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Research Article

Effect of Growth Promoting Fungi on Morphological and Physiological Properties of Sesame in Drought Condition

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Abstract

Drought has been recognized as a primary constraint in limiting the growth and development of plants. It impairs normal growth, disturbs water relations, and reduces water use efficiency in plants. Drought stress at any growth stage also, poses detrimental effects on morphological and physiological criteria in plants. To maintain growth and productivity, plants must adapt to stress conditions and exercise specific tolerance mechanisms of stress agents. Plant modification for enhanced tolerance is mostly based on gene transformation, however, the nature of the genetically complex mechanisms of abiotic stress tolerance, and the potential detrimental side effects, make this task extremely difficult. A promising alternative for improving plant drought tolerance is using the beneficial soil microorganisms including Plant-growth-promoting fungi (PGPF). This research was undertaken to investigate the effect of two PGPFs on some morphological and physiological indices and nutritional status of sesame plant under drought conditions. For this purpose, a field experiment using a completely randomized blocks with three replications and treatments including fungal inoculation (non-inoculation, *Piriformospora indica* and *Rhizophagus irregularis*) and drought levels include 55, 75 and 85% of SAW (Soil Available water) depletion on the basis of combined analysis in Lavark Field (Isfahan Province) was conducted. The results showed that the comparison of studied fungi indicates that only *R. irregularis* increased the concentration of phosphorus in the shoot part of plants, significantly. Each of the studied fungi showed their positive effect on different characteristics and also different drought stresses. So that, *R. irregularis* increased phosphorus content at 75% of SAW depletion, relative water content of leaf and nitrogen content at 85% of SAW depletion and catalase activity, membrane stability, iron concentration, shoot biomass and number of leaves at 75% and 85% of SAW depletion. However, P. indica increased iron concentration and height of shoot at 75% of SAW depletion, shoot biomass and nitrogen content at 85% of SAW depletion and catalase activity, membrane stability, relative water content of leaf and number of leaves at 75% and 85% of SAW depletion. The studied fungi increased the activity of catalase enzyme in all drought treatments. In general, the obtained results of this research indicate that drought is a limiting factor for plants, and PGPFs can improve plant tolerance to this environmental stress by improving plant nutritional status, water content and activity of anti-oxidant enzymes.

Keywords: Drought; Plant Growth promoting fungi (PGPFs) and Sesame

Introduction

To assuring crops quality and quantity in the future, proceedings must be directed to the adaptation of plants toward environmental issues namely drought stress (IPCC, 2o14) [26]. Drought is one of the main constraint factors for crop productivity in Iran, and it is anticipated that more than 50% of the arable lands in the world endure its serious problems by 2050 (Kasim et al., 2013; Sabbaghpour, 2006) [31, 49]. The global demand for vegetable oils is boosting, and among of them sesame (Sesamum indicum L.), which belongs to *Pedaliaceae* family, has been evaluated as it is a great source of oil (Pathak N, 2014) [44]. Sesame seed is one of the most drought-tol-

erant species among *Asteridae* subclass (Dossa et al., 2016) [11], and it is mainly grown in arid and semiarid areas where specified by high temperature and the long period of drought (Zhai et al., 2017) [81]; however, its productivity affected by severe water deficiency (Bahrami et al., 2012) [5]. Destructive effects of drought, which depends on crop species, crop developmental stages, and drought regime, alter morphological and physiological processes (Srivastava and Srivastava, 2014) [58]. To illustrate, drought conditions impair growth and development of the plant, reduction yield following decrease in the number of capsule and leaf properties (Bayoumi et al., 2017; Eskandari et al., 2009; Sun, 2010; Yasser Hussein, 2015) [7, 14, 59, 79]. Diminishing nutrient concentration in plants is another effect of drought that stems from reducing nutrient supply through mineralization and reduction of diffusion and mass flow in the soil (Bista et al., 2018) [8]. In drought conditions, reactive oxygen species produced which result in damage to cellular metabolisms and mechanisms as well as construction damages in lipids, proteins and nucleic acids (Koffler et al., 2014; Sunkar, 2010) [32, 60]. To mitigate the consequence of oxidative damage, plants encompass an effective antioxidant defense network increasing in the response to drought compare to optimum humidity conditions (Dossa et al., 2017; Kadkhodaie et al., 2014) [12, 30]. Another physiological index representing plant water relations is relative water content reducing under drought stress (Najafabadi and Ehsanzadeh, 2017) [41].

