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Research Article Effect of Salinity-NaCl and *Pseudomonas fluorescens* on the Germination of Wheat Genotypes (*Triticum durum* L.) Cultivated in Arid Regions of Algeria

Boualem Boumaaza

Biodiversity and Water and Soil Conservation, Department of Agronomy, University of Abdelhamid Ibn Badis, P.O. Box 300, 27000 Mostaganem, Algeria

Abstract

Background and Objective: Durum wheat (*Triticum turgidum* L. ssp. *durum* Desf.) is the most widely grown crop in the world with over more than 8% of wheat cultivated worldwide. The present study aimed to study the combined effect of salinity-NaCl and *Pseudomonas fluorescence* on germination of five genotypes of durum wheat cultivated in the arid regions of Algeria. **Materials and Methods:** Seeds are germinated in Petri dishes containing twice folded Whatman filter paper. Each Petri dish was provided with 5 mL of the respective salt concentration from 0-300 mM. There were 10 replicates, with 100 seeds in each replicate. Germination percentage varied significantly among various genotypes and between different concentrations of salt. **Results:** The five genotypes have been shown to be tolerant under 50 and 150 mM levels of salt stress. These results showed that Vitron genotype were more tolerant to salinity (83% of germination), with increasing NaCl solutions up to 300 mM, while, Boussellam, Simeto, GTA dur and Ofanto genotype were shown to be sensitive with a germination rate from 35-53%. The effect of *P. fluorescence* does not induce any change in the attenuation of the effect of salinity on the final percentage of germination. At the same time, this microbial agent did not provide any protective effect in the presence of moderate salinity (50-150). **Conclusion:** *Pseudomonas* strain does not attenuate the negative effect of the salinity on seeds germination and growth of wheat.

Key words: Durum wheat, germination, P. fluorescence, salt stress, Triticum durum L.

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Corresponding Author: Boualem Boumaaza, Biodiversity and Water and Soil Conservation, Department of Agronomy, University of Abdelhamid Ibn Badis, P.O. Box 300, 27000 Mostaganem, Algeria

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Durum wheat (*Triticum turgidum* L. ssp. *durum* Desf.) is the most widely grown crop in the world with over more than 8% of wheat cultivated worldwide, with annual crop production about 33 million t^1 . In Algeria more than 3.15 million t are produced per year and a cultivated surface of about 1.59 million² ha in 2018.

However, durum wheat yield showed high uctuations through Algeria and were attributed mainly to poor soil and crop management. 60% of the Algerian durum wheat cultivation has produced in arid and semi arid regions. These areas where water deficit is the main limiting factor for winter wheat production, especially during grain filling, when high evaporative demand coincides with low rainfall. In addition, one of the common features of the Algerian semiarid regions, soil salinity is one of the major environmental factors significantly reduces agricultural and crop productivity³.

Soil salinity lead to a deterioration wheat production by affecting growth and yield parameters, plant height and leaf area³. On the other hand, this problem is one of the major stresses that affect grain yield and its components, in particular the number of spike per meter square, the number of kernels per spike and the thousand kernels weight⁴.

Presence of NaCl outside the cell occurs from both osmotic stress due to low water potentials which may depressing the plant growth and development⁵. On the other hand, all stages of plant were affected by the salt stress, with different mechanisms such as leaf respiration, photosynthesis rate, water content, transpiration, cellular metabolism and solute accumulation⁶.

Salinity of soil or water is one of the main germination reducing factors for most of the plant species. After exposure to salt salinity, the seeds are faced with ion toxicity, osmotic stress and oxidative stress⁷.

The sensitivity to salt stress of numerous wheat species differs at seed germination and also a significant variation is observed from plant cultivars to cultivars^{5,8}. The rate of germination of seeds does necessarily correlate with number of plant/m² and therefore number of ear/m². Inter-cultivar variation under NaCl salinity at the germination has been assessed in wheat, *Triticum aestivum* L.and *Hordeum vulgare* L.⁹⁻¹¹. In Algerian arid regions many durum wheat varieties have been developed based on field assessment for higher yield and drought resistance. Unfortunately, the salty soils in these regions could be negatively exploited. Earlier only improvements by selection were used to create varieties resistant to abiotic stress, the use of biologic material was highly important¹².

Among these safe methods, the use of plant growth promoting bacteria such as *Pseudomonads*, played an important role against abiotic and biotic stress.

