



The Effect of *Streptomyces* sp. SF5 and Aqueous Extract of *Salsola vermiculata* on the Growth of *Triticum durum* L. under Salinity

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Abstract: The present study analyzed the effect of NaCl, *Salsola vermiculata* (halophyte) and *Streptomyces* sp. SF5 on the growth of MBB variety of durum wheat *Triticum durum* L. The experiment consisted of twelve treatment combinations carried out in three replications. NaCl solutions (0, 100 and 200 mM) were added to sterile soil with or without aqueous extract of *S. vermiculata* and *Streptomyces* sp. alone or in combination. The results showed that this variety was moderately halotolerant. Germination rates at 0, 100 and 200 mM NaCl were 100%, 80% and 53.2% respectively. Combination of *S. vermiculata* and *Streptomyces* sp. affected germination rates significantly at different levels of NaCl (46.66%, 40% and 26.6%). Sole application of the halophyte or *Streptomyces* sp. restored germination to 86.6%, and 93.2% (in 200 mM NaCl) and improved growth of the wheat. Furthermore, the length and the dry weight of roots and stems, chlorophyll a, b and carotenoids contents were also improved significantly. In the presence of 100 and 200 mM NaCl, proline reached levels of 45.3 and 20.31 $\mu\text{g g}^{-1}$ of fresh material respectively. They were however, reduced in the presence of *S. vermiculata* and/or *Streptomyces* sp. SF5. The aqueous extract of *S. vermiculata* and application of *Streptomyces* sp. SF5 to a saline soil partially alleviates its adverse effects and improves growth and performance of wheat seedlings.

Key words: Salt stress, *Triticum durum* L., *Salsola vermiculata*, *Streptomyces* sp. SF5, plant growth, osmoprotection.

Soil salinity is one of the most important factor limiting plant growth and secondary metabolism (Zhou *et al.*, 2018). According to the United Nations Environment Program 20% of agricultural land is affected by salinity (Naveed *et al.*, 2020; Flowers and Yeo, 1995). In the presence of high levels of sodium chloride, various physiological processes in plants are significantly affected such as germination, growth, photosynthesis, metabolism and nutrient availability (Ayala-Astorga and Alcaraz-Meléndez, 2012). Among the most widely consumed crops, wheat occupies a major place. It adapts to different types of stress and is considered moderately salt tolerant. However, when salinity exceeded 100 mM, germination and other parameters related to growth are significantly inhibited in wheat (Maghsoudi and Maghsoudi, 2008).

Indeed, to solve the problem of salinity and to limit the deleterious effects of NaCl on plants specially in arid and semi-arid zones, methods such as drainage and the use of irrigation water are applied, but they are quite difficult and costly and hence this limits their use.

The biological pathway is the most consistent strategy, through the use halophytes or biofertilizers (bacteria: *Azospirillum*, *Rhizobium*, *Pseudomonas* and actinomycetes). Actinomycetes are considered the dominant group of the soil microorganisms (Küster, 1968) that are involved in the decomposition of organic matter (Hoster *et al.*, 2005), the production of antibiotics, vitamins and enzymes (de Boer *et al.*, 2005), solubilisation of phosphorus (Crawford *et al.*, 1993) and production of indole acetic acid (Matsukawa *et al.*, 2007), which in turn is responsible for regulating many cellular processes such as cell division, elongation and differentiation (Hussein *et al.*, 2011).

On the other hand, halophytes are plants adapted to saline environments which accumulate osmoprotectants mainly in form of soluble sugars, proline and glycine betaine (Driouch *et al.*, 2001; Denden *et al.*, 2005). Significant intracellular levels of glycine betaine are found in many halophytes belonging to the Chenopodiaceae: such as *Salsola*, *Sarcocornia* sp. *Atriplex* sp. *Halosarcina* sp. (Rhodes and Hanson, 1993). Furthermore, different types of halophytes have different strategies to cope

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with high ionic concentrations (Meng *et al.*, 2018).

The aim of our study is to use an actinomycete strain isolated from the native soil which can alleviate the adverse effects of salt stress. The effect of the supply of the halophyte extract alone or in combination with *Streptomyces* sp. on germination and growth of durum wheat (*Triticum durum* L.) in the presence of inhibitory salt concentrations is also examined. The study is an initiative to analyze and to compare the effect of bacteria and/or halophyte on the growth of durum wheat under different levels of salinity.

Materials and Methods

This study was conducted using a variety of durum wheat named Mohamed Ben Bachir (MBB) which is a local selection from ITGC-Institut Technique des Grandes Cultures- Sétif (Algeria) obtained by the National Center for Control and Seed and Plant Certification.

Preparation of halophyte aqueous extract

S. vermiculata was collected from A sebkha of Ain Oulmen (Oued Chbel), South West of Sétif; Algeria (Latitude 36°11'28.03" N, Longitude 5°24'49.43" E). It was rinsed with distilled water (conductivity = 0.05 µS/cm), dried and ground. It was then sterilized (110°C/30 min) prior to its use at a concentration of 2% in control and saline solutions (NaCl 100 and 200 mM).

Inoculum preparation

Five ml of ISP2 (International *Streptomyces* Project) medium (g/l, yeast extract, 4, malt extract, 10 and glucose, 10) were inoculated from fresh cultures of *Streptomyces* sp. SF5. The strain was isolated from a fertile soil of El Ourissia region, North of Sétif, using a Starch-Casein agar medium. After incubation at 26°C for 72 hours, the bacterial suspensions were then centrifuged at 3000 rpm for 15 min, washed three times with 5 ml of physiological saline (NaCl: 9 g/l). The standard inoculum was of the order of 7x10⁷ CFU/ml.

