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# Improving the performance of the photosynthetic apparatus of *Citrus sinensis* with the use of chitosan-selenium nanocomposite (CS + Se NPs) under salinity stress

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## Abstract

**Background** Abiotic stress, such as salinity, affects the photosynthetic apparatus of plants. It is reported that the use of selenium nanoparticles (Se NPs), and biochemical compounds such as chitosan (CS) increase the tolerance of plants to stress conditions. Therefore, this study aimed to elucidate the potential of Se NPs, CS, and their composite (CS + Se NPs) in improving the photosynthetic apparatus of *C. sinensis* under salt stress in greenhouse conditions. The grafted seedlings of *C. sinensis* cv. Valencia after adapting to the greenhouse condition, were imposed with 0, 50, and 100 mM NaCl. After two weeks, the plants were foliar sprayed with distilled water (control), CS (0.1% w/v), Se NPs (20 mg $L^{-1}$), and CS + Se NPs (10 and 20 mg $L^{-1}$). Three months after treatment, the levels of photosynthetic pigments, leaf gas exchange, and chlorophyll fluorescence in the treated plants were evaluated.

**Results** Under salinity stress, total chlorophyll, carotenoid, and SPAD values decreased by 31%, 48%, and 28% respectively, and Fv/Fm also decreased compared to the control, while the ratio of absorption flux (ABS), dissipated energy flux ($DI_0$) and maximal trapping rate of PSII (TR0) to RC (a measure of PSII apparent antenna size) were increased. Under moderate (50 mM NaCl) and intense (100 mM NaCl) salinity stress, the application of CS + Se NPs significantly increased the levels of photosynthetic pigments and the Fv/Fm value compared to plants treated with distilled water.

**Conclusions** It may be inferred that foliar treatment with CS + Se NPs can sustain the photosynthetic ability of *C. sinensis* under salinity stress and minimize its deleterious effects on photosynthesis.

**Keywords** Chlorophyll fluorescence, JIP Test, Nanoparticles, Orange seedling, Plant gas exchanges, Sodium chloride

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## Background

Citrus trees belong to the Rutaceae family and grow in tropical and subtropical regions [1]. According to FAOSTAT (2022), oranges, contributing about 50% of the world's total citrus fruits. The global production of Orange fruits was 76.4 million tons in 2022. *Citrus sinensis* L. is the most popular species of *Citrus* genus in the whole world. Global production of *C. sinensis* L. is about 50% of all citrus fruit. Also, it constitutes more than 40% of global exports [2]. Sweet oranges are extensively consumed as fruit juice or fresh fruit all over the world, and they are high in antioxidant components like vitamin C, as well as other critical elements [3]. However, in recent years, gardeners have been worried about the future of citrus orchards, which are at risk of destruction, and among these factors, biotic and abiotic stressors can be mentioned [4]. Salinity is a major abiotic stress that adversely affects plant growth and development. Citrus trees are highly susceptible to salinity when compared to other garden crops since a relatively low salt level can affect root water conductivity, stomatal conductance, transpiration, and photosynthesis, resulting in decreased fruit development and production [5].

Salinity tolerance in citrus species varies depending on the rootstock type [6]. The harmful effects of salinity on agricultural productivity are due to various reasons, including natural events, inefficient irrigation methods, and excessive evaporation in arid and semi-arid regions, which cause nutritional imbalance, oxidative stress, and ionic toxicity, and ultimately growth and they limit plant production [7]. It is estimated that about 20% of the world's agricultural fields are exposed to large amounts of salt [8].

The closing of stomata in moderate and intense salinity stress is the most critical agent in limiting photosynthesis of plants. One of the most main parameters for investigating stress tolerance in plants is plant gas exchange [9]. Salinity stress has a negative and significant effect on the rate of electron transport in the donating part of photosystem II (PSII) and prevents the transport of electrons from the reaction centers to plastoquinone [10] and also reduces the interference in the electron transport chain and the photosynthesis efficiency [11]. Chlorophyll fluorescence analysis is a non-invasive photosynthesis measurement that is one of the most potent and extensively used tools for studying the influence of stressors on the photosynthetic process [12]. We may use this approach to calculate the amount of PSII response center damage induced by a stress source. As a susceptible indicator of plant photosynthetic ability, the peak quantum yield of the PSII ($F_v/F_m$) ratio has been utilized. A reduction in this index suggests a reduction in the photoinhibition effectiveness of PSII. The peak quantum yield of the PSII ($F_v/F_m$) values generally vary between 0.75 and 0.85, and this proportion is related to photochemistry's quantum efficiency [12]. There is a considerable association between plant growth and variations in chlorophyll fluorescence characteristics in response to stressors. It is also shown that under salt stress, the chlorophyll value and photosynthetic efficiency index decline [4].

Recent studies have explored the use of various bioactive compounds to mitigate the deleterious effects of salinity on crops. In response to this problem, researchers have explored the potential of various bioactive compounds, such as the trace mineral selenium (Se), to enhance the tolerance of crop plants to salinity stress. A study investigated the effects of selenium application on the physiological and biochemical responses of wheat plants under salinity stress. The results showed that exogenous selenium supplementation significantly improved the plants' antioxidant defense system, increased the activity of enzymes involved in osmoregulation, and reduced the accumulation of toxic ions, such as sodium, in the leaves. Furthermore, selenium application helped maintain photosynthetic efficiency and chlorophyll content, ultimately leading to enhanced growth and biomass production of the wheat plants under saline conditions. The authors concluded that the beneficial effects of selenium in alleviating salinity stress were primarily attributed to its role in regulating ionic homeostasis, modulating osmotic adjustment, and mitigating oxidative damage in the plants [13]. One promising approach involves the application of selenium (Se) and chitosan, two naturally occurring substances with demonstrated plant growth-promoting and stress-alleviating properties. A study by Alyafi et al. investigated the impact of selenium and chitosan on tomato plants exposed to salinity stress. The results showed that exogenous application of Se and chitosan significantly improved various physiological and biochemical parameters, including photosynthetic rate, antioxidant enzyme activity, and ion homeostasis, thereby enhancing the plants' tolerance to high salinity conditions. The authors suggested that the synergistic effects of these compounds on osmoregulation, oxidative stress management, and nutrient uptake contributed to the observed amelioration of salinity-induced damage in the tomato plants. These findings suggest that the strategic application of selenium could be a promising approach to improve the salinity tolerance of important crop species [14].

When nanoparticles are used on plants to cope with environmental problems like salinity stress, plant resistance is improved [15–17]. With the advancement of nano-technology, the use of nano-fertilizers has become popular as a better alternative to chemical fertilizers [18]. Using nano-fertilizers improves plant growth parameters and increases salinity stress resistance [19]. Selenium, on the other hand, promotes plant tolerance to salinity

stress by increasing enzyme antioxidant activity, antioxidant strength, and secondary metabolite metabolism [20]. Furthermore, Se increases the nutritional value and growth characteristics, indicating that Se is an essential element for plants [20]. Selenium NPs exhibit greater bioactivity, mobility, and solubility than the usual form of Se due to their increased surface-to-volume ratio [21].

One of the plant growth regulators and stress tolerance inducers is Chitosan or CS (2-amino-2-deoxy-β-D-glucosamine), which has recently been used in plant protection research. It is a naturally degradable semi-acetylated form of chitin that was initially discovered as a plant response stimulator because it enhances the formation of phytoalexin as a proteinase inhibitor [6]. The molecular weight of chitosan influences its physicochemical characteristics, such as crystallinity, surface charge density, and hydrophobicity of CS-based nanoparticles [21].

Our study introduces a pioneering method to enhance the photosynthetic efficiency of *C. sinensis* under saline conditions. This study investigates the application of a chitosan-selenium nanocomposite (CS + Se NPs), a novel biostimulant, to mitigate the detrimental effects of salinity stress on the photosynthetic machinery of the plant. The research highlights the synergistic properties of chitosan and selenium. Because chitosan has biocompatibility and capacity to form protective films and selenium has antioxidant capabilities and role in stress alleviation. Considering the beneficial effects of Se NPs and CS on plant development, physiological and biochemical components, and CS's capacity to act as a carrier and release control matrix, their combination is both desired and feasible. We hypothesis that Se NPs and CS can increase plant growth and development by increasing the photosynthetic system and the electron transport chain's performance. Our findings can aid in the development of guidelines for the use of NPs and CS in plants, as well as providing fresh insights into how these compounds influence plants. This innovative approach offers a potential strategy to improve crop productivity and stress tolerance in saline-affected agricultural areas.