Pseudomonas fluorescens are important plant growth-promoting rhizobacteria and can adapt to a variety of environments^{13,14}. Several mechanisms direct or indirect of *Pseudomonas* have been proposed on plant growth and development¹⁵, include, the ability to produce plant hormones such as gibberellins¹⁶, cytokinins¹⁷, auxins¹⁸ and ethylene¹⁷. The *P. fluorescens* play a role to proliferation of root hairs by increasing the absorption of nutrients such as phosphorus, nitrogen and iron¹⁹.

The aim of this research was to determine the effects of *P. fluorescens*, salt stress levels and their interaction on seed germination of five cultivars of durum wheat produced in Algeria arid and semi arid regions and to access if it may be a viable alternative to current management strategies of salinity.

MATERIALS AND METHODS

The research was conducted between December and March 2018 at Laboratory of Agrobiotechnology and Nutrition in Semi-arid regions, Faculty of Natural and Life Sciences, Ibn Khaldoun University, Tiaret, Algeria.

Seeds of 5 wheat genotypes Vitron, Boussellam, Simeto, GTA dur and Ofanto were obtained from National Center for Control and Certification of seeds and plants (CNCC), Algeria is presented in Table 1.

Microbial strain used during study: The *Pseudomonas* strain used in this study was provided by the Plant Pathology Laboratory at Es-Sénia University, Oran, Algeria, in the period of 2016. The bacterial strain was grown in tubes containing a King B medium additionally supplemented with 25% glycerol. Then, after growth at 28°C, the bacterial was stored at -4°C. The bacterial concentration is determined by using a dilution method. After counting, the bacterial suspension is adjusted with sterile distilled water in same to obtain a population concentration of 10⁸ CFU mL⁻¹.

Germination test: One hundred sterilized seeds of each cultivar were placed on two layers of filter paper as seed beds in petri dishes in ten replicates. The filter paper was moistened with either distilled water or different levels of sodium chloride solution. The salinity levels ranged with four concentrations 50,100, 150 and 300 mM. These concentrations are close to the salinity rates of soils in the western Algerian regions. The control corresponds to 0 Mm. The dishes were incubated at

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Cultivars	Origin	Agronomic and technological characteristics
Boussellam	Syria	High yield, high 1000 seed weight, good mitadinage, protein content 15.01%
GTA dur	Mexico	High yield, high 1000 seed weight
Ofanto	Italy	High yield, 1000 seed weight moyen, sensitive mitadinage, protein content 15.64%
Simeto	Italy	High yield, high 1000 seed weight, resistant mitadinage, protein content 15.80%
Vitron	Spain	High yield, high 1000 seed weight, resistant mitadinage, protein content 13.50%

Table 1: List of cultivars and their agronomic and technological characteristics used in this study

25°C. The experiment was repeated three times. The petri dishes are placed in the dark. Observations are made daily to assess the first germinations recorded as soon as the radicle appears. The germination percentage (GP) was calculated as described by Coolbear *et al.*²⁰.

Inoculation of seeds: Sterile durum wheat seeds were soaked in 50 mL of the bacterial suspension $(10^8 \text{ CFU mL}^{-1})$ for a period of 20 min. This inoculation procedure achieves a bacterial concentration of 10^7 CFU/seed . Control seeds were immersed in sterile distilled water for the same duration.

Influence of Pseudomonas on plant growth under saline

conditions: The seeds were sown in plastic pots of 10 cm diameter and 20 cm in height trays filled with peat at the rate of 5 seeds/pots at a depth of 1 cm. The seeds were then inoculated prepared according to the protocol as previously described. The pots were placed in greenhouses with a 14 h light (24°C) and 10 h dark period (20°C). The plants were irrigated regularly to 30% of the substrate holding capacity. After 25 days, the plants were exposed for one day to irrigation with four water salinity levels. The control plants were irrigated with 0 mM of NaCl. A week after salinity application, the lengths of the wheat seedlings were measured with a rectilinear scale. All the experiments are carried out in triplicates.

RESULTS

Effect of salinity on seed germination: The effect of salts at different concentration on seed germination of 5 wheat cultivars was observed after 24 h. It was found that salinity levels had a marked significant inhibitory effect on seed germination of 5 cultivars of wheat (Table 2). In the absence of salt (Table 2), wheat cultivars do not present the same profile of the germination percentage, the optimum percent for germination was from 97 and 96.5% of GTA dur and Vitron, respectively. Data in Table 2 indicate that, application of sodium salt caused a significant increase the germination inhibition at various concentrations tested compared with control (p>0.01). At moderate levels of NaCl (150 mM), Vitron and GTA have been identified as holder of resistance to salinity

with lowest percent inhibition of germination as 11.5 and 14% respectively. By application of highest concentration of salt (300 mM), the inhibition on seed germination 88 ± 4.32 (88%) was recorded in genotype Simeto.

