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Impact of A Selected Mycorrhizal Complex and A Rhizobacterial Species on Tomato Plants' Growth under Water Stress Conditions

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ABSTRACT

Background: Plant strategies for adapting to drought could be improved by associations between plant roots and soil microorganisms, including arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR). In this study, the impact of a selected AMF complex and a selected PGPR species on the growth of tomato (*Lycopersicum esculentum* Mill.) under induced water stress was evaluated.

Methods: Three different inoculation treatments were applied to tomato seedlings (a complex of AMF composed mainly of *Glomus* genus a *Bacillus* sp. PGPR treatment and a combination of both) and three different water levels (75%, 50% and 25% of field capacity).

Result: A significant damaging impact of drought on tomato growth parameters and root mycorrhizal colonization, although the presence of microbes stimulated tomato plants growth and decreased the impact ofdrought stress. Indeed inoculated plants presented greater heights, fresh and dry weights, leaves number and area; greater water status; and greater proteins, sugars and chlorophylls contents either with the AMF complex or the *Bacillus* sp. in normal and drought stress conditions compared to the non-inoculated plants. However dual inoculation recorded the highest values under all water levels treatments.

Key words: AMF, Drought stress, Growth, Inoculation, PGPR, Tomato (*Lycopersicum esculentum* Mill.).

INTRODUCTION

Drought is one of the most important abiotic stresses that impact meaningfully crop growth and productivity (Fahad *et al.,* 2017; Zadražnik *et al.,* 2020); it causes substantial negative impacts on plant growth, physiology and reproduction through many constraints such as nutritional and hormonal imbalances, physiological disorders and high susceptibility to diseases (Nadeem *et al.,* 2014).

This situation is made worse by the harsh global climate change seen over recent decades (Lesk *et al.,* 2016), especially in the mid-continental and Mediterranean climate areas where drought is expected to be more recurrent and acute (IPCC, 2013), indeed, the percentage of the agricultural area of the planet affected by drought has more than doubled in the last 40 years (FAO, 2017).

Tomato plants (*Lycopersicum esculentum Mill.*) are considered among the most cultivated and consumed plants all over the world (Sainju *et al.,* 2003) and also one of the main affected by water scarcity, as they require high water demand (Calvo-Polanco *et al.,* 2016; Nangare *et al.,* 2016).

AMF and PGPR symbiosis are widely believed to enhance host plants growth and development under drought conditions (Alizadeh *et al.,* 2011; Prudent *et al.,* 2015) and non-drought conditions (Bowles *et al.,* 2016; Glick, 2014). These microbes represent a significant portion of soil rhizosphere microflora, they colonize plant roots and stimulate their physiology (Fouad *et al.,* 2014; Islam *et al.,* 2014) with a detectable effect on root surface area and therefore enables the plant to absorb more water and nutrients from large soil volume, they also induce cell turgor maintain by accumulation of different compounds such as soluble sugars and proteins and enhancing chlorophyll and water content (Borkowska, 2002; Heidari *et al.,* 2011), in addition they improve crop production quality (Baum *et al.,* 2015; Berta *et al.,* 2013; Gholami *et al.,* 2009).

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Most studies evaluated the effect of separate inoculation of AMF and PGPR on tomato seedlings under drought stress conditions, however, to our knowledge, no study has been made to evaluate the effect of a co-inoculation of an AMF complex and a *bacillus* sp. on tomato plants growth under drought conditions.

The aims of this study are to examine: the effects of mycorrhizal inoculation with a complex of AMF; the effects of PGPR inoculation with a *bacillus* sp.; and the effects of both the previous treatments combination (AMF+ a PGPR strain), on growth physiological parameters, total sugars, proteins and chlorophyll content, of tomato plants subjected to various water levels.

MATERIALS AND METHODS

Soil used in this experiment for trapping AMF was collected from a cypress forest, located in the N'Fis valley (Haut Atlas, Morocco), composed mainly from a complex of *Glomus (G.)* species that were previously identified morphologically by Ouahmane *et al.,* (2007). The soil physico-chemical characteristics (Table 1) were formerly determined by Ouahmane *et al.,* (2007).

