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Piriformospora indica SYMBIOSIS AND IRON OXIDE NANOPARTICLES ALLEVIATES DROUGHT STRESS IN SOYBEAN PLANTS THROUGH IMPROVED ON PHOTOSYNTHETIC GAS EXCHANGE AND SUCROSE PHOSPHATE SYNTHASE AND ACID PHOSPHATASE

Ehsan Ghorbannia Delavar[1], Ali Faramarzi[1*], Jalil Ajalli[1], Naser Nazari[2], Mehrdad Abdi[1]

[1]Department of Agronomy and Plant Breeding, Miyaneh Branch, Islamic Azad University, Miyaneh, Iran
[2]Department of Soil Science, Miyaneh Branch, Islamic Azad University, Miyaneh, Iran
[*]Corresponding author. E-mail: ali.faramarzi1299@gmail.com

ABSTRACT

Drought stress is one of the most destructive environmental stresses which affect the performance of soybean photosynthetic apparatus and can reduce plant productivity. This study aimed to evaluate the effects of iron oxide nanoparticles (NPs), *Piriformospora indica* and drought effects on gas exchange, sucrose phosphate synthase (SPS) and acid phosphatase (ASP) activities. Treatments included inoculation and non-inoculation of *P. indica*, NPs (0, 0.5 and 0.75 gL^{-1}) and three irrigation regimes (control, irrigation after 50% and 75% depleting of soil water content). This study showed that drought stress reduced SPAD values and increased membrane electrolyte leakage. It also had negative effect on photosynthetic functional parameters. Nevertheless, inoculation of soybean with *P. indica* can provide drought-tolerance by improving the net photosynthesis, carboxylation efficiency, P, SPS and ASP parameters, keeping membrane stability and raising chlorophyll content. In addition, similar to *P. indica*, NPs treatment alleviated the negative effects of drought stress on gas exchange by altering physical biochemical responses, which caused a low internal CO$_2$ concentration, sucrose and leaf damage. The above findings show that, *P. indica* and NPs can mitigate the photosynthetic limitations through promoting photosynthetic capacity under drought stress in soybean.

Keywords: photosynthesis, SPS, ASP, drought stress, iron oxide nanoparticles, *P. indica*.

INTRODUCTION

Soybean (*Glycine max* L.) is one of the most economically valuable crops, and a source of oil and high-quality protein, which its demand has grown dramatically in recent decades. It is used as food and pharmaceutical industries, raw material in animal feed industry, biodiesel production, as well as the production of biodegradable materials as an alternative to plastics (Candeia et al., 2009; Masuda and Goldsmith, 2009; Song et al., 2011). However, the sustainability of soybean yields is adversely affected by climate change in many parts of the world (Ohashi et al., 2006; Li et al., 2013; Bilal et al., 2020).

Drought is one of the most devastating environmental stresses limiting crop productivity worldwide (Mafakheri et al., 2010). Drought stress causes broad range of physiological changes and disruption of several important plant mechanisms, such as photosynthesis and osmotic stress, which markedly reduces growth and plant yield (Gavili et al., 2019). The effects of drought on the photosynthesis is well established. Mainly, photosynthetic disturbance reduces carbon dioxide fixation and photosynthetic activity because of CO$_2$ emission limitation to chloroplasts and the closure of stomata and photosystem II disturbance (Ohashi et al., 2006; Li et al., 2013).

The use of rhizosphere microorganisms is an important environmental strategy to mitigate damage to physiological processes and photosynthetic efficiency of plants under drought stress. *Piriformospora indica* is one of the most important microorganisms that form a symbiosis with the roots of many plant species and increase their growth and yield (Sherameti et al., 2008; Shahabivand et al., 2017), nodulation (Mansotra et al., 2015), and promotes nutrient uptake (Wu et al., 2018). Several studies have shown that *P. indica*

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enhanced photosynthetic efficiency of plants such as sunflower (Shahabivand et al., 2017) tomato (Ghorbani et al., 2018) and Arabidopsis (Sherameti et al., 2008) through improving water and nutrient uptake, increasing antioxidant enzyme activity and improving electron transport chain activity under environmental stress. However, in studies of the efficacy of *P. indica*, little attention has been paid to the significant role of *P. indica* in improving physiological processes including photosynthesis and its components.

Crop nutrition management under stress conditions is one of the most important issues in crop production. Adequate intake of nutrients in plant will increase resistance to biological and non-biological stresses (Waraich et al., 2011; Waraich et al., 2012). On the other hand, calcareous soils of arid and semi-arid regions decrease the solubility of micronutrients in the soil, which leads to a decrease in micronutrients absorption by plant (Janmohammadi et al., 2016). Iron is one of the essential elements and plays a key role in many physiological processes including chlorophyll biosynthesis, oxidation and reduction in energy exchange cycles, activation of enzymes which are involved in photosynthetic and mitochondrial electron transfer, respiration, nitrogen fixation, lignin production and metabolism of sulfur substances (Qureshi et al., 2010). Due to the low efficiency of conventional chemical fertilizers and their harmful effects on the environment as well as the quality of agricultural products, application of novel technologies such as nano-fertilizers can be an effective step towards achieving sustainable and environmentally friendly agriculture (Kalia and Kaur, 2019). Moreover, previous studies showed that the nano-Fe application has a positive effect on growth, protein content, photosynthetic pigments and potassium and phosphorus uptake in Catharanthus Roseus (Askary et al., 2017), yield and yield components of safflower (Janmohammadi et al., 2018).

There is a little information on the application of iron nanoparticles in photosynthetic changes in soybean under drought stress. Hence, studying the effect of iron nanoparticles and water deficit stress will help us to understand physiological changes in soybean. In addition, no comprehensive studies have been conducted to determine the results and effects of interaction between two factors of iron nanoparticles and *P. indica* coexistence. Accordingly, the present study aimed to evaluate the effect of these two factors on water deficit stress tolerance using photosynthesis parameters in soybean.

