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Comparative Analysis of Plant Growth-Promoting Rhizobacteria (PGPR) and Chemical Fertilizers on Quantitative and Qualitative Characteristics of Rainfed Wheat

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Abstract: The indiscriminate use of hazardous chemical fertilizers can be reduced by applying eco-friendly smart farming technologies, such as biofertilizers. The effects of five different types of plant growth-promoting rhizobacteria (PGPR), including Fla-wheat (F), Barvar-2 (B), Nitroxin (N1), Nitrokara (N2), and SWRI, and their integration with chemical fertilizers (50% and/or 100% need-based N, P, and Zn) on the quantitative and qualitative traits of a rainfed wheat cultivar were investigated. Field experiments, in the form of randomized complete block design (RCBD) with four replications, were conducted at the Qamloo Dryland Agricultural Research Station in Kurdistan Province, Iran, in three cropping seasons (2016–2017, 2017–2018, and 2018–2019). All the investigated characteristics of rainfed wheat were significantly affected by the integrated application of PGPR chemical fertilizers. The grain yield of treated plants with F, B, N1, and N2 PGPR plus 50% of need-based chemical fertilizers was increased by 28%, 28%, 37%, and 33%, respectively, compared with the noninoculated control. Compared with the noninoculated control, the grain protein content was increased by 0.54%, 0.88%, and 0.34% through the integrated application of F, N1, and N2 PGPR plus 50% of need-based chemical fertilizers, respectively. A combination of Nitroxin PGPR and 100% of need-based chemical fertilizers was the best treatment to increase the grain yield (56%) and grain protein content (1%) of the Azar-2 rainfed wheat cultivar. The results of this 3-year field study showed that the integrated nutrient management of PGPR-need-based N, P, and Zn chemical fertilizers can be considered a crop management tactic to increase the yield and quality of rainfed wheat and reduce chemical fertilization and subsequent environmental pollution and could be useful in terms of sustainable rainfed crop production.

Keywords: dryland; biofertilizers; PGPR; grain protein; grain yield; soil fertility management; sustainable agriculture; wheat

1. Introduction

As the main staple food, wheat (*Triticum aestivum* L.) is an important crop contributing to food security [1]. In the world, especially in developing countries, the cultivatable land for cereals and legumes has declined significantly. This and climate change and the enhanced demands of an increasing population for food and feed all threaten food security, national security, and human survival [2–4]. Application of chemical fertilizers is the most common agricultural practice to improve soil nutrients and subsequently increase crop yield. However, increasing production costs, exhaustion of mineral sources, consumption of energy, environmental risks, destroying the soil structure and breaking the soil's microecosystem, and groundwater pollution are the problems associated with the excess use of chemical fertilizers [5]. An important alternative soil management to reduce the harmful effects of chemical fertilizers is the use of biological approaches, such biofertilizers [6]. Improving soil fertility, soil bacterial diversity, water and nutrient uptake, and plant tolerance to stress promotes plant growth, and subsequently, increasing crop production is the advantage of biofertilizer application [7–9].