Mycorrhizal inoculums production

Corn (*Zea mays* L.) and barley (*Hordeum vulgare*) were used as host plants for trapping mycorrhizal fungi. Seeds were surface sterilized in sodium hypochlorite 1%, then rinsed several times with sterile distilled water and germinated in 2L plastic pots containing the experimental soil. Mycorrhization intensity was estimated at different time intervals following the method developed by Phillips and Hayman (1970). After three months of culture, fresh roots colonized by AMF were harvested and cut into 1 centimeter small pieces and then used as inoculums for tomato seedlings (2 grams of roots per plant).

Bacterial strain

Bacterial strain tested is a *Bacillus Genus*, isolated from an arid rhizosphere surrounding wheat plants roots in Saada experimental field Marrakech and identified by Chrouqi *et al.,* (2017).

Bacterial strain was chosen based on its plant growth promotion traits (Table 2).

As described by Mayak *et al.,* (2004), a single bacterial colony was transferred to a liquid YT medium and incubated at 28°C for 24hours with continuous shaking (250rpm) to ensure proper aeration. Bacterial suspension was centrifuged at 4000xg for 10 minutes and then re-suspended in distilled water. This step was performed twice to assure its purification. Bacterial concentration was adjusted to 1.0 absorbance unit at 750nm; approximately 2.10^8UFC/ml, used for plants inoculation.

Experimental design and plant growth

Tomato seeds were surface sterilized with 10% sodium hypochlorite, then rinsed thoroughly with sterile distilled water and germinated in disinfected seedling trays containing sterilized peat. After 3 weeks, uniform seedlings (4-leaf stage) were selected and transplanted into 2L plastic pots, containing a mixture of sand and peat (3:1/v:v) previously sterilized for 3 hours at 121°C, on two consecutive days. The AMF inoculation was performed by supplying approximately 2 g (fresh weight) of barley and corn mycorrhized root fragments near the root system of each tomato plant; a second group of seedlings were treated with 30 ml of the bacterial suspension; a third group of seedlings were treated with a combination of both microbial inoculums and the remaining were considered as control.

Pots were placed in a greenhouse in the National Institute for Agricultural Research (INRA), Marrakech, during 2018, arranged in a randomized design block under natural lights:

Block 1: Plants without inoculation
Block 2: Plants inoculated with AMF
Block 3: Plants inoculated with PGPR
Block 4: Plants inoculated with AMF+PGPR

Day/night average temperature was 34/25 °C; relative humidity (RH) was 50/85 %. Plants received weekly 20 ml per plant of Hoagland's nutrient solution (Hoagland and Arnon, 1938) containing only 25% of Phosphorus, to prevent inhibition of AMF roots colonization.When plants reached the eight-leaf stage they were submitted to 16 treatments; four non-stressed treatments (AMF-inoculated, PGPR-inoculated, AMF+PGPR inoculated and non-inoculated),

Table 1: Physico-chemical characteristics of the experimental soil.

Experimental soil physico-chemical characteristics								
pH (H_2O)	Clay (%)	Fine silt (%)	Coarse silt (%)	Fine sand (%)	Coarse sand (%)	Carbon (%)	Total nitrogen (%)	Available phosphorus ($mg.kg^{-1}$)
7.3	4.6	30.8	13.3	30.1	20.9	2.33	0.11	16.1

Table 2: Bacterial strain traits related to plant growth promotion (Chrouqi *et al.,* 2017)

IAA production	PO_4 solubilization	NH_3 production	HCN production	Cellulase activity	Pectinase activity	N-fixation activity	Hydrolysis of starch	Catalase activity
+	+	+	+	+	-	+	+	+

which plants were watered regularly to 75% FC and four moderate drought stress treatments, in which plants were subjected to 50% FC and four severe drought stress treatments in which plants were exposed to 25% FC. Ten replicates per treatment were designed. Drought stress was applied for 30 days.

Growth parameters

After 30 days of drought stress application, plant growth was estimated by calculating shoot and root heights and biomass, leaf area and number of leaves per plant. Shoot and root fresh materials were separated, weighted and oven dried at 80°C for 48 hours to obtain dry weights. Leaf area was determined by image analyses using the Image J software (NIH).

