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DIFFERENTIAL RESPONSE OF *Rhizobium leguminosarum* INOCULATION FOR INDUCING WATER DEFICIT TOLERANCE IN *Triticum durum*

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ABSTRACT

Limited soil water availability is a major threat to agricultural productivity because it inhibits plant growth and yields. Soil microorganisms having the ability to alleviate abiotic stress and promoting plant development would be highly valuable tools in sustainable agriculture. To this purpose, two *Rhizobium leguminosarum* strains were tested for their potential to induce tolerance against water deficits in *Triticum durum* grown under two hydric conditions in greenhouse experiments. For this, physiological and biochemical parameters were measured as criteria to assess the effect of *R. leguminosarum* inoculation on *T. durum* under water deficiency. In the presence of rhizobia, wheat plants were able to withstand water stress more effectively than un-inoculated plant, as indicated by the recorded increases in relative water content of inoculated plants compared to un-inoculated plants, higher accumulation of osmoprotectant (soluble sugar), lower MDA and H_2O_2 accumulation, and increased in antioxidant responses (peroxidase). Under water-limited condition, co-inoculation of selected rhizobia was better than single application for one of studied genotype, which let us hypothesize that this variation in responsiveness indicates a possible genotype effect. As a result, rhizobia from various legumes have a huge potential for enhancing the water deficiency stress tolerance of cereals.

Keywords: antioxidants, *Triticum durum*, *Rhizobium leguminosarum*, water deficit tolerance.
Abbreviations: waha (WH), oued zenati (OZ), relative water content (RWC), malondialdehyde (MDA), catalase (CAT), gaiacol peroxidase (GPX).

INTRODUCTION

Crop production has been subjected to a multitude of abiotic stresses as a result of global warming and climate change events across the world (Hussain et al., 2018). Droughts is a serious challenge to agricultural productivity in arid and semi-arid parts of the world (Ullah et al., 2017). Durum wheat (*Triticum turgidum* L. var *durum* Desf.) is the 10th most important and commonly cultivated cereal worldwide with a yearly production average of 40 million tonnes (MT) (2016/17). Durum wheat cultivation typically accounts for 5% of total wheat production, with a global planting area of 16 million hectares [International Grains Council (IGC), 2020]. Drought stress adversely affects the development and growth of wheat such as flowering and physiological maturity (Farooq et al., 2012). Moreover, it inhibits plant growth by disturbing various biochemical and physiological processes, including nutrient metabolism, uptake of essential ions, respiration, translocation of carbohydrates, and photosynthesis (Farooq et al., 2008) and by lowering transpiration rate and stomatal conductance (Zhan et al., 2011). Additionally, plants submitted to drought stress are seriously affected by secondary damages caused by oxidative stresses. These oxidative damages are consequences of reactive oxygen species (ROS) that are able to rapidly react with a wide range of biomolecules causing therefore irreversible damages leading to cell necrosis and cell death (Raja et al., 2017). Keeping in view the problem, various researchers have tried different physical, chemical and biological strategies to ameliorate or reduce the impact of drought on plant growth and yield (Hussain et al., 2011).

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However, beneficial plant interactions with microbes can improve the fitness of crop plants under various environmental stresses (Khan et al., 2017), including water deficit stress (Nadeem et al., 2014). Additionally, the inoculation with plant beneficial bacteria remained advantageous over chemical or physical treatments whether in terms of economic returns or environmental sustainability (Hussain et al., 2018). *Rhizobium* is a large group of nodule-forming bacteria, known for their beneficial symbiotic association with legumes. They fix nitrogen by developing nodules on the roots of legumes, where they use nitrogenases to fix atmospheric di-nitrogen. *Rhizobium* were believed to be exclusively advantageous to legumes, but several researchers have recently demonstrated their potential to improve the growth of non-legumes (cereals) via indirect and direct mechanisms or strategies. Among this cereals we found wheat (Hussain et al., 2014a; Ullah et al., 2017), rice (Hussain et al., 2009), and maize (Hussain et al., 2016).

When rhizobia colonize the roots from non-legume plant in a nonspecific relationship, the strains from this genus may behave as Plant Growth-Promoting Rhizobacteria (PGPR) (Saharan and Nehra, 2011). Rhizobia ameliorate also adverse impacts of stresses and induce tolerance in plants by adopting different mechanisms, including induction of systemic tolerance by certain chemical or physical changes (Hussain et al., 2014b). These characteristics may include the production of organic compounds like trehalose, phytohormones, siderophores, up-regulation of enzymatic/ non-enzymatic antioxidant activity, biosynthesis of plant compatible solutes/ osmolytes and enhancing the availability of essential nutrients by mechanisms such as phosphate solubilization (Hansen et al., 2017).