Improving plant resistance to water stress and maintaining crop productivity are great challenges for achieving sustainable agriculture. To overcome drought stress remarkable attempts in agriculture practices have been done such as irrigation management that is not permanent in the long term because freshwater resources are narrow (Elliott et al., 2014) [13] or agrochemical attitude which endangers human health, terrestrial and aquatic ecosystems by over-using fertilizers. As well as acceptance of public and soil features are still the major challenges to genetic modification (Martínez-Ballesta et al., 2008) [38]. On the contrary biological propensities, such as the use of rhizospheric microorganisms have grasped more attention for a few decades. One such microorganism is endophytic fungi, remaining inside plant tissues without showing any disease symptoms such as *Rhizophagus irregularis* and *Serendipita indica* (Purahong and Hyde, 2011; Rodriguez et al., 2009) [45, 47].

In the midst of endophytic fungi, arbuscular mycorrhizal (AM) symbiosis is the most outstanding fungi residing the roots of almost 80% of plants (Yang et al., 2014) [78]. Due to the efficacy of AM fungi, modification of drought has been documented by many authors (Abbaspour et al., 2012; Zou et al., 2015) [1, 83]. Various mechanisms have been illustrated the role of *R. irregularis* symbiosis in drought conditions. For example, the contribution of mycorrhizal hyphae in uptake water through soil (Santander et al., 2017) [54], providing inaccessible nutrients (Nouri et al., 2014) [42], rising antioxidant enzyme against oxidative damages (Liu et al., 2016b) [36] and increasing water use efficiency (Farahani et al., 2013) [15].

P. indica, known as Piriformospora indica formerly (Weiß et al., 2016) [72], was obtained from the rhizosphere soils of the woody shrubs *Prosopis juliflora* (Swartz) DC and *Zizyphus nummularia* (Burm. fil.) Wt. & Arn. In the sandy desert soils of Rajasthan, where located in northwest India (Verma et al., 1998) [69]. Even though *S. indica* is resemblance to AM fungi in many features (Rai and Varma, 2005) [46], it can grow in axenic culture media without any host unlike mycorrhizal fungus that symbiotic partner is necessitated for their growth (Varma et al., 2001) [67]. *P. indica* can interact with many plant species (Weiß et al., 2016) [72] as well as it enhances plant resistance to biotic stresses and abiotic stresses (Lakshmipriya et al., 2017; Tyagi et al., 2017) [34, 65]. Evidence has supported that capability of *P.indica* to restriction consequences of drought derived from production and signaling of phytohormones such as ethylene, auxin, gibberellin and cytokinin (Xu et al., 2018) [76], improving nutrients uptake (Wu et al., 2018a; Wu et al., 2018b) [73, 74], enhancing yield and physiological traits such as chlorophyll concentration (Ahmadvand and Hajinia, 2018) [3], up-regulation drought-related genes, increasing proline content and antioxidative enzyme activity (Xu et al., 2017) [75], regulation of plant water relations (Hussin et al., 2017) [25], protecting the photosynthetic systems (Saddique et al., 2018a) [50] alteration in abundance of protein involving in the plant's primary metabolism (Ghaffari et al., 2019) [18].

To understand effects of drought issue on sesame and choose fungi as a considered method in sustainable agriculture, it is a necessity to know morphological, physiological and nutritional mechanisms that impacted by drought and investigate how *R. irregularis* and *P. indica* improve these traits; so the aim of this study measurement of some morphological, physiological indices and also nutrient

status in sesame plant inoculated by *R. irregularis* and *P. indica* in field conditions.

Methods and Material

Location and treatments

This project was done in the research field of the College of Agriculture, The Isfahan University of Technology, Lavark, located in Najafabad (Isfahan, Iran) in 2014. The desired region geographically situated 1,850 meters above sea level, its Latitude and longitude 31° 42' N, 51° 41' E and according to Köppen-Geiger has a cold semi-arid(steppe) climate classification (Kottek, 2006) [33]. Experimental factors included fungal treatment covering plants inoculated by *R. irregularis*, *P. indica* and control plants, and Irrigation treatments performed based on the maximum allowable depletion percentage(MAD) of the soil available water (SAW) (Allen et al., 1998) [4] and included I1, I2 and I3 which performed based on split-block design.

