



## Effect of *Piriformospora indica* inoculation on some morphophysiological parameters in licorice (*Glycyrrhiza glabra* L.) under drought stress

Ellahe Rezaei<sup>1</sup>, Mehdi Ghabooli<sup>1\*</sup>, Zahra Movahedi<sup>1</sup>, Ehsan Mohseni Fard<sup>2</sup>

1. Department of Agronomy and Plant Breeding, Faculty of Agriculture, Malayer University, Malayer, Iran

2. Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Zanjan, Zanjan, Iran

---

### Abstract

In the last years, there is a growing demand for plants used in traditional medicine. Licorice (*Glycyrrhiza glabra*) contains different phytochemicals, that have demonstrated various pharmacological activities. The endophytic fungus, *Piriformospora indica*, has been reported to enhance the growth of host plants and allow them to survive under abiotic and biotic stress. This study was undertaken to investigate the effect of *P. indica* on drought stress tolerance of *G. glabra*. The experiment was conducted in a completely randomized design with three fungus treatments (spore, mycelium and non-inoculated), and two levels of drought treatments (Field capacity and 50% F.C.) in three replications. Four weeks after stress induction (six weeks after inoculation), samples were collected and, growth and morpho-physiological traits were measured. The drought stress decreased shoot, and root dry weight, chlorophyll content, potassium, and phosphorus content and increased electrolyte leakage, H<sub>2</sub>O<sub>2</sub>, proline, and Na; but, the interaction of licorice with *P. indica* resulted in an overall increase in plant biomass. The photosynthetic pigment (Chl a, Chl b, total Chl, and carotenoid), proline, K, and P were significantly higher for inoculated seedling. Furthermore, the inoculated seedling had lower H<sub>2</sub>O<sub>2</sub>, electrolyte leakage, and Na content. In conclusion, our findings indicated that the symbiotic association of endophytic fungus *P. indica* had a positive effect on growth and morpho-physiological traits and also can help the plants to tolerate drought stress. These results are opening up a window of opportunity for application of this fungus in desert agriculture of medicinal plants.

**Keywords:** *Glycyrrhiza glabra*, Drought stress, Growth promotion, Symbiosis, K/Na.

**Rezaei, E. Ghabooli, M. Movahedi, Z. and E. Mohsenifard.** 2020. ' Effect of *Piriformospora indica* inoculation on some morphophysiological parameters in licorice (*Glycyrrhiza glabra* L.) under drought stress'. *Iranian Journal of Plant Physiology* 10(4), 3379-3389.

---

### Introduction

Licorice (*Glycyrrhiza glabra* L., Fabaceae) is one of the most popular medicinal plants and has extensively used in traditional medicine and pharmaceutical industry. Also, licorice can be used as a source for livestock feeding. In Iran, licorice is

among the most important raw herbal drug. Pharmacological characteristics of glycyrrhizin include anti-bacterial, insecticidal, anti-allergic properties, as well as the capacity to elicit a boost of the immune system (Davis and Morris, 1991; Karkanis et al., 2018).

Drought stress severely reduces plant growth and yield in arid and semi-arid regions, which include parts of Iran (Tabari and Talaee, 2011; Golian et al., 2015). Decrease in water

\*Corresponding author

E-mail address: m.ghabooli@malayeru.ac.ir

Received: January , 2020

Accepted: August , 2020

availability to plants leads to changes in the concentration of many metabolites, thus it can limit plant growth and development (Boyer, 1982). Stressed plants responses to drought stress by using different defensive strategies. A different approach to strengthening the plant capacity to tolerate drought involves exploiting their interaction with endophytic fungi (Achatz et al., 2010).

The endophytic fungi have played an important role in nutrient acquisition, stimulating plant growth, and tolerance to abiotic stress. *Piriformospora indica* is a plant-root colonizing endophytic fungus belonging to division Basidiomycetes that discovered in the Indian Thar desert. This fungus could be simply replicate in axenic culture (Varma et al., 1999). *P. indica* has attracted considerable attention due to its growth, and yield promoting ability, besides its potential to confer abiotic stress tolerance (Ghabooli et al., 2013; Kord et al., 2019; Li et al., 2017). The positive effects of *P. indica* on growth and development of several different medicinal plants such as *Bacopa monniera* (Prasad et al., 2013), *Stevia rebaudiana* (Kilam et al., 2017) *Withania somnifera* (Ahlawat et al., 2017) and *Valeriana officinalis* (Ghabooli et al., 2018) have been reported. The results indicate that the inoculation of medicinal plants with *P. indica* can improve plant growth and may help them overcome abiotic stress. *P. indica* inoculation may promote nutrient uptake into the hosts, increasing K/Na ratio, compatible solute accumulation and the overall performance of the plant.

Drought stress together with *P. indica* inoculation may influence the growth and development in licorice plants. In this research, the impact of *P. indica* on mitigating the adverse effects of drought stress on licorice plants was investigated. For this purpose, we evaluated some morpho-physiological responses of licorice plant inoculated with *P. indica* and its impact on drought stress tolerance via the measurements of biomass, photosynthetic pigments, proline, electrolyte leakage, elements contents, and relative water content.

