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DROUGHT STRESS ALLEVIATION: THE CONTRIBUTION OF A SOIL BACTERIUM AND AN ARBUSCULAR MYCORRHIZAL FUNGUS IN SCALLION

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ABSTRACT

Recent climate change and global warming have caused extreme heat and low rainfall which negatively affected soil nutrient, fertility status, decreased water content, and changed physio-chemical properties of agricultural soils in many parts of the globe. As a result of drought, crop production is seriously reduced. The beneficial effects of soil microorganisms including arbuscular mycorrhizal fungi and rhizospheric bacteria on crop production and soil health are vital for the sustainable management of stressed agricultural practices. The present study evaluated the effects of an arbuscular mycorrhizal fungus, *Rhizophagus irregularis,* and a rhizospheric bacterium, *Pseudomonas flourescens,* alone and in dual inoculation on the survival, growth, mycorrhizal colonization, nutrient uptake, glomalin production, and soil aggregation of *Allium fistulosum* plants. In water-stressed soil, seedling survival, growth, total biomass, nutrient uptake, glomalin production, and soil aggregation were significantly increased when inoculated with *R. irregularis* and *P. flourescens*. Dual inoculation with *R. irregularis* and *P. flourescens* produced vigorous seedlings which contained higher amount of nitrogen, phosphorus, potassium contents, glomalin production, and soil aggregation compared to single inoculation. The present results indicate that arbuscular mycorrhizal fungi and beneficial rhizospheric bacteria offer potential for biofertilizers and mitigating drought in *A. fistulosum* plants.

Keywords: Global warming, drought stress, arbuscular mycorrhizal fungi, rhizospheric bacteria, scallion.

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1. INTRODUCTION

Since the beginning of the 21st century our planet is experiencing an unprecedented change of climate and is getting warmer and warmer each year [1]. Agricultural plants are facing drought under field conditions that are lethal to plant survival and crop production world-wide. Because of drought global food supply will significantly affect food security and food availability worldwide [2]. Plant roots play a vital role by exploring the soil environment and taking up nutrient and water. Drought can affect root function by altering cell water permeability and influencing the growth and architecture of the root system [3]. Water channel proteins or aquaporins adjust root permeability in response to many stimuli, including drought stress [4]. Phytohormones such as auxin and abscisic acid are important in root growth and development by regulating entry of water during lateral root formation. Drought can impair normal plant growth, disturb water relations, and reduce water use efficiency in plants. During drought the rate of photosynthesis is reduced mainly by stomatal closure, membrane damage, and disturbed activity of various enzymes, especially those involved in ATP synthesis [5]. Drought can affect chemical, physical, and biological activities of soil that are essential for plant growth and soil health. Impacts may include lack of nutrient uptake by plants, increased soil temperatures, altered microbial activity, changes in organic matter decomposition, and increased release of carbon dioxide. Plants display a range of mechanisms to withstand drought, such as reduced water loss by increased diffusive resistance, increased water uptake with prolific and deep root systems, and smaller and succulent leaves to reduce transpirational loss [6-8]. It is reported that low molecular weight *organic compounds that influence the properties of biological fluids* including glycinebetaine, proline and other amino acids, organic acids, salicylic acid, auxins, gibberellins, cytokinins, abscisic acid, and polyols also play vital roles in sustaining cellular functions under drought condition [9-11]. There is a growing interest in recent years to use beneficial soil microorganisms to improve agricultural productivity under adverse soil conditions. Arbuscular mycorrhizal fungi are considered to be of particular importance for the sustainable management of agricultural ecosystems and mediating crop productivity [12-13]. Arbuscular mycorrhizal fungi are a specialized group of soil fungi that form symbiotic associations with higher plants [14]. In this association both the partners benefit from each other. Mycorrhizal fungi form symbiotic associations with host plans enhancing water and nutrient uptake from soil. Fungal hyphae can travel far beyond the reach of root systems [15- 16], increase resistance against diseases [17] and improve water-use efficiency [18-19]. In return, the plant provides the fungi with carbohydrates and sugars formed during photosynthesis for fungal growth and metabolism. Arbuscular mycorrhizal fungi are also known to provide plants greater tolerance to biotic and abiotic stresses by changing host-plant physiology and biochemistry along with secondary metabolites [20].

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Besides arbuscular mycorrhizal fungi, plant growth-promoting bacteria have recently attracted increasing attention because of their effect on promoting the growth in agricultural crops [21-23]. These beneficial bacteria enhance plant growth by producing growth hormones such as indole acetic acid, giberellic acid, cytokinins, fixing nitrogen from the atmosphere, and protects plants by producing antimicrobial compounds. Their application to field crops promotes the growth while reducing the need for chemical fertilizers [24]. In recent years, different plant growth-promoting bacteria have been studied including nitrogen fixing bacteria and and phosphate solubilizing bacteria for their effect on plant growth and nutrient uptake.

