

EFFECT OF PLANT GROWTH-PROMOTING RHIZOBACTERIA INOCULATION ON SEEDLING DEVELOPMENT OF THREE *TRITICUM DURUM* DSF. GENOTYPES GROWN IN DROUGHT CONDITIONS

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Abstract

Description of the subject: Durum wheat (*Triticum durum* Dsf.) is a species widely cultivated in Algeria and throughout the world. This crop is subject to unfavourable climatic conditions, causing significant water stress which is often a major constraint to its growth and improvement.

Objective : This work aims to study the morphological and biochemical responses of three genotypes of this species inoculated by four bacterial strains.

Methods : The study was carried out according to a split-split-plot design with three replicates for a total of 54 trials each pot contains four plants. The trials were conducted in greenhouses. The first inoculation treatment was *Frankia sp strain CcI3* and the second treatment was a combination of three strains (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) to severe water stress applied for 10 days.

Results : The results show that water stress has a depressing effect on the length and dry weight of the aerial part while the length and dry weight of the roots tends to increase. Stress influences the total chlorophyll and relative water content negatively; however, it increases the levels of total soluble protein and antioxidant enzymes. The Boussellem genotype appears to be more tolerant to stress than the other two genotypes Waha and Cirta. Bacterial inoculation revealed slight significant effects on morphological traits (length and dry weight of the root and aerial parts), physiological traits (total chlorophyll content and relative water content) and strong significant effects on biochemical traits such as the content of total soluble proteins and antioxidant enzymes (Catalase and Ascorbate peroxidase) compared to the control in all three genotypes

Conclusion : Indeed, the inoculation of plants by PGPBs (Plant Growth-Promoting Rhizobacteria) presents an alternative to improve the drought tolerance mechanisms that durum wheat faces.

Keywords : *Triticum durum* Dsf ; water stress ; inoculation ; PGPB.

EFFET DE L'INOCULATION DE RHIZOBACTÉRIES FAVORISANT LA CROISSANCE DES PLANTES SUR LE DÉVELOPPEMENT DES SEMIS DE TROIS GÉNOTYPES DE *TRITICUM DURUM* DSF. CULTIVÉS DANS DES CONDITIONS DE SÉCHERESSE

Résumé

Description du sujet : Le blé dur (*Triticum durum* Dsf.) est une espèce largement cultivée en Algérie et dans le monde. Cette culture est soumise à des conditions climatiques défavorables, provoquant un stress hydrique important qui est souvent une contrainte majeure à la croissance et l'amélioration de cette culture.

Objectifs : L'objectif de ce travail est d'étudier les réponses morphologiques, physiologiques et biochimiques de trois génotypes de cette espèce inoculés par quatre souches bactériennes.

Méthodes : L'étude a été réalisée sous serre selon un plan split-split-plot avec trois répétitions pour un total de 54 essais chaque pot contient quatre plantes. L'inoculation des variétés par *Frankia sp. strain CcI3* comme premier traitement d'inoculation et une combinaison de trois souches (*Azospirillum brasilense*, *Bacillus sp* et *Pseudomonas sp*) comme deuxième traitement, vis-à-vis d'un stress hydrique sévère appliqué pendant 10 jours.

Résultats : Les résultats montrent que le stress hydrique a un effet dépressif sur la longueur et le poids sec de la partie aérienne alors que la longueur et le poids sec des racines tend à augmenter. Le stress influence les teneurs de la chlorophylle totale et la teneur relative en eau négativement, cependant il augmente les teneurs des protéines totales solubles et les enzymes antioxydantes. Le génotype Boussellem paraît plus tolérant au stress que les deux autres génotypes Waha et Cirta. L'inoculation bactérienne a révélé des effets significatifs légers sur les caractères morphologiques (longueur et poids sec de la partie racinaire et aérienne), les caractères physiologiques (teneur de la chlorophylle totale et la teneur relative en eau) et des effets significatifs forts sur les caractères biochimiques comme la teneur des protéines totales solubles et les enzymes antioxydantes (Catalase et Ascorbate peroxydase) par rapport au témoin dans les trois génotypes.

Conclusion : En effet l'inoculation des plantes par les PGPB présente une alternative pour améliorer les mécanismes de tolérance à la sécheresse dont le blé dur est confronté.

Mots clés : *Triticum durum* Dsf ; stress hydrique ; water stress; PGPB ; inoculation.

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INTRODUCTION

One of the main missions of the agricultural sector in all countries of the world is to ensure food self-sufficiency for the population and to increase cereal production. Since 1970, cereal production in Algeria has no longer met the needs of the population. Cereal cultivation occupies an estimated area of 3 million hectares. It produces only a quarter of current needs, i.e., 80 million quintals. The relatively low level of productivity, averaging 7 quintals per hectare, is mainly attributed to the rainfall deficit, global climate change, rising temperatures and soil nitrogen and phosphorus deficiencies, which pose a new threat to crops. Therefore, it is necessary to quadruple cereal production to address the cereal production deficit, either by increasing the cereal area from 3 million hectares to 11.5 million hectares, or by increasing the yield to 27.5 quintals per hectare [1]. According to the US Department of Agriculture (USDA) cereal market forecast released in May 2020, Algeria's wheat imports are expected to increase by 15.3 percent to 7.5 million tonnes in the 2020/2021 season, but remains uncertain because of the country's declining foreign exchange reserves, linked to the economic crisis caused by the coronavirus. In this case, Algeria urgently needs to develop the management of agricultural production systems towards more productive, healthy and less costly sustainable practices. Within the framework of research aimed at improving cereals, particularly durum wheat, several approaches are used, including the method based on the use of Plant Growth Promoting Rhizobacteria (PGPR) microorganisms.

New biotechnological processes for the reproduction or multiplication of cereals and nitrogen fixers can, if they are well controlled, be of great value in this field. Plant-PGPR interaction provides beneficial and reciprocal associative symbioses. The use of biotechnological applications in the form of bio-inputs represent an interesting alternative for the development of environmentally friendly agriculture and ecosystem management.