## Materials and methods

### Fungal preparation, plant inoculation and growth condition

*P. indica* was cultured according to the method of Ghabooli et al. (2013), and after collection of fungal spores, their numbers were counted using Neobar slide and adjusted to  $5 \times 10^5$  spores per mL. To prepare the fungal mycelium, the active discs were placed in a liquid medium and then incubated in a shaker incubator at 28 °C and 150 rpm for 7-10 days. Next, mycelium was filtered and washed several times with distilled water (Bajaj et al., 2015).

Licorice seeds, obtained from Pakanbazar Co., were surface-sterilized by immersion in 95% v/v ethanol for 2 min, and then in 10% sodium hypochlorite (NaOCl) for 10 min. Then the seeds were rinsed in water and were germinated for seven days. Licorice seedlings were inoculated by immersing in the spore suspension solution with gentle shaking for 1–2 h. For mycelium treatment, 1% (w/w) of mycelium suspension was added to each pot. The mock-treated seedlings whether were dipped in sterile water or treated with autoclaved mycelium. The seedlings were later transferred into pots (1.5 kg plastic pots), filled with normal soil, and then placed in the research greenhouse of Agricultural faculty, Malayer University. The experiment was conducted in a completely randomized design with three fungus treatments (spore and mycelium *P. indica*-inoculated and non-inoculated), and two drought stress treatments [Filed capacity (F.C.) and 50% F.C.] in three biological replications. Drought was initiated by withholding water, and drought-stressed pots were re-watered, when the soil moisture reached 50% F.C. The well-watered treatment was maintained near F.C. (Ghaffari et al., 2016). The samples for physiological analysis were harvested four weeks after stress induction.

### Shoot and root dry weight, RWC, and Photosynthetic pigments

For dry weight measuring, the shoot and root samples were incubated in an oven at 70 °C for 48h. Leaf RWC (Relative Water Content) was determined, according to (Barr and Weatherley, 1962).

$RWC = [(FW - DW) / (TW - DW)] \times 100$ ; where FW=fresh weight, DW=Dry weight and TW= Turgid weight.

Photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) were extracted from leaf samples in 80% acetone (v/v) as described by Arnon (Arnon, 1949). After centrifugation, the optical density of supernatant was recorded at 645, 663 and, 470 nm using a UV-Vis spectrophotometer.

### Electrolyte leakage

EL from each treatment was measured to find out the membrane stability. In 50-ml vials, the young leaf pieces with same size were immersed in 40 ml of deionized water for 24 h on a shaker at 120 rpm, and electrical conductivity (EC1) was measured. After disrupting the cell membrane by boiling the samples at 100° C for 20 min, the electrical conductivity (EC2) was again measured (Ershadi et al., 2016). The following formula was used to define electrolyte leakage:

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

### Hydrogen peroxide and Proline

H<sub>2</sub>O<sub>2</sub> estimation was performed according to the modified protocol of (Velikova and Loreto, 2005). Shoot samples (1g) were crushed in 0.1% TCA solution and then centrifuged at 6000g for 15 min at 4 °C. Reaction mixture was added to 0.5 mL of 10 mM phosphate buffer (pH 7.0), 1.0 mL of 1M potassium iodide and 0.5 mL of supernatant. Absorbance was measured at 390 nm, and H<sub>2</sub>O<sub>2</sub> content was determined with the help of a standard curve.

According to Bates (1973) (Bates et al., 1973), proline was extracted from fresh leaf samples (0.5 g) using sulfosalicylic acid solution (3% w/v) and estimated spectrophotometrically by acid ninhydrin solution. The optical density of the supernatant was estimated at 520 nm against toluene using standard curve.

### Leaf elements (Na, K, and P)

Ions (P, K, and Na) were extracted from 0.2 g of dry leaves. Acid digestion was carried out by mixing samples with 4 ml HNO<sub>3</sub> and 1 ml HClO<sub>4</sub> and heated to 220 °C for 20 min. The resulting mixture

was extracted with 5 ml HNO<sub>3</sub> and adjusted to the final volume of 250 ml of distilled water. The ions content (P, K, and Na) in samples were analyzed by inductively coupled plasma spectrometry. The unit of element contents was expressed as mg/g F.W.

### Statistical analysis

Statistical analyses were done with JMP 13.2.0 software package, and mean comparison was made using Duncan's multiple range test at  $P \leq 0.05$ .

## Results

### Shoot dry weight, root dry weight, and RWC

The effect of *P. indica* co-cultivation on the licorice plant growth was assessed under normal and drought conditions. Colonization of *P. indica* significantly increased shoot biomass compared with un-colonized plants. The highest value was seen in P2 treatment (mycelium treatment- 0.29 gr per plant) (Fig. 1). Total biomass in normal conditions was significantly higher than stressed condition (0.27 g in normal versus 0.21 g in stressed), although the colonization increased total biomass accumulation.

The same positive effect of co-cultivation with *P. indica* was observed for root dry weight. The root dry weight of seedlings treated with endophytic fungus was significantly higher than un-treated. P1 treatment has the highest root weight (0.218 g) (Fig. 1). Drought stress increased root dry weight significantly (0.187 gr in normal versus 0.212 gr in stressed condition).