It is now known that arbuscular mycorrhizal fungi produce iron-containing glycoproteinaceous substance known as glomalin [25-26] that accumulate in the rhizospheric soil to concentrations of several mg per cm^3 of soil [27]. Glomalin produced in the spores and hyphae by arbuscular mycorrhizal fungi in the soil are beneficial for soil aggregation, water stability, protection of hyphae from water and nutrient loss [28-29], increase carbon sequestration in soil [30], plant growth and nutrition [31], resistance to detergents and acidic/base solvents, tolerance to chaotropic agents, heavy metal and other pollutant chelation [32-33]. It is now known that presence of glomalin increases water retention, reduces soil erosion, enhance nutrient cycling, contributes to the improvement of development of root system, increases soil porosity, soil enzyme activities, and overall plant growth [34]. This glycoprotein contains approximately 37% of carbon and can remain in the soil for several months to years [35]. Glomalin can be used as an effective indicator of soil quality and agricultural management systems [36]. The synergistic effect of arbuscular mycorrhizal fungi and other beneficial rhizospheric bacteria in the production of glomalin and soil aggregation in the water stress areas are limited.

The objectives of this study were to determine the interactions of an arbuscular mycorrhizal fungus (*Rhizophagus irregularis*) and a beneficial rhizospheric bacterium (*Pseudomonas flourescens*) in water stress conditions of scallion (*Allium fistulosum*) on (1) seedling survival, growth, nutrient uptake, and mycorrhizal colonization, and (2) production of glomalin and soil aggregation alone and in combination of *R. irregularis* and *P. flourescens.*

2. MATERIALS AND METHODS

2.1. Organisms

Seeds of *A. fistulosum* were obtained from Armstrong Garden Center, Glendora, California, USA. The arbuscular mycorrhizal fungus, *R. irregularis* (PL 1794RI) and a rhizospheric bacterium, *P. flourescens* (PL 009PF) were obtained from the culture collection bank of Microbiology Department, Pasteur Laboratory, Glendora, California, USA. The identity of these organisms was verified through DNA metabarcoding analysis of the rDNA ITS2 region. The inoculum of *R.*

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irregularis was maintained in the fragmented roots of carrots, whereas *P. flourescens* was maintained in tryptic soya broth nutrient medium.

2.2. Effect of *R. irregularis* and *P. flourescens* on drought tolerance of *A. fistulosum*

Garden soils were autoclaved at 121^0C for 15 minutes at 15 lb pressure. Once cooled, soils were placed on germination trays and covered with lids. Seeds of *A. fistulosum* were soaked in water overnight, washed with sterile distilled water, and then sown in germination trays containing moist sterile soil. The germination trays were then covered with a lid to prevent evaporation. The trays were kept in a growth chamber (25^0C ± 2^0C) and lightly sprayed with sterile distilled water daily to keep soil moist. Seeds of *A. fistulosum* started sprouting 4 days after sowing.

One week old *A. fistulosum* seedlings were transplanted in 5-inch plastic pots containing autoclaved soil. Electrical conductivity of the soil was 0.91 dSm^{-1} and pH was 6.3. For mycorrhizal treatment, seedlings were inoculated with 1 gm of soil containing approximately 100 spores of *R. irregularis* and were added to the soil surrounding roots. For bacterial treatment, 1 ml of *P. flourescens* (contains about $2X10^6$ cells per ml in sterile distilled water) was similarly added to the root zone. For dual inoculation, seedlings were simultaneously inoculated with 1 gm of soil containing *R. irregularis* and 1 ml of *P. flourescens*. Non-inoculated control seedlings received 1 gm of sterilized garden soil and 1 ml of sterilized distilled water without *P. flourescens*. There were 15 replicates plants for each treatment. Four treatments resulted: (1) non-inoculated control, (2) *R. irregularis*, (3) *P. flourescens*, and (4) a dual inoculation of *R. irregularis* plus *P. flourescens*. Three watering regimes were used. For each treatment, five pots received watering twice a week, five pots received watering once a week and other five pots received watering every 10 days. Seedlings were kept in the growth chamber at 25^0C ± 2^0C under LED growth light (Model X001NTBWA5) for 10 hours daily illumination. The pots were randomized in the growth chamber every 2 days. Seedling mortality was recorded for each treatment. Since garden soils were used, pots were not fertilized. The plants were harvested and evaluated 10 weeks after planting.

At harvest, seedlings from each treatment were carefully removed from the pots without damaging the root system. Roots were washed with water, shoot and root lengths were measured. Several randomly selected root lengths from each treatment were stored in test tubes with water for later quantification of mycorrhizal colonization, while the remaining root mass was dried along with shoots for quantifying total biomass and nutrient contents. Rhizospheric soil from each treatment were collected for estimation of glomalin. For calculating mycorrhizal colonization, roots from the test tubes were cleared in 10% potassium hydroxide, autoclaved for 10 minutes, acidified with 1% hydrochloric acid and stained with cotton blue [37-38]. Percentage of mycorrhizal colonization was evaluated in a randomly distributed 15 root samples from each treatment. The roots were oven-dried at 40^0C for 48 hours for measuring nutrient contents. Nitrogen concentration was measured

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using the automatic Kjeldahl apparatus method (K9840). Phosphorus concentration was measured using the molybdate-blue colorimetry method, and potassium concentration was determined with a flame photometer (Model 410, USA) [39-40].

