant differences were observed in the leaf chlorophyll a content according to the type of osmotic agents (Table 4). The lowest mean values were 0.12743 mg/g and 0.13658 mg/g recorded at 0.4 mol/L sucrose and 0.3 mol/L mannitol, respectively (Table 4). Similarly, increasing osmotic levels adversely affected the chlorophyll b content in quinoa leaves. The lowest mean values of chlorophyll b, 0.06507 mg/g and 0.07102 mg/g, were obtained at 0.4 mol/L sucrose and 0.3 mol/L mannitol, respectively (Table 4). The highest value, 0.39385 mg/g, was obtained under the control treatment of 0.1 mol/L sucrose. The amounts of total chlorophyll in the tested line were lowered by increasing osmotic stress. The lowest mean value, 0.19214 mg/g, was attained at 0.4 mol/L sucrose, while the highest value, 1.37090 mg/g, was acquired under the control treatment of 0.1 mol/L sucrose (Table 4). In contrast, exposing quinoa microshoots to different levels of Ag NPs resulted in a gradual increase in the content of chlorophyll a in the leaves. The highest value of chlorophyll a, 0.5639 mg/g, was observed at 0.4 mol/L sucrose + 75 mg/L Ag NPs, and the lowest values, 0.1328 mg/g, 0.1723 mg/g, and 0.1358 mg/g, were recorded at 0.4 mol/L sucrose, 0.3 mol/L sorbitol, and 0.3 mol/L mannitol, respectively, with 0.0 mg/L Ag NPs treatment (Table 5). Increasing Ag NP levels positively affected the chlorophyll b content in quinoa leaves. The data revealed that the amount of total chlorophyll in the leaves increased significantly with increasing Ag NP levels in the nutrient medium (Table 5).

3.2.2. Effect on protein and proline

According to our data, the highest mean value of protein content (49.10 mg/g) was obtained at 0.1 mol/L sucrose, while the lowest value of protein (15.50 mg/g) was observed at 0.3 mol/L mannitol (Table 6). In general,

Table 3. The Impact of Ag NP Levels on Q6 Microshoot Length, FW, DW, and Leaf Number.

Highest Osmotic levels mol/L +Ag NPs mg/L	Length (cm)	Leaf Number	FW (mg)	DW (mg)
0.4 sucrose	1.22e*	5.70def	151.0cde	9.5def
0.4 sucrose +25 Ag NPs	2.45cd	6.40cde	178.4bc	11.0bcde
0.4 sucrose +50 Ag NPs	2.98abc	6.60bcde	271.3a	14.6abcd
0.4 sucrose +75 Ag NPs	3.52a	7.90abc	270.9a	16.45ab
0.3 sorbitol	1.16e	3.90f	83.8e	5.45f
0.3 sorbitol + 25 Ag NPs	2.16d	5.00ef	134.8cde	10.0cdef
0.3 sorbitol +50 Ag NPs	3.17abc	8.60ab	248.8ab	157.6ab
0.3 sorbitol +75 Ag NPs	3.58a	8.80a	262.6a	152.1abc
0.3 mannitol	1.06e	5.10ef	98.7de	58.0ef
0.3 mannitol +25 Ag NPs	2.40cd	5.70def	153.2cde	11.3bcd
0.3 mannitol +50 Ag NPs	2.74bcd	6.40cde	163.2cd	9.9cdef
0.3 mannitol +75 Ag NPs	3.35ab	7.30abcd	297.9a	16.9a
P-values				
TRT	**<.0001**	**<.0001**	**<.0001**	**<.0001**

*Means in the same columns characterized by the same letter were not different significantly at the P ≤ 0.05 probability levels (according to the Tukeys HSD).

Table 4. The Content of Chlorophyll a, Chlorophyll b, and Total Chlorophyll for Q6 Microshoots (FW Basis) Under Different Osmotic Levels.