Soil Properties

Before planting, a composite sample was taken from 30 cm of the soil surface, the sample was air-dried and passed through a 10-mesh (2-mm opening) sieve. The soil texture was clay-loam (Gee, 1986) [16] and Typic Haplargids taxonomically and other soil properties measured, including pH 7.8 (Thomas, 1996) [62], Electrical conductivity (EC) 1.8 $dS.m^{-1}$ (Topp, 1993) [63], bulk density of 1.34 $gr.cm^{-3}$ (Blake and Hartge, 1986) [9], water-holding at field capacity (FCm) 23%, permanent wilting point (PWPm) 10% (Veihmeyer and Hendrickson, 1949) [68], organic carbon by 0.27 % (Walkley and Black, 1934) [71], total N (Bremner, 1965) [10], available P (Olsen, 1982) [43] and available K (Hald, 1947) [20] of 300, 14.9 and 250 $mg.kg^{-1}$ respectively.

Fungal inoculum preparation

P. indica was cultured on CM (complex medium) at 24°C for 4 weeks. To induce sporulation given petridishes for 2-4hour shock at $_{+}$4°C, and incubate them for 1 day at 24°C, then 10-15 ml of sterilized tap water containing 0.05% Tween-20 was added on the surface of CM plates and scratched with a spatula for dispersing spores in suspension. The spore suspension was filtered through miracloth to remove the mycelium, and the filtrate containing spores was transferred to 50 ml tubes. In order to get rid of spore aggregations, spores were washed with tween-water, vortexed and sonicated three times repeatedly. After each washing step, spores were collected by centrifugation at 5,000 rpm for 7 min at room temperature. Finally spores re-suspended in dH_2O and adjusted to $5\times10^{+5}$ per milliliter using a hemacytometer and a microscope (Huong Pham et al., 2008) [24].

R. irregularis prepared by the trap culture method. To illustrate *zea mays L.* seedlings were disinfected by shaking them in 0.5% Sodium Hypochlorite (NaClO) solution for 10min and rinsed successively 10 times for 5min in sterile water, and germinated in petridishes. The seeds were planted in plastic pots (25cm diameter and 30cm depth) containing autoclaved sand as well as 25gr *R. irregularis*, and placed in the greenhouse (temperature 28±2°C and 60% relative humidity). Plants were nourished by Johnson solution (Johnson et al., 1957) [29], which used 1:8 ratios for the first month and increased its ratio by appearing nutrient deficiency. After 90 days plants cut in the shoot, and root plus sand used as an inoculum (Ruiz-Lozano and Azcón, 1996) [48].

Application of Treatments

To apply treatments, 9 main plots considered by an area of 9 square meters included 3 row for irrigation treatment (I1, I2 and I3), which separated by 2 meters distance, and 3 plots in every row to fungal treatments (R. irregularis, S. indica and control), and each main plot splitted for three replications. Sesame seeds, which had provided by College of Agriculture, The Isfahan University of Technology, sterilized with 70% ethanol for 2 min followed by 5 min in 75% NaClO solution and washed thoroughly with sterile water by 6 times (Varma et al., 1999) [66]. Finally, seeds were sown on the ridge by 5 cm interval and fungal inoculum added to sesame seeds directly, considered 5 gr *R. irregularis* inoculum and 3 ml *P. indica* inoculum.

To irrigation and creating drought condition, plots were irrigated normally till four-leaf stage and after that irrigation procedure was

performing just after depletion of 55 % (I1), 75 % (I2) and 85 % (I3) of SAW. A TDR probe (TDR Trase System, Model 6050X3K1mini-trase kit) was used to the scheduling of irrigation by following equation:

$$\theta_{irig} = \theta_{fc} - (\theta_{fc} - \theta_{pwp}) \times p$$

which θ_{irig} indicating water content, θ_{fc}, and θ_{pwp} are the soil water contents at field capacity (%) and wilting point (%), respectively, and P is the fraction of SAW (55, 75 and 85 %) that can be depleted through the root depth. For similarity of irrigation, water volume used a counter by 1-liter accuracy.