These rhizosphere microorganisms can provide several benefits to cereals by inducing tolerance mechanisms in plants subjected to various abiotic stresses [2]. Water stress generally leads to oxidative stress due to stomatal closure [3], which increased formation of reactive oxygen species (ROS) in chloroplasts and mitochondria [4]. To this end, a comparative study of the morphological, physiological and biochemical aspects of 3 varieties (Bousselem, Waha and Cirta) of durum wheat (*Triticum durum* Dsf.) inoculated by different rhizobacteria (*Azospirillum brasilense*, *Bacillus* sp., *Pseudomonas* sp. and *Frankia Cc13*) was carried out under water deficit conditions. In addition, in this study, the role of PGPRs applied alone or in combination on wheat physiology was evaluated as well as their role in wheat drought tolerance.

MATERIAL AND METHODS

1. Plant and bacterial material

The plant material used consists of three varieties of durum wheat. Table 1 lists the genotypes provided by the experimental station in El-Khroub (Constantine) of the Algerian ITGC (*Institut Technique des Grandes Cultures*).

Table 1. Characteristics of the durum wheat varieties used

Variety	Origin	Pedigree	Tolerance to drought
Waha	Cimmyt-Icarda	Plc/Ruff//Gta/3/Rolette CM 17904	Low [5]
Boussellem	Heider/Martes//Huevos de Oro	Cimmyt-Icarda	Good [6]
Cirta	ITGC, FDPS, Khroub, Algeria	KB214-0KB-20KB-OKB-OKB-1KB-0KB	Low [7]

2. Culture of *Frankia* sp. strain Cc13 *Azospirillum brasilense*, *Bacillus* sp. and *Pseudomonas* sp.

Frankia sp. strain Cc13 culture was prepared according to the method of Diem [8]. The three strains (*Azospirillum brasilense*, *Bacillus* sp. and *Pseudomonas* sp.) were cultured in LB

medium (10g/L Tryptone, 10 g/L NaCl, 5 g/L Yeast Extract) for 48 h at 25°C. Then, one loop of each culture was transferred separately into 250 mL of liquid LB medium), and incubated at 25°C while stirring (120 rpm) for 48 h. After incubation, the recovered bacterial suspension was used to inoculate the durum wheat seeds.

Table 2. Origine of the bacterial strains

Bacteria	Origin
<i>Frankia sp. strain CcI3</i>	Laboratory of Genetics, Biochemistry and Plant Biotechnology, Frères Mentouri University, Constantine, Algeria. (isolate form casuarina trees)
<i>Azospirillum brasilense</i>	Laboratory of Genetics, Biochemistry and Plant Biotechnology, Frères Mentouri University, Constantine, Algeria. (isolate form wheat field)
<i>Bacillus sp.</i>	Laboratory of Genetics, Biochemistry and Plant Biotechnology, Frères Mentouri University, Constantine, Algeria. (isolate form wheat field)
<i>Pseudomonas sp.</i>	Laboratory of Genetics, Biochemistry and Plant Biotechnology, Frères Mentouri University, Constantine, Algeria. (isolate form wheat field)

3. Sterilization, germination and inoculation of durum wheat seeds

The durum wheat seeds were first sterilized in 70% ethanol for 20 seconds, then immersed in a 10% sodium hypochlorite solution for 20 minutes followed by several rinses with sterile distilled water. Seeds were germinated by placing them in Petri dishes containing Wattmann paper soaked in water and left in the dark for 48 hours at 25° C. After germination, the durum wheat seeds were inoculated with bacterial suspensions (1×10^9 UFC) of *Frankia CcI3* and the combination of three bacteria (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) at ($1,6 \times 10^9$ UFC) to provide 1mL for each seed.

4. Experimental framework and test setting

The study was carried out according to a split-split-plot design with three replicates for a total of 54 trials each pot contains four plants. The trials were conducted in greenhouse conditions under natural light, air temperature 25 ± 4 °C (night/day), $45 \pm 80\%$ of relative humidity and 16 h photoperiod. The seeds were transplanted into 0.5 liter container, filled with an equivalent mixture of soil (soil texture Loam clay, pH=7,9 these characteristics are favorable for the development of PGPR) and sand previously disinfected. The inoculation was done as follows

Factor 1 (TB): corresponds to the bacterial treatment according to 3 levels

Level 1: No bacterial treatment

Level 2: Treatment with *Frankia cci3*

Level 3: Treatment by the combination of 3 bacteria

Factor (RH): corresponds to the water regime according to 2 levels

Level 1: No stress

Level 2: Severe stress

Factor (G): corresponds to the genotypes of durum wheat

Level 1: Waha.

Level 2: Bousselem.

Level 3: Cirta.

Water stress was caused by stopping irrigation completely from the 3-leaf stage for 10 days until the plant started to wilt. The pots under stress were irrigated each time as soon as plant wilting was observed, until the end of the stress-affected phase.

5. Morphological and biochemical parameters studied

5.1. Length of the root part (LR)

Measurements were performed at the 3-leaf stage to see the effect of bacterial strains on root elongation of the different stressed and unstressed inoculated genotypes.

5.2. Water retention capacity (water status)

Leaf turgidity was estimated by determining the Relative Water Content (RWC) [9]. The weight of wheat leaf segments was measured immediately after sampling (fresh weight) then incubated for 24 h in distilled water (fully turgid weight). The leaves were then dried in an oven at 80°C for 24 hours to obtain their constant dry weight. The RWC was calculated using the following formula :

$$RWC = \frac{(\text{fresh weight} - \text{dry weight})}{(\text{fresh weight of complete turgidity} - \text{dry weight})} \times 100 \quad (1)$$

5.3. Catalase dosage and enzyme activity determination

The activity of catalase was measured by the modified method Dory [10]. The catalase

activity was expressed in $\mu\text{mol H}_2\text{O}_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein according to the following formula :

$$\text{Activity } (\mu\text{mole of H}_2\text{O}_2 \text{ min}^{-1} \cdot \text{mg}^{-1} \text{ prot}) = \frac{(\Delta DO \cdot \text{min}^{-1} \times 1000)}{(36 \times \text{mg proteins/mL})} \quad (2)$$

5.4. Ascorbate peroxidase dosage and enzyme activity determination

Cytosolic APX activity (without ascorbate) was measured according to the method of Nakano and Asada [11].

$$\text{Activity } (\mu\text{mole of oxidised asc.} \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \cdot \text{prot.}^{-1}) = \frac{\Delta DO \cdot \text{min}^{-1} \times 1000}{(2.88 \times \text{mg proteins/mL})} \quad (3)$$

6. Data processing

The results were interpreted statistically by an analysis of variance (ANOVA with two factors: inoculation factor, treatment factor and their interaction). XLSTAT (2014.5.03) software (Addinsoft, Paris, France) was used to generate

Enzyme activity was expressed in nmoles of oxidised ascorbate per minute and per mg of protein. The conversion of the initial rate to specific activity of APX was expressed by the following formula :

the mean values and their deviations. The Newman-Keuls test classifies the means with a significance level of 5% and analysis of the correlations between the parameters measured. The correlation matrix was established on the basis of the coefficients of Pearson.

