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ORIGINAL PAPER

A Mix of *Agrobacterium* Strains Reduces Nitrogen Fertilization While Enhancing Economic Returns in Field Trials with Durum Wheat in Contrasting Agroclimatic Regions

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Abstract

Today, using plant growth–promoting rhizobacteria is an important eco-friendly management approach for improving crop productivity. This study aimed to select efficient native rhizobia from white lupin nodules and assess their potential for improving the production and economic returns of *Triticum turgidum* spp. *durum* L. var. Razzek under field conditions in arid and semi-arid environments affected by salinity and drought stress. Seventy-two strains were assessed for their plant growth-promoting activities. Of them, three compatible strains of *Agrobacterium* spp. were selected and used individually and in mixes. The efficiency of seven inocula applied to the soil was assessed in a pot experiment at varying levels of synthetic nitrogen fertilization (0, 50, and 100%N). The experiment was set up in a completely randomized design with four replications. At the flowering stage plant height, flag leaf area, shoot dry weight, leaf chlorophyll content, chlorophyll fluorescence, and the normalized difference vegetation index were estimated. Subsequently, the most efficient inoculant was tested in a field trial under four different agro-environmental conditions contrasting in terms of salt and drought stress, respecting the same N fertilization rates and experimental design, with three replications. Yield components, nitrogen (N) and phosphorus (P) contents, and economic returns were estimated at harvest time. In the pot experiment, a combined application of the mix of three *Agrobacterium* spp. at 50%N proved to be the best treatment ensuring the highest values for all the measured parameters, which were statistically similar or higher than those recorded in uninoculated plants receiving 100%N. Field inoculation trials confirmed the results obtained in the pot experiment. In non-stressed environments, application of the bacterial mix at 50%N was as effective as the 100%N treatment. Meanwhile, under drought and salt stress conditions, the same treatment significantly increased grain yield by 33 to 42% and straw yield by 13 to 22% compared to uninoculated plants at 100%N. Furthermore, this treatment significantly enhanced N and P contents in wheat straw and grain in stressed environments. An economic analysis revealed that application of the selected mix with 50%N increased net-returns per hectare, ensuring a financial gain ranging from 26 to 106% compared to the control plants receiving a full N dose. The positive effect of the native *Agrobacterium* sp. mix on growth, yield, nutrition, and economic returns of durum wheat var. Razzek, when combined with 50%N, was proved for the first time. However, future studies should now focus on examining the behavior of this mix with other durum wheat varieties.

Keywords Cereals · Plant growth–promoting rhizobacteria · Abiotic stress · Economic analysis · Biofertilizer

Abbreviations

PGPR	Plant growth–promoting rhizobacteria
CAS	Chrome azurol S
IAA	Indole acetic acid
ACC	1-Aminocyclopropane-1-carboxylate
SMS	Sucrose minimal salts
CFU	Colony-forming units

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1 Introduction

Improving crop productivity is a major challenge and a global goal for meeting the food needs of a growing world population. Wheat is one of the most important staple foods in the world. In terms of world production, it is ranked second after rice. In particular, durum wheat (*Triticum turgidum* spp. *durum*) is one of the main crops grown in the Mediterranean basin, playing a major role in the agricultural economy (Araus et al. 2013; Shewry and Hey 2015). In these regions, durum wheat is regarded as a strategic crop for food security and social stability. However, its production is held back by several abiotic factors mainly related to (i) low and irregular annual and seasonal rainfall (Laatiri et al. 2010; Ben Zekri-Mghirbi 2017), and (ii) soil and water salinity, particularly in arid areas, where salinity can reach 8.5 dS m^{-1} and 10 dS m^{-1} in reservoirs and wells, respectively (Slama 2004).

Given this situation, expected climate changes will increase the frequency and the severity of these factors in the future (Araus et al. 2008; Laatiri et al. 2010) and, in the search for suitable biotechnological approaches to improve cereal crop productivity, introducing plant growth–promoting rhizobacteria (PGPR) is an eco-friendly and economically worthwhile approach (Ijaz et al. 2019). Within the wide diversity of PGPRs, this study focused on rhizobia. In addition to their ability to fix atmospheric nitrogen when combined with legumes, most rhizobia exhibit one or more PGP effects (Garcıa-Fraile et al. 2012; Abdelkrim et al. 2018). They promote plant growth directly, either by increasing nutrient availability and acquisition, including nitrogen (N), phosphorus (P), potassium (K), iron (Fe), and zinc (Zn) (Yasmeen and Bano 2014; Kumar et al. 2016), or by regulating various plant hormones, such as indole-3-acetic acid (IAA), abscissic acid, gibberellic acid, and cytokinin (Kumar et al. 2016; Abdelkrim et al. 2018). They can also stimulate growth indirectly by producing antibiotics (Labuschagne et al. 2010), siderophores (Glick, 2012; Kumar et al. 2016), and induced systemic resistance (Ramamoorthy et al. 2001), which limits the growth of soil-borne phytopathogens. Moreover, rhizobia can produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which stimulates plant defense mechanisms under stressed conditions, such as drought and salinity stress (Kumar et al. 2016).

Generally, rhizobia have been isolated from root nodules of legumes. However, many studies have shown the occurrence of endophytic rhizobia in the root or stem tissues of non-legume crops, such as sugarcane (*Saccharum* spp.) (Burbano et al. 2011; Menezes Junior et al. 2019), maize (*Zea mays*) (Youseif 2018; Cavalcanti et al. 2020), wheat (Santos et al. 2020), and rice (*Oryza sativa*) (Biswas et al. 2000; Santos et al. 2019). Although legume-rhizobia interaction is well documented, the beneficial effects of rhizobia on non-legume plants have so far remained poorly investigated. Most researchers have reported that greater PGPR efficiency was found when bacteria were used on the same host plant from which they were isolated. However, it was reported that PGPR isolated from a plant host can also promote the growth of many different other plants (Afzal et al. 2019; Hayat et al. 2010; Ma et al. 2011; Tsunoda and Van Dam 2017).

In such a context, this study aimed to (i) select native and non-antagonistic rhizobia from a collection of 72 locally trapped strains previously isolated from nodules of *Lupinus albus* L.; (ii) investigate their potential as single strains, and in mixes, for promoting the growth of durum wheat in a pot experiment with varying levels of synthetic N fertilizers; and (iii) assess the effect of the selected inoculant on durum wheat growth, yield, grain nutrient content and economic returns under actual conditions in arid and semi-arid environments affected by salinity and drought stress. The outcome of this study may be useful for sustainable durum wheat production, to achieve food security and promote environmental health.

2 Material and Methods

2.1 In vitro *Screening of PGPR*

With a view to finding efficient plant growth–promoting rhizobial strains with multiple beneficial activities, 72 bacteria belonging to *Rhizobium* spp., *Agrobacterium* spp., and *Neorhizobium* spp. were isolated from root nodules trapped with *Lupinus albus* L. in 15 different Tunisian soils (Tounsi-Hammami et al. 2019). Their ability to solubilize inorganic phosphate was investigated on National Botanical Research Institute Phosphate (NBRIP) medium (Nautiyal 1999). Siderophore production was examined on chrome azurol S (CAS) medium according to Schwyn and Neilands (1987). Ammonia production was tested in peptone water following the Cappuccino and Sherman (1992) method. Production of indole acetic acid was detected using the method described by Abdelkrim et al. (2018).

2.2 Antagonistic Test and Tolerance of Abiotic Stress

Out of the 72 tested strains, only isolates possessing multiple plant growth–promoting traits were selected and tested for their antagonistic behavior against each other, using the overlay plate technique (Oresnick et al. 1999). Three replications were carried out for each strain pair. Selected strains were also tested for abiotic stress tolerance, namely their ability to grow at variable levels of pH (from 4 to 11), in the presence of variable concentrations of NaCl, ranging from 0.6 M to 1.5 M (salt stress), and of polyethylene glycol (PEG 6000), ranging from 15 to 50% (drought stress). Four replicates were used for each treatment.

2.3 Microbial Inoculant Preparation

Only three strains, namely S1: LAa26, S2: LAa56, and S3: LAb21, were selected to prepare the inocula. The strains were used individually and in mixes of two and three strains, with a total of seven inocula defined as S1, S2, S3, S1 + S2, S1 + S3, S2 + S3, and their mix, M. The strains were grown individually in Erlenmeyer flasks containing YEM medium on a rotating shaker (150 rpm) for 48 h at 28 °C to late exponential phase, to obtain a final concentration of 10^9 CFU mL^{-1}. The bacterial suspensions were adjusted to 10^9 CFU mL^{-1} using a flow cytometer (Sysmex). The mixes of two and three strains were then prepared by combining equal volumes of the individual bacterial suspensions, just prior to their application, and corresponded to a final concentration of 10^9 CFU mL^{-1}.