MATERIAL AND METHODS

Plant material

A field experiment was conducted as a factorial based on a randomized complete block design with three replications in Sari city during the years 2017 and 2018. Treatments included inoculation of fungi (inoculated and non-inoculated control), three irrigation regimes (irrigation after 20%, 50% and 70% soil moisture depletion) and three levels of iron oxide Nano particles (0, 0.5 and 0.75 g L^{-1}). Soybean seeds were inoculated by dipping in fungal suspension for 3 hours. Then the seeds were sown in plots by dimensions of 5×3.5 m^2. NPs foliar spraying was performed on the six-leaf-stage of the plants, and drought stress was applied before flowering and continued until physiological maturity. NPs were obtained from Pishgaman Mashhad Company. Prasad et al. (2012) method was used to prepare iron oxide nanoparticles. The mixture of materials and distilled water was placed in an ultrasonic device for half an hour (ultrasonic disperses the nanomaterials). Then, in order to dissolve the nanoparticles and to prevent the nanomaterials from sticking together, a magnet was inserted into the shaker for 4 hours.

Root Colonization Measuring

The root colonization was measured by the method described by Vierheilig et al. (1998). The root-pieces were examined by optical microscope.

Membrane permeability

Membrane permeability was measured as described by Lutts et al. (1996). Leaf samples were placed in tubes containing 30 ml of deionized water at room temperature for 24 h. After the samples were incubated for 20 min at 95°C, the electrical conductivity was measured. Finally, EL was calculated using the following formula:

$$EL (\%) = (EC1/EC2) \times 100$$

Gas exchange

The photosynthesis parameters in the fresh leaf samples were measured using IRGA. All measurements were performed at 10-12 am by placing the middle part of the leaf inside the gas exchange chamber for one minute. The carboxylation efficiency was obtained by dividing photosynthesis rate per unit area by intracellular CO_2 concentration. Also, photosynthetic water use efficiency was obtained by dividing photosynthetic rate by stomatal conductance.

SPAD

A SPAD Chlorophyll Meter (SPAD-502 Minolta, Japan) was used to rapidly and accurately evaluate leaves' relative chlorophyll concentrations. Chlorophyll concentration changes were measured in the middle section of the leaves.

Phosphorus

The mineral Phosphorus (P) was extracted from the shoot of soybean according to Ntatsi et al. (2014).

Acid phosphatase

Acid phosphatase activity according to Li et al. (2017), the absorbance of the reaction product ρ-nitrophenol at 405 nm was measured spectrophotometrically following reaction with the substrate para-nitrophenylphosphate (ρ-NPP).

Sucrose

The sucrose concentration in leaves was determined by spectrophotometry at A_{480} nm (Xu et al., 2015).

Sucrose phosphate synthase

Sucrose phosphate synthase (SPS) activity assay was measured according to Hubbard et al. (1989). Reaction solution contained 40 μ L of dialyzed supernatant, 5 mM fructose-6-phophate, 10 mM uridine diphosphate glucose, 15 mM $MgCl_2$, 15 mM glucose-6-phophate, 1 mM EDTA, and 50 mM Hepes-KOH buffer (pH 7.5). The mixture incubated at 30°C for 30 min, then Samples mixed with 0.2 mL 5 mM NaOH at 100°C for 10 min. Subsequently, mixture mixed with 3.5 mL anthrone solution (0.15 g anthrone + 100 mL 81% H_2SO_4) at 40°C for 20 min.

Statistical analysis

Data were analyzed using SAS (9.2) statistical program and means were compared using an LSD (Least Significant Difference) test ($P<0.05$). Person's correlation analysis was used to evaluate Correlation coefficients among the measured traits, also principal component analysis (PCA) were analyzed by XLSTAT software.

RESULTS AND DISCUSSION

Colonization

In microscopic inspection, the chlamydospores and hyphae of *P. indica* were observed in the root cortex, as an extensive network of hyphae was formed around the roots (Figure 1).

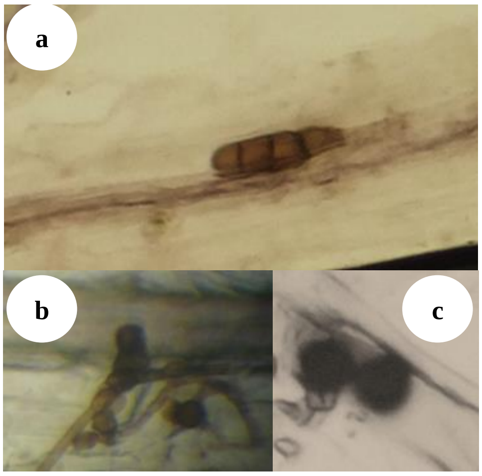

Figure 1. In microscopic analysis, development of *P. indica* [intracellular chlamydospores (a), hyphal growth (b) and spores (c)] was observed in root cells and around the root of soybean.
The root-pieces were examined under light microscope at the magnification of 10-40 X.

Leaves gas exchanges parameters

Stomatal conductance (gs)

The findings of this study showed that drought stress significantly reduced stomatal conductance compared to control (Table 1); and the reduction was significantly higher under severe stress. In both controlled and stress conditions, symbiosis relationship with *P. indica* had a significant effect on leaf stomatal conductance. Therefore, stomatal conductance was significantly increased in the fungal infected treatment (16.12% higher than the control treatment in medium drought stress). On the other hand, foliar spray with iron oxide nanoparticles (NPs) significantly increased stomatal conductance, but the difference between 0.5g L^{-1} and 0.75g L^{-1} concentration was not significant.

Photosynthesis net (Pn)

The results show that, the decrease in gs and the limitation of CO_2 availability in the chloroplasts were accompanied with a decrease in photosynthesis. As shown in Table 1, the decrease of photosynthesis was even greater when plants were exposed to severe drought. Under drought stress (Figure 4), there was no significant difference in photosynthetic rate between 2017 and 2018, whereas, leaf photosynthetic rate of control plants was higher in 2018 than in 2017. In both control and stress conditions (Table 1), fungal coexistence reduced the negative effects of stress on leaf photosynthesis (13.21 and 13.12% higher than control treatment under well watering and depleting 50% of soil water content, respectively). However, the positive effect of the fungus was not significant in severe drought. Application of NPs significantly increased photosynthesis rate of soybean leaf in control treatment. The highest increase was observed in NPs at 0.75g L^{-1} (30% higher than control treatment). Under stress conditions, both concentrations of NPs increased the photosynthetic rate of soybean leaf. However, the positive effect of NPs was significant only at medium stress level (13.21 and 13.12% higher than the control treatment in the application of 0.75 and 0.5 g L^{-1} of NPs, respectively) (Table 2).