RESULTS

1. Root length

Table 3 summarises the ANOVA results; it can be noted that the water stress treatment was significant ($p < 0,05$) and the genotype responded positively to the stress and showed an increase in root length. Moreover, significant values can be observed for the combination treatment of the 3 strains (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) with wheat variety Waha (21.7 ± 1.55 cm)

($p < 0,05$). The *Frankia sp.* strain *CcI3* treatment with t wheat variety Cirta (21.9 ± 1.48 cm) ($p < 0,05$) provided the best root length compared to the controls in the absence of water stress (Figure 1). While the Boussellem variety (17.3 ± 1.25 cm) gave better rooting in the case of the non-stressed non-inoculated plants compared to the other genotypes, plants inoculated with the stressed *Frankia sp.* strain *CcI3* with Boussellem (28.5 ± 1.48 cm) showed the highest root length compared to the others.

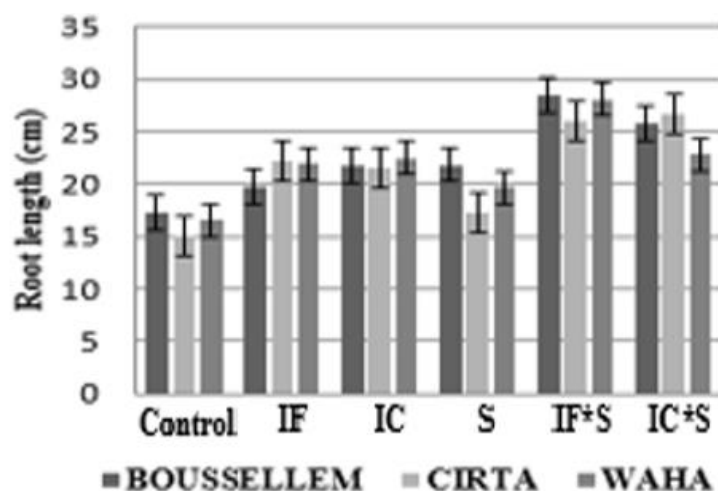


Figure 1. Variation of root length par of durum wheat varieties inoculated by *Frankia sp.* strain *CcI3* and the combination of PGPRs (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) under different conditions. Control: unstressed plant not inoculated, S: stressed plant, IF: plant inoculated with *Frankia sp.* strain *CcI3* without water stress, IC: plant inoculated with the combination (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) without water stress. IF*S: plant inoculated with *Frankia sp.* strain *CcI3* under water stress. IC*S: plant inoculated with the combination (*Azospirillum brasilense*, *Bacillus*

2. Dry weight of the root part

The ANOVA results (Table 2, Figure 2) also show a significant effect of genotype, treatment and water regime ($p < 0,05$). For the dry root weight of the plants, the analysis of the data indicates that significant values were observed for the stressed control compared to the unstressed control for all 3 varieties. Significant values can be observed for the combination treatment of the 3 strains with Boussellem wheat variety (0.19 ± 0.03 g) ($p < 0,05$) compared to the controls and the different treatments in the presence or absence of water stress.

In the case of the non-inoculated, non-stressed plants, the dry root weight of Boussellem variety ($0.13 \pm 0.025\text{g}$) ($p < 0,05$) is the highest compared to the other genotypes. However, in the case of stressed inoculated plants, the dry root weight of Boussellem and Cirta varieties

($0.088 \pm 0.04\text{g}$) ($p < 0,05$) is the highest compared to the others. On the other hand, treatment with *Frankia CcI3* increased the dry root weight for the varieties better than the combination of the 3 strains. *sp.* and *Pseudomonas sp.*) under water stress.

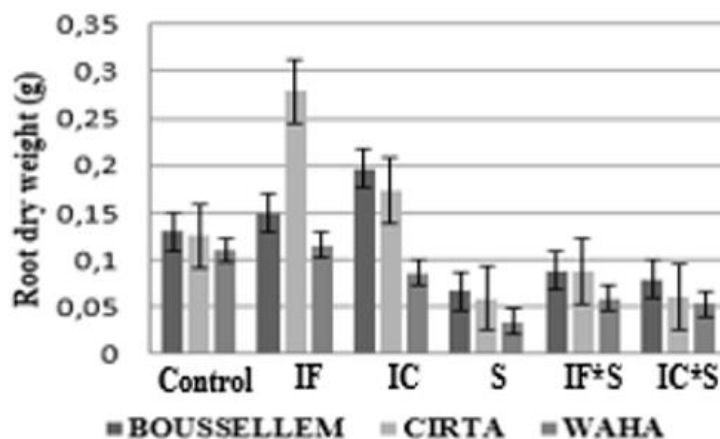


Figure 2. Variation of dry weight of the root part of durum wheat varieties inoculated by *Frankia sp.* strain *CcI3* and the combination of PGPRs (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) under different conditions. Control: unstressed plant not inoculated, S: stressed plant, IF: plant inoculated with *Frankia sp.* strain *CcI3* without water stress, IC: plant inoculated with the combination (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) without water stress. IF*S: plant inoculated with *Frankia sp.* strain *CcI3* under water stress. IC*S: plant inoculated with the combination (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) under water stress.