2.4 Pot Experiment and Selection of an Efficient Inoculant

All inocula were assessed for their efficiency in promoting durum wheat growth and their potential for reducing nitrogen fertilization. The most widespread wheat variety in Tunisia, Razzek, was selected to be used in the pot experiment and for further studies. Seeds were obtained from the National Agricultural Research Institute of Tunisia (INRAT). They were surface-sterilized by treatment with 95% ethanol for 1 min, followed by 25% sodium hypochloride for 3 min and finally rinsed ten times with sterile distilled water, then soaked in sterile water for 30 min. The effectiveness of this disinfection was ascertained by incubating ten seeds on YEM agar plates at 28 °C for 72 h and checking for the absence of bacterial contamination.

The surface-sterilized seeds were immediately sown in sterilized 21-L plastic pots filled with non-sterilized soils collected from the top soil (0–25 cm in depth) of a non-cultivated area at the National Agricultural Institute of Tunisia (INAT) (Table 1) at a rate of 10 seeds per pot. At full emergence of the first leaf, only four plants were kept, then the inocula were applied directly to the soil at a rate of one milliliter per seedling. Uninoculated plants were used as a control (S0). Three levels of synthetic nitrogen fertilization were applied: 0%, 50%, and 100% of the full recommended dose (150 kg ha^{-1}). The highest nitrogen dose in this study, 150 kg ha^{-1}, was the recommended rate in Tunisia for a yield target of 5–6 T ha^{-1}. Nitrogen fertilization was applied as ammonium nitrate (34% N) and dispensed in three application rates (30% at tillering, 40% at the beginning of stem elongation and 30% at the flowering stage). In all, 24 treatments were set up in four replications arranged in a complete

Table 1 Physicochemical properties of the soil used in the pot and field experiments

Location	Clay (%)	Silt (%)	Sand (%)	pH	EC (dS m^{-1})	CaCo3 (%)	Active CaCo3 (%)	OM (%)	Corg (%)	Nt (%)	AP (ppm)
Pot experiment											
Tunis (INAT)	37	44	19	7.81 ± 0.01	0.599 ± 0.01	40.56 ± 0.45	18.42 ± 0.24	2.56 ± 0.04	1.48 ± 0.02	0.08 ± 0.01	30 ± 0.03
Field experiment											
Medenine (MED)	20	24	56	6.77 ± 0.01	0.01 ± 0.00	4.68 ± 0.25	3.50 ± 0.31	0.90 ± 0.06	0.52 ± 0.03	0.02 ± 0.00	4.2 ± 0.01
Kairouan (KAI)	21	66	13	8.12 ± 0.01	0.122 ± 0.01	19.42 ± 0.31	16.25 ± 0.16	2.53 ± 0.05	1.46 ± 0.03	1.2 ± 0.01	9.4 ± 0.01

EC electrical conductivity, *CaCo3* total lime content, *OM* organic matter, *Corg* organic carbon, *Nt* total nitrogen, *AP* available phosphorus randomized design (details are shown in Table 2) under natural conditions during the 2016–2017 cropping season. Supplemental irrigation was provided as and when needed. At the flowering stage, the different inocula were judged according to their effect on plant height (PH) and on the leaf chlorophyll content index (LCCI) recorded using a chlorophyll meter (SPAD, soil plant analysis development, 502, Konica Minolta, Tokyo, Japan). Chlorophyll fluorescence characteristics (F_v/F_m) were measured using a portable multimode chlorophyll fluorometer (Model OS5P, Optisciences Inc., Winn Avenue, Hudson, USA) and the Normalized Difference Vegetation Index (NDVI) of the canopy of each pot was calculated with an active hand-held Greenseeker spectrometer (NTech Industries, Ukiah, CA, USA). Wheat plants were then harvested. The flag leaf area (FLA) was measured in cm^2 using a portable laser area meter (Model No. CI-202-CID, Inc). Plants were dried in an oven at 65 °C until constant weight was achieved, to determine their corresponding shoot dry weight (SDW).

2.5 Field Inoculation Experiment

Given that drought and salinity are the main factors limiting wheat production in Mediterranean areas, the effectiveness of the best inoculant was assessed under actual field conditions during the 2017–2018 cropping season in two locations, Echbika-Kairouan (KAI) and El-Fje-Medenine (MED) (Fig. 1) naturally affected by drought and salinity stress, respectively. Before sowing, five soil samples were taken in each location from the 0 to 45 cm horizon. They were then homogenized and used to assess soil physical and chemical characteristics by standard methods (Table 1).

In each location, we defined two environments differing by the level of salinity in the irrigation water at Mednine (MED-1.5 dS m^{-1}: drinkable water and MED-13.3 dS m^{-1}: groundwater) and by two levels of irrigation crop requirements (KAI-100% ETc and KAI-50% ETc) at Kairouan. In each environment, the experiment had a completely randomized block design with a factorial arrangement of three levels of nitrogen fertilization (0%, 50%, and 100% of 150 kg N ha^{-1}) and two inoculation levels (without inoculation and with inoculation). Three replications were respected for each treatment. The Razzek variety was also used in the four different field experiments. Sowing took place the first week in December 2017 at a seeding rate of 300 viable seeds m^{-2}. Individual plots (2 × 3 m) were made up of 10 rows measuring 3 m in length, spaced 20 cm apart.

Table 2 Treatments used in the experiment, strain combinations and levels of nitrogen fertilization

T1: S0-0% N	T5: S1 + S2-0% N	T9: S0-50% N	T13: S1 + S2-50% N	T17: S0-100% N	T21: S1 + S2-100% N
T2: S1-0% N	T6: S1 + S3-0% N	T10: S1-50% N	T14: S1 + S3-50% N	T18: S1-100% N	T22: S1 + S3-100% N
T3: S2-0% N	T7: S2 + S3-0% N	T11: S2-50% N	T15: S2 + S3-50% N	T19: S2-100% N	T23: S2 + S3-100% N
T4: S3-0% N	T8: M-0% N	T12: S3-50% N	T16: M-50% N	T20: S3-100% N	T24: M-100% N

S0, uninoculated plants; M, plants inoculated with the mix of three strains: S1 = LAa26, S2 = LAa56, S3 = LAb21

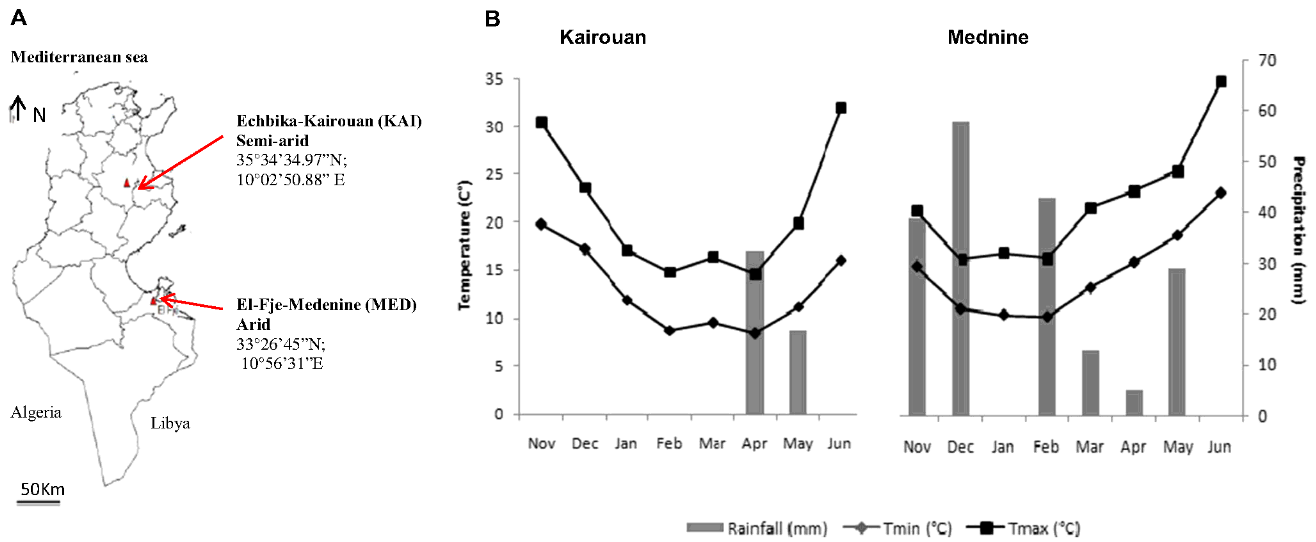

Fig. 1 Experimental site locations and climate (**A**). Average monthly minimum and maximum temperature and precipitation at the experimental sites during the 2017–2018 cropping season (**B**)

All the plots were separated by a distance of 2.5 m to avoid bacterial contamination. Phosphorus fertilization consisted of 46 kg ha^{-1} of P_2O_5 incorporated directly into the soil by harrowing just before sowing. Nitrogen fertilization was applied as ammonium nitrate (34% N) and dispensed in three application rates (30% at tillering, 40% at the beginning of stem elongation and 30% at the flowering stage) with the respective dose for each treatment. The crop was protected from weeds, insects and fungal pathogens by specific treatments, following local recommendations. A drip irrigation system was installed. In order to ensure a uniform water supply, line source emitters were installed on each planting row, with a spacing of 33 cm between emitters on the same row. Emitter discharge was 2 L h^{-1} at a 1.0 bar operating pressure. Irrigation water was provided from planting up to the grain filling stage. The total water supplied was calculated according to the climatic and soil data of each site to achieve wheat water requirements. The rainfall and temperature data were collected from the weather station at each site (Weathereye, Leicestershire, England).

