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Silver Nanoparticles Enhance Salt-Stress Tolerance of In Vitro-Grown Strawberry

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Silver Nanoparticles Enhance Salt-Stress Tolerance of In Vitro-Grown Strawberry

Abstract

Salinity severely limits strawberry (Fragaria × ananassa) production and micropropagation. This study evaluated whether exogenous silver nanoparticles (AgNPs) improve the salt tolerance of in vitro-grown strawberry cv. Sweet Charlie. Shoot clusters were cultured on MS medium under six treatments: control, 50 mM NaCl, 25 or 50 µM AgNPs alone, and 25 or 50 µM AgNPs combined with 50 mM NaCl. After six weeks, shoot proliferation, fresh weight, catalase (CAT) and peroxidase (POD) activities, proline, Na^+/K^+ contents and ratio, and chlorophylls were assessed. NaCl alone suppressed shoot number and fresh weight by 58% and 30%, doubled the Na^+/K^+ ratio (1.28 to 2.04), and triggered oxidative and osmotic responses. Adding 25 µM AgNPs under salinity restored shoot number and fresh weight to 96% and 125% of control values, maintained K^+ uptake, lowered the Na^+/K^+ ratio to 1.05, and further enhanced CAT, POD, and proline accumulation. The 50 µM dose was less effective and partially growth-inhibitory, indicating a hormetic response. These results demonstrate that a low, calibrated AgNPs dose (25 µM) protects tissue-cultured strawberry against NaCl stress through coordinated strengthening of antioxidant defense, osmotic adjustment, and K^+/Na^+ homeostasis.

Keywords: *micropropagation; antioxidant enzymes; osmotic adjustment; ion homeostasis; oxidative damage; NaCl; plant tissue culture*

1. Introduction

Soil and water salinization is one of the most pervasive constraints on world agriculture and its extent continues to expand as irrigation practices water scarcity and climate change converge. For high value horticultural crops the problem is compounded by their general salt sensitivity and by the premium placed on uniform vigorous planting material. Strawberry (Fragaria × ananassa Duch.) ranks among the most economically important berry crops worldwide yet it is classified as a salt sensitive species whose yield fruit quality and vegetative performance decline sharply as root zone salinity rises (Passa et al. 2026). A recent comprehensive review of biological intervention strategies against strawberry salinity stress confirms that the genus possesses only limited intrinsic tolerance mechanisms so that exogenous protective treatments remain the most practical route for sustaining production on marginally saline substrates (Passa et al. 2026).

The cellular basis of salt injury is well established. Elevated Na^+ competes with K^+ for uptake and binding sites disrupts membrane potential and inhibits potassium-dependent enzyme activities while Cl accumulation in shoots adds a second often underestimated toxic component. Because K^+ is required for protein synthesis enzyme activation and stomatal control a rising cytosolic Na^+/K^+ ratio is widely used as a physiological index of salt injury (Wahid et al. 2020). Salinity also imposes an osmotic component that reduces water availability and a secondary oxidative burst in which reactive oxygen species (ROS) damage membranes pigments and nucleic acids. Crops differ widely in their capacity to keep the Na^+/K^+ ratio low and to scavenge ROS and it is precisely these two traits that most protective treatments seek to reinforce (Khan et al. 2020; Khan et al. 2021).

Strawberry responds to salinity through a combination of ion exclusion osmotic adjustment and antioxidant defense but the capacity of most commercial cultivars is insufficient under moderate to high salt loads. In vitro selection studies have repeatedly shown that strawberry genotypes vary in NaCl tolerance and that tolerant soma clones can be recovered through step-wise selection on saline media underscoring both the genetic potential and the practical difficulty of improving the crop (Dziadczyk et al. 2003; Husaini and Abdin 2008). Husaini and Abdin (2008) further demonstrated that salt-tolerant strawberry plants can be obtained through genetic transformation yet transgenic solutions face regulatory and public-acceptance hurdles that limit their near term deployment.

During in vitro micropropagation the challenge is especially acute. Explants and micro shoots are cultured for weeks on sugar rich media in closed vessels and any salinity carried over from the donor environment the water used for medium preparation or the rooting acclimatization stage is transmitted directly to the most vulnerable stage of the plant's life cycle. Strawberry in vitro cultures respond to NaCl with reduced shoot proliferation chlorosis and poor rooting and these symptoms have been documented across a range of cultivars and basal media (Zhao et al. 2016; Mozafari et al. 2019). Any treatment that stabilizes in vitro performance under saline conditions therefore has direct commercial value for certified nursery production.

Nanotechnology offers a new toolkit for such stabilization. Engineered nanoparticles because of their high surface to volume ratio reactivity and capacity to traverse plant barriers at low doses can act as nanoscale elicitors that modify stress signalling with far smaller quantities of active material than conventional treatments (Siddiqi and Husen 2022; Alabdallah and Hasan 2021). Silver nanoparticles occupy a distinctive position within this toolkit. At appropriate doses they stimulate seed germination root elongation and antioxidant enzyme activity while their well-known antimicrobial activity simultaneously reduces the biotic load that frequently compounds abiotic stress in culture vessels (Siddiqi and Husen 2022).

The protective action of AgNPs under salinity has now been documented in a range of species. In wheat low dose AgNPs regulated abscisic acid balance improved ion homeostasis and mitigated oxidative damage under salt stress (Wahid et al. 2020). Seed priming with AgNPs in pearl millet alleviated salinity toxicity by strengthening antioxidant defenses and stabilizing the Na^+ /K^+ ratio (Khan et al. 2020) and follow up physiological and transcriptomic work showed that AgNPs reprogram stress responsive gene expression (Khan et al. 2023). In rice AgNPs treatment of seeds triggered metabolic and transcriptional reprogramming that improved both salt tolerance and disease resistance (Yan et al. 2023). Biosynthesized AgNPs priming improved wheat seedling performance under salt stress (Abou-Zeid and Ismail 2018) Phyto synthesized AgNPs enhanced the salt tolerance of wheat seedlings (Kesbi et al. 2025) and citrate-capped AgNPs combined with indole-3-acetic acid modulated salt responses in a range of plant systems (Afridi et al. 2023). Collectively these studies establish AgNPs as a credible dose-dependent mitigator of salinity stress.