Osmotic Agent Level (mol/L)	Chl a (mg/g FW)	Chl b (mg/g FW)	Total Chl (mg/g FW)
0.1 sucrose	0.97946 a*	0.39385 a	1.37090 a
0.2 sucrose	0.50384 b	0.36973 a	0.87180 b
0.3 sucrose	0.36618 cd	0.17136 de	0.53656 cd
0.4 sucrose	0.12743 g	0.06507 f	0.19214 e
0.1 sorbitol	0.40286 c	0.35851 ab	0.75975 b
0.2 sorbitol	0.29128 de	0.23131 cd	0.52151 cd
0.3 sorbitol	0.14831 fg	0.09155 ef	0.23939 e
0.1 mannitol	0.33062 cd	0.28060 bc	0.60993 c
0.2 mannitol	0.22585 ef	0.19128 d	0.41624 d
0.3 mannitol	0.13658 g	0.07102 f	0.20721 e
P- values			
TRT	<.0001	<.0001	<.0001
Sugar	<.0001	<.0001	<.0001
TRT*Sugar	<.0001	0.0432	<.0001

*Means in the same columns characterized by the same letter were not different significantly at the P ≤ 0.05 probability levels according to the Tukeys HSD.

Table 5. Effect of the highest osmotic levels with different levels of silver NPs on the content of chlorophyll a, chlorophyll b, and total chlorophyll to Q6 microshoots.

Highest Osmotic levels mol/L +Ag NPs (g/L)	Chl a (mg/g FW)	Chl b (mg/g FW)	Total Chl (mg/g FW)
0.4 sucrose	0.1328 f*	0.06308 d	0.19555 h
0.4 sucrose +25 Ag NPs	0.3393 bc	0.19259 c	0.53089 cd
0.4 sucrose +50 Ag NPs	0.3742 b	0.33781 b	0.71052 b
0.4 sucrose +75 Ag NPs	0.5639 a	0.48504 a	1.0467 a
0.3 sorbitol	0.1723 ef	0.08015 d	0.25201 fgh
0.3 sorbitol + 25 Ag NPs	0.1919 def	0.15587 c	0.34614 ef
0.3 sorbitol +50 Ag NPs	0.2643 cd	0.26886 b	0.53196 cd
0.3 sorbitol +75 Ag NPs	0.3880 b	0.33949 b	0.72597 b
0.3 mannitol	0.1358 f	0.06912 d	0.20452 gh
0.3 mannitol +25 Ag NPs	0.1730 ef	0.16616 c	0.33841 efg
0.3 mannitol +50 Ag NPs	0.2463 de	0.18997 c	0.43533 de
0.3 mannitol +75 Ag NPs	0.3473 bc	0.28744 b	0.63338 bc
P-values			
TRT	<.0001	<.0001	<.0001

*Means within the columns characterized by the same letter were not different significantly at the P ≤ 0.05 probability levels according to the Tukeys HSD.

osmotic stress caused an increase in proline levels in the explants, with proline levels tending to increase with higher sucrose, sorbitol, and mannitol concentrations in the medium, as shown in Table 6. The highest mean values of proline content (67.64, 66.07, and 58.14 µmol/g) were obtained at osmotic levels of 0.4 mol/L sucrose, 0.3 mol/L mannitol, and 0.3 mol/L sorbitol, respectively. The lowest value of proline was 0.67 mmol/kg at 0.1 mol/L sucrose (Table 6). Data in Table 7 indicated an improvement in protein and proline results due to the presence of silver nanoparticles. The highest protein content (49.1 mg/g) was recorded in explants treated with 0.3M mannitol plus 0.75 g NPs, while the maximum proline level was obtained in explants treated with 0.4 M sucrose (Table 7).

Table 6. Protein content and proline content of quinoa Q6 microshoots under various osmotic levels.

Osmotic agents level mol/L	Content of Protein (mg/g FW)	Content of Proline (mmol/kg FW)
0.1 sucrose	49.10a*	0.67c
0.2 sucrose	42.10b	19.44b
0.3 sucrose	34.44c	32.32b
0.4 sucrose	25.62d	67.64a
0.1 sorbitol	44.10b	21.75b
0.2 sorbitol	22.35e	28.77b
0.3 sorbitol	21.22e	58.14a
0.1 mannitol	44.10b	25.48b
0.2 mannitol	22.53e	31.61b
0.3 mannitol	15.50f	66.07a
P-values		
TRT	**<.0001**	**<.0001**
Sugar	**<.0001**	**<.0001**
TRT*Sugar	**<.0001**	**0.0102**

*Means in the same columns characterized by the same letter were not different significantly at the P ≤ 0.05 probability levels. According to the Tukeys HSD

Table 7. Effect of Ag NPs on protein and proline content of quinoa Q6 microshoot.

