



A study on the effect of endophyte fungus, *Piriformospora indica*, on the yield and phytochemical changes of globe artichoke (*Cynara scolymus* L.) leaves under water stress

Shiva Rahimi Tanha¹, Azim Ghasemnezhad², Valiollah Babaeizad³

¹*M.Sc student at Horticulture Faculty of Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran*

²*Assistance Professor at Horticulture Faculty of Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran*

³*Assistance Professor at Plant Protection Faculty of Sari University of Agricultural Sciences and Natural Resources, Sari, Iran*

ABSTRACT

Piriformospora indica, a cultivable root colonizing endophytic fungus of Sebaciales, promotes plant growth and confers resistance against biotic and abiotic stress. The fungus has also the ability to colonize the roots of many varieties of plant species. The goal of present study was to clarify the colonization ability of *P.indica* in artichoke roots and the respond of plant to water stress. Results showed that Globe artichoke (*Cynara scolymus* L.) was strongly colonized by the fungus. The fungus retarded the drought-induced malfunctioning in the photosynthetic pigments. Proline, an osmotic signal adjustment was clearly increased during water stress in colonized plants. Total phenol, flavonoid contents, radical scavenging activities, and photosynthetic pigments were influenced by treatments. Based on the obtained results and observations, it can be concluded that *P.indica* as an elicitor enhances the plant growth efficiency under sever conditions. Thus, *P.indica*-colonized *Cynara scolymus* L., provides a good model system to study root-to-shoot communication in secondary metabolites production.

Key words: *Cynara scolymus* L., Globe artichoke, *Piriformospora indica*, Proline, radical scavenging.

INTRODUCTION

Drought is one of the major abiotic stress limiting plants growth and productivity in many areas of the world (Tanji, 1990). Expansion of drought leads to osmotic stress, specific ions toxicity and ionic imbalances (Munns, 2003). Limited water supply is a major environmental constraint in productivity of medicinal plants; e.i. moisture deficiency induces various physiological and metabolic responses like stomatal closure and decline in growth rate or photosynthesis (Flex et al, 2004). The results of Idrees et al. (2011) showed that water stress significantly reduced the plant height, shoot and root dry weight and long term water stress led to gradual decrease in photosynthetic activity, antioxidant metabolism, total alkaloids and ascorbic acid of *Cathanthous roseous*. *Piriformospora indica*, (Basidiomycota, Sebaciales) just the same as arbascular-mycorrhizal-fungi (AMF) colonizes the roots of many plants (Harrison, 2005; Oldroyd, 2006), but it is an axenic fungus. The fungus was discovered by Verma et al.(1998) from Thar desert in Rajasthan. *P.indica* tremendously improves the growth and overall biomass of diverse hosts including legumes (Verma, 1998; Varma 1999). *Arabidopsis Thailania* (Peřkan-Berghöfer et al.,2004; Shahollari et al., 2005; Sheramti et al., 2005), barely (Waller et al., 2005 and 2008; Baltruschat et al., 2008), tobacco (Barazanni et al., 2007 for *sebacina vermifera*), medicinal and other industrial crops (Rai et al. 2001; Singh et al.2011). Globe artichoke (*Cynara scolymus* L.) is a perennial

vegetable that flower petals and fleshy flower bottoms are eaten throughout the world (Rottenberg, 1996). The plant first-year serrated grey leaves are rich sources of phenolic compounds, flavonoid and antioxidant like Cynarin or hydroxycinnamic acid which is a biological active chemical constituent of globe artichoke, stimulating liver function and lowering cholesterol level (Walker et al., 2001). The, some other essential phenolic derivatives like chlorogenic acid and caffeic acid are flavonoids that must be mentioned as secondary metabolites. One of the main adaptation of this plant to drought stress is toxic ions compartmentalize (control delivering Na^+ and Cl^- in vacuole of the cells). A good fungal symbiosis relation also was observed in this plant as a perennial herb from Asteraceae family (Warcub et al, 1983). The aim of the present study was to clarify whether *P.indica* as a symbiotic endophyte, increases the tolerance of artichoke against drought stresses or not?

MATERIALS AND METHODS

Priformospora indica Growth and cultivation conditions

To have enough spores for plant root contamination, *P.indica* was cultured in Petri dishes on a CM (complex medium), (Peškan-Berghöfer, 2004) Plates were placed in the incubator for 27 days at 25°C. After approximately one month the culture was kept at 4°C till inoculation.

Seed preparation

Globe artichoke seeds were surface sterilized by Garlicon under laminar hood and then they were cultured in glass tubes containing 1/2 concentration of MS (Murashige and skoog, 1962) nutrient medium under sterile conditions, then they stored in 26°C in a dark chamber for seed germination.

P.indica inoculation and treatment

Seedlings were stabilized in glass tubes in one week. A mixture of sterile distilled water 20 ml, with 10 μ l tween-20 poured in *P.indica* culture petridish. To disperse spores in suspension, the surface of petridishes was scratched gently. Then the suspension was filtered through cheesecloth to remove the excess medium and washed three times with distilled water. After each washing step, the spores were collected by centrifugation at 4000 g for 7 min. The spores pellet was finally suspended again in distilled water and adjusted to 5 \times 10⁵ spores per ml. The seedlings roots were soaked in spore suspension and shook (75 rpm) about 3h. The same was done for control samples with no fungi suspension. To have a better sporadic establishment in rootlets, 24 hours after inoculation, seedlings were cultured in small pots (5 \times 7 cm) containing a mixture of sterile perlite, coco peat and pumices (1:1:1) for two weeks. Irrigation was done using distilled water and nutrition Hoagland solution. After 15 days the seedlings were transferred to plastic bags (30 \times 17cm) containing sterilized loamy soil (1plant/pot) for further phytochemical analyses.