This study aimed to investigate the role of two different *Rhizobium leguminosarum* strains on the drought tolerance of two *Triticum durum* genotypes. This could potentially lead to explore a side of culture rotations of cereals-leguminous sequence. Thus, there is considerable potential for residual rhizobia to colonize the root system of germinating wheat seedlings (Ullah et al., 2017). We proposed to investigate the role of *R. leguminosarum* inoculation under drought and its effect on physiological and biochemical responses in leaves of *T. durum*. We measured physiological parameters to determine water status (RWC and stomatal conductance), osmolyte accumulation (soluble sugar), oxidative stress markers (MDA and H_2O_2 Content), enzyme activities (Catalase and peroxidase) as parameters to assess the effect of *R. leguminosarum* inoculation on *T. durum* under water deficiency in greenhouse experiments.

MATERIAL AND METHODS

1. Biological material and growth conditions

In this study, two durum wheat (*Triticum Durum*) genotypes, Waha (WH) and Oued Zenati (OZ), inoculated with two selected *Rhizobium leguminosarum* strains were evaluated in pot experiment for inducing water deficit stress tolerance. The experiment was conducted under greenhouse conditions at the laboratory of Plant Genetics, Biochemistry and Biotechnology, University of Mentouri Brothers, Constantine I, Algeria, during the winter of 2020 under natural light, air temperature between 22°C±4°C (night/day), 50±80% of relative humidity and 16 h of photoperiod. The two genotypes were received from the Technical Institute of Field Crops (ITGC, Constantine, Algeria).

Before inoculation, seeds of the two genotypes were surface sterilized with 70% Ethanol. After eliminating the ethanol solution, the seeds were immerged in 3% sodium hypochlorite solution, and then were rinsed with sterile deionized water. The two selected rhizobial strains were evaluated in a pot experiment for inducing water deficit stress tolerance in durum wheat. The first Rhizobial strain was *Rhizobium leguminosarum bv. viciae* 3841, able to nodulates legumes in the Tribe *Viciae* - *Vicia*, *Pisum*, *Lathyrus*, *Lens*. It was received from the Sophia Agrobiotech Institute, Nice,

France. The second rhizobial strains was isolated from the nodule of *Lens culinaris* from a semi-arid algerian region, strain OL13 (Riah, 2015). Rhizobial cells were harvested by centrifugation at 5000 × g for 20 min (twice 10 min) after culturing in Yeast Mannitol Broth. Then, bacterial cells (pellets) were washed and suspended in 0.9% NaCl solution and uniform bacterial cell density (10^8 CFU.mL^{-1}) was achieved by maintaining the optical density at a wavelength of 600 nm by a spectrophotometer. This rhizobial cells suspension was used as inoculum.

Wheat seeds were inoculated by soaking for 20 min having 5% of soluble starch as an adhesive agent. Control wheat seeds (un-inoculated plants) were soaked with the same adhesive agent, but sterilized saline solution (0.9% NaCl) was used instead of living bacterial cells. The seeds of the two genotypes were inoculated individually with the two rhizobial strains. In the case of co-inoculation, the two inoculums of the rhizobial strains were mixed in equal proportions and vortexed for 5 min to ensure cell density homogenization before seeds soaking. The inoculated and uninoculated (control) wheat seeds were left to germinate separately in sterile cotton at 23°C for four days. Seedlings with homogenous stages of development were selected and sown at 3-cm depth in 7 L plastic pots (seven seeds per pot), filled with 5 kg of sterilized soil, passed through a 2-mm sieve. Soil was classified as sandy clay loam. The soil moisture was kept at 80% field capacity (FC) in all pots by applying water on alternate days to replace the loss of water due to evaporation until the induction of water deficit treatment.

At the end of ear emergence stage GS 50-59 (Zadoks Growth Stage); 60-65 days after sowing (DAS), water deficit was applied by maintaining the soil moisture at 40% of substrate (FC) for the stressed plants, and 80% (FC) for the well-watered plants. With six replicates per treatment and genotype, the experimental pots were set up in a completely randomized design.

2. Assessment of physiological and biochemical parameters

2.1. Relative Water Content (RWC) and Stomatal conductance

After 20 days of water deficit (end of flowering stage (80-85 DAS), measurements and flag leaves harvesting were taken between 11:00 and 13:00 local time. Stomatal conductance was measured using a porometer (AP4 Delta-T Devices, Cambridge, UK) on the abaxial surface of the flag leaf once per plant. Flag leaves were harvested and used to determine the relative water content (RWC %) by using the equation (1):

$$\text{RWC}\ (\%) = \frac{(\text{Fresh weight} - \text{Dry weight})}{(\text{Turgid weight} - \text{Dry weight})} * 100 \quad (1)$$

Three replicates per plant and treatment were performed. Relative water content (RWC) is commonly used to characterize a plant's water balance.

After 20 days of water deficit (end of flowering stage), flag leaf of plants were harvested and immediately frozen in liquid nitrogen, and conserved in -80°C for total soluble sugar, oxidative stress markers and enzymes measurements.