2.3. Isolation of glomalin from rhizosphere of *R. irregularis* and *P. flourescens* on drought stress conditions of *A. fistulosum*

From each treatment, the amount of total glomalin-related soil protein (T-GRSP) and easily extrable glomalin-related soil protein (EE-GRSP) were determined [41]. One gm of rhizospheric soil from different treatments was air dried, incubated with 8 ml of 20 mM sodium citrate solution (pH 7.0), autoclaved at 121^0C and 103 kPa for 30 min and then centrifuges at 1000 rpm for 15 min to extract EE-GRSP. T-GRSP was extracted with 8 ml of 50 mM sodium citrate solution (pH 8.0) by autoclaving at 121^0C and 103 kPa for min. The procedure was replicated 5 times and all suspensions were collected. The T-GRSP and EE-GRSP concentrations were determined spectrophotometrically by the Bradford dye-binding assay using bovine serum albumin as the standard [42].

2.4. Soil aggregate stability

Soil aggregate for different treatments were conducted using Slakes method [43]. Rhizospheric soil (5 gm) from different treatments were collected, filtered through sieves (10 mm, 2 mm, and 1 mm) and soaked in distilled water. Soaked soils were filtered through 0.5 mm sieve and oven-dried for 48 hours at 90^0C. Percent soil aggregate stability were calculated for each treatment.

2.4. Statistical analysis

Data were subjected to analysis of variance [44]. Individual means were compared using Scheffe's test for multiple comparisons using SAS software [45]. Means followed by the same letters (a,b,c and so on) on bars in the graphs and tables for a particular treatment are not significantly (P = 0.05) different from each other by Scheffe's test for multiple comparison.

3. RESULTS

3.1. Seedling mortality

When *A. fistulosum* seedlings were watered once and twice a week, no mortality was recorded in both inoculated and non-inoculated control seedlings (Figure 1). When seedlings were watered every 10 days, non-inoculated control seedlings had 29% mortality and seedlings inoculated with *P. flourescens* had 9% mortality (Figure 1). Seedlings inoculated with *R. irregularis* and dual inoculation with *R. irregularis* plus *P. flourescens* had no mortality when watered every 10 days (Figure 1).

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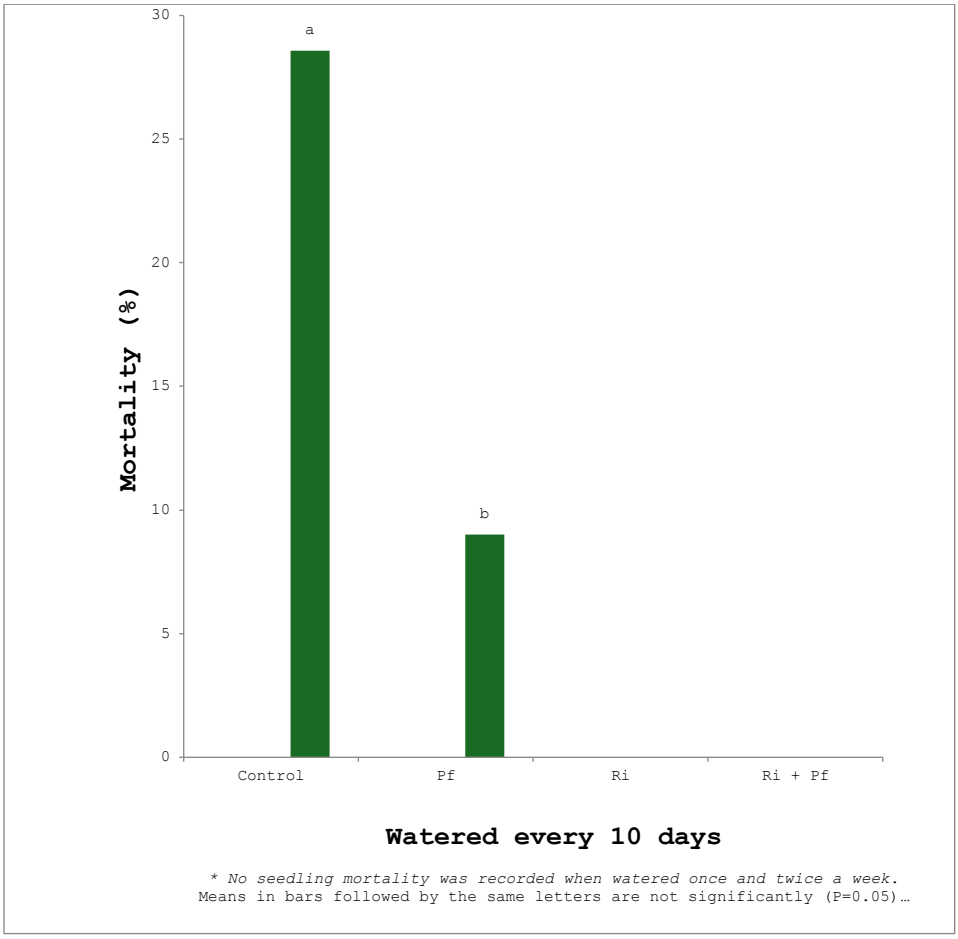