Staining fungal spores

To monitor root colonization, small parts of the roots of two-week contaminated seedlings were stained according to Vierheilg (1987) method. For that, root slices were transferred to 10% potassium hydroxide and were boiled for 6 min. The roots were treated with a solution containing 19cc acetic acid and 1cc black stamp ink. After 5min, excess dye was washed with water prior to microscopy. The spores were detected and pictured by Olympus BX51 fluorescence microscope (DIC imaging, USA).

PCR ASSAY

After 14 days of inoculation, four plants of each CP and NCP, examined for the presence of *P.indica* within the host root. For PCR comparison 0.15 g root tissue of each sample was used to isolate 2 μ l genomic DNA (10-50 ng) by the Cethyl Trimethyl Ammonium Bromide (cTAB) method (Bousquet et al, 1990). PCRs were carried out in a final volume of 25 μ l, containing 2.5 μ l PCR buffer 10X, 0.8 μ l MgCl_2 (500 mM), 0.8 μ l dNTPmix(10mM), 1 μ l of each primer(10pmol/ μ l), 0.3 μ l taq DNA polymerase (5U/ μ l), 16.4 μ l deionized water. The following primer pairs for *P.indica*, Pi tef forward: (5'-TCGTCTGCTGCAAGATG-3') and Pi tef reverse :(5'-GAGGGCTCGAGCATGTTGT-3'). Reactions

were performed in a thermal cycler (EPPendorf) set to the following reaction conditions: initial denaturation at 94°C for 4 min and one cycling, for 35 cycles, denaturation at 94°C for 30s, annealing at 54°C for 30s, and 72°C in 30s for elongation and extension was at 72°C in one cycling. Tubes with primers were used as positive controls.

Drought stress induction

In present study, three irrigation periods with four replications were used to study the effect of drought stress on contaminated and non contaminated plants. An irrigation interval of 3 days(B1), was used as control to compare with 6 days(B2) and 12 days(B3) of irrigation periods around 120 days. Pots were set up in Gorgan agricultural faculty greenhouse, based on the randomized design under 65% humidity, 27-32°C temperature and day longevity of 16h (220 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ photon flux density). In each irrigation time, plants were irrigated with 200 \pm 50 cc of tap water.

Determination of biomass parameters

After 120 days of water stress induction, in both contaminated (CP) and non-contaminated plants (NCP) various biomass characters including, fresh and dry weight of leaves (g), root length (cm), and root volume (mm^3), over ground biomass (Fresh weight/ Dry weight of leaves) and total fresh weight of plant (roots+ leaves) were measured. Before chemical analysis, samples were dried in room temperature (27°C) over 15 days (Catchpole *et al.* 1992).

Proline assay

The amount of proline was measured according to Bates *et al.* (1973). To proline estimation, 0.5 g of plant material was extracted in 10 ml of 3% aqueous sulphosalicylic acid and was homogenized. The homogenate aqueous was then centrifuged in 15000 rpm for 15 min at 4°C. Then it was kept in ice bath. Around 2 ml of glacial acetic acid and 2ml acid ninhydrin were added to the extract. This mixture was heated in boiling water bath for 1h. The reaction was terminated by placing the tubes in ice bath. After cooling the mixture, 4 ml of toluene was added and vortexed well for 20-30 seconds. The toluene layer was separated and absorbance was measured using 2800UV/Vis Spectrophotometry, at 520nm. Standard graph of proline was drawn by using standard proline and the amount of proline was calculated on a fresh weight basis as follows: $[(\mu\text{g proline}/\text{ml} \times \text{ml toluene}) / 115.5 \mu\text{g}/\mu\text{mol}] / [(g \text{ sample}/5)] = \mu\text{moles proline} / g \text{ of freshwt.}$

Photosynthetic pigments measurements

The photosynthetic pigments i.e. chlorophyll (Chl) a; chlorophyll b; Total chlorophyll and carotenoid compounds were determined by Arnon method (1956). Chlorophyll extract was prepared from 0.5 g fresh leaves by grinding in a mortar and pestle together with 20 ml 80% acetone. The mixture was centrifuged in 6000 rpm for 10 min. The supernatant was saved and pellet was re-extracted twice. All the supernatant were pooled and saved. The volume of the extraction became 50 ml by adding extra 80% acetone. The absorbance of the extract was recorded at 663 nm, 645 nm and the concentration of chlorophyll a, chlorophyll b and total chlorophyll was calculated using Arnon's equations as follow: Chl.a mg/g FW=[12.7 (A663) – 2.69 (A 645)] \times V/W, Chl.b mg/g FW=[22.9 (A645) – 4.68 (A 663)] \times V/W, Chl. total mg/g FW=[20.2 (A645) + 8.02 (A 663)] \times V/W. Carotenoid absorbance of the extract was recorded at 480 nm and 510 nm; Car.mg/g FW= [7.6(A480) - (1.49(A510)] \times V/W ,respectively.

Sodium and Potassium ions in leaves

Leaves are important source for minerals. Potassium and sodium are the two most important elements especially during stresses (Graifenberg *et al.*, 1995). The leaves were oven-dried at 75°C and grounded. Then grounded samples were dry-ashed at 550°C for 12 hours mixed with 2M hot HCl, filtered and then brought to a final volume of 50 ml with distilled water. Potassium and Sodium were determined in these

sample solutions using a PFP7/JENVAY (Made in UK) model flame photometer (Chapman and Pratt, 1982).

Determination of total phenolic compounds

Total phenolic compounds were determined by the Folin-ciocalteu method with some modifications (Ragazzi and Veronese, 1973). For that 20 μ l of methanolic extract sample was mixed with 1.16 ml of distilled water and 100 μ l of Folin-ciocalteu, the mixture was kept in dark for 1-8 minutes. Then, 300 μ l of sodium carbonate was added. After 2 h of incubation at room conditions, the absorbance of reaction was measured at 760 nm. The standard curve was prepared using 50 to 250 mg/ml solutions of Gallic acid in methanol: water (1:1 v/v). The value of total phenol was expressed in terms of gallic acid equivalent (mg/g).