Growth of *Triticum durum* L. and treatment application

The seeds were disinfected with sodium hypochlorite and rinsed with distilled water. They were planted at the rate of 5 seeds in pots

containing sterile soil (110°C/30 min). Sterile solutions of NaCl 0, 100 and 200 mM and/or halophyte extract, or bacterial solution were added every two days and were incubated for 30 days at 20°C. The study consisted of twelve treatments carried out in triplicate. During this period, different parameters related to wheat growth (shoots and roots lengths and dry weight) and metabolism (chlorophylls a, b and total, carotenoids and proline contents) were determined.

Shoots and roots dry weight measurements: Samples were washed and rinsed with distilled water, dried at 78°C for 48 hours and measured gravimetrically (g/plant) as two portions i.e. the root and the shoot, respectively..

Measurement of chlorophyll pigments, carotenoids and proline contents: Fully expanded leaves were sampled at 30 days after salt application for total leaf pigment and proline analyses. Fresh samples were used for these analyses. For total chlorophyll content analysis, each sample of 0.1 g was extracted with 10 ml of 80% (v/v) acetone and filtered. Then, absorbance was determined with a spectrophotometer at 470, 645 and 663 nm. The concentrations of chlorophylls a, b and total and carotenoids were calculated as follows (Shahba *et al.*, 2010).

$$Chl_a (\mu g/ml) = 12.5A_{663.2} - 2.79 A_{646.8}$$

$$Chl_b (\mu g/ml) = 21.51A_{646.8} - 5.1 A_{663.2}$$

$$Chl_{total} (\mu g/ml) = Chl_a + Chl_b$$

$$Car(\mu g/ml) = (1000 A_{470} - 1.8 Chl_a - 85.02 Chl_b)/198$$

Extraction and determination of proline (µmol g⁻¹ fresh weight-FW-) was done spectrophotometrically (Bates *et al.*, 1973). Leaf samples of 0.5 g were extracted with 5 ml of sulphosalicylic acid (3%). Extracts of 2 ml were held for 1 h in boiling water by adding 2 ml ninhydrin and 2 ml glacial acetic acid, after which cold toluene of 4 ml was added. Proline content was measured by a spectrophotometer at 520 nm and then calculated as µmol g⁻¹ FW against standard proline.

Statistical study

Statistical study included a separate analysis of *Triticum durum* L. growth parameters (ANOVA) between the twelve treatments (p<0.05). Principal Component Analysis (PCA)

was carried out; which involved the correlation study of the different parameters for each experiment. The PCA analysis is a multivariate technique that reduces the dimension of a data table by converting inter-correlated quantitative variables into few major components or factors known as principal components (PCs). These PCs are orthogonal to each other. Loading factors of original variable to a particular PC indicates the importance of the variable on that particular PC. PCA can be generalized as correspondence analysis in order to handle qualitative variables and as multiple factor analysis in order to handle heterogeneous sets of variables (Abdi and Williams, 2010). In our study, PCA analyzes the inter-correlation between 10 measured physiological and growth variables under different treatment and these are germination rate (GR), shoots length (SL), roots length (RL), shoot dry weight (SDW), root dry weight (RDW), chlorophyll a content (Chla), chlorophyll b content (Chlb), total chlorophyll content (Chl tot), carotenoid content (Car) and proline content (Pro).

Results

Effect of NaCl, S. vermiculata, Streptomyces sp. SF5 and S. vermiculata+ Streptomyces sp. SF5 on germination rate of T. durum L.

The germination of *Triticum durum* L. was monitored for 30 days. Maximum number of germinated seeds were obtained for the treatment 1 (control), while a decrease was observed in treatment with 200 mM NaCl. Combination of *S. vermiculata* and *Streptomyces* sp. SF5 also inhibited germination at 100 mM and also at 200 mM (Fig. 1).

Effect of NaCl, S. vermiculata, Streptomyces sp. SF5 and S. vermiculata+ Streptomyces sp. SF5 on root and shoot lengths and dry weight of T. durum L.

The dry weight and root length are significantly affected mainly at 200 mM NaCl. The inhibitory effect is also noted in the presence of *S. vermiculata* + *Streptomyces* sp. SF5. *S. vermiculata* stimulated roots dry weight considerably compared to *Streptomyces* sp. SF5 at 200 mM NaCl (Fig. 2). On the other hand, shoots dry weight was significantly decreased compared to the roots. Individually, *S. vermiculata* and *Streptomyces* sp. SF5 stimulate these parameters mainly at 0 and 100 mM NaCl.