A well-known class of biofertilizers are plant growth-promoting rhizobacteria (PGPR). These are the bacterial strains that reside in the plant rhizosphere, interact with plants roots, and affect their growth and productivity. Seed-applied PGPR have positive effects on the different characteristics of their host plants. Biological nitrogen fixation; dissolving inorganic phosphate compounds and mineralization of organic phosphate compounds; secretion of various phytohormones, vitamins, and growth regulators; restriction of ethylene synthesis; and secretion of antibiotic and fungicidal compounds are the mechanisms by which PGPR enhance plant growth, water and nutrient uptake, drought tolerance, and disease resistance [8,10]. *Acinetobacter*, *Aeromonas*, *Agrobacterium*, *Allorhizobium*, *Arthrobacter*, *Azoarcus*, *Azorhizobium*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Delftia*, *Enterobacter*, *Flavobacterium*, *Frankia*, *Gluconacetobacter*, *Klebsiella*, *Mesorhizobium*, *Micrococcus*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Streptomyces*, and *Thiobacillus* are the most reported bacteria genera that have been applied as PGPR-based biofertilizer [11]. The positive effects of PGPR on both the quantitative and qualitative characteristics of plants have been reported. PGPR treatment (*Bradyrhizobium* sp. and *Bacillus subtilis*) led to improved shoot length, root length, number of branches, plant dry weight, leaf area index (LAI), chlorophyll content, nutrient uptake, seed yield, total proteins, carbohydrates, fats, starch, and guaran contents of guar (*Cyamopsis tetragonoloba* L.) plants compared with the control plants in [12]. *Bacillus* sp. *Azospirillum lipoferum* and *Azospirillum brasilense* PGPR improved the drought tolerance of wheat by improving relative water content, gas exchange attributes, increased nutrient uptake, osmolyte production, and upregulating of the antioxidant enzymes superoxide dismutase, peroxidase, and catalase in [13]. Khan et al. [14] applied *Bacillus subtilis*, *Bacillus thuringiensis*, and *Bacillus megaterium* PGPR strains in chickpea (*Cicer arietinum* L.) and reported a significant increase in the chlorophyll, protein, and sugar content, along with an enhanced accumulation of riboflavin, L-asparagine, aspartate, glycerol, nicotinamide, and 3-hydroxy-3- contents in PGPR-treated plants compared with irrigated and drought conditions. Dal Cortivo et al. [15] reported an upregulated content of two high-quality protein subunits, including the 81 kDa high-molecular-weight glutenin subunit and the 43.6 kDa low-molecular-weight glutenin subunit, in wheat flour by the application of *Azospirillum* + *Azoarcus* + *Azorhizobium* biofertilizers, in comparison with noninoculated controls.

Drought stress is one of the most important abiotic stresses that hampers the wheat growth and production in arid and semiarid agricultural regions of world, including Iran [3]. Long-lasting use of chemical fertilizers is a harmful factor that adversely affects the soil ecosystems of these regions. Microbial biotechnologies, as a smart farming technology, can reduce the indiscriminate use of chemical fertilizers and increase plant production in an environmentally friendly manner [16,17]. PGPR are one of the microbial-based approaches with great potential to increase plant growth under abiotic stress conditions [18]. The present study was conducted to investigate the effects of five different types of PGPR and their integration with need-based N, P, and Zn chemical fertilizers on the quantitative and qualitative characteristics of an Iranian rainfed wheat cultivar. We tried to find how PGPR will allow us to reduce chemical fertilization inputs in a drought stress context.

2. Materials and Methods

2.1. Experimental Design and Treatments

Three field experiments were conducted to investigate the effects of biofertilizers and their integrative effects with the need-based chemical fertilizers on the quantitative and qualitative characteristics of the Azar-2 rainfed wheat cultivar in three continuous growing seasons (2016–2017, 2017–2018, and 2018–2019). Biofertilizers consisted of four commercial brands of PGPR, including Fla-wheat® (F) containing *Microbacterium* sp., Nitroxin® (N1) containing the airborne nitrogen-fixing bacteria *Pseudomonas* and *Enterobacter cloacae*, Nitrokara® (N2) containing *Azorhizobium caulinodans*, and Barvar-2® (B) containing soil phosphate solubilizing *Pseudomonas putida* and *Pantoea agglomerans* bacteria along with new PGPR introduced by the Soil and Water Research Institute of Iran (SWRI) containing *Pseudomonas* bacteria R169. A randomized complete block design (RCBD) with four replications was applied to assess the application of F, N1, N2, B, and SWRI PGPR and their combinations with 50% and/or 100% of need-based nitrogen (N), phosphorus (P), and zinc (Zn) chemical fertilizers, based on soil test (ST), and compared their effects with untreated controls. Open-field experiments were carried out in the Arid Land Agricultural Research Station of Qamloo in Kurdistan Province, Iran (47°29′ E longitude and 35°9′ N latitude). The physicochemical properties of the experimental site's soil, during the investigated growing seasons, are presented in Table 1.