Highest osmotic levels mol/L +Ag NPs mg/L	Content of Protein (mg/g FW)	Content of Proline (μmol/g FW)
0.4 sucrose	27.68 fg*	67.64 a
0.4 sucrose +25 Ag NPs	31.10 ef	12.21 b
0.4 sucrose +50 Ag NPs	33.16 de	11.11 b
0.4 sucrose +75 Ag NPs	49.10 a	9.85 b
0.3 sorbitol	25.80 g	58.14 a
0.3 sorbitol + 25 Ag NPs	38.19 c	12.32 b
0.3 sorbitol +50 Ag NPs	45.53 ab	11.62 b
0.3 sorbitol +75 Ag NPs	44.10 b	9.65 b
0.3 mannitol	28.80 efg	66.07 a
0.3 mannitol +25 Ag NPs	36.53 cd	13.32 b
0.3 mannitol +50 Ag NPs	43.22 b	12.09 b
0.3 mannitol +75 Ag NPs	49.10 a	10.98 b
P-values		
TRT	**<.0001**	**<.0001**

*Means in the same columns characterized by the same letter were not different significantly at the P ≤ 0.05 probability levels. According to the Tukeys HSD.

3.2.3. Effect on ion content

Regarding ion contents, a significant decline in calcium (Ca) and phosphorus (P) was observed as the osmotic levels in the media were elevated (Figure 1). On the other hand, sodium (Na) content was significantly affected by osmotic stress; increasing the concentrations of sucrose, sorbitol, and mannitol resulted in higher Na content (Figure 1). Elevated levels of Na^+ were reported to play a significant role in plant adaptation to osmotic stress by altering the biosynthesis of osmolytes and osmoprotectants such as proline.

Meanwhile, potassium (K) content in both shoots and roots decreased as the concentrations of sucrose, sorbitol, and mannitol increased. The highest potassium levels were obtained with 0.1 mol/L sucrose, 0.2 mol/L sucrose, and 0.1 mol/L sorbitol, while the lowest potassium content was found at 0.3 mol/L mannitol (Figure 1). Conversely, our results revealed a notable enhancement in ion content after the addition of silver nanoparticles (AgNPs) (Figure 2).

4. Discussion

According to our data, the microshoots showed better growth performance when exposed to sucrose compared to sorbitol or mannitol (Table 2). This can be related to the fact that plants grown *in vitro* are highly influenced by the type of carbon source and other components in the culture medium (Du et al., 2012).

The reduction in growth in response to high sugar levels in the media was also observed by Kulpa et al. (2018), who revealed that when sorbitol was added to the culture medium at a concentration of 200 mmol/L, it caused growth inhibition and dry matter reduction (Kulpa et al., 2018). The induction of mannitol at 150 and 200 mmol/L in the culture medium of *in vitro*-grown soybean (G*lycine max* L.) caused growth inhibition. Many reports indicate that elevated levels of sorbitol in the culture medium of *in vitro*-grown plants can reach toxic levels because carbohydrates accumulate continuously in the cells (Tahtamouni et al., 2001; Moges et al., 2003; Shibli et al., 2001; El-Homosany and Noor El-Deen, 2019). This can be attributed to the fact that sucrose, sorbitol, and mannitol are sugar alcohols. The addition of these agents to the culture medium can restrict the available water to the explant and decrease the water potential (Shibli et al., 2001; Du et al., 2012; Taiz et al., 2015). Hence, they act in the culture medium as both sources of carbon and osmotic stressors that can lower the water potential and the available water to the *in vitro*-grown plants (Shibli et al., 2001).