Staining fungal hyphae

To monitor root colonization, small parts of the roots from seedlings that were inoculated with *P. indica* and *R. iregularis* were transferred to 10% potassium hydroxide and were boiled for 10 min. After washing with water for 1 min, the roots were put into a 0.01% acid fuchsin-lactic acid solution and were boiled again for 10 min. Excess dye was removed with water prior to microscopy (Zhang and Guo, 2007) [82].

Plant analysis

At the end of the farming season parameters such as shoot dry weight (48 hours in 65°C in the oven), leaf number and shoot height were measured. To measure nutrient concentration, plant tissue digested by Baker method (Baker et al., 1964) [6], and atomic absorption system (Perkin Elmer, Model 3030) and spectrophotometer (PD-303) used to determine the content of iron (Fe) and phosphorus (P) concentration respectively. Total nitrogen content (N) was measured by Micro-Kjeldahl method described by Isaac and Johnson (Isaac and Johnson, 2018) [27].

Enzyme extraction to measure catalase activity was performed by homogenizing 0.1 gr Leaf textures with 1 mL NaH_2PO_4/Na_2HPO_4 buffer (PBS, 50 mM, pH 7.8) containing NaH_2PO_4/Na_2HPO_4 buffer 50 mM; pH=7.0 (100ml), EDTA (2 mM), Triton X-100 (0.1% v/v), Tris–HCl (0.605 gr) and DDT (2mM). The homogenate was centrifuged at 12,000 rpm for 30 min at 4°C, the supernatant (enzyme extraction) was collected and immediately used for the enzyme assays. All steps were done at 4°C. Eventually catalase activity (EC 1.11.1.6) was assayed by the method described by (Aebi, 1984) [2]. The 50 µl of leaf enzyme extract was mixed with 3ml of the reaction medium containing 50 mM PBS (pH 7.0) and after pre-incubation at 25°C for 5 min, 6 mM H_2O_2 was added to initiate the reaction. The changes of the absorbance at 240 nm (extinction coefficient of 0.036 $mM^{-1}cm^{-1}$) within 2 min were recorded by a spectrophotometer UV-160A (Hitachi, Japan).

The leaf relative water content was determined using the fully expanded leaf. To illustrate, the fresh weight of 10 leaf discs, which punched from a set of leaves was weighted (F.W) and immersed for several hours on distilled water in covered petri-dishes until the discs become fully turgid (T.W). Afterward, disc samples oven dried at 80 for 3h and the dry weight determined (D.W). Finally, leaf relative water content was calculated using the following formula (Turner, 1981) [64]:

$$RWC\ (\%) = F.W\text{-}D.W T.W\text{-}D.W \times 100$$

For leaf membrane stability index, leaf discs were thoroughly washed by tap and double distilled water and placed in 10 ml of double distilled water at 40°C for 30 min, thereafter electrical conductivity was measured by a conductivity meter (C_1). After the expiry of the period, their electrical conductivity was recorded by a conductivity meter (C_1). Subsequently, the same samples were placed in a boiling water bath (100°C) for 10 min and their electrical conductivity recorded again (C_2). The membrane stability index was calculated a (Sairam et al., 1997) [53]:

$$MSI = (1 - C_1C_2) \times 100$$

Statistical analysis

Data were subjected to combined analysis by using SAS statistical software (SAS, ver. 9.1). When the main effect was significant ($P < 0.05$), differences between means were evaluated by using the least significant difference (LSD).

Results

Morphological traits

The results indicated that the shoot dry biomass of non-inoculated plants (controls) was decreased significantly with decreasing irrigation level. At I1, inoculation of *P. indica* resulted in significant increase of shoot dry biomass (more than 13% higher than the control), but inoculation of *R. irregularis* had no significant effect than the control (Fig. 2). On the contrary, at I2 inoculation of *R. irregularis* led to an increase in shoot dry biomass compared to control treatment, while there was no significant difference between control and treatment of *P. indica*. At I3, inoculated plants with of *P. indica* and *R. irregularis* increased shoot dry biomass by 14% and 13% compare to control, respectively, however, no significant difference was found between these two fungal treatments.