Mycorrhizal analysis

Approximately 0.5 g of root samples of tomato plants were cleared in 10% Potassium hydroxide and stained in 0.05% trypan blue as described by Phillips and Hayman (1970). Root mycorrhizal colonization frequency (F%) was determined by visual observation of roots fragments under an optical microscope (40X magnification) using a few drops of glycerol (Trouvelot *et al.,* 1986). AMF infection percentage (F) was calculated using the following equation:

$$\%F = \frac{\text{Root length infected}}{\text{Root length observed}} \times 100$$

Root colonization intensity (M%) was determined observing 10 root fragments, each root fragment was represented by a class note (from 0 to 5), corresponding to the level estimation of mycorrhizal colonization: 0 (absence of root colonization); 1 (less than 1 %); 2 (1 to 10%); 3 (10 to 50%); 4 (50 to 90%) and 5 (more than 90%). It was calculated using the following formula [28]:

$$M\%(95*n5)+(70*n4)+(30*n3)+(5*n2)+n1)/N;$$

where N represents total number of observed fragments; n5, n4, n3, n2 and n1 are the number of noted fragments respectively, 5, 4, 3, 2 and 1. Mycorrhizal Efficiency Index (MEI) was estimated according to Bagyaraj (1992) formula:

$$MEI = \frac{\text{DW of inoculated plant - DW of non-inoculated plan}}{\text{DW of inoculated plant.}} \times 100$$

This parameter is essential to evaluate crop growth improvement due to mycorrhizal fungi (Bagyaraj, 1992).

Relative water content

Relative water content (RWC) was measured using the methoddeveloped by Barrs and Weatherly (1962).

$$RWC = \frac{FW\text{-}DW}{TW\text{-}DW} \times 100$$

where FW, DW and TW correspond toFresh weight,dry weight and turgid weight respectively. The turgid weight (TW)was determined after fully submerging the leaves in waterfor 24 hours at 4°C.

Total soluble sugars

The total soluble sugars concentration was assessed following Dubois *et al.,* (1958) method. The finely ground powder was extracted with 80% ethanol, centrifuged then treated with phenol and sulfuric acid. Absorbance was measured at 625 nm and soluble sugars concentration was derived from a standard curve using glucose.

Chlorophyll (Chl) content

Chlorophylls a and b were extracted and analyzed according to Geider and Osborne (1992). Fresh leaves were ground in 0.5 ml of 90% acetone in a mortar kept cold on ice. The homogenate was centrifuged for 5 minutes at 10,000×g. The supernatants were then placed in dark at 4°C for 2 hours to allow complete extraction of pigments. Absorbance was measured at 664 and 647 nm.

Chlorophylls a and b amounts ($\mu g.mg^{-1}$) were calculated using following equations:

- Chl(a) = 11.93 × DO664 - 1.93 × DO647
- Chl(b) = 20.36 × DO647 - 5.5 × DO664

And then sum (chl a + chl b) was estimated.

Total proteins

Total proteins were measured using the protein dye-binding method of Bradford (1976). Using bovine serum albumin (BSA) as a standard.

Statistical analysis

All results were analyzed statistically with SPSS 20.0. The statistical processing is performed on interaction of different microbial strains with different water levels imposed on soil, using variance analysis (ANOVA) followed by the post-hoc Tukey test. Signiûcant differences between factors were calculated at 5%. All values shown in the ûgures are means (n=4).

RESULTS AND DISCUSSION

The purpose of this paper was to evaluate the response of tomato plants inoculated with a complex of AMF and a *Bacillus* sp. (PGPR), alone or combined, to drought stress based essentially on their physiological parameters.

Drought stress application induces a significant decrease in physiological and biochemical parameters of tomato plants (English-Loeb *et al.,* 1997; Sanchez-Rodriguez *et al.,* 2009), which is in agreement with the results obtained in this study; there was a significant impact of drought on tomato growth parametersespecially under severe drought stress (25% FC). Generally plants use some specific mechanisms to face stresses, which are more noticeable when colonized by beneficial microbial populations of rhizosphere that alleviate drought intensity (Benabdellah *et al.,* 2011; Ortiz *et al.,* 2014). Same ascertainment has been obtained in the present study that showed for the first time resistance of tomato plants to water shortage inoculated with a complex of AMF and/or a *Bacillus* sp.