Determination of Flavonoids contents

Flavonoid content of the extraction was determined by Ebrahimzadeh et al.(2008c). Briefly, 0.5 ml of plant extraction solution (10% w/v) was separately mixed with 1.5 ml of methanol, 0.1ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate, and 2.8 ml of distilled water and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm; the calibration curve was prepared by quercetin solutions at concentrations of 12.5 to 100 μ g/ml.

DPPH radical-scavenging activity

The stable 1,1-diphenyl-2-picryl hydroxyl radical (DPPH) was used to determine free radical scavenging activity of the extract (Ebrahimzade et al., 2008a,b). One milliliter of methanolic concentration was added to an equal volume to methanol solution of DPPH (100 μ M). After 15 min at room temperature the absorbance was recorded at 517 nm. Control sample also, contains 1 ml of methanol and 1 ml of DPPH. The percentage of DPPH radical-scavenging activity was calculated according to the formula bellow:

$$\text{DPPH radical scavenging activity(\%)} = \frac{A_c - A_s}{A_c} \times 100$$

A_c = control sample, A_s =extract sample

2.14 Chlorogenic acid and Caffeic acid (HPLC analysis)

1.2.14 sample treatment

According to Schutz et al,(2006) with a modification, about 0.5 g of fresh artichoke leaves were crushed in a mortar mixing with (1:10) HPLC grade methanol (Made in Belgium).The obtained solution was sonicated for 10 min at room temperature. Then the mixture shook for 12h. And then the mixture was centrifuged for 10 min in 3500 rpm. Finally the supernatant was filtered through 0.2 μ m syringe filter and stored at -20°C until further analysis.

HPLC analysis

The analyses were repeated three times using HPLC, Merck-Hitachi-L7100, consisting of anUV detector Hitachi system and column oven L-2300 Hitachi. The instrument was equipped with an Reverse Phase (RP)-18: (250 \times 4.6mm,5 μ m particle size). Acidified water was 1ml (0.5% acetic acid v/v) and 10 ml acetonitrile, 89 ml of deionized distilled water were used as a mobile phase. The flow rate was set at 1 ml/min throughout the isocratic phase, The system was set on 320 nm. The column temperature was maintained at 25°C and the injection volume was 10 μ l.Quantification was performed by calibration curve, using the available standards. Particularly, Caffeic acid (hydroxycinnamic acid) standard curve was considered by10,25,50,100mg/ml concentrations of Caffeic acid (Sigma-aldrichHPLC grade). 5-O-caffeoylquinic acid (Chlorogenic acid) standard curve was calculated by 100,200,500,600 mg/ml of different concentrations of Chlorogenic acid (Sigma-aldrichHPLC grade).

2.15 statistical analyses

Samples were evaluated with factorial experiment based on a complete randomized design, with four repetitions, the significance level was treated with $P > 0.05$. Analysis of variance was done using SAS. 9.0. and the mean comparison was done using LSD test.

RESULTS

Root colonization stimulates biomass production of artichoke

Fourteen-day roots of contaminated (CP) artichoke seedlings were microscopically examined for the colonization ratio. plus, a gradual increase in the band intensity in control positive and *P.indica* contaminated plants during the course of the tef gene experiments was observed. no band intensity for non contaminated plants was observed (Fig.1a). Results showed that the development of the lateral roots was strongly promoted by the fungus (Fig.1b). As data show in (Table-1), *P.indica* had a significant influence on growth parameters of CP plants, (e.g. top biomass and total fresh weight) under stress. Root volume of CP was obviously more than non contaminated plants (NCP). Microscopic analysis revealed that the degree of the root colonization was quite high ($>90\%$). Spores were detected in almost all areas of the roots (Fig.1c). It seems that drought stress had less effect on contaminated plants.

Proline accumulation of contaminated and non contaminated plants under water stress condition

Proline accumulates in plants in response to osmosis pressure of the soil during drought stress. As presented in table 2 under low drought stress the proline accumulation of NCP was higher than CP. As drought stress increased from low to moderate proline production of both plant groups was relatively similar but a change happened when drought stress shifts to severity. In this case, contaminated plants showed a better potency in proline accumulation.

Photosynthesis pigments, K^+ and Na^+ concentrations in different conditions

Since chlorophyll (Chla, Chlb) and carotenoid play an important role in photosynthesis and photo protection, the concentrations of them were measured in Contaminated and non contaminated plants. As data shown in Table-2, photosynthetic pigments were safely maintained in contaminated plants under whole drought conditions. K^+ and Na^+ are the two most important indicator ions in plant tolerance against salinity and drought stress. As presented in (table -2), in low stress conditions potassium accumulation of CP was nearly two times higher than NCP. Opposite to that, sodium accumulation (as a bad ion) was significantly low in contaminated plants. Surprisingly the sodium accumulation of non contaminated plants was three times higher than contaminated plants.

The content of total phenol, flavonoid and radical scavenging activity of contaminated and non contaminated artichoke leaves and roots under water stress conditions.