Table 1. Results of physical and chemical analysis of soil (0–30 cm) of the Qamloo Dryland Agricultural Research Station during the 2016–2017, 2017–2018, and 2018–2019 growing seasons.

Year	Mn	Fe	Cu	Zn	K_{av}	P_{av}	Texture	Sand	Silt	Clay	N_{Tot}	T.N.V.	OC	Sp	pH	Ec × 10^{-3}
	(mg/kg)							(%)								
2016–2017	**13.4**	**6.62**	**0.68**	**0.67**	**363**	**8.6**	**Clay Loam**	**29**	**33**	**38**	**0.10**	**18**	**0.87**	42.24	7.91	0.618
2017–2018	14.2	5.98	0.73	0.65	299	11.72	Clay Loam	20	42	38	0.07	18	0.73	40.24	8.20	0.559
2018–2019	12.2	5.32	0.78	0.81	334	10.1	Clay Loam	30	32	39	0.11	16	0.65	40.11	7.62	0.512

In autumn, a plot, 1090 m^2 (24.5 × 44.5 m), with a fallow–wheat rotation system was selected. For the cultivation of rainfed wheat, land preparation consisted of tillage operations with a plow, followed by a disc. The field experiment was divided into four equal blocks (44.5 × 5 m). Then, each block was divided into 15 plots (5 × 2.5 m) with six rows of 5 m length, 25 cm row spacing, and 50 cm between plots in a block. Treatments, including control; F; B; N1; N2; SWRI; F + 100% ST need-based N, P, and Zn fertilizers; F + 50% ST need-based N, P, and Zn fertilizers; B + 100% ST need-based N, P, and Zn fertilizers; B + 50% ST need-based N, P, and Zn fertilizers; N1 + 100% ST need-based N, P, and Zn fertilizers; N1 + 50% ST need-based N, P, and Zn fertilizers; N2 + 100% ST need-based N, P, and Zn fertilizers; N2 + 50% ST need-based N, P, and Zn fertilizers; and 100% ST need-based N, P, and Zn fertilizers, were randomly assigned to plots (Figure 1).

Soil test need-based fertilizers (100% ST) included 60 kg/ha nitrogen (N) from urea, 75 kg/ha phosphorus (P) from triple superphosphate, and 20 kg/ha zinc sulfate (Zn). Seeds of the Azar-2 rainfed wheat cultivar, disinfected with the Dividend fungicide, were inoculated by F (1 l/100 kg of seeds), N1 (2 l/100 kg of seeds), N2 (1 l/100 kg of seeds), B (2 l/100 kg of seeds), and SWRI (1 l/100 kg of seeds) PGPR. For inoculation, PGPR was added to the plastic bags containing the seeds; then the inoculated seeds were dried in an aseptic condition. Chemical fertilizers (both 50% and 100% need-based) were used during seed sowing. Wheat seeds were sown at a depth of 5–7 cm with a density of 350 seeds per m^2. Control (noninoculated) seeds were first sown; then the inoculated seeds were sown in their assigned plots.

