

SELENIUM NANOPARTICLE PROTECTED STRAWBERRY AGAINST SALT STRESS THROUGH MODIFICATIONS IN SALICYLIC ACID, ION HOMEOSTASIS, ANTIOXIDANT MACHINERY, AND PHOTOSYNTHESIS PERFORMANCE

REZA SOLEYMANZADEH¹, ALIREZA IRANBAKSH^{1*}, GHADER HABIBI²
AND ZAHRA ORAGHI ARDEBILI³

¹Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

²Department of Biology, Payame Noor University (PNU), Tehran, Iran

³Department of Biology, Garmsar Branch, Islamic Azad University, Garmsar, Iran

Received September 24, 2019; revision accepted December 18, 2019

Since plant responses to selenium nanoparticles (nSe) had not been clarified, this study was carried out to evaluate the effects of nSe (10 and 100 μM) on photosynthesis performance, ion homeostasis, antioxidant system, and phenylpropanoids in strawberry exposed to salt stress. Inductively Coupled Plasma-Mass Spectroscopy analyses indicated that foliar-applied nSe can be taken up by leaves and trans-located to roots. Salinity led to an increase in Na concentration and reductions in Ca and K contents which were relieved by the nSe applications. Moreover, the nSe treatment at 10 μM alleviated the NaCl-induced lesion to PSII functioning, contributing to improvement in water-splitting complex (F_v/F_o) under salinity. The exposure to nSe at a concentration of 100 μM exhibited a moderate stress, determined by the increases in hydrogen peroxide (H_2O_2) and lipid peroxidation rate (membrane integrity index). The nSe10 treatment increased catalase activity and phenylpropanoid derivatives contents (salicylic acid, catechin, and caffeic acid) and decreased the content of oxidants under salinity condition. Consequently, nSe utilization at a suitable dose can be an effective method to alleviate signs of salt stress via improvements in photosynthesis, ion homeostasis, photosynthesis performance, salicylic acid (a vital signaling defensive hormone), and antioxidant machinery.

Keywords: nutrition, phenylpropanoid derivatives, photosynthesis, salicylic acid, selenium nanoparticle

INTRODUCTION

Among various physicochemical stresses, salinity is a highly serious issue that restricts growth and yield of crops (Jiang et al., 2017). It is worth noting that reactive oxygen species (ROS) can provoke significant harm to the membrane structure, macromolecules, and photosynthetic pigments, resulting in considerable decreases in photosynthetic capacity. Salt stress is associated with

impairment in photosynthesis events through prevention of photosynthetic electron transport activities, chlorophyll degradation, oxidative stress, and damages in the membrane integrity (Mittal et al., 2012; Jiang et al., 2016).

Human need to Se as an essential micronutrient is estimated to be 55 $\mu\text{g day}^{-1}$ (Hu et al., 2018). Taking human needs into account, enrichment of agricultural products with Se without reducing plant yield efficiency is of particular

* Corresponding author, e-mail: iranbakhsh@iau.ac.ir

importance (Mimmo et al., 2017). Previous studies have manifested that Se supplementation at low concentrations may enhance plant growth, physiology, and protection (Diao et al., 2014; Zhu et al., 2017; Djanaguiraman et al., 2018; Hernández et al., 2019; Quiterio-Gutiérrez et al., 2019). However, classification of Se as a plant's essential micronutrient and its phytotoxicity are controversial issues which are being disputed. Moreover, Se at higher levels may trigger oxidative burst and exhibit a phytotoxic effect (Lima et al., 2018). Furthermore, it has been stated that Se pretreatment may improve postharvest life of fruits via delaying fruit ripening process (Zhu et al., 2017). Some convincing research confirmed that Se has significant potency to ameliorate salt-induced stress via modifying antioxidant system and maintaining photosynthetic capacity (Diao et al., 2014; Jiang et al., 2017).

Taking account of nanotechnology, sustainable agriculture, and environmental issues, it is essential to study the different ways in which nanoproducts may modify plant growth, development, and metabolism. Nowadays, Se nanoparticle (nSe) has been introduced as highly stable nano-elemental Se for application in medicinal industry and as a fertilizer in agricultural and food industries (El-Ramady et al., 2016; Babajani et al., 2019). It has higher biological activity and lower toxicity than selenite or selenate (Prasad et al., 2013; Djanaguiraman et al., 2018). Recently, it has been reported that the spray of nSe protected sorghum plants exposed to high-temperature stress by improved antioxidative defense mechanisms (Djanaguiraman et al., 2018). Also, there is molecular evidence that nSe modulated the expression rates of heat shock factor A4A (HSFA4A) gene (an apoptotic agent) in *Triticum aestivum* (Safari et al., 2018). The nSe treatment exhibited no phytotoxic effects on photosynthetic machinery in *Nicotiana tabacum* (Zsiros et al., 2019). Babajani et al. (2019) found that nSe acted as an elicitor to trigger secondary metabolism in *Melissa officinalis*. However, the effects of exogenous nSe supply on photosynthesis apparatus, antioxidant system, and secondary metabolism in plants counteracted with salinity remains elusive. To address this issue, we evaluated the biochemical strategies by which nSe may modify the plant growth, photosynthesis, antioxidative system, phenolic metabolism, and ion homeostasis in NaCl-treated strawberry (*Fragaria × ananassa*) plants. Considering the importance of Se bio-fortification toward human intake,

strawberry has been introduced as a suitable target without significant impairments in plant growth and yield characteristics (Mimmo et al., 2017). Strawberry plant is a crop sensitive to salts, and soil salinity as a key limiting factor which reduces its growth and productivity; therefore, understanding of the mechanism of strawberry plant response to exogenous nSe under salt toxicity may be crucial for sustainable strawberry production. Herein, we evaluate the possible benefits of nSe pretreatment on growth and physiology of NaCl-treated strawberry plants. There is especial focus on assessing the phytotoxicity of nanoparticles, while quantitative ways for determining nanoparticles in plant cells have not been considered. Hence, in the current research, the uptake and accumulation rates of nSe were quantified by Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) method. We hypothesize that nSe can mitigate the risk associated with salinity conditions. Therefore, the main objective of the current research was to investigate the effectiveness of nSe in alleviation of toxicity signs of NaCl in strawberry.