In the present study, the application of *Pseudomonas* did not inhibit the effect of salinity on seeds germination. According to results, it was found that *Pseudomonas* exacerbated a reduction on seeds germination. However, germination percentages were very low in all salinity levels. Also the inoculation of seeds in the absence of NaCl caused a double reduction on germination in all the cultivars as compared to control. The germination inhibition kept going from: 7-77.5% (77.5±5.26) (Vitron), 12-90% (GTA dur), 28.5-87% (Ofanto), 14.5-96.5 (96.5±2.82) (Boussellam) and 14-92.5 (92.5±1.91) (Simeto) as presented in Table 3. At the same time, our studies have demonstrated negative effect of pseudomonas on seed germination.

Effect *in vivo* of salinity on seed germination: The effect *in vivo* of salinity stress on germination is exposed in Table 4. Evaluation of germination of seeds carried after 1 week showed a variation between the cultivars and the salinity levels. The obtained results showed that seed germination of Simeto and GTA dur cultivars were inhibited at heavy salt stress. Of the five wheat cultivars tested, only three were confirmed to be resistant R" to salt stress (Bousellam, Vitro and Ofonto), two cultivars (Simeto and GTA dur) were sensible to higher salinity.

Effect *in vivo* of salinity and *Pseudomonas* on seed germination: As reported in Table 5, the application of concentrations from 50-150 mM with bacterization of wheat seeds did not inhibit their germination. However, increase in salinity from 150-300 mM drastically inhibited the germination for Vitron, Simeto and GTA dur. Comparable to than the single treatment (salt stress), germination was not found in seeds inoculated and irrigated by water salinity (300 mM). It was observed from this study that NaCl application at higher levels associated with microbial inoculation can wholly inhibits the seeds germination. Here, we report that 2 cultivars (Bousellam and Ofonto) were confirmed to be resistant R^{*m*} to combined effect of *P. fluorescens* and salt stress, three

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Table 2: Effect of salinity on seed germination (Inhibition on seed germination %) after 24 h

Salinity level (mM NaCl)	Wheat cultivars					
	Vitron	GTA dur	Ofanto	Boussellam	Simeto	
Control	3.5±1.91	3.0±1.15	7.0±1.15	8.5±2.43	7±1.46	
50	4.0±1.00	4.0±2.82	12.5±4.22	9.0±3.03	10±1.32	
100	4.0±1.63	8.0±1.32	20.0±5.88	14.5±5.00	14±3.11	
150	11.5±3.78	14.0±2.16	43.0±3.66	61.0±5.25	52±8.48	
300	77.0±1.91	81.5±6.60	82.0±4.63	85.5±3.00	88±4.32	

Table 3: Effect of salinity and Pseudomonas on seed germination (Inhibition on seed germination %) after 24 h

Salinity level (mM NaCl)	Wheat cultivars					
	Control	7.0±3.83	12.0±1.10	28.5±5.0	14.1±4.34	14.0±3.65
50	8.0±2.30	21.0±2.72	31.0±7.6	25.0±6.21	16.0±4.32	
100	9.0±3.77	33.5±1.20	33.5±4.82	28.5.5±9.58	23.5±4.43	
150	14.5±5.16	35.5±2.16	48.0±10.86	61.5±2.58	57.0±4.76	
300	77.5±5.26	90.0±6.06	87.0±3.83	96.5±2.82	92.5±1.91	

Table 4: In vivo effect of salinity on seed germination after 1 week

Salinity levels (mM)	Vitron	GTA dur	Ofonto	Bousellam	Simeto
0	+	+	+	+	+
50	+	+	+	+	+
100	+	+	+	+	+
150	+	+	+	+	+
300	+	-	+	+	-

-: Ungerminated seed, +: Germinated seed

Table 5: In vivo effect of salinity and Pseudomonas on seed germination after 1 week

Salinity levels (mM)	Vitron	GTA dur	Ofonto	Bousellam	Simeto	
0	+	+	+	+	+	
50	+	+	+	+	+	
100	+	+	+	+	+	
150	+	+	+	+	+	
300	-	-	+	+	-	