2.2. Soluble sugar contents

After 20 days of water stress, soluble sugar content was determined using the method described by Dubois et al. (1956). The soluble sugar content was revealed through absorbance at 485 nm. Soluble sugar content was estimated by referring to a standard curve, constructed using glucose, and was expressed as mg soluble sugar. g^{-1} FW. Three replicates per genotype per treatment were performed.

2.3. Lipid peroxidation and hydrogen peroxide content

After 20 days of water stress, flag leaves were harvested for oxidative stress indicator measurements. Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) produced by the

thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). The MDA concentration was calculated from the absorbance at 532 nm (unspecific turbidity was corrected by subtracting the absorbance at 600 nm) by using an extinction coefficient of 155 mM^{-1}. cm^{-1} and the results were expressed as nmol MDA. g^{-1} FW. Three replicates per genotype per treatment were performed.

Hydrogen peroxide (H_2O_2) was measured as described by Velikova et al. (2000). The absorbance of the supernatant was measured at 390 nm. The molar extinction coefficient (0.28 $\mu M^{-1}.cm^{-1}$) was used to calculate the H_2O_2 concentration, which was reported as nmol H_2O_2. g^{-1} FW. Each treatment included three replicates.

2.4. Antioxidant enzymes activities

For protein and antioxidant enzyme assays, 0.25 g of frozen flag leaf samples from control and treated plants were ground to a fine powder in liquid nitrogen and homogenized on ice in 2 ml extraction buffer of 50 mM sodium phosphate buffer (pH 7.8) containing 0.1 mM EDTA, 0.1 % (v/v) Triton X-100, 1 mM phenylmethanesulfonyl fluoride (PMSF). The homogenate was centrifuged at 13,000 × g for 20 min, at 4°C, and the supernatant was used for enzyme activity and protein determinations. The concentration of soluble protein was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as a standard. All enzyme activities were expressed as Units. min^{-1}. mg^{-1} of protein. Catalase (CAT) (EC 1.11.1.6) activity was measured by the decomposition of H_2O_2 as the decrease in absorbance at 240 nm according to Cakmak and Marschner (1992). The reaction mixture contained 25 mM phosphate buffer (pH 7.0), 10 mM H_2O_2 and enzyme extract. Guaiacol Peroxidase (GPX) activity (EC 1.11.1.7) was determined at 470 nm by its ability to convert guaiacol to tetraguaiacol in the presence of H_2O_2, using the method of Fielding and Hall (1978). The reaction mixture contained 25 mM phosphate buffer (pH 7.0), 9 mM guaiacol, 5 mM H_2O_2 and enzyme extract.

3. Statistical analysis

Statistical analysis was performed using XLSTAT version 2016 software and two-ways analysis of variance (ANOVA II). The data were expressed as the mean ± standard error. Means were statistically compared using Student-Newman-Keuls's multiple-range test at the level of $p<0.05$.

RESULTS AND DISCUSSION

1. Relative water content (RWC%) and stomatal conductance

Growth performances of two different durum wheat genotypes inoculated individually and co-inoculated with two different *Rhizobium leguminosarum* strains were analyzed under well-watered and limited-water conditions. The results showed that the water deficit decrease significantly the RWC, for both studied genotypes, and for all bacterial treatments. However, under hydric conditions, the inoculated plants showed an increase of RWC comparing to un-inoculated plants, for both studied genotypes (Figure 1). Moreover, between the two hydric conditions, the percentage of decreasing is more important in WH un-inoculated plants then in OZ un-inoculated plants, with -47.54% and -31.95%, respectively. When comparing the effect of *Rhizobium* isolates on WH and OZ genotypes, the percentage of decrease between the two hydric conditions is lower in co-inoculated plants then in single-inoculated plants. For both studied genotypes, the lower decrease is recorded in co-inoculated plants.