In both inoculated and non-inoculated plants, the most positive effect of NP_S in Pn and MC was observed in high levels of NPs. however, there was no significant difference in Pn and MC between two NPs concentration (Table 2).

Internal CO_2 concentration (Ci)

The internal CO_2 concentration increased in response to drought stress. It is noteworthy that leaves which were sprayed with iron NPs showed lower Ci at both controlled and stress conditions (Table 2). This reduction was more noticeable at medium drought stress (25.46 and 20.29% lower than the control treatment under depleting 50% and 75% of soil water content, respectively). Fungi symbiosis remarkably decreased internal CO_2

concentration in control treatments, and it is interesting to note that NPs application (Table 3) remarkably reduced internal CO_2 concentration in both inoculated and non-inoculated plants. The most positive effect of NPs on Ci was observed in inoculated plants treated with 0.75 g L^{-1} of NPs.

There was no significant difference in Ci between 2017 and 2018 in the Application of NPs (Figure 3) and fungus inoculation (Figure 2) treatments, whereas, internal CO_2 concentration of control plants was higher in 2018 than in 2017.

Carboxylation efficiency (MC) and Transpiration rate (E)

According to the results, drought stress significantly decreased plant carboxylation efficiency (Table 1), and it reached to the lowest level in severe drought (83.3% decrease compared to control). Nevertheless, the symbiotic relationship with *P. indica* helped soybean plant to mitigate the negative effect of drought on carboxylation efficiency; compared with the control, the greatest effect of symbiosis was observed at a moderate level of drought (15.38% improvement compared to the control).

Under both controlled and stress conditions, NPs application remarkably increased carboxylation efficiency (MC) in soybean leaves. In normal condition, the highest values were recorded in 0.75 g L^{-1} of NPs (30% improvement compared with control plants). However, in stress condition, NPs significantly enhanced MC, especially in medium levels of drought. The highest increase was observed in 0.75 g L^{-1} of NPs (Table 3) .

In addition, drought significantly reduced transpiration value (Table 44). Transpiration value reached to the lowest level in severe water deficit (84.76% compared with the control). Inoculation with *P. indica* and application of NPs increased transpiration rate compared to control plants. The highest increase was observed in 0.5 g L^{-1} of NPs (21.72 % higher than control plants) (Table 2). However, there was no significant difference in transpiration rate between fungi coexistence and NPS application.

Photosynthetic water use efficiency (WUE)

The results in (Table 5) show that photosynthetic water use efficiency (WUE) was significantly affected by the three-way interaction of drought stress, *P. indica* and NPs treatments. Compared to the control, a rise of 30% was recorded in WUE when water deficit reached to 75% decrease in the soil water content. As expected, *P. indica* and NPs treatments increased this value. In severe drought stress, symbiotic relationship with *P. indica* was more effective in increasing the WUE (8.60% increase compared to control) compare to the other treatments. The highest value of WUE was observed in the 0.75g L^{-1} NPs in the medium drought stress and the lowest one was recorded in the control treatment.

Chlorophyll content

As shown in (Table 1), SPAD value sharply declined under increasing water deficit. The decline was more noticeable in severe drought with 31.06% reduction. However, symbiosis relationship with *P. indica* significantly ameliorate the negative effect of drought stress on SPAD value, the improvement was more significant when water deficit was increased to 75% depletion of soil water content (23.33% higher compared to uninoculated control, whereas the improvement in the well-watered plants was only 5.51%).

SPAD was also affected by NPs separately, as well as their interaction with fungi-inoculated treatment. NPs increased SPAD in uninoculated plants and plants treated with *P. indica* (there was no significant difference in SPAD value between two NPs concentrations). The highest and the lowest values were observed in fungi-inoculated treatments with 0.75 g L^{-1} of NPs and control plants, respectively (Table 2).

Membrane electrolyte leakage (EL)

The findings show that cell membrane considerably damaged by drought stress (Table 5) which led to increased membrane electrolyte leakage (62.7%) under 75% of soil water content depletion; however, cell membrane damages ameliorated by NPs, *P. indica* and their interaction. Under severe drought stress conditions, symbiosis relationship with *P. indica* separately was more effective than NPs and their interactions for reducing the negative effect of drought stress. The highest membrane permeability was observed when plants were colonized by *P. indica* in the three level of irrigation regime (Table 5).

Phosphorus

The results of Table 5 show that water deficiency resulted in a significant decrease in soybean leaf phosphorus concentration (about 40% decrease in FC 30% compared to the control). However, in both drought and non-drought conditions, inoculation with fungi and application of NPs significantly increased phosphorus uptake. In both drought and non-drought conditions, the increase was even greater when inoculated plants were exposed to NPs, especially in 0.75 gL^{-1} concentration of NPs. The highest and lowest levels of phosphorus were observed in normal (*P. indica* * NPs) and severe drought stress (in non-symbiotic plants and without application of NPs), respectively. Application of 0.5 gL^{-1} NPs (Figure 3) and fungus inoculation (Figure 2) significantly increased leaf phosphorus in 2017 compared to 2018; whereas, leaf phosphorus content of control plants was higher in 2017 than in 2018.

Acid phosphatase

Acid phosphatase activity increased when plants were exposed to drought stress (Table 5). The increase was even greater when severe drought stress (FC 30%) was applied. Also, fungal symbiosis significantly increased acid phosphatase level under normal condition. Under drought stress, acid phosphatase activity was significantly higher in plants treated with 0.5 and 0.75 gL^{-1} NPs and fungal inoculation compared to control plants. This difference was even greater when higher drought levels (30% FC) were applied. The highest acid phosphatase activity was observed in NPs treatments (0.5 gL^{-1} and 0.75 gL^{-1}) under severe drought stress.