The bacterial inoculant was applied at the early tillering stage. Fresh preparations of individual strains were produced (10^9 bacteria mL^{-1}) and mixed equally to obtain the mix of the three selected bacteria (2 L ha^{-1}), then diluted with well water (1/20) and finally sprayed manually along the seedling line using a portable hand sprayer. Inoculation was carried out in the late afternoon, in order to minimize UV light interference with bacterial survival. The volume applied ensured good soil wetness and an expected bacterial density of 4×10^7 CFU m^{-2} of ground.

At flowering, ten plants from the center of each plot were used to determine plant height, the leaf chlorophyll content index, the normalized difference vegetation index and shoot dry weight using the same equipment as that in the pot experiment. Two weeks after physiological maturity, 1 m^2 of each plot was harvested by hand. Grains were collected using a thresher (Wentersteiger, LD-180, Germany). The number of spikes per square meter (NS), straw yield (SY), grain yield (GY), and thousand-grain weight (TGW) were measured per 1 m^2 then converted to per ha. N and P concentrations in straw and grains were determined using the methods of Kjeldahl (1883) and Mendes Filho et al. (2010), respectively. Finally, the economic returns from wheat cropping were calculated for all the treatments by subtracting total production costs (purchase and inputs) from the total benefits (sale of wheat grain and straw). The prices used in this economic study were the common market prices prevailing in 2018. Tunisian Dinar values were converted into American Dollar (US $) based on the exchange rate prevailing during 2018 (1 DT = US $0.34). To better understand the experimental process, the schematic representation in supplementary Fig. S1 shows the analytical steps of our study.

2.6 Statistical Analysis

The data of the various parameters measured in the pot and field experiments underwent an analysis of variance (ANOVA) in accordance with the experimental design using R statistical software version 3.5.1, to quantify and evaluate the source of variation. The treatment means were compared using LSD tests at a significance level of 5%. The ranking of each treatment was denoted by letters.

3 Results

3.1 In vitro *Plant Growth–Promoting Traits of the Isolates*

The results for the 29 most interesting strains are presented in Table 3, while complete data can be found in Supplementary Table S1. The results indicated that 45 strains (65%) were able to produce siderophores. The highest values were found in *Agrobacterium* sp. LAa56, with a diameter of 15.1 mm. In contrast, the smallest diameter was observed for the *Rhizobium* sp. LAa37 strain (0.63 mm). Only 21 (29%) formed a visible halo indicating a positive phosphate solubilizing activity, with a maximum recorded for *Agrobacterium* sp. LAb21 (9.03 mm) and a minimum found for *Rhizobium* sp. LAa34 (0.5 mm). The tested strains were found to present a very wide range of IAA production. Indeed, it ranged from 10.46 to 341.23 μg mL^{-1} recorded by the *Rhizobium* sp. LAa25 and *Agrobacterium* sp. LAa26 strains, respectively. Eleven strains did not produce IAA: LAa8, LAa9, LAa38, LAa51, LAa53, LAb4, LAb8, LAb9, LAb13, LAb14, and LAa3. In all, 55 strains (76%) were classified as ammonium producers. Of them, five agrobacterial strains, LAa13, LAa21, LAa38, LAa56, and Lab31, and one rhizobial strain, LAa22, exhibited strong ammonium production ability.

3.2 Selection of Compatible Strains and Tolerance of Abiotic Stress

In vitro antagonistic assays showed that the three strains LAa26, LAa56, and LAb21 were compatible and had no antagonistic effects on each other (Table S2), as no growth inhibition zone was seen on the intersection areas. Consequently, their use, either independently or together as a mix of two or three strains, was assumed not to cause any undesirable interference. These strains appeared to be resistant to salinity and could grow in a range of 600 mM to 900 mM of NaCl. Only strain LAa56 was able to grow in 1 M of NaCl. When considering tolerance of acidic and alkaline conditions, LAa26, LAa56 and LAb21 were resistant to a wide pH range of 4 to 11. Under osmotic stress, all the tested strains

Table 3 Plant growth–promoting abilities of the 29 pre-selected root nodule strains

Strains	Siderophore production (mm)	Phosphate solubilization (mm)	IAA (µg mL^{-1})	NH_3
Rhizobium sp.				
LAa22	0.00	0.00	143.57 lm ± 9	+ + +
LAa23	0.00	0.00	191.80 gh ± 11	+ +
LAa24	0.00	0.00	192.83 gh ± 14	+ +
LAa34	1.80 lmn ± 0.10	0.50 j ± 0.15	107.13 qrs ± 9	+ +
LAa37	0.63 n ± 015	3.87 efg ± 0.56	234.67 e ± 11	+ +
LAa40	0.83 mn ± 0.18	4.00 ef ± 0.52	133.97 mno ± 5	+
LAa43	10.27 bcdefgh ± 0.92	0.00	62.67 xyz ± 2	+ +
LAb19	2.67 ijklmn ± 0.63	0.00	261.47 c ± 10	-
LAb26	0.00	6.20 c ± 0.72	291.47 b ± 11	+
LAa46	12.27 bcdef ± 0.93	3.30 fgh ± 0.45	36.80 BC ± 5	+ +
LAa53	13.57 bcd ± 0.45	3.23 gh ± 0.25	0.00	+
LAa5	1.17 lmn ± 0.23	1.37 i ± 0.25	330.27 a ± 12	+
LAa12	7.77 cdefghijklmn ± 0.45	0.00	105.43 rs ± 5	+ +
LAa13	9.37 bcdefghijk ± 0.57	0.00	91.37 stu ± 5	+ + +
LAa26	6.27 efghijklmn ± 0.64	0.00	341.23 a ± 8	+ +
LAa42	12.33 bcde ± 0.64	0.00	63.00 xyz ± 2	+ +
LAa44	10.33 ± 0.86	0.00	58.37 yzA ± 4	-
LAa45	14.33 bc ± 1.05	0.00	55.50 zA ± 2	+
LAa47	9.43 bcdefghij ± 0.56	0.00	261.27 cd ± 9	+
LAa55	10.10 bcdefgh ± 0.86	0.00	60.03 xyz ± 8	-
LAa56	15.10 a ± 0.15	0.00	25.43 CDE ± 2	+ + +
LAb6	9.13 bcdefghijk ± 0.51	0.00	107.67 qrs ± 10	+ +
LAb7	6.80 defghijklmn ± 0.67	0.00	246.20 de ± 10	+ +
LAb17	5.23 efghijklmn ± 0.68	0.00	130.37 mnop ± 14	-
LAb21	6.10 efghijklmn ± 0.71	9.03 a ± 0.57	196.73 fgh ± 6	+ +
LAb22	7.63 cdefghijklmn ± 0.45	4.03 ef ± 0.23	173.80 ij ± 13	+
LAb23	2.07 lmn ± 0.57	0.00	200.03 fgh ± 12	+ +
LAb29	8.10 bcdefghijkl ± 0.76	0.00	261.13 cd ± 14	+ +
LAb30	9.43 bcdefghij ± 0.64	0.00	75.43 vwx ± 2	+ +

Values are the mean of three separate experiments in triplicate; ammonia production (based on intensity of color): absent (−), weak (+), moderate (++), strong (+++). Different letters indicate significant differences according to the LSD test ($p \leq 0.05$)

were tolerant of a PEG 6000 concentration ranging from 15 to 50% (Table S3).

3.3 Pot Experiment and Selection of an Efficient Inoculant

The seven inocula exerted positive effects on almost all the measured parameters (Table 4). However, their efficiency was modulated by the level of N fertilization. For instance, significant increases in durum wheat growth parameters were observed without N fertilization and with N fertilization at a medium level, when compared to their respective controls S0-0%N and S0-50%N. At these two levels, the mix of the three strains showed the best results, followed by the mixes of two strains, compared to the single inoculated and uninoculated treatments.