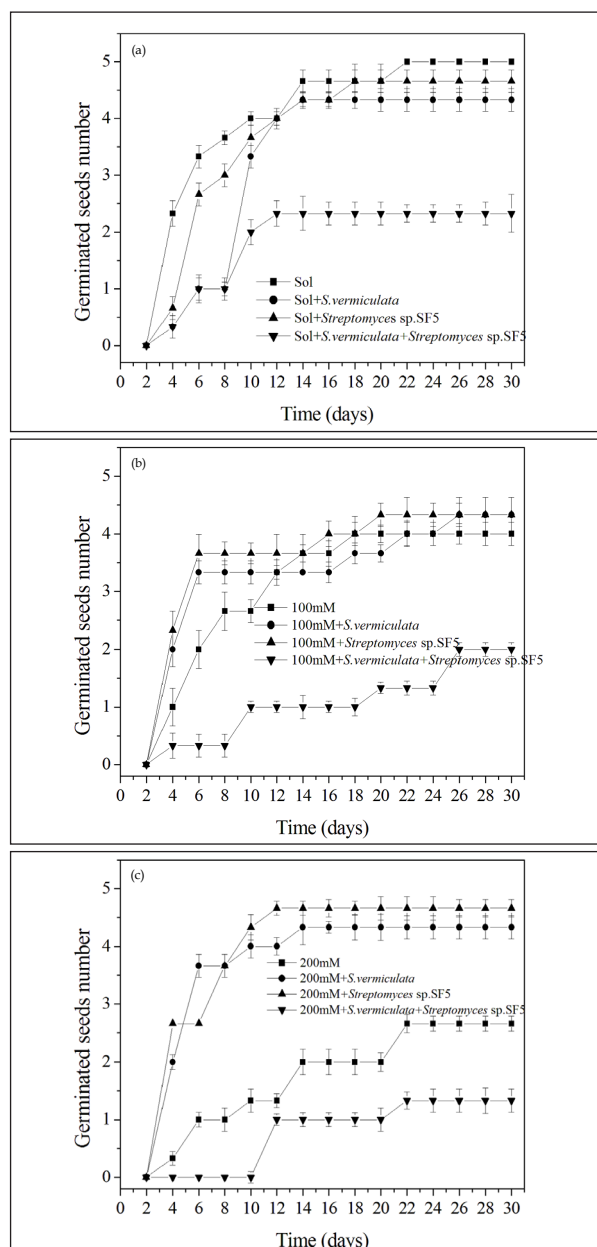


Fig.1. Effect of different concentrations of NaCl, *S. vermiculata*, *Streptomyces* sp. SF5 and *S. vermiculata* + *Streptomyces* sp. SF5 on *Triticum durum* L. germination: Control-0 mM NaCl (a); 100 mM NaCl (b); 200 mM NaCl (c).

(1: control: soil, 2: control + *S. vermiculata*, 3: control + *Streptomyces* sp. SF5, 4: control + *S. vermiculata* + *Streptomyces* sp. SF5, 5: 100 mM, 6: 100 mM + *S. vermiculata*, 7: 100 mM + *Streptomyces* sp. SF5, 8: 100 mM + *S. vermiculata* + *Streptomyces* sp. SF5, 9: 200 mM, 10: 200 mM + *S. vermiculata*, 11: 200 mM + *Streptomyces* sp. SF5, 12: 200 mM + *S. vermiculata* + *Streptomyces* sp. SF5)

The osmoprotective action of the halophyte was important at NaCl 200 mM. Similar effect was observed with *Streptomyces* sp. SF5. However, it is noteworthy that the inhibitory action of

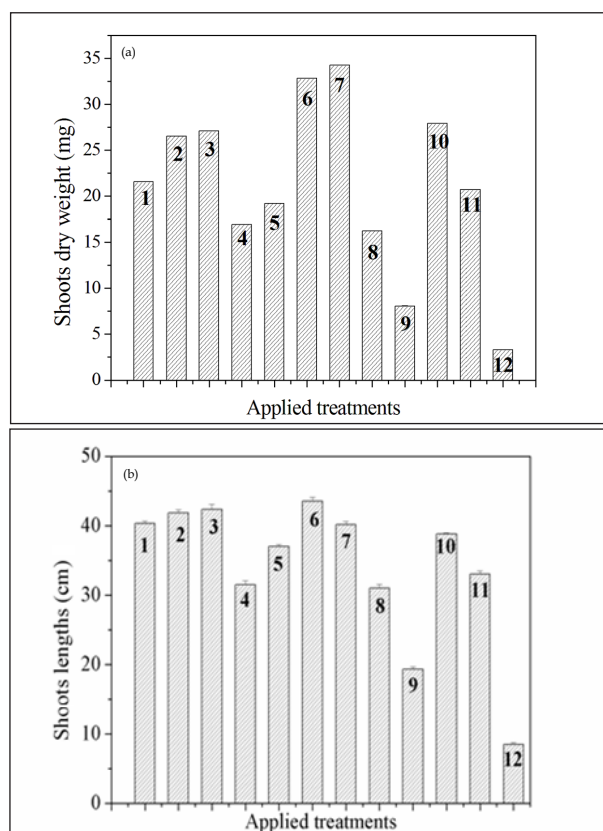


Fig. 2. Effect of different concentrations of NaCl, *S. vermiculata*, *Streptomyces* sp. SF5 and *S. vermiculata* + *Streptomyces* sp. SF5 on roots dry weight (a) and lengths (b) in *T. durum* L.

(1: control: soil, 2: control + *S. vermiculata*, 3: control + *Streptomyces* sp. SF5, 4: control + *S. vermiculata* + *Streptomyces* sp. SF5, 5: 100 mM, 6: 100 mM + *S. vermiculata*, 7: 100 mM + *Streptomyces* sp. SF5, 8: 100 mM + *S. vermiculata* + *Streptomyces* sp. SF5, 9: 200 mM, 10: 200 mM + *S. vermiculata*, 11: 200 mM + *Streptomyces* sp. SF5, 12: 200 mM + *S. vermiculata* + *Streptomyces* sp. SF5).

the combination of both *S. vermiculata* and *Streptomyces* sp. was also noted for treatments 4, 8 and 12 (Fig. 3).