Interaction of fungal treatments (*P.indica*, *R.irregularis*) and irrigation levels (I1, I2 and I3) on shoot height showed that drought condition declined shoot height significantly in plants inoculated with *P.indica*; however, there was not significant difference in treatment of *R.irregularis*. At I1, treatment of *P.indica* enhanced shoot height compare to control plants by 9%, but there was no significant difference between *R.irregularis* and control plants. At I2, plants inoculated by *P.indica* and *R.irregularis* enhanced shoot height significantly compare to treatment of control, but no significant change was detected between fungal treatment. At I3, control plants had the lowest shoot height and statically treatment of *P.indica* had not significant difference with control plants, but plants inoculated *with R.irregularis* incremented shoot height by 10% compare to control plants (Fig.2).

Results revealed that leaf number in plants inoculated with *P.indica* and *R.irregularis* was the same significantly at I1, I2 and I3 (Fig.3). At I1, not significant difference was detected among fungal treatments and control plants. At 75% of SAW depletion, treatments of *P.indica* and *R.irregularis* enhanced sesame leaf number by 44% compared to control plants. At I3, plants inoculated with *P.indica* and *R.irregularis* enhanced leaf number markedly in comparison with control plants (38% and 43% increment respectively).

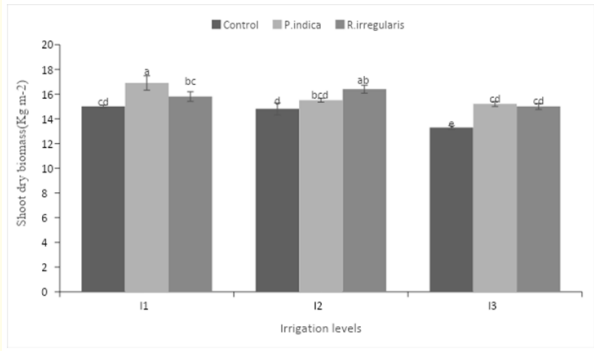


Figure 1: Effect of inoculation of *P. indica* and *R.irregularis* on shoot dry biomass of sesame plant grown under 3 different irrigation conditions (I1, I2 and I3 representing depletion of 55, 75 and 85% of Soil Available Water respectively). Values are expressed as means± SD and different small letters indicate significant difference at 0.05 level.

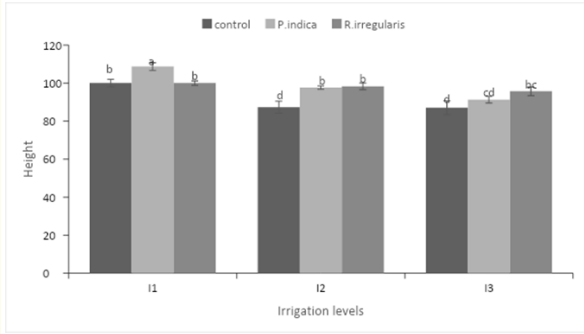


Figure 2: Effect of inoculation of *P. indica* and *R.irregularis* on shoot height of sesame plant grown under 3 different irrigation conditions (I1, I2 and I3 representing depletion of 55, 75 and 85% of Soil Available Water respectively). Values are expressed as means± SD and different small letters indicate significant difference at 0.05 level.

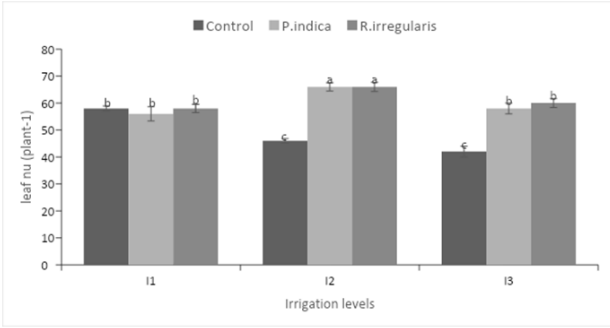


Figure 3: Effect of inoculation of *P. indica* and *R.irregularis* on leaf number of sesame plant grown under 3 different irrigation conditions (I1, I2 and I3 representing depletion of 55, 75 and 85% of Soil Available Water respectively). Values are expressed as means± SD and different small letters indicate significant difference at 0.05 level.

Shoot concentrations N, P and Fe

The results indicated that shoot nitrogen concentration in fungal treatments remained stable statically by depletion of SAW. At 55 and 75% of SAW depletion, there was not significant change between control plants and treatments of *P.indica* and *R.irregularis*. At 85% of SAW depletion, treatments of *P.indica* and *R.irregularis* enhanced shoot nitrogen concentration by 33% and 23% compare to treatment of control respectively (Fig. 4).