Fig. 1: Seedling mortality of *A. fistulosum* inoculated with *R. irregularis* (Ri) and *P. flourescens* (Pf) and non-inoculated control seedlings when watered every 10 days.

3.2. Shoot length and root length

Shoot and root length of *A. fistulosum* seedlings were significantly higher when inoculated with *R. irregularis* alone and in combination with *P. flourescens* (Figures 2 and 3) in all 3 water regimes compared to non-inoculated control seedlings (Figure 2 and 3). Dual inoculation with *P. flourescens* and *R. irregularis* produced highest shoot and length (Figure 2 and 3).

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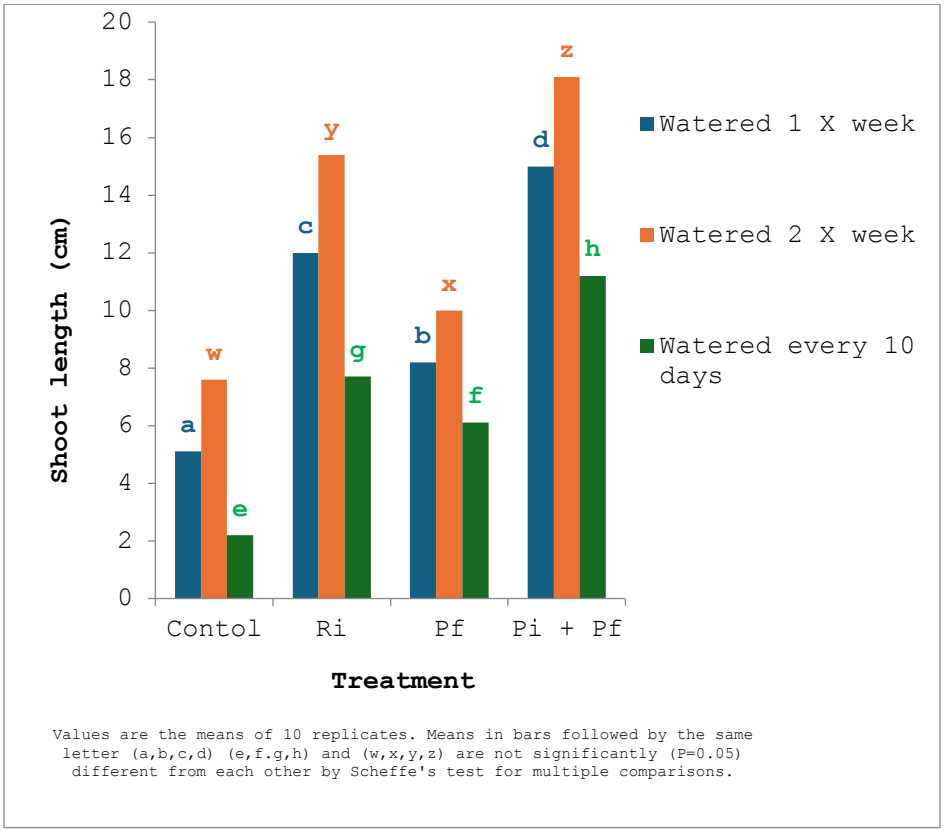

Fig. 2: Shoot length of *A. fistulosum* inoculated with *R. irregularis* (Ri) and *P. flourescens* (Pf) and non-inoculated control seedlings.

