NARDI FUNDULEA, ROMANIA ROMANIAN AGRICULTURAL RESEARCH, NO. 41, 2024
www.incda-fundulea.ro; doi.org/10.59665/rar4112 First Online: December, 2023. DII 2067-5720 RAR 2024-45

Effect of Metal Nanoparticles on Photosynthetic and Antioxidant Enzyme Activities of Soybean

Phan Thi Thu Hien[1*], Luu Thi Tam[2], Nguyen Cam Ha[2*], Dinh Thai Hoang[4], Dang Diem Hong[2,3]

[1]Hanoi Pedagogical University 2, Phuc Yen, Vinh Phuc, Vietnam
[2]Institute of Biotechnology, Vietnam Academy of Science and Technology, Hanoi, Vietnam
[3]Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Hanoi, Vietnam
[4]Vietnam National University of Agriculture, Hanoi, Vietnam
[*]Corresponding authors. E-mail: phanthithuhien@hpu2.edu.vn; hanguyen.hou@gmail.com

ABSTRACT

This study presents the effects of nanoscale zerovalent cobalt (NZVC) on the photosynthetic activity and expression of genes related to the photosynthetic apparatus as well as the activity of antioxidant enzymes in soybean (*Glycine max* L. Merr). Treatment of soybean seeds with NZVC solution (at concentrations of 0.17; 0.33 and 100 mg/kg seed) has increased the efficiency of light energy absorbed by photosystem II, improved photosynthetic efficiency, and maintained the function of photosynthetic apparatus when plant growth is reduced by enhancing the value of photosynthetic parameters (Fo, Fm, Fv/Fm, ETR, FPSII) compared with the control. The expression of genes involved in photosynthesis in soybean leaves was changed by growth time and NZVC treatment concentration. The *psa*A, *Lhca*, *psb*A, *and psb*B genes had increased expression levels at 17 and 32 days after sowing, then decreased at 70 days after sowing. The expression of the *psb*E, *and Cyt b6f* genes was greater than the control during the growth period. Treatment of soybean seeds with high concentrations of NZVC (0.33 and 100 mg/kg seed) did not negatively affect the photosynthetic function of soybean plants. The activity of nitrate and nitrite reductases and antioxidant enzymes including superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase of cobalt-treated plants were better than those of the control. This is a defense mechanism of plants to minimize the harmful effects of these stresses. This result has elucidated the mechanism of increasing the yield of soybean when its seeds were treated with NZVC.

Keywords: antioxidant enzymes, chlorophyll fluorescence, cobalt zerovalent nanoparticles, soybean, nanotechnology.

INTRODUCTION

Soybean is one of the most important and widely grown crops in the world. It is the main source of protein and vegetable oil, as well as a highly nutritious food source for livestock and poultry. Moreover, growing soybeans could improve arable lands, especially infertile soil. In soybean and leguminous crops, cobalt plays an essential role in plant growth and development by regulating water utilization, especially in nodule formation and nitrogen fixation (DalCorso et al., 2014; Akeel and Jahan, 2020). Gad et al. (2013) reported that soybean yield increased by 42.5% when the plants were treated with a cobalt solution at a concentration of 12 mg/L. Cobalt has been demonstrated to affect plant growth and metabolism at different levels depending on its concentration and state. Jayakumar et al. (2009) showed that soybean yield increased when cobalt was added at a low concentration of 50 mg Co/kg soil, but decreased at higher concentrations of 100 to 250 mg Co/kg soil.

The productivity of soybeans is mainly contributed by the amount of photosynthetic products during the growth stages. In particular, chlorophyll is the main and essential photosynthetic pigment. The chlorophyll content at 680 nm in the reaction center of photosystem II (PSII) is an effective tool to quickly determine the physiological state of plants (Guo et al., 2005; Hussain and Reigosa, 2011). Furthermore, under adverse conditions, plants always have a self-defense mechanism by altering the expression levels of genes involved in PSI and PSII activity to

Received 27 October 2023; accepted 28 November 2023.

protect the photosynthetic apparatus (Wang et al., 2014). The core of PSII consists of two proteins D_1 and D_2 (encoded by *psb*A and *psb*D genes) linked to a heterodimer located in the center of the PSII reaction center, denoted by the fluorescent molecule P680, which is the primary electron acceptor in the photosynthetic electron transport chain. Along with some pigment molecules such as chlorophyll a, β-carotene, and iron, they form the reaction center of PSII. Meanwhile, two other proteins (encoded by *psb*B and *psb*C genes) bind an array of chlorophyll and β-carotene molecules as light-harvesting complexes, known as CP47 and CP43 (Barber et al., 1997). Changes in the expression levels of these genes will affect photosynthetic functions such as the quantum efficiency of photosynthesis and photosynthetic activity (Luciński and Jackowski, 2006). Then, PSI accepts electrons from the cytochrome b6f complex and transfers them to ferredoxin for the production of high-energy molecules such as NADPH. After that, they are further utilized in dark reactions to fix CO_2 in the Calvin-Benson cycle. Components of PSI are encoded by chloroplast and nuclear genes such as *psa*A, *psa*B, and *Lhca*, which exhibit function to absorb light and transport absorbed energy to the core of PS II (Berry et al., 2013). Thus, the expression levels of several genes encoding proteins in reaction center of PSI and PSII, the light-harvesting complex, and the electron transport chain in the photosynthetic transport chain are closely related to the activity of *psa*A, *psa*B, *psb*A, *psb*B, *psb*C, *psb*D, *psb*E, *Cyt b6f* and *Lhca* genes in plants (Teixeira et al., 2016).

To contribute to the explanation of the positive effect of cobalt nanoparticles on increasing soybean productivity through increased photosynthetic activity, we evaluated the expression levels of several genes encoding proteins located in the reaction center of PSI and PSII, the light-harvesting complex and the electron transport chain in photosynthesis pathway. In addition, it is necessary to evaluate the changes in the activity of antioxidant enzymes of soybeans at different growth stages in the presence and absence of cobalt nanoparticles. The research results will help elucidate the mechanism of action of cobalt nanoparticles on the physiological and biochemical processes of soybean plants.