($p < 0,05$) compared to the controls and the different genotypes in the presence of water stress. On the other hand, treatment with *Frankia sp.* strain *CcI3* increased the length of the aerial part of Boussellem wheat variety ($32.93 \pm 1.48\text{cm}$) in water deficit conditions. In the case of non-stressed non-inoculated plants and stressed inoculated plants, the results show that the aerial length of Boussellem variety ($35.63 \pm 1.25\text{cm}$ and Waha $31.3 \pm 1.48\text{cm}$ respectively) is the highest compared to the other genotypes. The treatment with *Frankia sp.* strain *CcI3* strain allowed to increase the length of the aerial part for the 2 varieties Waha and Boussellem better than the combination of the 3 strains. On the other hand, the Waha variety inoculated with the combination of the 3 strains stimulated the growth of the aerial part with a greater length than that inoculated with *Frankia sp.* strain *CcI3*.

3. Length of the aerial part

Analysis of the data (Figure 3) on the length of the aerial part of the plants shows that significant values were observed for the stressed control compared to the unstressed control for all 3 varieties. Inoculation with the combination of the 3 strains significantly increased the aerial length of Boussellem wheat variety ($32 \pm 1.6\text{cm}$)

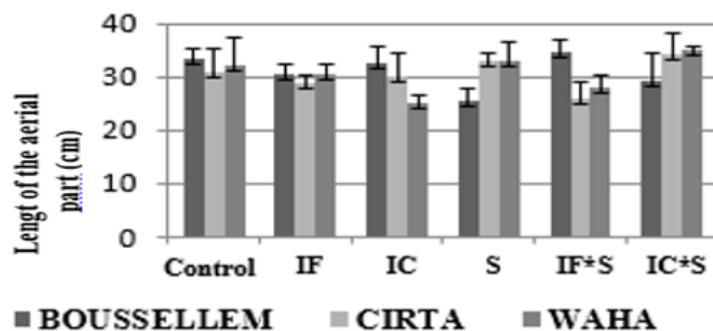


Figure 3. Variation of length of the aerial part of durum wheat varieties inoculated by *Frankia sp.* strain *CcI3* and the combination of PGPRs (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) under different conditions. Control: unstressed plant not inoculated, S: stressed plant, IF: plant inoculated with *Frankia sp.* strain *CcI3* without water stress, IC: plant inoculated with the combination (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) without water stress. IF*S: plant inoculated with *Frankia sp.* strain *CcI3* under water stress. IC*S: plant inoculated with the combination (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) under water stress

4. Dry weight of the aerial part

According to the ANOVA results (Table 2), significant values were found with the stressed control compared to the unstressed control for all 3 varieties. The inoculation treatment results with *Frankia sp.* strain *CcI3* show significant values with the wheat variety Bousselem (0.28 ± 0.04 g) compared to the stressed control. Also under water stress *Frankia sp.* strain *CcI3* treatment with Bousselem wheat variety (0.16 ± 0.03 g) ($p < 0,05$) shows significant dry weight values compared to the controls and the different treatments under water stress.

In the case of non-stressed, non-inoculated plants, it can be seen that the aerial dry weight of the Bousselem variety (0.25 ± 0.025 cm) ($p < 0,05$) is the highest compared to the other genotypes. However, in the case of stressed inoculated plants, the aerial dry weight of the Cirta variety (0.12 ± 0.04 cm) ($p < 0,05$) inoculated with the combination is the highest compared to the others, and the treatment with *Frankia sp.* strain *CcI3* increases the aerial dry weight for Waha variety better than the combination of the 3 strains.

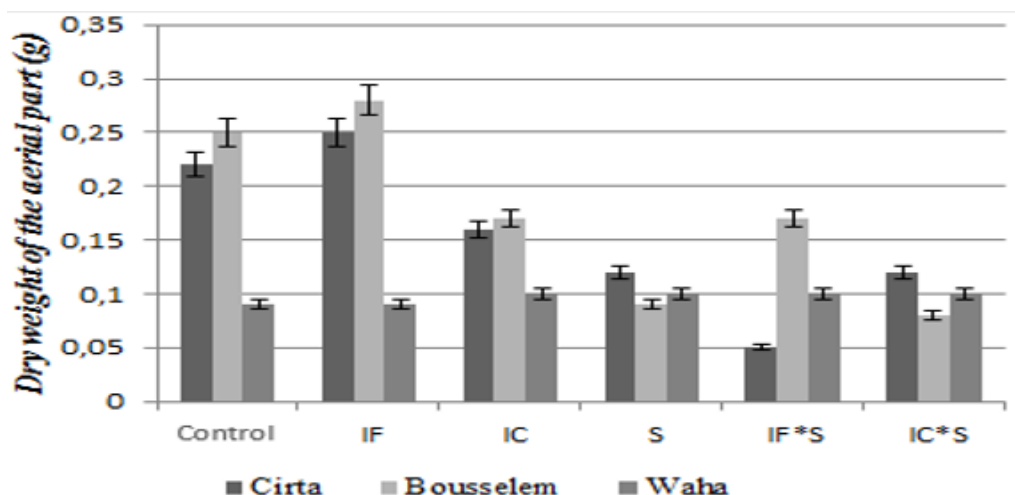


Figure 4. Variation of dry weight of the aerial part of durum wheat varieties inoculated by *Frankia sp.* strain *CcI3* and the combination of PGPRs (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) under different conditions. Control: unstressed plant not inoculated, S: stressed plant, IF: plant inoculated with *Frankia sp.* strain *CcI3* without water stress, IC: plant inoculated with the combination (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) without water stress. IF*S: plant inoculated with *Frankia sp.* strain *CcI3* under water stress. IC*S: plant inoculated with the combination (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) under water stress.

5. Relative water content (RWC)

RWC data (Figure 5) show significant values for the stressed control compared to the unstressed control for all 3 varieties. However, when the 3 strains were treated together, significant values are observed for Waha wheat variety (81.8 ± 1.8 cm) ($p < 0,05$), compared to the stressed control in the presence of water stress. While *Frankia sp.* strain *CcI3* treatment showed significant values with the Cirta wheat variety (80.07 ± 1.48 cm) ($p < 0,05$) compared to the controls stressed in the presence of water stress.