Effect of NaCl, S. vermiculata, Streptomyces sp. SF5 and S. vermiculata + Streptomyces sp. SF5 on proline, chlorophylls a, b and total and carotenoids contents

The proline content did not change significantly at 0 mM NaCl, under different treatment (1, 2, 3 and 4). It reached maximum at 100 mM NaCl but decreased in the presence of *S. vermiculata* or *Streptomyces* sp. SF5 alone. The combination of *S. vermiculata* and *Streptomyces* sp. SF5, however, decreased it further (Fig. 4).

High contents of chlorophylls a, b and total were observed in treatments 1, 2 and 3

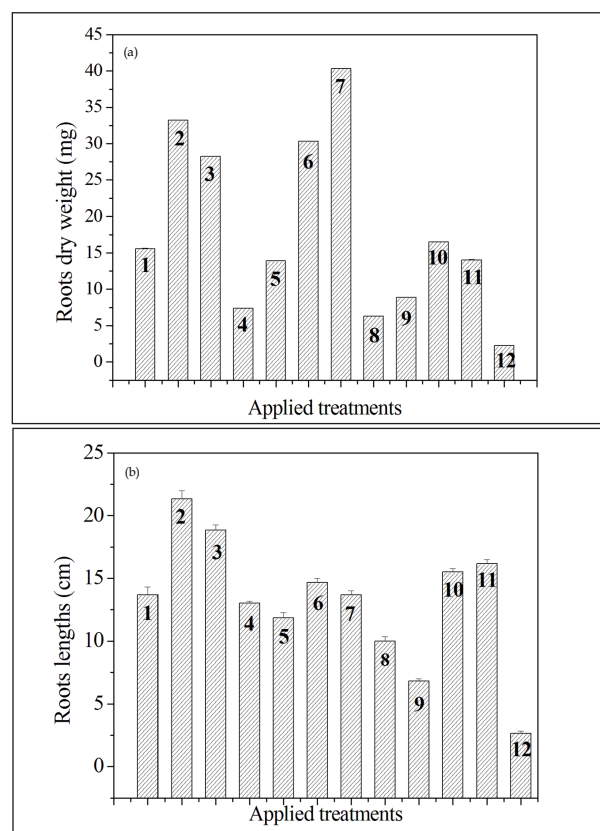


Fig. 3. Effect of different concentrations of NaCl, *S. vermiculata*, *Streptomyces* sp. SF5 and *S. vermiculata* + *Streptomyces* sp. SF5 on shoots dry weight (a) and lengths (b) in *T. durum* L.

(1: control: soil, 2: control + *S. vermiculata*, 3: control + *Streptomyces* sp. SF5, 4: control + *S. vermiculata* + *Streptomyces* sp. SF5, 5: 100 mM, 6: 100 mM + *S. vermiculata*, 7: 100 mM + *Streptomyces* sp. SF5, 8: 100 mM + *S. vermiculata* + *Streptomyces* sp. SF5, 9: 200 mM, 10: 200 mM + *S. vermiculata*, 11: 200 mM + *Streptomyces* sp. SF5, 12: 200 mM + *S. vermiculata* + *Streptomyces* sp. SF5).

(Fig. 5). They were significantly reduced in the presence of NaCl 200 mM and improved by the supply of *S. vermiculata* and *Streptomyces* sp. SF5. The halophyte combined to the bacterial strain strongly reduces chlorophylls pigments contents. They are greatly enhanced in the presence of *S. vermiculata* and *Streptomyces* sp. added separately. NaCl (100 and 200 mM) reduced the carotenoids synthesis but not significantly. Individually, the halophyte and *Streptomyces* sp. SF5 restored effectively their synthesis but their combination reduced their content.

Statistical analysis

Results of PCA analysis of physiological and growth parameters under different salinity

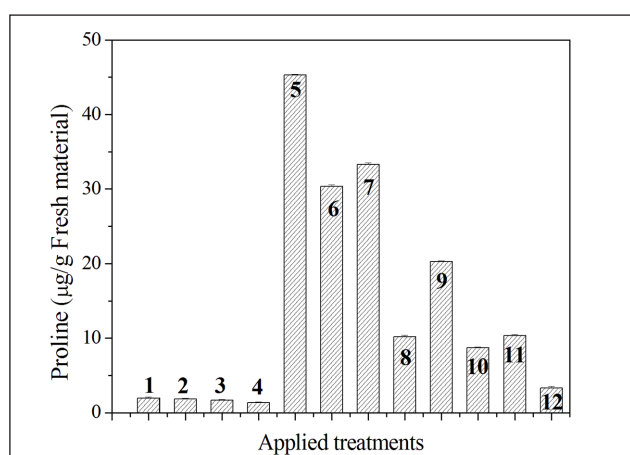


Fig. 4. Effect of different concentrations of NaCl, *S. vermiculata*, *Streptomyces* sp. SF5 and *S. vermiculata* + *Streptomyces* sp. SF5 on proline content in *T. durum* L.

(1: control: soil, 2: control + *S. vermiculata*, 3: control + *Streptomyces* sp. SF5, 4: control+*S.vermiculata* + *Streptomyces* sp. SF5, 5: 100 mM, 6: 100 Mm + *S. vermiculata*, 7: 100 mM + *Streptomyces* sp. SF5, 8: 100 mM + *S. vermiculata* + *Streptomyces* sp. SF5, 9: 200 mM, 10: 200 mM + *S. vermiculata*, 11: 200 mM + *Streptomyces* sp. SF5, 12: 200 mM + *S. vermiculata* + *Streptomyces* sp. SF5).