We observed a significant increase in the measured parameters when subjected to 75 mg/L AgNPs under the highest levels of sucrose, sorbitol, and mannitol (Table 3). Our results are consistent with those of Aghdam et al. (2016) and Azmat et al. (2020). It was found that TiO_2 NPs significantly improve the agronomical parameters of wheat under water-deficient stress conditions (Jaberzadeh et al., 2013). The application of appropriate concentrations of TiO_2 NPs to flaxseed (*Linum usitatissimum* L.) plants under water stress significantly increased plant tolerance and improved physiological processes (Aghdam et al., 2016).

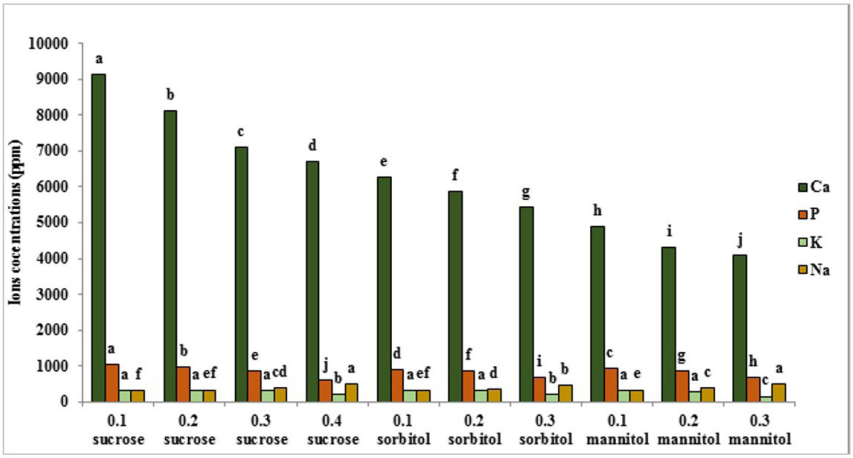

Figure 1. Effect of different level of osmotic stress on ion content; Ca, P, K, and Na. Lowercase letters represent the degree of significance of the means of ion content. Means of the bars with same letter are not significantly different at the P ≤ 0.05 probability levels.

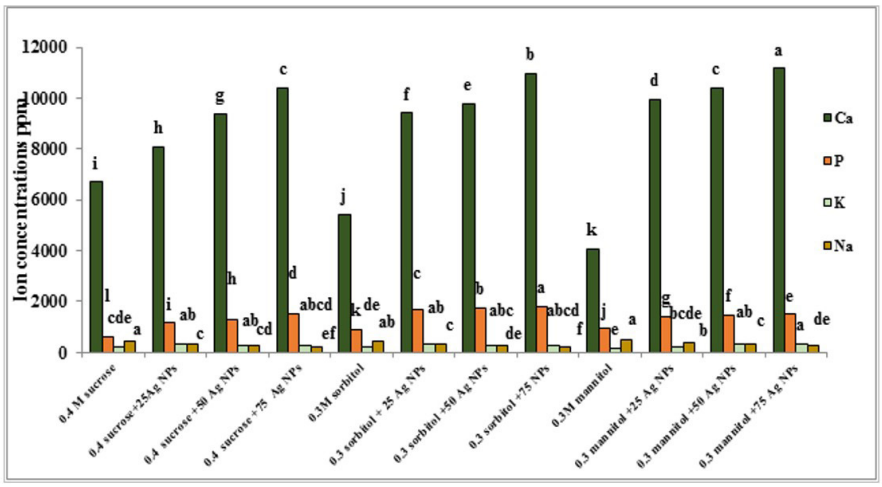

Figure 2. Effect of different Ag NPs levels on ion contents; Ca, P, K, and Na of Q6 Quinoa plantlets. Lowercase letters represent the degree of significance of the means of ion content after adding NPs. Means of the bars with same letter are not significantly different at the P ≤ 0.05 probability levels.

The growth characteristics of maize subjected to TiO_2 NPs under water stress were significantly enhanced in a dose-dependent manner (Yaqoob et al., 2020). A previous report demonstrated that *Araucaria excelsa* grown in MS medium induced with AgNPs exhibited adequate growth and maintained their green color compared to MS medium without AgNPs (Sarmast and Salehi, 2016).