MATERIALS AND METHODS

PLANT MATERIAL AND TREATMENTS

Strawberry (*Fragaria × ananassa* Duch. cv. 'Gaviota') plants at the same development stage were chosen and kept in a greenhouse located near the city of Malekan (relative humidity: 55–58%; temperature: 20–30/17–19°C; light intensity: 350–400 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The plants were grown in pots containing sandy loam soil (pH 7.1; EC 1.07 dS m^{-1} ; field capacity (FC) 20%) and irrigated with water every 4 days to keep humidity at 90% FC. NH_4NO_3 (200 mg kg^{-1} soil) and KH_2PO_4 (62.5 mg kg^{-1} soil) were added. Afterwards, pretreatments were started, and nSe (Iranian Nanomaterials Pioneers, Mashad, Iran) at three concentrations (0, 10, and 100 μM) was foliarly applied two times with a week interval. The size of the elemental nSe was 10–45 nm with a corresponding surface area of 30–50 $\text{m}^2 \text{g}^{-1}$, density of 3.89 g cm^{-3} , 99.95% purity, and spherical morphology.

The volume of the spray was 200 ml per plot. Tween 20 was utilized as surfactant. At pretest experiments, the effects of different concentrations of NaCl ranging from 1, 4, 5, 7, 8, and 16 g kg^{-1} were examined. Then, based on the findings, the concentrations having a 50% inhibition rate were

chosen for the main experiment. At day 7 after the nSe pretreatments, the plants were subjected to two salinity levels. NaCl was applied to acquire the EC of 5 dS m⁻¹. To avoid osmotic shock, NaCl was gradually utilized during 4 days. The EC amount in the control soil was 1.07 dS m⁻¹. Ten days after the salinity treatment, the treated plants were subjected to physiological determinations.

CHLOROPHYLL A FLUORESCENCE MEASUREMENT

Chlorophyll *a* (Chl *a*) fluorescence transients (*OJIP* transients) were estimated with a Packet-PEA chlorophyll fluorimeter (Plant Efficiency Analyser, Hansatech Instruments Ltd., King's Lynn, Norfolk, PE 32 1JL, England). Moreover, the *JIP*-test was applied for analyzing fluorescence in Chl *a*. The following characteristics based on the *JIP*-test were represented (Strasser et al., 2004):

- F_v/F_m – the maximum photosystem II (PSII) photochemical efficiency, i.e., the maximum quantum yield of primary photochemistry;
- F_m or F_{max} is maximum fluorescence intensity determined when all reaction centers (RCs) in PSII are closed;
- F_v is variable Chl fluorescence ($F_m - F_o$); F_o – minimum fluorescence (nearly all RCs located in PSII are open);
- F_v/F_o – the efficiency in the water-splitting complex on PSII (the donor side); F_v – variable Chl fluorescence ($F_m - F_o$) and F_o – minimal fluorescence; nearly all RCs in PSII are open;
- PI_{abs} – the performance index was calculated as: $(RC/ABS) \times (\varphi_{Po}/(1 - \varphi_{Po})) \times (\psi_o/(1 - \psi_o))$; ABS – absorption flux; φ_{Po} – maximum quantum yield for primary photochemistry; ψ_o – the quantum yield of electron transport.

ASSAY OF PHENYLALANINE AMMONIA-LYASE (PAL) ACTIVITY AND RELATED METABOLITES

To extract the enzyme, a leaf sample was homogenized in 50 mM sodium phosphate buffer (pH 7.0) supplemented with 2% (w/v) polyvinylpyrrolidone (PVPP), 18 mM β -mercaptoethanol, 2 mM EDTA, and 0.1% (v/v) Triton X-100. Formation of cinnamic acid was recorded by a spectrophotometer at 290 nm based on the method previously described by Zucker (1965). The PAL

activity was estimated according to the production rate of cinnamate. In addition, total soluble phenolics were quantified accordingly using the Folin-Ciocalteu reagent method and a standard curve of Gallic acid.

MEASUREMENTS OF CA, NA, K, AND SE CONTENTS

0.5M HCl solvent was utilized to resolve the prepared ash (dry-ash method; 550°C for 8 h). Ca, Na, K, and Se concentrations were estimated based on Inductively-Coupled Plasma-Atomic Emission Spectrometry (ICP-AES; INTEGRA XL2, GBC; Australia).

ASSAY OF ANTIOXIDATIVE ENZYMES

The activities of antioxidant enzymes, including superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6) were analyzed according to methods reported previously (Habibi and Hajiboland, 2012).

LIPID PEROXIDATION AND HYDROGEN PEROXIDE (H₂O₂)

The rate of lipid peroxidation was assessed from the levels of malondialdehyde (MDA) production quantified based on the standard curve of 1,1,3,3-tetraethoxypropane (Sigma). In addition, H₂O₂ content in leaves was monitored (Velikova et al., 2000).

HPLC ANALYSIS OF PHENYLPROPANOID DERIVATIVES

The leaf methanolic extract (filtered prior to HPLC analysis) was subjected to determination of the concentrations of several phenylpropanoid derivatives through the HPLC assessment (Knauer liquid chromatography apparatus; Shimadzu HPLC instrument; a 1000 Smartline Pump, a 5000 Smartline Manager Solvent Organizer and a 2800 Smartline Photodiode Array Detector). It should be noted that an injection of the extract into an HPLC column was performed through a 3900 Smart-line Autosampler injector equipped with a 100 μ L loop. Under the running time of 55 min, phenylpropanoid derivatives were separated by 0.02% trifluoro acetic acid in water (elution A) and methanol (elution D) at a flow rate of 0.5 ml min⁻¹ (oven temperature: 20°C). The levels

of ascorbic acid, salicylic acid, catechin, and coumaric acid were finally quantified according to the standard curves of each substance.