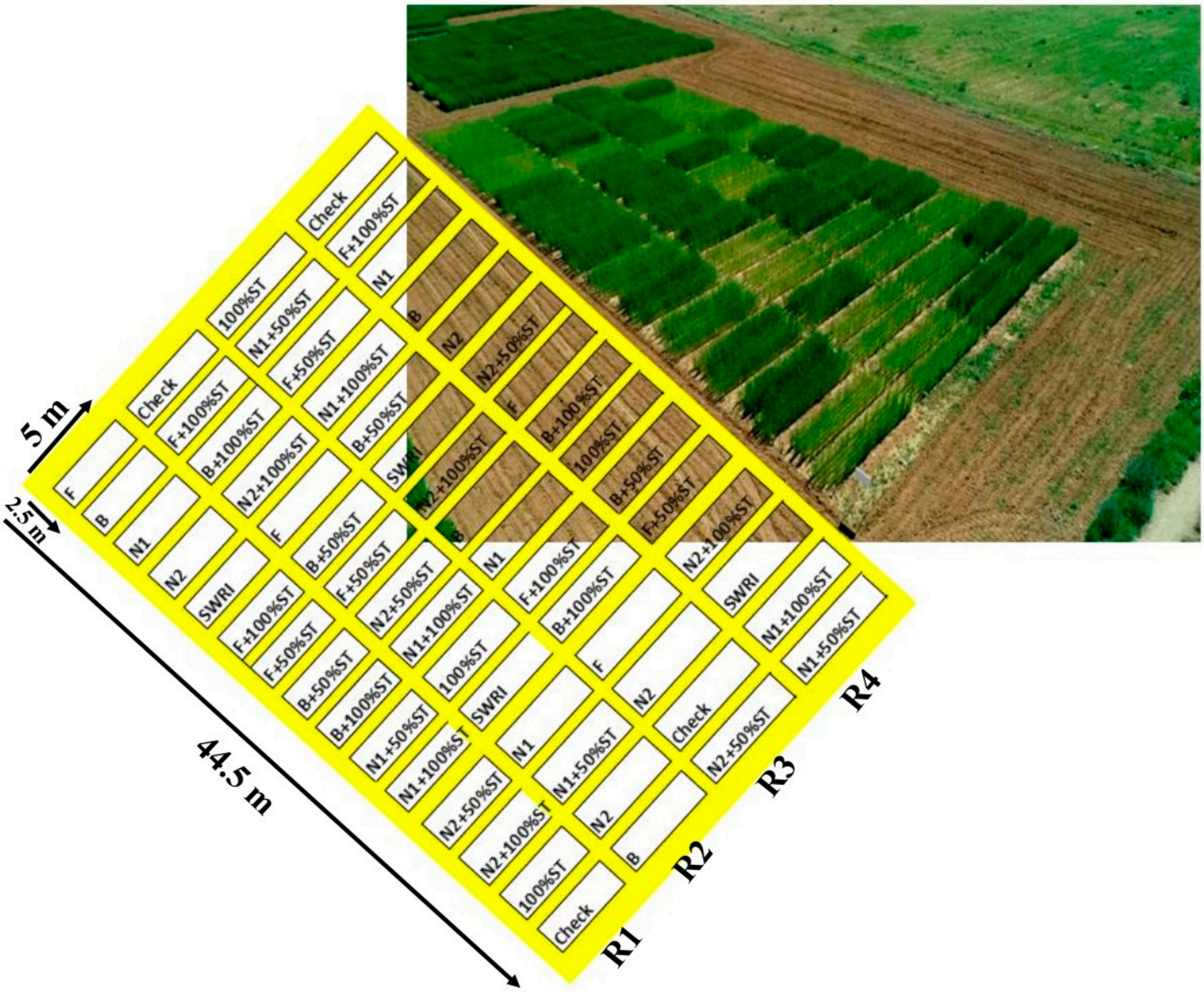

Figure 1. Schematic presentation of an applied randomized complete block design to assess the effects of PGPR biofertilizers and their combinations with 50% and 100% of soil test (ST) need-based N, P, and Zn chemical fertilizers on the quantitative and qualitative characteristics of the Azar-2 rainfed wheat cultivar during the 2018–2019 growing season. F (Fla-wheat), B (Barvar-2), N1 (Nitroxin), N2 (Nitrokara), SWRI (Soil and Water Research Institute growth stimulant), ST (soil test chemical fertilizers).