Sucrose

The results demonstrated that sucrose content increased with increasing water deficit stress severity (46.39% and 58% increase when plants were subjected to the medium severe water deficit stress (Table 4). *Piriformospora indica* symbiosis with soybean had significant effect on leaf sucrose content. Fungi coexistence reduced sucrose content as 7.9% compared to the uninoculated control. On the other hand, NPs foliar spray increased sucrose content. The highest and the lowest values were recorded in 0.75 g L^{-1} of NPs and control treatment, respectively (Figure 5).

Sucrose phosphate synthase

The results (Table 5) show that SPS activity in soybean leaves significantly increased when plants exposed to drought stress. SPS activity increased to a peak at severe drought level (21.54% increase compare to the control). In both control and drought conditions, inoculated plants showed a significant increase in SPS activity in their leaves compared to the control. The application of iron oxide nanoparticles also increased SPS activity under drought stress conditions. However, increase in SPS activity depends on the intensity of stress as well as the concentration of NPs. At both drought stress levels (50 and 30% Fc), SPS activity was higher in the application of 0.75 gL^{-1} than 0.5 gL^{-1} NPs. However, under non-stress conditions, SPS activity decreased in the NPs application treatment compared to the control. In addition, in the present study, the positive effect of the fungus on SPS activity in fungal inoculation treatments was greater compared to the treatments in which inoculated plants were sprayed with different concentrations of NPs.

Principle Component Analysis (PCA)

The PCA results of data obtained from all photosynthetic parameters, Sucrose, SPS, APS, P, relative chlorophyll content (SPAD reading) and cell membrane electrolytic leakage of soybean leaf exposed to irrigation regimes are shown in Figure 6. It shows that the first component with 66.30% relative variance and the second component with 15.75% had the highest variation among the obtained components; and the two main components together accounted for 82.05% of the total variation in traits in this study (Figure 6). In the first and second components of the biplot, it was shown that the treatments were divided in two distinct groups. Based on the results of the biplot, the control treatments were placed into one group. On the other hand, moderate and severe stress treatments were included in one set. The biplot results showed that sucrose, SPAD, P, Pn, CM, gs and E traits had a strong positive correlation with non-stress treatments, while, WUE, APS, SPS, EC and Ci had a strong positive correlation with moderate and severe stress treatments.

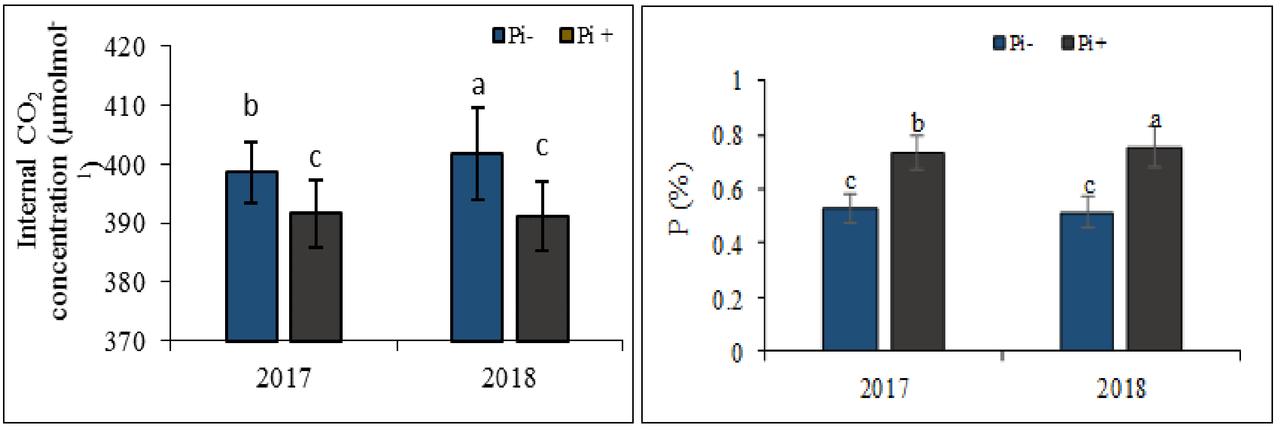

Figure 2. Effects of *P. indica* and iron oxide nanoparticles (NPs) on internal CO_2 concentration (Ci) and phosphorus (P) in 2017 and 2018, in soybean. In each figure, means with the same letter are not significantly different according to LSD test at $P < 0.05$. Bars represent means±SD (n=3).

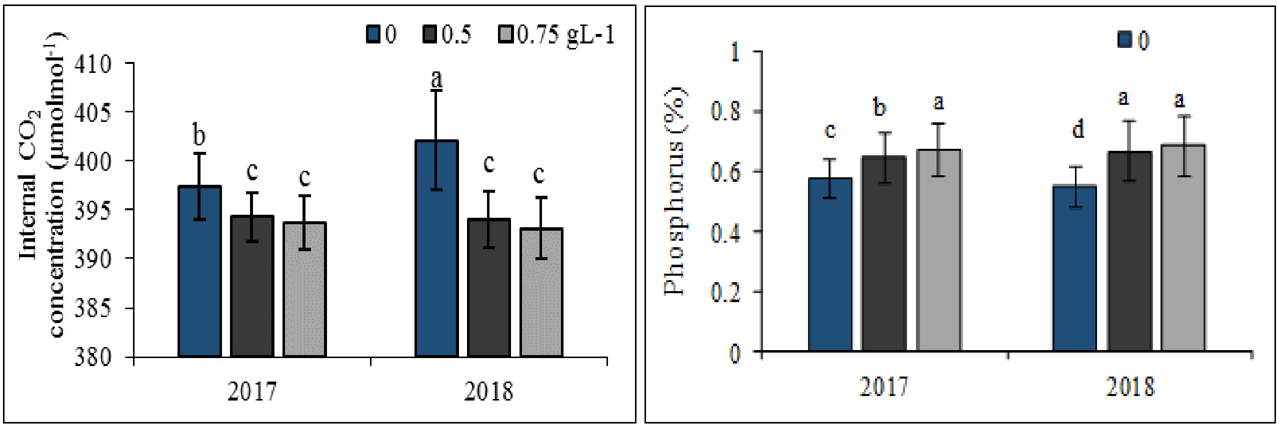

Figure 3. Effect of iron oxide nanoparticles (NPs) on internal CO_2 concentration (Ci) and phosphorus (P) in 2017 and 2018, in soybean. In each figure, means with the same letter are significantly different according to LSD test at $P < 0.05$. Bars represent means±SD (n=3).