The effect of treatments of *P.indica* and *R.irregularis* on shoot phosphorus concentration in different levels of irrigation revealed that rising drought dropped phosphorus concentration markedly in treatments of *P.indica* and control plants at I2 and I3 compare to I1. At I1 shoot phosphorus concentration in plants inoculated with *P.indica* and *R.irregularis* did not changed significantly compare to control plants. By depletion of 75% of SAW, phosphorus concentration in plants inoculated with *R.irregularis* incremented by 194% compare to non-inoculated plants, but no significant difference detected between treatment of *P.indica* and control. At I3, there was not significant difference among fungal treatments compare to non-inoculated plants (Fig. 5).

The results indicated that shoot iron concentration declined in treatments of *R.irregularis* and control by decreasing irrigation level at I1 to I3 , and the highest amount of shoot iron concentration was measured in plants inoculated with *P.indica* at I3 (Fig. 6). At I1,

inoculation of *R.irregularis* resulted in 7% increment of shoot iron concentration, but inoculation of *P.indica* had not significant effect on shoot iron concentration compare to control plants. At I2, inoculation of *P.indica* and *R.irregularis* led to increase in shoot iron concentration by 45% and 15% respectively compared to treatment of control. By depletion of 85% of SAW, plants inoculated with *R.irregularis* increased shoot iron concentration 7% compare to control plants, but no significant difference was detected in plants inoculated with *P.indica*.

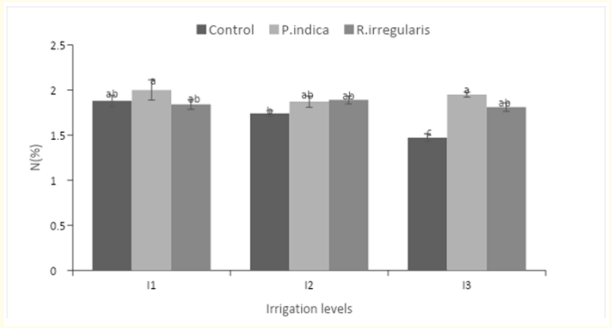
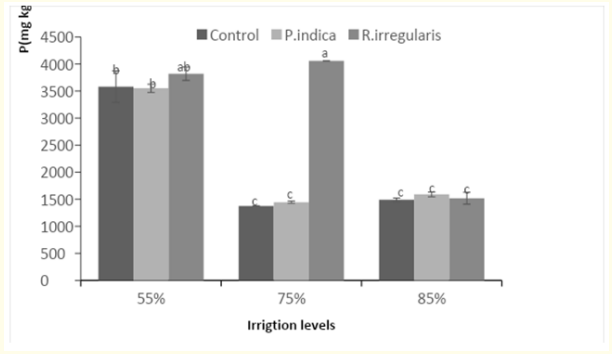


Figure 4: Effect of inoculation of *P. indica* and *R.irregularis* on Nitrogen concentration (N) of sesame plant grown under 3 different irrigation conditions (I1, I2 and I3 representing depletion of 55, 75 and 85% of Soil Available Water respectively). Values are expressed as means± SD and different small letters indicate significant difference at 0.05 level.



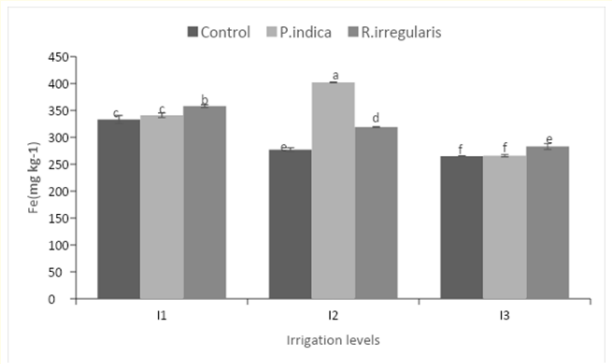


Figure 6: Effect of inoculation of *P. indica* and *R.irregularis* on Iron concentration (Fe) of sesame plant grown under 3 different irrigation conditions (I1, I2 and I3 representing depletion of 55, 75 and 85% of Soil Available Water respectively). Values are expressed as means± SD and different small letters indicate significant difference at 0.05 level.