Durum wheat plants inoculated with the mix M but without N fertilization showed significant increases in PH, FLA and SDW, by 49%, 38%, and 60%, respectively, compared to the control plants without inoculation and N fertilization. Not surprisingly, these increases did not reach the maximum growth recorded with a complete dose of N fertilization. Indeed, plants treated with the mix M and 50% N fertilization showed significant increases in PH (37%), FLA (45%), and SDW (89%) when compared to the control plants. Interestingly, at this treatment level, the growth parameters were statistically equivalent to, or higher than, those recorded in the control plants with 100% N fertilization. In contrast, at the highest dose of N fertilization, inoculant application only had neutral effects on the growth parameters when compared to the application of synthetic N fertilizer alone.

For their part, the vegetation indices of the durum wheat plants, LCCI, F_v/F_m, and NDVI, followed the same trend as the growth

Table 4 Crop growth parameters and vegetation indices of durum wheat plants under the influence of combined doses of synthetic nitrogen fertilization and bacterial inocula

Treatments	PH (cm plant^{-1})	FLA (cm^{-2})	SDW (g plant^{-1})	LCCI	F_v/F_m	NDVI
S0-0% N	34.00 j ± 2.00	19.33 i ± 0.00	1.55 l ± 0.04	24.95 h ± 1.22	0.78 g ± 0.01	0.34 jk ± 0.02
S1-0% N	37.16 hij ± 4.44	21.22 hi ± 0.40	1.85 kl ± 0.02	27.26 h ± 1.13	0.78 g ± 0.00	0.32 k ± 0.02
S2-0% N	36.00 hij ± 4.00	21.72 h ± 0.60	1.87 kl ± 0.02	32.46 g ± 1.60	0.78 g ± 0.02	0.37 ijk ± 0.02
S3-0% N	35.83 ij ± 3.17	24.22 g ± 1.06	1.82 kl ± 0.04	27.88 h ± 1.45	0.78 g ± 0.00	0.37 ijk ± 0.02
S1 + S2-0% N	42.16 gh ± 2.17	24.75 fg ± 0.41	1.93 k ± 0.05	33.80 g ± 1.37	0.79 fg ± 0.00	0.38 ij ± 0.04
S1 + S3-0% N	41.50 ghi ± 2.17	26.51 f ± 0.00	2.11 jk ± 0.08	33.56 g ± 2.85	0.79 fg ± 0.01	0.39 ij ± 0.04
S2 + S3-0% N	47.01 fg ± 2.66	25.85 fg ± 0.33	2.29 ij ± 0.46	32.71 g ± 2.24	0.78 g ± 0.00	0.37 ijk ± 0.04
M-0% N	50.78 ef ± 2.02	26.04 fg ± 0.67	2.48 i ± 0.32	34.33 g ± 1.83	0.79 ef ± 0.00	0.40 i ± 0.02
S0-50% N	55.61 de ± 3.41	30.53 e ± 0.74	3.71 h ± 0.10	41.33 f ± 1.74	0.80 de ± 0.00	0.48 h ± 0.03
S1-50% N	59.50 d ± 2.67	36.71 cd ± 0.17	5.91 g ± 0.05	44.18 ef ± 4.05	0.80 de ± 0.00	0.56 g ± 0.04
S2-50% N	66.51 c ± 2.82	35.82 d ± 0.37	6.00 fg ± 0.10	44.63 def ± 2.42	0.80 de ± 0.01	0.59 efg ± 0.05
S3-50% N	70.18 bc ± 5.25	37.53 cd ± 1.10	6.10 fg ± 0.04	45.85 de ± 3.43	0.81 d ± 0.00	0.58 fg ± 0.03
S1 + S2-50% N	74.33 ab ± 5.33	41.06 b ± 2.33	6.87 ab ± 0.02	50.25 abc ± 2.30	0.82 bc ± 0.00	0.65 bcd ± 0.05
S1 + S3-50% N	73.83 ab ± 4.50	40.61 b ± 0.18	6.78 abc ± 0.03	46.06 de ± 2.89	0.83 abc ± 0.00	0.65 abcd ± 0.02
S2 + S3-50% N	73.83 ab ± 4.83	41.03 b ± 0.00	6.65 bcd ± 0.04	45.93 de ± 3.54	0.81 cd ± 0.00	0.63 bcde ± 0.03
M-50% N	76.50 a ± 4.33	44.13 a ± 0.37	7.09 a ± 0.08	51.33 ab ± 1.74	0.84 a ± 0.00	0.70 a ± 0.01
S0-100% N	71.33 abc ± 1.67	41.43 b ± 0.00	6.63 bcd ± 0.20	50.55 abc ± 2.08	0.83 abc ± 0.00	0.66 abc ± 0.04
S1-100% N	69.83 bc ± 2.50	40.14 b ± 0.38	6.52 cde ± 0.06	49.78 abc ± 2.27	0.82 bc ± 0.00	0.63 cdef ± 0.04
S2-100% N	74.25 ab ± 2.42	40.30 b ± 0.18	6.32 def ± 0.12	48.00 bcd ± 2.92	0.82 abc ± 0.02	0.60 defg ± 0.06
S3-100% N	74.33 ab ± 7.00	38.10 c ± 0.37	6.27 ef ± 0.12	47.30 cde ± 1.30	0.82 abc ± 0.00	0.62 cdefg ± 0.06
S1 + S2-100% N	74.00 ab ± 4.33	41.02 b ± 0.90	6.58 bcde ± 0.10	50.50 abc ± 2.35	0.82 abc ± 0.00	0.64 cde ± 0.02
S1 + S3-100% N	71.50 abc ± 3.16	40.32 b ± 1.03	6.57 bcde ± 0.13	50.23 abc ± 2.27	0.82 abc ± 0.00	0.65 bcd ± 0.04
S2 + S3-100% N	73.66 ab ± 3.16	40.99 b ± 1.78	6.54 bcde ± 0.14	50.16 abc ± 2.25	0.83 ab ± 0.00	0.65 abcd ± 0.04
M-100% N	75.00 ab ± 2.33	41.10 b ± 0.47	6.60 bcd ± 0.09	52.05 a ± 1.50	0.83 ab ± 0.00	0.69 ab ± 0.05
Nitrogen fertilization (N)	***	***	***	***	***	***
Bacterial inoculation (BI)	***	***	***	***	***	***
N x BI interaction	***	***	***	**	Ns	***

S0, uninoculated plants; M, plants inoculated with the mix of three strains: S1 = LAa26, S2 = LAa56, and S3 = LAb21. Different letters indicate significant differences according to the LSD test ($p \leq 0.05$)

PH plant height, *FLA* flag leaf area, *SDW* shoot dry weight, *LCCI* leaf chlorophyll content index, F_v/F_m chlorophyll fluorescence, *NDVI* normalized difference with vegetation index

ns: not significant, *, **, ***significance at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, respectively

parameters, as the efficiency of the different bacterial inocula was higher when the durum wheat plants received the half dose of synthetic N fertilization. In particular, inoculation with the mix, M, resulted in significant increases in the LCCI (+24%), in F_v/F_m (+3%) and in NDVI (+45%) compared to the control plants supplemented with only 50% of N fertilization. Interestingly, these increases reached averages of 51.33, 0.83, and 0.7 for LCCI, F_v/F_m and NDVI, respectively, which were statistically similar to those recorded in the control plants receiving a full dose of N fertilization.

3.4 Field Experiments

3.4.1 Growth Parameters

In the four field environments, the growth of the wheat plants was significantly affected by the level of synthetic N fertilization, as well as by bacterial inoculation (Table 5). As expected, the growth parameter values increased with the level of nitrogen fertilization. Indeed, the efficiency of the mix of three strains depended on the level of synthetic nitrogen fertilization applied. It is important to note that significant interactions between nitrogen fertilization and bacterial inoculations were found for all the growth parameters in all the field environments.