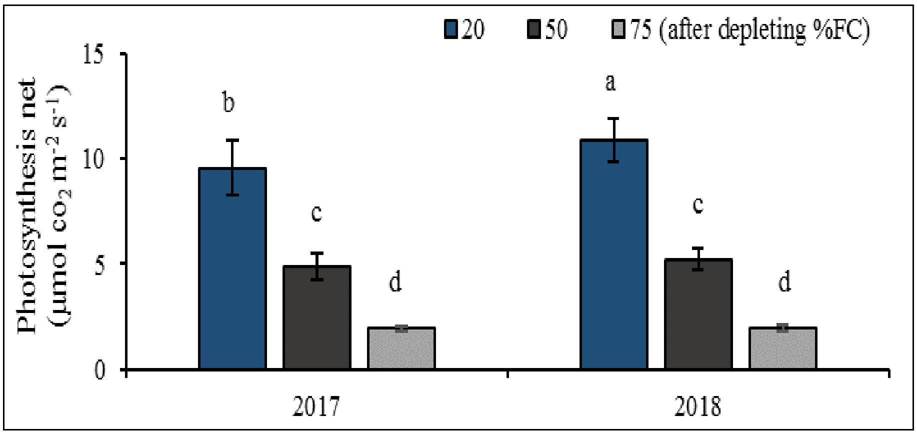

Figure 4. Effect of drought stress on photosynthesis net (Pn) in 2017 and 2018, in soybean. In each figure, means with the same letter are significantly different according to LSD test at P < 0.05. Bars represent means±SD (n=3).

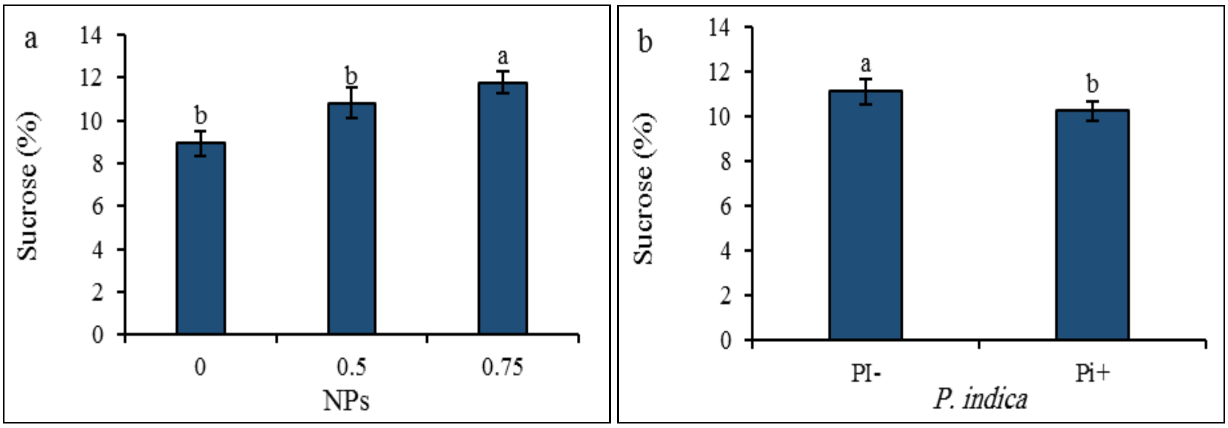

Figure 5. Effects of iron oxide nanoparticles (NPs) (a) and *P. indica* (b) on sucrose in soybean. In each figure, means with the same letter are significantly different according to LSD test at P < 0.05. Bars represent means±SD (n=3).

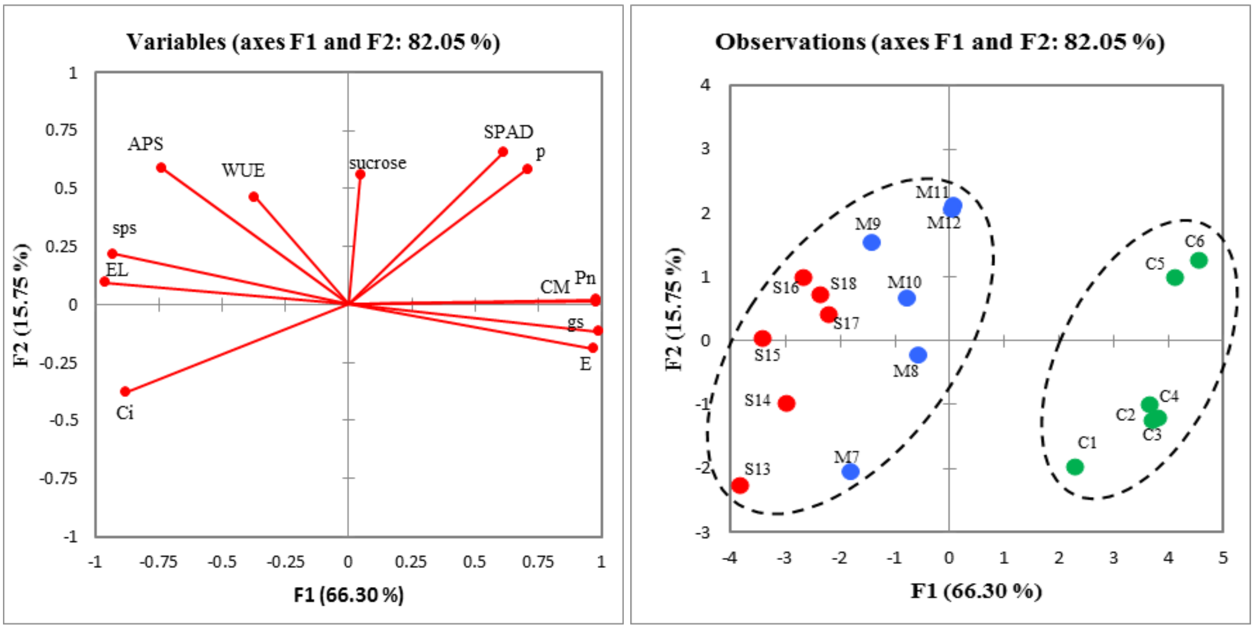

Figure 6. Biplot of principle component analysis for the first two principle components of all parametersdrought stress, *P. indica* and iron oxide nanoparticles (NPs). PCA biplot for traits studied under drought stress and *P. indica* and NPs treatment (a) and parameters (b). Abbreviations: C (non-drought stress), M (medium drought stress), S (severe drought stress), 1 (control treatment), 2 (0.5 g L^{-1} of NPs), 3 (0.75 g L^{-1} of NPs), 4 (*P. indica*), 5 (0.5 g L^{-1} of NPs + *P. indica*) and 6 (0.75 g L^{-1} of NPs + *P. indica*).