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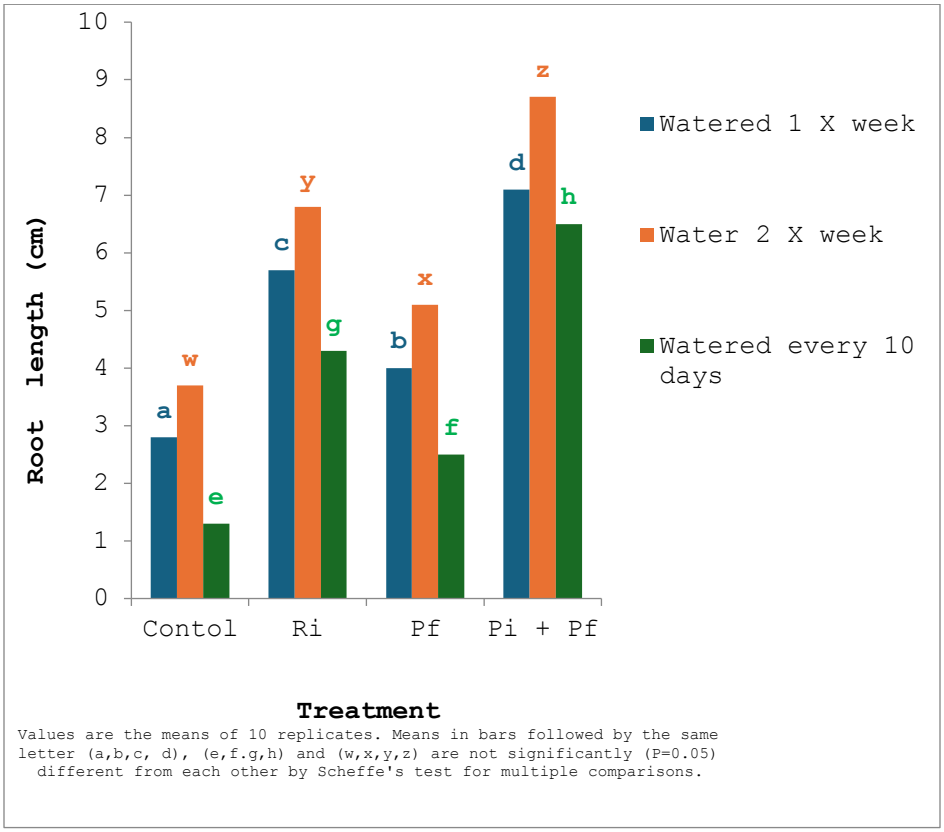

Fig. 3: Root length of *A. fistulosum* inoculated with *R. irregularis* (Ri) and *P. flourescens* (Pf) and non-inoculated control seedlings.

3.3. Biomass production

Seedlings produced higher biomass when inoculated with *R. irregularis* alone and in combination with *P. flourescens* (Figure 4) compared to non-inoculated control seedlings when watered once, twice, and every 10 days (Figure 4). Dual inoculation with *P. flourescens* and *R. irregularis* produced highest biomass compared to single inoculation. Non-inoculated control seedlings had the lowest biomass in all three different watering regimes (Figure 4).

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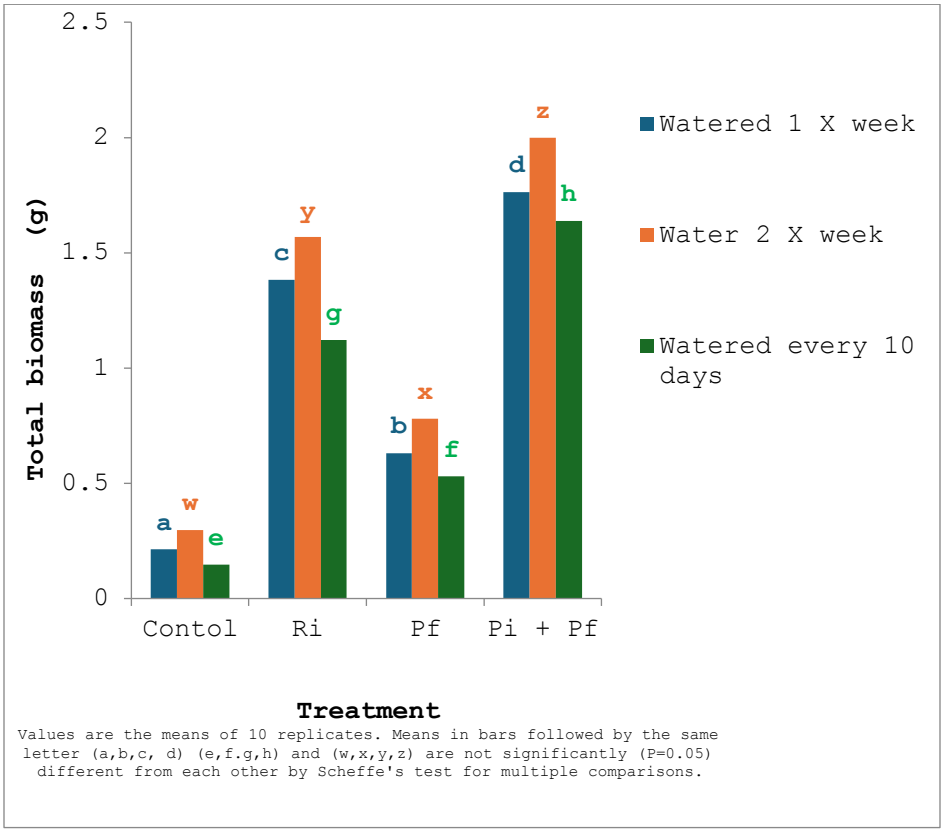

Fig. 4: Total biomass of *A. fistulosum* inoculated with *R. irregularis* (Ri) and *P. flourescens* (Pf) and non-inoculated control seedlings.