Mycorrhizal analysis

Microscopic root observations conûrmed absence of mycorrhizal structures in non-inoculated plants and those inoculated only with bacteria. Mycorrhizal colonization was successful in all the AMF inoculated treatments (M and BM). AMF infection frequency (F) in tomato roots plants is slightly affected by soil water deficiency, it remained high in all inoculated treatments (F>50%). The used PGPR strain did affect significantly AMF infection frequency especially among stressed inoculated plants (25, 50% FC) (Fig 1A). Drought stress signiticantly reduced AMF colonization intensity, however it was more pronounced among plants inoculated with the AMF complex only (M treatments) compared to plants with combined inoculation (BM treatments) (Fig 1B). Indeed highest root colonization was observed within BM-inoculated plants, regardless of water regimes. These data agrees with several studies in which mycorrhizal symbiosis decreased when applied drought stress to host plants (Islam *et al.,* 2014). Drought can reduce AMF colonization by inhibiting spores germination and reducing growth and spread of hyphae (Abbaspour *et al.,* 2012).

The MEI parameter increased significantly within inoculated tomato plants subjected to severe and moderate drought stresses compared to non-stressed ones(25% FC> 50% FC> 75% FC). that confirm that the AMF complex used, did improve tomato growth under drought stress conditions, although Baslam *et al.,* (2014), have found that drought stress had no significant effect on the MEI parameter in inoculated date palm plants. However the highest level of root colonization was recorded among plants inoculated with AMF+PGPR treatments and under all the used water levels, compared to the M treatments (Fig 1C).

Growth parameters

Severe drought stress (25%FC) decreased biochemical parameters evaluated in the present study (Chlorophyll content, total soluble sugars and proteins), regardless of inoculation treatment (Table 3), inoculated plants with BM treatment showed the highest values. Drought effects (25% and 50% FC) were alleviated by inoculations. Significant increase was noted within inoculated plants compared to the non-inoculated control plants (C) under drought stress conditions.

One of the most important effects of drought stress is the decrease of plant photosynthesis that affect soluble sugars status (Reddy *et al.,* 2004) which is in agreement with our results.(it measured from 0.33±0.04 mg/ml of total soluble sugars in the control under 25% FC to 0.87±0.06 mg/ml under normal conditions 75% FC and from 7.73±0.55 µg/ml of Total Chl a+Chl b in the control under 25% FC to 33.33±1.51 µg/ml under normal conditions). Improvement of soluble sugars and chlorophyll content found in leaves of inoculated plants may be a consequence of enhanced photosynthetic rates induced by the effect of the AMF and bacterial demand for sugars from leaves to roots (Baset Mia *et al.,* 2010; Feng *et al.,* 2002) Increase in sugar concentration may be due to hydrolysis of starch to sugars (Nemec, 1981). The enhanced chlorophyll content found in plants dually inoculated affects the translocation of soluble sugars to host roots, thus increasing fungal growth and activity in the root (Vivas *et al.,* 2003).