Exposure to drought stress greatly influenced plant water potential and reduced relative water content (RWC) significantly (75.26% versus 64.96%). RWC was higher in *P. indica* co-cultivated plants than the un-inoculated plants at whatever drought level assessed (Table 1). The highest RWC value was seen in P1 treatment (72.37%). Overall, these results showed that *P. indica* considerably increased plant performance under normal and stressed conditions.

### Photosynthetic pigments

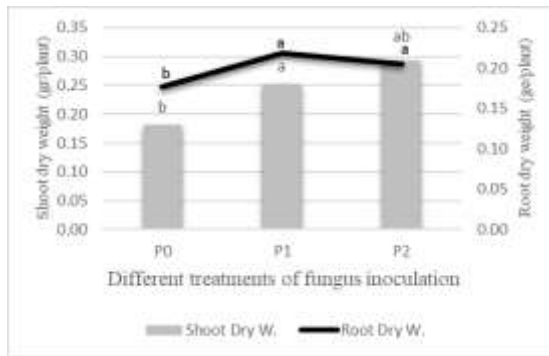


Fig. 1. Comparing shoot and root dry weight in inoculated plants and control plants. Means with different letters are significantly different based on Duncan's multiple range test ( $\alpha=0.05$ ).

The results showed that drought stress significantly decreased the chlorophyll content in both groups of plants (colonized and uncolonized). However, a significant increase in chlorophyll a, b and total chlorophyll content was found in leaves of colonized plants under normal and drought stress treatments. The highest value of total chlorophyll was found in P1 treatments (0.433 mg/g). Based on our results, carotenoid content was increased in stressed condition significantly as well (0.374 mg/g in normal versus 0.385 mg/g in drought stress condition). A similar pattern with chlorophyll content was also observed in carotenoid in endophyte treatments. Inoculation of licorice plants with spore and mycelium (P1 and P2 treatments) increased carotenoid content in normal and stressed conditions significantly (Table 1).

### Electrolyte leakage

Drought increased electrolyte leakage significantly from 15.86 % under normal conditions to 26.41 % under drought stress. *P. indica* prominently reduced the electrolyte leakage in licorice in different treatments, as compared to uninoculated plants (Table 2). The highest and lowest value was seen in P0 (normal) and P1 (fungus spore) treatments, respectively (23.13 % versus 19.74 %).

### H<sub>2</sub>O<sub>2</sub> and Proline

Drought stress increased peroxide hydrogen in different treatment of *P. indica*. Under drought stress, whether *P. indica* colonized in roots of licorice seedlings or not, their H<sub>2</sub>O<sub>2</sub>

change significantly; so that hydrogen peroxide content increased from 0.182 µg / ml in normal conditions to 0.311 µg / ml under drought stress. Although, the effect of *P. indica* on this trait wasn't significant, the fungus decreased hydrogen peroxide content; the highest amount of hydrogen peroxide was observed in P0 treatment (uninoculated plants) (0.27 µg/ml).

Drought stress increased leaf proline content (18.79 to 27.30 µmol/gr F.W.). However, *P. indica*-inoculated plants had higher proline content. Inoculation with *P. indica* spores (P1 treatment) significantly increased the proline content, as compared to uninoculated plants (24.64 µmol/gr F.W.) (Table 2).

### Leaf elements (Na, K, and P)

To examine whether different treatments of *P. indica* inoculum resulted in alteration of ion accumulation in licorice, the level of Na, K, and P ions were measured in treated experimental plants. Drought stress significantly increased Na content (1.22 to 1.91 mg/g D.W.). The concentration of shoot Na was not found to be significantly different between *P. indica* co-cultivated and uninoculated plants; however, the level of Na was reduced by *P. indica* mycelium compared with the uninoculated plants. The results of mean comparison showed that drought had a decreasing effect on leaf potassium content, so stress reduced the leaf potassium content from 10.35 mg/g under normal conditions to 7.79 mg/g D.W. under stress conditions.

The K content of seedlings treated with *P. indica* spores (P1 treatment) was significantly higher than untreated ones (Table 2). Like K, the result showed that drought stress decreased P content (3.81 mg/g D.W. versus 3.30 mg/g D.W.). The same positive effect of co-cultivation with endophytic fungus *P. indica* was observed for P content. *P. indica* colonization affected the phosphate concentration in licorice seedling, and significantly increased phosphorus levels, compared with un-inoculated treatments. P1 treatment has the highest P content (3.92 mg/g D.W.) (Table 2).

Here, drought stress was accompanied by an apparent increase of Na concentration, combined with low K concentrations of both colonized and non-colonized plants. When

**Table 1**

Mean and SD of RWC, Chl. a, Chl. b, total Chl and Carotenoid in different treatments of fungus.

