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Soil microorganisms alleviates the negative effect of salinity on morphophysiological characteristics during growth stages of durum wheat genotypes

K. BOUDABOUS[1*], N. BEN AISSA[1], Y. TRIFA[1], A. SAHLI[1], H. SLIM AMARA[1]

[1] National Institute of Agriculture of Tunisia, 43, Avenue Charles Nicole, 1082 Tunis, Tunisia.

*Corresponding author: Khaoula_boudabbous@yahoo.fr

Abstract - The rhizosphere is a biologically active zone in the soil around the roots of plants. Root microorganism interaction in the rhizosphere can be beneficial to the plant mainly in stress conditions. In order to investigate their interactions, contrasted tests were carried out. Four durum wheat varieties, too are landraces (Agili glabre and Bayadha) and too are improved (Razzek and Maali) were planted in greenhouse under sterilized and non-sterilized soil to evaluate their physiological comparative performance under water saline and non-saline irrigation conditions. Morpho-physiological responses to salinity (0 and 12dSm^{-1}) were evaluated for contrasting genotypes: the chlorophyll fluorescence (Fv/Fm); relative water content (RWC) and leaf area (LA), during vegetative and reproductive stages. Results showed that salinity affected negatively all parameters mainly in sterilized conditions. The leaf area index was much less affected by salinity. However, (Fv/Fm) and WRC were significantly affected by salinity ($P<0.01$) and microbial activity mainly at vegetative stage. Agili glabre and Maali found to be the most tolerant even in sterilized conditions. Correlations studies showed significant positive correlations ($r=0.54$, $P<0.01$; $r=74$, $P<0.01$) between MBC and both the Fv/Fm and WRC respectively. These results suggested that microorganisms might be usefull for salinity tolerant genotypes primarily at vegetative stages.

Keywords: Salinity, durum wheat, microbial biomass, growth stages, physiological parameters.

1. Introduction

Soil salinity is one of the major abiotic stresses affecting productivity especially in areas of low rainfull. This problem is very severe in arid and semi-arid regions due to unusual precipitation and high temperature (Latiri et al., 2010). Poor quality of irrigation are the main causes of production decreasing mainly in arid and semi-arid conditions. Tunisia is one of the countries that suffer from severe salinity problems. About 80% of land areas are affected by salinity (AM, 2015). Improving the salt tolerance of crop reveals the prime way to overcome the limitation of crops production in a salinized area. In Tunisia, durum wheat (*Triticum durum* Desf.) is one of the three major cereals that play a key role in food system. It holds the most important place and occupies about 50% of all cereals areas. Salinity leads to different morphological and physiological changes that adversely affect durum wheat growth and productivity (Gerosa, 2014).Water stress, photosynthesis and leaf areas have been used as one of the sensitive indicator of salinity. Previous studies showed that salinity decreases the osmotic potential and consequently causes the water stress, hence declines the leaf water potential (Munns et al. 2005). In fact, leaves is considered the main organ of photosyntéhtis, thus, the leaf size seems to reduce the chlorophyll fluorescence. Actually, this parameter is used in studies of stress physiology of selected cereal crops (Sayed, 2003). It can provide insights into the ability of plants to tolerate stress (Yu-fang and Shi-qing, 2011). Some authors reported that chlorophyll fluorescence is a more reliable technique to sense environmental stress including salt stress for durum wheat (Yousfi et al. 2010; Karmous et al. 2013) because salinity has a direct effect on photosynthetic electro transport (Mehta et al. 2010). In durum wheat there is a large variation for salt tolerance at critical stages (Din et al. 2008). In general, cereals are more sensitive to salinity at vegetative stages than reproductive one (Elhandawy et al. 2005).

Since in a soil plant system, the soil's energy powerhouse is the rhizosphere, any stress will have a strong impact on the plant productivity. The biological processes occurring in the rhizosphere are

mainly attributed to the activity of soil microorganisms (Hinsinger et al. 2011). The rhizosphere is the narrow region of soil that is affected by root secretion and associated soil microorganisms (Tang. 2013). It contains many microorganisms affected by root activities. Screening for salt tolerance of different cultivars and its validation in salt affected fields are still required to more understand the mechanism of tolerance and to improve salt tolerance in durum wheat. For this purpose, physiological measurements on four durum wheat cultivars were studied. However, one or two of the above factors were conducted in previous studies (Naveed et al. 2014), and to our knowledge, effect of microorganisms growth stages and genotypes under saline conditions on physiological response of durum wheat cultivars has been less reported as so far.