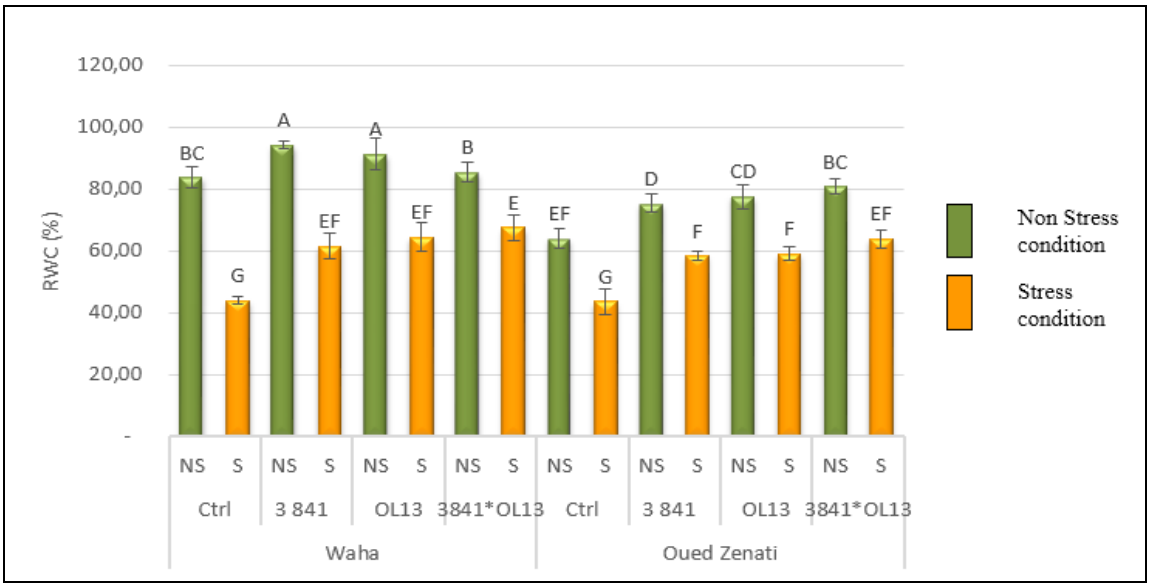

Figure 1. Effect of water stress and rhizobium inoculation on relative water content of flag leaves in studied durum wheat genotypes
Eighty day-old *Triticum durum* plants, previously inoculated with *rhizobium strains* (Ctrl: un-inoculated, 3841: inoculated with strain 3841, OL13: inoculated with strain OL13, 3841*OL13: co-inoculated with the two strains).
The values shown are the mean ± SD of three independent replicates.

Induction of water stress reduced significantly stomatal conductance in both studied genotypes (Figure 2). Stomatal conductance was not significantly different between the *Rhizobium* inoculations and un-inoculated plants under limited-water conditions (Figure 2). In contrast, under well watered conditions, the inoculated plants showed a higher stomatal conductance, for both studied genotypes, when comparing with un-inoculated plants. However, no significant differences were recorded between the single and co-inoculated plants, for both genotypes.

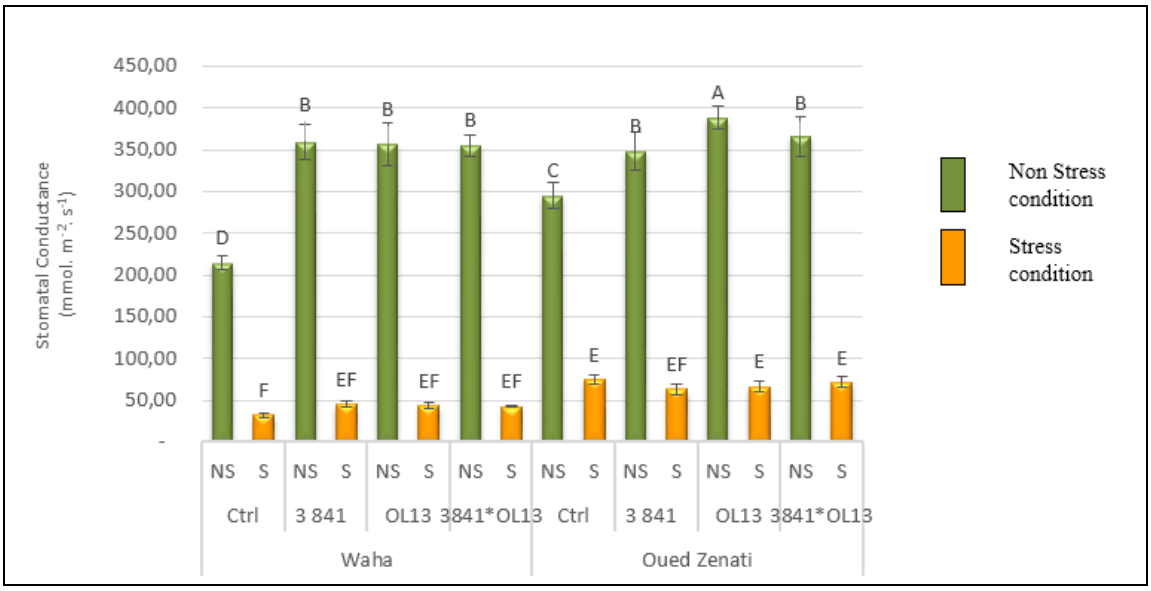

Figure 2. Stomatal conductance (mmol. m^{-2}. s^{-1}) as influenced by *R. Leguminosarum* inoculation and water deficit in two durum wheat genotypes
Eighty day-old *Triticum durum* plants, previously inoculated with *rhizobium strains* (Ctrl: un-inoculated, 3841: inoculated with strain 3841, OL13: inoculated with strain OL13, 3841*OL13: co-inoculated with the two strains).
The values shown are the mean ± SD of three independent replicates.