Treatments	RWC (%)	Chl. a mg/g F.W.	Chl. b mg/g F.W.	Total Chl. mg/g F.W.	Carotenoid mg/g F.W.
P <sub>0</sub> (non-colonized)	67.0±6.85 <sup>b</sup>	0.250±0.023 <sup>b</sup>	0.118±0.016 <sup>b</sup>	0.368±0.037 <sup>b</sup>	0.371±0.010 <sup>b</sup>
P <sub>1</sub> (fungus spore)	72.4±7.05 <sup>a</sup>	0.286±0.018 <sup>a</sup>	0.147±0.022 <sup>a</sup>	0.433±0.040 <sup>a</sup>	0.383±0.008 <sup>a</sup>
P <sub>2</sub> (fungus mycelium)	71.0±4.89 <sup>a</sup>	0.279±0.018 <sup>a</sup>	0.124±0.015 <sup>b</sup>	0.403±0.031 <sup>ab</sup>	0.387±0.009 <sup>a</sup>

Means with different letters are significantly different based on Duncan's multiple range test ( $\alpha=0.05$ ).**Table 2.**

Mean and SD of electrolyte leakage, proline, potassium and phosphorus content in different treatments of fungus

Treatments	Electrolyte leakage (%)	Proline $\mu\text{mol/gr F.W.}$	P mg/g D.W.	K mg/g D.W.
P <sub>0</sub> (non-colonized)	23.13±7.169 <sup>a</sup>	22.02±4.418 <sup>b</sup>	2.99±0.63 <sup>b</sup>	8.61±1.56 <sup>b</sup>
P <sub>1</sub> (fungus spore)	19.74±5.426 <sup>b</sup>	24.64±5.566 <sup>a</sup>	3.92±0.44 <sup>a</sup>	9.83±1.57 <sup>a</sup>
P <sub>2</sub> (fungus mycelium)	20.55±5.476 <sup>b</sup>	22.48±4.653 <sup>b</sup>	3.77±0.62 <sup>a</sup>	9.23±1.64 <sup>a</sup>

Means with different letters are significantly different based on Duncan's multiple range test ( $\alpha=0.05$ ).

compared to non-colonized plants, plants colonized with *P. indica* accumulated less Na, but more K. This pattern was confirmed by calculating K/Na ratios in colonized and non-colonized plants. Under drought stress, colonization with *P. indica* increased K/Na ratios in licorice (Fig. II). Collectively, P<sub>1</sub> and P<sub>2</sub> treatments (spore and mycelium of *P. indica*) had a positive effect of measured parameters; so, it can be concluded that both fungus inoculum can colonize the licorice and enhance plant growth and drought stress tolerance.

## Discussion

In this study, different inoculums of *P. indica* were co-cultivated with licorice in drought stress and well-watered conditions. Drought stress had been adversely affected the growth rates of both *P. indica*-colonized and non-colonized plants. Reduced growth in licorice plants has reported under drought stress (Nasrollahi et al., 2014). The symbiotic efficiency of *P. indica* was measured in terms of plant biomass production

and some morpho-physiological traits under drought conditions, which demonstrated its stimulating effect on the growth of licorice. *P. indica* increased shoot and root dry weight and, hence the biomass was higher in *P. indica*-inoculated plants. *P. indica* has been reported in antagonizing abiotic stress conditions like drought stress in *Valeriana officinalis* (Ghabooli et al., 2018), rice (Ghabooli et al., 2015) and, barley (Ghaffari et al., 2016). The results showed that the leaf relative water content of non-inoculated plants significantly reduced by drought stress. However, this reduction was lower in inoculated plants. This research showed that *P. indica* inoculation promotes water uptake and relative water content in the water stressed plants. Some reports pointed out that *P. indica*-mediated drought resistance has been mainly attributed to ameliorated water and nutrient uptake. The *P. indica* mycelia form a network around the roots of inoculated plants, which extend into the rhizosphere and support prompt water and nutrients uptake (Sherameti et al., 2005).

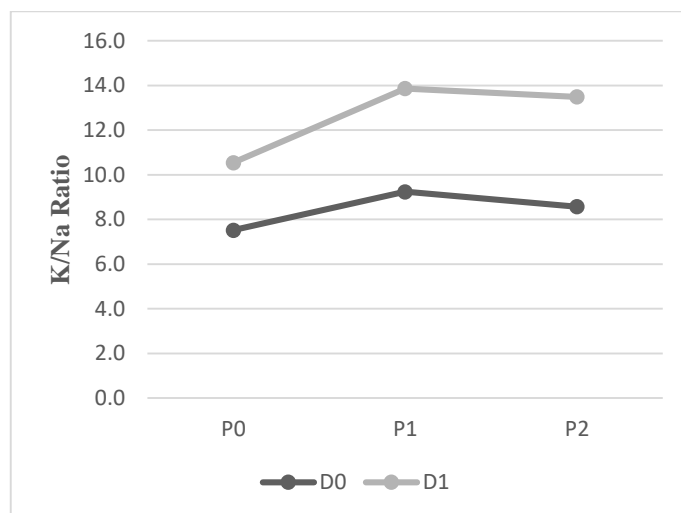


Fig II. Comparing K/Na ratio in inoculated plants compared with control plants

Additionally, the presence of this network in the rhizosphere may stabilize the soil, hence increasing its water holding capacity (Rillig and Mummey, 2006). This would elucidate the improved water balance under drought stress. Moreover, symbiotic fungi by increasing the level of compatible osmolytes, such as proline and soluble sugars, regulate osmotic pressure, and improve the absorption of water under abiotic stress (Ruiz-Lozano et al., 1995).