STATISTICAL ANALYSIS

The experiments were undertaken in a complete randomized block design (RBD). Chl fluorescence evaluation was done on eight different replications ($n = 8$). The achieved data on Chl fluorescence were assessed using the PEA Plus V1.10 software. In addition, Statistical analysis was conducted by sigma stat (3.5) with the Tukey test ($P < 0.05$). Furthermore, a correlation analysis using Spearman Rank Order Correlation in sigma stat (3.5) was applied.

RESULTS AND DISCUSSION

NUTRITIONAL STATUS AND ION HOMEOSTASIS

The nSe treatments changed ionic responses of strawberry during NaCl stress (Fig. 1). Under NaCl treatment, a significant rise in Na level was recorded in leaves, which was mitigated by the nSe treatment (Fig. 1). Reductions in K and Ca concentrations were also detected in leaves of the salt-treated plants (Fig. 1). Moreover, the utilization of nSe at both 10 and 100 μM caused substantial increases in the Ca and K contents, and a decrease in Na in leaves under salt stress (Fig. 1). In line with our results, nSe improved the nutritional status of K, Fe, and Zn in *Melissa officinalis* (Babajani et al., 2019). The same results were recorded by Jiang et al., (2017) in maize exposed to salinity. It should be noted that reinforcement in cellular essential nutrients, like Fe and S has been mentioned as an important mechanism through which Se may improve plant growth and protection. Moreover, it has been stated that Se up-regulates expression of genes implicated in sulfur assimilation process and stress in hyper-accumulator species (Lima et al., 2018). However, the high levels of Se in plants and method-dependent manners may result in toxicity, mainly owing to its resemblance to sulfur and generation of nonfunctional biological molecules as well as oxidative stress (Lindblom et al., 2013; Babajani et al., 2019). As the Se element was traced in both leaves and roots, the uptake and transport of nSe from leaves to roots, most probably via phloem conducting tissue, were

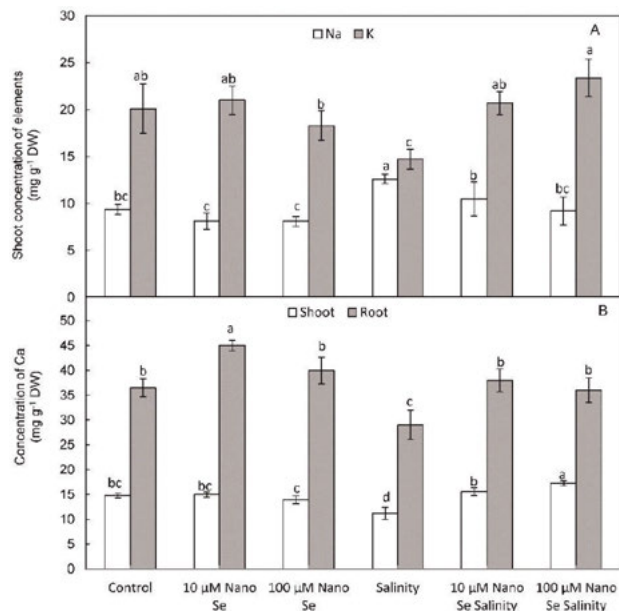


Fig. 1. The nSe-associated changes in the concentration of Na, K (A), and Ca (B) in strawberry plants exposed to two salinity levels. Error bars indicate the standard deviation.

manifested (Table 1). In this experiment, detecting Se in control plants exhibited the presence of mineral Se in the applied local soil. It has been reported that strawberry has high potency to accumulate Se (Mimmo et al., 2017). Therefore, the nSe-mediated enhancement in the nutritional status may be considered as a critical mechanism contributing to mitigation of the toxicity sign of salinity in strawberry. Since nSe has an excellent bioavailability and low toxicity for rectifying crop productivity (Prasad et al., 2013; Quiterio-Gutiérrez et al., 2019), nSe was utilized to reduce negative impacts of salt stress in strawberry.

OXIDATIVE STRESS AND ANTIOXIDANTENZYMES

It is well documented that salinity causes ROS accumulation in plants, resulting in membrane lipid peroxidation and ion leakage (Jiang et al., 2017). In this study, short-term salt stress caused oxidative stress in strawberry plants, based on the H_2O_2 and MDA accumulation (Fig. 2). The plants supplemented with nSe at a concentration of 100 μM exhibited an extreme stress for strawberry, as demonstrated by accumulation of MDA which was similar to that observed in salinity treatment (Fig. 2). Consequently, nSe of 10 μM was a more efficient treatment

TABLE 1. Concentration of Se in strawberry plants under two salinity levels.

| Treatment | | Leaf Se content (mg kg ⁻¹ DW) | Root Se content (mg kg ⁻¹ DW) |
|-----------|----------------|---|---|
| -Salinity | 0 μM Nano Se | 0.10±0.05 ^c | 0.13±0.01 ^c |
| | 10 μM Nano Se | 0.70±0.02 ^b | 0.21±0.03 ^b |
| | 100 μM Nano Se | 0.90±0.03 ^a | 0.38±0.02 ^a |
| +Salinity | 0 μM Nano Se | 0.12±0.01 ^c | 0.10±0.03 ^c |
| | 10 μM Nano Se | 0.68±0.03 ^b | 0.20±0.02 ^b |
| | 100 μM Nano Se | 0.87±0.05 ^a | 0.37±0.05 ^a |