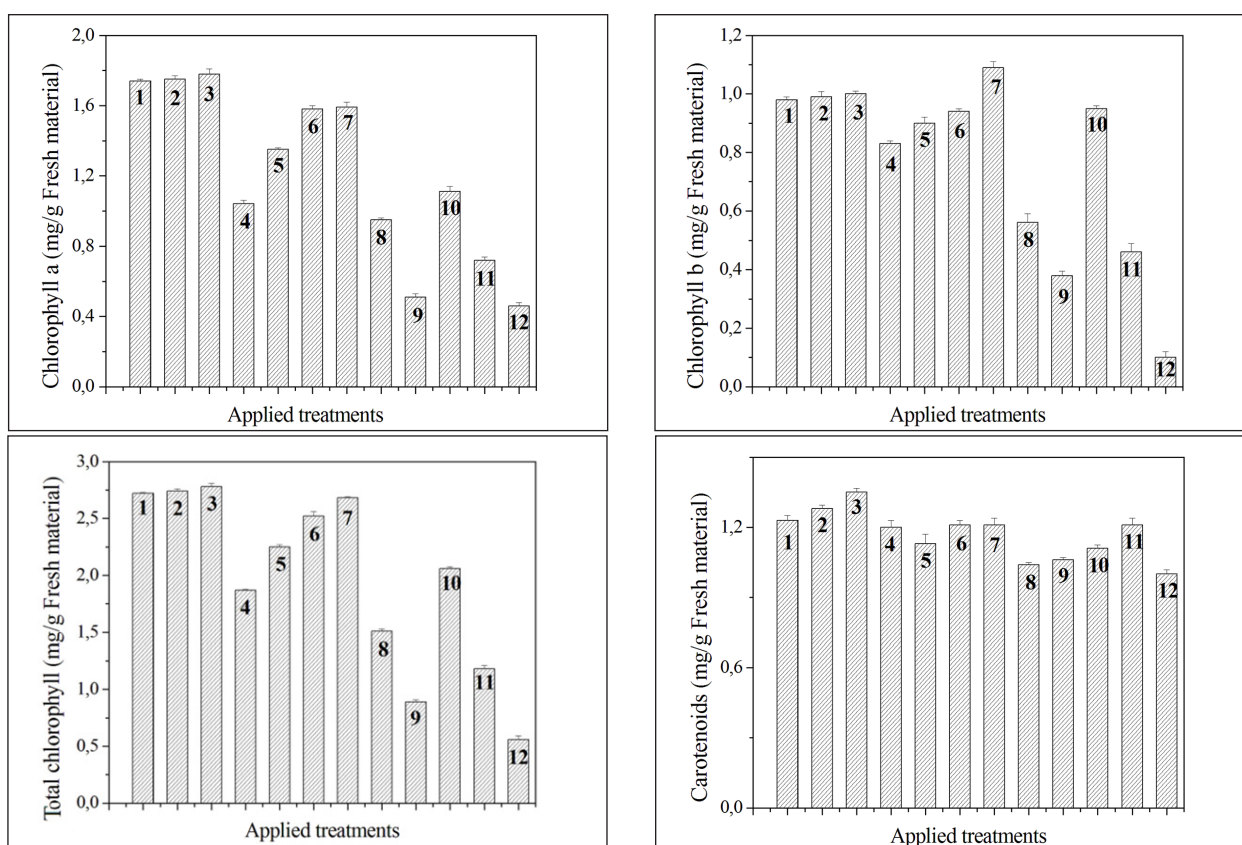


Fig. 5. Effect of different concentrations of NaCl, *S. vermiculata*, *Streptomyces* sp. SF5 and *S. vermiculata* + *Streptomyces* sp. SF5 on chlorophylls a, b and total and carotenoids contents in *T. durum* L.

stress treatments are presented in Fig. 8a-8d. It was observed that the first principal component (refer Factor 1 in Figures 8a-8d) describes 70-74% variation in the multivariate data whereas second principal component (refer Factor 2 in

Figures 8a-8d) described 11-14% variation in the data. Thus, two major PCs together described 87-90% variation in data (Table 1). Therefore, the dimension of data was reduced from 10 to 2. The biplot between PC1 and PC2 in absence of

Table 1. Variation of PC1 and PC2 under different treatments

Treatments	Factor 1: PC1	Factor 2: PC2
NaCl 0 mM	74.71% Car: positive GR: negative	13.16% Pro: negative
NaCl 100 mM	73.41% RL: negative Chl a: positive	13.98% SL: negative RDW and GR: positive
NaCl 200 mM	78.27% Chl b and Car: positive	11.86% Pro: positive
NaCl 0, 100 and 200 mM	70.55% GR: positive Pro: positive	13.12% SL: positive Chl b: negative

NaCl showed that Car and GR were located far from the origin and the eigenvector of these two parameters are almost parallel to X-axis, which is PC1 or Factor 1 (Fig. 8a). It indicates that these two parameters have the highest loading factors (eigen values) on PC1 as compared to other parameters and thus majorly control the value of PC1. However, the parameter Car was located on the right side of origin whereas the variable GR was located on the left side of the origin which indicates that Car has positive loading factor and GR has negative loading factor on PC1. On the other hand, the parameter Pro was very near to vertical axis, and thus the PC2 was majorly controlled by this parameter. In the presence of 100 mM NaCl, the biplot of PCA analysis showed that Chla and RL are major parameters to control PC1 however with positive and negative influence on PC1 (Fig. 8b). In contrary, SL, RDW and GR have major control on the value of PC2 whereas SL negatively influenced PC2 and RDW and GR positively influenced PC2. (Fig. 8b). At 200 mM NaCl, we noted that Car and

Chl b have major and positive influence on PC1 whereas Pro is the major dominant parameter to positively influence PC2 (Fig. 8c). The pooled PCA showed that GR was located on the right side of origin, so it has a positive influence on PC1 values and the highest loading factors comparing to other parameters. On the other hand, SL and Chl b are major parameters to control PC2 positively or negatively (Fig. 8d).