Low concentrations of photosynthetic pigments can directly reduce photosynthetic potential and primary production. Enhancement in photosynthesis rate due to the elevation of chlorophyll content and leaf area is a significant phenomenon in photosynthetic potential and plant's primary productivity (Battie-Laclau et al., 2013). Abiotic stress such as drought leading to chlorophyll peroxidation and protein synthesis alteration, and hence a decrease in chlorophyll concentrations is a typical symptom of oxidative stress (Levitt, 1980). Therefore, the amount of chlorophyll content changes under drought stress can be considered as a biological index to evaluate stress tolerance (Ashraf and Harris, 2013). Under drought stress, reactive oxygen species damage to chloroplast and decrease chlorophyll level (Smirnov 1995).

Our results showed a reduction of chlorophyll contents in leaves of licorice by drought stress. *P. indica* colonization had a positive effect and did increase chlorophyll a, b, and total chlorophyll levels, which is directly linked to the rate of photosynthesis (Ma et al., 2011). It

seems that *P. indica* alleviated the destructive effects of osmotic stress according to the former results (Sheng et al., 2008; Jogawat et al., 2013). The increase in chlorophyll content may improve photosynthetic capability of inoculated plants and have resulted in increased biomass accumulation. Results, also showed that *P. indica* significantly improved the carotenoid content. Carotenoids protect photosynthetic apparatus against free radicals, inhibit lipid peroxidation, stabilize cell membranes and also play a critical role in the assembly of the light-harvesting complex (Farooq et al., 2009). Meanwhile, inoculation with *P. indica* helps the plants to overcome photo-destruction and photo-inhibition of pigments under water-stress conditions by increasing the content of carotenoids.

Accumulation of proline in response to drought stress may assist plants in the regulation of available N and stabilizing the membrane against its disruption in abiotic stress conditions. Increasing proline concentration, and other compatible solute under drought stress can help maintain a favorable water potential gradient. Therefore, proline content and electrolyte leakage measurements can be a powerful tool to determine drought stress and its effects on plants (Mansour, 1998). In present study, proline content was increased in drought-stressed plant leaves compared with the control ones. Also, the level of proline content was found to be moderately increased after inoculation with *P. indica*. The proline accumulation in inoculated plants is a hint of endophyte-induced proline biosynthesis which

leads to higher osmoregulatory and detoxification capacity compared with non-inoculated plants. Electrolyte leakage is a precious measure to identify the degree of cell membrane injury. Indeed, preserving the integrity of cellular membranes is essential for plants to cope with stress (Stevens et al., 2006). The results of this study suggested a reduced electrolyte leakage in drought treated plants, which were inoculated with *P. indica* compared to their corresponding controls. In non-colonized plants, drought stress increased the electrolyte leakage. According to these results, symbiont *P. indica* fungus helps the host plant to encounter stress by increasing the proline and decreasing the rate of electrolyte leakage. Similar results were reported for maize (Estrada et al., 2013) and pepper (Kaya et al., 2009).

Recently, H<sub>2</sub>O<sub>2</sub>, as a common ROS, has been regarded as a signaling molecule (Orozco-Cárdenas et al., 2001). Drought treatment significantly increased H<sub>2</sub>O<sub>2</sub> content in licorice seedlings, but colonization with *P. indica* significantly decreased H<sub>2</sub>O<sub>2</sub> accumulation. The decrease in H<sub>2</sub>O<sub>2</sub> content might be due to increased activities of CAT, APX, and GPX. Similar results have also been reported in *Arabidopsis thaliana* seedlings (Camehl et al., 2011). The stability of the membrane is necessary for normal physiological functions in the cell (Liu et al., 2014). The osmotic adjustment can be achieved by either de novo synthesis of compatible solutes or by increased uptake of inorganic ions (like K and Na). Na accumulation in the cytosol results in Na toxicity; thus, relying on K for the osmotic adjustment in the shoot is the most preferred option. K plays a key role in plant vital processes, including photosynthesis, osmotic regulation, enzyme activation, and protein synthesis. One of the strategies that plants use to cope with drought stress is to reduce the accumulation of toxic Na ions in the root and shoot systems. Another way is to counterbalance the entry of Na ions into cells is by increasing intracellular K concentrations. Therefore, it is essential to determine the content of Na and K.

It has been proposed that beneficial fungi prevent the transfer of Na ions from root to shoot and store it in vesicles, inside vacuole of root cells or in intra-radical fungal hyphae (Evelin et al.,

2013). In this study, *P. indica* showed an effect on Na content accumulation in the leaves of licorice with considerable impact on K content. These findings probably show that this fungus can regulate root to shoot translocation of Na and K ions. Low K/Na ratio in plants and higher accumulation of Na can cause growth retardation. Maintenance of cellular homeostasis, higher concentration of K, and K/Na ratio by *P. indica* can play an essential role in plant tolerance to drought stress. Our results showed that inoculation of licorice with *P. indica* increased K content, whereas decreased Na content; so, *P. indica* significantly increased K/Na ratio in the shoot of plant under drought stress, which is an index for drought tolerance. Higher K/Na ratio in inoculated plants could prohibit the cellular functional disorders as a result of K-activated processes maintenance and the ionic homeostasis. Our findings were in consistent with Amanifar et al., (2019).