Physiological properties

The results showed that catalase enzyme activity soared significantly by decreasing irrigation level specially in plants inoculated with *P.indica* and *R.irregularis* (Fig. 7). At I1, no significant difference was detected among fungal treatments (*P.indica* and *R.irregularis*) and non-inoculated plants. At I2, inoculation of *P.indica* resulted in 9% increase in catalase enzyme activity compared to control treatment, but there was no significant difference between treatments of *R.irregularis* and control. At I3, inoculated plants with of *P.indica* and *R.irregularis* increased catalase enzyme activity by 15% and 13% compare to control, respectively.

Interaction of fungal treatments (*P.indica*, *R.irregularis* and control) and irrigation levels (I1, I2 and I3) showed that drought condition declined MSI in treatments of control, and MSI in treatment of *R.irregularis* had not changed statically by decreasing irrigation levels. By depletion of 55% of SAW, MSI in plants inoculated with *P.indica* and *R.irregularis* had not changed significantly in comparison with non-inoculated plants. At I2, plants inoculated with *P.indica* and *R.irregularis* rose MSI significantly compare to non-inoculated plants (15% and 12% respectively). At I3, treatments of *P.indica* and *R.irregularis* enhanced MSI by 16 and 17% respectively compare to control plants (Fig. 8).

The results showed that RWC did not changed in plants inoculated with *R.irregularis* by decreasing irrigation levels. At I1, there was no significant difference among inoculated and control plants. Depletion of 75% of SAW caused RWC incremented in plants inoculated with *P.indica*, however there was no significant difference between treatments of *R.irregularis* and control plants. At I3, treatments of *P.indica* and *R.irregularis* increased RWC by 13% and 17% respectively (Fig. 9).

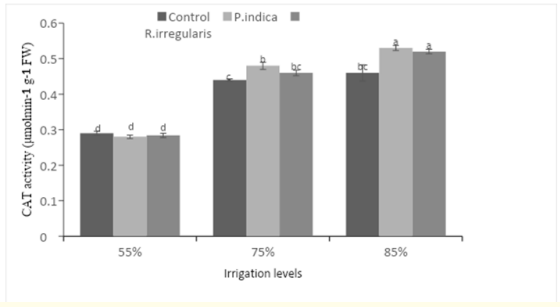


Figure 7: Effect of inoculation of *P. indica* and *R.irregularis* on Catalase Enzyme Activity (CAT) of sesame plant grown under 3 different irrigation conditions (I1, I2 and I3 representing depletion of 55, 75 and 85% of Soil Available Water respectively). Values are expressed as means± SD and different small letters indicate significant difference at 0.05 level.

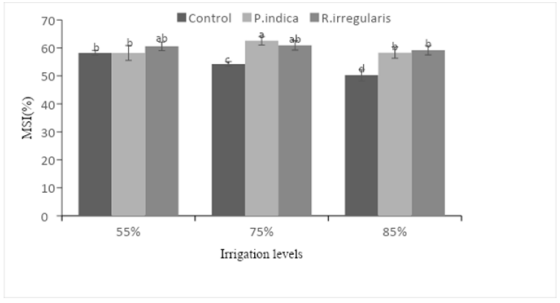


Figure 8: Effect of inoculation of *P. indica* and *R.irregularis* on Membrane Stability Index (MSI) of sesame plant grown under 3 different irrigation conditions (I1, I2 and I3 representing depletion of 55, 75 and 85% of Soil Available Water respectively). Values are expressed as means± SD and different small letters indicate significant difference at 0.05 level.

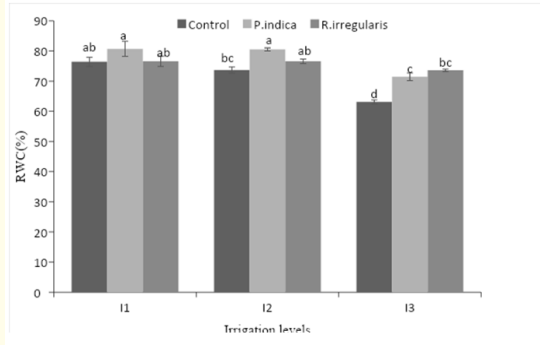


Figure 9: Effect of inoculation of *P. indica* and *R.irregularis* on Relative water content (RWC), of sesame plant leaves grown under 3 different irrigation conditions (I1, I2 and I3 representing depletion of 55, 75 and 85% of Soil Available Water respectively). Values are expressed as means± SD and different small letters indicate significant difference at 0.05 level.