MATERIAL AND METHODS

The study was carried out using soybean *Glycine max* (L.) Merr. DT96 variety which was provided by the Agricultural Genetics Institute, Vietnam Academy of Agricultural Sciences.

Nanoscale zerovalent cobalt (NZVC) with a size <50 nm were synthesized and supplied by the Institute of Environmental Technology, Vietnam Academy of Science and Technology (Ngo et al., 2014). These particles were dispersed in RO water by Sonic & Materials machine (USA) with power of 375 W, frequency of 20 KHz for 3 minutes and 30 seconds, using CMC protectant and $NaBH_4$ reducing agent to reduce Co^{2+} ions into Co^{o}.

The bright yellow and uniform size soybean seeds were treated in different cobalt nanoparticle concentrations following the process described by Dang et al. (2019). The mixture was shaken to evenly distribute the cobalt nano solution within the seeds. The seeds were incubated with NZVC solution for 30 minutes at room temperature. Then, seeds were removed and spread evenly over absorbent paper at room temperature until seeds were seed thoroughly dry before sowing them into well-prepared soil beds. The selected cobalt nanoparticle concentrations based on the previous results in Phan et al. (2018a, b) consisted of four treatments: Control- seeds treated with RO water (water filtered through reverse osmosis), CT1- 0.17 mg NZVC/kg seed, CT2- 0.33 mg NZVC/kg seed and CT3- 100 mg NZVC/kg seed. All treatments were performed in triplicate.

The soil was fertilized with 360 kg manure, 3 kg urea, 10 kg superphosphate, and 5 kg potassium chloride per Northern Vietnamese acre (360 m^2) before sowing seeds.

Chlorophyll fluorescence measurement

The measurements of chlorophyll fluorescence parameters including minimum fluorescence (Fo), maximum fluorescence (Fm), maximum photochemical efficiency (Fv/Fm), electron transport rate (ETR), photochemical efficiency of Photosystem II (PSII) were carried out using Photosynthesis Yield Analyzer - Mini PAM (Walz, Germany) as described by Dang et al. (2019). The dark incubation time was 7 minutes.

Determination of antioxidant enzyme activity

Soybean leaves obtained at different growth stages were extracted with 5 ml phosphate buffer including 50 mM potassium phosphate buffer (pH=7.0), 1 mM phenylmethylsulfonyl fluoride, 0.2 mM EDTA, 1% (w/v) polyvinylpyrrolidone. The mixture was centrifuged at 4^{o}C for 15 minutes. The supernatant was transferred to a fresh falcon tube, adjusted to 5 ml, and stored at -20^{o}C in a deep freezer to determine protein content and activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POX). The protein content in leaves was extracted according to the method of Bradford (1976). The activities of the SOD enzymes SOD, CAT, APX, and POX were determined according to methods of Asada (1992), Amako et al. (1994), Scebba et al. (1999) and Chen et al. (2000), respectively.

Determination of the expression of main genes involved in photosynthesis

At 17 days after sowing (DAS), 32 DAS (the time when photosynthesis reaches maximum), and 70 DAS (the time when photosynthesis is drastically reduced), the fully expanded leaves were collected and stored at -20°C until use.

Total RNA extraction

Total RNA from sampled leaves was extracted using the RNAiso plus kit (Takara, Tokyo, Japan) according to the manufacturer's instructions.

Synthesis of cDNA from a total RNA template

cDNA was synthesized on an RNA template using the RevertAid First Strand cDNA kit (Singapore), according to the instructions of the manufacturer.

Semi-quantitative RT-PCR amplification of genes

Semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) was performed with a cDNA template synthesized from RNA in leaf samples. Specific primer pairs for main genes (*psbA, psbB, psbE, psaA, psaB, Cytb6f,* and *Lhca*) are detailed in Luu et al. (2019). The PCR reaction was performed with a total volume of 20 µL consisting of 1 µL cDNA, 10 µL Master Mix (10X), 1 µL primer F (10 pmol), 1 µL primer R (10 pmol), and 7 µL H_2O. The thermal cycling was performed as follows: Step 1: 95^{o}C for 5 minutes; Step 2: 95^{o}C for 30 seconds; Step 3: 49^{o}C for 30 seconds for *psaA, psbA, psbE* genes and 52^{o}C for 30 seconds for *psbB, Cyt b6f, Lhca* genes; Step 4: 72^{o}C for 1 minute 30 seconds; Step 5: repeat 35 cycles for *psaA, psbA, psbE* genes and 45 cycles for *psbB, psb E, Cyt b6f, Lhca* genes from step 2 to step 4; Step 6: 72^{o}C for 5 minutes; Step 7: keep PCR product at 15^{o}C until use. β-actin was used as the reference gene for data normalization. The conditions for β-actin gene multiplication are similar genes mentioned above. PCR products were checked by electrophoresis on 2% agarose gel, stained with ethidium bromide, and then imaged and processed using Gel pro32 Analyzer software.

Expression levels of genes were assessed by analyzing the area and intensity of the bands after electrophoresis using the Gel pro32 Analyzer software to indirectly quantify the content of the bands. The ratio of the band intensity of the genes *psa*A, *Lhca*, *psb*A-B-E, and *Cytb6f* at each time point in each experimental formula will be normalized with the corresponding β-actin gene and compared with the control formula.

Statistical analysis

Data were processed using the Microsoft Office Excel program. The difference between the experimental treatments was

evaluated by one–way analysis of variance (ANOVA) with significance $p<0.05$.

RESULTS AND DISCUSSION

Effect of nanoscale zerovalent cobalt **on chlorophyll fluorescence**

Our results showed that Fo increased gradually from 17 to 32 DAS, then stabilized until 40 DAS and decreased slightly at later periods. At 32 DAS, Fo of the NZVC treatment at the dose of 0.17 mg/kg and 0.33 mg/kg was lower than that in the control treatment ($p<0.05$). The decrease in Fo showed the effectiveness of applying NZVC to reduce damage in photosystem II of soybean plants. However, at the higher dose of NZVC (100 mg/kg), Fo was not significantly higher than that of the control treatment.