Phosphate plays a series of important roles, such as energy transmission and other regulatory mechanisms (Balemi and Negisho, 2012). Higher phosphate uptake can maintain the cell membrane integrity under abiotic stress conditions, reduce electrolyte leakage, and stabilize vacuolar membrane (Evelin et al., 2013). Enhanced P uptake by *P. indica* inoculation was reported earlier (Shahollari et al., 2005; Yadav et al., 2014). In this experiment, phosphate content was observed to be higher in inoculated plants under drought and well-watered conditions. This result is in accordance with Gosal et al. (2010), who stated that in seedlings inoculated with fungi, the uptakes of P were increased. Moreover, Achatz et al. (2010) showed that P content was improved in *P. indica*-inoculated barley plants (Achatz et al., 2010). In this experiment, there was a significant increase in the contents of P and K in licorice leaves, suggesting that *P. indica* indeed stimulated the nutrition absorption and transporting to the aerial parts. The plant-growth-promoting ability of *P. indica* became evident based on the significantly enhanced N, P, and K uptake in chickpea plant tissue (Nautiyal et al., 2010) and Brassica napus (Su et al., 2017). Based on these results, *P. indica* stimulated the nutrition absorption and improved the nutritional status of the host plant. The enhanced nutritive elements

uptake by this fungus may contribute to the beneficial effects for licorice, such as promoting vegetative growth and increased biomass.

We demonstrate that licorice seedlings colonized by *P. indica* spore and mycelium enhanced several vital parameters, including growth, photosynthesis, and ion uptake under drought stress compared with non-colonized plants. Many reports during the last decade confirm that *P. indica* mediates improvements in the growth and yield of various plants, including crop plants, horticultural, and medicinal plants.

### Conclusion

The most advantage of *P. indica* compared with arbuscular mycorrhizal (AM) fungi is that this endophytic fungus can be easily cultured on various synthetic media; also, it can colonize a wide range of plants. The results presented in this study confirm that drought stress disrupts nutrient and water acquisition, resulting in reduced growth and biomass of licorice plantlets. However, plant tolerance to drought stress is improved by its colonization with symbiotic fungus *P. indica*. Based on our results, *P. indica* regulates plant growth, and at the same time, also increases photosynthetic pigments, proline, K and P content and decreased H<sub>2</sub>O<sub>2</sub>, Na content, and electrolyte leakage which may help plants resisting stress conditions. Thus, the current study provides insight into encounter drought stress by the application of beneficial fungi in the culture of medicinal plants like licorice. The potential of *P. indica* in reducing the problems caused by drought stress and protecting the crops in arid and semi-arid agricultural regions is worthy of more detailed research.

### References

- Achatz, B., S. Von Rüden, D. Andrade, E. Neumann, J. Pons-Kühnemann, K.-H. Kogel, P. Franken and F. Waller, 2010. 'Root colonization by *Piriformospora indica* enhances grain yield in barley under diverse nutrient regimes by accelerating plant development'. *Plant and soil*. 333, (1-2) 59-70.
- Ahlawat, S., P. Saxena, A. Ali, S. Khan and M. Z. Abdin, 2017. 'Comparative study of withanolide production and the related transcriptional responses of biosynthetic genes in fungi elicited cell suspension culture of *Withania somnifera* in shake flask and bioreactor'. *Plant physiology and biochemistry*. 114, 19-28.
- Amanifar, S., M. Khodabandeloo, E. M. Fard, M. S. Askari and M. Ashrafi, 2019. 'Alleviation of salt stress and changes in glycyrrhizin accumulation by arbuscular mycorrhiza in liquorice (*Glycyrrhiza glabra*) grown under salinity stress'. *Environmental and experimental botany*. 160, 25-34.
- Arnon, D. I., 1949. 'Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*'. *Plant physiology*. 24, (1) 1-15.
- Ashraf, M. and P. J. Harris, 2013. 'Photosynthesis under stressful environments: an overview'. *Photosynthetica*. 51, (2) 163-190.
- Bajaj, R., W. Hu, Y. Huang, S. Chen, R. Prasad, A. Varma and K. E. Bushley, 2015. 'The beneficial root endophyte *Piriformospora indica* reduces egg density of the soybean cyst nematode'. *Biological control*. 90, 193-199.
- Balemi, T. and K. Negisho, 2012. 'Management of soil phosphorus and plant adaptation mechanisms to phosphorus stress for sustainable crop production: a review'. *Journal of soil science and plant nutrition*. 12, (3) 547-562.
- Barr, H. and P. Weatherley, 1962. 'A re-examination of the relative turgidity technique for estimating water deficit in leaves'. *Australian Journal of Biological Sciences*, 15(3) 413 – 428.
- Bates, L. S., R. P. Waldren and I. Teare, 1973. 'Rapid determination of free proline for water-stress studies'. *Plant and soil*. 39, (1) 205-207.
- Battie-Laclau, P., J.-P. Laclau, M. De Cassia Piccolo, B. C. Arenque, C. Beri, L. Mietton, M. R. A. Muniz, L. Jordan-Meille, M. S. Buckeridge and Y. Nouvellon, 2013. 'Influence of potassium and sodium nutrition on leaf area components in *Eucalyptus grandis* trees'. *Plant and soil*. 371, (1-2) 19-35.
- Boyer, J. S., 1982. 'Plant productivity and environment'. *Science*. 218, (4571) 443-448.
- Camehl, I., C. Drzewiecki, J. Vadassery, B. Shahollari, I. Sherameti, C. Forzani, T. Munnik, H. Hirt and R. Oelmüller, 2011. 'The