Dose calibration is critical. Nanoparticle effects in plants classically follow a hermetic pattern in which low concentrations are stimulatory and high concentrations become inhibitory or phytotoxic. Almutairi (2016) reported that silver nanoparticles improved the salt resistance of tomato during germination whereas Younes and Nassef (2015) found no enhancement of

salt tolerance in tomato transplants at 10-40 ppm illustrating that the outcome depends on species stage and concentration. In strawberry itself Mozafari et al. (2018) obtained positive responses of in vitro explants to combined salicylic acid and iron nanoparticle application under salinity and Abu Zeid et al. (2023) showed that ZnO nanoparticles at a moderate dose enhanced the in vitro growth ion balance and leaf anatomy of two strawberry cultivars under NaCl stress. Silver nanoparticles by contrast have scarcely been examined in strawberry and particularly not in an in vitro salinity context leaving a clear knowledge gap.

The present study was designed to fill that gap. Using in vitro shoot clusters of strawberry cv. Sweet Charlie we tested the hypothesis that exogenous AgNPs supplied at two concentrations in the culture medium improve tolerance to 50 mM NaCl by (i) preserving shoot proliferation and biomass accumulation (ii) reinforcing the antioxidant enzymes catalase and peroxidase (iii) promoting proline based osmotic adjustment and (iv) restricting Na^+ accumulation while maintaining K^+ uptake thereby keeping the Na^+ /K^+ ratio low. The low-dose treatment (25 µM) was expected to be protective whereas the higher dose (50 µM) was included to locate the stimulatory versus inhibitory boundary of the hermetic response.

2. Materials and Methods

2.1 Plant material and in vitro culture

Stock plants of strawberry (Fragaria × ananassa Duch.) cv. Sweet Charlie were maintained in a greenhouse and used as the source of runner-tip explants. Surface sterilization followed standard strawberry micropropagation practice: runner tips were washed in running water disinfected in 70% ethanol for 30 seconds then immersed in 1% sodium hypochlorite for 12-15 minutes with two drops of Tween-20 and rinsed three times with sterile distilled water. Single shoot tips (1-1.5 cm) were inoculated into glass jars containing 40 ml of Murashige and Skoog (MS) basal medium supplemented with 3% sucrose and solidified with 0.7% agar. The pH of all media was adjusted to 5.7 before autoclaving at 121°C for 20 minutes. Cultures were incubated at 22 ± 2°C under a 16-h photoperiod provided by cool-white fluorescent lamps and fully proliferated shoot clusters were obtained after six weeks.

2.2 Experimental treatments and design

Six treatments were arranged in a completely randomized design (CRD) with three replicates (jars) each jar containing five excised shoot clusters as the experimental unit. The treatments were: (1) control (MS medium without amendments) (2) 50 mM NaCl (3) 25 µM silver nanoparticles (4) 50 µM silver nanoparticles (5) 25 µM AgNPs + 50 mM NaCl and (6) 50 µM AgNPs + 50 mM NaCl. Silver nanoparticles were added to the medium before autoclaving and NaCl was included at medium preparation. After six weeks of culture jars were assessed for shoot number per cluster root number and shoot fresh weight (SFW). Representative cultures were photographed under uniform lighting for the morphological record (Figures 1-3).

2.3 Biochemical analyses

Proline content of shoot tissues was estimated by the acid ninhydrin method after extraction in aqueous sulfosalicylic acid with absorbance read at 520 nm and proline expressed as mg g 1 fresh weight. Catalase (CAT) activity was assayed spectrophotometrically by

monitoring the decomposition of hydrogen peroxide at 240 nm and peroxidase (POD) activity was determined using guaiacol as the hydrogen donor with the increase in absorbance at 470 nm recorded over time; both enzymes were expressed as mU mg-1 protein.

2.4 Ion content and Na^+ /K^+ ratio

Dried shoot material was extracted and Na^+ and K^+ concentrations were measured by flame photometry expressed as µmol g-1 dry weight. The Na^+ /K^+ ratio was calculated for each replicate and expressed as the mean of three replicates per treatment.

2.5 Photosynthetic pigments

Chlorophyll a and b were extracted from fresh leaf tissue in 80% acetone and absorbance was recorded at 663 and 645 nm; pigment contents were calculated using standard extinction equations and expressed as mg g 1 fresh weight.

2.6 Statistical analysis

Data were analyzed as a one-way classification of a completely randomized design using analysis of variance (ANOVA). Treatment means were separated by Duncan's multiple range test (DMRT) at the 5% probability level and the least significant difference (LSD 0.05) and coefficient of variation (CV%) are reported with each trait. Letters in tables and figures denote significant differences among treatment means at $P < 0.05$.

3. Results

3.1 Morphological responses and culture performance

The visual performance of the cultures after six weeks reflected the numerical data closely. Control and AgNPs treated cultures remained vigorously green and well proliferated whereas NaCl only cultures developed clear chlorosis leaf margin burn and stunted sparse shoots (Figure 1). Cultures receiving 25 µM AgNPs together with NaCl remained substantially greener and more turgid than salt-stressed cultures without nanoparticles indicating a visible protective effect at the lower dose.



control | Salinity 50 mM | AgNPs 25 µm | AgNPs 50 µm | AgNPs 25 µm + Salinity 50 mM | AgNPs 50 µm + Salinity 50 mM

Figure 1. General view of in vitro strawberry cv. Sweet Charlie cultures after six weeks on MS medium under the six treatments non-saline control ,50 mM NaCl, 25 µM AgNPs ,50 µM AgNPs, 25 µM AgNPs + 50 mM NaCl and 50 µM AgNPs + 50 mM NaCl.

Shoot proliferation was strongly affected by treatment ($F = 86.06$; $P < 0.001$). The control produced 13.42 shoots per cluster and the 25 µM AgNPs treatment was statistically comparable (12.71). Salt stress alone reduced shoot number to 5.69 a decline of 58% relative to the control. Supplementation with 25 µM AgNPs under salinity raised shoot number to 11.76 recovering 96% of the control value whereas 50 µM AgNPs under salinity gave only 10.39 shoots (Figure 2). A similar pattern held for shoot fresh weight ($F = 87.58$; $P < 0.001$): 50 mM NaCl depressed fresh weight to 610.27 mg (30% below the 865.59 mg control) 25 µM AgNPs alone produced the highest value of all treatments (1240.99 mg) and 25 µM AgNPs + NaCl yielded 1082.14 mg exceeding the non-saline control by 25% (Figure 4). Root number declined under salinity from 10.71 to 5.27 roots per cluster and was partially restored to 7.30 by 25 µM AgNPs ($F = 20.52$; $P < 0.001$ Figure 3). The 50 µM dose whether alone or with salt consistently produced intermediate values confirming a hermetic dose response in which the lower nanoparticle concentration was the more effective protectant. The corresponding treatment means are presented in Table 1.