Recently, findings on the micropropagation of *Tecomella undulata* (Roxb.) Seem revealed that culture vessels containing ethylene during its *in vitro* growth resulted in a lower number of leaves and reduced chlorophyll content. Meanwhile, the addition of AgNPs to the MS medium of *T. undulata* increased the mean number of shoots and their length, as well as the survival rate (Saha and Gupta, 2018). Saha and Gupta (2018) noted that the presence of AgNPs in the culture medium enhances plant growth by significantly reducing ethylene production in the culture vessels. Thus, the enhancement effects of AgNPs observed in our results can be attributed to their impact on ethylene action (Sarmast et al., 2015). Ethylene, present in the gaseous state under ambient conditions, can adversely impact microshoot growth and development due to limited space in sealed vessels (Taiz et al., 2015; Sarmast et al., 2015). Nano-sized silver acts as an effective inhibitor of ethylene, suppressing its action due to its higher surface area-to-mass ratio and physiochemical properties (Lin et al., 2009; Taiz et al., 2015). Another explanation for our findings is that plants typically develop a defense system in response to oxidative damage and stress conditions, including enhanced antioxidant enzyme activity. It was found that AgNPs induction to the culture medium significantly elevated redox enzyme activity in the shoots and roots of *Lycopersicon esculentum* (Mehrian et al., 2016). Similarly, a remarkable increase in

dry weight (DW) and redox enzyme activity was observed in *Triticum aestivum* L. plants treated with AgNPs under heat stress conditions (Iqbal et al., 2019). Saha and Gupta (2018) reported that AgNPs induced *in vitro* cultures of *Swertia chirata* enhanced growth by activating antioxidant enzymes, which subsequently stimulated shoot proliferation and increased the number of shoots per explant. Ali et al. (2019) suggested that AgNPs act as chemical elevators affecting cell growth and secondary metabolite production. Thus, plants activate their antioxidant mechanisms to cope with stressful conditions. Recent studies have shown that AgNPs, especially, induce the activation of antioxidant enzyme-producing genes in response to abiotic stress (Kaveh et al., 2013; Nair and Chung, 2014). Our results suggest that AgNPs induction in the *in vitro* culture medium has the potential to activate antioxidant enzymes and scavenge free radicals as a defense mechanism (Jadczak et al., 2020).

4.1. Effect of osmoticum on microshoots physiological responses before and after adding AgNPs

4.1.1. Effect on chlorophyll content

Reduction in chlorophyll content can be attributed to several factors. One possible cause is the activation of chlorophyllase, an enzyme induced under osmotic stress and other stress conditions (Britto et al., 2003). Osmotic agents may elevate growth inhibitory substances, initiating signaling events related to stress response pathways and gene expression (Zou et al., 2010). For instance, abscisic acid (ABA) degrades chlorophyll by stimulating chlorophyllase, one of the chlorophyll-degrading enzymes (Bhusal et al., 2019). These findings align with those of Aghaie et al. (2018), Iqbal et al. (2018), Chaudhry et al. (2021), Muhammad et al. (2021) and Tafreshi et al. (2021). Our results imply that chlorophyll content decreased due to osmotic stress, while AgNPs alleviated this adverse impact (Tables 4 and 5) either by enhancing plant growth or increasing nutrient uptake (Naumann et al., 2008). Nanoparticles positively impacted chlorophyll content, possibly by reducing the uptake of Na^+ and other toxic ions (Shakeel Ahmed et al., 2016). In contrast, Thiruvengadam et al. (2015) found no significant effect of AgNPs on total chlorophyll content at lower concentrations (1.0 mg/L), while higher concentrations of AgNPs led to a significant reduction in total chlorophyll (Dewez et al., 2018). ZnO-NPs have been suggested to increase chlorophyll content and improve photosynthetic pigment efficiency, enhancing the photosynthesis rate (Narendhran et al., 2016). This may be due to NPs amplifying the efficiency of the photosynthetic process, promoting light absorption by chlorophyll and transferring energy to NPs (Mohsenzadeh and Moosavian, 2017).