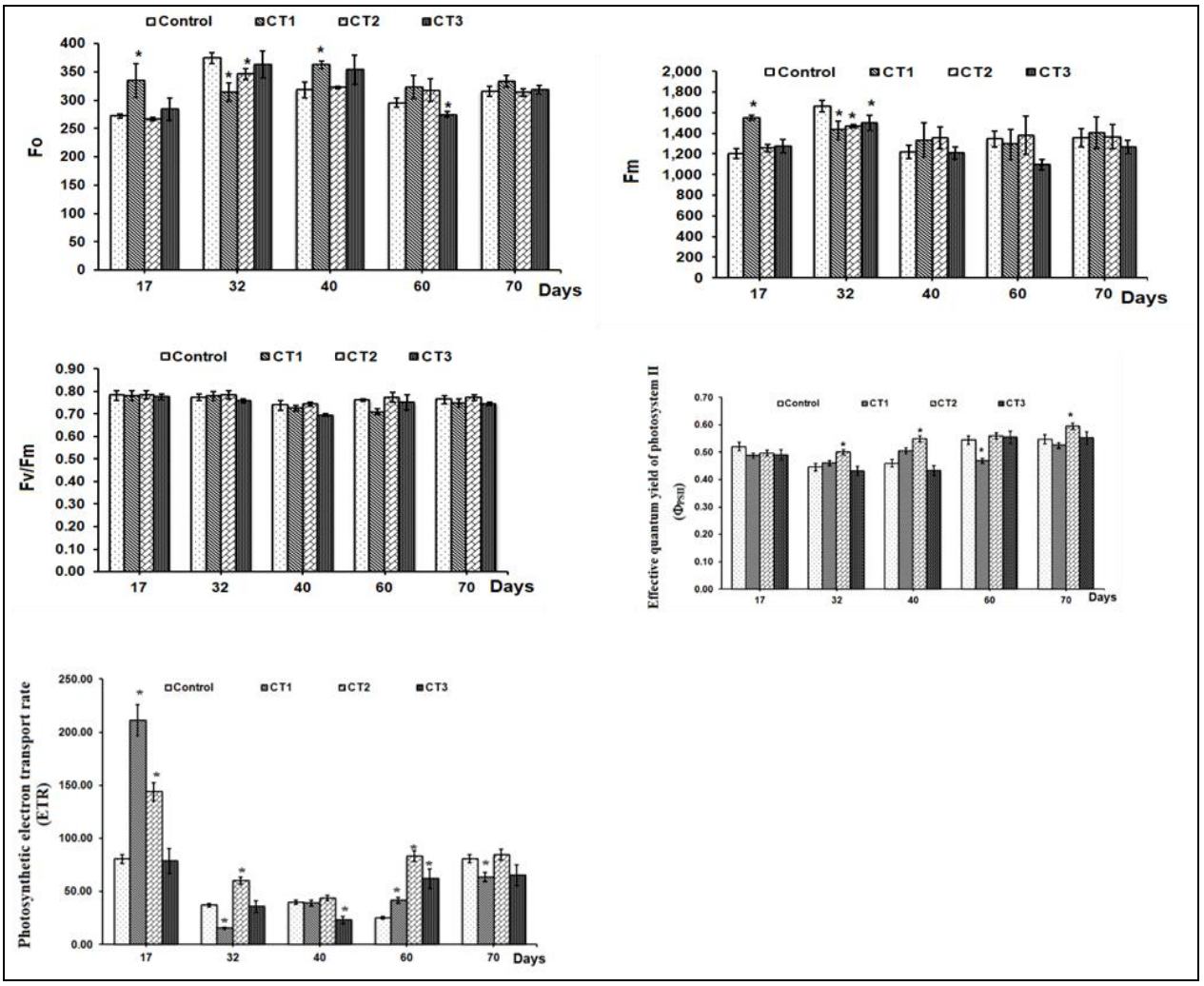


Figure 1. Chlorophyll fluorescence parameters of soybean leaves treated with different levels of NZVC

There was a similar trend in the change of Fm as the Fo, which increased gradually and reached maximum values at the 32 DAS, before decreasing uniformly in all treatments. The Fm value of the NZVC treated treatment was higher than the control (except for 32 DAS). The gradual increase in Fm values indicates an important role of NZVC in reducing the sensitivity of the photosynthetic apparatus in soybean to photoinhibition.

The Fv/Fm ratio is a characteristic of the efficiency of light energy absorbed by PSII used in the photochemical reaction. The results showed that Fv/Fm increased in all treatments to a maximum at 32 DAS and then decreased slightly in the later periods. In particular, the treatment treated with NZVC at a dose of 0.33 mg/kg was higher than that of the control from 10 to 70 DAS. The period of 32-40 days is an important and decisive

time for the photosynthesis and productivity of soybeans. The higher Fv/Fm ratio in the cobalt nanoparticle-treated treatment compared to the control treatment could be the result of the decrease in Fo and increase in Fm during the period from 10 to 32 DAS. Applying NZVC at the rate of 0.33 mg/kg showed the highest value for Fv/Fm of 0.784. Higher Fv/Fm values showed better photosynthetic efficiency in using the light energy absorbed by PSII for the photosynthetic reaction. The treatment of 100 mg NZVC/kg seed showed the lowest value for Fv/Fm of 0.758. It indicated that applying NZVX at a concentration of 100 mg/kg inhibited plant growth.

The ETR increased gradually throughout the plant growth stages. At the same period, the ETR treatments with NZVC were higher than the control. The ΦPSII also increased gradually from 32 DAS with higher values at treatments of 0.17 and 0.33 mg NZVC/kg seed compared to the control treatment. The ΦPSII had the highest value at the dose of 0.33 mg/kg seed and was significantly higher than that of other treatments. During the period from 60 to 70 DAS, treatment of 100 mg NZVC/kg seed had the lowest values for ΦPSII. This result is consistent with the report of Sarropoulou et al. (2016) that high cobalt concentrations cause stress in plants leading to a reduction in the actual photosynthetic rate of tomatoes. This may be a result of the decrease in stomatal conductance and intracellular CO_2 concentration, along with the decrease in photosynthetic pigment and carbonic anhydrase activity.