2.2. Plant Analysis

The effects of applied biofertilizers and their combinations with chemical fertilizers were assessed on the qualitative (concentrations of N, P, and K in flag leaves; concentrations of N, P, K, and protein in seeds) and quantitative (harvest index, 1000-seed weight, straw yield, grain yield, and biological yield) characteristics of the Azar-2 rainfed cultivar.

To measure the N, P, and K contents in flag leaves, leaf samples (30 flag leaves) were gathered at the heading stage, washed with distilled water, dried at room temperature (20–25 °C), and then placed in an oven at 70 °C for 48 h. For seed analyses, 100 g of seeds from each plot was used. Fine powder of a composed sample (five replicates) was taken for analysis. The N content of flag leaf and seed samples was determined according to the Kjeldahl method. The P concentration of flag leaf and ground grain samples was determined using microwave plasma emission spectroscopy after hot sulfuric acid digestion [19]. The potassium concentration of flag leaf and seed samples was determined according to Yoshida et al. [20] using a flame photometer. The protein content of seeds was estimated after determining the concentration of nitrogen in the seeds and applying a coefficient of 6.25 [21].

Quantitative characteristics, including harvest index (HI), 1000-seed weight (TSW), straw yield (SY), grain yield (GY), and biological yield (BY), were measured at the harvest stage. The potential edge effects were reduced by removing two side rows and 0.5 m from both end sides of each plot. Then, the aboveground crop biomass of center rows was harvested using hand sickles. Both grain and straw yields were calculated from dried aboveground crop biomass samples using a digital scale.

2.3. Data Analysis

Analysis of variance (ANOVA), followed by post hoc least significant difference (LSD), was carried out using the SAS® (SAS Institute Inc., Cary, NC, USA) software. LSD at 5% ($p \leq 0.05$) probability levels was used for means comparison analysis. Principal component analysis (PCA) was performed using the SAS® software to determine which climatic factors influence the quantitative characteristics of the rainfed wheat cultivar during the three studied growing seasons. A correlation matrix obtained from mean data was used for PCA, and two principal components were extracted using eigenvalues [22].

3. Results

3.1. Effects of Applied Biofertilizers and Their Combinations with Chemical Fertilizers on Yield Components of the Azar-2 Rainfed Cultivar

The results of the combined ANOVA of the effect of the applied PGPR and their combination with chemical fertilizers on the yield components of the Azar-2 wheat cultivar are shown in Table S1. The effect of year on biological yield, grain yield, straw yield, harvest index, and 1000-seed weight was statistically significant at a 1% probability level. Applied treatments, including PGPR and PGPR + chemical fertilizers, showed significant effects on BY, GY, SY, and TSW at a 1% probability level; however, there was no significant effect on HI (Table S1). The interaction effect of year and treatment was only significant on SY and TSW at a 1% probability level (Table S1).

Means comparison analysis of yield components showed that the highest and lowest means of BY were obtained during the 2018–2019 and 2017–2018 cropping seasons, respectively (Table S2). The highest means of GY and SY were observed from the 2018–2019 growing season; however, the lowest means of these two yield components were obtained during the 2017–2018 cropping season (Table S2). For the harvest index, the highest and lowest means were obtained during the 2017–2018 and 2018–2019 cropping seasons, respectively (Table S2). The highest and lowest means of TSW were observed during the 2016–2017 and 2018–2019 cropping seasons; however, there was no significant difference between the 2016–2017 and 2017–2018 cropping seasons (Table S2).

Means comparison analysis of investigated yield components under the applied PGPR and t need-based N, P, and Zn chemical fertilizers using LSD test, at the 5% probability level, showed that the highest and lowest means of BY were obtained by the sole application of chemical fertilizers and SWRI PGPR, respectively. There was no significant difference between F, B, N1, N2, and SWRI PGPR and the control treatment at the 5% probability level (Table 2). There was no significant difference between the chemical fertilizers and their combination (100% soil test need-based) with F, B, N1, and N2 PGPR at the 5% probability level (Table 2).