As can be seen in table-3, most measured phytochemical parameters involved in antioxidant activity of plant extract were significantly influenced by both fungus contamination and water availability. Under low and moderate water stress (B1 and B2), no significant difference was observed between contaminated and non contaminated plant leaves phenol content. On the other hand by increasing the irrigation interval and under high water stress (B3) the total phenol content of leaves in non contaminated plants tend to decrease (0.27 and 0.40 mg/Fw, in NCP and CP, respectively). Opposite of leaves, no significant differences was observed on the phenol content of roots under different water stress conditions. The same changes were observed in the content of flavonoids in leaves and roots. As presented in table 3, under low and moderate levels of water stress no significant differences was observed in flavonoid content of both leaves and roots. As water stress increased by increasing the distance of irrigation time, both in roots and leaves the flavonoid content of contaminated plants was significantly higher than that of non contaminated plants (2.31 and 3.08 mg/g Fw in NCP and CP, respectively). Antioxidant activity of plant extract followed a similar path. Under low water stress conditions no significant difference was observed in antioxidant activity of NCP and CP. When the water availability reduced by increasing the distance of

irrigation, the antioxidant activity of CP leaves was significantly higher than that of NCP, both in moderate and high water stress conditions (48.1% and 84.9% in roots of NCP and CP, respectively).

Total phenolic compounds variations in CP and NCP

To compare the variation of caffeic and chlorogenic acids of contaminated and non contaminated plants under water stress only the plants which were treated with low and high water stress regimes were used. Results showed that the amount of chlorogenic acid of leaves of non stressed and contaminated plants was significantly (<0.05) higher than that of non stressed and non contaminated plants. Although the content of chlorogenic acid of contaminated plants under severe stress tended to be higher than that of non contaminated plants, but the difference was not significant (Figure 2:2a). Caffeic acid under mild stress conditions was significantly higher (>0.05) regardless of contamination or not (Figure 2b). Opposite to chlorogenic acid, the amount of caffeic acid of CPs grown under severe water stress, was relatively lower than non contaminated plants but not in a significant way.

DISCUSSION

Piriformospora indica enhances plant biomass and photosynthetic activity under water stress

The fungal mycelium penetrates in epidermis and cortical cell; pear-shaped chlamidospores were also colonized intercellularly in roots cortical tissue (Kost et al. 2013). In present study, compared with control plants, different morphological changes in artichoke colonized with *P.indica* was observed. This conclusion is in agreement with Jogwart et al., (2013); Sun et al., (2012). Because of the pharmaceutical and agricultural importance of this perennial vegetable, and the drought as a global warming, we investigated these interactions in details. According to finding of Sun et al. (2013), drought stress causes a general lesion of plastid functions. Plus, *P.indica* creates an atmosphere in the cell that prevents degradations in plastid functions. This ideal atmosphere in stress conditions may let the ATP⁺ase cytoplasmic pumps to substitute potassium and calcium ions with sodium and chlor instead, so that the plant can tolerate the stressful conditions easier. As it mentioned in result, root volume largely increased in CP providing that fungi mycelia spread in root cellular peacefully. Hypothetically this significant growth of lateral roots may refer to phytohormonal (e.g auxin) involvement. It has been shown that Arabidopsis-*P.indica* interactions involve specific molecular pathway and signaling signature as apparent from the requirement of auxins, while auxin level in various Arabidopsis mutant affected plant response to *P.indica*, this phytohormone manipulations did not show any effect on Solanaceous plants (Long et al, 2008). So, recent transcriptome analyses asserted a biphasic lifestyle trait in *P.indica* (Zuccaro et al, 2011). In unpublished data of us, auxin level in the leaves of contaminated plants was significantly higher than that of non-contaminated ones. While auxin means root enhancement, proline, is an osmoprotectant factor that has been shown to accumulate in plants in response to salinity, drought, water flooded, etc. stresses (Delauney, 1993). Accumulation of proline under stress conditions in many plants species has been correlated with stress tolerance and its concentration is generally high in stress tolerant plants (Ashraf et al, 2007). Proline has an important role in enzyme protection (Peng and Verma, 1996), Bagheri et al.(2013) and Jogowat et al. (2013). It is also a nitrogenic amino acid which can be a source of cellular carbon and nitrogen (Walton and Podivinsky, 1998). Mattioni et al.,(1997) states that proline content in leaves transferred to meristemic tissues as an osmoregulator. Thus, the plant stays alive and food supplements are accessible. This could be a reason why the biomass production of contaminated plants is higher than that of non contaminated one.

***P.indica* contaminated artichoke produce a higher amount of certain secondary metabolite under water stress conditions**

There are astonishing evidences that plant enzymes involved in secondary metabolism have been associated with plant stresses such as drought (Ali et al., 2006; Kulchesk, 2011). Consistently, the results of the present study correspond to other studies indicated that arbuscular mycorrhizal induces flavonoid

production, correlating biosynthesis of isoflavonoids or accumulation of cell-wall-bound phenolics cinnamic acid amide derivatives in the roots of tobacco (Nascimento, 2011). However, it is conceivable that, phenolic compounds and flavonoids are powerful antioxidants against free radical scavenging agents (Rice-Evans *et al.*, 1997). Furthermore, flavonoids and phenolic contents were known to be involved in defense system. In higher caffeic acid accumulation in contaminated compare to non-contaminated plants may refer to the fact that this phenolic chemical could be changed to its other homologues contents, like cynarin, a hepatoprotective agent, that is suggested to be determined in future studies.

CONCLUSIONS

The estimations suggest that the fungal hyphae propagated well in globe artichoke root cells and the induced water stress in all three levels respectively, affected dehydration in globe artichoke's leaves. *P. indica*, in inoculated plants can ease the potassium ions entrance into cytoplasm and excrete toxic ions like sodium to make an equivalent tonoplastic balance, thus plant is immunized against drought stress and photosynthetic pigments remain efficient. The other benefit of this mutuality relation is that an enlargement surface of the roots, support minerals and water absorption for plant. Plants become ubiquitously armed against drought and grow in healthier and safer conditions like when proline accumulation increases. Interaction between *P.indica* and water stress caused the activation of signal oxidation during stress process to prevent cell damages and helping the plant in maintaining the osmotic pressure. Thus, more water stress levels in host-plants might attribute to increase the content of phenolics and flavonoids.