- OXI1 kinase pathway mediates *Piriformospora indica*-induced growth promotion in *Arabidopsis*'. *PLoS pathogens*. 7, (5) e1002051.
- Davis, E. A. and D. J. Morris**, 1991. 'Medicinal uses of licorice through the millennia: the good and plenty of it'. *Molecular and cellular endocrinology*. 78, (1-2) 1-6.
- Ershadi, A., R. Karimi and K. N. Mahdei**, 2016. 'Freezing tolerance and its relationship with soluble carbohydrates, proline and water content in 12 grapevine cultivars'. *Acta physiologiae plantarum*. 38, (1) 2.
- Estrada, B., R. Aroca, J. M. Barea and J. M. Ruiz-Lozano**, 2013. 'Native arbuscular mycorrhizal fungi isolated from a saline habitat improved maize antioxidant systems and plant tolerance to salinity'. *Plant science*. 201, 42-51.
- Evelin, H., B. Giri and R. Kapoor**, 2013. 'Ultrastructural evidence for AMF mediated salt stress mitigation in *Trigonella foenum-graecum*'. *Mycorrhiza*. 23, (1) 71-86.
- Farooq, M., A. Wahid, N. Kobayashi, D. Fujita and S. Basra**. 2009. 'Plant drought stress: effects, mechanisms and management'. In *Sustainable agriculture*:153-188: Springer. Number of 153-188 pp.
- Ghabooli, M., B. Khatabi, F. S. Ahmadi, M. Sepehri, M. Mirzaei, A. Amirkhani, J. V. Jorrín-Novo and G. H. Salekdeh**, 2013. 'Proteomics study reveals the molecular mechanisms underlying water stress tolerance induced by *Piriformospora indica* in barley'. *Journal of proteomics*. 94, 289-301.
- Ghabooli, M., S. Lorestani, Z. Movahedi and R. Karimi**, 2018. 'An evaluation of fungus *Piriformospora indica* effects on some morphophysiological traits of valerine under drought stress'. *Journal of Iranian Plant Ecophysiological Research*. 13, (50) 41-52.
- Ghabouli, M., G. Hosseini Salekdeh and M. Sepehri**, 2015. 'The Effect of Mycorrhiza-like Fungus *Piriformospora indica* on Some Morphophysiological Traits of Rice under Normal and Drought Stress Conditions. *Environmental plant physiology*,13(50).41-52.
- Ghaffari, M. R., M. Ghabooli, B. Khatabi, M. R. Hajirezaei, P. Schweizer and G. H. Salekdeh**, 2016. 'Metabolic and transcriptional response of central metabolism affected by root endophytic fungus *Piriformospora indica* under salinity in barley'. *Plant molecular biology*. 90, (6) 699-717.
- Golian, S., O. Mazdiyasn and A. Aghakouchak**, 2015. 'Trends in meteorological and agricultural droughts in Iran'. *Theoretical and applied climatology*. 119, (3-4) 679-688.
- Gosal, S., A. Karlupia, S. Gosal, I. Chhibba and A. Varma**, 2010. 'Biotization with *Piriformospora indica* and *Pseudomonas fluorescens* improves survival rate, nutrient acquisition, field performance and saponin content of micropropagated *Chlorophytum* sp. *Indian Journal of Biotechnology* 9(3):289-297.
- Jogawat, A., S. Saha, M. Bakshi, V. Dayaman, M. Kumar, M. Dua, A. Varma, R. Oelmüller, N. Tuteja and A. K. Johri**, 2013. '*Piriformospora indica* rescues growth diminution of rice seedlings during high salt stress'. *Plant signaling & behavior*. 8, (10) e26891.
- Karkanis, A., N. Martins, S. Petropoulos and I. C. Ferreira**, 2018. 'Phytochemical composition, health effects, and crop management of liquorice (*Glycyrrhiza glabra* L.): A medicinal plant'. *Food reviews international*. 34, (2) 182-203.
- Kaya, C., M. Ashraf, O. Sonmez, S. Aydemir, A. L. Tuna and M. A. Cullu**, 2009. 'The influence of arbuscular mycorrhizal colonisation on key growth parameters and fruit yield of pepper plants grown at high salinity'. *Scientia Horticulturae*. 121, (1) 1-6.
- Kilam, D., M. Saifi, M. Abdin, A. Agnihotri and A. Varma**, 2017. 'Endophytic root fungus *Piriformospora indica* affects transcription of steviol biosynthesis genes and enhances production of steviol glycosides in *Stevia rebaudiana*'. *Physiological and Molecular Plant Pathology*. 97, 40-48.
- Kord, H., B. Fakheri, M. Ghabooli, M. Solouki, A. Emamjomeh, B. Khatabi, M. Sepehri, G. H. Salekdeh and M. R. Ghaffari**, 2019. 'Salinity-associated microRNAs and their potential roles in mediating salt tolerance in rice colonized by the endophytic root fungus *Piriformospora indica*'. *Functional & integrative genomics*. 1-14.
- Levitt, J.** 1980. *Responses of Plants to Environmental Stress, Volume 1: Chilling*,