2. Materials and methods

2.1. Plant material

Four durum wheat genotypes were selected, two are improved (Razzek and Maali) and two are landraces (Bayadha and Agili Glabre).Razzek and Bayadha are considered sensitives, Whereas, Maali and Agili Glabre are tolerants.

2.2. Experimental design

Eighty pots experiments were conducted during the season 2013 in the semi controlled greenhouse of the National Institute of Agronomy of Tunisia. The experiments were performed in a completely randomized design with four factors: four durum wheat genotypes, two landraces (Bayadha and Agili Glabtre) and two improvement (Razzek and Maali), two levels of salinity [0.2 dS m−1 (non-saline) and 14 dS m−1 (saline)], sterilized and non-sterilized soil. For each pot (containing 7 kg of the natural soil). In order to assess the impact of soil microbial activities on plant response, ten sterilized durum wheat seeds were respectively sown into the sterilized soils and into the non-sterilized soils. Ten grains thinned to five after germination were planted on Decembre 23 in 2013.

2.3. Plant growth condition

The soil used was a loam clay (sand 14,82%, silt ,33,77:%, clay 49,10%) (Table 1), collected from the National Research Center, Experimental Station, Barrouta sieved (pore size, 2 mm), and divided into two portions, one portion was non sterilized and the second portion was sterilized by steaming the mixture at 121°C for 20mn on three consecutive days. Before planting, soil chemical analysis was determined according to pauwels et al. (1992) and presented in Table 1. Fertilization was carried out by adding ammonitre (33, 5%N), at the rate of 0,7g pot−1, respectively, at stages three leafs (Z13), six leafs (Z19) and at two nodes (Z32),. All pots were irrigated to soil saturation before planting. After planting, irrigation was applied at the appropriate times with tap water to maintain soil moisture near maximum water-holding capacity. The concentration of NaCl in the soil was increased gradually by 50 mM per day two times per week to avoid an osmotic shock. Plants were maintained under these conditions for six months. To standardize the experiment conditions, the position of the pots was interchanged every two days.

2.4. Physico-chemical analysis

Physio-chemical properties were determined. Soil available N was extracted with 1M KCL for 1 h and determined by Kjeldahl method (Warning and Bremner, 1964), Available Phosphorus was extracted with Olsen reagent (1 M NaHCO3) (pH 8.5) at soil extractant ratio of 1: 20 shaken for 30 mn and quantified by blue- colorometry (Olsen et al., 1954). Available K was extracted with neutral normal ammonium acetate (Ph7), shaken for 1h and measured by flame photometry, Soil organic matter (SOM) was determined by dichromate oxidation.

2.5. Soil biological analysis

During growth stages of each treatment, soil was sampled on the depth 0-20 cm. For each sterilized and non sterilized soil microbial biomass carbon determined using chloroform fumigation-extraction method (Brooks et al, 1985).The fumigation step is supposed to kill the microbial cells that thereafter release their content in the soils. Soil was fumigated with chloroform vapour for 24h. Then the soil (fumigated and parallel non-fumigated soil) was extracted with 0.5M K_2SO_4 to measure the MBC.

2.6. Plant analysis

2.6.1. The relative water content (RWC)

In determining RWC, flag leaf samples were washed in distilled water and weighted after drying by filter paper to obtain their fresh weights (f wt.), the turgid weights of leaf samples were taken (turgid wt) by incubating leaf sample in 100 ml of distilled water under darken conditions for 6h, All the leaf samples were then dried at 80 C° for 24 h, with their dry weights (d wt) being determined. Leaf RWC in different stages were calculated by the following equation.

RWC = (Fresh wt-dry wt) / (turgid wt- dry wt)

2.6.2. Leaf area (LA)

Leaf area was measured by CI-203 portable laser leaf area analyzer (CID Company, USA), which was calibrated by conventional manual method.