3.4. Mycorrhizal colonization

Seedlings inoculated with *R. irregularis* alone or in combination with *P. flourescens* had a higher arbuscular mycorrhizal colonization when watered twice a week (Figure 5) compared to seedlings watered once a week and every 10 days. Mycorrhizal colonization was stimulated when co-inoculated with *P. flourescens* (Figure 5).

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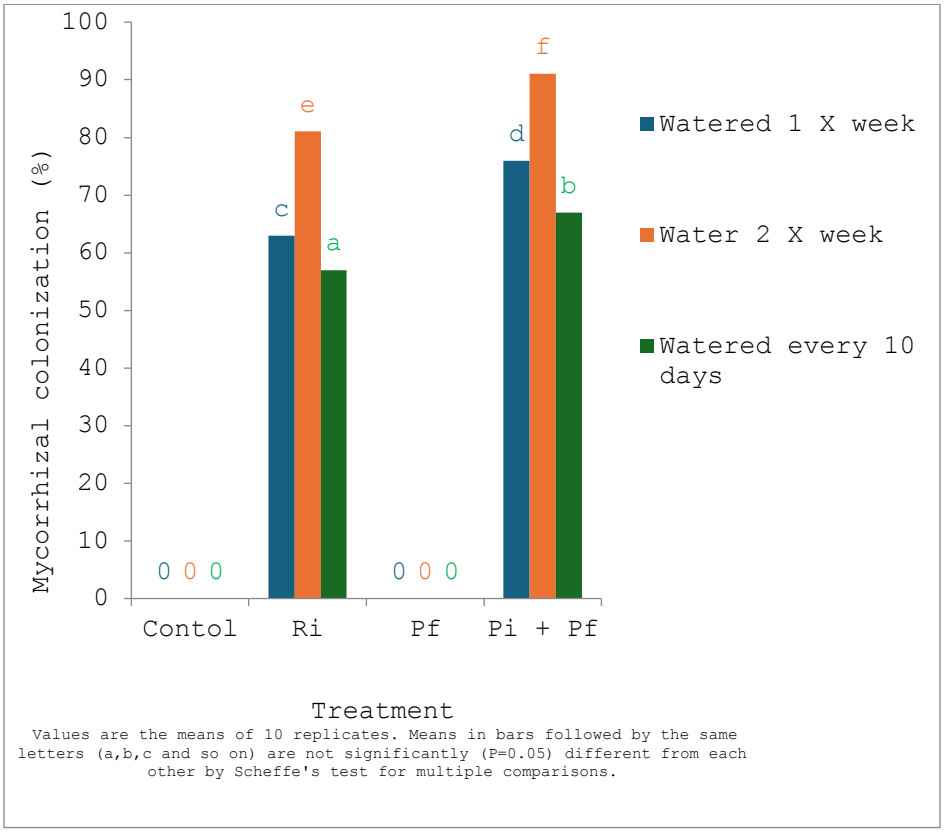

Fig. 5: Mycorrhizal colonization of *A. fistulosum* inoculated with *R. irregularis* (Ri) and *P. flourescens* (Pf) and non-inoculated control seedlings.

3.5. Nutrient contents

The amount of nitrogen, phosphorus and potassium contents of *A. fistulosum* was significantly reduced when seedlings were watered once a week and every 10 days (Table 1). Seedlings watered twice a week had the highest accumulation of nitrogen, phosphorus and potassium when inoculated with both *P. flourescens* and *R. irregularis* alone and in combinations (Table 1). Non-inoculated control seedlings had lowest amount of nitrogen, phosphorus, and potassium contents when received water every 10 days (Table 2).

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Table 1: The effect of *Pseudomonas flourescens* and *Rhizopagus irregularis* on nutrient contents of *A. fistulosum* seedlings grown under three different water regimes.

Treatment	Watered 1 time per week	Watered twice per week	Watered every 10 days
Nitrogen (g/kg):			
Non-inoculated control	0.443$_a$	1.131$_a$	0.331$_a$
P. flourescens (*P.f*)	0.991$_b$	2.671$_b$	0.881$_b$
R. irregularis (*R.i*)	2.341$_c$	5.116$_c$	1.710$_c$
Dual inoculation of *P.f* + *R.i*	4.191$_d$	6.911$_d$	2.430$_d$
Phosphorus (g/kg):			
Non-inoculated control	0.021$_a$	0.071$_a$	0.014$_a$
P. flourescens (*P.f*)	0.187$_b$	0.387$_b$	0.187$_b$
R. irregularis (*R.i*)	0.631$_c$	0.931$_c$	0.431$_c$
Dual inoculation of *P.f* + *R.i*	0.831$_d$	1.306$_d$	0.671$_d$
Potassium (g/kg):			
Non-inoculated control	0.061$_a$	0.341$_a$	0.173$_a$
P. flourescens (*P.f*)	0.103$_b$	0.531$_b$	0.213$_b$
R. irregularis (*R.i*)	1.031$_c$	1.872$_c$	0.779$_c$
Dual inoculation of *P.f* + *R.i*	1.972$_d$	2.311$_d$	1.431$_d$

Values are the means of three replicates. Means followed by the same letters (a,b,c and so on) in columns for a particular element are not significantly (P=0.05) different from each other by Scheffe's test for multiple comparison.