In this study, drought stress caused damage to membrane system and chlorophyll content of soybean leaves (Table 1, 5). One of the most important consequences of drought stress is thylakoid membrane lipids peroxidation in chloroplast which is caused by overproduction of reactive oxygen species (ROS) (Tian et al., 2013; Shao et al., 2016). Interestingly, the application of NPs reduced the damage of chloroplast membrane system caused by drought stress, and maintained the relative stability of the soybean leaf membrane system (Table 5). This oxidative damage reduction can be attributed to ROS scavenger activation such as catalase, peroxidase and isoform of superoxide dismutase which help to remove excessive ROS produced under drought stress and also improve plant cell membrane integrity leading to increased drought tolerance (Akbari et al., 2013). We also found that NPs played a positive role in enhancement of photosynthetic pigments under drought stress; this can be explained by the role of iron in the chlorophyll biosynthesis (Miller et al., 1984), which is consistent with a previous study of NPs - positive effects on photosynthesis pigments in Arabidopsis by Yoon et al. (2019).

As it is expected, the results showed that endophytic fungi helped soybean to improve cell membrane integrity and to protect cellular chlorophyll by reducing the oxidative damage caused by drought (Table 1, 5). Some studies have suggested that mycorrhizal fungi are involved in the detoxification of free radicals through the accumulation of free radicals in the fungal arbuscules, resulting in lower membrane lipid and protein oxidation (Wu et al., 2009; Fester and Hause, 2005). On the other hand, some studies showed that endophytic fungi helped plants to ameliorate the negative effects of reactive oxygen species on the membrane system by enhancing the ROS scavenger activity (Khalvandi et al., 2019).

Meanwhile, in this study gas exchange parameters showed that drought stress had a negative effect on soybean photosynthetic system. It reduced stomatal conductance, photosynthesis, transpiration, carboxylation efficiency and photosynthetic efficiency of water use. We noticed that there was a direct correlation between photosynthetic parameters, for example: stomatal closure was associated with simultaneous reduction in carboxylation efficiency, water use efficiency and photosynthesis rate. In consistent with our results, previous studies reported that stomatal restriction is associated with impaired metabolism and reduced photosynthesis (Sarabi et al., 2019). This could indicate that the photosynthetic parameters are completely intertwined.

It was also seen that NPs played a positive role in reducing photosynthetic system disturbances under drought stress in soybean. Moreover, as evidenced by mitigated inhibitory effect of drought stress on stomatal conductance and CO_2 uptake (Table 4), they might have affected cytochromes in the electron transport chain, leading to an increase in CO_2 uptake and improved photosynthesis (Qureshi et al., 2010). Consistent with these results, an increase in photosynthetic activity and growth in exogenous application of zero-valent nano-iron in the Arabidopsis plant has been reported. They showed that iron nanoparticles Causes stomata openings and facilitate CO_2 uptake through over-expressing of H+ - ATPase, and eventually, it increases photosynthesis (Yoon et al., 2019).

In this study, we provided evidence that fungal symbiosis increased stomatal conductance and subsequently increased photosynthesis at different levels of drought stress, suggesting that the presence of fungi can help to improve carbon availability for photosynthesis (Table 1). This is consistent with a previous study of the positive effects of *P. indica* on *Solanum lycopersicum* L. photosynthesis by Ghorbani et al. (2018). The reason that the photosynthetic rate increases in inoculated plants under drought stress conditions could be improved water uptake and changes in the expression of aquaporin genes which leads to maintenance of cellular water and mesophilic conduction improvement (Chen et al., 2017); upregulating the RbcL gene expression (encoding the large Rubisco subunit) in symbiotic plants can be another strategy for reducing metabolic limitation of CO_2 uptake and inhibiting CO_2 accumulation in intercellular areas (Chen et al., 2017).

The results show that phosphorus uptake in *P. indica* plants increased significantly under both normal and stress conditions. It is well documented that endophytic fungi can release P from sources which are not accessible for plant root in normal condition. It transforms P to the available forms, which are transported to host plants through fungal hyphae directly or taken up by roots (Wu et al., 2018). This can be attributed to the ability of *P. indica* to increase the activity of phosphorus transporters and also to stimulate phosphatase activities in the plant as well as in the rhizosphere soil. In our study, the acid phosphatase enzyme activity in leaves was strongly stimulated by *P. indica*, when plant subjected to drought stress. Consistent with these results, Wu et al. (2018) reported that the fungus stimulated the acid phosphatase activity in *B. napus*, and the accumulation of organic acids such as oxalic, malic and citric acids in the rhizosphere, ultimately increased the solubilization of inorganic P and improved its uptake by *B. napus* root. We demonstrate that high P concentration in plants exposed to NPs can be due to the proton pump activity which is induced by NPs. Activation of PM H + -ATPase induced by NPs leads to proton leakage and acidification of the rhizosphere, which ultimately increases the P availability for plants (Yoon et al., 2019).

Drought stress decreased carbon uptake while increased sucrose accumulation in leaves. Previous studies have shown that increasing soluble sugars, mainly sucrose, increases plant tolerance to drought stress. One reason is that sucrose is an important source of energy for maintaining metabolism and growth of plant cells under drought stress. In addition, sucrose is an osmoprotectants which is important to protect the cell membrane against dehydration (Schubert et al., 2004: Wu et al., 2017). However, the amount of sucrose decreased in *P. indica* soybean leaves. This may be due to the effect of fungal root colonization on phloem loading rate and carbon allocation to soybean root system; also, physiological changes and changes in genes expression related to carbon metabolism through increasing P concentration in shoots (Zhang et al., 2020). Previous studies also reported higher sucrose allocation in AM roots for nutrient and phosphorus exchange (Bücking and Shachar-Hill, 2005; Zhang et al., 2020).