- Freezing, and High Temperature Stresses. Academic Press.
- Li, L., L. Li, X. Wang, P. Zhu, H. Wu and S. Qi,** 2017. 'Plant growth-promoting endophyte *Piriformospora indica* alleviates salinity stress in *Medicago truncatula*.' *Plant physiology and biochemistry*. 119, 211-223.
- Liu, J. J., Z. Wei and J. H. Li,** 2014. 'Effects of copper on leaf membrane structure and root activity of maize seedling'. *Botanical studies*. 55, (1) 47.
- Ma, Q., L.-J. Yue, J.-L. Zhang, G.-Q. Wu, A.-K. Bao and S.-M. Wang,** 2011. 'Sodium chloride improves photosynthesis and water status in the succulent xerophyte *Zygophyllum xanthoxylum*.' *Tree Physiology*. 32, (1) 4-13.
- Mansour, M. M. F.,** 1998. 'Protection of plasma membrane of onion epidermal cells by glycinebetaine and proline against NaCl stress'. *Plant Physiology and Biochemistry*. 36, (10) 767-772.
- Nasrollahi, V., A. Mirzaie-Asl, K. Piri, S. Nazeri and R. Mehrabi,** 2014. 'The effect of drought stress on the expression of key genes involved in the biosynthesis of triterpenoid saponins in liquorice (*Glycyrrhiza glabra*)'. *Phytochemistry*. 103, 32-37.
- Nautiyal, C. S., P. S. Chauhan, S. M. Dasgupta, K. Seem, A. Varma and W. J. Staddon,** 2010. 'Tripartite interactions among *Paenibacillus lentimorbus* NRRL B-30488, *Piriformospora indica* DSM 11827, and *Cicer arietinum* L.'. *World Journal of Microbiology and Biotechnology*. 26, (8) 1393-1399.
- Orozco-Cárdenas, M. L., J. Narváez-Vásquez and C. A. Ryan,** 2001. 'Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate'. *The Plant Cell*. 13, (1) 179-191.
- Prasad, R., S. Kamal, P. K. Sharma, R. Oelmüller and A. Varma,** 2013. 'Root endophyte *Piriformospora indica* DSM 11827 alters plant morphology, enhances biomass and antioxidant activity of medicinal plant *Bacopa monniera*.' *Journal of basic microbiology*. 53, (12) 1016-1024.
- Rillig, M. C. and D. L. Mummey,** 2006. 'Mycorrhizas and soil structure'. *New Phytologist*. 171, (1) 41-53.
- Ruiz-Lozano, J., R. Azcón and M. Gomez,** 1995. 'Effects of arbuscular-mycorrhizal glomus species on drought tolerance: physiological and nutritional plant responses'. *Appl Environ Microbiol*. 61, (2) 456-460.
- Shahollari, B., A. Varma and R. Oelmüller,** 2005. 'Expression of a receptor kinase in *Arabidopsis* roots is stimulated by the basidiomycete *Piriformospora indica* and the protein accumulates in Triton X-100 insoluble plasma membrane microdomains'. *Journal of plant physiology*. 162, (8) 945-958.
- Sheng, M., M. Tang, H. Chen, B. Yang, F. Zhang and Y. Huang,** 2008. 'Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress'. *Mycorrhiza*. 18, (6-7) 287-296.
- Sherameti, I., B. Shahollari, Y. Venus, L. Altschmied, A. Varma and R. Oelmüller,** 2005. 'The endophytic fungus *Piriformospora indica* stimulates the expression of nitrate reductase and the starch-degrading enzyme glucan-water dikinase in tobacco and *Arabidopsis* roots through a homeodomain transcription factor that binds to a conserved motif in their promoters'. *Journal of Biological Chemistry*. 280, (28) 26241-26247.
- Stevens, J., T. Senaratna and K. Sivasithamparam,** 2006. 'Salicylic acid induces salinity tolerance in tomato (*Lycopersicon esculentum* cv. Roma): associated changes in gas exchange, water relations and membrane stabilisation'. *Plant Growth Regulation*. 49, (1) 77-83.
- Su, Z.-Z., T. Wang, N. Shrivastava, Y.-Y. Chen, X. Liu, C. Sun, Y. Yin, Q.-K. Gao and B.-G. Lou,** 2017. '*Piriformospora indica* promotes growth, seed yield and quality of *Brassica napus* L.'. *Microbiological research*. 199, 29-39.
- Tabari, H. and P. H. Talaei,** 2011. 'Temporal variability of precipitation over Iran: 1966–2005'. *Journal of Hydrology*. 396, (3-4) 313-320.
- Varma, A., S. Verma, N. Sahay, B. Bütehorn and P. Franken,** 1999. '*Piriformospora indica*, a cultivable plant-growth-

promoting root endophyte'. *Appl Environ Microbiol.* 65, (6) 2741-2744.

**Velikova, V. and F. Loreto,** 2005. 'On the relationship between isoprene emission and thermotolerance in *Phragmites australis* leaves exposed to high temperatures and during the recovery from a heat stress'. *Plant, Cell & Environment.* 28, (3) 318-327.

**Yadav, R. K., R. S. Sangwan, F. Sabir, A. K. Srivastava and N. S. Sangwan,** 2014. 'Effect of prolonged water stress on specialized secondary metabolites, peltate glandular trichomes, and pathway gene expression in *Artemisia annua* L.'. *Plant Physiology and Biochemistry.* 74, 70-83.