In the case of the non-stressed non-inoculated plants, it can be seen that the relative water content of Waha variety (84.04 ± 1.25 cm) ($p < 0,05$) is the highest compared to the other genotypes. However, in the case of stressed inoculated plants, the relative water content of Waha variety (69.01 ± 1.48 cm) ($p < 0,05$) inoculated with *Frankia sp.* strain *CcI3* is the highest compared to the other genotypes. Furthermore, the treatment with the combination of the 3 strains increases the relative water content for the other 2 varieties better than *Frankia sp.* strain *CcI3*.

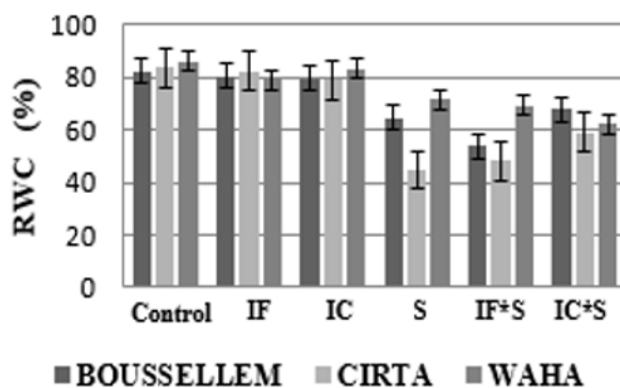


Figure 5. Variation of root length par of durum wheat varieties inoculated by *Frankia sp.* strain *CcI3* and the combination of PGPBs (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) under different conditions. Control: unstressed plant not inoculated, S: stressed plant, IF: plant inoculated with *Frankia sp.* strain *CcI3* without water stress, IC: plant inoculated with the combination (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) without water stress. IF*S: plant inoculated with *Frankia sp.* strain *CcI3* under water stress. IC*S: plant inoculated with the combination (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) under water stress

6. Total chlorophyll rate

The results of the total chlorophyll analysis showed variations in chlorophyll according to genotype. It can be observed that the Waha genotype inoculated with combinaison has the highest rate as a control (45.77 SPAD unit). The analysis of variance (Table 2) shows that the water stress treatment is significant ($p < 0.05$) on this criterion; the genotypes responded positively to the stress. Therefore, an increase in chlorophyll content compared to the control was obtained. According to Streb and Feierabend [12], plants under water stress become less

green and show wilting on principle, which is not the case in this study. The interaction of the 3 treatments (water regime, genotype and inoculation) was not significant ($p > 0.1$). However, the results show that the treatment with *Frankia sp.* strain *CcI3* bacteria under water deficit conditions is the most important for the Waha (42.1 SPAD unit) and Boussellem (41.3 SPAD unit) genotypes. These findings are explained by the establishment of a symbiosis between these two genotypes and *Frankia sp.* strain *CcI3*; consequently, the plants benefited from this symbiosis.

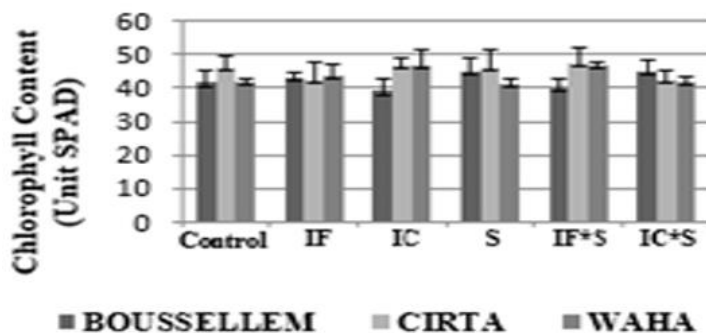


Figure 6. Variation of Total chlorophyll rat eof durum wheat varieties inoculated by *Frankia sp.* strain *CcI3* and the combination of PGPBs (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) under different conditions. Control: unstressed plant not inoculated, S: stressed plant, IF: plant inoculated with *Frankia sp.* strain *CcI3* without water stress, IC: plant inoculated with the combination (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) without water stress. IF*S: plant inoculated with *Frankia sp.* strain *CcI3* under water stress. IC*S: plant inoculated with the combination (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) under water stress

7. Total soluble protein

The increased production and accumulation of solute-compatible products to osmotically adjust and protect against oxidative stress are among the plant responses to abiotic stresses such as drought and salinity [13]. The results reported in (Figure 7) suggest that the protein

content in the control batches is higher in Waha variety (12.48 mg/g MF) than its counterparts Boussellem and Cirta. The ANOVA results (Table 2) indicate that the water stress treatment was significant ($p < 0.05$) the total soluble protein content of the stressed plants increased significantly,

indicating that drought stress accelerates soluble protein accumulation. Furthermore, the results indicate that inoculation had a significant effect ($p < 0.05$); the inoculated plants have a higher protein content than the controls, noting that inoculation by the combination *Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.* gave better results. The interaction of the 3 treatments (genotypes, water

diet and inoculation) is significant ($p < 0.05$). Cirta genotype inoculated by the combination *Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.* gave the best protein content under water stress conditions (15.58 mg/g FVM) followed by Bousellem (13.66 mg/g FVM); Waha genotype gave best results with *Frankia sp.* strain *CcI3* inoculation.

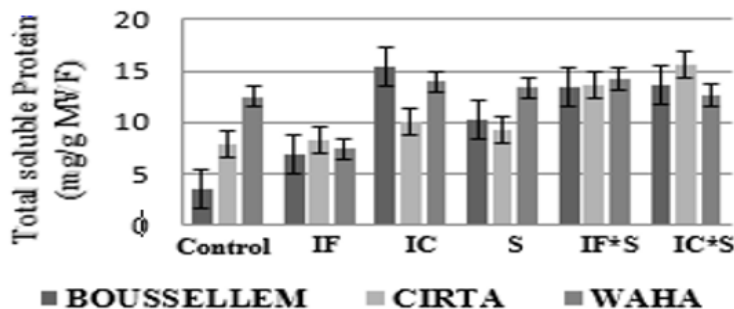


Figure 7. Variation of total soluble protein of durum wheat varieties inoculated by *Frankia sp.* strain *CcI3* and the combination of PGPBs (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) under different conditions. Control: unstressed plant not inoculated, S: stressed plant, IF: plant inoculated with *Frankia sp.* strain *CcI3* without water stress, IC: plant inoculated with the combination (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) without water stress. IF*S: plant inoculated with *Frankia sp.* strain *CcI3* under water stress. IC*S: plant inoculated with the combination (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) under water stress.