ACKNOWLEDGEMENT

This is a part of the author thesis, we greatly thankful to Medicinal plant production horticultural Faculty of Gorgan Agricultural University for laboratories and fundamental supplements.

REFERENCES

- Ali, G.M., Komatsu, S. (2006). Proteomic analysis of rice leaf sheath during drought stress. *Journal of proteomic research*. 2:396-403.
- Arnon, DJ., (1956). Chlorophyll absorption spectrum and quantitative determination. *Biochemical and biophysical Acta*.20: 449-461.
- Ashraf, M., Foolad, MR.,(2007). Roles of Glycine betaine and proline in improving to abiotic plant resistance, *Environmental and experimental botany*.59: 206-216.
- Bagheri, AA., Saadatmand, S., Niknam, V., Nejadstari, T., Babaeizad, V. (2013). Effect of endophytic fungus, *Piriformospora indica* on growth and activity of antioxidant enzymes of rice (*Oryza sativa* L.) under salinity stress. *International journal of advanced biology and biomedical research*.11:1337-1350
- Bates, LS., Waldron, RP., Teare, I.D. (1973). Rapid determination of free proline for water stress studies. *Plant soil*. 39:205-208.
- Baltruscht, H., Fodor, J., Harrach, BD., Niemczyk, E., Barna, B., Gullner, G., *et al.*(2008). Salt tolerance of barely induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. *New phytol*. 180: 501-510.

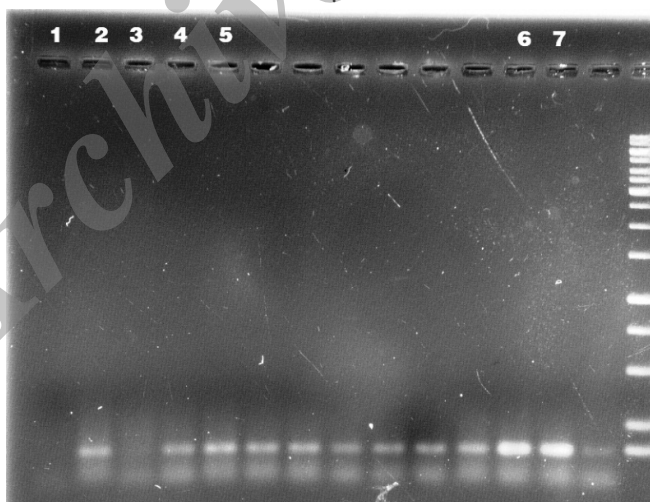
- Barazani, O., Von Dahl, C., Baldwin, I.T.(2007). *sebacina vermifera* promotes the growth at the expense of herbivore and fitness of *Nicotiana attenuata* by inhibiting ethylene signaling. *Plant physiol*, 144:1223-1232.
- Bousquet, J., Simon, L., LaLonde, M. (1990). DNA amplification from vegetative and sexual tissues of trees using polymerase chain reaction. *Can J Res* 20: 254–257.
- Catchpole WR, Wheeler CJ(1992). Estimating plant biomass: a review of techniques. *Australian Journal of Ecology*. 17(2): 121-131.
- Chapman ,H., Pratt, PE. (1961). *Methods of analysis for soils, plants, and waters*. University of California, Division of Agricultural sciences. California. USA. Riverside Press.
- Delauney, AJ., Verma, DPS.(1993). Proline biosynthesis and osmoregulation in plants. *Plant J*. 4:215-223.
- Ebrahimzade, MA., Hossinimehr, SJ., Hamidinia, A., Jafari, M. (2008a). Antioxidant and free radical scavenging activity of *Fijoa sallowiana*. Fruits peel and leaves. *Pharmacology online*. 1: 7-14.
- Ebrahimzade, MA., Pourmorad, F., Hafezi, S.(2008b). Antioxidant activities of Iranian corn silk. *Turk.J.Biol*. 32:43-49.
- Ebrahimzade, MA., Pourmorad, F., Bekhradnia, AR. (2008c). Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. *Afr.J.Biotechnol*. 7:3188-3192.
- Flexas, J., Bota, J., Loreto, FC., ornig, G., Sharkey, TD. (2004). Diffusive and metabolic limitation to photosynthesis under drought and salinity in C3 plants. *Plant biology*6:249-279.
- Graifenberg, A., Giustiniani, L., Temperini, O., Lipucci, DP. (1995). Allocation of Na, Cl, K and Ca within plant tissue in *Globe Artichoke (Cynara scolymus L.)* under Saline- sodiac condition. *Scientia horticulture*.63: 1-10.
- Harrison, MJ.(2005). Signaling in the arbuscular mycorrhizal symbiosis. *Annu Rev Microbiol* 59:19–42
- Idrees, M., Khan, MA., Naeem, M., Aftab, T., Hashemi, N., Alam, M.(2011). Modulation of defense responses by improving photosynthetic activity. Antioxidant metabolism and vincristine and vinblastine accumulations in *Catharethus roseous(L.)* G. Don through salicylic acid under water stress. *Russian agricultural sciences*. 6:474-483.
- Jogwart, A., Saha, S., Bakshi, M., Dayaman, V., Dua, M., Varma, A. (2013). *Piriformospora indica* rescues growth diminution of rice seedlings during high salt stress. *NCBI pub Med*.9(1).
- Kost, G., Rexer, KH. (2013). *Piriformospora indica*: Morphology and ultra structure of *Piriformospora indica*. Varma A Kost, G OelmullerR (Ed) Springer Marburg Germany.
- Kulchesk, FR., Oliveira, L., Molina, LG., Almerag, P., Rodrigues, F., Margolino, J., Barbosa, J., Stolf-Moreira, R., Nepomuceno, A., Marcelino-Guimaraes, F., et al.(2011). Identification of noval soybean microRNA involved in abiotic and biotic stresses. *BMC Genomics*. 12:307.
- Long, H.H., Schmidt, D.D., Baldwin, I.T.(2008). Native bacterial endophytes promote host growth in species-specific manner. Phytohormone manipulations do not result in common growth responses. *PLoS ONE*. 3,e2702.