This work aimed to understand better the mechanism of photosynthetic apparatus response to salinity stress under the use of chitosan-selenium nanocomposite. By this method, it is possible to determine the level of photosystem II (PSII) reaction centers damages caused by stress factor. Research on the efficiency of PSII in different plant species under stress conditions can help us in accurate evaluating the photosynthetic apparatus and as a result the growth and development of plants. The most accurate analysis of the effect of a stressor on plants can be made by simultaneous measurements of gas exchange and chlorophyll a fluorescence. In this way, the photochemical efficiency of plant photosynthesis can be analyzed and valuable information can be obtained about the components involved, especially PSII, in electron transport in the photosynthesis process. Photosystem II (PSII) is a part of the photosynthetic apparatus and is most sensitive to environmental stress. It plays a vital role in responding to the photosynthetic apparatus to stress conditions.

## Materials and methods

### Experimental design

This research was conducted in 2022 in the research greenhouse at the University of Jiroft, Iran. The greenhouse temperature day/night was 30/20 ± 5°C, and relative humidity was 60 ± 10%. The experiment included three levels of salinity (0, 50, and 100 mM). Five levels of foliar application were used: distilled water (control), chitosan (0.1% w/v), Se nanoparticles (20 mg $L^{-1}$), and the combination of Se NPs (10 and 20 mg $L^{-1}$) with CS.

One-year grafted seedling of sweet orange (*C. sinensis*) on sour orange (*C. aurantium*) rootstock were purchased from a certified citrus seedling production company (Khazai), and transferred to the research greenhouse of University of Jiroft, and after four weeks of adaptation to greenhouse conditions, salinity treatment was applied. Plants were watered twice a week with saline water. To avoid osmotic shock, NaCl concentration was gradually increased. To prevent salt accumulation, the pots were washed with tap water every two weeks. Two weeks after applying salinity stress, application of the experimental foliar sprays began (5 times with an interval of every three days). Freshly prepared CS, Se NPs and composite nanoparticle solutions were applied using a hand sprayer. Tween 20 was also used at a low concentration to break solution surface tension. Spray was applied to runoff. During the growing period, EC of the pot drainage was evaluated weekly, and to prevent excessive accumulation of salt, the pots were rinsed every two weeks based on the seedling's washing needs. The seedlings were stressed for 90 days. At the end of the experiment, sampling was done from fully developed leaves at the end of the branch to evaluate performance of photosynthetic apparatus.

### Preparation of CS + Se NPs

For preparation the composite of CS/Se NPs, chitosan (CS), ascorbic acid (Vit C), and $Na_2SeO_3$ was used as a stabilizing template, reducing agent, and Se source, respectively. To obtain the CS/Se NPs, 10 mL CS solution (0.1%, w/w) were dispersed in acetic acid (4% w/w) at room temperature and were stirred until completely dissolved. Then, 10 mL of $Na_2SeO_3$ (10 and 20 mg $L^{-1}$) solution was added to the stirring mixture, dropwise. After 30 min, Vit C solution (4 mL, 0.1 M) was added dropwise to the mixture reaction and stirred for another 30 min. The reaction process could be clearly monitored by

observing color changes. To prepare the CS/Se NPs composite, 10 mL of CS solution (0.1%, w/w) was dispersed in 4% acetic acid and stirred until completely dissolved. Then, 10 mL of Na2SeO3 solution (10 and 20 mg $L^{-1}$) was added dropwise. After 30 min, 4 mL of Vit C solution (0.1 M) was added dropwise and stirred for another 30 min.

#### Characterization of CS + Se NPs

Powder X-ray diffraction (XRD) patterns were investigated using Cu K radiation (wavelength = 1.54) on a Panalytical X'PertPro diffractometer (Almelo, the Netherlands). The XRD pattern of CS + Se NPs exhibited the peaks at 23.22°, 29.53°, 41.14°, 43.60°, 45.50°, 51.48°, 61.40°, 64.88°, 71.40° attributed to (100), (101), (110), (102), (111), (201), (112), (202), (210) and (113) planes of Se-NPs shown in Fig. 1A and in assent with the standard JCPDS data (JCPDS No. 06-0362). Furthermore, the large peak between 10° and 40° correlates to chitosan. SIGMA VP from Carl Zeiss Inc., Jena, Germany) was used for field emission scanning electron microscopy (FESEM) and energy-dispersive X-ray spectroscopy (EDS). The shape of CS + Se NPs was found to be spherical with a smooth surface. The particle size was determined to be between 50 and 150 nm. The presence of selenium in the CS + Se NPs was verified by EDS analysis, as shown in Fig. 1(B-C).

#### Measurement of photosynthetic pigments

The amounts of photosynthetic pigments were evaluated according to Lichtenthaler and Buschmann method [22]. Using this approach, 0.02 g of frozen leaves were pulverized in a Chinese mortar with 2 mL of 80% acetone and centrifuged at 6000 rpm for 10 min. Finally, the absorbance of the samples was measured using a spectrophotometer (UV/VIS, Perkin Elmer, USA) at 475, 645, and 663 nm.

#### Assessment greenness index (SPAD)

It was used of a Minolta SPAD-502 (Japan) leaf chlorophyll meter to assess the greenness index of developed leaves in the middle of shoots.

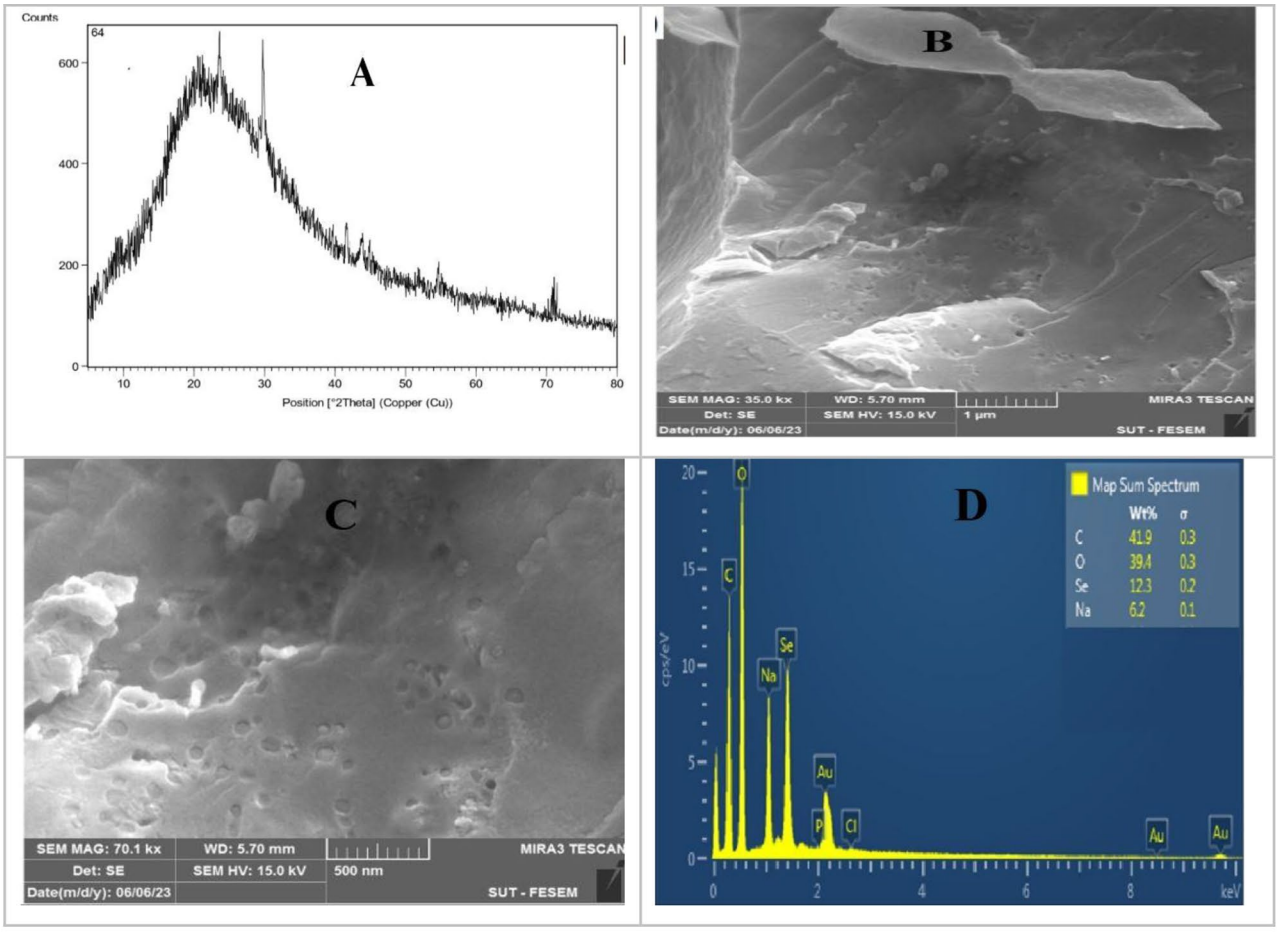


**Fig. 1** **A**: The X-ray powder diffraction (XRD) pattern of CS + Se NPs; **B** and **C**: FESEM images of CS + Se NPs; D: EDS pattern of CS + Se NPs