2.6.3. Chlorophyll fluorescence (Fv/Fm)

Chlorophyll fluorescence of durum wheat functional leaves was measured using portable Fluorescence Induction Monitor (FIM 1500, Analytical Development Company Limited, ADC) at Z13, Z21, Z30, Z65 and Z87. Leaves were dark-adapted for 30 min and then illuminated with a modulated measuring beam to obtain. The maximal photochemical efficiency of PSII (Fv/Fm); Fv: the variable fluorescence and Fm: the maximal fluorescence.

2.7. Statistical analysis

The experiment consisted of a randomized block of two treatments with four different durum wheat genotype per replicate. Five replications for each treatment were performed. Statistical analysis of data was processed using analysis of variance (ANOVA) in the General Linear Model procedure of SPSS (Ver. 16). ANOVA. Pearson's correlation test was also carried out to study the relationships among all parameters and duration of growth stage. Differences between treatments and cultivars were considered significant if $P < 0.05$

3. Results and discussion

3.1. Basic physical chemical property of the tested soil

Physicochemical characteristics of collected soil are presented in Table 2. Soil textured analysis indicated that it was loamy-clayy, It characterized by a medium rate of organic carbon and slightly higher level of available phosphorus.

Table.1. Physical and chemical characteristics of collected soil

EC(dSm^{-1})	pH	CaCO3 (mg g^{-1})	C org (mg g^{-1})	AP (mg kg-1)	Total N (mg kg^{-1})	C:N	Sand (%)	Silt (%)	Clay (%)	Bulk density (g cm^{-3})
0.8	7.9	3.18	15	533	1.2	12.5	13.25	65	21.75	1.5

Data are presented as mean ± standard deviation. The means were obtained from three replicates (n =3).AP: available phosphorus, EC: Electrical conductivity, $CaCO_3$: Total calcareous, C: Carbone, N: Nitrogen, C/N ratio carbon on Nitrogen.

3.2. Rhizosphere microbial biomass carbon (MBC)

Analysis of variance showed significant variations among genotypes (G), soil (S), salinity (Sl) growth stages (GS) and S × G for MBC. Moreover, (G × S), (G × GS), (S × G) interaction showed highly significant ($P < 0.01$) effect on MBC. A significant triple interaction (GS × G × S) was also observed (Table 2).

Table.2. Effect of salinity, autoclaved soil, genotypes and growth stages on leaf area (LAI), relative water content (RWC), chlorophyll fluorescence (Fv/Fm) and microbial biomass carbon (MBC). Genotype means are calculated from 60

measurements (two soils, three stages, two water irrigation salinity and five replicates). Soils and irrigation water salinity values are the means of 120 measurements. Growth Stages are the means of 80 measurements. Analysis of variance for the same variables is shown for the irrigation water salinity (S), genotype (G), Soils , durum wheat growth stage(GS) and the respective interaction effects.

	MBC	RWC	LA	Fv/Fm
Soil (S)				
Soil 1(sterilized)	-	62.31b	7.93b	0.719b
Soil 2 (non-sterilized)	291.40	82.22a	10.57a	0.782a
Irrigation water salinity				
Sl1(0dSm-1)	333.17a	78.18a	9.69a	0.768a
Sl2(12dSm-1)	249.63	66.35b	8.80b	0.733b
Growth Stages(GS)				
6 –leaves(S1)	271.46b	79.20a	6.91c	0.71b
Tillering(S2)	307a	71.80b	9.07b	0.769a
Flowering(S3)	314.03a	65.69c	11.76a	0.766a
Maturity(S4)	106.92c	-	-	-
Landraces genotypes				
Bayadha	241.63b	65.94b	9.13a	0.721c
Agili Glabre	333.011a	79.18a	10.73b	0.782a
Improved genotypes				
Razzek	224.92c	68.46b	8.14b	0.742c
Maali	313.01a	75.47a	8.99a	0.757b
ANOVA				
Soil(S)	-	**	**	**
Salinity(Sl)	**	**	**	**
Stage(St)	**	**	**	**
Genotypes(G)	**	**	**	**
SxSl	-	**	**	**
SxSt	**	**	**	**
SxG	**	**	**	**
SlxSt	-	**	**	**
GxSt	**	**	**	**
GxStxS	**	**	**	**
GxStxSxSl	-	**	**	**