-: Ungerminated seed +: Germinated seed

Table 6: Effect of salinity levels on plant growth

Cultivars	Salinity level (mM NaCl)					
	Control	50	100	150	300	
Boussellam	39.8±7.9	34.6±7.1	34.2±6.06	30.5±4.3	6.4±0.09	
Vitron	37.6±4.7	40.8±3.2	32.3±4.3	27.9±2.5	8.0±3.1	
Simeto	43.6±12.2	35.2±2.7	33.1±8.2	29.4±4.4	-	
GTA dur	38.3±4.6	38.3±0.6	31.9±1.6	29.0±3.8	-	
Ofanto	39.2±6.1	39.8±2.2	37.9±1.8	28.7±3.4	6.7±0.2	

±: Standard deviation, -: Ungerminated seed

cultivars (Simeto, Vitron and GTA dur) were sensible compared to the plants non-inoculated and irrigated by water salinity.

Effect of salinity levels on plant growth: As compared of the responses of the different cultivars, plant length of evaluated varieties was significantly affected by different salinity levels (Table 6), decrease in plant length with increasing salinity level. With the increase in salinity level (150-300 mM) reduction in plant height was observed by 6.4, 8 and 6.7 cm for Boussellam, Vitron and Ofanto, respectively (Table 6).

When seeds of wheat were irrigated by water of low salinity (50 mM), a significant enhanced in plant height from 37.6-40.8 for Vitron cultivars.

Influence of *Pseudomonas* on plant growth under saline conditions: Combined effect of salinity and bacterization was the highest inhibited comparable to than the sole treatment by salinity. Applications *P. fluorescens* give very negative influence on wheat plant seedling height. In absence of salt stress, plants seedling height were decreased from 39.8, 37.6,

Cultivars	Salinity level (mM N	Salinity level (mM NaCl)					
	Control	50	100	150	300		
Boussellam	34.2±12.5	32.4±1.9	27.9±7.1	28.05±2.7	3.7±2.8		
Vitron	38.5±4.6	32.0±10.9	29.5±8.8	26.2±3.1	-		
Simeto	34.1±6.7	31.3±6.1	28.4±2.5	24.4±3.4	-		
GTA dur	32.3±8.0	24.2±6.4	20.4±7.5	13.05±0.7	-		
Ofanto	33.2±2.9	36.1±8.6	27.9±11.6	26.2±3.2	2.7±0.1		

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±: Standard deviation, -: Ungerminated seed

43.6, 38.3 and 39.2 to 34.2, 38.5, 34.1, 32.3 and 33.2 for Boussellam, Vitron, Simeto, GTA dur and Ofanto respectively, compared to seeds inoculated and irrigated by sterile distilled water as presented in Table 7. It is important to not that reduction due to water salinity stress was increased in P. fluorescens inoculated plants, especially in 300 mM.

DISCUSSION

Various Agronomic characters are measured to quantify the level of salinity stress effects on wheat plants. The Agronomic traits like the height of plant, number of tillers/m², number of ear/m², number of ear/plant number of grain/ear and thousand grain weight are known to be affected under saline conditions. Germination is an important stage in the asses of yield process and has to be concluded before harvest. Plant responses to salinity stress mainly by three important mechanisms, salt tolerance, salt resistant and salt sensitivity. To fight the salinity-induced plant growth reduction, there is a great solution to this problem such as physical remediation, chemical remediation, bioremediation and phytoremediation²¹.

The idea of using PGPR to stimulate plant growth under saline conditions has been experienced by Banaei-Asl et al.22, Wang et al.23 and Shahid et al.24. PGPR can enhance plants tolerate salinity by numerous mechanisms²⁵, such as overcome osmotic stress by produce compatible osmolytes like trehalose, proline and glycine betaine²⁶, alleviate the toxicity of Na⁺ and nutrient deficiency by maintaining ion homeostasis caused by the high influx of Na⁺ and Cl⁻ ions²⁷ and dampen the oxidative stress by increasing of antioxidants enzymes^{27,28}.

The results of this study show that the increasing of levels of NaCl significantly decrease seed germination of five cultivars of wheat. High salt concentration causing significant length reduction. However, in cultivar 'Vitro' there was noticed a significant increase of plant growth occurred only at low salt concentrations (50 mM NaCl).