### Relative water content (RWC)

The RWC of the leaves was assessed using Barres's and Weatherly's technique [23]. The fresh weight (*fw*) was determined by weighing fresh completely expanded leaves immediately after harvest. The leaves were rehydrated by soaking the samples in distilled water for 24 h, weighing them (*tw*), and then oven drying them for 24 h at 60 °C to determine the dry weight (*dw*). Finally, the RWC was computed using Eq. (5):

Equation (5): RWC= ((fw-dw)/(tw-dw))×100.

### Assessment chlorophyll fluorescence apparatus

The PSII photochemical activity of *C. sinensis* leaves was measured 90 days following the application of salt stress, between 9:00 AM and 12:00 AM, using a portable photosynthetic analyzer (Pocket PEA, Hansatech instruments Ltd, Po King's Lynn, UK). The mature leaves from the middle shoots were exposed to darkness for 15 min by attaching specific clips to each upper leaf blade. The saturating light (3500 µmol $m^{-2}$ $s^{-1}$ and 650 nm) induced transient of chlorophyll fluorescence extending from $F_0$ (minimal fluorescence, when all PSII RCs are open to $F_m$ (maximal fluorescence, when all PSII RCs are closed), ($F_t$, fluorescence at time t after the start of the actinic lighting; $F_0$=F30µs, minimum fluorescence intensity; $F_j = F_{2ms}$, fluorescence intensity at the Jstep; $F_i = F_{30ms}$, fluorescence intensity at the I-step; $F_p = F_m$, maximum fluorescence intensity, at the peak P of OJIP) for all treatments. The PSII parameters from the OJIP transient were investigated [24]. The PSII parameters obtained from the OJIP transient were evaluated based on the Strasser et al. [25] methods. JIP test parameters are listed in Additional file 1.

### Assessment leaf gas exchange

The photosynthetic parameters such as stomatal conductance ($g_s$: mol $H_2O$ $m^{-2}$ $s^{-1}$), net $CO_2$ assimilation rate ($A$: µmol $CO_2$ $m^{-2}$ $s^{-1}$), transpiration rate ($E$: mmol $H_2O$ $m^{-2}$ $s^{-1}$), sub-stomatal $CO_2$ concentration ($C_i$: µmol $CO_2$ $mol^{-1}$), instantaneous carboxylation efficiency ($A/C_i$), and water-use efficiency (*WUE*: µmol $CO_2$ mol $H_2O^{-1}$), were assessed using a portable photosynthesis system (ADC-Bio Scientific Ltd, LCi-SD, Hoddesdon U.K.) 90 days after seedling were imposed to salinity stress. During the measurements, both the air temperature and the light intensity in the cuvette were at room temperature. The $CO_2$ content was 400 ppm.

### Data analysis

Statistical analysis software (SAS) version 9.4 was used for statistical data analysis. To evaluate the difference between treatment means, Duncan's multiple range test was used at $P \leq 0.05$. Different letters indicate significant differences. Pearson correlation coefficient, and dendrogram clustering were carried out by using R v3.4.3 (www.r-project.org). Biophysical parameters were calculated using "PEA Plus" software version 1.12.

## Results

### Photosynthetic pigments

The results of this study demonstrated significant effects of salinity stress, CS+Se NPs, and their interaction on various Photosynthetic parameters of *C. sinensis*. According to Table 1, salinity stress, CS+Se NPs, and their interaction significantly affected Chlorophyll a (Chl *a*), Chlorophyll b Chl *b*, the ratio of Chlorophyll a to b (Chl *a/b*), total Chlorophyll (Chl), and total Carotenoids (Car). Under moderate salinity stress, Chl *a*, *b*, *a/b*, total

**Table 1** Effect of nanocomposites CS + Se NPs on photosynthetic pigments and RWC of *C. Sinensis* under salinity stress

| Salinity | Treatment | Chl a (mg $g^{-1}$ FW) | Chl b (mg $g^{-1}$ FW) | Chl a/b | TChl (mg $g^{-1}$ FW) | Car (mg $g^{-1}$ FW) | SPAD | RWC (%) |
|---|---|---|---|---|---|---|---|---|
| **Control** | WT | $0.39 \pm 0.05^{def}$ | $0.15 \pm 0.01^{b-e}$ | $2.55 \pm 0.18^{b-e}$ | $0.54 \pm 0.07^{cde}$ | $0.23 \pm 0.04^{ab}$ | $46.50 \pm 3.32^{ab}$ | $83.67 \pm 0.91^{ab}$ |
| | CS | $0.38 \pm 0.02^{efg}$ | $0.14 \pm 0.02^{c-f}$ | $2.77 \pm 0.34^{bc}$ | $0.53 \pm 0.04^{def}$ | $0.16 \pm 0.02^{cd}$ | $36.27 \pm 3.52^{c-f}$ | $83.50 \pm 1.13^{ab}$ |
| | Se | $0.49 \pm 0.04^{ab}$ | $0.19 \pm 0.02^{a}$ | $2.55 \pm 0.10^{b-e}$ | $0.68 \pm 0.05^{a}$ | $0.20 \pm 0.04^{abc}$ | $48.70 \pm 3.50^{a}$ | $83.35 \pm 3.27^{ab}$ |
| | CS + Se NPs10 | $0.52 \pm 0.03^{a}$ | $0.16 \pm 0.02^{a-d}$ | $3.24 \pm 0.34^{a}$ | $0.68 \pm 0.05^{a}$ | $0.25 \pm 0.03^{a}$ | $42.56 \pm 2.79^{bc}$ | $79.12 \pm 3.38^{bc}$ |
| | CS + Se NPs20 | $0.47 \pm 0.04^{ab}$ | $0.16 \pm 0.02^{bcd}$ | $2.92 \pm 0.18^{ab}$ | $0.63 \pm 0.05^{ab}$ | $0.23 \pm 0.03^{ab}$ | $38.85 \pm 3.55^{bcd}$ | $84.18 \pm 3.70^{ab}$ |
| **Moderate (50 mM)** | WT | $0.25 \pm 0.03^{i}$ | $0.12 \pm 0.02^{f}$ | $2.18 \pm 0.28^{def}$ | $0.37 \pm 0.05^{g}$ | $0.12 \pm 0.03^{e}$ | $33.57 \pm 1.53^{f}$ | $79.64 \pm 3.44^{bc}$ |
| | CS | $0.35 \pm 0.02^{e-h}$ | $0.14 \pm 0.02^{b-f}$ | $2.43 \pm 0.15^{cde}$ | $0.49 \pm 0.04^{ef}$ | $0.14 \pm 0.03^{de}$ | $36.11 \pm 2.52^{c-f}$ | $83.55 \pm 0.43^{ab}$ |
| | Se | $0.33 \pm 0.02^{gh}$ | $0.13 \pm 0.01^{def}$ | $2.55 \pm 0.06^{b-e}$ | $0.46 \pm 0.03^{f}$ | $0.16 \pm 0.02^{cde}$ | $37.07 \pm 1.89^{c-f}$ | $87.48 \pm 0.95^{a}$ |
| | CS + Se NPs10 | $0.40 \pm 0.02^{c-f}$ | $0.17 \pm 0.01^{ab}$ | $2.32 \pm 0.10^{cde}$ | $0.57 \pm 0.03^{bcd}$ | $0.23 \pm 0.02^{ab}$ | $35.53 \pm 1.93^{def}$ | $77.25 \pm 3.22^{cd}$ |
| | CS + Se NPs20 | $0.45 \pm 0.03^{bc}$ | $0.1 \pm 50.02^{b-e}$ | $2.90 \pm 0.12^{ab}$ | $0.60 \pm 0.05^{bcd}$ | $0.20 \pm 0.01^{abc}$ | $40.63 \pm 2.16^{b-e}$ | $81.70 \pm 0.98^{cd}$ |
| **Intense (100 mM)** | WT | $0.34 \pm 0.04^{fgh}$ | $0.14 \pm 0.02^{def}$ | $2.49 \pm 0.27^{b-e}$ | $0.48 \pm 0.06^{ef}$ | $0.19 \pm 0.02^{bcd}$ | $34.87 \pm 3.72^{ef}$ | $73.03 \pm 4.72^{d}$ |
| | CS | $0.31 \pm 0.03^{h}$ | $0.15 \pm 0.01^{b-f}$ | $2.15 \pm 0.23^{ef}$ | $0.46 \pm 0.02^{f}$ | $0.19 \pm 0.02^{bcd}$ | $27.85 \pm 0.45^{g}$ | $84.49 \pm 2.90^{ab}$ |
| | Se | $0.24 \pm 0.02^{i}$ | $0.13 \pm 0.03^{ef}$ | $1.89 \pm 0.28^{f}$ | $0.37 \pm 0.05^{g}$ | $0.15 \pm 0.02^{de}$ | $17.60 \pm 2.94^{h}$ | $80.00 \pm 1.11^{bc}$ |
| | CS + Se NPs10 | $0.44 \pm 0.00^{bcd}$ | $0.17 \pm 0.03^{abc}$ | $2.60 \pm 0.24^{bcd}$ | $0.61 \pm 0.03^{abc}$ | $0.20 \pm 0.03^{abc}$ | $41.97 \pm 0.92^{bcd}$ | $84.12 \pm 0.03^{ab}$ |
| | CS + Se NPs20 | $0.40 \pm 0.03^{cde}$ | $0.17 \pm 0.01^{ab}$ | $2.30 \pm 0.10^{def}$ | $0.57 \pm 0.03^{bcd}$ | $0.23 \pm 0.03^{ab}$ | $38.23 \pm 3.40^{c-f}$ | $83.78 \pm 2.76^{ab}$ |