2. Soluble sugar accumulation

As many environmental stresses, drought lead to major alterations in carbohydrate metabolism and the sugar signaling pathways interact with stress pathways to modulate metabolism. Sugars play an indirect role in controlling carbohydrate metabolism during plant growth and development under abiotic stress (Jha and Subramanian, 2018). In our study, a significant accumulation of soluble sugar was observed under water-limited conditions in both un-inoculated and inoculated plants when compared with well-watered conditions. Intense soluble sugar accumulation was observed under drought stress conditions in both genotypes, being significantly higher in OZ than in WH genotype (Figure 3). However, the inoculated plants showed a significantly higher increase in soluble sugar accumulation compared with un-inoculated plant, for both genotypes. Nevertheless, the single and co-inoculated plants recorded the same increase under limited-water conditions. These findings show that sugar metabolites play a substantial role in maintaining osmotic potential during drought stress under the current experimental settings.

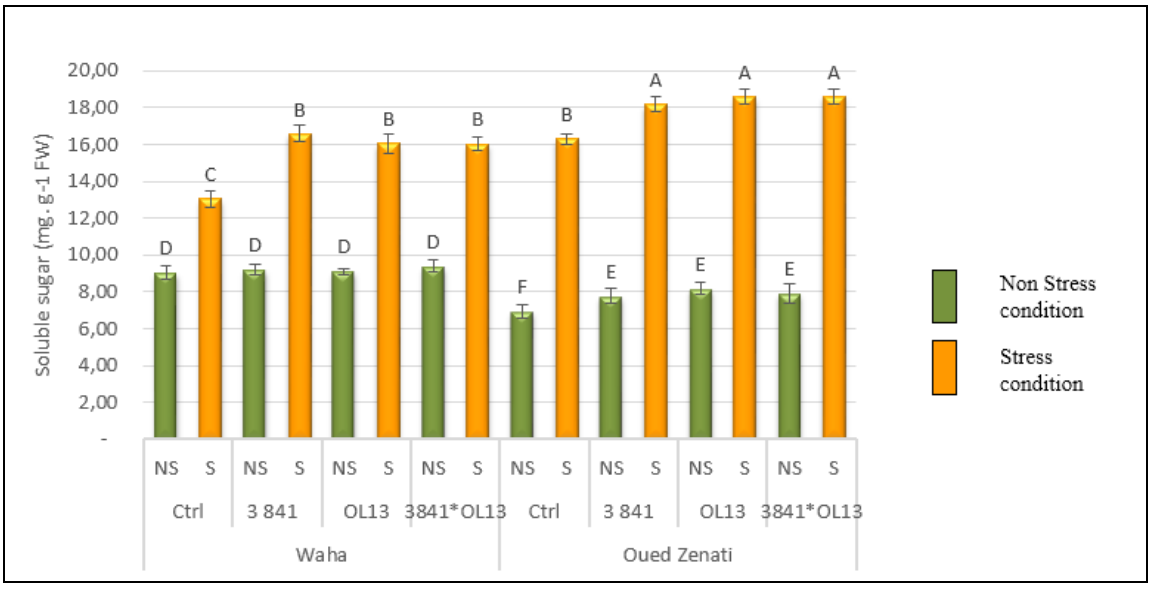

Figure 3. Soluble sugar content in flag leaves of two durum wheat genotypes as influenced by *R. Leguminosarum* inoculation and water deficit

Eighty day-old *Triticum durum* plants, previously inoculated with *rhizobium strains* (Ctrl: un-inoculated, 3841: inoculated with strain 3841, OL13: inoculated with strain OL13, 3841*OL13: co-inoculated with the two strains).
The values shown are the mean ± SD of three independent replicates.

3. Lipid peroxidation and hydrogen peroxide content

Plants exposed to drought stress produce and over accumulate reactive oxygen species (ROS), such as superoxide radical (O_2), hydroxyl radicals (OH), and hydrogen peroxide (H_2O_2), which cause oxidative damage to cellular ingredients including carbohydrates, proteins, lipids, DNA, etc. (Raja et al., 2017; Hasanuzzaman et al., 2020). The MDA and H_2O_2 content are determinants of oxidative stress in plants under drought stress conditions, hence we measured the content of MDA and H_2O_2 in flag leaves (Table 1). In well-watered conditions, the un-inoculated and inoculated plants showed similar levels of MDA and H_2O_2 contents, however when plants were exposed to water deficit conditions, there was a considerable increase in both MDA and H_2O_2 content (Table 1). MDA accumulation was significantly lower in leaves of OZ than in WH, with 113.26% and 656.27%, respectively. Concerning H_2O_2 content, this increase was also significantly lower in OZ than in WH with 76.78% and 105.61%, respectively. Furthermore, for both MDA and H_2O_2 content, a significant increase was observed in un-inoculated plants compared to inoculated plants for both studied genotypes.

Moreover, the two studied strains decrease similarly H_2O_2 content under water-limited conditions, whereas, the lower increase was register in co-inoculated plants of OZ genotype.