Overall, the treatment of NZVC at a concentration of 0.33 mg/kg had a positive effect on the photosynthetic parameters of the DT96 soybean variety at 70 DAS. Fo, Fm, and Fv/Fm increased and reached the peaks at 32 DAS, followed by a stable period until 40 DAS before a slight decrease at 60 and 70 DAS. In addition, Fv/Fm, ΦPSII, and ETR of NZVC treatment at the dose of 0.33 mg/kg were significantly higher than those of the control. However, the changes in Fo and Fm values did not follow a clear pattern. The effects of NZVC on the growth and photosynthetic parameters of soybean in this study are similar to Dang et al. (2019), and Phan et al. (2018a, b). Therefore, treating soybean seeds with NZVC at a concentration of 0.33 mg/kg of seed can increase and maximize the efficiency of using light energy absorbed by photochemical system II (PSII) in photochemical reaction, enhance photosynthetic efficiency, and maintain the good photosynthetic capability when plant growth in reducing, thereby contributing to the improvement of soybean productivity.

Effect of nanoscale zerovalent cobalt **on activities of antioxidant enzymes**

The activities of antioxidant enzymes in soybean leaves at different NZVC-treated doses are shown in Figure 2.

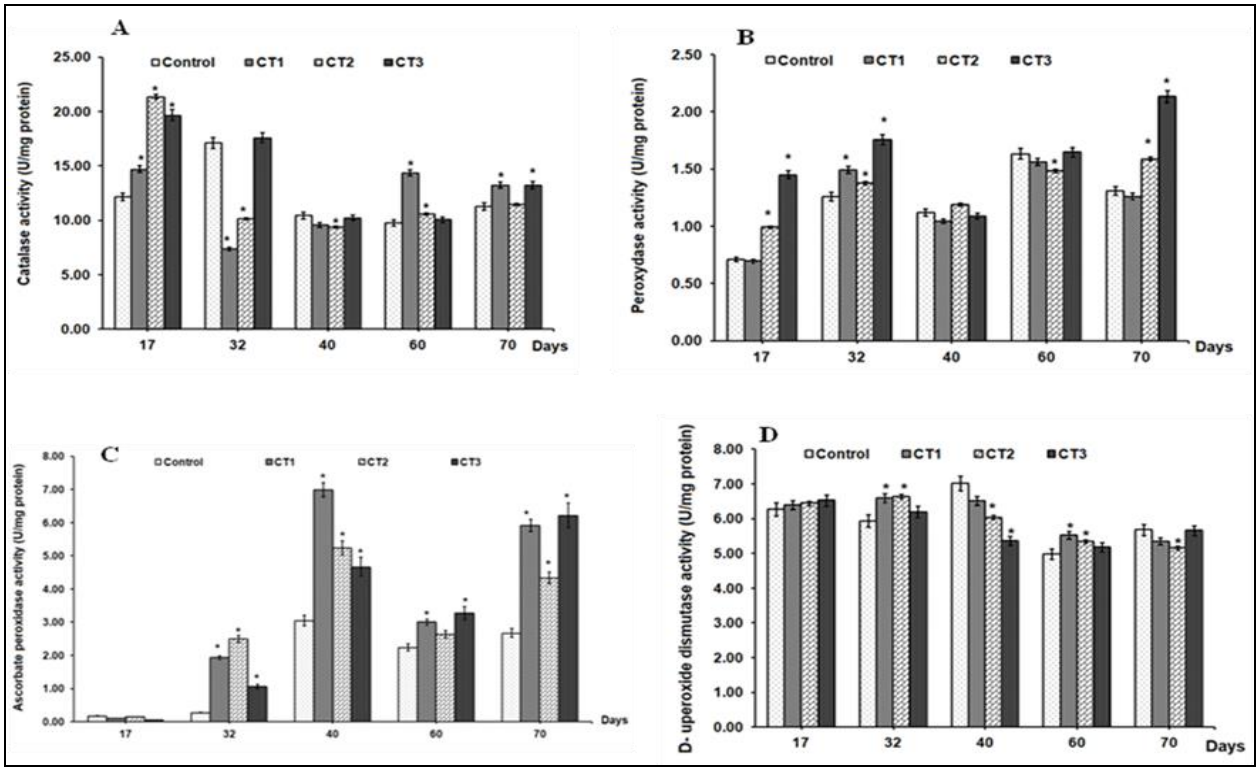


Figure 2. Activities of antioxidant enzymes: catalase (A), peroxidase (B), ascorbate peroxidase (C), and superoxide dismutase (D) of soybean leaves treated with different levels of NZVC

For CAT: with the function of breaking down H_2O_2 into H_2O and O_2, a strong and continuous increase in CAT activity will contribute to reducing the H_2O_2 content in soybean leaves. The study results presented in Figure 2A showed that the catalase activity reached the highest value at 17 DAS, then decreased and stabilized in the later periods. At the same time, the CAT activity in the control was lower than those in the NZVC-treated treatments.

For POX: the POX activity increased gradually from 17 to 70 DAS. There was a significant difference in POX activity between NZVC-treated treatments and control treatment. At 70 DAS, CT3 (100 mg/kg) achieved the highest value (2.13 U/mg protein/min) which was 1.6 times higher than that in the control treatment (Figure 2B).

For APX: Applying NZVC promoted a continuous increase in APX activity in soybean leaves with significantly higher values of APX activity in the NZVC treatment compared to the control. The highest APX activity of 6.98 U/mg protein (2.29 times higher than that in the control treatment) was obtained in soybean leaves in the CT1 treatment (0.17 mg/kg) at 40 DAS (Figure 2C). APX is an enzyme that catalyzes the detoxification of peroxide compounds in the cytoplasm. Therefore, the change in APX activity directly affects the variation of endogenous H_2O_2 in plants. This relationship was demonstrated in soybean plants under the influence of NZVC, when APX maintained high activity, H_2O_2 content in leaves was maintained at a low level.