The different letters in each columns indicate significant differences between treatments. WT, water; CS, chitosan (0.1%); Se, selenium; CS + Se NPs10, CS + Se NPs (10 mg $L^{-1}$); CS + Se NPs20, CS + Se NPs (20 mg/ $L^{-1}$); Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; TChl, total chlorophyll. car carotenoids; RWC, relative water content

Chl, Car, and SPAD value decreased significantly by 36, 20, 15, 31, 48, and 28%, respectively, compared to normal conditions (Table 1). Under moderate salinity stress, foliar application of CS, Se, CS+Se NPs (10 and 20 mg $L^{-1}$) significantly improved chlorophyll *a* by 40, 32, 60, and 80%, respectively, compared to the control group. And, foliar application of CS+Se NPs (10 and 20 mg $L^{-1}$) improved significantly Chl *b* by 42 and 25%, total Chl by 54 and 62%, carotenoids by 92 and 67%, and CS+Se NPs (20 mg $L^{-1}$) increased ratio of Chl *a/b* and SPAD value by 33 and 21%, respectively, compared to control plants under the same salinity conditions. Also, under intense salinity stress, foliar application of CS+Se NPs (10 and 20 mg $L^{-1}$) significantly improved Chl *a* by 21 and 29%, Chl *b* by 21 and 21%, total chlorophyll by 27 and 19%. Nanocomposite CS+Se NPs (10 mg $L^{-1}$) improved SPAD value by 20%, respectively, compared to the control group (Table 1).

#### Relative water content (RWC)

The interaction of salinity and NPs significantly affected RWC (Table 1). Moderate and intense salinity stresses decreased RWC by, 5 and 13%, respectively, compared to normal conditions and foliar application of CS, Se, and CS+Se NPs (10 and 20 mg $L^{-1}$) under salinity stress conditions significantly increased RWC (Table 1). Under the intense salinity stress foliar application of CS, Se, and CS+Se NPs (10 and 20 mg $L^{-1}$) significantly increased RWC by 16, 10, 15 and 15%, respectively compared to control group (Table 1).

#### Leaf gas exchange analyses

The results of this study demonstrated significant effects of salinity stress, CS+Se NPs, and their interaction on various leaf gas exchange parameters of *C. sinensis*. Under moderate and intense salinity stress conditions, stomatal conductance ($g_s$) decreased by 57 and 86%, $CO_2$ assimilation rate (*A*) by 68 and 80%, Transpiration rate (*E*) by 27 and 74%, *WUE* by 56 and 25%, *A/Ci* by 68 and 83% while $C_i$ enhanced by 6 and 21% compared to the control, respectively. At all salinity stress levels, foliar applications of CS, Se, CS+Se NPs10, and 20 increased amounts of *gs*, *A*, *E*, *WUE*, and *A/Ci*, and decreased *Ci* compared to control, with CS+Se NPs20 having the stronger effect (Fig. 2). Under moderate salinity stress spraying with CS+Se NPs20 increased $g_s$, *A*, *E*, *WUE* and $A/C_i$ by 87, 41, 37, 4, and 79% while decreased $C_i$ by 22% compared to water sprayed plants, respectively. Under intense salinity stress spraying with CS+Se NPs20 increased $g_s$, *A*, *E*, *WUE* and $A/C_i$ by 75, 82, 80, 4, and 159%, while decreasing $C_i$ by 30% compared to water sprayed controls, respectively (Fig. 2).

#### Prompt chlorophyll a fluorescence

The results of this study demonstrated significant effects of salinity stress, CS+Se NPs, and their interaction on fluorescence transient curve of *C. sinensis*. Salinity stress and NPs had a significant effect on the fluorescence transient curve, according to the findings. Chitosan treatment had the greatest effect on increasing the fluorescence induction curve at all points under non-stress situations. Under 50 and 100 mM stress conditions, NPs (20 mg $L^{-1}$) and CS treatments had the greatest influence on enhancing the fluorescence induction curve, particularly in J, I, and P points. The results revealed that as the stress intensity increased, the fluorescence induction curve reduced, and salinity stress had a negative influence on this curve (Fig. 3).

#### JIP-test parameters, calculated from chlorophyll fluorescence transients

The following biophysical parameters have been derived from OJIP transients: Specific energy fluxes (per $Q_A$ decreased PSII reaction center), quantum yield for primary photochemistry, slopes and integrals, and performance indices are calculated from the extracted and normalized data. The measured parameter values have been standardized to those of non-stress plants. The departure of the activity pattern of plants under stress and nanocomposite from non-stress plants was revealed on radar plots (Fig. 4). The findings of this research, showed that various nanocomposite and salinity stress treatments had an effect on JIP-test parameters. According to the findings, foliar application of CS has a significant influence on increasing the parameters of $PI_{ABS}$, $PI_{total}$, $S_m$, and N and decreasing $dVG/dt_0$ and $dV/dt_0$ under non-stress situations. Foliar application of CS+Se NPs (10 mg $L^{-1}$) enhanced $F_m$ and $F_0$ significantly. The quantum performance parameters (ϕP0, ϕE0, ϕR0, ΨE0, δR0) did not affect significantly by the nanocomposite treatments (Fig. 4A). Foliar treatment of CS and CS+Se NPs (20 mg $L^{-1}$) had a substantial influence on enhancing the performance indexes (PIABS and PItotal) and quantum yield (ϕP0, ϕE0, ϕR0, ΨE0, δR0) parameters under salinity stress conditions. Treatments containing CS had a greater effect on reducing the effects of stress under 100 mM salinity stress conditions (Fig. 4B, C). The results showed that salinity stress conditions had a significant effect on increasing F0/Fm, ABS/RC, and DI0/RC parameters (Fig. 4D). The increase in Fv/Fm observed in CS+Se treated plants under salinity stress (Fig. 4) suggests improved photochemical efficiency and protection against stress-induced damage.