3.6. Total glomalin-related soil protein (T-GRSP-mg per g), easily extrable glomalin-related soil protein (EE-GRSP - mg/g), and aggregate stability

T-GRSP, EE-GRSP, and soil aggregate stability were highest in the rhizosphere of seedlings when co-inoculated with both *P. flourescens* and *R. irregularis* followed by single inoculation with *R. irregularis* (Table 2). No glomalin was reported in non-inoculated control and seedlings inoculated

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with *P. flourescens* in the rhizosphere of *A. fistulosum* (Table 2). Soil aggregate stability was highest in the rhizosphere of seedlings when co-inoculated with both *P. flourescens* and *R. irregularis,* followed by single inoculation with *R. irregularis, P. flourescens* and non-inoculated control seedlings in the rhizosphere of *A. fistulosum* (Table 2).

Table 2: Total glomalin-related soil protein (T-GRSP-mg per g), easily extrable glomalin-related soil protein (EE-GRSP - mg/g), and soil aggregate stability (%) in the rhizosphere of *A. fistulosum* under different treatments.

Treatment	Watered once / week		Watered twice / week		Watered every 15 days		Soil Aggregate stability (%)
	T-GRSP	EE-GRSP	T-GRSP	EE-GRSP	T-GRSP	EE-GRSP	
Non-inoculated control	0.0_a	0.0_a	0.0_a	0.0_a	0.0_a	0.0_a	4.6_a
***P. flourescens* (*P.f*)**	0.0_a	0.0_a	0.0_a	0.0_a	0.0^a	0.0_a	5.1_a
***R. irregularis* (*R.i*)**	3.51_b	3.01_b	4.87_b	4.93_b	2.77_b	1.06_b	68.06_b
Dual inoculation of *P.f* + *R.i*	6.87_c	5.97_c	7.31_c	6.31_c	3.98_c	2.01_c	81.06_c

Values are the means of five replicates. Means followed by the same letters (a,b,c and so on) in columns for a particular glomalin are not significantly (P=0.05) different from each other by Scheffe's test for multiple comparison.

4. DISCUSSION

Our agricultural industry is dependent on sunlight, temperature, and precipitation. Any change of these factors can significantly affect agricultural production. An increase in global temperature can significantly influence rainfall which might lead to drought condition. Soil properties such as water holding capacity, reduced amount of organic carbon, lower rate of decomposition, higher rate of evaporation, and lesser movement of water in the soil layers, can make soil infertile. Low rainfall in many parts of the globe is seriously affecting agricultural lands and crop productivity is significantly reduced. Modern agriculture practices should therefore need to implement and integrate other management practices that can reduce or avoid irreversible damage to the crop production caused by drought [46].

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Beneficial soil microorganisms offer potential for alleviating detrimental effects of drought in agricultural practices. Among beneficial microorganisms' arbuscular mycorrhizal fungi and rhizospheric bacteria are potential candidates for mitigating these stresses. Arbuscular mycorrhizal fungi are an important beneficial soil-inhabiting fungi in natural ecosystems that form symbiotic associations with most land plants. They form fungal networks connecting plant roots and soil and can influence plant growth, nutrient uptake, combat against other root pathogens, and form plant-microbe-soil interactions [14,47]. Several studies have shown that arbuscular mycorrhizal fungi can alleviate drought and this is mainly due to the combination of nutritional, physiological, biochemical changes, and microbial associations with plants [48-52].

In our study, growth of *A. fistulosum* seedlings were significantly stunted and showed higher rate of mortality in water stressed conditions when grown without any microbial treatment. The efficiency of the arbuscular mycorrhizal fungi and rhizospheric bacteria is determined in terms of plant growth and biomass production under water stress condition. It was found that *A. fistulosum* seedlings inoculated with mycorrhizal fungi always outperformed non-mycorrhizal seedlings when subjected to water stress condition. Mycorrhizal fungal hyphae are much thinner than plant roots and can facilitate absorption of water-filled pores, which otherwise are inaccessible to roots. Both mycorrhizal fungi and rhizospheric bacteria boosted overall plant growth and nutrient uptake which is consistent with the results of other studies showing mycorrhizal fungi can alleviate the negative effects of drought and improve plant growth [53-58]. This could be attributed to the fact that improvement in drpught-affected *A. fistulosum* leaves had higher carbon assimilation rate, photosynthesis, and the antioxidant capacities. Mycorrhizal fungi produce hyphae that can extend far beyond the reach of plant roots and transport the elemental nutrients to intracellular arbuscles to the colonized roots. Rhizospheric bacteria on the other hand, produce various plant growth hormones such as indole acetic, gibberellic acid, and cytokines which can enhance overall healthier plant growth. Superior plant growth and nutrient uptake (nitrogen, phosphorus and potassium) by *A. fistulosum* seedlings were evident in this study when seedlings were inoculated with *P. flourescens,* and *R. irregularis,* alone and in combinations.