In each of the four environments, stressed or unstressed, the treatment involving the PGPR mix with the 50% rate of synthetic nitrogen fertilizer significantly increased the studied growth parameter values compared to those recorded for uninoculated plants receiving only the half dose of synthetic nitrogen fertilizer. In the unstressed environment, KAI-100% ETC and MED-1.5 dS m^{-1}, these increases varied from 13 to 38% for LCCI, from 19 to 36% for NDVI, and from 29

Table 5 Crop vegetation indices and plant height of durum wheat plants under the influence of combined doses of synthetic nitrogen fertilization and the mix of three *Agrobacterium* sp. (M)

Treatment	LCCI				NDVI				PH (cm plant^{-1})			
	KAI-100% ET_C	KAI-50% ET_C	MED-1.5 dS m^{-1}	MED-13.3 dS m^{-1}	KAI-100% ET_C	KAI-50% ET_C	MED-1.5 dS m^{-1}	MED-13.3 dS m^{-1}	KAI-100%ET_C	KAI-50% ET_C	MED-1.5 dS m^{-1}	MED-13.3 dS m^{-1}
S0-0% N	38.23c ± 1.78	32.83d ± 0.16	30.33d ± 0.46	27.66d ± 1.11	0.46d ± 0.01	0.32d ± 0.01	0.23e ± 0.06	0.20e ± 0.01	47.66e ± 1.78	43.00e ± 0.05	58.33c ± 1.11	48.00d ± 1.33
M-0% N	40.66c ± 1.29	39.23c ± 1.09	33.33c ± 0.89	29.33d ± 0.44	0.50d ± 0.04	0.48c ± 0.02	0.35d ± 0.00	0.34d ± 0.03	55.66d ± 2.89	53.33d ± 0.89	68.33b ± 1.11	65.00c ± 0.67
S0-50% N	46.66b ± 0.71	41.06c ± 0.25	45.33b ± 0.44	37.56c ± 0.36	0.59c ± 0.01	0.49c ± 0.00	0.59c ± 0.00	0.45c ± 0.02	68.66c ± 2.22	55.66d ± 0.39	73.33b ± 2.22	61.00c ± 2.67
M-50% N	64.40a ± 1.47	60.90a ± 0.73	51.00a ± 0.67	50.66a ± 0.96	0.80a ± 0.07	0.76a ± 0.01	0.70a ± 0.01	0.67a ± 0.01	91.66a ± 0.89	86.00a ± 1.00	94.66a ± 3.78	90.66a ± 0.67
S0-100% N	61.40a ± 1.13	52.86b ± 0.73	51.00a ± 0.67	45.66b ± 1.11	0.76ab ± 0.01	0.58b ± 0.01	0.66ab ± 0.02	0.58b ± 0.02	84.66b ± 2.44	69.00 c ± 0.86	92.00a ± 0.67	75.00b ± 4.00
M-100% N	61.13a ± 4..18	54.56b ± 2.56	52.00a ± 2.00	46.00b ± 1.33	0.72bc ± 0.01	0.59b ± 0.01	0.64bc ± 0.00	0.61b ± 0.00	85.00b ± 1.33	75.66b ± 2.89	92.66a ± 5.11	85.00a ± 2
Nitrogen fertilization (N)	***	***	***	***	***	***	***	***	***	***	***	***
Bacterial inoculation (BI)	***	***	***	***	*	***	***	***	***	***	***	***
N x BI interaction	***	***		***	*	***	**	***	***	***	***	***

S0, uninoculated plants; M, plants inoculated with a mix of three strains: S1 = LAa26, S2 = LAa56, and S3 = LAb21. Different letters indicate significant differences according to the LSD test ($p \leq 0.05$)

LCCI leaf chlorophyll content index, *NDVI* normalized difference with vegetation index, *PH* plant height, ns: not significant, *, **, ***significance at $p \leq 0.1$, $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, respectively to 33% for PH when compared to control plants at the same level of N fertilization. However, under drought stress in KAI-50% ETC, the effect of combining PGPR with 50%N increased the LCCI, NDVI and PH by 48%, 55%, and 54%, respectively, compared to the plants receiving only 50% of synthetic N fertilization. Moreover, under saline conditions in MED-13.3 dS m^{-1}, the abovementioned treatment M-50%N increased the LCCI by 35%, NDVI by 49%, and PH by 49% compared to the half dose of synthetic nitrogen fertilization. Interestingly, the treatment M-50%N resulted in the highest values compared to the other treatments, and usually significantly, similar to plants receiving the highest level of synthetic nitrogen.

3.4.2 Durum Wheat Yield

Similar to the growth parameters, yield components were significantly affected by the level of synthetic N fertilization, by bacterial inoculation and by their interactions (Table 6, Table S4a,b). Across all four field environments, the combined application of the bacterial mix with 50%N significantly increased all the studied yield components. In the unstressed environments, KAI-100% ETC and MED-1.5 dS m^{-1}, located in the semi-arid and arid regions, respectively, bacterial inoculation along with 50%N increased the NS (41–75%), GY (34–38%), BY (41–57%), and TGW (9–10%) compared to the half dose of N fertilizer, and ensured values similar to or higher than those of plants receiving a full dose of nitrogen.

However, larger increases were recorded in the stressed environments. For instance, inoculation of plants receiving only 50% of their water and nitrogen requirements at KAI-50% ETC resulted in significant improvements amounting to 28% for the NS, 42% for GY, and 13% for BY, compared to treatment S0-100%N. In addition, when irrigated with saline water at MED-13.3 dS m^{-1}, bacterial inoculation combined with 50%N induced significant increases amounting to 43% for the NS, 33% for GY, and 22% for BY compared to plants treated exclusively with 100%N. For the TGW, the combination of bacterial inoculation with 50%N assured statistically similar averages to those recorded in control plants fertilized with 100%N in all environments. Except for KAI-50% ETC, the abovementioned treatment induced a significant increase (6%) compared to S0-100%N.

For the N and P contents in the grain and straw of wheat plants (Table 7, Table S5a,b), across all the environments, stressed or not, bacterial inoculation combined with 50%N performed as well as the 100%N treatment. Exceptionally, under saline conditions, the inoculated plants fertilized with a half dose of N displayed grain N and P contents that were statistically higher than those recorded in control plants S0-100%N. Also under drought stress, the combination of a bacterial mix with 50%N resulted in the highest accumulation of P in the grains of wheat plants compared to the 100%N treatment.

3.5 Economic Benefits Obtained Using the Selected Mix, M, Combined with a Reduced Application of Synthetic N Fertilizer

Total costs, total benefits, as well as net returns, for all treatments in the four environments are presented in Table 8. The results showed that, when compared to the net return from the control plants fertilized with 100%N, applying the mix, M, with 50% of synthetic nitrogen fertilization was the only positive solution and the best way of increasing the potential financial return from the wheat crop. Thus, the combined application of the selected mix with 50%N helped to create a net return per hectare of $ 1250 at KAI-100% ETc, $ 942 at KAI-50% ETc, $ 715 at MED-1.5 dS m^{-1}, and $ 587 at MED-13.3 dS m^{-1}, amounting to a percentage gain of 51%, 78%, 26%, and 106%, respectively, compared to the net return from the control plants supplemented with the full recommended dose.

4 Discussion

In Mediterranean areas, such as Tunisia, increasing durum wheat production is a challenging objective for scientists given the severity of climate change, the low nitrogen fertility of soils, together with the increasing cost of synthetic nitrogen fertilizers (Laatiri et al. 2010). This study was designed to explore the in vitro PGP potential of 72 native rhizobial strains previously isolated from root nodules of *Lupinus albus* L. (Tounsi-Hammami et al. 2019), with a view to selecting native elite strains as potential biofertilizers for durum wheat in Tunisia.

When screened, many of the tested rhizobial strains exhibited more than one plant growth–promoting trait that might promote plant growth directly and indirectly. About 86% of strains were able to synthesize IAA in a range of 10.47 to 341.23 μg mL^{-1} using tryptophan as a precursor. Such variation is mainly related to the distinct synthetic pathways, regulatory strategies and key genes of different bacteria (Li et al. 2018). Generally, IAA is considered as the most important phytohormone that coordinates several developmental processes in plants. Indeed, a low concentration of IAA induces primary root elongation, while a high concentration stimulates lateral and adventitious root development (Duca et al. 2014). In the current study, the amounts of IAA produced by the native strains were considerably higher than those produced by endophytic bacteria previously isolated from nodules of other legumes grown in Tunisian soils, with values ranging from 0.67 to 74.51 μg mL^{-1} (Saïdi et al. 2013; Abdelkrim et al. 2018; Ferchichi et al.