#### Correlation analysis

The correlation plot (Fig. 5) provides the correlations between photosynthesis pigments, gas exchange, and

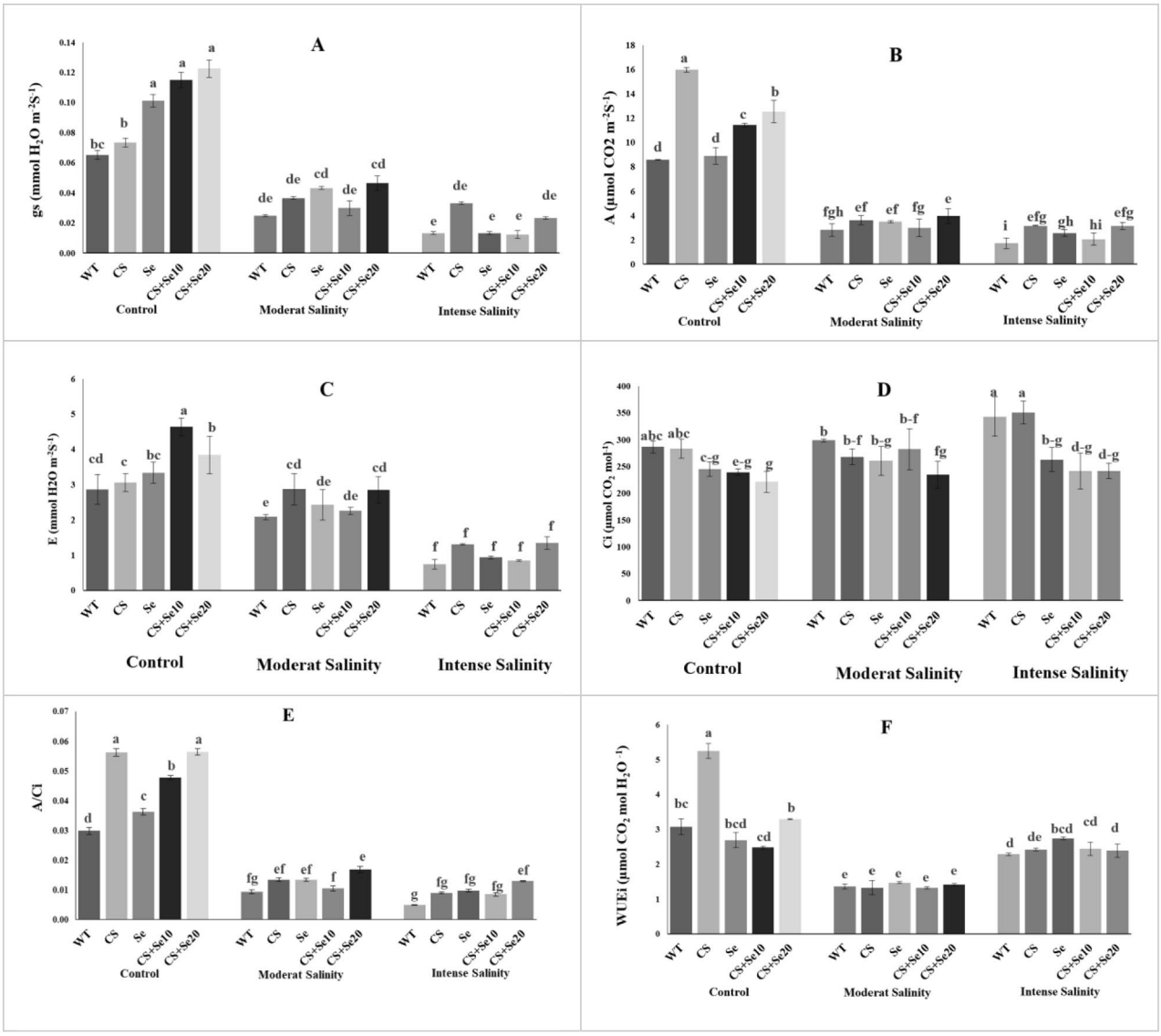


**Fig. 2** Leaf gas exchange parameters of different treatments on orange seedlings cv. Valencia under salinity stress conditions. **A**: Stomatal conductance ($g_s$); **B**: $CO_2$ assimilation rate ($A$); **C**: Transpiration rate ($E$); **D**: Sub-stomatal $CO_2$ concentration ($C_i$); **E**: Instantaneous carboxylation efficiency ($A/C_i$); **F**: water-use efficiency ($WUE$) Means followed by the same letter for a parameter, are not significantly different according to the Duncan's ($p \leq 0.05$). Vertical bars indicate the standard deviation of three replicates

prompt fluorescence parameters. The size and color severity of squares is proportionate to Pearson's correlation coefficient at $p<0.01$. Red squares show positive correlations, while blue show negative correlations. The results showed that the parameter of $F_v/F_m$, is an indicator of the maximum quantum efficiency of PSII, with lower values indicating photoinhibition or damage to the photosynthetic apparatus, had a strong negative correlation with ABS/RC and $DI_0$/RC parameters. Photosynthetic pigments were negatively correlated with ABS/RC, $DI_0$/RC, and $TR_0$/RC parameters. Total chlorophyll was positively correlated with chlorophyll *a*. $F_v$ parameter was positively correlated with Fm. The performance indexes ($PI_{ABS}$ and $PI_{total}$) and quantum yield ($\phi_{P0}$, $\phi_{E0}$, $\phi_{R0}$, $\Psi_{E0}$, $\delta_{R0}$) parameters had positive correlation with $F_m$, $F_v$, and $F_v/F_m$ parameters and negative correlation with ABS/RC, $DI_0$/RC, $TR_0$/RC. We performed a cluster analysis to separate citrus seedlings based on different NP and salinity treatments and obtained three clades as shown in the dendrogram (Fig. 6). Clade I contained citrus seedlings treated with CS+Se NPs 10 and 20 $mg^{-1}$ and Se-NPs in non-saline conditions. While clade II citrus seedlings treated with CS+Se NPs 20 $mg^{-1}$ under moderate and intense salt levels; and the plants grown under no salinity, moderate and severe salinity conditions with CS foliar spraying as well as plants grown under moderate salinity