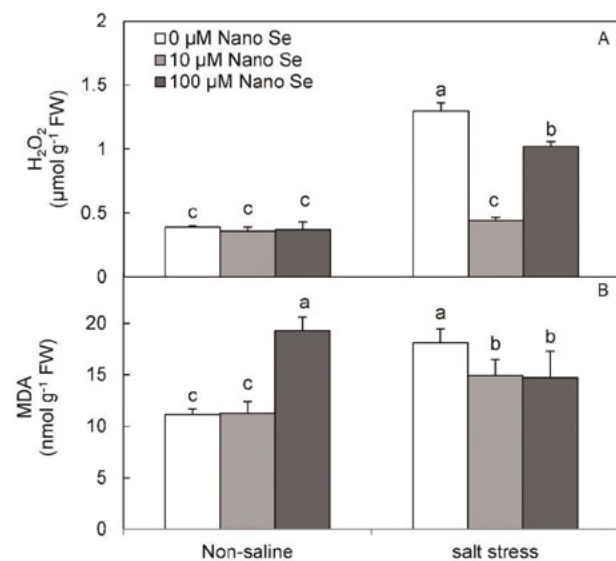


Fig. 2. Effects of nSe on the concentration of hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) in strawberry plants under salt stress. Error bars indicate the standard deviation.

than 100 μM in the alleviation of salt stress. Pervious literature demonstrated that Se may enhance the activity of antioxidant enzymes and tolerance of plants subjected to various stresses (Djanaguiraman et al., 2018; Safari et al., 2018; Babajani et al., 2019; Hernández et al., 2019; Quiterio-Gutiérrez et al., 2019). Under salt stress, nSe improved CAT activities in the nSe-treated plants compared with untreated controls (Tab. 2). These observations were consistent with the results of Djanaguiraman et al. (2018) in sorghum plants exposed to high temperature. Since CAT predominantly contributes to remedy oxidative stress,

improvement in activity of this enzyme corresponds to a considerable decrease in the damage to cell membranes in the Se-applied samples under salt stress when compared to salinity control. However, the SOD activities were not found statistically significant (Table 2), which may be occurred later. As molecular evidence, the nSe application modified the expression rates of HSFA4A gene (a heat shock factor) in *Triticum aestivum* which is known as an apoptotic factor and has close relation with MAPK signaling cascade and the antioxidant system (Safari et al., 2018). Thus, the application of nSe at 10 μM exhibited advantages to ameliorate toxicity signs of salt stress through improvement of antioxidant machinery.

PAL ACTIVITY AND PHENYLPROPANOID DERIVATIVES

The exogenous nSe application at a concentration of 10 μM provoked a rise in leaf soluble phenolics as well as in the PAL activity under salt stress conditions (Fig. 3). Babajani et al. (2019) addressed the nSe-mediated upregulations in transcriptions of genes involved in secondary metabolism in *Melissa officinalis*. These modifications in phenolic compounds may exhibit the antioxidant capacity and efficiency of radical scavenging process, thereby reducing lipid peroxidation of cellular membrane (Chu et al., 2010). Moreover, salicylic acid, a well-known signaling bioactive hormone, is derived from the phenylpropanoid pathway. It has been well illustrated that salicylic acid protects plant cells against diverse stress conditions. Therefore, we aimed to evaluate the nSe10- associated changes in the profile of phenols. We found that increases in

TABLE 2. Effects of nSe on the activities of superoxide dismutase (SOD) and catalase (CAT) enzymes in strawberry counteracting salt stress. Measurements were performed 10 days after the salt treatment. Data of each column indicated by the same letter are not significantly different ($P < 0.05$, Tukey test). Values are the mean \pm SD.

| Treatment | | SOD activity (U mg ⁻¹ protein) | CAT activity (μ mol mg ⁻¹ protein min ⁻¹) |
|-----------|---------------------|--|--|
| -Salinity | 0 μ M Nano Se | 2.12 \pm 0.11 ^a | 0.61 \pm 0.18 ^c |
| | 10 μ M Nano Se | 2.09 \pm 0.16 ^a | 0.58 \pm 0.07 ^c |
| | 100 μ M Nano Se | 2.29 \pm 0.18 ^a | 1.45 \pm 0.14 ^b |
| +Salinity | 0 μ M Nano Se | 2.20 \pm 0.18 ^a | 1.49 \pm 0.22 ^b |
| | 10 μ M Nano Se | 1.94 \pm 0.20 ^a | 2.12 \pm 0.56 ^a |
| | 100 μ M Nano Se | 2.07 \pm 0.21 ^a | 1.40 \pm 0.23 ^b |

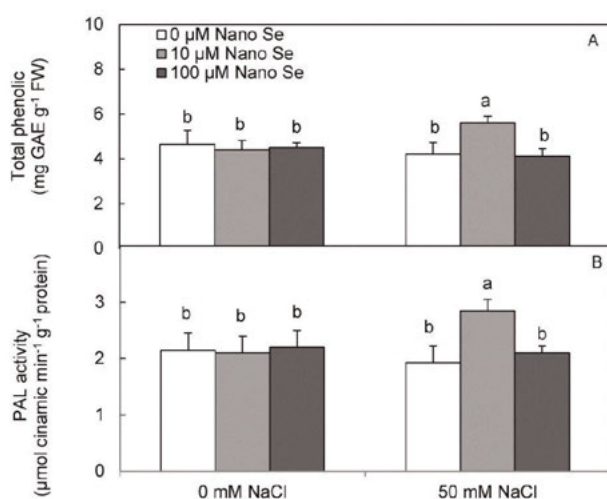


Fig. 3. Effects of nSe on the content of leaf phenolic, anthocyanin and flavonoids, and the activity of phenylalanine ammonia-lyase (PAL) in strawberry under salt stress. Error bars indicate the standard deviation.