The means followed by different letters were significantly different (P<0.05) by Duncan's test. (significant at **P<0.01)

Microbial activity seems to play a crucial role on in their maintenance during crop growth (Qui et al. 2008) (Table.2). Compared saline water irrigation, salinity reduced the MBC by 25%. These results could be attributed to the negative effects of salts on microbial communities (Reitz and Haynes, 2003) and by the decreased of organic matter inputs (crop residue, litter and fine roots) in the soil caused by the toxics effects of salts on vegetation (Singh et al., 2015). Soil MBC activity ascended gradually with durum wheat growth at early stage and reached the highest value at tillering and flowering (S2) by 307 and 314 mg kg^{-1} respectively, then decreased slowly until sudden drop at maturity by 106.92 mg kg^{-1} in non-sterilized soil (Figure 1.) . This may be attributed to increase in the root exudates in the tillering and flowering stages (Islam and Borthakur. 2016) and may also be attributed to sufficient moisture availability due to rainy period. Overall, the average highest MBC was obtained for Maali and Agili Glabre by 191mg kg^{-1} and 182 mg kg^{-1} respectively (Figure 1.). According to Zuo et al. (2014) a difference wheat genotypes was found during crop growth caused by chemicals compound

that excreted by each genotypes. Similarly, (Corneo et al. 2016) suggested that wheat genotypes could affect MBC resulted by specific root length and by root diameter. In addition, the highest MBC in some genotypes relative to others showed that plant genotypes could affect their own performance and consequently the rhizosphere microbial activities (Shweizter et al. 2014).

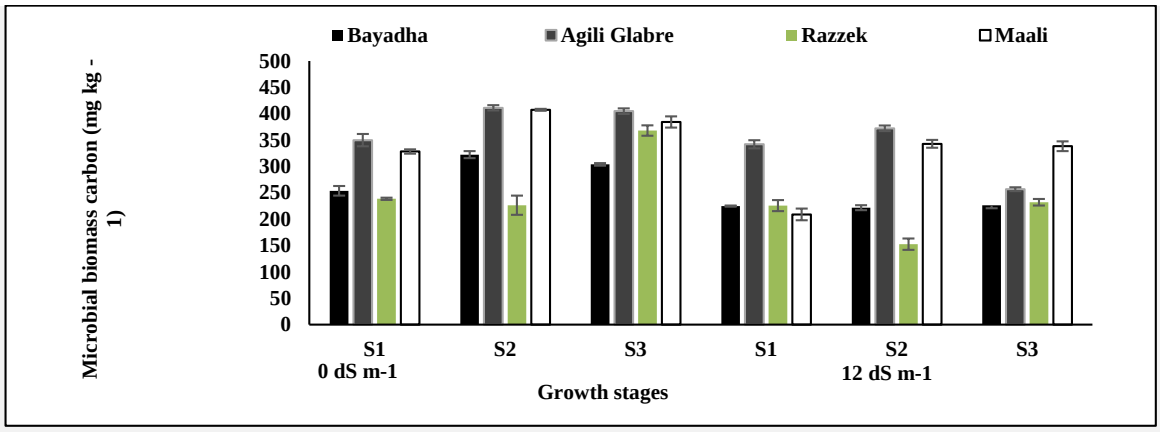

Figure 1. Changes of microbial biomass carbon (MBC) during growth stages(S1. 6 leaves S2; Tillering and S3; Flowering) of four durum wheat genotypes under non saline (0dSm^{-1}) and saline (12 dSm^{-1}) water irrigation