8. Catalase dosage

The results indicate (Figure 8) that in non-stressed conditions, the activity of the antioxidant enzyme Catalase varies little between inoculated and non-inoculated treatments. The ANOVA results (Table 2) show that water stress has a highly significant effect ($p < 0.01$), there is a significant increase in enzyme activity in inoculated and non-inoculated treatments of the antioxidant enzyme catalase in all three genotypes compared to the non-stressed controls noting that the Waha genotype had the highest enzyme activity under stress (14 $\mu\text{mol H}_2\text{O}_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein).

A rapid and continuous increase in CAT activity could indicate that CAT is a major enzyme detoxifying hydrogen peroxide (H_2O_2) which accumulates in water deficiency [14]. Inoculation with the combination (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) significantly increased the enzymatic activity of catalase for all three genotypes, indicating the synergy of the two treatments. On the other hand, inoculation with the *Frankia CcI3* strain has a slight effect on the enzymatic activity of the Cirta genotype.



Figure 8 : Variation of the enzymatic activity of catalase of durum wheat varieties inoculated by *Frankia sp.* strain *CcI3* and the combination of PGPRs (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) under different conditions. Control: unstressed plant not inoculated, S: stressed plant, IF: plant inoculated with *Frankia sp.* strain *CcI3* without water stress, IC: plant inoculated with the combination (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) without water stress. IF*S: plant inoculated with *Frankia sp.* strain *CcI3* under water stress. IC*S: plant inoculated with the combination (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) under water stress.

9. Ascorbate peroxidase dosage

The results of the enzymatic activity of ascorbate peroxidase are similar to the results of the catalysis activity. Figure 9 shows that the Bousselem control presents the highest activity of APX antioxidant enzymes, which translates into a high level of total protein content, without stress. The enzymatic activity is important with the bacterial treatment of the combination of three strains (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*). A remarkable increase in activity with *Frankia sp.* strain

CcI3 with the three genotypes can be observed. This increase reflects the beneficial effect of PGPBs on the antioxidant defence of plants, despite the fact that they are not under water stress or oxidative stress. Furthermore, under water stress, a significant increase in enzymatic activity is observed and with the two treatments with *Frankia sp.* strain *CcI3* and with the three bacterial strains. This is confirmed by the ANOVA (Table 2) where a highly significant effect ($p < 0.001$) can be observed.

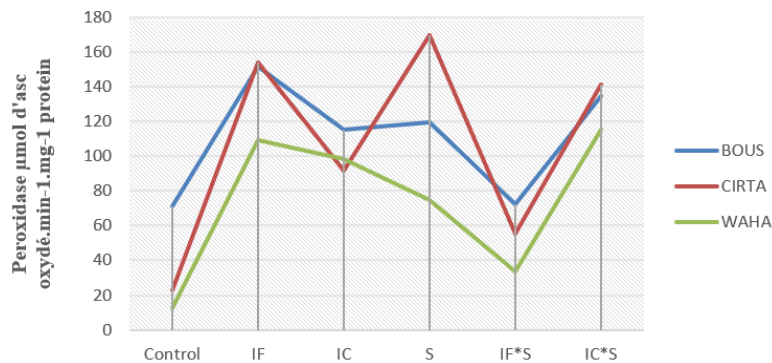


Figure 9. Variation of the enzymatic activity of ascorbate peroxidase of durum wheat varieties inoculated by *Frankia sp.* strain *CcI3* and the combination of PGPBs (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) under different conditions. Control: unstressed plant not inoculated, S: stressed plant, IF: plant inoculated with *Frankia sp.* strain *CcI3* without water stress, IC: plant inoculated with the combination (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) without water stress. IF*S: plant inoculated with *Frankia sp.* strain *CcI3* under water stress. IC*S: plant inoculated with the combination (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) under water stress

Table 3. Roots length, length of the aerial part, rate of chlorophyll (SPAD), root dry weight, leaf dry weight, RWC, Soluble protein, Catalase, Peroxydase, wheat plants Cirta, Bousselem and Waha inoculated or not stressed or not with *Frankia sp.* strain *CcI3* strains or combinaison (*Azospirillum brasilense*, *Bacillus sp.* et *Pseudomonas sp.*).

Variables	Treatments (T) ¹						Genotypes (G)			Significance (p value) ²		
	Control	TS	IF	IC	IF*S	IC*S	Cirta	Bousselem	Waha	T	G	T*G
Length of the aerial part (cm)	32,2 a	30 a	29,3 a	30,5 a	29,7 a	32,9 a	30,6 a	31 a	30,7 a	0,104	0,931	0,001 **
Roots length(cm)	22,6 ab	22,1 ab	19,3 b	21,7 ab	21,6 ab	24,7 a	20,3 b	23,3 a	22,3 ab	0,069	0,040	0,000***
Weight of the aerial part (g)	0,2 ab	0,1 b	0,1 b	0,1 ab	0,1 ab	0,2 a	0,2 a	0,2 a	0,1 a	0,005	0,142	0,002 **
Root dry weight(g)	0,2 a	0,1 b	0,1 ab	0,1 ab	0,1 ab	0,1 ab	0,1 b	0,1 a	0,1 a	0,045	0,002	0,005**
RWC (%)	84,1 a	64,9 b	68,4 ab	76,7 ab	61,8 b	69,8 ab	69,9 a	72,4 a	70,5 a	0,002	0,798	0,001**
SPAD (SPAD Unit)	43,3 a	43,2 a	44,1 a	44 a	44,8 a	43 a	45,3 a	42,2 b	43,7 ab	0,869	0,032	0,041**
Soluble protein (mg/g MVF)	10,4 ab	12,2 a	13,5 a	12,3 a	11,5 ab	7,4 b	10,5 b	11,9 a	11,2 a	0,006	0,451	0,007**
Catalase(µmol H ₂ O ₂ .min-1.mg-1 protéine)	3,1 e	9,6 b	4,7 c	2,7 e	7,6 c	11,6 a	6 b	6 b	7,6 a	0,000	0,000	0,000***
Peroxydase(µmol of oxidised asc.min-1.mg-1 protéine)	35,5 e	138,3 a	101,7 c	121,3 b	53,7 d	130,4 ab	105,7 a	110,9 a	73,8 b	0,000	0,000	0,000***

¹TS: control stressed; IF: inoculation with *Frankia* sp. strain Cc13, IC: inoculation with combination (*Azospirillum brasilense*, *Bacillus* sp et *Pseudomonas* sp), IF*S: Stressed plant inoculated with *Frankia* sp. strain Cc13, IC*S: Stressed plant inoculated with (*Azospirillum brasilense*, *Bacillus* sp et *Pseudomonas* sp), G: genotype, T:Treatment, T*G: interaction Treatment genotype. ²Significances: <0.1: *, <0.05: **, <0.001: ***, <0.0001: **** the results in the same line followed by the same letter are not significantly different, according to the test Newman-Keuls test ($p < 0.05$).