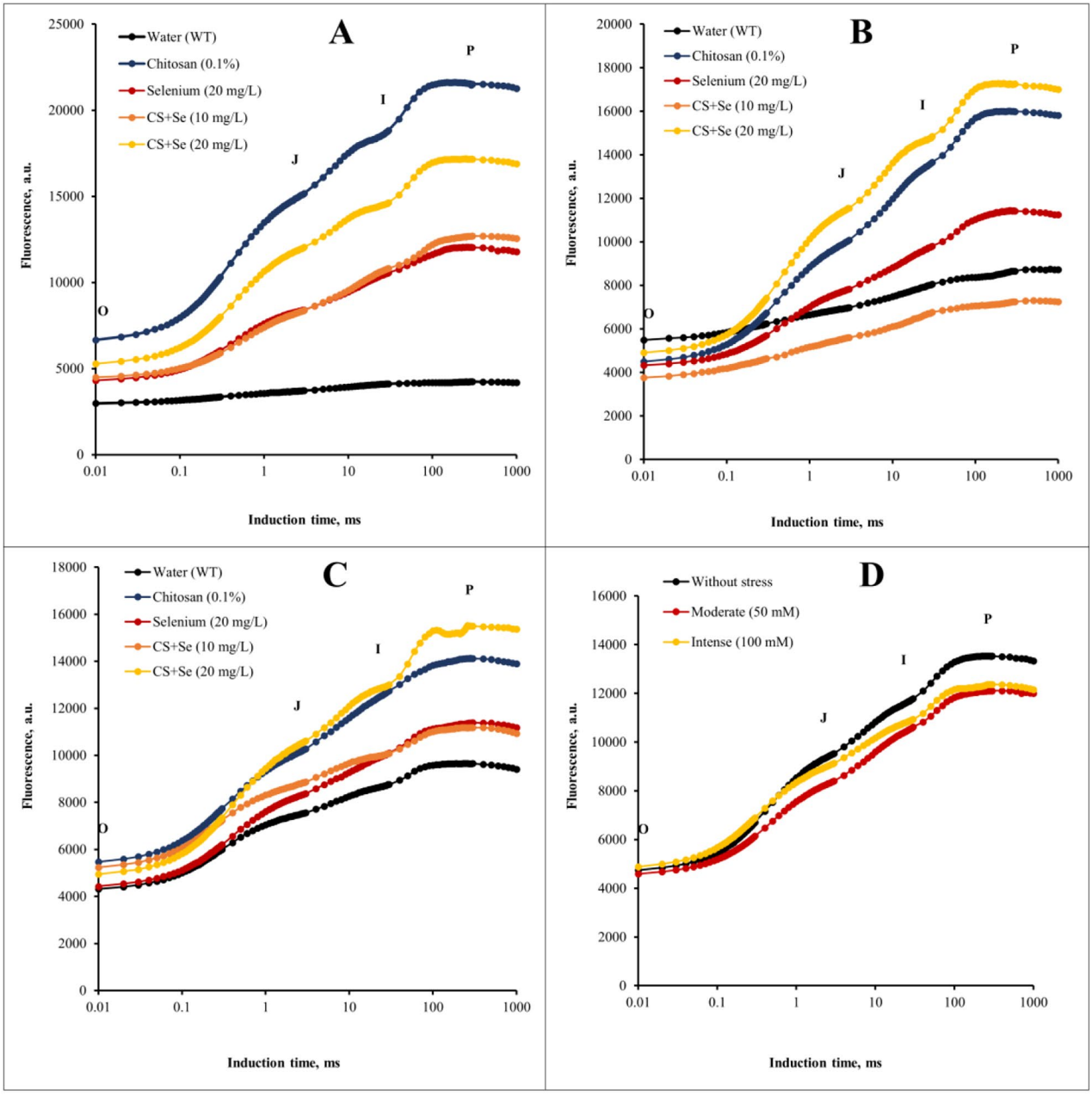


**Fig. 3** Induction curves of chlorophyll *a* fluorescence. The effect of foliar application (WT, Cs, Se, CS + Se NPs (10 and 20 mg $L^{-1}$)) under salinity stress (**A**: control; **B**: Moderate salinity (50 mM); **C**: Intense salinity (100 mM)); **D**: Independent effects of salinity stress condition under different treatments on induction curves of chlorophyll *a* fluorescence. WT, distilled water; CS, Chitosan; Se, Selenium; CS + Se NPs, nanocomposite of Chitosan + Selenium; control, without salinity stress

stress conditions with CS + Se NPs 10 and $mg^{-1}$ application were included. Clade III of citrus seedlings treated with moderate and severe salt levels; and plants grown under moderate and severe salinity conditions with Se-NPs foliar spraying, as well as plants that were grown under severe salinity stress conditions with the application of CS + Se NPs 10 $mg^{-1}$.

## Discussion

### Mechanisms of salinity stress

Salinity stress leads to the accumulation of reactive oxygen species (ROS), which cause oxidative damage to chlorophyll molecules, resulting in their degradation. Reduction of photosynthesis due to salinity stress occurs due to various factors including disruption of chlorophyll production, change in enzyme activity, stomatal closure, reduction of $CO_2$ availability and disruption of

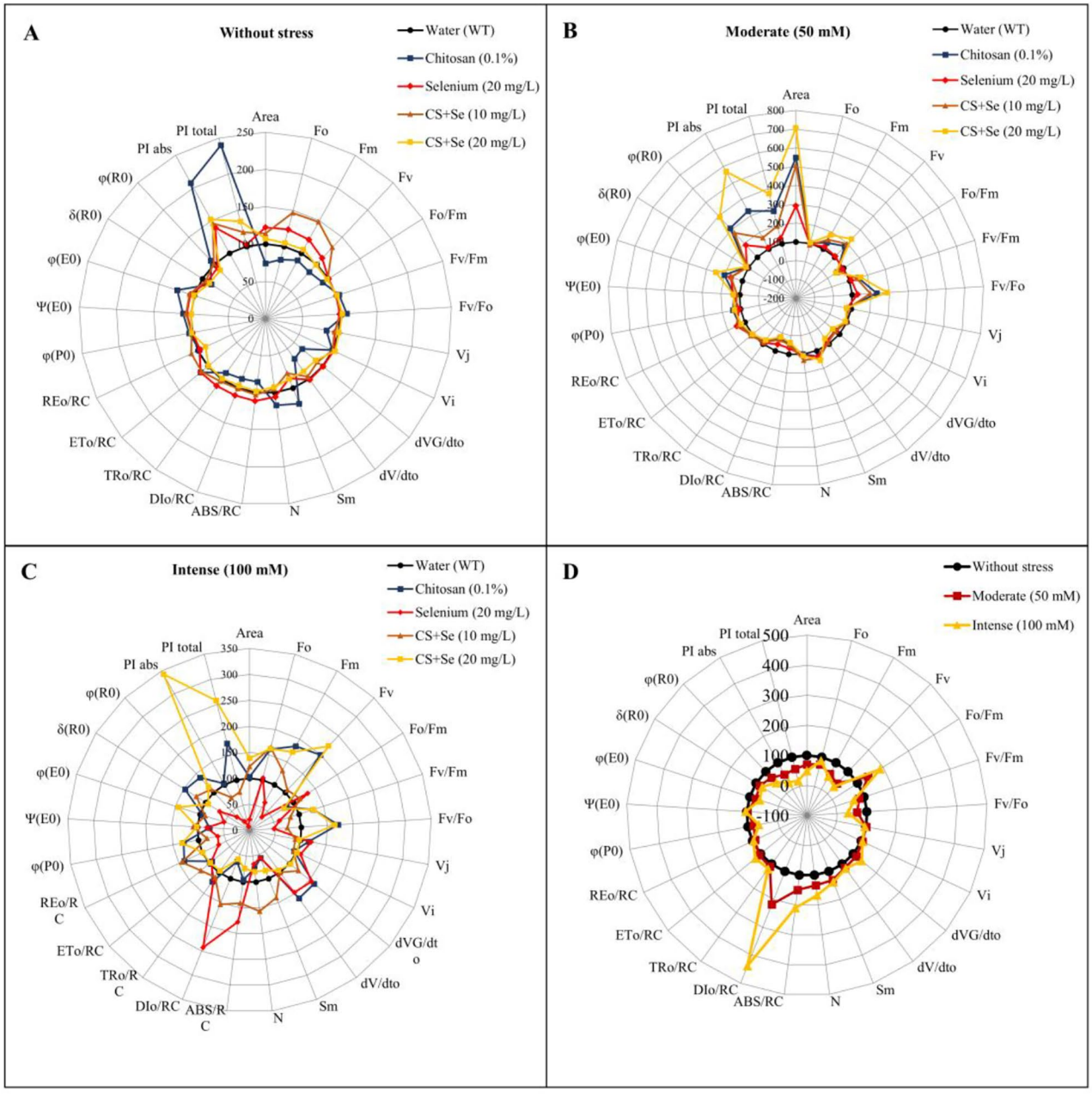


**Fig. 4** JIP-test parameters normalized on radar plots. The effect of foliar application (WT, CS, Se, CS + Se NPs (10 and 20 mg $L^{-1}$) under salinity stress (**A**: control; **B**: Moderate (50 mM); **C**: Intense (100 mM)); **D**: Independent effects of stress condition under different treatments on JIP-test parameters. WT, distilled water; CS, Chitosan; Se, Selenium; CS + Se NPs, nanocomposite of Chitosan- Selenium; control, without salinity stress

photosynthetic machinery. Our findings that chlorophyll content decreases under salinity stress (Table 1) are consistent with previous studies showing enhanced oxidation due to ROS accumulation. Excessive ROS generation is generated by pseudocyclic electron transport, which is created by blocking the electron transport chain. As a result, ROS alter photosynthetic proteins and photosystem assembly [26]. According to previous researchers, Se can accelerate Chl biosynthesis by interacting with porphobilinogen deaminase and 5-aminolevulinic acid dehydratase, facilitating electron transport in the respiratory chain and respiration [27, 28].

### Role of chitosan and selenium

Chitosan acts as a biostimulant, enhancing the formation of protective proteins like phytoalexins, which improve the plant's defense mechanisms [6].