For SOD: There was a statistically significant difference in SOD enzyme activity between the control and NZVC treatments at concentrations of 0.17 and 0.33 mg/kg seed at 32 DAS ($p<0.05$) (Figure 2D). As SOD is an enzyme that converts O_2- into H_2O_2 and O_2, increasing the activity of this enzyme helps to reduce the amount of endogenous O_2- in soybean leaves.

Effect of nanoscale zerovalent cobalt **on activities of nitrate reductase and nitrite reductase**

The changes in nitrate reductase and nitrite reductase activities in soybean leaves under the effects of different NZVC rates are presented in Figure 3.

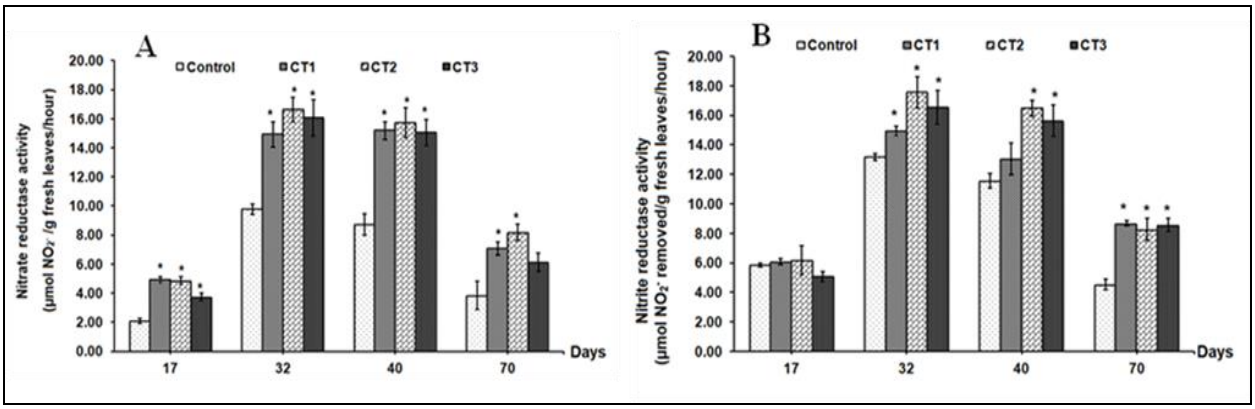


Figure 3. Activities of nitrate reductase (A) and nitrite reductase (B) in DT96 soybean leaves treated with different levels of NZVX

The results in Figure 3A showed that nitrate reductase activity increased from 17 to 32 DAS, then remained stable until 40 DAS and decreased sharply at 70 DAS. The nitrate reductase activity values in the treatments treated with NZVC were higher than those of the control. There was a statistically significant difference between the control and the NZVC-treated treatments at experimental times. The nitrate reductase activity was highest in the NZVC treatment of 0.33 mg/kg seed (CT2) which was 1.7 times higher than the corresponding value in the control formula at 32 DAS. However, there was no significant difference in nitrate reductase activity between the NZVC treatment of 0.33 mg/kg seed (CT2) and 100 mg/kg seed (CT3).

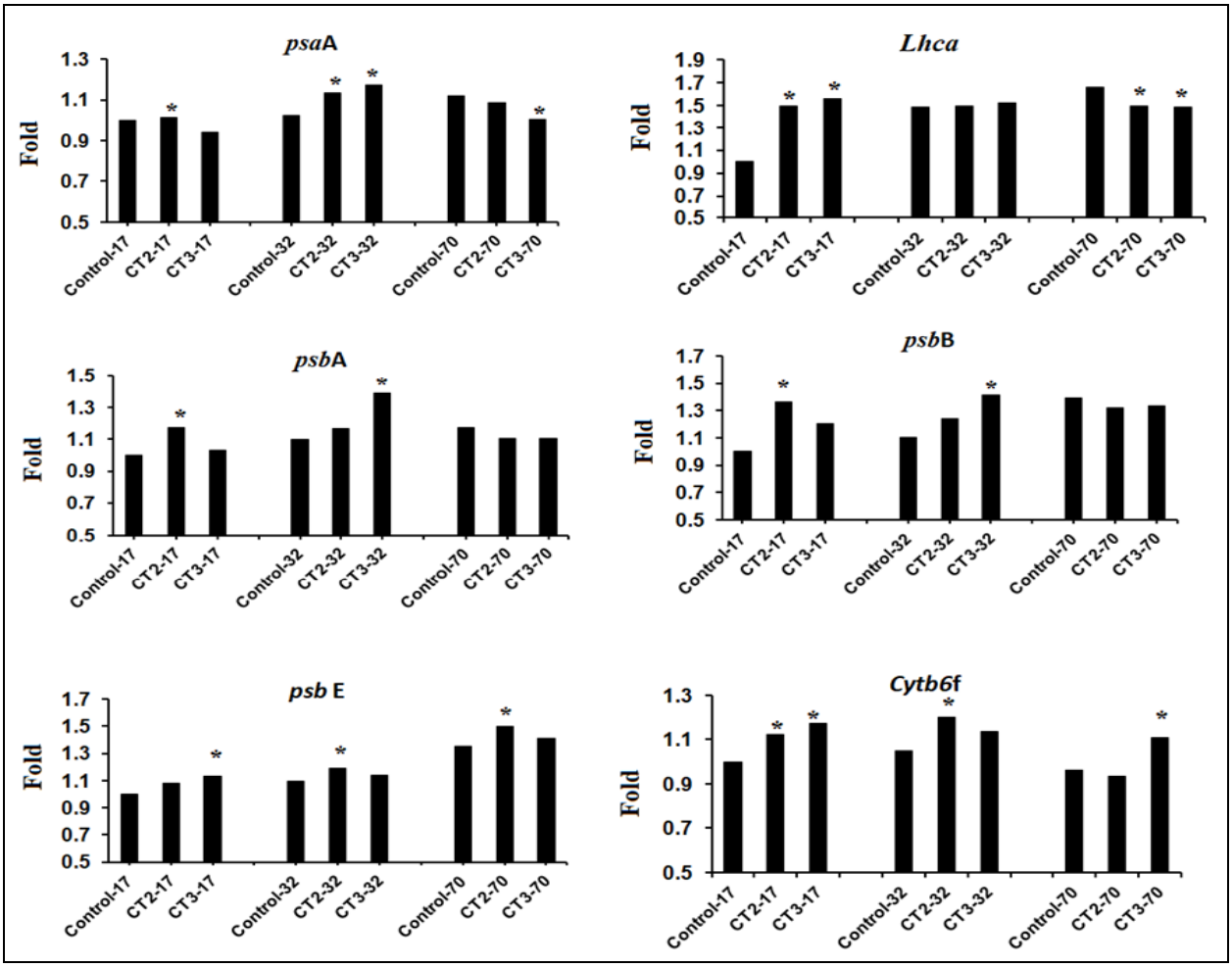
The change in nitrate reductase activity was correlated with the photosynthesis of soybean plants when treated with NZVC. Photosynthetic parameters of soybean plants treated with NZVC were higher than those of the control, also gradually increasing to 32 DAS, then remaining stable until DAS and gradually decreasing in later periods. Kaiser and Kaiser and Förster (1989) also demonstrated that nitrate reductase in spinach leaves rapidly decreased within a few minutes when the photosynthesis rate decreased due to stomatal closure or decreased CO_2 concentration in the external environment. The inactivation of nitrate reductase in response to CO_2 deficiency suggests that CO_2 fixation during photosynthesis requires nitrate reductase to function optimally (Kaiser and Brendel-Behnisch, 1991).