Table 4. Correlation matrix of measured durum wheat parameters

Variables	Rate of chlorophyll (Unit SPAD)	Aerial length (cm)	Root length (cm)	Dry weight of aerial part (g)	Root dry weight (g)	Total soluble Protein (mg/g MVF)	RWC%	Catalase (μmol H2O2.min-1.mg-1 protein)	Peroxidase (μmol d'asc oxydé.min-1.mg-1 protein)
Rate of chlorophyll (Unit SPAD)	1								
Length of aerial part (cm)	-0,296**	1							
Root length (cm)	-0,364**	0,436***	1						
Dry weight of aerial part (g)	-0,214	0,720***	0,554**	1					
Root dry weight (g)	-0,382**	0,390**	0,351**	0,397**	1				
Total soluble Protein (mg/g MVF)	0,301**	-0,356*	-0,347*	-0,519***	-0,3446	1			
RWC (%)	-0,291**	0,526***	0,3180	0,380**	0,402**	-0,250	1		
Catalase (μmol H2O2.min-1.mg-1 protein)	0,000	0,109	0,210	0,149	-0,113	-0,218	-0,261	1	
Peroxidase (μmol of oxidised asc. min-1.mg-1 protein)	-0,028	0,036	0,023	0,028	-0,192	-0,013	-0,103	0,2714**	1

Significances :< (0.1): *, < (0.05): **, < (0.001): ***, < (0.0001): ****.

10. Correlation matrix

Analysis of the correlations between the parameters measured shows that there are positive and negative relationships ranging from low to high correlation (Table 3). The knowledge of the relationships between the different traits allows the selection of the most discriminating variable. This is the significant correlation (Table 3) between the length of the aerial part and the dry weight of the aerial part (r=0.720) and the length of the roots was positively and significantly correlated with the dry weight of the root (r=0.554). The results for the dry weight of the aerial and root parts show that dry matter production was improved by inoculation. The inoculation resulted in an increase in the dry weight of the aerial and root parts of the durum wheat varieties compared to the controls.

These results have been mentioned by several authors on various crops. For example, the work of Pedraza [15] on strawberries shows that root inoculation with strains of *Azospirillum brasilense* has a high rate of infection of the root system, resulting in high dry matter production in the aerial part and especially in the root part. This explains the accumulation of root dry weight in unstressed inoculated plants, as well as in plants inoculated in the presence of water stress. However, it has a negative correlation with the dry weight of the aerial part and total protein (r=-0.519). Other significant positive correlations were observed between the length of the aerial part and the ERR (r=0.526) and

between the dry weight of the roots and the ERR (r=0.4024). Moisture content is considered to be one of the most widely used and significant dehydration tolerance indexes. Tolerant genotypes have a high ERR in contrast to susceptible genotypes [16]. Stressed plants lose more water than those in favourable conditions. Indeed, the greater the assimilative surface area, the greater the loss and show a large decrease in ERR [17]. The most common adaptation technique used by plants is leaf curling to minimise the rate of water loss during stress [18].

DISCUSSION

1. Effect of PGPBs on root length and aerial length of plants under water stress conditions

Root length results of plants inoculated with bacterial strains significantly improved shoot length, root length, fresh and dry biomass of wheat seedlings at all field capacity levels compared to the uninoculated control. The efficiency of water extraction from the soil by the roots is one of the types of adaptation that allows the plant to avoid or, more precisely, to delay the dehydration of its tissues. Ashrafuzzaman [19] reported that phytohormones can be produced by PGPBs, which can stimulate plant development by affecting root elongation, division and cellular differentiation. In addition, they play a very important role in the plant's response to biotic and abiotic stresses.

Some bacteria can produce auxins such as *Azospirillum*, *Pseudomonas*, *Xanthomonas* and *Rhizobium*. In addition to auxin, *Azospirillum* can synthesize cytokinin and gibberillin. These results were reported by Singh [20] and Egamberdieva [21]. The authors showed that inoculation of wheat and rice plants with PGPBs (*Pseudomonas sp.* strain) under stress conditions (salt stress) resulted in an increase in root length, fresh and dry weight of roots.

Comparing the length of the aerial part of the witness and the stressed witness, it can be noticed that they are almost the same length. This is due to a reduction in growth, reorientation of cellular energy and organic elements produced by the plant towards lengthening the roots to increase the absorption network and capture water and mineral elements according to several mechanisms [22]. This can be done by minimising the leaf surface area, closing the stomata to prevent water loss through evapotranspiration and reducing CO₂ absorption. Consequently, this can lead to a reduction in photosynthesis resulting in reduced leaf expansion, damage to the photosynthetic apparatus and degradation of total chlorophyll. Comparing the stressed control, the inoculated control and the stressed inoculated control, a more or less significant increase in the length of the aerial part of the inoculated plants can be observed. This is due to the maintenance of growth in the aerial part and stimulation of the production of organic elements by the plant inoculated with the bacteria by maintaining the opening of the stomata for the increase of CO₂ absorption, consequently it can cause a stimulation of photosynthesis.

2. Effect of PGPBs on the dry root weight and the dry weight of the plant aerial part in water stress conditions

The results of the root dry weight are similar with those reported by Jaleel [23]. Therefore, those rhizobacteria (*Pseudomonas fluorescens*) can directly stimulate plant growth under water deficit stress by increasing the availability of soil nutrients, such as atmospheric nitrogen fixation, solubilisation of minerals by phosphorus and iron, production of siderophors and enzymes, induction and production of growth regulators and activation of induced resistance mechanisms.