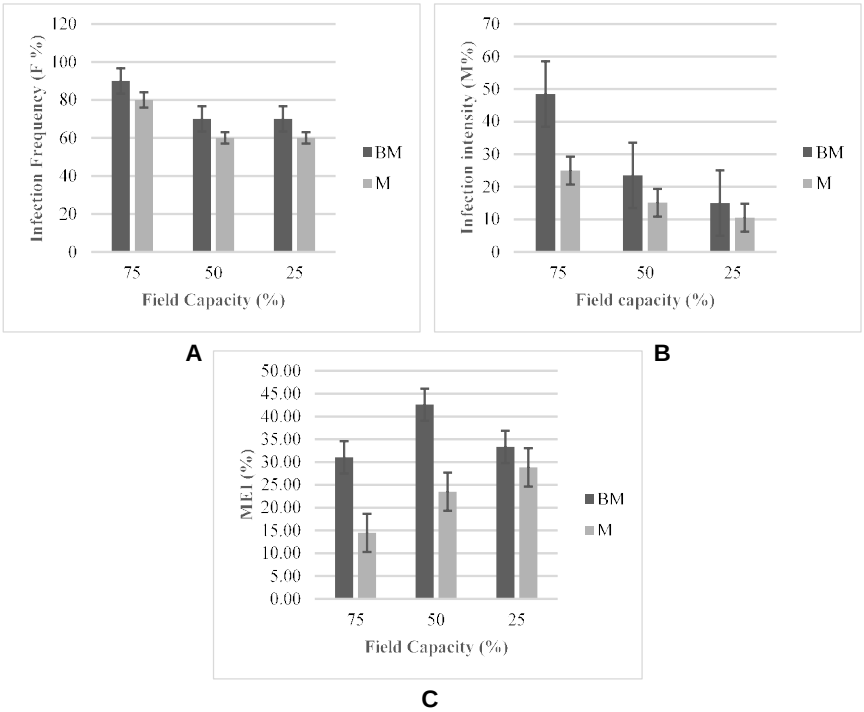

Fig 1: Effect of water levels, AMF complex inoculation (M) and AMF+PGPR inoculation (BM) on (**A**) root mycorrhizal colonization frequency (F %), (**B**) root mycorrhizal colonization intensity, (**C**) MEI of tomato plants.

A significant increase in proteins content was observed as a consequence of inoculation treatments, drought stressed AMF plants and PGPR plants showed higher content of proteins in leaves than drought stressed non inoculated plants, under 25%FC, the per cent increase in total proteins was 51% for B treatment; 55.9% for M treatment and 57.32% for BM treatment, while under 50% FC, the per cent increase was 49.33% for B treatment; 55.21% for M treatment and 57.02% for BM treatment (Table 3). Similar results have been pointed out by previous reports indicating that AMF could mitigate or decrease disassembly of RNA and could increase capacity of the non-enzymatic antioxidant defense system with soluble proteins (Manoharan *et al.,* 2010) in addition to PGPR ability in Nitrogen incorporation which increases proteins formation (Baset Mia *et al.,* 2010).

Overall physiological results are summarized in Table 4 and 5, plants subjected to drought stress showed significant decrease of growth and biomass accumulation, whether inoculated or notcompared to those exposed to normal water levels.

Inoculated plants present greater heights, fresh and dry weights, either inoculated with the AMF complex or the *Bacillus* sp. in normal and drought stress conditions compared to the non-inoculated plants. However dual inoculation recorded the highest values under all water levels treatments. Shoot and root fresh weight (SFW, SDW) and length (SH, RL) were significantly decreased due to drought, especially under 25% FC compared to normal conditions (75% FC). Reduction rate of SFW, RFW, SH, RL, was 44.53%; 36%; 27.16%; 37.5% respectively in non inoculated plants. When inoculated, the drought stress effect was alleviated, for example in SFW the per cent increase was at 53.48% in the B treatment; at 68.4% in the M treatment and the highest was recorded in the BM treatment at 97% (Table 4). Leaf Number and area (LN, LA) were significantly affected under drought stress (25, 50% FC) compared to normal conditions (75%) and significantlty improved by inoculations (Table 5).

These whole results agree with other studies that have proved positive effect of AMF and/or PGPR inoculation on physiological parameters of different plant species under drought or normal conditions (Bona *et al.,* 2017; Porcel and Ruiz-Lozano, 2004; Vivas *et al.,* 2003). Ameliorative effects due to AMF colonization can be explained by a number of mechanisms. It has been shown that mycorrhizal plants increase surface area of roots for nutrient acquisition (Artursson *et al.,* 2006) and absorb water more efficiently under water deficit environment compared to non-inoculated plants (Khalvati *et al.,* 2005) that might be due to modification in root plants architecture and formation of extramatricial hyphae which results in better root growth (Berta *et al.,* 2005) and facilitates absorption and translocation of more nutrients compared to non mycorrhizal plants (Guo *et al.,* 2010). According to Ahmad *et al.,* (2006),promotion of plants growth by PGPR is either by providing plants with some growth promoting substances like IAA that are synthesized by bacterium and improves significantly roots elongation (Patten and Glick, 2002) and can enhance drought tolerance (Bhattacharyya and Jha, 2012) or facilitating nutrient uptake from rhizosphere by solubilizing mineral phosphates and other nutrients. *Bacillus* sp. used in our study hold these two PGPR traits and many others (mentioned earlier in Table 2).