Table 1. Maondialdehyde and Hydrogen peroxide contents in leaves as influenced by *R. Leguminosarum* inoculation and water deficit in two durum wheat genotypes

Genotypes	Rhizobium Treatment	MDA contents (nMol. g_{-1} FW)		H_2O_2 contents (nMol. g^{-1} FW)	
		Control	Water stress	Control	Water stress
Waha	Un-inoculated	$17,10 \pm 1,32^{F}$	$129,33 \pm 6,35^{A}$	$28,02 \pm 2,32^{EFG}$	$57,62 \pm 1,62^{A}$
	3841	$17,39 \pm 0,87^{F}$	$91,36 \pm 4,88^{B}$	$28,95 \pm 1,70^{EF}$	$52,26 \pm 3,36^{B}$
	OL13	$17,97 \pm 0,53^{F}$	$65,28 \pm 3,48^{D}$	$28,95 \pm 3,69^{EF}$	$50,00 \pm 1,99^{B}$
	3841*OL13	$16,87 \pm 1,06^{F}$	$66,14 \pm 1,48^{D}$	$29,57 \pm 1,78^{EF}$	$50,24 \pm 1,97^{B}$
Oued Zenati	Un-inoculated	$42,84 \pm 4,93^{E}$	$91,36 \pm 2,09^{B}$	$23,38 \pm 1,21^{GH}$	$41,33 \pm 2,21^{C}$
	3841	$43,94 \pm 5,37^{E}$	$82,96 \pm 4,60^{C}$	$24,57 \pm 0,71^{FGH}$	$32,67 \pm 1,94^{DE}$
	OL13	$46,03 \pm 1,76^{E}$	$76,75 \pm 6,18^{C}$	$24,57 \pm 1,17^{FGH}$	$38,67 \pm 4,21^{D}$
	3841*OL13	$39,48 \pm 6,06^{E}$	$60,06 \pm 2,90^{D}$	$22,00 \pm 1,59^{H}$	$27,38 \pm 0,97^{EFG}$

Eighty day-old *Triticum Durum* plants, previously inoculated with *rhizobium strains* (Ctrl : un-inoculated, 3841: inoculated with strain 3841, OL13: inoculated with strain OL13, 3841*OL13 : co-inoculated with the two strains), were grown under control conditions (80% FC) or under water limited conditions (40% FC). WH : Waha ; OZ : Oued Zenati. The values shown are the mean ± SD of three independent replicates.

4. Enzymatic activities responses to water deficit

Plants possess antioxidant defense systems that include both enzymatic and non-enzymatic components to defend themselves from the impacts of drought stress (Hasanuzzaman et al., 2020). Antioxidants are defined as chemicals capable of blocking or quenching free radical processes, delaying or preventing cell damage, and considerably delaying or hindering the oxidation of prospective substrate in lower concentrations than the potential substrate to be oxidized (Dumont and Rivoal, 2019). The activities of antioxidant enzymes catalases (CAT) and gaiacol peroxidase (GPX) were measured as parameters of antioxidant defense in flag leaves. CAT and GPX enzyme convert H_2O_2 to H_2O (Rasool et al., 2013).

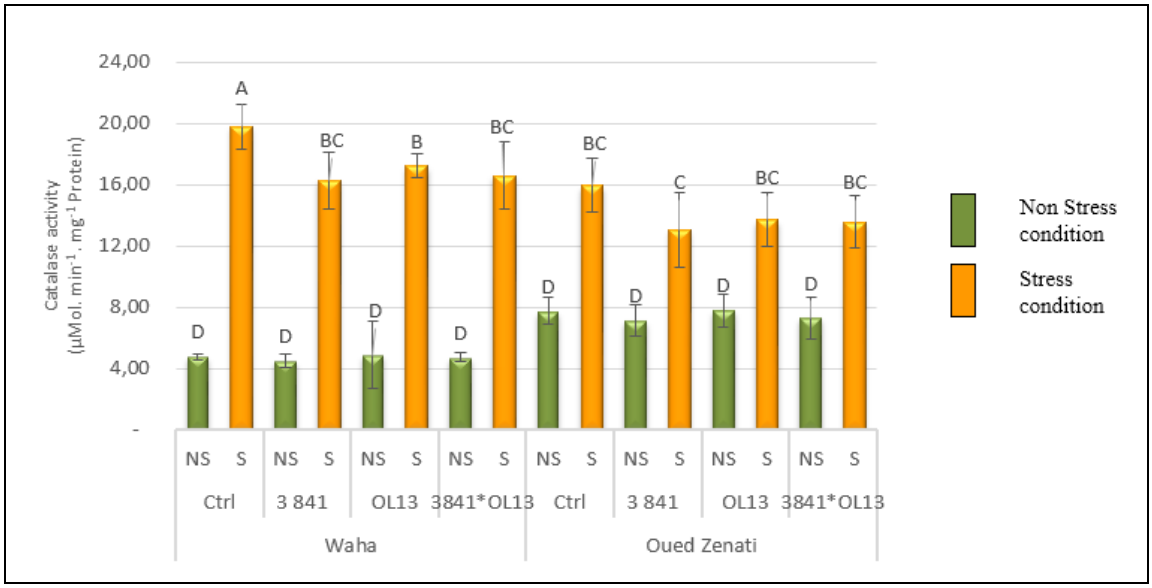

Figure 4. Catalase activity in two durum wheat genotype flag leaves as influenced by *R. Leguminosarum* inoculation and water deficit