Se enhances antioxidant activities by increasing glutathione peroxidase (GSH-Px) activity, which helps neutralize free radicals and reduce oxidative damage, Se

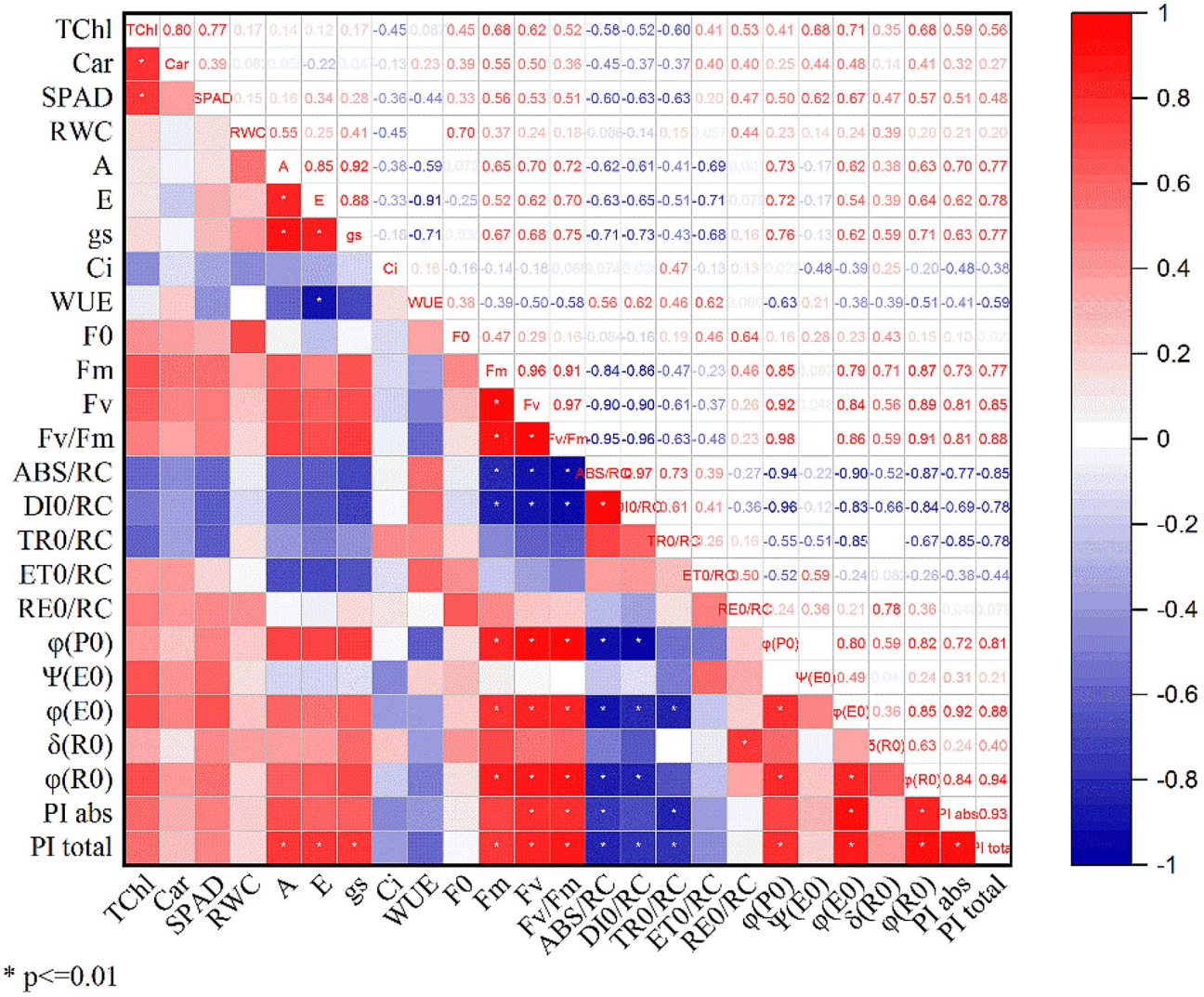


**Fig. 5** Pearson correlation analysis of treatments (salinity stress and foliar application of **CS**, Chitosan; **Se**, Selenium; CS + Se NPs, nanocomposite of Chitosan- Selenium; **WT**, distilled water) with variable trait relationship of photosynthetic apparatus in *C. sinensis* seedlings

can help plants resist abiotic stress by reducing the large amount of free radicals that plants release under stress conditions. Se addition can promote protein synthesis by directly affecting it through amino acid forms that include sulfur substituted with Se, and it can also stimulate chlorophyll synthesis by controlling the biosynthesis of porphyrins in plants. Porphyrins have been reported to be involved in chlorophyll synthesis [29].

Because of the high salt content in the soil, the osmotic potential of plant cells be more negative during salt stress, creating an osmotic gradient that sucks water from plant cells, declines turgor pressure. The degree of leaf water potential decreases and the osmotic potential of plant cells are determined by the osmotic potential in the root environment and the level of salt stress. Under transpiration conditions, water flows of the soil to the xylem of root by an apoplastic route carried by a hydrostatic pressure gradient. Water travels largely through the cell-to-cell channel across membranes when transpiration is restricted by salt. When plants are exposed to short-term salt stress, their RWC, water absorb, and transpiration level decrease [26]. Se can maintain water homeostasis in plants under salinity stress condition and improve RWC in plants under this condition [30].

### *Photosynthetic efficiency*

Such reductions in photosynthetic efficiency under salinity conditions strongly suggests the need for salinity reduction strategies to prevent disruption at the metabolic level with final agents on production. The current study also reports the effect of using selenium nanocomposite as foliar spray on the photosynthetic apparatus under different salinity treatments. The decline in $CO_2$ assimilation is related to the decline in stomatal

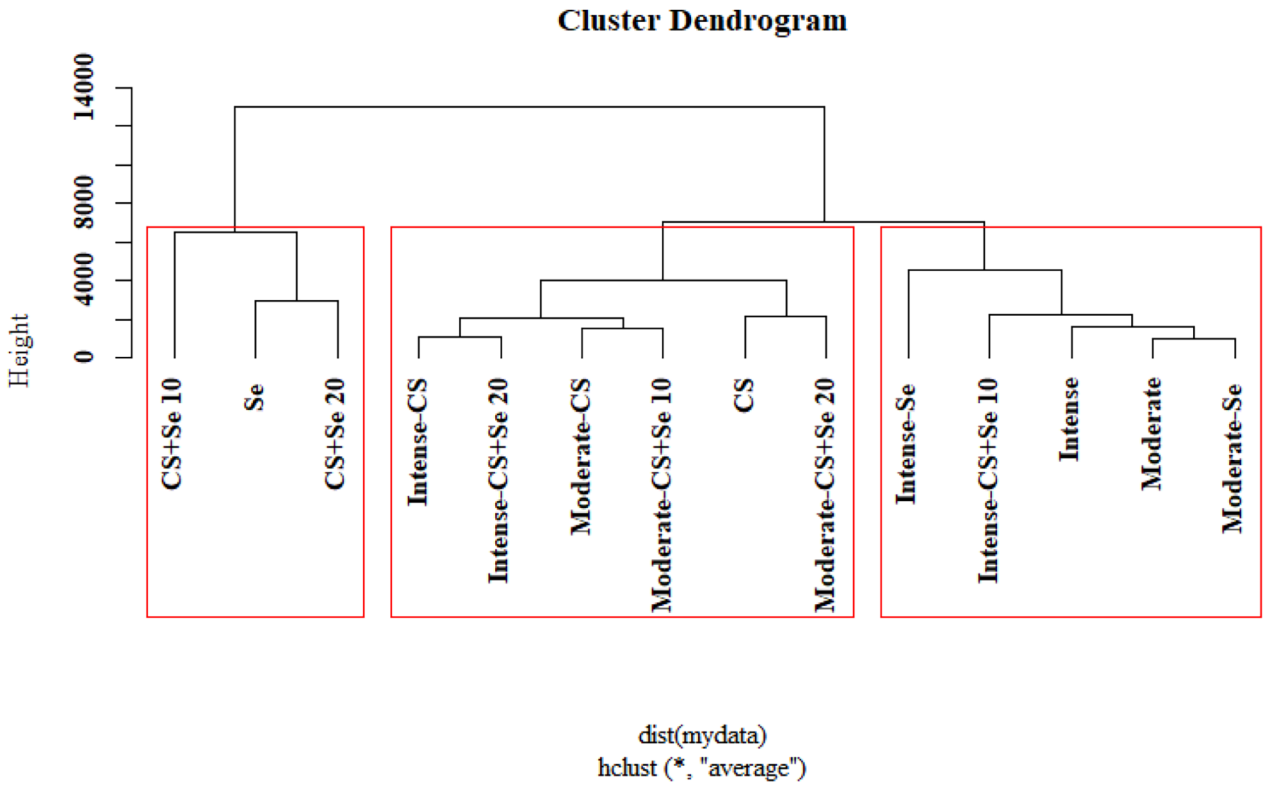


**Fig. 6** Dendrogram clustering of foliar application treatment of Chitosan (CS), Selenium (Se), nanocomposite of CS + Se NPs (10 and 20 $mg^{-1}$) in citrus seedlings exposed to non-saline (control, 0 mM NaCl), moderate (50 mM) and intense (150 mM) salinity stresses

conductance. The decrease in photosynthesis was considered to be due to stomatal factors such as stomatal conductance reduction and non-stomatic factors such as chlorophyll degradation. As mentioned earlier, the optimal internal $CO_2$ or $C_i$ and *A/Ci* are also related to the stomatal nature, among which stomatal conductivity ($g_s$) can be mentioned. An increase in $g_s$, by spraying CS/Se20 as seen in Fig. 2, provides more gas exchange, which means more transpiration (*E*) and more $CO_2$ entry in the leaf mesophyll (*Ci*), thus a higher $CO_2$ assimilation rate (*A*) resulting in higher instantaneous efficiency of carboxylation (*A/Ci*). Salinity destroys chloroplast structure and the instability of pigment-protein compounds. The quantum performance parameter of PSII is a good characteristic to determine the difference between control and stress. The flow of electrons in the photosystem is indicative of the overall rate of photosynthesis, and the measurement of chlorophyll fluorescence allows us to estimate how photosynthesis works. Salinity stress reduces photosynthetic rates at low salinity stress. Severely damaged chloroplast structures and photosynthetic systems at moderate to intense salinity stress result in loss of crop productivity [26].