Table 1. Effects of silver nanoparticles (AgNPs) and NaCl on shoot number, root number, and shoot fresh weight of in vitro grown strawberry cv. Sweet Charlie.

Treatment	AgNPs	NaCl	Shoot number per cluster	Root number per cluster	Shoot fresh weight (mg)
control	0	0	13.42±.57a	10.71±1.49a	865.59±47.13c
salinity	0	50 mM	5.69±.43e	5.27±.65c	610.27±26.50e
AgNPs 25 µM	25 µM	0	12.71±.98ab	9.70±.61a	1240.99±53.82a
AgNPs 50 µM	50 µM	0	8.27±.32d	8.03±.38b	928.22±47.18c
AgNPs 25 µM	25 µM	50 mM	11.76±.15b	7.30±.36b	1082.14±23.66b
AgNPs 50 µM	50 µM	50 mM	10.39±.47c	7.17±.15b	787.87±38.67d

Note: Values are means ± standard deviation (n = 3). Means within a column followed by different letters differ significantly according to Duncan's multiple range test ($P < 0.05$).

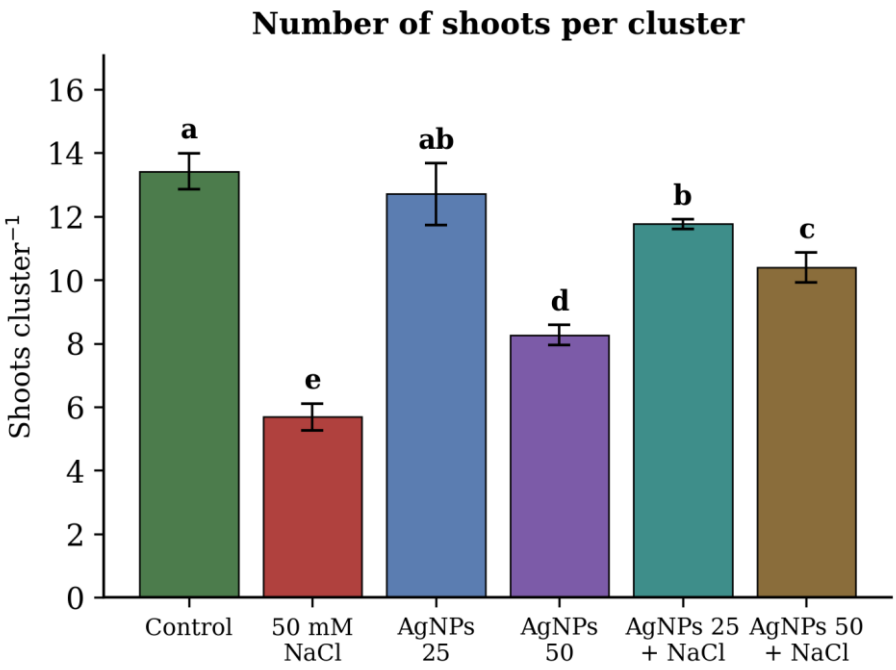


Figure 2. Effect of AgNPs and NaCl on the number of shoots per cluster of in vitro strawberry cv. Sweet Charlie. Bars are means of three replicates ± standard deviation; different letters indicate significant differences (DMRT P < 0.05).

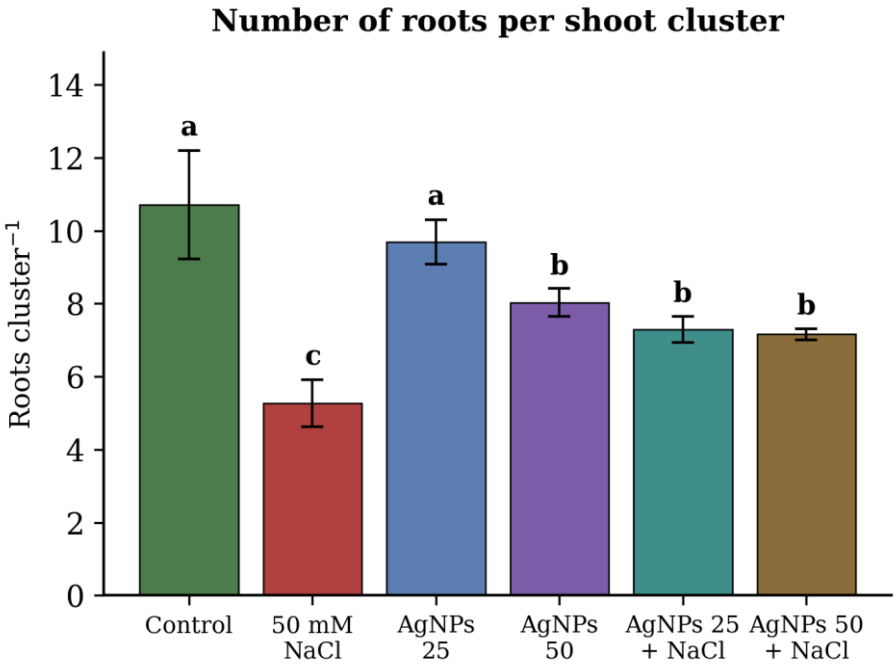


Figure 3. Effect of AgNPs and NaCl on the number of roots per shoot cluster. Bars are means ± SD (n = 3); different letters indicate significant differences (DMRT P < 0.05).