The major impact of drought on plant growth is non-availability of water. Relative water content (RWC) was strongly influenced by the microbial inoculations, it was increased significantly by all microbial inoculants applied, either bacterial or fungal going from 88.07% to 95.01% and 95.69 in the bacterial and mycorrhizal treatment respectively to 97.37% in the combined treatment (BM) at the severe water stress (25% FC). Although no significant differences were observed between inoculated plantlings and the non-inoculated ones in the absence of stress (75% FC)(Table 5), that involve various natural processes to help plants to sustain their development under drought. Plants inoculated with BM treatments have shown the better water status and would be less damaged

Table 3: Effects of water levels [Field Capacity (FC%)] on total soluble sugars, total proteins, total chlorophyll a + b content of non-inoculated control tomato plants (C), mycorrhizal (M), Bacterial (B) and inoculated with combination of both (BM).

FC %	Treatment	Total soluble sugars mg/ml	Total proteins (µg/ml)	Total Chl a + Chl b (µg/ml)
25	C	0.33±0.04 Aa	9.74±0.04 Aa	7.73±0.55 Aa
	B	0.92±0.03 Ba	19.86±0.81 Ba	27.53±0.74 Ba
	M	0.99±0.03 Bb	22.08±0.15 Ca	39.96±1.32 Ca
	BM	1.85±0.09 Ca	22.82±1.53 Ca	56.43±2.32 Da
50	C	0.68±0.06 Ab	11.77±0.14 Ab	10.06±0.50 Aa
	B	0.99±0.10 Ba	23.23±0.30 Bb	53.92±1.10 Bb
	M	0.97±0.12 Bb	26.28±0.27 Ca	42.19±1.98 Ca
	BM	2.39±0.04 Cb	27.32±0.17 Db	61.72±1.17 Db
75	C	0.87±0.06 Ac	12.34±0.22 Ac	33.33±1.51 Ab
	B	1.32±0.17 Bb	26.04±0.39 Bc	56.25±0.90 Bb
	M	1.29±0.03 Bc	26.54±1.32 Ba	54.69±0.87 Ba
	BM	4.03±0.59 Cd	30.92±0.40 Bb	70.80±1.29 Cc

Different capital letters indicate significant differences ($p<0.05$) between treatments within the same FC %.

Different small letters indicate significant differences ($p<0.05$) between FC % for the same treatment.

Table 4: Effects of water levels [Field Capacity (FC%)] onShoot and root fresh weights (SFW, RFW), shoot heights (SH) and root lengths(RL) and dry weights (DW) of non-inoculated control tomato plants (C), mycorrhizal (M), bacterial (B) and inoculated with combination of both (BM).

FC %	Treatment	SFW (g)	RFW (g)	SH (cm)	RL (cm)
25	C	10.79±1.31Aa	3.74±0.37 Aa	33.33±1.08 Aa	11.67±1.08Aa
	B	16.56±0.52 Ba	7.72±0.27 Ba	48.83±1.61 Ba	20.67±0.08Ba
	M	18.17±1.95Ba	9.31±1.38 Ca	54.00±1.00 Ca	19.00±1.00Ca
	BM	21.26 ±1.08 Ca	11.45±1.19 Ca	55.23 ±1.12 Ca	23.70±0.98Ba
50	C	13.80 ±1.38 Aa	4.91±0.57 Ab	41.00±1.00Ab	16.00±1.00Ab
	B	17.47±1.23Aa	8.07±0.51 Ba	51.83±1.25 Ba	27.07±1.03Aa
	M	19.51 ±1.30 Ba	9.74±0.85 Bb	54.33±1.52BCa	22.33±0.58Aa
	BM	24.87±1.15Cab	13.61±0.72 Db	58.83±1.57Ca	24.17±1.75Bb
75	C	19.45 ±0.89 Ab	5.91±0.32 Ab	45.67±0.23Ab	18.67±1.15Ab
	B	24.40 ±1.14 Bb	10.10±0.99 Ba	62.83±0.80Bb	29.33±0.58Ba
	M	24.45 ±0.52 Bb	13.17±0.72 Ca	61.00±1.65Bb	24.00±1.61Ba
	BM	27.79 ±1.22 Cb	16.07±1.51 Cc	63.67±1.56Bb	27.33±1.89Ac

Different vapital letters indicate significant differences ($p<0.05$) between treatments within the same FC %.
Different small letters indicate significant differences ($p<0.05$) between FC % for the same treatment.

Table 5: Effects of water levels [Field Capacity (FC%)] on relative water content (RWC), leaves number (LN) and leaf area (LA) of non-inoculated control tomato plants (C), mycorrhizal (M), bacterial (B) and inoculated with combination of both (BM).