The effects of salinity stress are primarily attributed to the loss of active reaction centers [31]. The water splitting complex (OEC) is an important and sensitive part of the electron transport chain of the photosynthetic apparatus [32]. Its efficiency decreases due to the disruption of the electron transfer process caused by the dissociation of light-receiving complexes (LHCII). Salt stress has been shown to reduce photosystem II activity by affecting the Mn cluster and photosystem I activity [33] and induce reactive oxygen species (ROS) production and D1 protein degradation [34] by sequestering plastocyanin from Cytochrome C553 showed that the photosynthetic electron transport chain and plant gas exchange parameters are vulnerable under stress conditions [35]. Increased salinity imposes a multifaceted stress on plants and negatively affects the performance of photosystem II (PSII) and thus the overall efficiency of photosynthesis. Increasing salt concentration disrupts cellular ion homeostasis in chloroplast. This disrupts the electrostatic interactions that are critical for enzymatic activity in the PSII reaction centers. Disruption of these interactions can lead to denaturation of key proteins involved in electron transport and photochemistry [36]. The thylakoid membrane, where PSII is embedded, is rich in net negatively charged phospholipids. High salt concentration can lead to increased influx of sodium (Na+) and displacement of essential cations such as potassium (K+).

This alters membrane fluidity and disrupts the activity of membrane-bound enzymes involved in electron transport within PSII [37]. PSII contains OEC, a cluster of manganese (Mn) clusters responsible for water oxidation and oxygen evolution. Salinity can directly inhibit OEC by changing the oxidation state of Mn, compromising its ability to donate electrons to the plastoquinone pool, a critical step in the electron transport chain [38]. Salt stress can cause the production of reactive oxygen species (ROS) that damage chlorophylls, the pigments responsible for light absorption in PSII. This reduction in chlorophyll content reduces the efficiency of light absorption and limits electron flow through PSII [39]. As a response to water stress, plants may close their stomata in response to salinity. While this conserves water, it also limits the release of CO2 into the leaf mesophyll, the site of carbon fixation. Reduced CO2 availability acts as a bottleneck for the Calvin-Benson cycle and further limits the electron transfer efficiency of PSII [40]. These mechanisms collectively reduce the efficiency of PSII. Decreased efficiency of PSII leads to a lower rate of electron transfer, reduced ATP (adenosine triphosphate) production, and ultimately reduced NADPH (nicotinic adenine dinucleotide phosphate) production. This limits the energy and reducing power available for CO2 fixation, thereby hindering the overall efficiency of photosynthesis [41].

Chitosan and NPs (Se) have been found in recent research to be engaged in photosynthesis, particularly when plants are stressed [6, 19], while some studies have concentrated on the effect of their composite on plants. Chlorophyll fluorescence characteristics are frequently employed to indicate changes in plant photosystems caused by environmental stress, and the measurement is simple, precise, and sensitive [42]. Usually, plants grown in stress conditions have lower $F_v/F_m$ than non-stressed conditions [43]. The significantly higher values of $F_v/F_m$ in treated seedlings with CS+Se NPs (10 and 20 mg $L^{-1}$) under salinity stress suggested that may successfully preserve photochemical efficiency in *C. sinensis*. The increase in ABS/RC in *C. sinensis* after salinity treatment can be attributed to a decrease in the number of active reactive centers (RC) of PSII, which may function as a defensive mechanism to minimize the burden on its systems when salt stress occurs [23]. The results of our study showed that exposure to salinity stress leads to a decrease in the flow of electrons towards the receptor in photosystem I. This decrease can be attributed to the deactivation of ferredoxin NADP reductase on the receptor side, which is shown by the decrease of performance index parameters ($PI_{abs}$ and $PI_{total}$) and the increase of heat dissipation ($F_0$/Fm) in Fig. 3-D. The conversion of absorbed light energy into heat is another effective protective mechanism for plants under salinity stress [44], as evidenced by significantly higher values of non-photochemical quenching (NPQ) per reaction center of PSII ($DI_0$/RC) in *C. sinensis* under salinity stress (Fig. 3). Furthermore, TR0/RC was much greater in plants exposed to salinity stress, indicating that *C. sinensis* exposed to salinity had enhanced the efficiency of the remaining active reaction centers. In contrast to salinity-treated seedlings, CS+Se NPs treatment increased PSII activity in *C. sinensis* (Fig. 4), as evidenced by greater $F_v/F_m$ and reduced ABS/RC, $DI_0$/RC, and $TR_0$/RC. However, the addition of a NO scavenger (L-name) reduced this impact, resulting in a drop in Fv/Fm and an increase in ABS/RC, $DI_0$/RC, and $TR_0$/RC values (Fig. 4). These findings demonstrate that salinity stress reduces photosynthetic rates by causing damage to chloroplast structures and disrupting pigment-protein complexes.

## Conclusions

This study demonstrated that chitosan-selenium nanocomposite (CS+Se) significantly enhances the photosynthetic efficiency and overall performance of *C. sinensis* under salinity stress. CS+Se NPs effectively alleviated the adverse effects of salinity by enhancing chlorophyll content, improving the performance of the photosynthetic apparatus, and reducing oxidative damage. Although salinity stress significantly increased dissipated energy in photosystem II, chitosan and selenium treatments improved the performance of photosynthetic apparatus under stress conditions by reducing dissipated energy and significantly increasing performance index parameters. These results underscore the potential of CS+Se NPs as a biocompatible and efficient agent for mitigating salinity-induced stress in citrus. Further investigations should focus on elucidating the underlying mechanisms of CS+Se NPs action, optimizing application protocols, and assessing its long-term impacts and applicability across diverse agricultural contexts. This research provides valuable insights into developing innovative strategies to enhance crop resilience and productivity in saline environments, contributing to sustainable agricultural practices. Further research should also explore the scalability and economic feasibility of CS+Se NPs application in large-scale agricultural practices. These findings suggest that CS+Se NPs can be used as a biocompatible and efficient treatment to mitigate salinity-induced stress, potentially enhancing citrus crop resilience and productivity. The use of CS+Se NPs nanocomposites as foliar sprays could be a viable strategy to mitigate the effects of salinity stress in citrus orchards, thereby improving crop yield and quality. The application of CS+Se NPs may also benefit other crops affected by salinity stress, indicating broader agricultural applications. Future studies should investigate the molecular mechanisms underlying CS+Se NPs action, optimize application protocols for different

crop species, and evaluate long-term effects on soil health and crop productivity.

## Supplementary Information

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Supplementary Material 1

### Author contributions

In the collaborative effort leading to the research findings, R. S. was pivotal in conducting both the greenhouse and laboratory experiments, collecting vital data. A.S., M.E., and S.M.Z. played critical role in managing the research group, ensuring that the project's objectives were met efficiently. N.S. prepared nanoparticles and related graphs. M.R.M. participated in the recording and analysis of the photosynthetic analyzer data and prepared the relevant graphs. A.S., M.R.M., and S.M.Z. participated in writing and editing of the manuscript, ensuring the scientific integrity and accuracy of the final document. All authors have reviewed the completed manuscript and given consent for its publication, indicating their agreement with the content and findings reported in the study.

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### Data availability

The data used in this study is openly available, and the data used is available upon request from the corresponding authors A.S.

## Declarations

### Ethic approval and consent to participate

Not applicable.

### Compliance with ethical standards

The presented manuscript represents honest research work of authors.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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