Similar to nitrate reductase, soybean plants with seeds treated with NZVC had higher activity of nitrite reductase than control plants without NZVC treatment. The activity of this enzyme gradually increased to 32 DAS, then gradually decreased at 40 and 70 DAS (Figure 3B). In most NZVC treatments, the activity of nitrite reductase was always stronger than that of nitrate reductase. This may be because NO_2^-, which is a product of the reduction process of nitrate reductase, is toxic to cells and should not be allowed to accumulate. Therefore, the production of a large amount of NO_2^- also requires the stronger activity of nitrite reductase to convert and homogenize this product so that the cells can use it.

The results demonstrated that NZVC treated before sowing improved the ability to fix, convert, and absorb nitrogen in soybean variety by activating the activities of nitrate reductase and nitrite reductase.

Effect of NZVC on photosynthesis-related gene expression

The expression levels of photosynthesis-related genes in soybeans are shown in Figure 4.



** Indicate a significant difference between NZVC treatments and control at p<0.05. Control- non-NZVC treated; CT2 and CT3 - treated with 0.33 and 100 mg NZVC/kg seed, respectively. The number of 17, 32, and 70 were the time of sampling at 17, 32, and 70 DAS, respectively.*

Figure 4. Expression of photosynthesis-related genes of soybean treated with different levels of NZVC

The results showed that the expression levels of *Lhca*, *psa*A genes of photosystem I increased slightly by an increased treated dose of NZVC at 17 DAS. When comparing the expression levels of these genes in soybeans at 32 DAS, the NZVC treatments also had higher values than the control. However, at 70 DAS the expression levels of the *psa*A in the CT2 (0.33 mg/kg seed) were equal, whereas it in CT3 (100 mg/kg seed) was lower than the control.

The change of expression levels of photosynthesis-related genes of photosystem II such as *psb*A, *psb*B, *psb*E, and *Cytb6f* had similar trends to the *psa*A at 17 and 32 DAS. Treated with NZVC led to an increase in expression levels of all investigated genes compared to the control. For the *psb*A and *psb*B, when treated with NZVC of 0.33 mg/kg seeds, the expression levels of these genes were higher than those of the control but not statistically significant. Meanwhile, treated at NZVC concentration of 100 mg/kg seed, the expression levels of *psb*A and *psb*B genes showed a significant difference compared to the control. In contrast, differences in the expression levels of *psb*E and *Cytb6f* genes were only observed in the treatment of NZVC at a concentration of 0.33 mg/kg seeds.

At 70 DAS, the expression levels of *psa*A, *Lhc*A, *psb*A, and *psb*B of NZVC treatments were lower, while the *psb*E and *Cytb6f* genes still expressed higher than that in the control. The results obtained in this study are different from the result of Luu et al. *(*2019) on the photosynthetic gene expression, in which the expression levels of genes related to photosynthesis were higher than those in the control treatment at 70 DAS. This difference may be due to the genetic characteristics of different used soybean

varieties, different concentrations of treated NZVC, and different sowing conditions.

Cobalt directly affects the P680 complex of the electron transport circuit in PSII. There is a change in the distribution of excitation energy in favor of the PSI system. This may also increase ATP synthesis through cyclic electron transport in the photosynthetic electron transport circuit, leading to enhanced/stimulated accumulation of inorganic carbon in growing cells at low cobalt concentrations. Previous studies showed that cobalt at low concentrations has a positive effect on the growth and electron transport rate in the photosynthetic electron transport chain (Mohanty et al., 1989; Ali et al., 2010). El-Sheekh et al. (2003) investigated the effect of cobalt concentration on growth, pigment content, and electron transport chain in the photosynthesis of two algae species of *Monoraphidium minutum* and *Nitzschia perminuta* also found that cobalt stimulated the growth and pigment content of these two algae at low concentrations (0.1-0.5 ppm).

Action mechanism of metal nanoparticles on physiological process

There was little report on the absorption of cobalt in plants at the cellular and subcellular levels. The amount of cobalt absorbed into plants is usually distributed mainly in plant roots (Young, 1979). The amount of cobalt in plants depends on the concentration and chemical form of cobalt in the soil and the plant species. The use of cobalt by fertilizing the soil, seed pretreatment, or foliar spraying at the appropriate doses has high efficiency in promoting plant growth and productivity. For legumes, the benefit of cobalt supplementation may be due to increased nitrogen fixation by *Rhizobia*. For other crops, the benefits of cobalt may be due to interactions with other metal ions. Cobalt also stimulates seed germination and seedling growth, prolongs the shelf life of agricultural products through inhibition of ethylene production and inhibition of ACC oxidase activity (1-aminocyclopropan-1- carboxylic acids), etc.