These results are in agreement with some earlier findings which confirmed that seed germination of wheat genotypes exposed to NaCl treatments have been decreased^{9,29,30}. Reduced and delayed seed germination under NaCl stress in barley and Oryza sativa have also been reported by El-Sharkawy et al.³¹ and Fogliatto et al.³². The reduction in seed germination at high salt concentrations is caused by disturbances in osmotic regulation, which leads to difficulties in the absorption of water in saline conditions or is the result of toxic effects of Na⁺ and Cl⁻ ions³³.

Salinity leads to the reduction in cell division and cell elongation, primarily because of the difficulty to absorb nutrients mostly due to the accumulation of large amounts of reactive oxygen species, inhibition of cellular enzymes, loss of turgor and hormone imbalance which affects plant growth and later has a negative effect on the production of biomass and yields³⁴.

At moderate levels of NaCl (50-150 mM), Vitron and GTA have been identified as holder of resistance to salinity with lowest percent inhibition of germination but Boussellam, Simeto and Ofanto showed high sensitivity at this salt stress level.

It is also stated that application of different concentrations of NaCl reduced the seedling growth of wheat. Similar results were reported by Naeem et al. 35 and Borawska-Jarmulowicz et al.³⁶.

Based on the results obtained, Pseudomonas strains have, manifestly, the capacity to reduce durum wheat seed germination. There are some contradictory reports on the effect of Pseudomonas species on seed germination. In some studies, inoculation with bacteria has enhanced seed germination, another reports it has been found to decrease seedgermination³⁷⁻³⁹. For example, Banowetz et al.⁴⁰ reported that *P. fluorescens* WH6 significantly suppressed germination of annual bluegrass seeds and some other poaceae species.

The inhibitory action of bacteria on seed germination could be due, presumably, to the production of 4-formylaminooxyvinylglycine in the inoculated seeds. Some previous studies showed that high levels of indole-3-acetic acid produced by PGPR-inoculated seeds were almost certainly the main cause inhibition on seeds germination of wheat^{37,41}.

From the present results, it is concluded that *Pseudomonas fluorescens* strain did not affect plant height for all wheat cultivars. Concerning plant height, seedlings that received inoculants at sowing had lower plant length values compared to seedlings non-inoculated and irrigated by water salinity. The negative effect of *Bacillus* sp. is confirmed by Mosimann *et al.*⁴², has shown that strain *Pseudomonas fluorescens* Pf 153 had a negative effect on plant biomass, although its improved ability to colonize the roots. The negative action of bacteria may be related to their physiological and metabolic adaptability, bacteria in the plant root zones play important roles in changing soil agroecosystems⁴³.

The production of cyanide by *Pseudomonas fluorescens* may have negatively affected plant growth directly^{44,45}. In other studies, that same strain has shown ability to reduce the growth of indigenous beneficial microorganisms living in the maize rhizosphere and thereby negatively affected plant growth indirectly⁴⁶. However as reported in the literature, *Pseudomonas fluorescens* was able to increase yield components of *Zea mays*⁴⁷, *Oryza sativa*⁴⁸, wheat⁴⁹ and tomato¹⁵. One of the recommendations is the evaluation of other strains of bacteria that could be effectively used as biofertilizers for wheat

CONCLUSION

Durum wheat is widely cultivated in Mediterranean area especially in arid and semi-arid regions. Unfortunately, the production of wheat could be negatively influenced by soil salinity. In durum wheat, response differences between cultivars against salt stress are strongly dependent upon their genetic composition for their tolerance, resistant and sensitivity. Assessment the performance of local cultivars of durum wheat against soil salinity in order to identify salt-tolerant cultivars seems to be valuable for a wide range of genotypes. The research on methods and mechanisms for improving the salt resistance of wheat by using *P. fluorescens* has great significance for its future commercial use.

SIGNIFICANCE STATEMENT

Soil salinity lead to a deterioration wheat production by affecting growth, grain yield and their components. This problem is one of the major stresses in the arid region of Algeria. To reduce the negative effects of salt stress on the growth and yield of plant with plant growth promoting bacteria (PGPR) are previously studied. The principal aim of PGPR is the reduction of negative impact of salinity stress. In addition, the effectiveness of PGPR sometimes influenced by many factors such as high temperature and salinity. To date, there are limited studies carried on the PGPR activity under salinity stress. This study discovered that salinity and pseudomonas have adverse effect on seeds germination of wheat. This study will help the researcher to uncover the effects of PGPR under saline conditions, which this issue still appears to be a valuable future objective.

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