Notably, in this study, a positive correlation was observed between SPS activity and sucrose content. Many previous studies showed that sucrose accumulation under stress conditions is associated with increased activity of SPS and decreased activity of sucrose cleavage enzymes (Wu et al., 2017). In stress conditions, increased SPS activity is associated with ABA- signaling which increases leaf sucrose content through UDPG and fructose free recycling (Ruan, 2012). On the other hand, reducing sucrose and increasing SPS activity in inoculated plants supports the hypothesis that symbiotic plants may have a higher sucrose distribution from shoots to roots for reducing drought stress effects. In addition, increased activity of sucrose cleavage enzymes in the roots of symbiotic plants can results in higher sucrose demand in roots under drought stress (Schubert et al., 2004). Increased SPS in inoculated soybean under drought stress indicates its functional contribution to increasing the efficiency of sucrose utilization and sufficient carbon pools maintenance for several important pathways, such as secondary metabolites and better growth (Al-Arjani et al., 2020). The positive effect of endophytic root colonization reported on carbon allocation to the soybean root system (Wang et al., 2016) and increased activity of SPS in trifoliate orange (Wu et al., 2017).

Table 1. Effect of *P. indica* and drought stress on photosynthesis parameters in soybean plants

Drought stress (after depleting %FC)	*P. indica*	Photosynthesis net (µmol co$_2$ m^{-2} s^{-1})	Stomatal conductance [mmol (H$_2$O) m^{-2} s^{-1}]	Carboxylation efficiency (m^{-2}s^{-1} mmol)	SPAD
20	Pi-	9.506 (±0.633) ^{b}	0.682 (±0.015) ^{b}	0.024 (±0.002) ^{b}	47.983 (±1.473) ^{bc}
20	Pi+	10.960 (±0.356) ^{a}	0.786 (±0.015) ^{a}	0.029 (±0.001) ^{a}	54.166 (±1.114) ^{a}
50	Pi-	4.709 (±0.275) ^{d}	0.265 (±0.016) ^{d}	0.012 (±0.001) ^{d}	39.184 (±1.739) ^{d}
50	Pi+	5.411 (±0.145) ^{c}	0.311 (±0.019) ^{c}	0.014 (±0.000) ^{c}	51.041 (±1.090) ^{b}
75	Pi-	1.770 (±0.074) ^{e}	0.122 (±0.007) ^{e}	0.004 (±0.000) ^{e}	33.842 (±1.925) ^{e}
75	Pi+	2.087 (±0.023) ^{e}	0.134 (±0.010) ^{e}	0.005 (±0.000) ^{e}	50.096 (±1.083) ^{c}

Means in a column followed by the same letter are not significantly different according to LSD test at $P \leq 0.05$ (n=3).

Table 2. Effect of iron oxide nanoparticles (NPs) foliar spray and *P. indica* on photosynthesis parameters in soybean plants

P. indica	NPs (gL^{-1})	Internal CO$_2$ concentration (µmolmol^{-1})	Photosynthesis net (µmol co$_2$ m^{-2} s^{-1})	Transpiration rate (m^{-2}s^{-1} mmol)	Carboxylation efficiency (m^{-2}s^{-1} mmol)	SPAD
Pi-	0	406.333 (±2.986) ^{a}	3.793 (±0.567) ^{c}	3.171 (±0.633) ^{c}	0.009 (±0.001) ^{c}	31.856 (±1.990) ^{d}
Pi+	0.5	396.743 (±1.946) ^{b}	5.958 (±0.855) ^{ab}	4.056 (±0.789) ^{a}	0.015 (±0.002) ^{b}	43.981 (±1.591) ^{c}
Pi-	0.75	397.369 (±2.254) ^{b}	6.235 (±1.006) ^{ab}	3.553 (±0.771) ^{bc}	0.016 (±0.003) ^{ab}	45.174 (±1.420) ^{c}
Pi+	0	393.122 (±2.085) ^{c}	5.813 (±0.810) ^{b}	3.863 (±0.756) ^{ab}	0.015 (±0.002) ^{b}	48.063 (±1.052) ^{b}
Pi-	0.5	391.521 (±2.490) ^{cd}	6.179 (±0.928) ^{ab}	3.907 (±0.682) ^{ab}	0.016 (±0.002) ^{ab}	52.682 (±0.856) ^{a}
Pi+	0.75	389.412 (±2.451) ^{d}	6.467 (±0.991) ^{a}	3.867 (±0.707) ^{ab}	0.017 (±0.003) ^{a}	54.558 (±0.991) ^{a}

Means in a column followed by the same letter are not significantly different according to LSD test at $P \leq 0.05$ (n=3).

Table 3. Effect of iron oxide nanoparticles (NPs) and drought stress on photosynthesis parameters in soybean plants

Drought stress (after depleting %FC)	NPs (gL^{-1})	Internal CO$_2$ concentration (µmolmol^{-1})	Photosynthesis net (µmol co$_2$ m^{-2} s^{-1})	Carboxylation efficiency (MC) (m^{-2}s^{-1} mmol)
20	0	387.86 (±3.84) e	8.35 (±1.29) c	0.021 (±0.0034) c
	0.5	386.9 (±2.97) ef	10.58 (±1.13) b	0.027 (±0.0029) b
	0.75	384.02 (±4.2) f	11.76 (±0.56) a	0.03 (±0.0013) a
50	0	400.18 (±5.62) c	4.30 (±0.63) e	0.01 (±0.0016) e
	0.5	392.06 (±3.96) d	5. 29 (±0.455) d	0.013 (±0.0011) d
	0.75	389.81 (±2.83) de	5.58 (±0.307) d	0.014 (±0.0007) d
75	0	411.13 (±5.31) a	1.75 (±0.244) f	0.0042 (±0.0006) f
	0.5	406.25 (±1.38) b	1.99 (±0.055) f	0.0049 (±0.0001) f
	0.75	404.08 (±3.53) b	2.03 (±0.053) f	0.005 (±0.0001) f

Means in a column followed by the same letter are not significantly different according to LSD test at $P \leq 0.05$ (n=3).