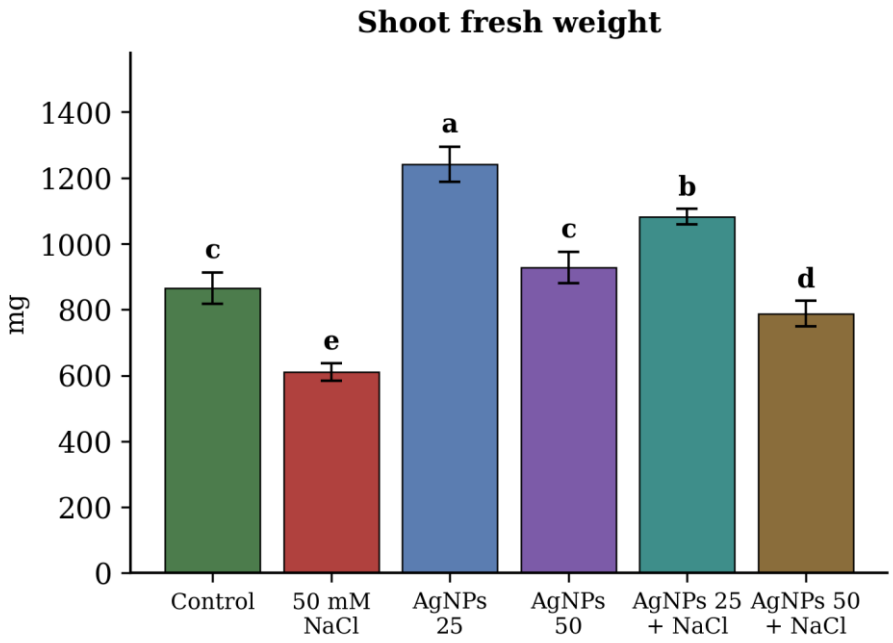


Figure 4. Effect of AgNPs and NaCl on shoot fresh weight. Bars are means ± SD (n = 3); different letters indicate significant differences (DMRT P < 0.05).

3.2 Antioxidant enzyme activities

Both assayed enzymes responded sharply to treatment. Catalase activity was highest in the 25 µM AgNPs + NaCl treatment (189.02 mU mg-1 protein) followed by 50 µM AgNPs + NaCl (175.30) and NaCl alone (175.24) all significantly above the control (104.92) ($F = 92.60$; $P < 0.001$ Figure 5). Peroxidase showed its maximum under NaCl alone (97.94 mU mg-1 protein) with the combined 25 µM AgNPs + NaCl treatment at 69.46 the 50 µM AgNPs + NaCl at 77.53 and the control at 52.44 ($F = 94.02$; $P < 0.001$ Figure 6). The pattern indicates that salt stress triggered a strong endogenous antioxidant response and that co-supplied AgNPs sustained or further amplified CAT while allowing POD to remain well above control levels jointly maintaining a high ROS-scavenging capacity in the protected tissues. The corresponding treatment means are presented in Table 2.

Table 2. Effects of silver nanoparticles (AgNPs) and NaCl on catalase (CAT) and peroxidase (POD) activities and proline content in shoots of in vitro grown strawberry cv. Sweet Charlie.

Treatment	AgNPs	NaCl	CAT (mU mg^{-1} protein)	POD (mU mg^{-1} protein)	Proline (mg g^{-1} FW)
control	0	0	104.92±9.59d	52.44±5.02d	94.66±2.94b
salinity	0	50 mM	175.24±8.90b	97.94±3.41a	130.16±4.12a
AgNPs 25 µM	25 µM	0	94.46±7.89d	44.30±.90e	55.81±2.38d
AgNPs 50 µM	50 µM	0	141.49±4.93c	65.74±2.18c	85.57±3.30c
AgNPs 25 µM	25 µM	50 mM	189.02±3.23a	69.46±2.84c	96.05±1.96b
AgNPs 50 µM	50 µM	50 mM	175.30±6.33b	77.53±4.29b	93.34±5.85b

Note: Values are means ± standard deviation (n = 3). Means within a column followed by different letters differ significantly according to Duncan's multiple range test ($P < 0.05$).

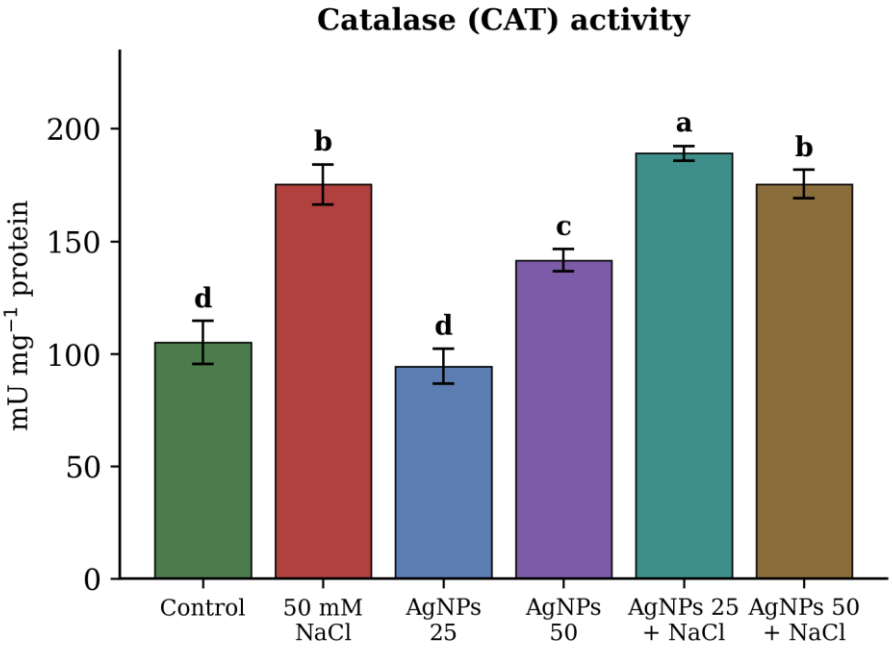


Figure 5. Catalase (CAT) activity in shoots of in vitro strawberry under AgNPs and NaCl treatments. Bars are means ± SD (n = 3) different letters indicate significant differences (DMRT P < 0.05).