Table 6 Yield components of durum wheat plants under the influence of combined doses of synthetic nitrogen fertilization and the mix of three *Agrobacterium* sp. (M)

Treatment	Number of spikes per square meter				Grain yield (qx ha^{-1})				Biomass yield (T ha^{-1})				Thousand-grain weight (g)			
	KAI-100%ET_C	KAI-50% ET_C	MED-1.5 dS m^{-1}	MED-13.3 dS m^{-1}	KAI-100%ET_C	KAI-50%ET_C	MED-1.5 dS m^{-1}	MED-13.3 dS m^{-1}	KAI-100%ET_C	KAI-50%ET_C	MED-1.5 dS m^{-1}	MED-13.3 dS m^{-1}	KAI-100%ET_C	KAI-50% ET_C	MED-1.5 dS m^{-1}	MED-13.3 dS m^{-1}
S0-0%N	233.33d	169.00d	107.33c	90.66d	21.98c	15.83d	8.39e	5.70d	3.07e	2.62d	2.03c	1.56d	39.30d	37.57e	35.86b	32.44d
M-0%N	290.33d	234.00 cd	128.00c	105.66d	25.09c	19.19 cd	13.22d	14.33c	3.80d	2.89 cd	2.29c	2.49 cd	41.22 cd	40.99d	37.52b	36.94c
S0-50%N	355.00c	296.00bc	180.00b	158.16c	43.96b	24.90bc	27.01c	18.56c	4.41c	3.65bc	3.70b	3.42c	43.08bc	41.66 cd	47.09a	41.96b
M-50%N	501.33a	429.00a	315.83a	317.00a	58.96a	45.33a	37.47a	34.20a	6.26a	5.10a	5.84a	5.85a	47.25a	46.46a	51.17a	50.73a
S0-100%N	432.00b	334.66b	320.00a	221.66b	46.56b	31.71b	33.18b	25.68b	5.02b	4.50ab	5.88a	4.79b	45.68ab	43.67bc	50.76a	47.58a
M-100%N	436.00b	359.33b	298.33a	234.66b	48.92b	32.71b	32.29b	28.76b	5.09b	4.34ab	5.94a	5.19ab	45.44ab	44.84ab	50.81a	48.50a
Nitrogen fertilization (N)	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Bacterial inoculation (BI)	**	**	***	***	**	**	***	***	***		***	***		***	*	***
N*In interaction	*		***	***	*	**	***	**	***		***	*	ns		ns	*

S0, uninoculated plants; M, plants inoculated with a mix of three strains: S1 = LAa26, S2 = LAa56, and S3 = LAb21. Different letters indicate significant differences according to the LSD test ($p \leq 0.05$)

ns: not significant, *, **, ***significance at $p \leq 0.1$, $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, respectively

Table 7 Nitrogen and phosphorus content in grain and straw of durum wheat plants under the influence of combined doses of synthetic nitrogen fertilization and the mix (M)

Treatment	Nitrogen concentration in grains (%)				Nitrogen concentration in straw (%)				Phosphorus concentration in grains (%)				Phosphorus concentration in straw (%)			
	KAI-100%ET_C	KAI-50%ET_C	MED-1.5dSm^{-1}	MED-13.3 dS m^{-1}	KAI-100%ET_C	KAI-50%ET_C	MED-1.5 dS m^{-1}	MED-13.3 dS m^{-1}	KAI-100%ET_C	KAI-50%ET_C	MED-1.5 dS m^{-1}	MED-13.3 dS m^{-1}	KAI-100%ET_C	KAI-50% ET_C	MED-1.5 dS m^{-1}	MED-13.3 dS m^{-1}
S0-0% N	2.413c	2.115d	1.414c	1.316d	0.700d	0.672b	0.290b	0.266c	0.649d	0.591e	0.526c	0.401e	0.270e	0.239d	0.112d	0.099b
M-0% N	2.517bc	2.477c	1.484bc	1.444 cd	0.742 cd	0.717b	0.302b	0.277bc	0.758c	0.716d	0.568c	0.526d	0.300d	0.303d	0.120d	0.103b
S0-50% N	2.637b	2.475bc	1.638b	1.489c	0.756bcd	0.664b	0.35ab	0.291bc	0.981b	0.803c	0.781b	0.610c	0.395c	0.318 cd	0.212c	0.155b
M-50% N	3.040a	2.949a	1.904a	1.866a	0.870a	0.829a	0.38ab	0.386a	1.271a	1.186a	1.078a	0.986a	0.533a	0.498a	0.347a	0.331a
S0-100% N	2.991a	2.643ab	1.845a	1.552bc	0.848abc	0.784a	0.398a	0.344a	1.206a	1.024b	1.009a	0.834b	0.510a	0.387ab	0.330a	0.257a
M-100% N	2.956a	2.785bc	1.817a	1.680b	0.855ab	0.815a	0.389ab	0.375ab	1.202a	0.988b	0.983a	0.898b	0.483ab	0.460bc	0.303b	0.280a
Nitrogen fertilization (N)	***	*	***	***	***	**	*	***	***	***	***	***	***	***	***	***
Bacterial inoculation (BI)	**	***	*	***		***	ns	*	***	***	*	***	***	***	***	**
N*BI interaction	***	**		*	ns	*	ns	ns	**	***	**	***	***		***	**

S0, uninoculated plants; M, plants inoculated with a mix of three strains: S1 = LAa26, S2 = LAa56, and S3 = LAb21. Different letters indicate significant differences according to the LSD test ($p \leq 0.05$)

ns: not significant, *, **, ***significance at $p \leq 0.1$, $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, respectively

Table 8 Benefits, costs and net returns for wheat production using the selected mix, M, in combination with synthetic nitrogen fertilizer

Environments	S0-0%N	M-0%N	S0-50%N	M-50%N	S0-100%N	M-100%N
KAI-100%ETc						
Variable costs (US $ ha^{-1}) (a + b + c)	299.20	343.40	337.45	381.65	375.70	419.90
N fertilization (US $ ha^{-1}) (a)	0.00	0.00	38.25	38.25	76.50	76.50
Bacterial inoculation (US $ ha^{-1}) (b)	0.00	44.20	0.00	44.20	0.00	44.20
Irrigation cost (US $ ha^{-1}) (c)	299.20	299.20	299.20	299.20	299.20	299.20
Fixed cost (US $ ha^{-1}) (d)	428.74	428.74	428.74	428.74	428.74	428.74
Total cost (US $ ha^{-1}) (a + b + c + d)	727.94	772.14	766.19	810.39	804.44	848.64
Benefit (grain) (US $ ha^{-1}) (A)	612.80c ± 83	699.60c ± 71	1225.60b ± 172	1643.80a ± 159	1298.09b ± 104	1363.89b ± 109
Benefit (straw) (US $ ha^{-1}) (B)	204.42e ± 15	253.16d ± 31	293.39c ± 7	416.13a ± 17	333.78b ± 12	338.82b ± 13
Total benefits (US $ ha^{-1}) (A + B)	817.22c ± 99	952.76c ± 59	1519.00b ± 169	2060.10a ± 162	1631.87b ± 105	1702.71b ± 117
Net returns (US $ ha^{-1})	89.28c ± 99	180.62c ± 59	752.81b ± 169	1249.71a ± 162	827.43b ± 105	854.07b ± 117
% increases	− 89.21	− 78.17	− 9.02	51.03		3.22
KAI-50%ETc						
Variable costs (US $ ha^{-1}) (a + b + c)	149.60	193.80	187.85	232.05	226.10	270.30
N fertilization (US $ ha^{-1}) (a)	0.00	0.00	38.25	38.25	76.50	76.50
Bacterial inoculation (US $ ha^{-1}) (b)	0.00	44.20	0.00	44.20	0.00	44.20
Irrigation cost (US $ ha^{-1}) (c)	149.60	149.60	149.60	149.60	149.60	149.60
Fixed cost (US $ ha^{-1}) (d)	428.74	428.74	428.74	428.74	428.74	428.74
Total cost (US $ ha^{-1}) (a + b + c + d)	578.34	622.54	616.59	660.79	654.84	699.04
Benefit (grain) (US $ ha^{-1}) (A)	441.43c ± 11	634.92c ± 73	852.38b ± 60	1263.70a ± 73	883.98b ± 221	912.04b ± 209
Benefit (straw) (US $ ha^{-1}) (B)	179.39d ± 19	192.15 cd ± 12	242.83bc ± 25	339.27a ± 24	299.50ab ± 59	288.95ab ± 46
Total benefits (US $ ha^{-1}) (A + B)	615.83c ± 22	727.07c ± 68	1095.21b ± 84	1602a ± 67	1183.48b ± 234	1201.00b ± 256
Net returns (US $ ha^{-1})	37.49c ± 22	104.53c ± 68	478.62b ± 84	942.16a ± 67	528.64b ± 234	501.96b ± 256
% increases	− 92.91	− 80.23	− 9.46	78.23	–	− 5.05
Med-1.5 dS m^{-1}						
Variable costs (US $ ha^{-1}) (a + b + c)	244.80	289.00	283.05	327.25	321.30	365.50
N fertilization(US $ ha^{-1}) (a)	0.00	0.00	38.25	38.25	76.50	76.50
Bacterial inoculation (US $ ha^{-1}) (b)	0.00	44.20	0.00	44.20	0.00	44.20
Irrigation cost (US $ ha^{-1}) (c)	244.80	244.80	244.80	244.80	244.80	244.80
Fixed cost (US $ ha^{-1}) (d)	428.74	428.74	428.74	428.74	428.74	428.74
Total cost (US $ ha^{-1}) (a + b + c + d)	673.54	717.74	711.79	755.99	750.04	794.24
Benefit (grain) (US $ ha^{-1}) (A)	234.13e ± 15	368.57d ± 43	753.13c ± 19	1044.81a ± 10	925.11b ± 39	900.27b ± 16
Benefit (straw) (US $ ha^{-1}) (B)	135.31c ± 7	152.31c ± 5	246.26b ± 41	338.48a ± 24	391.17a ± 33	396.46a ± 4
Total benefits (US $ ha^{-1}) (A + B)	369.45e ± 19	521.24d ± 40	999.39c ± 60	1433.29a ± 33	1316.28b ± 22	1296.73b ± 73
Net returns (US $ ha^{-1})	− 304.08f ± 19	− 234.74e ± 40	287d ± 60	715.55a ± 33	566.24b ± 22	502.49c ± 13
% increases	− 153.70	− 141.46	− 49.21	26.37	–	− 11.26
Med-13.3 dS m^{-1}						
Variable costs (US $ ha^{-1}) (a + b + c)	244.80	289.00	283.05	327.25	321.30	365.50
N fertilization(US $ ha^{-1}) (a)	0.00	0.00	38.25	38.25	76.50	76.50
Bacterial inoculation (US $ ha^{-1}) (b)	0.00	44.20	0.00	44.20	0.00	44.20
Irrigation cost (US $ ha^{-1}) (c)	244.80	244.80	244.80	244.80	244.80	244.80
Fixed cost (US $ ha^{-1}) (d)	428.74	428.74	428.74	428.74	428.74	428.74
Total cost (US $ ha^{-1}) (a + b + c + d)	673.54	717.74	711.79	755.99	750.04	794.24
Benefit (grain) (US $ ha^{-1}) (A)	158.95d ± 31	399.52c ± 74	517.61c ± 62	953.57a ± 63	715.85b ± 70	801.74b ± 134
Benefit (straw) (US $ ha^{-1}) (B)	104.13d ± 21	166.03 cd ± 10	227.40c ± 45	389.17a ± 25	319.08b ± 47	345.44ab ± 47
Total benefits (US $ ha^{-1}) (A + B)	263.08e ± 12	565.55d ± 85	745.01c ± 22	1342.75a ± 44	1034.94b ± 116	1147.19b ± 76
Net returns (US $ ha^{-1})	− 410.45e ± 26	− 152.18d ± 85	33.22c ± 22	586.76a ± 44	282.90b ± 116	352.95b ± 96
% increases	− 239.82	− 153.41	− 88.33	105.95	–	23.88