- Munns, R.(2003). Comparative physiology of salt and water stress. *Plant cell Environ.* 25: 239-250.
- Murashige, T., Skoog, F.(1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures, *plant physiol.* 15: 473-497.
- Mattioni, C., Lacerenza, N., Troccoli, A., De Leonardis, A., Di Fonzo, M.(1997). Water and salt stress – induced alternation in proline metabolism of *Triticum durum* seedlings. *Physiologia plantarum.* 101:787-792.
- Nascimento, NC., Cannes, N., Frett-Neto, AG. (2010). Plant secondary metabolisms and challenges in modifying its operation: an overview. In *Plant secondary metabolism engineering.* Human press. 1-13
- Oldroyd, G. downie, J.A. (2006). Nuclear Calcium Changes at the Core of Symbiosis Signaling. *current opinion. Plant biology.* 9: 351–357.
- Peng, Z., Verma, L.(1996). Reciprocal regulation of pyrroline-5-carboxylate synthetase and proline dehydrogenase genes controls proline levels during and after osmotic stress in plants. *Mol Gen Genet.* 253:334-341.
- Peškan-Berghöfer, T., Shahollari, B., Giang, PH., Hehl, S., Markert, C., Blanke, V., et al.(2004). Association of *Piriformospora indica* with *Arabidopsis thaliana* roots represents a novel system to study beneficial plant-microbe interactions and involves early plant protein modifications in the endoplasmatic reticulum and at the plasma membrane. *Physiol plant.* 122:465-477.
- Ragazzi, E., & Veronese, G. (1973). Quantitative analysis of phenolic compounds after thin-layer chromatographic separation. *Journal of Chromatography A.* 77:369-375.
- Rai, M., Acharya, D., singh, A. (2001). Positive growth response of the medicinal plants *Spilanthes calva* and *Withania somnifera* to inoculation by *Piriformospora indica* in a field trial. *Mycorrhiza.* 11: 123-128.
- Rice-Evans, C., Miller, N., Paganga, G. (1997).Antioxidant properties of phenolic compounds. *Trends in plant science.*4 :152-159.
- Rottenberg, A., Zohary,D. (1996). The wild ancestry of the cultivated artichoke. *Genetic resources crop evolution.*43:53-58.
- Shahollari, B., Varma, A., Oelmüller, R.(2005). Expression of a receptor kinase in *Arabidopsis* roots stimulated by the basidiomycete *Piriformospora indica* and the protein accumulates in TritonX-100 insoluble plasma membrane microdomains. *J plant physiol.* 162:945-958.
- Sherameti, I., Tripathi, S., Varma, A., Oelmüller, R. (2005). The root-colonizing endophyte *Piriformospora indica* confers drought tolerance in *Arabidopsis* by stimulating the expression of drought stress-related genes in leaves. *Mol plant-Microb interact.*21:799-807.
- Schutz, K., Muks, E., Carle, R., Schieber, A. (2006). Quantitative determination of phenolic compounds in artichoke-based dietary supplements and pharmaceutical by high-performance liquid chromatography. *Journal of agricultural and food chemistry.* 54: 8812-8817.
- Singh, LP., Singh, Gill, S., Tuteja, N.(2011). Unrevealing the role of fungal symbionts in plant abiotic stress tolerance. *Plant signaling and behavior.* 6: 175-191.
- Sun, C., Johnson, JM., Cai, D., Sheramti, I., Oelmüllre, R., Lou, B.(2010). *Piriformospora indica* confers drought tolerance in chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. *J plant physiol.* 167:1009-1017.

- Tanji, K.,K.(1990). Nature and extent of agricultural salinity.In: Tanji,K,K.(ed), Agricultural Salinity assessment and management .pp: 1-13. ASCE, New York.
- Varma, A., Verma, S., SudhaSahay, NA., Butehorn, B., Franken, P.(1999). Piriformospora indica a cultivable lant growth promotinh root endophyte. Appl Environ Microbiol. 65:2741-2744.
- Verma, SA., Varma, A., Rexer, K-H., Hassel, A., Kost, G., Sarbhoy, A., et al.(1998). Piriformospora indica gen. et. Sp. nov., a new root- colonizing fungus. Mycologia. 90:898-965.
- Vierheilig, H., coughlan, AP., Wyss, U., Piche, Y. (1998). Ink and vinegar, a simple staining technique for arbascular mycorrhizal fungi. Application of Environmental Microbiology. 64: 5004-5007.
- Walker, A.F., Middleton, RW., Petrowicz, O. (2001). Artichoke leaf extract reduces symptoms of irritable bowel syndrome in a post marketing surveillance study. Phytotherapy Reasearch.15: 58-61.
- Waller, F., Achatz, B., Bultrusch, H., Fodor, J., Becker, K., Fischer, M., et al.(2005). The endophytic fungus Piriformospora indica reprograms barely to salt-stress tolerance. Disease resistance and higher yield. Proc Natl Acad Sci USA. 102: 13386-13391.
- Waller, F., Mukherjee, K., Deshmukh, SD., Achatz, B., Sharma, M., Schafer, P., et al.(2008). Systemic and local modulation of plant responses by Piriformospora indica and related Sebaciniales species. J Plant Physiol. 179: 675-686.
- Warcup, JH., Mcgee, PA.(1983). The mycorrhizal associations of some Australian Asteraceae. New Phytol. 4: 667-672.
- Walton, E., Podivinsky, E.(1998). Regulation of proline biosynthesis in Kiwifruit buds with and without hydrogen cynammide treatment. Physiologia Plantarum. 102: 171-178.
- Zuccaro, A., Lahrmann, U., Guldener, U., Langen, G., Pfiffi, S., Biedenkopf, D., Wong, P., Samans, P., Grimme, C., Basiewicz, M., et al.(2011). Endophytic life strategies decoded by genome and transcriptome analyses of the mutualistic root symbiont Piriformospora indica. PLoS Pathog. 7,e1002290.