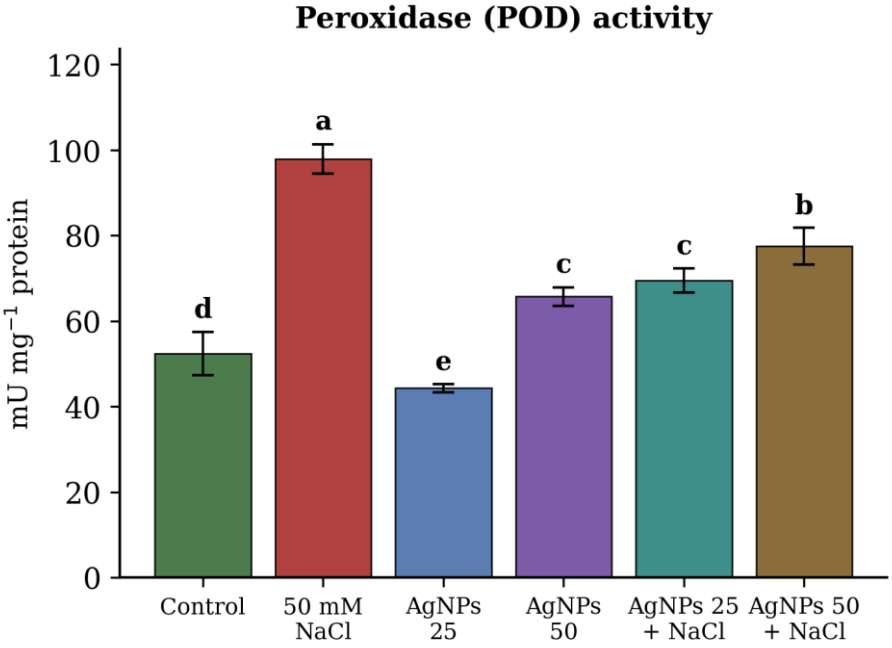


Figure 6. Peroxidase (POD) activity in shoots of in vitro strawberry under AgNPs and NaCl treatments. Bars are means ± SD (n = 3) different letters indicate significant differences (DMRT P < 0.05).

3.3 Proline accumulation

Proline content ranged from 55.81 mg g-1 FW in the 25 µM AgNPs treatment to 130.16 mg g-1 FW under NaCl alone with a highly significant treatment effect ($F = 127.15$; $P < 0.001$; Figure 7). Relative to the control (94.66) salt stress raised proline by 37% reflecting osmotic adjustment and ROS buffering. The 25 µM AgNPs + NaCl combination maintained proline at 96.05 statistically comparable to the control and well above the non-saline AgNPs treatments.

The osmolyte pool therefore remained mobilized under the combined treatment without the runaway accumulation that marks severe stress injury.

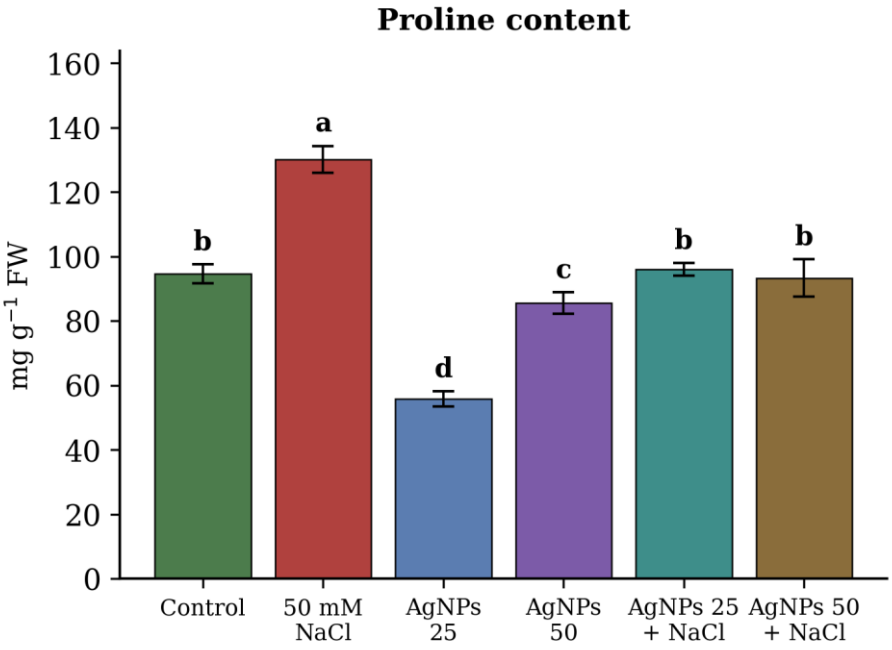


Figure 7. Proline content of in vitro strawberry shoots under AgNPs and NaCl treatments. Bars are means ± SD (n = 3) different letters indicate significant differences (DMRT P < 0.05).

3.4 Ion homeostasis and Na^+ /K^+ ratio

Potassium content was significantly affected by treatment (F = 21.10; P < 0.001; Figure 8). The 25 µM AgNPs treatment gave the highest K^+ accumulation (61.94 µmol g-1) followed by 25 µM AgNPs + NaCl (61.52) and the control (50.60) while NaCl alone reduced K+ to 42.96. Sodium content was likewise treatment-dependent (F = 27.66; P < 0.001; Figure 9): NaCl alone elevated Na^+ to 87.34 µmol g-1 whereas all AgNPs containing treatments remained at or below 65.26 and the 25 µM AgNPs treatment recorded the lowest Na+ content (55.76). The Na^+ /K^+ ratio (F = 36.71; P < 0.001 Figure 10) consequently doubled under salt stress (2.04 versus 1.28 in the control) but fell to 1.05 with 25 µM AgNPs + NaCl a value statistically indistinguishable from the control and from the 25 µM AgNPs treatment (0.90). AgNPs therefore protected the shoots not only by limiting Na^+ entry but equally importantly by preserving K^+ acquisition under saline conditions. The corresponding treatment means are presented in Table 3.

Table 3. Effects of silver nanoparticles (AgNPs) and NaCl on potassium (K^+) and sodium (Na^+) contents and the Na^+/K^+ ratio in shoots of in vitro-grown strawberry cv. Sweet Charlie.

Treatment	AgNPs	NaCl	K^+ (µmol g^{-1})	Na^+ (µmol g^{-1})	Na^+/K^+
control	0	0	50.60±4.12d	**64.54±3.93b**	1.28±.16b
salinity	0	50 mM	42.96±2.95e	87.34±3.73a	2.04±.19a
AgNPs 25 µM	25 µM	0	61.94±1.06a	55.76±2.47c	0.90±.05e
AgNPs 50 µM	50 µM	0	53.06±1.47cd	62.46±2.16b	1.18±.08bc
AgNPs 25 µM	25 µM	50 mM	61.52±2.25ab	64.43±3.62b	1.05±.03cd
AgNPs 50 µM	50 µM	50 mM	56.78±3.23bc	65.26±4.66b	1.15±.09bc

Note: Values are means ± standard deviation (n = 3). Means within a column followed by different letters differ significantly according to Duncan's multiple range test ($P < 0.05$).