Net returns (total benefit − total cost); % increases = (net return of treatment − net return of S0 + 100%N) × 100/net return of S0 + 100%N. Different letters indicate significant differences according to the LSD test ($p \leq 0.05$)

US $ ha^{-1} American dollar per hectare

2019). Larger amounts exceeding 400 μg mL^{-1} produced by two cowpea strains related to *Agrobacterium radiobacter* were recently reported by Valdez-Nunez et al. (2019) in Peru. In addition to IAA, siderophore production presents an important PGP trait. It was reported that siderophores might promote plant growth directly by supplying Fe to plants (Tariq et al. 2017) and indirectly by inhibiting the establishment of pathogenic agents through the sequestration of Fe from the root environment (Ahemad and Kibret 2014). In the current study, under in vitro conditions with iron limitation, 65% of the native strains were siderophore producers. Twelve strains of our bacterial collection seemed to be better producers of siderophores (9–15 mm) than *R. leguminosarum*, *S. meliloti*, *Pseudomonas* sp., *P. fluorescens*, *Luteibacter* sp., *Variovorax* sp., *B. simplex*, and *B. megaterium* isolated from root nodules of grass pea (*Lathyrus sativus*) grown in Tunisia, with a halo zone ranging from 4.3 to 9 mm (Abdelkrim et al. 2018). Furthermore, 29% of the strains showed their ability to solubilize insoluble forms of phosphorus (tricalcium phosphate), which might be due to the release of organic acids that chelate calcium associated with phosphate and thus make P more available (Satyaprakash et al. 2017). This PGP trait is an important characteristic, particularly in environments where P is a limiting factor for crop production. Another important PGP trait exhibited by almost all of the strains (76%) was ammonia production, with ammonia being released into the rhizospheric soil and serving as a plant nutrient source.

Based on the antagonistic test between strains and on their PGP trait, only the three compatible strains LAa26, LAa56, and LAb21 were selected for further studies and seemed to be good candidates to act as durum wheat PGPRs. The choice of these strains to be trialed as a biofertilizer was designed to maximize the solubilization of P and Fe and the production of ammonia and IAA in rhizospheric soil. These strains were previously identified as *Agrobacterium* sp. (Tounsi-Hammami et al. 2019). It is well known that the members of *Agrobacterium* are widespread in soil and plant habitats (Zahradník et al. 2018). Despite the general perception that most of them are phytopathogenic, causing plant diseases, numerous studies have shown that many strains of *Agrobacterium tumefaciens* isolated from the root nodules of several legumes, rhizospheric soils, or cereal roots, are not pathogenic (Youseif et al. 2014a, b; Youseif 2018; Tounsi-Hammami et al. 2019) and exhibit PGP potentiality (Youseif 2018; Valdez-Nunez et al. 2019; Cavalcanti et al. 2020).

Given this background, the efficiency of the selected three *Agrobacterium* sp. strain candidates (LAa26, LAa56 and LAb21) for durum wheat growth (Razzek variety) was examined in this study. The selected strains were used individually and in mixes of two and three strains in a pot experiment under natural conditions, varying the level of synthetic nitrogen fertilization (0%, 50%, and 100% of the recommended dose) with a view to selecting the most efficient combination. It is important to note that the soil used in the pot experiment was not sterilized, because one of the most important factors to consider during screening of new PGP rhizobacteria is their ability to compete with well-adapted native microorganisms (Ross et al. 2000; Rana et al. 2011; Buragohain et al. 2018).

The results provided some promising data on the use of the selected PGP rhizobia, which displayed a better agronomic performance on durum wheat plants var. Razzek when compared to the uninoculated plants. In fact, the improved PH, FLA, and SDW in inoculated durum wheat plants could probably be attributed to greater photosynthetic activity compared to the control plants. Our findings support those of Mesa-Marin et al. (2018), who reported that PGPRs are able to improve photosynthetic activity directly by improving the efficiency of photosystem II, which was reflected in the current study in a higher LCCI, F_v/F_m, together with the NDVI. In the same context, Yaghoubi Khanghahi et al. (2021) confirmed that the inoculation of durum wheat plants with a PGPR mix significantly improves the quantum yield of PSII photochemistry, the electron transport rate of PSII, photosynthesis capacity, transpiration rate and stomatal conductance. It was also reported that photosynthetic activity can be improved indirectly by enhancing the availability of several nutrients, such as N, P, and Fe in the root soil environment (Mia et al. 2010). Moreover, our findings highlighted that bacterial mixes, especially the mix of three strains, provided greater benefits for plant growth than single strains, regardless of the nitrogen dose applied. Our results are consistent with previous studies by Saleem et al. (2021) and Pereira et al. (2020), carried out on other cereal crops such as maize. It is important to note that the selected agrobacterial strains LAa26, LAa56 and LAb21 were non-antagonistic to each other and had the highest production of IAA, siderophores, and phosphate solubilization, respectively. In addition, all of them were ammonia producers. It could be assumed that the complementarity and the synergistic relationship between these PGP traits lay behind the efficiency of the mix of three strains (Dutta et al. 2017; Molina-Romero et al. 2017). On the other hand, strong interactions were observed between the bacterial inocula and the synthetic nitrogen fertilizer, showing that the efficiency of these inocula depended on the nitrogen rate applied to the soil. Applying bacterial inocula alone, without synthetic nitrogen input, induced significant increases in all the measured growth parameters when compared to the control plants. However, these increases were not sufficient to achieve the optimum growth recorded by plants treated with the full recommended dose (150 kg ha^{-1}). This result means that the efficiency of bacterial inocula alone was not found to equal that of the synthetic fertilizer for achieving maximum crop yields, which is in line with several earlier studies

(Titah et al. 2013; Kumar et al. 2014; Wang et al. 2020). In contrast, the efficiency of the tested inocula was reduced and not significant when combined with the highest dose of synthetic N fertilization (150 kg ha^{-1}). This might be explained by the fact that synthetic agricultural inputs may negatively affect bacterial populations in the root environment (Thilagar et al. 2016). Coherently, Reid et al. (2021) reported that the abundance of rhizobacteria that potentially benefit plants by mobilizing insoluble nutrients, such as N, P, K, Fe, and Zn in soil, was significantly reduced in wheat grown in soils treated with synthetic NPK fertilizers. In the same context it has been reported that synthetic N fertilization decreases the occurrence of IAA-producing bacteria in rhizospheric soils (Martinez et al. 2011) and thereby reduces plant root growth (Dal Cortivo et al. 2017). The most important result obtained from the pot experiment was that the PGPR inoculation was able to replace the loss of 50% of synthetic N fertilizer. Indeed inoculated plants provided with 50%N showed greater vegetative indices and growth parameters than those of plants given 100%N. This can be attributed to greater availability of nutrients in the rhizosphere, which supports the growth of rhizobia and host plants equally (Zarei et al. 2012). It is important to note that, during our experiment, plant roots were analyzed for any abnormal root growth or cortical hypertrophy (data not shown) and the three selected strains affiliated to the *Agrobacterium* genus did not produce abnormal tumors in durum wheat plants, which indicated that these strains should be safe and suitable for application in the natural environment.