specific phenols like salicylic acid, catechin, and caffeic acid occurred in response to salinity exposure, while total phenols were slightly changed. The differential HPLC chromatograms of treatment groups clearly confirmed the modifications in phenylpropanoid derivatives in response to nSe and/or salinity treatments. The nSe10 treatment led to slight (but not significant) increases in ascorbate content by 5%, compared to the untreated control (Table 3). The nSe10 supplementation caused a drastic augmentation in catechin concentration by 2.67 folds when compared to the control (Table 3). The individual salinity

treatment also increased catechin concentration by 51% in comparison to the control, while this rate reached up to 2.2 folds in nSe10 + salinity group (Table 3). The salinity, nSe10, and nSe10 + salinity groups exhibited slightly higher amounts of coumaric acid by a mean of 17%, compared to the control (Table 3). The nSe utilizations in both non-saline and saline conditions drastically improved the concentrations of salicylic acid (Table 3). Hereby, the convincing evidence is provided on the nSe-mediated modifications in the concentrations of catechin, coumaric acid, and salicylic acid. Among these metabolites, salicylic acid is known as a vital hormone-like compound which effectively contributes to activation and modulations of the defense-related system (especially antioxidant machinery) and stress-responsive genes. Therefore, the nSe-associated changes in salicylic acid may be considered as an underlying mechanism through which the nSe application at optimum concentrations may mitigate the toxicity signs of abiotic stresses. Several lines of evidence indicated that salicylic acid displayed outstanding potency to alleviate risks associated with salinity stress (Palma et al., 2013; Sheteiwy et al., 2019). In line with our results, the foliar application of nSe led to induction in PAL activity in peppermint (Nazerieh et al., 2018) and tomato (Hernández et al., 2019)

PHOTOSYNTHESIS PERFORMANCE

The results revealed that the nSe10 treatment improved photosystem performance under salinity condition (Fig. 4). The maximum quantum yield of

TABLE 3. Effects of nSe on the content of phenolic compounds in strawberry under salt stress. Measurements were performed 10 d after salt treatment. Data of each column indicated by the same letter are not significantly different ($P < 0.05$, Tukey test). Values are the mean \pm SD.

| | Ascorbate | Catechin | Caffeic acid | Coumaric acid | Salicylic acid |
|------------------------------|-------------------------------|------------------------------|------------------------------|------------------------------|-------------------------------|
| Control | 1.25 \pm 0.05 ^a | 0.83 \pm 0.04 ^d | 0.77 \pm 0.01 ^b | 0.52 \pm 0.01 ^b | 0.31 \pm 0.005 ^c |
| Salinity | 1.18 \pm 0.001 ^a | 1.26 \pm 0.09 ^c | 0.79 \pm 0.02 ^b | 0.60 \pm 0.06 ^a | 0.33 \pm 0.005 ^c |
| 10 μ M Nano Se | 1.31 \pm 0.01 ^a | 2.22 \pm 0.09 ^a | 0.93 \pm 0.05 ^a | 0.62 \pm 0.01 ^a | 1.13 \pm 0.006 ^a |
| 10 μ M Nano Se +Salinity | 1.08 \pm 0.28 ^a | 1.84 \pm 0.03 ^b | 0.82 \pm 0.08 ^b | 0.61 \pm 0.01 ^a | 0.67 \pm 0.02 ^b |

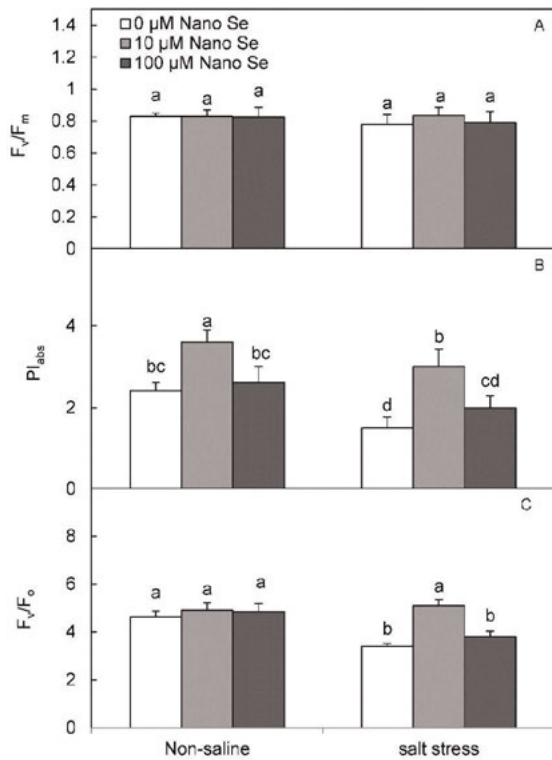


Fig. 4. Effects of nSe on the maximum quantum yield of PSII (F_v/F_m), the Performance Index (PI_{abs}), and the efficiency of the water-splitting complex on the donor side of PSII (F_v/F_o) in strawberry under salt stress. Error bars indicate the standard deviation.