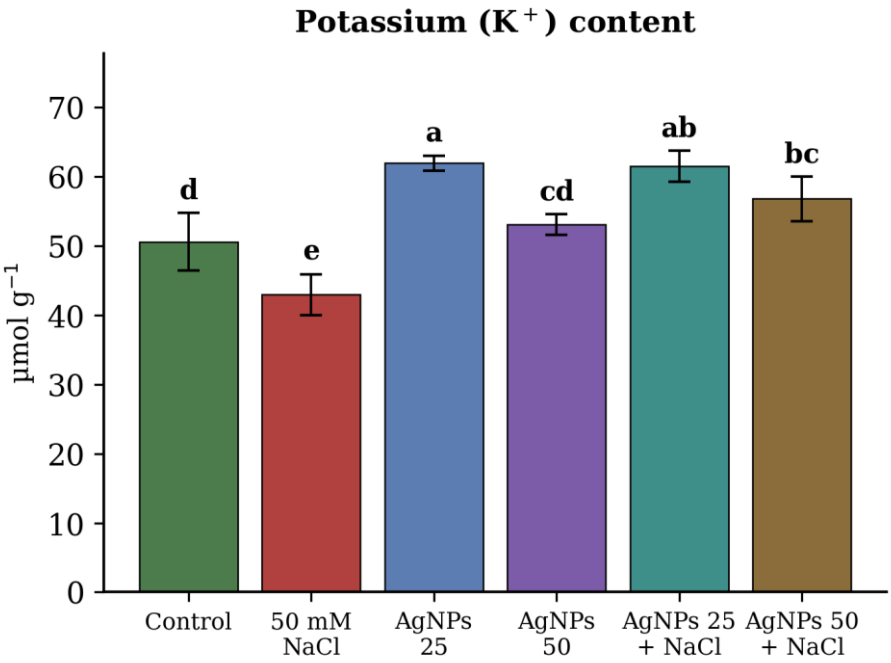


Figure 8. Potassium (K^+) content of in vitro strawberry shoots under AgNPs and NaCl treatments. Bars are means ± SD (n = 3) different letters indicate significant differences (DMRT $P < 0.05$).

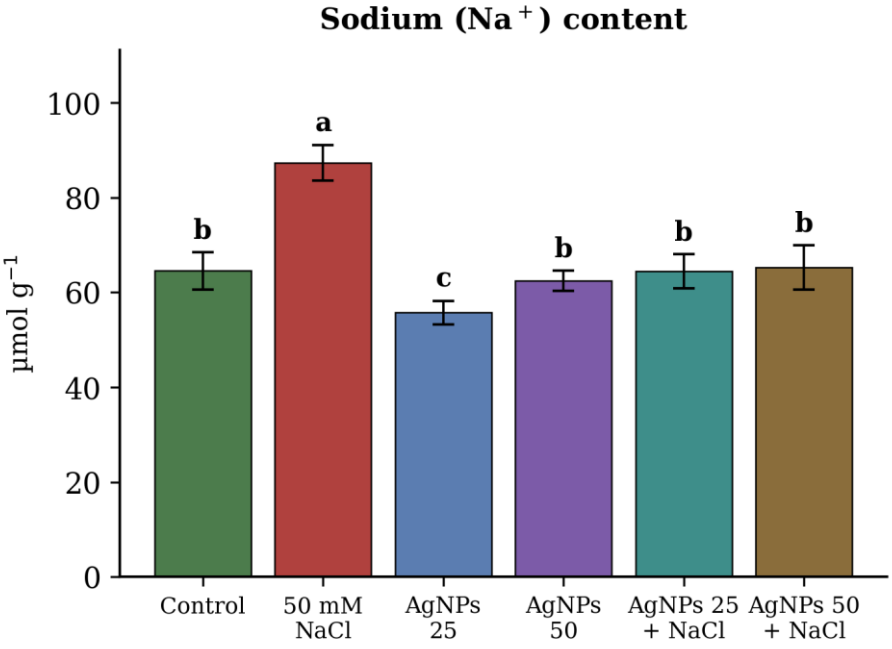


Figure 9. Sodium (Na^+) content of in vitro strawberry shoots under AgNPs and NaCl treatments. Bars are means ± SD (n = 3) different letters indicate significant differences (DMRT $P < 0.05$).

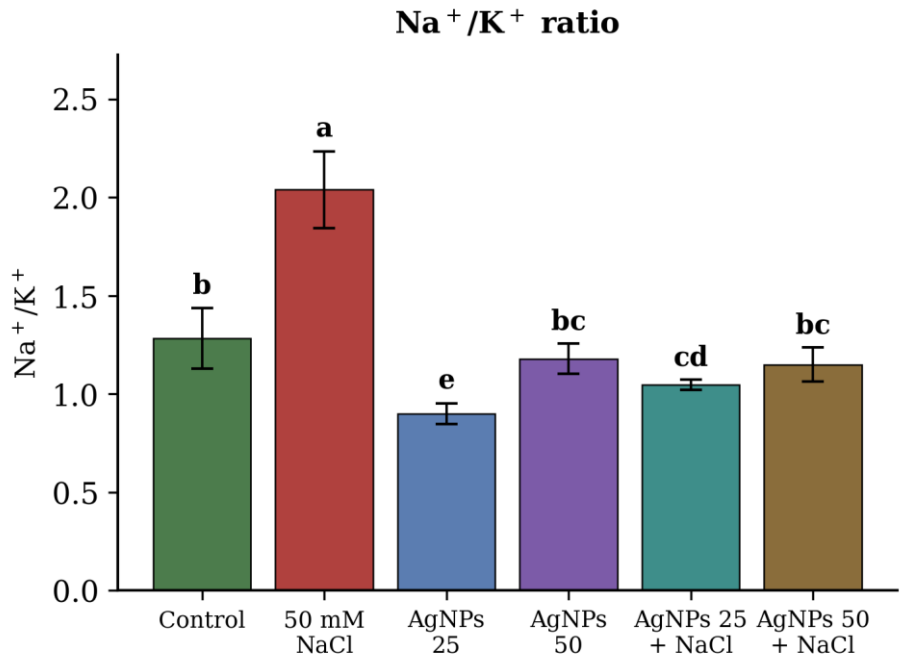


Figure 10. Na^+ /K^+ ratio of in vitro strawberry shoots under AgNPs and NaCl treatments. Bars are means ± SD (n = 3) different letters indicate significant differences (DMRT P < 0.05).