Discussion

Salinity stress limits and inhibits strongly plant growth mainly in arid and semi arid areas. It reduces water availability induces toxicity of sodium and chloride and also decreases the availability of essential nutrients (Carpici *et al.*, 2010). In the present study, all the treatments applied had variable effect on the germination of seeds of *Triticum durum* L. Germination rates are calculated to analyze and compare the effect and the influence of the different treatments (Dantas *et al.*, 2005). In the absence of salt stress, the germination rate was 100%. In the presence

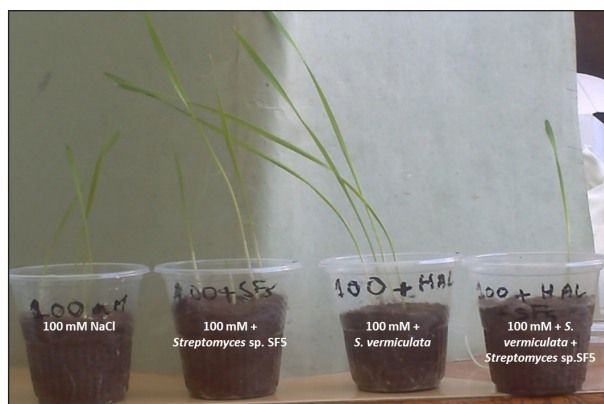


Fig. 6. Growth of *Triticum durum* L. in the presence of NaCl 100 mM, *Streptomyces* sp. SF5 + 100 mM and *S. vermiculata* + 100 mM.



Fig. 7. Growth of *Triticum durum* L. in the presence of NaCl 200 mM, *Streptomyces* sp. SF5 + 200 mM and *S. vermiculata* + 200 mM.

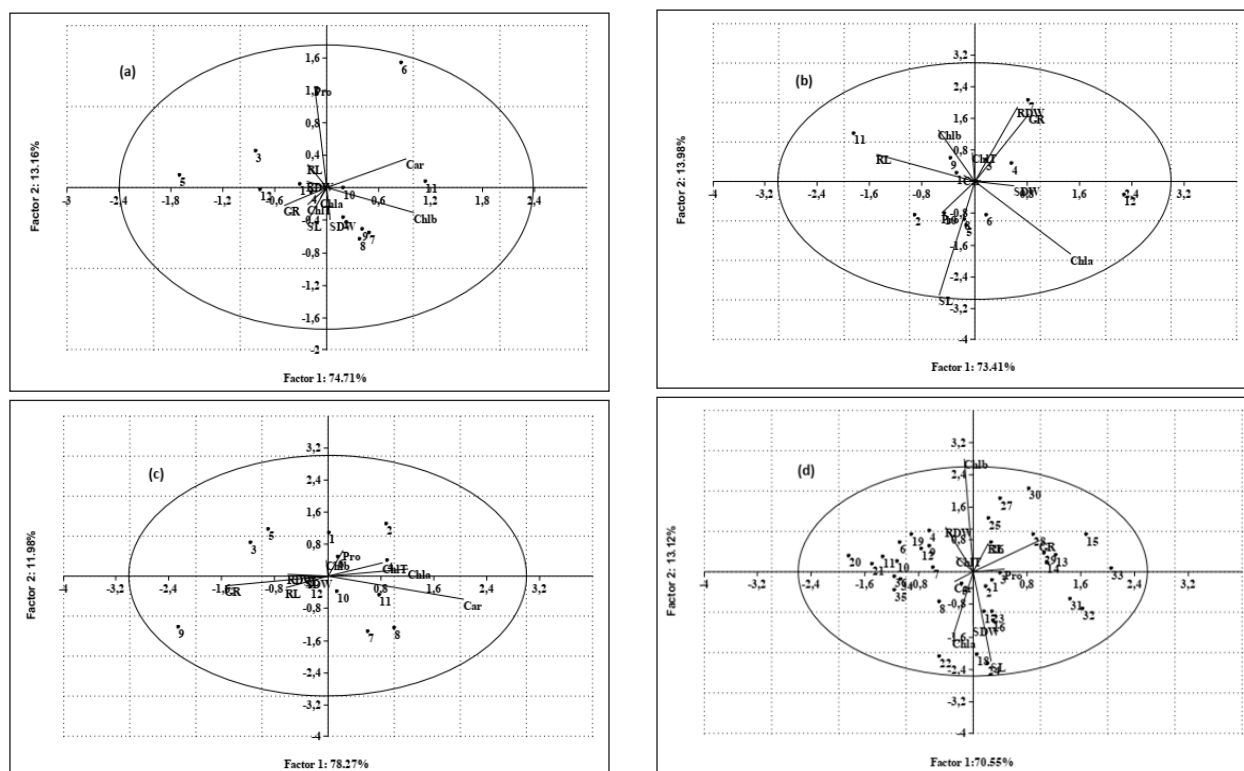


Fig. 8. PCA: inter-correlation of the different parameters: Control - 0 mM NaCl (a); 100 mM NaCl (b); 200 mM NaCl (c) and Pooled PCA (d).

Proline: Pro; Chlorophyll a: Chla; Chlorophyll b: Chlb; Total Chlorophyll: Chl T; Carotenoids: Car; Shoot Dry Weight: SDW; Root Dry Weight: RDW; Root Length: RL; Shoot Length: SL; Germination rate: GR.

of 100 mM NaCl, reduction in germination was observed (80%).