PSII (F_v/F_m) was not influenced by NaCl stress (Fig. 4). The photosystem performance index (PI_{abs}) and the efficiency of the water-splitting complex located on the donor side of PSII (as inferred from F_v/F_o) exhibited a larger decrease under salt-stress conditions, which may have resulted from the negative impact of stress on this section of the

electron transport chain. However, the application of 10 μ M nSe alleviated these adverse effects under salinity. In contrast, the nSe application at a concentration of 100 μ M had no significant effect on the photosystem performance index and the efficiency of the water-splitting complex under salt stress. The Se-induced improvement of photosynthesis in tomato plants counteracting with salinity has also been reported (Diao et al., 2014). Se had considerable potency to maintain ultrastructure of chloroplast and mitochondria through which it improved photosynthesis capacity and acclimation to salt stress in sorrel plant (Kong et al., 2005). The nSe exhibited beneficial effect toward photosystem performance in sorghum plants under high-temperature stresses (Djanaguiraman et al., 2018). Under non-saline conditions, the nSe pretreatment increased Chl fluorescence in the I-P section of the induction curve (Fig. 5a), which may be connected with the decrease of electron transporters of the PSI acceptor side, including intermediary acceptors, ferredoxin, and NADP. Under saline conditions, a general decrease in the I-P section of the induction curve of salt-stressed plants was observed (Fig. 5b), which may be related to the lesion to the reaction center and, consequently, severe decline in photochemistry. The application of nSe at 10 μ M diminished the adverse impacts of salt stress on the PSII functioning. Thus, the application of nSe at 10 μ M significantly increased the photochemical activity of strawberry leaves counteracting salt stress. It should be noted that a negative linear correlation was found between H_2O_2 content and PI_{abs} ($r = -0.68$, $P < 0.05$) as well as between H_2O_2 content and F_v/F_o ($r = -0.81$, $P < 0.01$) in plants supplemented with nSe of 10 μ M under salinity conditions (Fig. 6). The nSe treatment at 100 mg l^{-1} was not associated with

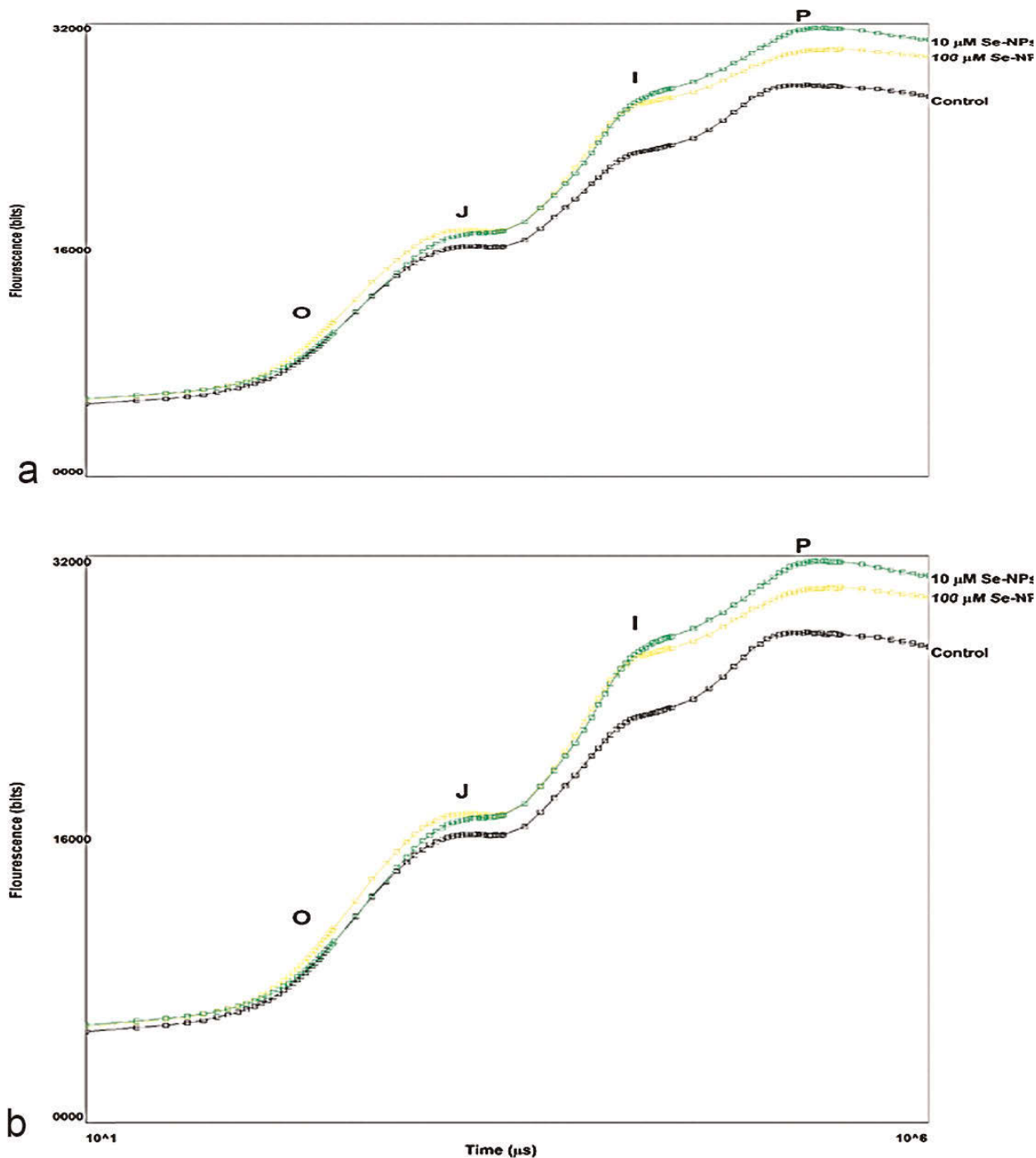


Fig. 5. Effects of nSe on the Chl *a* fluorescence induction curve of strawberry under non-saline (a) and salt stress (b) conditions. Measurements were performed 10 d after salt treatment.

impairments in the structure and function of the photosynthetic machinery in *Nicotiana tabacum* (Zsiros et al., 2019). Exogenously-applied Se improved maize resistance against salt stress via modulation of Na^+ homeostasis, promotion of photosynthetic capacity, and enhancement of the

antioxidant system (Jiang et al., 2017). Interestingly, the early toxicity signs of salinity on nutritional status and photosynthesis yield were mitigated by the nSe of 10 μ M which may be partly attributed to the nSe-associated increases in salicylic acid and induction in the antioxidant system.