Mycorrhizal colonization was reduced but not completely eliminated when seedlings were watered once a week or every 10 days indicating that low duration of drought does not completely discourage colonization. Similar observations were made by other researchers who reported that long-term watering cessation reduced the root colonization by arbuscular mycorrhizal fungi [59-60]. This phenomenon could be attributed to the less carbon availability from host plants. During unfavorable conditions, both mycorrhizal fungi and rhizospheric bacteria can go in dormant state for a long period of time and become active again when the conditions are favorable for their growth and activities. However, the recovery process after an extended period of drought and the role played by mycorrhizal fungi and rhizospheric bacteria is not known.

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Glomalin (glomalin-related soil protein and easily extractable glomalin-related soil protein) is a special class of glycoprotein, released by spores and hyphae of arbuscular mycorrhizal fungi in the soil (61]. Glomalin play an important role in improving soil water and thermal conditions, not easily degradable in soil, improving soil aggregates, regulating plant growth, and improving accumulation of soil organic carbon [62]. In our study, dual inoculation with *P. flourescens* and *R. irregularis* significantly increased glomalin production in the rhizosphere of *A. fistulosum* compared to *R. irregularis* alone. It is interesting to note that *P. flourescens* alone did not produce any glomalin production; however, glomalin production was significantly increased in the presence of *R. irregularis*. The synergistic effect of *P. flourescens* and *R. irregularis* in glomalin production is unknown and needs further study. For non-inoculated control and seedlings inoculated with *P. flourescens*, no glomalin production was recorded since there were no spores or hyphae of *R. irregularis* in the rhizosphere, indicating that glomalin is produced only in the presence of arbuscular mycorrhizal fungi.

Similar results were obtained with our soil aggregate stability study. Soil aggregate stability is a way to determine the ability of a soil to maintain good water infiltration rates, good tilth and proper aeration for plant survival and growth [62]. In our study, dual inoculation with *P. flourescens* and *R. irregularis* significantly increased the formation of soil aggregates in the rhizosphere of *A. fistulosum* compared to *R. irregularis* alone. Least amount of soil aggregates were observed in the rhizosphere when seedlings were inoculated with *P. flourescens* and non-inoculated control seedlings. Glomalin plays an important role in the formation of soil aggregates that bind soil particles together and then gradually form macro-aggregate structures by arbuscular mycorrhizal fungal hyphae and improving soil aggregates [29,34,63-65]. Both non-inoculated control and inoculated with *P. flourescens* seedlings had very low soil aggregates since there was no glomalin or *R. irregularis* fungal hyphae to bind the soil.

Climate change has negatively affected soil nutrient, fertility status, decreased water content and changed physio-chemical properties of agricultural soils throughout the globe. Exploitation of mycorrhizal fungi and rhizospheric bacteria could be an eco-friendly approach to promote modern and sustainable agriculture. Further studies are needed under field conditions using different field crops to see if these microorganisms offer similar beneficial effects in water-stressed and drought soils since so many unknown variables are operating in the field at the same time.

5. CONCLUSION

Global warming and climate change have significant negative impacts on our agricultural lands; including reduced water contents, fertility, and changed physio-chemical properties of soil. Beneficial soil microorganisms such as arbuscular mycorrhizal fungi and rhizospheric bacteria offer potential for reducing these adverse effects. Arbuscular mycorrhizal fungi form symbiotic

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associations with host plants enhancing water and nutrient uptake from soil. Fungal hyphae can travel far beyond the reach of root systems and improve water-use efficiency. Arbuscular mycorrhizal fungi also produce glycoproteins (glomalins) which helps soil aggregation, water and nutrient loss, reduce soil erosion, increase soil porosity, soil enzyme activities, and overall plant growth. Beneficial rhizospheric bacteria enhance plant growth by producing growth hormones, fixing nitrogen from the atmosphere, and can solubilize phosphate in the rhizosphere of plants. Together, these microorganisms could be an environmentally friendly approach to promote modern and sustainable agriculture in the lands affected by global warming.

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AUTHORS CONTRIBUTION

The corresponding author (P.C.) carried out the experiment, collected the empirical data, and wrote the paper. C.Z. performed chemical analysis of the samples and analyzed data. Both the authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

DATA AVAILABILITY STATEMENT

Available from the corresponding author upon request.

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