Eighty day-old *Triticum durum* plants, previously inoculated with *rhizobium strains* (Ctrl: un-inoculated, 3841: inoculated with strain 3841, OL13: inoculated with strain OL13, 3841*OL13: co-inoculated with the two strains).
The values shown are the mean ± SD of three independent replicates.

In the present experiment, for both studied antioxidant enzymes, no significant differences was recorded between the un-inoculated and inoculated plants for both studied genotypes under well-watered conditions (Figures 4 and 5). However, the highest CAT activity was detected in un-inoculated plants in both studied genotypes, subjected to water-limited condition (Figure 4). In contrast, GPX activity was highly induced in inoculated plants compared to un-inoculated plants for both studied genotypes, under water-limited condition. However, this increase was more important in co-inoculated plants for OZ genotype with 119.68%, 129.77% and 162.52% for single inoculation (3841 and OL13) and co-inoculation, respectively (Figure 5).

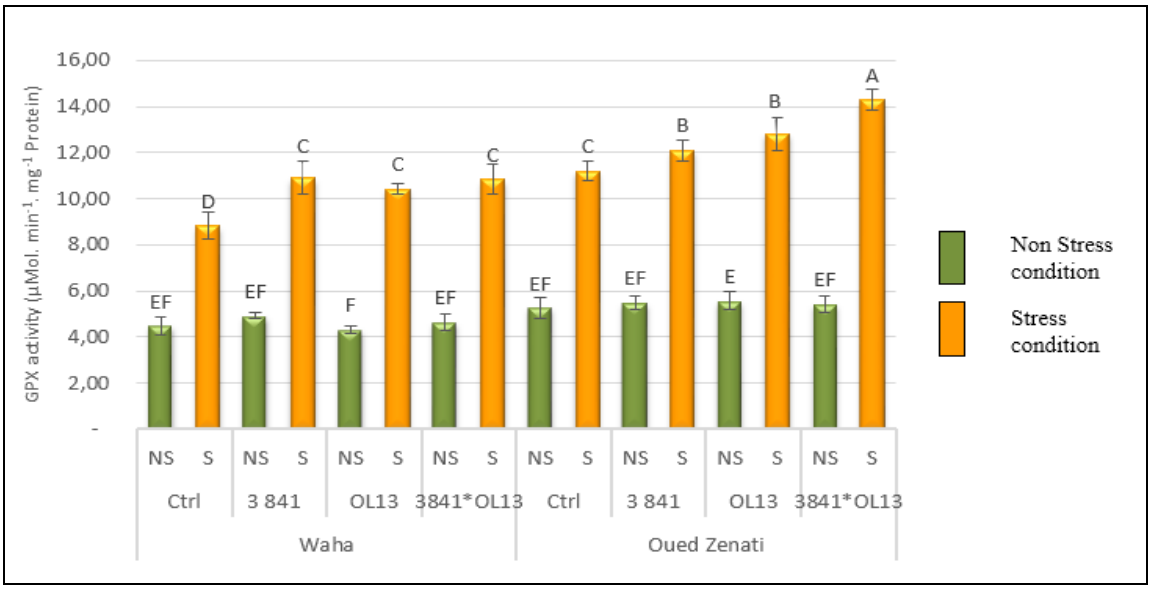

Figure 5. Peroxidase activity in two durum wheat genotype flag leaves as influenced by *R. Leguminosarum* inoculation and water deficit
Eighty day-old *Triticum durum* plants, previously inoculated with *rhizobium strains* (Ctrl: un-inoculated, 3841: inoculated with strain 3841, OL13: inoculated with strain OL13, 3841*OL13: co-inoculated with the two strains).
The values shown are the mean ± SD of three independent replicates.