FC %	Treatment	RWC (%)	LN	LA (cm^2)	FW (g)	DW (g)
25	C	88.07±0.48 Aa	31.00±1.65 Aa	4.84±0.57 Aa	14.53±0.60 Aa	2.81±0.22 Aa
	B	95.01±0.95 Bb	76.00±1.00 Ca	5.52±2.61 Ba	25.29±0.63 Bb	3.95±0.58 Bb
	M	95.69±1.02 Bb	54.00±1.00 Ba	7.33±2.67 Ca	31.48±1.72 Bc	4.22±1.03 Bb
	BM	97.37±0.88 Cc	83.33±1.05 Da	13.58±1.25Da	36.70±1.67 Bc	4.57±0.25 Bb
50	C	90.21±1.09 Aa	51.33±1.09 Ab	5.61±2.25 Ab	18.71±0.91 Aa	3.33±0.14 Aa
	B	95.48±0.59 Bb	87.33±1.53 Cb	7.03±0.54 Bb	26.54±1.63 Bb	5.37±0.06 Bc
	M	96.65±0.67 Cb	68.00±1.00 Bb	8.60±1.61 Bb	32.68±1.76 Bc	5.30±0.85 Bc
	BM	97.13±0.56 Cc	83.67±3.06 Cb	15.35±0.85 Cb	40.48±1.49 Cd	5.96±0.24 Bc
75	C	96.25±1.75 Cb	77.00±3.08 Cc	6.25±1.48 Ac	25.36±1.65 Bb	5.63±0.73 Bb
	B	97.30±0.34 Cc	91.00±2.29 Dc	7.11±2.72 Ac	33.50±1.61 Bc	7.20±0.43 Dc
	M	97.31±0.90 Cc	89.67±2.08 Dc	9.18±3.61 Bb	34.19±1.37 Bc	7.92±0.23 Dd
	BM	98.70±0.23 Cc	99.67±0.51 Dc	17.53±2.52 Cb	42.86±2.72 Cd	8.22±0.31 Dd

Different capital letters indicate significant differences ($p<0.05$) between treatments within the same FC %.
Different small letters indicate significant differences ($p<0.05$) between FC % for the same treatment.

by the water stress imposed. The upkeep of water relations in the plant under drought conditions is likewise enormously reliant on the osmotic adjustment in the plant cell, which consists of the accumulation of ions and osmotic molecules that bring down the osmotic potential in the cell, making water move into the cell and increase cell turgor (Farooq *et al.,* 2009).

Dually inoculated plants were better protected against drought stress imposed and this is due to the synergic interactions between microbes, that not only promote plant growth but also enhance the population of each other (Yusran *et al.,* 2009). Indeed, ability of *Bacillus* sp. to increase AMF colonization suggests a direct bacterial effect on the metabolic status of AMF. Bacteria can produce compounds (such as indole acetic acid) to increase cell permeability that could directly enhance root exudation rate stimulating hyphal growth and facilitating root penetration by fungus (Jaderlund *et al.,* 2008; Jeffries *et al.,* 2003). Dual application of fungus and bacteria improved root colonization of lettuce by AM fungus while it was reduced under drought stress (Vivas *et al.,* 2003). Bacterium appears to act as a mycorrhiza-helper microorganism (Fitter and Garbaye, 1994; Jaderlund *et al.,* 2008).

CONCLUSION

Negative impact induced by water deficit on tomato plants growth is very serious. However, it can be attenuated and/ or minimized by microorganisms' symbiosis including bacteria and mycorrhizal fungi found naturally in almost all types of soil, whether applied alone or in combination. Tomato plants inoculation with any of the three treatments (B, M and BM) alleviated significantly the deleterious effects of drought stress; the positive effect was especially evident in BM treatment.

Mycorrhizal and bacterial symbiosis always benefited growth of plants, independently of water level; they could be very effective for enhancing plant growth and development under normal as well as stress conditions.

This offers an alternative ecological strategy, reducing use of chemicals. Such microbial soil populations need a systematic strategy for their potential to be used effectively. More investigations into the mechanisms by which PGPR alone or associated to AMF elicit tolerance to drought stress would improve our knowledge on the use of these rhizobacteria in agriculture to provide induced systemic tolerance to water stress.

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