A similar trend was observed for GY as the lowest means were obtained by the application of the investigated PGPR, and there was no significant difference between the PGPR and the control treatment. Although the highest mean of GY was obtained from the sole application of chemical fertilizers (100% ST), F + 100% ST, B + 100% ST, N1 + 100% ST, N2 + 100% ST, and 100% ST had similar effects on this treat (Table 2).

Table 2. Means comparison analysis of the effect of applied PGPR and their combinations with soil test need-based N, P, and Zn chemical fertilizers on the yield components of the Azar-2 rainfed wheat cultivar.

Treatment		Biological Yield	Grain Yield	Straw Yield	1000-Seed Weight
1	Control	4716 e	1822 e	2894 d	41.86 bc
2	F	4856 e	1810 e	4034 ab	41.54 bcd
3	B	5034 e	1885 e	3525 bcd	43.28 a
4	N1	4974 e	1920 e	3597 bc	41.50 bcd
5	N2	5089 e	1896 e	3536 bc	42.64 ab
6	SWRI	4485 e	1678 e	3287 cd	41.03 cd
7	F + 100% ST	7088 abcd	2765 ab	3980 ab	39.65 effg
8	F + 50% ST	6338 cd	2337 d	4313 a	40.51 cde
9	B + 100% ST	7320 ab	2705 abc	4304 a	38.97 fg
10	B + 50% ST	6266 d	2334 d	4276 a	39.55 efg
11	N1 + 100% ST	7177 abc	2846 a	3853 abc	39.00 fg
12	N1 + 50% ST	6560 bcd	2489 bcd	3729 abc	39.55 efg
13	N2 + 100% ST	7400 ab	2847 a	4010 ab	40.45 cde
14	N2 + 50% ST	6406 cd	2422 cd	3608 bc	40.21 def
15	100% ST	7593 a	2877 a	3728 abc	38.31 g
LSD 5%		1186	319	639	1.36

Means followed by the same letters within columns are not significantly different at the 5% level. F (Fla-wheat), B (Barvar-2), N1 (Nitroxin), N2 (Nitrokara), SWRI (Soil and Water Research Institute growth stimulant), ST (soil test).

For the harvest index, the highest and lowest means were obtained from F + 50% ST and the control treatment, respectively (Table 2). There was no significant difference between F and F + 50% ST, according to LSD test at the 5% probability level (Table 2).

The highest and lowest means of 1000-seed weight were obtained from the B and 100% ST treatments, respectively (Table 2). There was no significant difference between B and N2 PGPR in terms of TSW at the 5% probability level, according to the results of the LSD test (Table 2).

A summary of the climatic conditions during the three studied cropping seasons and the effect of the applied PGPR and chemical fertilizers on the grain yield of the Azar-2 rainfed cultivar and grain yield change (kg/ha) compared with noninoculated control is presented in Table 3. The application of 100% N, P, and Zn chemical fertilizers led to a 1056 kg/ha increase in the grain yield of the Azar-2 rainfed cultivar, compared with the control treatment (Table 3). In the PGPR group, their most positive effects on the grain yield, compared with control, were observed when combined with 100% of the soil test need-based chemical fertilizers (969 kg/ha) (Table 3). The integrated application of the PGPR and 50% of the chemical fertilizers resulted in 574 kg/ha higher GY than the control treatment (Table 3).

Table 3. Summary of climatic factors and changes in the grain yield of the Azar-2 rainfed wheat cultivar during three cropping seasons (2016–2017, 2017–2018, and 2018–2019) and under the effects of the PGPR and chemical fertilizers.