Salinity delayed germination of many plant species but did not reduce significantly the rate of germination (Ayers and Westcot, 1985). In the presence of 200 mM NaCl, the inhibitory effect of NaCl was more pronounced (53.2% germination). NaCl delayed germination through hormones and enzymes alteration (Botia *et al.*, 1998). α -amylase is the most enzyme involved in the plant germination process and it is inhibited by salt stress by altering its structure (Saboury and Karbassi, 2000). Salinity induced an imbalance of metabolic processes resulting in phenolic compounds formation (Ayaz *et al.*, 2000). In saline conditions, competition and interaction between ions causes nutritional imbalance. Ions such as Na^+ reduce the availability of Ca^{+2} and K^+ and their transport to the different parts of plants, which affects the structure and the composition of vegetative and reproductive organs (Cerdà and Martinez, 1988). The presence of NaCl around the roots leads to the

degradation of specific proteins that have been reported to be involved in germination and in root and shoots growth (Khan and Gulzar, 2003). Roots and shoots lengths of *T. durum* were reduced by 1.15%, 2% and 1.09% and 2.08% at 100 and 200 mM NaCl, respectively. According to Jamil and Rha, 2004, these two parameters are very important in the study of plant responses to salt stress. The roots are in direct contact with soil and absorb water and other elements and shoots distribute them to the different parts of plants. The dry weight is reduced by 1.75% and 2.68% (200 mM NaCl). According to (Datta *et al.*, 2009), roots and shoots development reduction is due to the toxic effects of NaCl and the imbalance in the availability of nutrients. Salinity has also been reported to decrease shoots length and inhibits the seedlings growth of *Vicia faba* (variety Alfia 5) by about 75.6% (Benidire *et al.*, 2015).

Salsola vermiculata effectively restored roots and shoots dry weight by 2.12%, 2.17%, 1.80% and 1.23%, 1.70% and 3.47%, respectively at 0, 100 and 200mM NaCl. Lengths were

correspondingly improved by 1.56%, 1.23%, 2.26% and 1.03%, 1.17% and 2%. As halophytes are a source of mineral and organic matter, thus they are likely to promote plant growth. In *Kochia sieversiana*, for example, many solutes (Na^+ , K^+ , Ca^{+2} , Mg^{+2} , Cl^- , SO_4^{-2} , NO_3^- , H_2PO_3^- , glycine betaine, proline, soluble sugars and organic acids were determined (Yang *et al.*, 2007). *Suaeda* sp., *Acacia* sp., *Salicornia* sp., *Pandanus tectorius*, *Glycyrrhiza uralensis*, *Asparagus persicus* also contained starch, sugars and proteins. *Salsola imbricata* and *S. soda* also accumulated ions like: Na^+ , K^+ , Ca^{+2} , Fe^{+2} , Cu^{+2} and Zn^{+2} . It is noteworthy that these mineral and organic moieties play an important role in plant nutrition and growth.

Combination of *S. vermiculata* and *Streptomyces* sp. SF5 reflected an antagonist effect which exceeds the inhibitory action of NaCl. Roots and shoots lengths decreased by 5.13% and 4.74%, respectively. Dry weights, however, dropped by 7% and 6.52% (200 mM NaCl). This inhibition is due to the antagonist effect of phenolic compounds accumulated by halophyte which reversed the stimulatory action of indole acetic acid (IAA) due to presence of *Streptomyces* sp. SF5 (Ameur and Ghoul, 2014). Similar results have also been reported by Ray *et al.* (1980) and Sharma *et al.* (1986) wherein abscisic acid considered as a plant growth inhibitor combined with some phenolic compounds, constituted a way of regulating plant growth.

Streptomyces sp. SF5 enhanced roots and shoots lengths which may be due to indole acetic acid production (Fig. 2, 3, 6 and 7). In *Triticum aestivum* L. indole acetic acid accelerates the emergence of roots and increases shoots growth, it also improves the length and dry weight of coleoptiles (Akbari *et al.*, 2007). The study also aimed to analyze the effect of NaCl on the accumulation of proline, chlorophyll pigments and carotenoids. Proline accumulated in the presence of NaCl (100 and 200 mM). However, very low levels of proline were obtained at 0 mM NaCl and in presence of *S. vermiculata* and *Streptomyces* sp. SF5. Proline synthesis is considered as an adaptive mechanism in plants mainly in several wheat varieties and its content is correlated to NaCl tolerance (El-Jaafari, 1993). The ability to increase proline synthesis in salt stress conditions can determine the level of plant tolerance and this may regulate many

processes necessary for survival under saline conditions (Meng *et al.*, 2018).

Chlorophylls a and b levels are significantly attenuated at 200 mM NaCl. In wheat, salt stress disrupts many cellular processes including photosynthesis (Gobinathan *et al.*, 2009). The contents of chlorophylls a and total chlorophyll are significantly reduced by 32% and 36% under salt stress in *Capsicum annuum* L. (R'him *et al.*, 2013). Similar results were obtained for *Zea mays* L. (Aliu *et al.*, 2015) and for *Alfalfa* (Biswas *et al.*, 2020). The deleterious effects of NaCl on leaf senescence is related to the accumulation of toxic ions (sodium and chloride) and reduction of the absorption of Mg^{+2} ions thus affecting the chlorophyll content (Leidi *et al.*, 1991). The decrease in chlorophyll content may also be due to lipid peroxidation in chloroplasts membranes, formation of hydro-peroxides of fatty acids and an increase in chlorophyllases activity (Jaleel *et al.*, 2007).