3.3. The effect of salinity, sterilized soil, growth stage and genotypes on chlorophyll fluorescence (Fv/Fm)

Results showed that sterilized soil affected negatively the fluorescence chlorophyll by 10 % (Table 2), Such results suggesting the presence of beneficial microorganisms that could affected positively the photosystem II. According to data showed in (Figure 2A, 2B). A significant difference between growth stages was obtained and we accorded the highest Fv/Fm in S2 and S3. Salinity affected negatively the Fv/Fm (Figure 2A, 2B), suggesting that photo-inhibition may have occurred, however these results is in contradiction with Plazek et al. (2013) who showed the highest disturbance of PSII in salines conditions and suggesting that plant tolerance to salinity may be demonstrated by the a higher dissipation of energy. Analysis of chlorophyll fluorescence induction showed that inhibition of the activity of reaction center in photosystem Π is the main effects of salt stress in sensitives durum wheat cultivars (Kahrizi et al. 2014). Moreover, analysis of chlorophyll fluorescence clearly divided the plant response to salt stress into two genotypes groups: Maali and Agili Glabre which showed the highest values of Fv/Fm during the three growth stages by 8% than Bayadha. In non-sterilized soil, Agili Galbre showed the highest Fv/Fm evenwith saline water irrigation by more than 0.82.

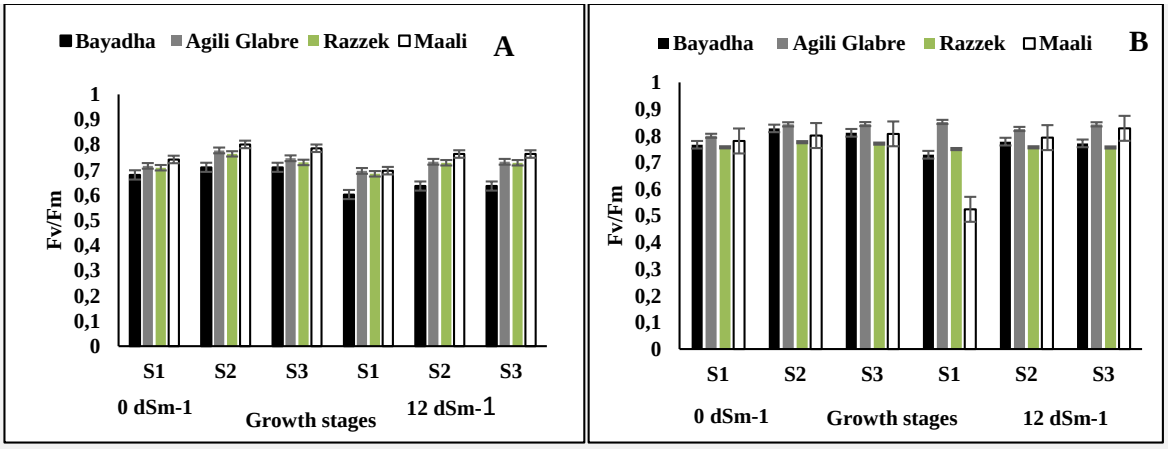

Figure 2. Changes of fluorescence chlorophyll (Fv/Fm) during growth stages (S1. 6 leaves, S2; Tillering and S3; Flowering) of four durum wheat genotypes in sterilized (A) and non steril soils(B) under non-saline (0dSm^{-1}) and saline (12dSm^{-1}) water irrigation

3.4. The effect of salinity, sterilized soil, growth stage and genotypes on relative water content (WRC)

Relative water content (WRC) in leaves showed a significant effect of different treatments: Salinity, sterilized soil, growth stages, genotypes and their interaction ($P<0.01$) (Table 2). Compared in non-saline water irrigation, WRC declined under increasing salinity in all genotypes by 15 % (Table 2; Figure 2A, 2B). These finding is in contradiction with those obtained by (Fercha. 2011). However, In non-sterilized soil these decline is less pronounced with 25 % than sterilized soil (Table 2). This may be due to a reduction of inhibitory effect of salinity on root and the developpement of a more effective root system in the presence of microorganisms (Naveed et al. 2014). During crop growth of all genotypes, WRC showed an increase at S1 and declined slightly to S2 with 13% and more pronounced at S3 by 22% (Table 2) (Goghdi et al. 2012) Taking into account the reduction of WRC of all genotypes. 'Bayadha' demonstrated the highest sensitivity to salinity among the studied genotypes that could be caused by negative effect of osmolyte concentrations such as Na^+ (Paustini et Siosmandeh. 2004). However, it is necessary to underline that, even at highest salt concentration, Agili Glabre genotype showed a higher WRC (85%) than others durum wheat genotypes followed by Maali (80%) (Table 2), such result could be explained by a decrease in membrane permeability increased tissue hydration (Plazek et al. 2013). The behavior of genotypes differed under non-sterilized soil, Agili Glabre seemed to be the most dependent genotype on microbial activities and accorded the highest WRC even in saline conditions (Figure 2B), such results could be explained by the most abundant microoganisms under its rhizodphere and consequently better activities which could affect positively the root system (Dodd et al. 2010).