Cobalt causes toxicity to plants at high concentrations. It causes leaf yellowing and inhibits photosynthesis by suppressing transport and uptake from roots to stems. The strong affinity of cobalt for the -SH group in proteins or polypeptides causes the inactivation of enzymes in plants. In addition, the presence of cobalt affects the ion transport channel on the cell membrane, changing the balance of essential nutrients inside the plant through direct interaction with nutrient absorption of the plant. The absorption of cobalt in plants depends on many factors such as the concentration of cobalt used, pH, light-dark cycle, and the presence of other metal ions (iron, zinc, copper, etc.).

Using zero valence metal nanoparticles has many advantages because these particles have much lower toxicity than their chelates and salt forms, are easily absorbed, and have the ability to stimulate physiological and biological processes even at low concentrations (<300 mg/ha). Furthermore, the bioactivity of these particles was enhanced when they were diffused in an aqueous solution using an ultrasound machine. In this case, the atoms on their surface will be oxidized to create free electrons to stimulate the metabolic pathways into the crop. In particular, seed treatment with zero valence nanoparticles before sowing positively affects plant respiration and germination because this is the stage when free electrons are needed to carry out exchange reactions (Ngo et al. 2014).

In this study, the results showed that the cobalt nanoparticle has a positive effect on plant growth, helping soybean plants to grow strong and healthy, enhancing tolerance to environmental conditions through improved growth indicators (such as plant height, root length, dry weight of rhizomes (Phan et al., 2022) as well as increasing photosynthetic efficiency and activity of antioxidant enzymes. Furthermore, the nitrogen fixation conversion and, absorption capacity in soybeans improved through the activation of the two enzymes nitrate reductase and nitrite reductase. In particular, the efficiency of

plant photosynthesis is enhanced by increasing the synthesis of chlorophyll in leaves, improving the efficiency of light energy absorption by PSII used in photochemical reactions, stimulating and electron transport in photosystem II. Simultaneously, it stimulates the expression levels of genes related to the structure of photosystems I and II to protect the photosynthetic apparatus and mitigate the influence of environmental factors that inhibit photosynthesis and cellular homeostasis. In addition, low concentrations of cobalt can directly affect the P_{680} complex of the electron transport circuit in Photosystem II leading to changes in changes energy distribution in favor of Photosystem I. Therefore, it helps to enhance and stimulate inorganic carbon accumulation in plant cells through cyclic electron transport in the photosynthetic electron transport circuit to enhance ATP molecule synthesis.

CONCLUSIONS

The treatment of soybean plants with nanoscale zerovalent cobalt led to an increase in the the efficiency of using light energy absorbed by photosystem II, increased photosynthetic efficiency, and maintained good operation of the photosynthetic apparatus operation when plant growth was reduced by increasing the value of photosynthetic parameters. Seed treatment with NZVC induced mild “oxidative stress” in soybeans and increased the activitie of antioxidant enzymes (catalase, superoxide dismutase, peroxidase, and ascorbate peroxidase). This is considered a defense mechanism of plants to minimize the harmful effects of these stresses. Higher activity of nitrate reductase and nitrite reductase enzymes in soybeans treated with NZVC than control proves that treatment with NZVC has improved the ability of soybean plants to fix, convert, and absorb nitrogen.

The expression of the main genes involved in photosynthesis of soybean leaves changed along with the change in NZVC treatment concentrations. However, expression levels of *psa*A and *Lhca* genes (involved in photosystem I) and *psb*A, and *psb*B genes (involved in photosystem II) under the effect of NZVC increased only at 17 and 32 DAS and decreased at 70 DAS. Meanwhile, *psb*E and *Cytb6f* genes (involved in photosystem II) showed higher expression levels than the control over growth time from 17 to 70 DAS. NZVC at high concentrations (0.33 and 100 mg/kg seed) did not negatively affect the photosynthetic function of soybean.

ACKNOWLEDGEMENTS

This research is funded by the Vietnam Ministry of Education and Training under grant number B.2021-SP2-04. The authors would like to thank Prof. Dr. Nguyen Hoai Chau, MSc. Dao Trong Hien, Institute of Environmental Technology, Vietnam Academy of Science and Technology helped produce zero valence cobalt nanoparticles used in the experiment.

REFERENCES

Asada, K., 1992 *Ascorbate peroxidase - A hydrogen peroxide-sca-venging enzyme in plants*. Physiol. Plantarum, 85: 235-241.

Akeel, A., and Jahan, A., 2020. *Role of cobalt in plants: Its stress and alleviation*. In: Naeem, M., Ansari, A., Gill, S. (eds.), Contaminants in Agriculture. Springer, Cham. https://doi.org/10.1007/978-3-030-41552-5_17

Ali, B., Hayat, S., Hayat, Q., Ahmad, A., 2010. *Cobalt stress affects nitrogen metabolism, photosynthesis and antioxidant system in chickpea (Cicer arietinum* L.*).* J. Plant Interact., 5(3): 223-231.

Amako, K., Chen, G.X., Asade, K., 1994. *Separate assays specific for ascorbate peroxidase and guaiacol peroxidase and for the chloroplastic and cytosolic isozymes of ascorbate peroxidase in plants*. Plant and Cell Physiology, 35: 497-504.

Barber, J., Nield, J., Morris, E.P., Zheleva, D., Hankamer, B., 1997. *The structure, function and dynamics of photosystem two.* Physiologia Plantarum, 100(4): 817-827.

Berry, J.O., Yerramsetty, P., Zielinski, A.M., Mure, C.M., 2013. *Photosynthetic gene expression in higher plants*. Photosynthesis Research, 117(1-3): 91-120.

Bradford, M.M., 1976. *A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding*. Analytical Biochemistry, 72(1-2): 248-254.