Discussion

Drought is one of the main abiotic stress which concomitantly impact plant morphological and physiological characteristics (Saeidi and Abdoli, 2018) [52]. Although evidence suggests that plant colonization with plant growth promoting fungi can alleviate the adverse effects of drought stress, there is less literature that clarifies the effects of *R. intraradices* and *P. indica* on sesame during drought stress and field natural conditions. Therfore, the aim of this study measurement of some morphological, physiological indices and also nutrient status in sesame plant inoculated by *R. irregularis* and *P. indica* during drought in field conditions.

In this study, sesame inoculated with *R. irregularis* and *P. indica* resulted in more shoot dry biomass, height and leaf number compare to non-inoculated plant at limited irrigation levels. Gong et al. (2015) [19] have showed that *G. intraradices* enhanced morphological indices such as plant height and grain weight. *P. indica* treatment also resulted in increasing shoot fresh and dry weights of barely under osmotic stress (Ghabooli, 2014) [17]. The positive effects are probably because *R. irregularis* and *P. indica* improve water and nutrient absorption, which can induce plants production (references).

Our results indicate that both *P. indica* and *R. intraradices* increased water content of sesame leaves in drought condition. The increase in the RWC due to the association with *P. indica* has been reported (Swetha and Padmavathi, 2019) [61], and it was considered to be a possible mechanism in which improves morphological traits., *R. irregularis* the increased RWC of poplar leaves under drought condition (Liu et al. 2016a) [37].

Inoculation of sesame with *R. intraradices* improved nutrient concentration in drought condition. *P. indica* treatment had highest N and Fe concentration at I3 and I2 respectively. In another study, reported that the different impact of mycorrhiza on nutrients uptake under drought stress may be attributed to the proportion of active structures of arbuscular (Marulanda et al., 2003) [39]. Sawers et al. (2017) [55] indicated that different behavior of AM fungi depending on both fungal and plant species. The lack of response in P concentration in the current study do not in accordance with findings of Saddique et al. (2018b) [51] who reported that rice inoculation with *P. indica* increased zinc and phosphorous concentration both in root and leave; therefor, inoculation of sesame seeds with *P. indica* under stress warranties further researches.

Water deficiency stress may induce a combination of negative effects on plants and consequently oxidative stress. Stress resistance in plants is related to more efficient antioxidative systems for effective removal of ROS. Antioxidant enzymes (e.g., APX, POD, CAT) act

not only as direct ROS scavengers but also as key sensors of the cellular redox status (Huang et al., 2014) [23]. The increase of antioxidant enzyme activity has been reported by the earlier researchers results during drought conditions (Hosseini et al., 2017; Lambais et al., 2003; Yaghoubian et al., 2014) [22, 35, 77]. In this study, *P. indica* treatment rose CAT activity at limited irrigation levels (I2 and I3). In contrast, *R. intraradices* enhanced CAT activity just at I3. This finding is consistent with finding of Rostami et al. (2013) that reported, severity and duration of stress, species and age of the plant cause alteration in the activity of the antioxidant enzymes. On the other hand, (Zarik et al., 2016) [80] proposed that increasing in activity a set of defense enzymes involving in the elimination of ROS can be the possible reason for low CAT activity in inoculated plants.

Drought stress induced loss of membrane function which disorder in growth of various plant species, and lower membrane index is a symptom for production of ROS molecules leading to lipid peroxidation. The ability of plants to maintain membrane integrity under drought is what determines tolerance towards drought stress (Sofo et al., 2016) [57]. It has been proven that PGPFs improve the stability of plant cell membranes by activating the antioxidant defense system, enhancing drought tolerance in plants (Mona et al., 2017) [40]. In the present study, *P. indica* and *R.irregularis* treatments increased MSI which result in protection toward membrane dysfunction. Previous results have shown that the associations of AMF protect membrane structure and its functional integrity by improving polyunsaturated fatty acid concentrations in cellular membranes (Hashem et al., 2019) [21]. Moreover, endophytic fungi stabilize cell membrane with enhancing concentration of antioxidant enzymes namely catalase as scavenger of ROS (Shukla et al., 2012) [56].

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