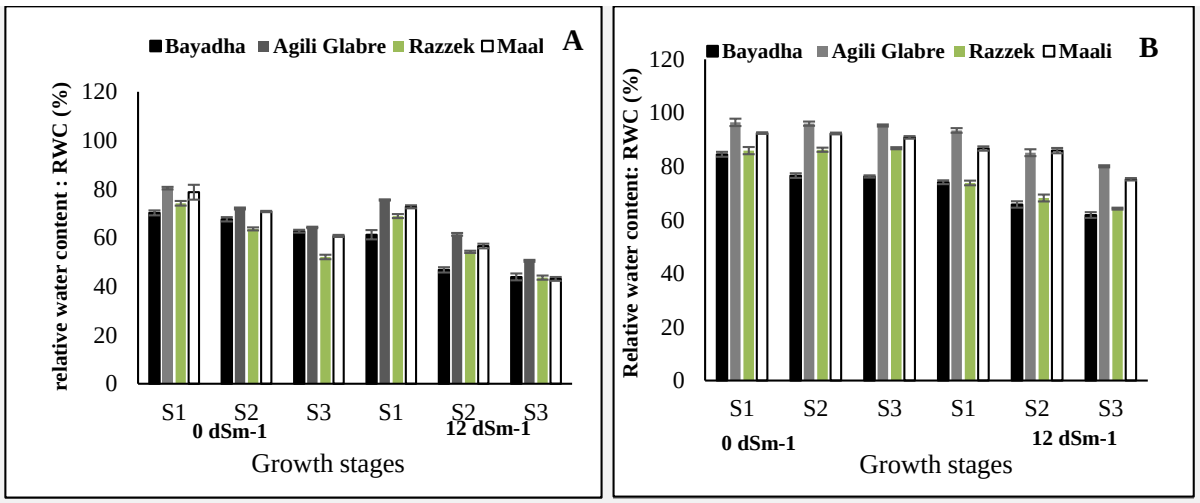

Figure 3. Changes of relative water content (RWC.: %) during growth stages(S1. 6 leaves, S2; Tillering, S3; Flowering) of four durum wheat genotypes in sterilized (A) and no- sterilized soils(B) under non-saline ($0dSm^{-1}$) and saline ($12dSm^{-1}$) water irrigation

3.5. Changes of leaf area (LA) by salinity, sterilized soil, growth stage and genotypes

Analysis of variance showed a significant effect of all treatment and their interaction on LA (Table 2). According to data showed in table 2, we observed 25% of LA in non-sterilized soil relative to sterilized conditions. Such results could be explained by the part of microorganisms on adaptation of genotypes to saline conditions. Moreover, salinity affected the LA negatively by 10%. These reduction can be caused by cell division and in some cases by the senescence and abscission of old leaves. Consequently the reducing of photosynthetic area observation is in accordance with previous reports that plant in salt stress reduces the cell divisions (Kharizi et al. 2013). A similar trend of LA at different growth stages was observed in genotypes (Maali and Agili Glabre) in S2. These genotypes showed greater leaf area by 24% compared with Razzek genotype (Table 2; Fig 3A, 3B). There was significant differences between genotypes, Bayadha and Agili Glabre accorded the highest LA during all growth stages as well as the salinity and the two soils (Soil 1 and Soil2) (Figure 3A, 3B).