3.5 Photosynthetic pigments

Chlorophyll a was significantly affected by treatment (F = 11.35; P < 0.001 Figure 11) the control recorded 1.27 mg g-1 FW salt stress reduced it to 0.90 and the AgNPs treatments clustered between 0.91 and 1.03 with 50 µM AgNPs + NaCl slightly higher (1.03) than the other AgNPs treatments. Chlorophyll b was also treatment-dependent (F = 7.52; P < 0.01 Figure 12) declining from 0.222 in the control to 0.145 under NaCl with AgNPs treatments maintaining 0.181-0.191. AgNPs thus buffered the salt-induced loss of photosynthetic pigments consistent with the greener phenotype of the protected cultures. The corresponding treatment means are presented in Table 4.

Table 4. Effects of silver nanoparticles (AgNPs) and NaCl on chlorophyll a and b contents in shoots of in vitro-grown strawberry cv. Sweet Charlie.

Treatment	AgNPs	NaCl	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)
control	0	0	**1.27±.14a**	**0.22±.02a**
salinity	0	50 mM	**0.90±.06b**	**0.15±.01c**
AgNPs 25 µM	25 µM	0	**0.95±.02b**	**0.18±.01b**
AgNPs 50 µM	50 µM	0	**0.91±.03b**	**0.18±.01b**
AgNPs 25 µM	25 µM	50 mM	**0.91±.07b**	**0.19±.02b**
AgNPs 50 µM	50 µM	50 mM	**1.03±.05b**	**0.19±.01b**

Note: Values are means ± standard deviation (n = 3). Means within a column followed by different letters differ significantly according to Duncan's multiple range test (P < 0.05).

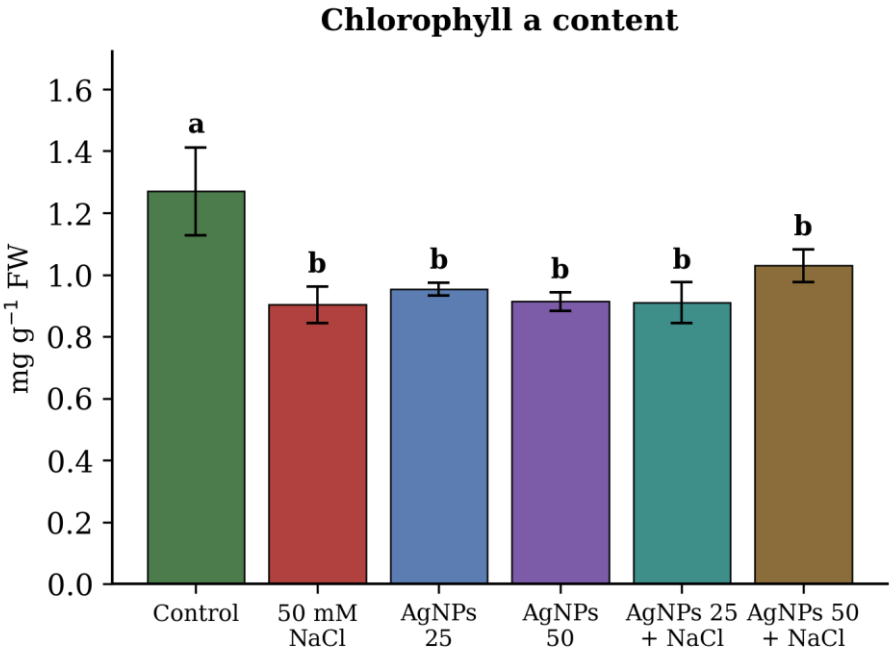


Figure 11. Chlorophyll a content of in vitro strawberry shoots under AgNPs and NaCl treatments. Bars are means ± SD (n = 3) different letters indicate significant differences (DMRT P < 0.05).

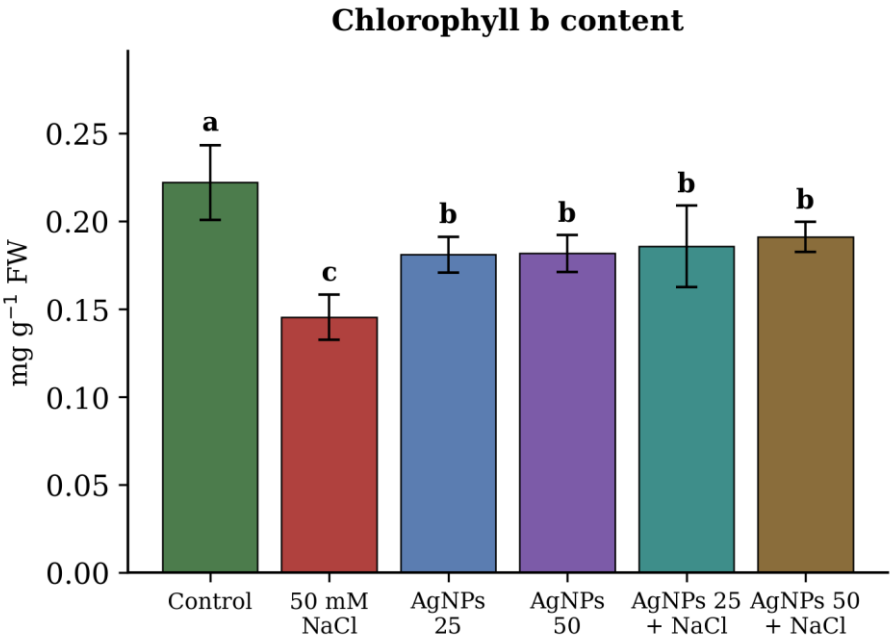


Figure 12. Chlorophyll b content of in vitro strawberry shoots under AgNPs and NaCl treatments. Bars are means ± SD (n = 3) different letters indicate significant differences (DMRT P < 0.05).

3.6 Analysis of variance

Analysis of variance confirmed significant treatment effects for all measured traits ($P < 0.01$ or $P < 0.001$). Treatment means, standard deviations, and Duncan's multiple range test groupings are presented in (Tables 1-4).

4. Discussion

The results obtained in this study provide clear evidence that silver nanoparticles when supplied at an appropriately low concentration in the culture medium substantially improve the performance of in vitro strawberry cultures exposed to NaCl stress. The three lines of defense measured here growth maintenance antioxidant activation and ion homeostasis control responded in a coordinated fashion and the magnitude of the protection was agronomically meaningful: cultures receiving 25 µM AgNPs together with 50 mM NaCl matched or exceeded the non-saline control for shoot number and fresh weight.