Climatic Factor	Year		
	2016–2017	**2017–2018**	**2018–2019**
Total rainfall (mm)	336	339.5	466.5
Rainfall in autumn (mm)	39	24.5	155
Rainfall in winter (mm)	150	157.5	149
Rainfall in spring (mm)	147	46.4	162.5
Mean temperature (°C)	9.53	10.2	8.3
Minimum of temperature (°C)	−26	−22	−18.6
Number of frosty days	128	103	92

Table 3. *Cont.*

Climatic Factor	Year					
	2016–2017	**2017–2018**	**2018–2019**			
		Grain yield (kg/ha)				
Noninoculated control	2017	1264	2184			
Fertilizer treatments	Grain yield change in treatments versus check (kg/ha)			Total grain yield change of treatments versus control during three cropping seasons (kg/ha)	Group *	Grain yield change in fertilizer groups versus noninoculated control (kg/ha)
F	+83	−195	+78	−11	I	16
B	+3	−38	+225	+63		
N1	+330	−37	+334	+98		
N2	−50	−214	+484	+78		
SWRI	+15	−291	−155	−144		
F+ 50% ST	+390	+826	+327	+514	II	574
B + 50% ST	+288	+814	+436	+513		
N1 + 50% ST	+345	+736	+920	+667		
N2 + 50% ST	+178	+738	+884	+600		
F + 100% ST	+865	+990	+974	+943	III	969
B + 100% ST	+695	+1015	+939	+883		
N1 + 100% ST	+968	+977	+1126	+1024		
N2 + 100% ST	+760	+1241	+1076	+1026		
100% ST	+793	+1065	+1309	+1056	IV	1056

F (Fla-wheat), B (Barvar-2), N1 (Nitroxin), N2 (Nitrokara), SWRI (Soil and Water Research Institute growth stimulant), and ST (soil testing) or application of chemical fertilizer based on soil test. * Group: I: Sole application of PGPR biofertilizers. II: Application of PGPR + 50% of soil test need-based N, P, and Zn chemical fertilizers. III: Application of PGPR + 100% of soil test need-based N, P, and Zn chemical fertilizers. IV: Application of 100% soil test need-based N, P, and Zn chemical fertilizers.

Results of PCA showed that grain yield, biological yield, and straw yield were positively associated with total rainfall, rainfall in spring, and rainfall in autumn, whereas these yield components were negatively affected by the number of frosty days (Figure 2). All quantitative characteristics were negatively affected by rainfall in winter (Figure 2).

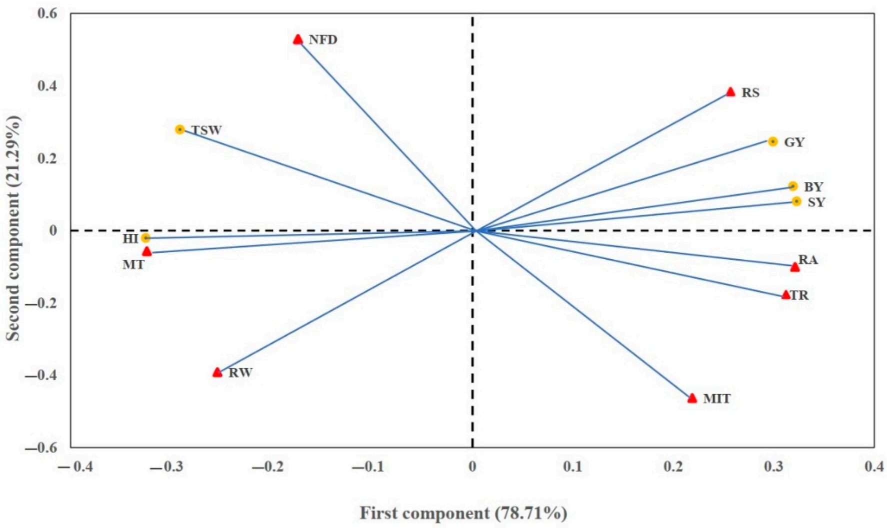

Figure 2. Biplot based on principal component analysis of the quantitative characteristics of the Azar-2 rainfed wheat cultivar during three studied growing seasons. BY (biological yield), GY (grain yield), HI (harvest index), MT (mean temperature), MIT (minimum of temperature), NFD (number of frosty days), RA (rainfall in autumn), RS (rainfall in spring), RW (rainfall in winter), SY (straw yield), TR (total rainfall), TSW (1000-seed weight).