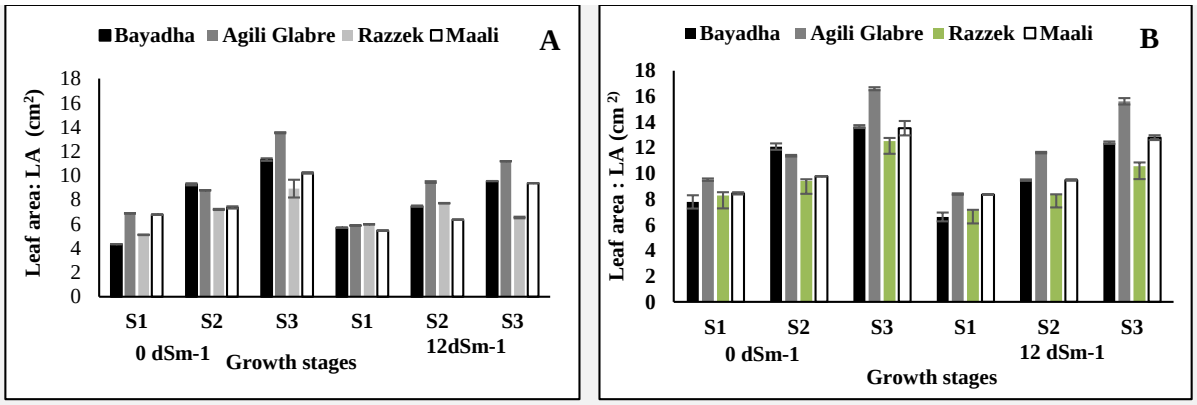

Figure 4. Changes of leaf area (LA.:cm^2) during growth stages(S1. 6 leaves, S2; Tillering and S3; Flowering) of four durum wheat genotypes in sterilized (A) and non-sterilized soils(B) under non saline (0dSm^{-1}) and saline (12dSm^{-1}) water irrigation

3.6. Correlation between MBC and physiological parameters

Correlation analyses among physiological parameters of soil and microbial biomass activities indicated several positive significant trends. The highest significantly correlation was found between WRC and MBC ($p<0.001$) (Table 3). Our results was agree with previous maize researches, that microbial activity such as bacterial increase the ability of plant to enhance water under saline stress (Saghafi et al. 2013). In addition, Mycorrhiza could interfere in osmotic adjustment by changes in water content and uptake the water with hyphae (Choochani et al. 2015). On other hand, data showed a significantly positive correlation between leaf area and relative water content. Previous studies have shown that water uptake by wheat genotypes could affect the expansion of leaves, thus in saline conditions, leaves lead to have smaller final size (Farouk. 2011). In addition the photosynthetic activity was showed highly related to microorganisms activities. These observance is in accordance with previous reports (Colla et al. 2015) that mycorrhiza were capable of maintaining a higher maximum quantum use efficiency of PSII (Fv/Fm) during tillering and anthesis stages of winter wheat due to associating with a higher net assimilation rate of CO2 and plant growth and also for somes researches microorganisms could improve accumulation of organic acid in leaves and consequently more photosynthetic activity.

Table.3. Correlation between MBC and physiological parameters of plant

Variables	MBC	WRC	LA	Fv/Fm
MBC	1			
WRC	0.705**	1		
LA	0.574**	0.52**	1	
Fv/Fm	0.51*	Ns	0.52**	1

MBC; Microbial biomass carbon, WRC; relative water content, LAI leaf area index; Fv/Fm; chlorophyll fluorescence. *, **correlations are significant at the 0.05, and 0.01 level, respectively

4. Conclusion

Our data showed that plant genotypes influenced significantly the microorganisms biomass. Plant growth increased significantly the microorganism biomass in non-sterilized soil. A choice of parameters, which clearly could be used for selection of salt tolerant plants is problematic. The ability to increase microbial activities in saline soil is a fundamental feature deciding salt tolerance. This suggested that the effect of wheat genotypes on MBC through their roots traits can have positive effect on morpo-physiological parameters. Taking into account the changes of Fv/Fm, LA, RWC and MBC under salinity, durum wheat genotypes 'Maali and Agili Glabre could be recognized as more salt tolerant and should be included in durum wheat breeding programs for developing salinity tolerant varieties. While 'Bayadha' is the most salt sensitive. This is why the interaction between genotypes and microorganims in rhizosphere should be used for plant selection.

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