The hermetic dose response observed between 25 and 50 µM is consistent with the wider nano literature. Stimulatory effects of silver nanoparticles at low concentrations and inhibitory or neutral effects at higher concentrations have been reported repeatedly (Siddiqi and Husen 2022; Almutairi 2016) and our data place strawberry firmly within this pattern. The 25 µM dose was stimulatory even without salt raising shoot fresh weight by 43% above the control whereas 50 µM was growth-neutral alone and only partially protective under salinity. This behaviour argues against a simple nutritional effect and supports the interpretation of AgNPs as a signal-modulating elicitor whose benefit is confined to a narrow concentration window.

The ion-balance data offer the most direct explanation for the growth protection. Salt stress alone doubled the shoot Na^+ /K^+ ratio driven by both Na+ accumulation (87.34 µmol g-1) and K^+ depletion (42.96 µmol g-1) The 25 µM AgNPs + NaCl treatment compressed the ratio to 1.05 by holding Na^+ to 64.43 and K^+ to 61.52 µmol g-1. A low Na^+ /K^+ ratio is a canonical hallmark of salt tolerance (Wahid et al. 2020) and its restoration here mirrors the AgNPs induced ion homeostasis reported in wheat (Wahid et al. 2020) pearl millet (Khan et al. 2020 2021) and quinoa (Shibli et al. 2022). The mechanism may involve nanoparticle-mediated modulation of membrane transporter activity and of stress hormone signalling since AgNPs exposure has been shown to alter abscisic-acid dynamics in salt stressed wheat (Wahid et al. 2020) and silver ions are long known to interfere with ethylene perception indirectly prolonging the growth promoting phase under stress.

Antioxidant defense formed the second pillar of protection. NaCl alone roughly doubled POD activity and raised CAT activity by 67% relative to the control confirming that in vitro strawberry shoots mount a substantial oxidative response to salinity as also documented in strawberry by Mozafari et al. (2019) The combined AgNPs + NaCl treatments sustained CAT at the highest values recorded (189.02 mU mg-1 protein at 25 µM) while POD remained well above control levels. Enhanced CAT and POD activity under AgNPs treatment is among the most reproducible findings in the AgNPs literature across species (Khan et al. 2020, Kesbi et al. 2025, Afridi et al. 2023) and our results extend it to a horticultural in vitro system Because CAT and POD jointly dismantle hydrogen peroxide their elevated activity in the protected treatments implies a reduced steady state ROS burden which is consistent with the better preserved chlorophyll and the absence of the chlorosis seen in NaCl only cultures.

Proline behaviour adds a third osmotic dimension. Salt stress raised shoot proline by 37% and the combined AgNPs + salt treatment held it at control level rather than allowing the excessive accumulation associated with severe injury. Proline serves both as an osmolyte and

as a ROS scavenger and redox buffer and nanoparticle treatments have repeatedly been shown to modulate its accumulation under salinity (Khan et al. 2020, Alabdallah and Hasan 2021). The moderate proline pool in the AgNPs protected shoots therefore indicates effective osmotic adjustment without the cost of runaway stress metabolism.

The pigment data complete the physiological picture. Chlorophyll a and b both declined under NaCl reflecting photooxidative damage to the photosynthetic apparatus and the AgNPs treatments buffered this decline. Given that in vitro shoots depend entirely on exogenous sugar for carbon but still require functional chloroplasts for Vigor and acclimatization pigment protection is directly relevant to the quality of micro propagated plants at the ex vitro transfer stage.

Comparison with related work on strawberry strengthens the credibility of these findings. Abu Zeid et al. (2023) reported that ZnO nanoparticles at a moderate dose improved the in vitro growth ion balance and leaf anatomy of strawberry cultivars Camaros and Sweet Charlie under NaCl stress and that the response was likewise dose dependent our results with AgNPs reproduce that dose dependence and the ion-homeostasis component in the same cultivar background. Mozafari et al. (2018) obtained positive responses of strawberry explants to iron nanoparticle and salicylic acid application under salinity and Mozafari et al. (2019) documented genotype dependent morpho-physiological and biochemical responses to salinity in this crop. The present study is to our knowledge the first to quantify AgNPs mediated salinity protection across growth antioxidant osmotic ionic and pigment traits simultaneously in in vitro strawberry.

Several limitations qualify these conclusions The experiment was conducted with a single cultivar a single salt level and two nanoparticle concentrations so the dose response surface remains only partially mapped; the nanoparticle size distribution and aggregation state in the autoclaved medium were not characterized although both are known to influence bioavailability (Siddiqi and Husen 2022); and molecular endpoints transcriptomic or hormonal were beyond the scope of the work. Follow up studies should test additional cultivars and salt levels characterize the nanoparticles physiochemically and examine the expression of ion transporters and antioxidant pathway genes following the transcriptomic approaches already demonstrated for AgNPs treated cereals (Khan et al. 2023,Yan et al. 2023). Ex vitro validation on saline substrates would establish whether the in vitro protection translates to whole plant performance.

From an applied perspective the findings are promising for certified strawberry nursery production. Micropropagation laboratories routinely struggle with latent salinity in water sources and media components a low dose AgNPs amendment that stabilizes shoot proliferation and rooting under such conditions while simultaneously suppressing microbial contamination through the well documented antimicrobial action of silver could simplify quality control at negligible material cost. The clear superiority of the 25 µM dose over 50 µM also supplies a practical calibration point since nanoparticle benefits in plant tissue culture are frequently lost through overdosing.

5. Conclusion

Silver nanoparticles at 25 µM supplied in the culture medium markedly improved the salt tolerance of in vitro-grown strawberry cv. Sweet Charlie under 50 mM NaCl. The protection operated through three complementary mechanisms preservation of K^+ uptake and suppression of the Na^+ /K^+ ratio to control values sustained activation of catalase and peroxidase together with balanced proline accumulation; and buffering of chlorophyll loss. Shoot proliferation and fresh weight under combined AgNPs + NaCl treatment reached or exceeded those of non stressed controls. The higher dose (50 µM) was clearly less effective confirming a hermetic response. Silver nanoparticles therefore represent a practical low dose nanotechnology option for stabilizing strawberry micropropagation under saline conditions and merit evaluation across cultivars salt levels and ex vitro production systems.

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