Table 4. Effect of drought stress on transpiration rate, sucrose and stomatal conductance in soybean plants

Drought stress (after depleting %FC)	Transpiration rate (m^{-2}s^{-1} mmol)	Sucrose (%)	Stomatal conductance (mmol (H$_2$O) m^{-2} s^{-1})
20	7.81 (±0.196) ^{a}	9.53 (±0.67) ^{c}	0.35 (±0.045) ^{b}
50	2.19 (±0.079) ^{b}	10.32 (±0.73) ^{b}	0.4 (±0.048) ^{a}
75	1.19 (±0.04) ^{c}	11.1 (±0.48) ^{a}	0.38 (±0.047) ^{a}

Means in a column followed by the same letter are not significantly according to LSD test at $P \leq 0.05$ (n=3).

Table 5. Effect of *P. indica*, iron oxide nanoparticles (NPs) and drought stress on soybean plants

Drought stress (after depleting %FC)	*P. indica*	NPs (gL^{-1})	Water use efficiency (mol^{-1} µmol co$_2$)	Membrane electrolyte leakage (%)	Phosphorus (%)	Sucrose phosphate synthase (mmol sucrose mg^{-1} protein h^{-1})	Acid phosphatase (mg h^{-1} g^{-1} FW)
20	Pi-	0	0.93 (±0.066) ^{k}	17.93 (±0.449) ^{h}	0.61 (±0.23)^{h}	6.59 (±0.04)^{hi}	3.40 (±0.21)^{h}
20	Pi-	0.5	1.175 (±0.051) $^{i-k}$	15.70 (±0.576) ^{i}	0.64 (±0.022)^{gh}	6.52 (±0.082)^{i}	2.95 (±0.30)^{i}
20	Pi-	0.75	1.37 (±0.030) $^{g-j}$	15.35 (±0.439) ^{i}	0.65 (±0.019)^{g}	6.92 (±0.124)^{g}	2.83 (±0.33)^{i}
20	Pi+	0	1.11 (±0.082) ^{jk}	13.90 (±0.215) ^{i}	0.74 (±0.015)^{d}	6.87 (±0.051)^{g}	4.15 (±0.02)^{g}
20	Pi+	0.5	1.30 (±0.087) $^{h-j}$	14.931 (±0.133) ^{i}	0.87 (±0.010)^{b}	7.02 (±0.267)^{g}	4.2 (±0.11)^{g}
20	Pi+	0.75	1.51 (±0.053) $^{f-i}$	14.62 (±0.160) ^{i}	0.91 (±0.016)^{a}	6.82 (±0.138)^{gh}	4.22 (±0.11)^{g}
50	Pi-	0	1.74 (±0.135) $^{d-f}$	34.70 (±0.455) ^{e}	0.42 (±0.013)^{j}	7.74 (±0.112)^{f}	4.18 (±0.06)^{g}
50	Pi-	0.5	2.50 (±0.146) ^{b}	28.90 (±0.761) ^{g}	0.54 (±0.018)^{i}	7.66 (±0.17)^{f}	5.25 (±0.24)^{f}
50	Pi-	0.75	4.28 (±0.286) ^{a}	36.16 (±0.636) ^{e}	0.55 (±0.018)^{i}	7.79 (±0.105)^{f}	6.11 (±1.15)^{e}
50	Pi+	0	2.18 (±0.058) ^{bc}	27.08 (±0.553) ^{g}	0.66 (±0.012)^{fg}	8.30 (±0.153)^{cd}	7.06 (±0.18)^{abc}
50	Pi+	0.5	2.07 (±0.105) ^{cd}	32.50 (±0.468) ^{f}	0.74 (±0.029)^{d}	7.66 (±0.199)^{f}	6.9 (±0.02)^{cd}
50	Pi+	0.75	2.26 (±0.234) ^{bc}	35.41 (±1.135) ^{e}	0.79 (±0.02)^{c}	7.89 (±0.18)^{ef}	6.33 (±0.05)^{de}
75	Pi-	0	1.46 (±0.019) $^{f-j}$	48.07 (±1.016) ^{b}	0.37 (±0.022)^{k}	8.40 (±0.213)^{bc}	5.04 (±0.08)^{f}
75	Pi-	0.5	1.68 (±0.092) $^{e-g}$	45.05 (±1.080) ^{c}	0.43 (±0.014)^{j}	8.34 (±0.27)^{bcd}	6.36 (±0.05)^{de}
75	Pi-	0.75	1.72 (±0.061) $^{e-g}$	51.21 (±0.719) ^{a}	0.43 (±0.016)^{j}	8.81 (±0.22)^{a}	6.69 (±0.19)^{cd}
75	Pi+	0	1.92 (±0.036) $^{c-e}$	42.14 (±0.523) ^{d}	0.54 (±0.013)^{i}	8.58 (±0.22)^{ab}	7.23 (±0.26)^{ab}
75	Pi+	0.5	1.58 (±0.079) $^{e-h}$	45.09 (±0.616) ^{c}	0.69 (±0.03)^{ef}	8.12 (±0.144)^{de}	7.42 (±0.16)^{a}
75	Pi+	0.75	1.79 (±0.054) $^{d-f}$	45.69 (±0.670) ^{c}	0.71 (±0.021)^{de}	8.46 (±0.24)^{bc}	7.44 (±0.28)^{a}

Means in a column followed by the same letter are not significantly different according to LSD test at $P \leq 0.05$ (n=3).

CONCLUSIONS

Photosynthesis is the major determinant of plant performance. The results of this study showed that drought stress caused several physiological and biochemical changes in soybean plants, including damage to the cell membrane and a decrease in P uptake and photosynthetic efficiency. Meanwhile, *P. indica* and NPs had a positive impact on photosynthetic functional parameters in soybean leaves; as inoculation of soybean with *P. indica* and the application of NPs helped the plant to maintain greater photosynthetic function, SPS and ASP and membrane stability.

As a result, they showed higher net photosynthesis rates. Effective use of latent ecological relationships in plant rhizosphere and potential of nano fertilizers can be a good solution for increasing plant resistance to environmental stresses and sustainability of field production.

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