Chen, W.P., Li, P.H., Chen, T.H.H., 2000. *Glycinebetaine increases chilling tolerance and reduces chilling-induced lipid peroxidation in Zea mays L*. Plant, Cell and Environment, 23: 609-618.

DalCorso, G., Manara, A., Piasentin, S., Furini, A., 2014. *Nutrient metal elements in plants*. Metallomics, 6: 1770-1788.

Dang, D.H., Hoang, T.L.A., Luu, T.T., Show, P.L., Leong, H.Y., 2019. *Effects of nanoscale zerovalent cobalt on growth and photosynthetic parameters of soybean Glycine max (L.) Merr. DT26 at different stages*. BMC Energy, 1(1): 1-6.

El-Sheekh, M.M., El-Naggar, A.H., Osman, M.E.H., El-Mazaly, E., 2003. *Effect of cobalt on growth, pigments and the photosynthetic electron transport in Monoraphidium minutum and Nitzchia perminuta*. Brazilian Journal of Plant Physiology, 15(3): 159-166.

Gad, N., El-Moez, A., Bekbayeva, L.K., Karabayeva, A.A., Surif, M., 2013. *Effect of cobalt supplement on the growth and productivity of soybean* (*Glycine max L. Merril)*. World Applied Sciences Journal, 26(7): 926-933.

Guo, D.P., Guo, Y.P., Zhao, J.P., Liu, H., Peng, Y., Wang, Q.M., Rao, G.Z., 2005. *Photosynthetic rate and chlorophyll fluorescence in leaves of stem mustard* (*Brassica juncea var. tsatsai) after turnip mosaic virus infection*. Plant Science, 168(1): 57-63.

Hussain, M.I., and Reigosa, M.J., 2011. *A chlorophyll fluorescence analysis of photosynthetic efficiency, quantum yield and photon energy dissipation in PSII antennae of Lactuca sativa L. leaves exposed to cinnamic acid.* Plant Physiology and Biochemistry, 49(11): 1290-1298.

Jayakumar, K., Jaleel, C.A., Azooz, M.M., Vijayarengan, P., Gomathinayagam, M., Panneerselvam, R., 2009. *Effect of different concentrations of cobalt on morphological parameters and yield components of soybean*. Global Journal of Molecular Sciences, 4(1): 10-14.

Kaiser, W.M., and Forster, J., 1989. *Low CO_2 prevents nitrate reduction in leaves*. Plant Physiology, 91(3): 970-974.

Kaiser, W.M., and Brendle-Behnisch, E., 1991. *Rapid modulation of spinach leaf nitrate reductase by photosynthesis. I. Modulation by CO_2-availability*. Plant Physiology, 96: 363-367.

Luciński, R., and Jackowski, G., 2006. *The structure, functions and degradation of pigment-binding proteins of photosystem II*. Acta Biochimica Polonica, 53(4): 693-708.

Luu, T.T., Hoang, T.M.H., Hoang, T.L.A., Phan, H.T., Dang, D.H., 2019. *The impact of zerovalent cobalt nanoparticle on photosynthesis-related genes expression in soybean leaves Glycine max (L.) Merr. (DT26)*. Biology, 41(1):141-152. (Abstract in English)

Mohanty, N., Vass, I., Demeter, S., 1989. *Impairment of photosystem 2 activity at the level of secondary quinone electron acceptor in chloroplasts treated with cobalt, nickel and zinc ions.* Physiol. Plant, 76: 386-390.

Ngo, Q.B., Dao, T.H., Nguyen, H.C., Tran, X.T., Van Nguyen, T., Khuu, T.D., Huynh, T.H., 2014. *Effects of nanocrystalline powders (Fe, Co and Cu) on the germination, growth, crop yield and product quality of soybean* (*Vietnamese species DT-51).* Advances in Natural Sciences: Nanoscience and Nanotechnology, 5(1): 015016.

Phan, H.T., Hoang, T.L.A., Luu, T.T., Ngo, T.H.T., Dao, T.H., Nguyen, H.C., Dang, D.H., 2018a. *Evaluating the effect of zero valence cobalt nanoparticles on growth and photosynthetic parameters of soybean plant Glycine max (L). Merril "DT51" at different growth stages.* National Biotechnology Science Conference, Natural Science and Technology Publishing House: 1331-1338. (Abstract in English)

Phan, H.T., Luu, T.T, Hoang, T.L.A., Ngo, T.H.T., Nguyen, H.C., Dang, D.H., 2018b. *Study on the effect of single and mixed cobalt nanoparticles on the change of photosynthetic parameters and antioxidant enzyme activity in soybean Glycine max Merr. DT26.* Journal of Biology, 40(2): 233-243. (Abstract in English)

Phan, T.T.H., Chu, D.H., Pham, T.P., Do, T.T., Bui, T.T., Ha, D.C., Kieu, T.H.M., Nguyen, V.D., 2022. *Effect of nano cobalt on some agro-biological characteristics of DT96 soybean cultivated in Vinh Phuc province*. Vietnam Science and Technology Journal, 64(7): 44-48. (Abstract in English)

Sarropoulou, V., Dimassi-Theriou, K., Therios, I., 2016. *Effect of the ethylene inhibitors silver nitrate, silver sulfate, and cobalt chloride on micropropagation and biochemical parameters in the cherryrootstocks CAB-6P and Gisela 6*. Turkish Journal of Biology, 40(3): 670-683.

Scebba, F., Sebastiani, L., Vitagliano, C., 1999. *Protective enzymes against activated oxygen species in wheat* (*Triticum aestivum* L.) *seedlings: Responses to cold acclimation*. Journal of Plant Physiology, 155: 762-768.

Teixeira, R.N., Ligterink, W., França-Neto, J.D.B., Hilhorst, H.W., da Silva, E.A., 2016. *Gene expression profiling of the green seed problem in soybean*. BMC Plant Biology, 16(1): 1-15.

Wang, B., Du, Q., Yang, X., Zhang, D., 2014. *Identification and characterization of nuclear genes involved in photosynthesis in Populus*. BMC Plant Biology, 14(1): 1-17.

Young, R.S., 1979. *Cobalt in biology and biochemistry*. Academic Press, London.