Table 1- Biomass production of artichoke affected by fungus and water stress

Total Fresh Weight (g)	Root		Leaf				Drought sets
	Volume (mm ³)	Length (cm)	Over ground biomass	Dry weight (g)	Fresh weight(g)		
41.78 ^b	192.25 ^{cd}	30.75 ^a	8.14 ^{ab}	3.57 ^a	29.06 ^b	-P.indica	B1
67.33 ^a	592.5 ^a	27.6 ^a	13.51 ^a	3.21 ^a	43.43 ^a	+P.indica	
33.48 ^{bc}	145.0 ^d	18.75 ^b	8.08 ^{ab}	2.88 ^b	23.33 ^b	-P.indica	B2
47.55 ^b	443.75 ^b	32.5 ^a	12.08 ^a	2.65 ^b	31.95 ^a	+P.indica	
18.74 ^c	177.14 ^d	17.75 ^b	6.24 ^b	1.97 ^b	12.32 ^b	-P.indica	B3
21.88 ^c	253.0 ^c	23.25 ^{ab}	8.68 ^{ab}	1.71 ^b	14.91 ^a	+P.indica	

**Figure-1a:** PCR assay (1-non contaminated plants, 2 to 4 Contaminated plants, 3 6 and 7: Positive controls)

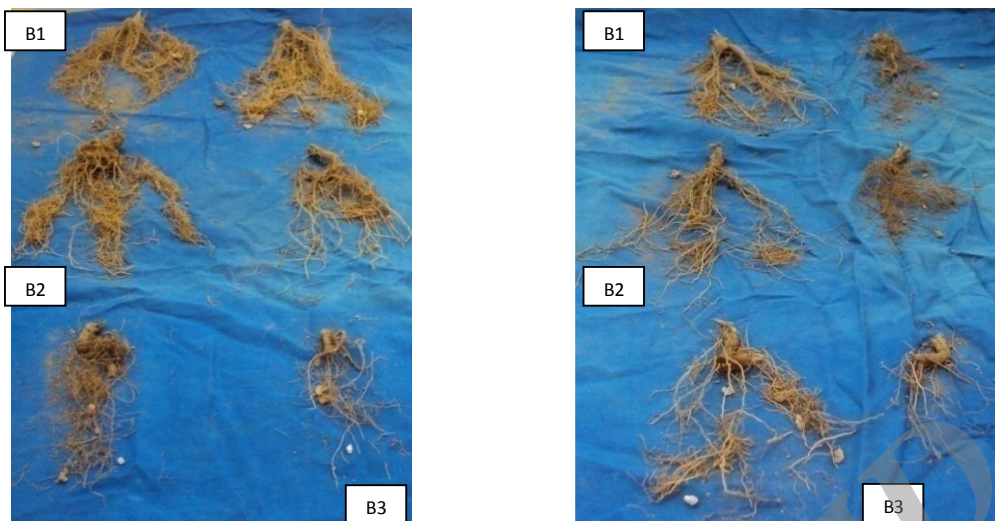


Figure-1b: Roots of *P.indica* contaminated (left) and non contaminated (right) under different levels of water stress

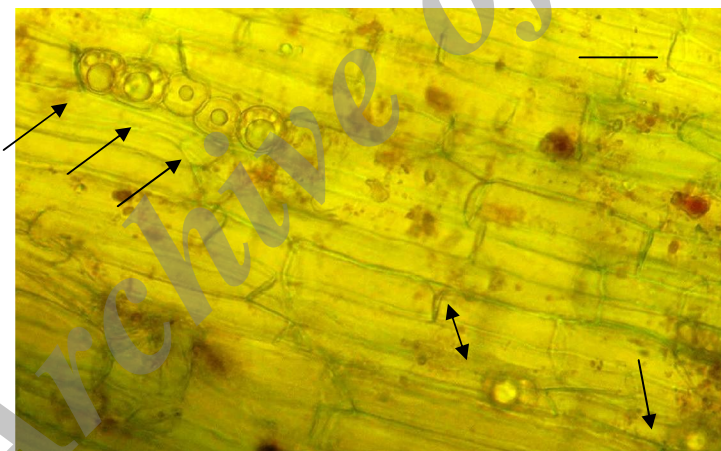


Figure-1c: *Piriformospora indica* intercellular chlamidospores in cortex of the *Cynara scolymus* roots 14 days after inoculation. Scale bar shows 30µm

Table-2: variation in the amount of proline, potassium, sodium and photosynthetic pigments of contaminated and non contaminated artichoke leaves under water stress

K ⁺ (meq/L)	Na ⁺ (meq/L)	Cart(m g/gFw)	Tchl(m g/gFw)	Chlb(m g/gFw)	Chla(mg/ gFw)	Proline (μmol/ gFw)		Drought set
53.16 ^b	13.21 ^b	0.267 ^c	0.818 ^b	0.163 ^b	0.656 ^b	6.8 ^c	-P.indica	B ₁
111.77 ^a	7.64 ^c	0.581 ^a	1.197 ^a	0.236 ^a	0.96 ^a	4.43 ^d	+P.indica	
39.96 ^b	15.3 ^b	0.244 ^c	0.713 ^b	0.186 ^b	0.515 ^b	7.38 ^{bc}	-P.indica	B ₂
61.62 ^a	16.95 ^b	0.424 ^b	1.183 ^a	0.258 ^a	0.925 ^a	7.3 ^{bc}	+P.indica	
56.01 ^b	47.9 ^a	0.267 ^c	0.585 ^b	0.13 ^b	0.438 ^b	7.8 ^b	-P.indica	B ₃
69.8 ^a	15.3 ^b	0.306 ^c	0.867 ^b	0.244 ^a	0.88 ^a	9.43 ^a	+P.indica	