Under actual field conditions, in semi-arid and arid regions, the results obtained were positively correlated with those obtained in the pot experiment. In fact, a combined application of the mix, M, and the half dose of nitrogen induced the highest vegetation indices as well as growth parameters. Interestingly, the improvements were transmitted to yield components, resulting in a number of spikes m^{-2}, grain yield (qx ha^{-1}), as well as biomass yield (T ha^{-1}), which were statistically similar to, or greater than, those recorded in uninoculated plants receiving the full dose of N fertilization. The results confirmed that the combined application of the selected mix and 50%N was more effective and induced greater increases in all the measured parameters under stressful conditions, KAI-50% ETC and MED-13.3 dS m^{-1}, than under normal conditions, which is in agreement with the results of Rubin et al. (2017). These findings demonstrated the beneficial role of the selected mix in attenuating the negative effects of drought and salt stress when combined with 50% of synthetic nitrogen fertilizer. The benefit obtained through the supplementation of PGP agrobacteria could be due to the interactive effect of nutrient availability (Wang et al. 2020), tolerance of severe environmental factors (Pereira et al. 2020; Saleem et al. 2021) and to the wheat variety (Razzek) (Inculet et al. 2019; Khan et al. 2019). Indeed, besides PGPR traits, the *Agrobacterium* strains used in the current study appeared to be highly tolerant of salinity and drought, and are able to grow under a widely varying pH (4–11), which enables these strains to act as a good biofertilizer, enhancing the resilience of wheat plants against drought and salinity (Ilyas et al. 2020; Redondo-Gómez et al. 2021). Marulanda et al. (2009) reported that strains well adapted to abiotic stress conditions contribute to increasing their survival in soil and allow them to express their plant growth–promoting activities. It has already been proved that PGPRs, including rhizobia, may help plants to cope with drought and salt stress by increasing photosynthetic activity, altering hormonal root–shoot signaling, increasing production of osmolytes, improving ion homeostasis and transport, as well as through the expression of specific genes (Nautiyal et al. 2013; Ilangumaran and Smith 2017; Liu et al. 2019). Many studies have confirmed the significant correlation of photosynthesis enhancement and biomass accumulation with increased soil water levels (Saleem et al. 2021). The boosting of photosynthetic activity, vegetative growth, and yield parameters under drought and saline conditions linked to the selected mix could therefore be attributed to greater water and nutrient availability (Aroca and Ruiz-Lozano 2009; Nehela et al. 2021), which directly affects the water status of leaves (Zhou et al. 2021) and results in the presence of a healthy photosynthetic system (Khan et al. 2019). It could also be suggested that the selected mix of IAA producer strains mediated alterations in root system architecture, optimizing the use of water and nutrients for better growth under water limitations (Araujo et al. 2021). Furthermore, studies by Nehela et al. (2021) and Akbari et al. (2020) on maize grown under saline conditions demonstrated that PGPR inoculation limits the uptake of toxic Na^+ while increasing that of K^+ and K^+/Na^+, resulting in greater osmotic adjustment.

The main result of this study was the finding that the durum wheat plants of this optimum treatment, M-50%N, exhibited similar or greater N and P accumulation in both grains and straw, when compared to uninoculated plants at the full fertilization rate in all four of the tested environments. N and P are considered as the main essential plant growth-limiting nutrients. According to Laatiri et al. (2010), Tunisian soils have low N fertility. In addition, about 95% of soil P is present in insoluble forms and therefore not available to plants. In the current study, the three selected strains of *Agrobacterium* sp. LAa26, LAa56, and LAb21 were ammonia producers and interestingly improved N uptake by wheat plants that ultimately enhanced N accumulation in the plant. While LAb21 was the best phosphorus solubilizer, it significantly increased P accumulation in the grains and straw of durum wheat plants. This could be related to the ability of this strain to release organic acids such as oxalic and citric acids and decrease the pH of the rhizosphere (Maliha et al. 2004), which led to the dissolution of the mineral phosphate (Wang et al. 2020). The enhancement of yields and nutritional status are also consistent with the known benefit of bacteria IAA production for the root surface area by increasing the number of root tips and ramifications (Dal Cortivo et al. 2017), even under stressful conditions (Naveed et al. 2014; Sadeghi et al. 2012; Timmusk et al. 2014; Saleem et al. 2021). In this way, faster root establishment enhances the surface area in contact with the soil and allows the plant to explore larger soil volumes and gain better access to nutrients and water, while also enhancing their uptake (Gutierrez-Manero et al. 2001; Ramos-Solano et al. 2008; Saleem et al. 2021).

As regards the literature consulted, the positive effect of *Agrobacterium* sp. strains isolated from root nodules of white lupin on durum wheat var. Razzek and their potential to reduce N synthetic fertilization by 50% is reported for the first time in this study. Considering the low N fertility of most farm soils, together with the increasing cost of synthetic N fertilizers, these savings are of great interest to producers. Although the beneficial effect of the tested mix on the growth and productivity of the Razzek variety was proved in this study, this mix M may have different effects on other wheat varieties. In particular, several studies have proved that the efficiency of PGPRs depends significantly on plant varieties (Inculet et al. 2019; Khan et al. 2019), as well as on environmental stress, such as drought (Akbari et al. 2020) and salinity (Inculet et al. 2019). Akbari et al. (2020) showed that the response of four commercial varieties of bread wheat (*Triticum aestivum*) to a defined PGPR differed on the physiological, phenotypic and molecular levels.

Adoption of new practices is totally related to their economic feasibility (Khan et al. 2012). In our research, the economic analysis of the data revealed that inoculating durum wheat plants with the bacterial mix in combination with half the synthetic nitrogen application resulted in higher net returns in non-stressed environments varying from 26 to 51% compared to plants treated with only a full dose application of synthetic nitrogen. It was shown that bacterial inoculation was more efficient in stressed environments resulting in greater net returns varying from 78% at KAI-50% ETC (drought) to 106% at MED-13.3 dS m^{-1} (salinity). The above-mentioned treatment gave the highest net income due to valuable increases in wheat grain and straw yields in the different environments. In contrast, no reduction in production costs was detected compared to the S0-100%N control treatment. It is important to note that production costs may decrease if the selected *Agrobacterium* spp. strains are able to persist in the soil for the next growing seasons. From an environmental point of view, the 50% reduction in synthetic nitrogen fertilizers without affecting economic yields is of great importance, which will help in reducing environmental pollution and improving soil quality with a view to achieving sustainable agriculture.

5 Conclusions

The current investigation revealed that the use of PGP rhizobia isolated from *Lupinus albus* L. may be a good strategy for enhancing the growth and production of durum wheat var. Razzek in semi-arid and arid areas, which are vulnerable to drought and salinity. Our findings demonstrated that, under stressed and non-stressed conditions, the combined application of the mix of three selected strains of *Agrobacterium* sp. and 50%N increased photosynthetic activity, improved growth, maintained optimum yields and enhanced N and P content in both grains and straw compared to uninoculated plants treated only with 100%N. Furthermore, this combination resulted in significantly better net economic returns, especially in stressed environments. Our study recommended the use of this mix as a biofertilizer to enhance the sustainability of durum wheat production in arid and semi-arid areas. However, further studies are needed to assess the positive effect of our proposed biofertilizer on other durum wheat varieties and other cereal crops, as well as on the soil microbial composition in different environments.

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Declarations

Conflict of Interest The authors declare no competing interests.

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