The decrease in chlorophyll content in saline conditions was reported by Khan *et al.* (2009) and da Conceição Gomes *et al.* (2017). *S. vermiculata* significantly improved the contents of chlorophylls a, b and total. The chlorophyll synthesis is influenced by mineral nutrition that included nitrogen, phosphorus and potassium (Bojovic and Stojanovic, 2005). Halophytes rhizosphere is a source of mineral matter, in *Salsola Nitraria* Pall., For example, the total contents of nitrogen, phosphorus and potassium are respectively 0.37, 0.733 and 15.09 g kg⁻¹ (Liangpeng *et al.*, 2007). Therefore, *S. vermiculata* provides not only a source of osmoprotection but also a nutritional source contributing subsequently to chlorophyll synthesis.

Streptomyces sp. improved chlorophylls a, b and total contents at 100 and 200 mM NaCl. Indole acetic acid synthesized by *Streptomyces* appears to have a protector role on *Triticum durum* L. photosynthetic system and also by improving minerals availability that have been reported to have primary role in synthesis of chlorophyll pigments. Simultaneously, carotenoid contents decreased in the presence of salt. It is well established that carotenoids are involved in the protection of photosynthetic system from the oxidative damage caused by salinity (Gadalla, 2009). Decrease in carotenoids content seems to have resulted in the reduction

of chlorophylls a, b and total contents. *S. vermiculata* and *Streptomyces* certainly enhances carotenoids contents (Fig. 5). This may be due to the induction of some important enzymes responsible for their synthesis or due to elimination of ROS. To prevent damage caused by ROS, plants have developed multiple detoxification mechanisms like the synthesis of certain antioxidant molecules such as α -tocopherols and carotenoids protecting membrane lipids (Falk and Munné-Bosch, 2010). However, it should be noted that the combination of *S. vermiculata* and *Streptomyces* sp. SF5 exerted an antagonist effect on proline, chlorophyll a, b and total carotenoids contents.

PCA results revealed that the inter-correlation between the different parameters was heterogeneous. Some of them were correlated, others are independents. The most important parameters are those that are near to one of the axes representing PCs, and far enough from the origin. These parameters are well correlated with the PCs and are the major explanatory parameters for the PC; their true distance from the origin is well represented on the factorial plane (Abdi and Williams, 2010). In the absence of NaCl, we can see that these parameters were carotenoids (Car), chlorophyll b (Chl b), and proline (Pro) which were located very far from the origin, so they are well presented on the factorial plane. The closed angle, starting from the origin formed by the points (Car) and (Chl b) indicated that these 2 variables were correlated. On the other hand, the right angle formed by (Car) or (Chl b) and (Pro) indicated that these variables were independents. Points located near the center: germination rate (GR), root dry weight (RDW), shoot dry weight (SDW) were therefore generally poorly represented by the factorial plane; consequently, their interpretation cannot be carried out with confidence. PCA showed that proline is very near to the vertical axis, so this axis corresponds to proline. On the other hand carotenoids and chlorophyll b were very near to the horizontal axis, so (Car) or (Chl b) presented this axis. We can say that proline was not correlated to the other parameters; this is justified by our results which showed that very low levels of proline were obtained at NaCl 0 mM and in presence of *S. vermiculata* and/or *Streptomyces* sp. SF5 compared to the other parameters. In the presence of 100 mM

NaCl, PCA showed that shoot length (SL), proline (Pro), chlorophyll b (Chlb) and total chlorophyll (Chl T) were near to the vertical axis, however, (SL) was so far from the origin. So, it corresponds to this axis. On the other axis, we noted that (RL) and (SDW) were near to the horizontal axis, but (RL) was far from the origin; therefore, it represented this axis. The right angle formed by (RL) and (SL) or (GR) indicated that these parameters were independents. Concerning results obtained at 200 mM NaCl, PCA showed that all the points except that of (Pro) were very near to the horizontal axis, but (Car) was very far from the origin which means that it is considered as the explicative point and it presented this axis. All these points formed a closed angle; this means that they were very correlated by the treatment applied. However, proline is presented on the vertical axis; it formed a right angle with the other points. We can conclude that it is independent to the other parameters. PCA of the different treatments (0, 100 and 200 mM NaCl) (Fig. 8d) showed that GR describes more than 70% variation in data which influenced PC1 values. On the other hand, SL was the principal component explaining the data variation (more than 11%). The variation in PC1 and PC2 mentioned in table 1 showed that some of the physiological parameters; GR and Pro influenced positively the variation data, this variation exceeds 70% (Fig. 8d). Consequently, we can say that these two parameters could be taken as the major physiological parameters that can be used as indicator to identify salinity stress. According to our results, it is noteworthy that all the physiological parameters were influenced by salinity; however the inhibitory effect was more evident at 200 mM NaCl, and more pronounced in treatments supplemented with *Salsola vermiculata* and *Streptomyces* sp. SF5

Conclusion

The main results confirm the potential osmoprotective effect of *Salsola vermiculata* and *Streptomyces* sp. SF5 applied separately on *Triticum durum* L. on germination and growth in the presence of salt stress by improving roots and shoots length and dry weight, chlorophyll pigments and carotenoids contents. However, a combination of *Salsola vermiculata* and *Streptomyces* sp. SF5 considerably inhibited the wheat growth which greatly exceeded the

inhibitory effect of NaCl. The aqueous extract of this halophyte or this bacterium to soil confronted by salinity problem can minimize the deleterious effects of salt and improve wheat biomass.

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