Archive of SID

Table 3- changes of antioxidant activity of contaminated and non contaminated artichoke under different levels of water stress

Root			Leaves				Drought set
Antioxidant (%)	Flavonoid (mg/g Fw)	Phenol (mg/gFw)	Antioxidant (%)	Flavonoid (mg/g Fw)	Phenol (mg/gFw)		
73.6 ^a	0.24 ^c	0.46 ^a	71.66 ^b	2.24 ^b	0.37 ^a	-P.indica	B ₁
84.54 ^a	0.38 ^a	0.46 ^a	74.33 ^b	2.52 ^b	0.43 ^a	+P.indica	
52.3 ^b	0.35 ^b	0.28 ^b	67 ^c	2.75 ^{ab}	0.36 ^a	-P.indica	B ₂
73.98 ^a	0.43 ^a	0.36 ^a	75.33 ^b	2.72 ^{ab}	0.43 ^a	+P.indica	
48.01 ^c	0.36 ^b	0.36 ^a	61.66 ^c	2.31 ^b	0.27 ^b	-P.indica	B ₃
84.9 ^a	0.44 ^a	0.32 ^a	82.66 ^a	3.08 ^a	0.40 ^a	+P.indica	

Archive of SID

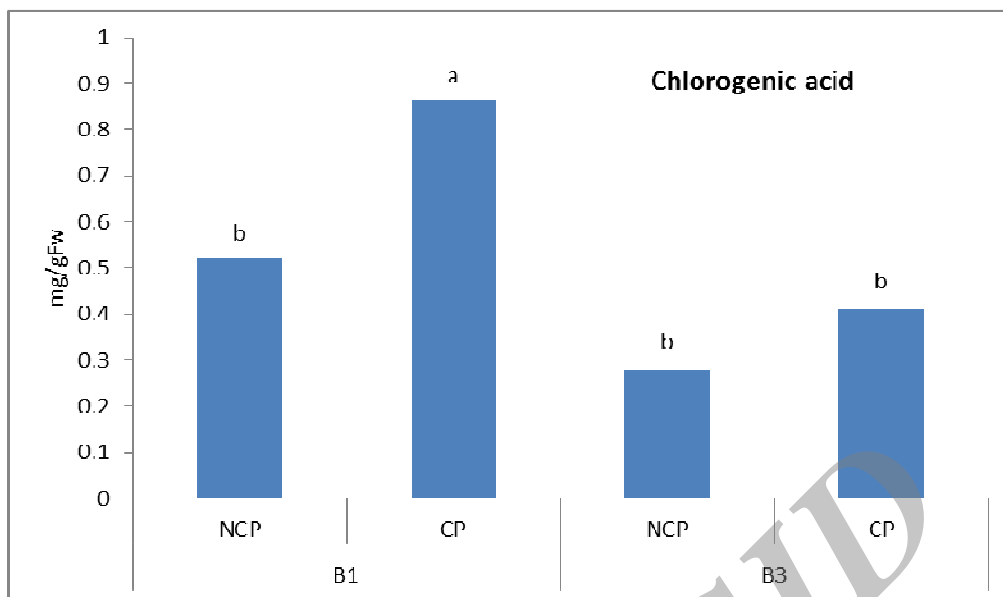


Figure 2a: NCP= Non contaminated plant, CP=Contaminated plant (B₁= each 3days of irrigation, B₃= each 12 days of irrigation)

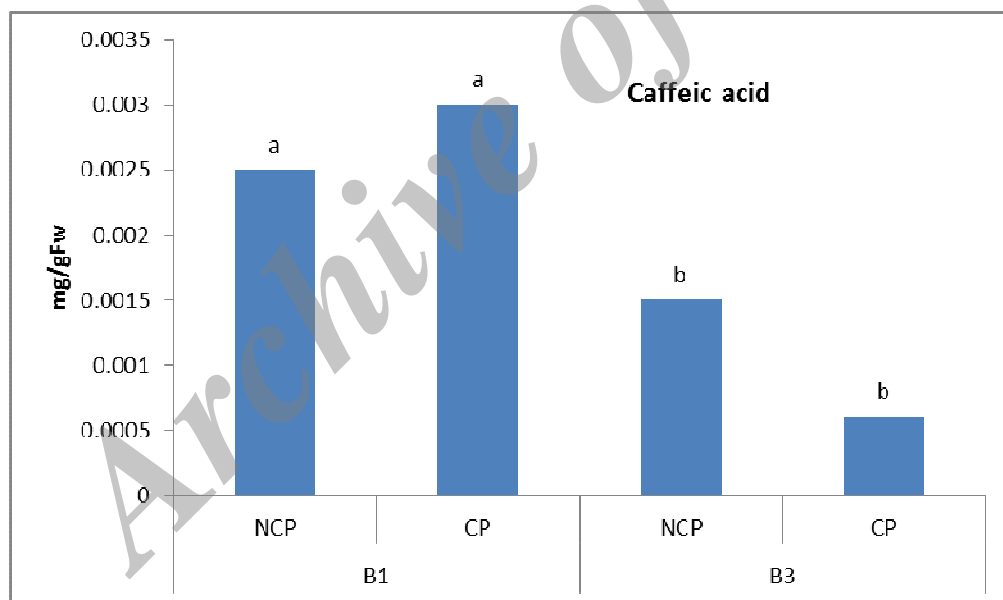


Figure 2b: NCP= Non contaminated plant, CP=Contaminated plant. (B₁= each 3days of irrigation, B₃= each 12 days of irrigation)