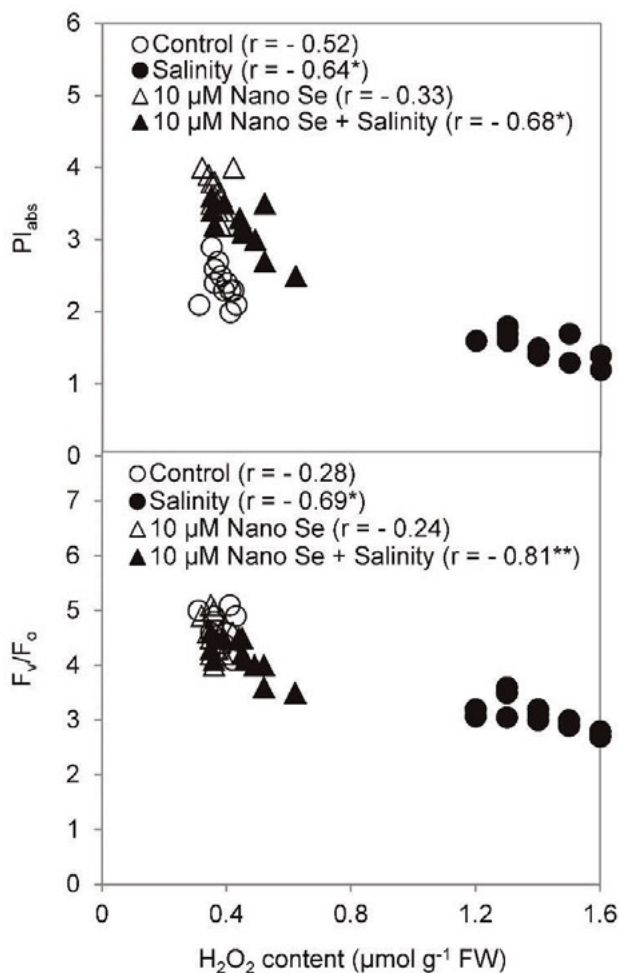


Fig. 6. Effects of nSe on the correlation between values of performance index (PI_{abs}) or efficiency of the water-splitting complex on the donor side of PSII (F_v/F_o) and hydrogen peroxide (H_2O_2) content recorded in strawberry plants grown under salinity: ns - non-significant, * and ** - significant at 5% and 1% levels of probability, respectively.

It is obvious that more convincing studies are required to elucidate the exact mechanisms involved.

CONCLUSION

Since strawberry is known as a plant species sensitive to salinity, its growth and productivity are severely decreased by salinity. In this study, the rapid changes in plant physiology following nSe pretreatment and salt stress were explored.

In contrast to nSe of 10 μM , the nSe of 100 μM and salinity caused oxidative stress. The nSe pretreatment modified the concentrations of phenylpropanoid derivatives among which salicylic acid is the most important. The photosynthesis was decreased due to salinity; however, this lesion was mitigated by application of low levels of nSe (10 μM), contributing to improvement in photosynthetic capacity by preservation of water-splitting complex, activation of the antioxidant defense system to prevent oxidative stress, and amendment of ion homeostasis under salinity stress. Overall, we have concluded that foliar spray of 10 μM nSe in strawberry is a protectant against salinity. The mechanisms for nSe to mitigate salt stress are currently unknown and need to be further explored.

AUTHOR'S CONTRIBUTION

AI, GH, and ZOA designed the experiments. RS prepared materials and performed treatments. RS and GH performed experimental analysis. ZOA and AI carried out the statistical analysis. AI, GH, and ZOA contributed to the interpretation of the findings. RS prepared the figures. AI took the lead in writing the manuscript. All authors have contributed, seen and approved the manuscript.

ACKNOWLEDGMENTS

This research was conducted at Razi laboratory department, Islamic Azad University, Science and Research Branch. The authors did not receive any grant. The authors would like to thank referees for their professional comments.

REFERENCES

- BABAJANI A, IRANBAKHSH A, ARDEBILI ZO, and ESLAMI B. 2019. Differential growth, nutrition, physiology, and gene expression in *Melissa officinalis* mediated by zinc oxide and elemental selenium nanoparticles. *Environmental Science and Pollution Research* <https://doi.org/10.1007/s11356-019-05676-z>.
- CHU J, YAO X, and ZHANG Z. 2010. Responses of wheat seedlings to exogenous selenium supply under cold stress. *Biology Trace Element Research* 136: 355–363.
- DIAO M, MA L, WANG J, CUI J, FU A, and LIU H. 2014. Selenium promotes the growth and photosynthesis of