Consequently, they indirectly stimulate plant growth through their antagonistic effect on harmful microflora by transforming toxic metabolites and through the production of antibiotics or hydrocyanic acid, competition for nutrients, production of extracellular enzymes. Concerning the aerial dry weight of the plants, several studies have reported results on various crops. These include the work of Pedraza [15] on strawberry plants where inoculation of the roots with *Azospirillum brasilense sp.* strains has a high rate of infection of the root system, resulting in a high production of dry matter in the aerial part. This explains the accumulation of aerial dry weight in unstressed inoculated plants, as well as inoculated plants under water stress. In addition, Goral and Alen [24] showed that the inoculation process with *Azospirillum* and *Bacillus spp.* showed a clear accumulation of these minerals in the plant tissues. Moreover, several studies have highlighted the phytobeneficial effect of rhizobacteria on plants subjected to abiotic stress.

3. Effect of PGPBs on the relative water content of plants under water stress conditions

The results of the effect of PGPBs on the relative water content of plants under water stress conditions can be explained by the action of PGPBs which triggers one of the most common stress resistance strategies in plants. This strategy is the overproduction of different types of organic solutes according to Streb [12]. PGPBs promote the production of compatible solutes which are highly soluble, low molecular weight compounds and are generally non-toxic, even at high cytosolic concentrations. Generally, they protect plants from stress by various means such as contributing to osmotic adjustment, detoxification of reactive oxygen species (ROSs), membrane stabilisation, and native enzyme and protein structures. Osmotic adjustment is a mechanism for maintaining water stress relationships under osmotic stress. It involves the accumulation of a range of osmotically active molecules/ions, including soluble sugars, alcohols, proline, glycinebetaine, organic acids, calcium, potassium, chloride ions, etc. Matos *et al.* [25] found that for plants of different crops subjected to water stress and PGPB inoculation and as a consequence of the accumulation of solutes, the osmotic potential of the cell is decreased, which attracts water into the cell and contributes with the maintenance of turgidity.

4. Effect of PGPBs on leaf chlorophyll content and soluble protein concentration

The increase in total chlorophyll content is the consequence of the reduction in leaf cell size under the effect of water stress which causes chlorophyll condensation [26]. In this study, treatment with *Frankia Cc13* bacteria under water deficit conditions was the most important for the Cirta (48.4 SPAD unit) and Waha (46.73 SPAD unit) genotypes, which is explained by the establishment of a symbiosis between these two genotypes and *Frankia Cc13* and consequently the plants benefited from this symbiosis. Chlorophyll levels for the 3 genotypes were influenced by several factors such as leaf age, leaf position, and environmental factors such as light, temperature and water availability and soil composition [27]. This result reflects the insignificance of the interaction of the 3 treatments. Plants subjected to water stress respond with protein accumulation to protect themselves against drought, protein accumulation influences various physiological mechanisms, such as osmotic adjustment through the synthesis of proline and other amino acids, detoxification of ROS by antioxidant enzymes (Catalase and Ascorbate peroxidase) [28]. These mechanisms are improved by inoculation with PGPBs, which explains the increase in total proteins in the inoculated plants.

5. Role of PGPBs in osmotic adjustment and attenuation of oxidative stress

On the one hand, the results of the catalase assay confirm the improving effect of PGPBs on antioxidant resistance. Many studies have reported the effectiveness of PGPBs under stressful conditions in protecting plants against the harmful effects of environmental stresses [29, 30, 31]. Inoculation of lettuce (*Lactuca stiva* L.) with *Pseudomonas mendocina* increased catalase activity under water stress conditions, suggesting that PGPBs can be used as a solution for oxidative damage caused by water stress. In another experiment the treatment of Basil plants (*Ocimum basilicum* L.) with *Pseudomonas sp.* under water stress significantly increased the activity of the catalase enzyme. PGPBs ensure drought tolerance by overcoming the deleterious effects posed by drought caused by hormonal changes such as the level of IAA (indole acetic acid) and ethylene which promote root elongation and increased water absorption which is further promoted by improving the accumulation of

solutes in the plant cell which prevents water loss.

On the other hand, exposure of plants to water stress leads to the generation of ROS, including anionic superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), and alkoxy radicals (RO). ROS react with proteins, lipids and deoxyribonucleic acid, causing oxidative damage and altering the normal functions of plant cells. Plants develop antioxidant defence systems comprising both enzymatic and non-enzymatic components to overcome these effects, which serve to prevent the accumulation of ROS and mitigate oxidative damage during drought stress [32]. Enzyme components include catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR). The non-enzymatic components contain cysteine, glutathione and ascorbic acid [33]. It is noteworthy that despite the significant interaction between drought stress and antioxidant enzyme activity, treatment with PGPB strains tends to reduce the drought stress effect on enzyme activity [34].

CONCLUSION

This study demonstrated that the inoculation of durum wheat varieties (*Triticum durum* Dsf.) with PGPB strains improves the morphological aspect of the plant under favorable or unfavorable conditions such as root and aerial length elongation, aerial part and dry weight. The inoculation of *Triticum durum* Dsf. with the *Frankia Cc13* strain considerably improved the size of the root and aerial system in favorable conditions. Therefore, it maximized water uptake into the soil during severe stress and thus contributed to maintaining increased dry matter production. The result demonstrates the effectiveness of PGPBs in improving the prevention of wheat dehydration during water stress. Moreover, the results indicate that the different genotypes responded differently depending on each parameter studied and the type of treatment. As an example, the *Frankia Cc13* inoculum improved root growth and functions contributing to dry matter production during drought periods in the Boussellem and Cirta varieties.

However, inoculations by the combination of the three strains of bacteria (*Azospirillum brasilense*, *Bacillus sp.* and *Pseudomonas sp.*) have a higher efficiency compared to the inoculation of a single bacterium in terms of production of soluble proteins and the antioxidant enzymes Catalase and Ascorbate peroxidase necessary for the maintenance of water status especially in the Cirta and Waha varieties.

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