4.1.2. Effect on proline and protein content

Proline can act as either a signaling molecule or a regulatory molecule, stimulating various responses to adapt and cope with stress conditions (Maggio et al., 2002). Ngara et al. (2018) used sorbitol treatments to induce osmotic stress in Sorghum (Sorghum bicolor L. Moench) and found an overall increase in protein secretion. Conversely, Jain et al. (2010) reported that different concentrations of sorbitol significantly decreased chlorophyll a, chlorophyll b, and protein content in Zea mays seedlings. On the other hand, using AgNPs significantly increased protein content and decreased proline concentrations in stressed plantlets, attributed to the amelioration role of AgNPs under stress conditions (Jasim et al., 2017). Other studies have shown that NPs positively affect plant cells under stress by improving their ability to cope with abiotic stress (Alabdallah et al., 2021; Faizan et al., 2021; van Nguyen et al., 2022; Rajput et al., 2021; Singh et al., 2021). In our study, AgNPs played an effective role in mitigating osmotic stress and improving overall plant condition. This effect may be due to their capacity to inhibit ethylene signaling (Mahendran et al., 2019). Ethylene production harms plant cells by deactivating auxin translocation, increasing acidity, and eventually causing cell death. Ag^+ ions compete with ethylene for binding sites, thereby blocking ethylene production (Qin et al., 2005).

4.1.3. Effect on ion content

There was a significant general reduction in nutrient contents, namely Ca and P (Figure 1). This can be attributed to disturbances in ion acquisition under osmotic stress, as high concentrations of osmotic agents in the nutrient medium obstruct the absorption of essential plant nutrients (mineral elements necessary for growth). This is particularly evident between potassium (K) and sodium (Na); as one increases, the plant's ability to absorb the other decreases (Abbas et al., 2021; Amini and Ehsanpour, 2005; Aghaie et al., 2018). Lower levels of Ca, P, and K^+ in response to elevated levels of sucrose, sorbitol, and mannitol were also reported by Slama et al. (2007), who noted that mannitol uptake by cells mitigates the osmotic gradient of the medium. The remarkable enhancement of ion content after adding AgNPs was observed (Figure 2). This is likely due to the ability of AgNPs to enhance stress tolerance in plants by increasing nutrient absorption and improving enzyme reactions (Zarei and Ehsanpour, 2023). Similar results were obtained by several publications (Abasi et al., 2022; Şener and Saygi, 2023).

These studies demonstrate a significant link between plant tissue culture and nanotechnology (AL-Mohusaien et al., 2022; Shibli et al., 2022; Al Gethami and El Sayed, 2020; Aghdam et al., 2016). Therefore, integrating biotechnology via *in vitro* growing of the Q6 quinoa line under induced osmotic stress conditions, and nanotechnology via Ag NPs, could be considered an attractive research scope. In this research, we studied the growth features and physiological responses of osmotically stressed quinoa (Q6 line) microshoots grown *in vitro*. Additionally, we examined whether adding different levels of silver NPs to the stressing mediums would improve the performance of the Q6 cultures under stress.

5. Conclusions

The growth features and physiological responses of quinoa (Q6 line) microshoots grown *in vitro* were adversely affected under osmotic stress conditions. Examining different levels of silver NPs in the stress mediums improved

the performance of the Q6 culture under stress conditions. Quinoa microshoots grown in high sugar-containing media to induce osmotic stress exhibited reduced growth and altered physiological responses. However, the impacts of osmotic stress were alleviated by using specific levels of silver NPs. The specific level of silver NPs (75 mg/L) significantly influenced plant growth and mitigated the adverse effects of induced stresses. The concentrations of AgNPs used (75 mg/L) significantly improved plant growth and growth indicators (shoot length, leaf number, fresh weight, and dry weight) compared to the stress treatments alone. For physiological indicators, the AgNPs concentrations used led to a significant increase in chlorophyll content, protein, Ca, P, and K content, while decreasing proline and measured ions (Na) compared to stress treatments. Thus, the use of silver NPs holds great potential for mitigating the negative impacts of stress on quinoa plants and improving growth and physiological responses. Further research is recommended to study the influence of AgNPs on quinoa plant quality and yield, as well as to explore the uptake capacity and permissible limits of AgNPs for other plant species.

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