- tomato seedlings under salt stress by enhancing chloroplast antioxidant defense system. *Journal of Plant Growth Regulation* 33: 671–682.
- DJANAGUIRAMAN M, BELLIRAJ N, BOSSMANN SH, and PRASAD PV. 2018. High-temperature stress alleviation by selenium nanoparticle treatment in grain sorghum. *ACS Omega* 3(3): 2479–2491.
- EL-RAMADY H, ABDALLA N, TAHA HS, ALSHAAL T, EL-HENAWY A, SALAH EDF, and ELHAWAT N. 2016. Selenium and nano-selenium in plant nutrition. *Environmental Chemistry Letters* 14: 123–147.
- HABIBI G, and HAJIBOLAND R. 2012. Comparison of photosynthesis and antioxidative protection in *Sedum album* and *Sedum stoloniferum* (Crassulaceae) under water stress. *Photosynthetica* 50: 508–518.
- HERNÁNDEZ-HERNÁNDEZ H, QUITERIO-GUTIÉRREZ T, CADENAS-PLIEGO G, ORTEGA-ORTIZ H, HERNÁNDEZ-FUENTES AD, CABRERA DE LA FUENTE M, VALDÉS-REYNA J, and JUÁREZ-MALDONADO A. 2019. Impact of selenium and copper nanoparticles on yield, antioxidant system, and fruit quality of tomato plants. *Plants* 8(10): 355.
- HU T, LI H, LI J, ZHAO G, WU W, LIU L, WANG Q, and GUO Y. 2018. Absorption and bio-transformation of selenium nanoparticles by wheat seedlings (*Triticum aestivum* L.). *Frontiers in Plant Science* 9: 597.
- JIANG C, CUI Q, FENG K, XU D, LI C, and ZHENG Q. 2016. Melatonin improves antioxidant capacity and ion homeostasis and enhances salt tolerance in maize seedlings. *Acta Physiologiae Plantarum* 38: 82.
- JIANG C, ZU C, LU D, ZHENG Q, SHEN J, WANG H, and LI D. 2017. Effect of exogenous selenium supply on photosynthesis, Na⁺ accumulation and antioxidative capacity of maize (*Zea mays* L.) under salinity stress. *Scientific Reports* 7: 42039.
- KONG L, WANG M, and BI D. 2005. Selenium modulates the activities of antioxidant enzymes, osmotic homeostasis and promotes the growth of sorrel seedlings under salt stress. *Plant Growth Regulation* 45: 155–163.
- LIMA LW, PILON-SMITS EA, and SCHIAVON M. 2018. Mechanisms of selenium hyperaccumulation in plants: A survey of molecular, biochemical and ecological cues. *Biochimica and Biophysica Acta (BBA) - General Subjects* 1862(11): 2343–2353.
- LINDBLOM SD, VALDEZ-BARILLAS JR, FAKRA SC, MARCUS MA, WANGELINE AL, and PILON-SMITS EA. 2013. Influence of microbial associations on selenium localization and speciation in roots of *Astragalus* and *Stanleya* hyperaccumulators. *Environmental and Experimental Botany* 88: 33–42.
- MIMMO T, TIZIANI R, VALENTINUZZI F, LUCINI L, NICOLETTO C, SAMBO P, SCAMPICCHIO M, PII Y, and CESCO S. 2017. Selenium biofortification in *Fragaria* × *ananassa*: implications on strawberry fruits quality, content of bioactive health beneficial compounds and metabolomic profile. *Frontiers in Plant Science* 8: 1887.
- MITTAL S, KUMARI N, and SHARMA V. 2012. Differential response of salt stress on *Brassica juncea*: Photosynthetic performance, pigment, proline, D1 and antioxidant enzymes. *Plant Physiology and Biochemistry* 54: 17–26.
- NAZERIEH H, ARDEBILI ZO, and IRANBAKHSH A. 2018. Potential benefits and toxicity of nanoselenium and nitric oxide in peppermint. *Acta Agriculturae Slovenica* 111(2): 357–68.
- PALMA F, LÓPEZ-GÓMEZ M, TEJERA NA, and LIUCH C. 2013. Salicylic acid improves the salinity tolerance of *Medicago sativa* in symbiosis with *Sinorhizobium meliloti* by preventing nitrogen fixation inhibition. *Plant Science* 208: 75–82.
- PRASAD S, PATEL H, PATEL T, PATEL K, and SELVARAJ K. 2013. Biosynthesis of Se nanoparticles and its effect on UV-induced DNA damage. *Colloids and Surfaces B: Biointerfaces* 103: 261–266.
- QUITERIO-GUTIÉRREZ T, ORTEGA-ORTIZ H, CADENAS-PLIEGO G, HERNÁNDEZ-FUENTES AD, SANDOVAL-RANGEL A, BENAVIDES-MENDOZA A, CABRERA-DE LA FUENTE M, and JUÁREZ-MALDONADO A. 2019. The application of selenium and copper nanoparticles modifies the biochemical responses of tomato plants under stress by *Alternaria solani*. *International Journal of Molecular Sciences* 20(8): E1950.
- SAFARI M, ARDEBILI ZO, and IRANBAKHSH A. 2018. Selenium nano-particle induced alterations in expression patterns of heat shock factor A4A (HSFA4A), and high molecular weight glutenin subunit 1Bx (Glu-1Bx) and enhanced nitrate reductase activity in wheat (*Triticum aestivum* L.). *Acta Physiologiae Plantarum* 40 (6): 117.
- SHETEVIWY MS, AN J, YIN M, JIA X, GUAN Y, HE F, and HU J. (2019) Cold plasma treatment and exogenous salicylic acid priming enhances salinity tolerance of *Oryza sativa* seedlings. *Protoplasma* 256: 79–99
- STRASSER RJ, TSIMILLI-MICHAEL M, and SRIVASTAVA A. 2004. Analysis of the fluorescence transient. In: George C, Papageorgiou C, Govindjee (eds.): *Chlorophyll fluorescence: A signature of photosynthesis. Advances in Photosynthesis and Respiration Series*, 321–362. Springer, Dordrecht
- VELIKOVA V, YORDANOV I, and EDREVA A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. *Plant Science* 151: 59–66.
- ZHU Z, CHEN Y, SHI G, and ZHANG X. 2017. Selenium delays tomato fruit ripening by inhibiting ethylene biosynthesis and enhancing the antioxidant defense system. *Food Chemistry* 219: 179–184.
- ZSIROS O, NAGY V, PÁRDUZ Á, NAGY G, ÜNNEP R, EL-RAMADY H, PROKISCH J, LISZTES-SZABÓ Z, FÁRI M, CSAJBÓK J, and TÓTH SZ. 2019. Effects of selenate and red Se-nanoparticles on the photosynthetic apparatus of *Nicotiana tabacum*. *Photosynthesis Research* 139(1-3): 449–60.
- ZUCKER M. 1965. Induction of phenylalanine deaminase by light and its relation to chlorogenic acid synthesis in potato tuber tissue. *Plant Physiology* 40: 779–778.