Certain microorganisms, such as bacteria, have complex processes that enable them to survive in adverse settings and improve crop growth and development through direct and indirect interactions with plants (Khan et al., 2017). According to our findings, drought stress has a considerable impact on physiological and biochemical parameters on the two-studied genotype, differentiated according to the presence of Rhizobium strains. RWC and stomatal conductance are important physiological parameters frequently employed to measure water status as they correspond to uptake of water by the roots, water lost by transpiration and stomatal closure. According to numerous studies on durum wheat (Aprile et al., 2013; Habash et al., 2014); RWC values under water deficit conditions are higher in drought tolerant genotypes. Thus, in this study, Rhizobium-inoculation increased RWC when compared to un-inoculated plants for both studied genotypes and under the two water conditions (Figure 1). This gave an indication that the Rhizobia could help wheat tolerate water deficit conditions by maintaining a better water status. The mechanisms associated with this increase in RWC by rhizobial inoculation maybe linked to their PGPR mechanism of making insoluble phosphorus available for plants and stomatal regulation through hormonal signals, as was reported by Zarik et al. (2016) for mycorrhizal application. Under well-watered condition, the inoculated plants showed a higher stomatal conductance compared to un-inoculated plants (Figure 2). According to Noel et al. (1996), the phytohormone synthesis, which includes cytokinins, is one of the *R. leguminosarum* plant growth mechanisms. Kaushal and Wani

(2016) reported that cytokinins stimulate cell division, cell enlargement, shoot growth and causes stomatal opening, this may explain the increase in stomatal conductance in inoculated plants over un-inoculated plants under well-watered conditions.

Production and accumulation of soluble sugars as osmolytes is another method of acclimatization towards osmotic adjustment under drought stress in order to limit water losses by promoting water retention in the plant without interfering with a normal metabolism (Gontia-Mishra et al., 2016; Camaille et al., 2021). In wheat, soluble sugars make the largest contribution to osmotic adjustment when subjected to the drought stress (Camaille et al., 2021). However, a significant increase in soluble sugar in inoculated plants was recorded in comparison to un-inoculated plants under water-limited condition (Figure 3). Observed results are consistent with Feng et al. (2002), who reported that in maize, mycorrhizal plants maintained higher total soluble sugar than non-mycorrhizal plants. These findings suggest that *Rhizobium*-inoculation help wheat plant to maintain a better osmotic adjustment, to cope with drought stress and a better development.

MDA contents, a product of lipid peroxidation, is reliable indicator of oxidative membrane damages in plants due to stress. Avoiding damages caused to cell membranes is a key point for plant to resist to drought stress (Camaille et al., 2021). In this study, a significant decline in MDA and H_2O_2 content was observed in inoculated plants in contrast to un-inoculated plants under drought stress, suggesting that *Rhizobium* helped to protect the integrity of plant cell membranes from the detrimental effects of drought (Table 1). Moreover, the most pronounced inoculation decrease effect on MDA and H_2O_2 content was recorded in co-inoculated plants of OZ genotype, under limited-water condition. These results were correlated with the high GPX activity recorded in co-inoculated OZ. Indeed, peroxidase production is a critical mode of H_2O_2 destruction, and these findings demonstrate the important role played by GPX in detoxifying plant cell of H_2O_2. In contrast, CAT activity increase was more pronounced in un-inoculated plants. The fact that *Rhizobium*-inoculated plants had low or equal CAT activity when compared to un-inoculated plants, under water-limited conditions rules out a direct involvement of this enzyme in oxidative damage prevention. In fact, the CAT activity was found to increase especially in drought-sensitive varieties of wheat (Simova-Stoilova et al., 2010). Thus, the difference in such antioxidant activities (high Peroxidase activity and low CAT activity) reflected a certain enzymatic balance within plant cells in order to detoxify them from ROS which allows plants to adapt and survive oxidative stress produced by drought stress. Our results are in accordance with earlier studies affirming that PGPR inoculation combats oxidative damage caused by drought stress (Gusain et al., 2015; Tiwari et al., 2016).

Taken together, these results allow us to confirm the drought tolerance of OZ genotype over WH genotype. Furthermore, both studied *Rhizobium leguminosarum* strains improved similarly physiological and biochemical parameters of both studied genotypes. This improvement was recorded not only for single-inoculation, but also in co-inoculation for OZ genotype. The co-inoculation effects being consistent only for OZ genotype under water-limited condition, let us suggest that this difference in response indicate a possible genotype effect.

CONCLUSIONS

Drought stress has been shown to have a deleterious effect on plant growth and development by altering nutritional and hormonal balances. However, the impact of such stress can be alleviated and/or minimized by naturally occurring microorganism, such as *Rhizobium leguminosarum*. As revealed in our study, *R. leguminosarum* can be used as plant growth promoting bacteria to induce systemic tolerance in *Triticum durum* under limited water condition. Alleviation of water stress is

suggested to be due to enhancement of antioxidants and higher accumulation of osmoprotectant such as soluble sugar. In addition, our research suggests that co-inoculation of various rhizobia (acquired from different legumes) may be a better alternative than single strain inoculation for increasing plant development under water limited conditions. Further studies on the characteristics of rhizobia strains and the relationship between rhizobia and cereals need to be done to better understand the mechanism of drought stress tolerance induction in order to better choose the most efficient couples. One of the implications of this study is to demonstrate the beneficial PGPR effect of rhizobia on durum wheat in the context of cereal-leguminous crop rotations in order to protect the environment by minimizing chemical intrans and to conserve crop biodiversity.

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