3.2. *Effects of Applied Biofertilizers and Their Combinations with Chemical Fertilizers on the Qualitative Characteristics of the Azar-2 Rainfed Cultivar*

The results of combined ANOVA showed the significant effects of cropping seasons (year) on the concentrations of P and K in the grain and flag leaves of the Azar-2 rainfed wheat cultivar at a 1% probability level (Table S3). The effect of applied biofertilizers and chemical fertilizers was only significant on the concentration of phosphorus and potassium in the flag leaves of the Azar-2 wheat cultivar at the 5% probability level (Table S3). The interaction effect of year and combinations of biofertilizers and chemical fertilizers was not significant in all the investigated qualitative characteristics (Table S3).

Means comparison analysis, using the LSD test at the 5% probability level, showed that the highest and lowest means of the K content in flag leaves were related to the control and N2 + 100 ST treatments, respectively (Table 4). There was no significant difference between the control, F, B, N1, N2, SWRI, F + 100% ST, F + 50% ST, B + 100% ST, and 100% ST treatments in terms of the K content of flag leaves (Table 4). The same trend was observed for the phosphorous content of flag leaves, and the highest and lowest means of this characteristic were obtained from the control and N2 + 100 ST treatments, respectively (Table 4).

Table 4. Means comparison analysis of the effect of the applied PGPR and their combinations with soil test need-based N, P, and Zn chemical fertilizers on the potassium and phosphorous contents in the flag leaves of the Azar-2 rainfed wheat cultivar.

No	Treatment	Concentration in Flag Leaves (%)	
		Potassium	**Phosphorous**
1	Control	1.94 a	0.36 a
2	F	1.92 ab	0.28 bcd
3	B	1.82 abc	0.27 bcd
4	N1	1.82 abc	0.27 bcd
5	N2	1.87 abc	0.28 bcd
6	SWRI	1.84 abc	0.27 bcd
7	F + 100% ST	1.85 abc	0.27 bcd
8	F + 50% ST	1.77 abcd	0.24 cd
9	B + 100% ST	1.81 abc	0.27 bcd
10	B + 50% ST	1.76 bcd	0.28 bcd
11	N1 + 100% ST	1.76 bcd	0.30 bc
12	N1 + 50% ST	1.75 bcd	0.29 bc
13	N2 + 100% ST	1.59 d	0.23 d
14	N2 + 50% ST	1.71 cd	0.25 bcd
15	100% ST	1.82 abc	0.33 ab
LSD 5%		0.175	0.063

Means followed by the same letters within columns are not significantly different at the 5% level. F (Fla-wheat), B (Barvar-2), N1 (Nitroxin), N2 (Nitrokara), SWRI (Soil and Water Research Institute formulation), ST (soil test).

Figure 3 shows the changing trend of the grain protein content in the Azar-2 rainfed cultivar under the effect of the investigated fertilizer groups. As is clear, the highest grain protein contents were obtained by the integrated application of F, B, N1, and N2 PGPR with 100% of N, P, and Zn chemical fertilizers (Figure 3). Among all the investigated treatments, the highest and lowest grain protein contents were obtained by F + 100% ST and noninoculated control, respectively (Figure 3).

The highest increase in grain protein content, compared with the control treatment, was related to the combination of F PGPR and 100% N, P, and Zn chemical fertilizers (1.02%), whereas B and B + 50% ST led to decreased contents (−0.28%) of grain protein compared with the control treatment (Figure 2).

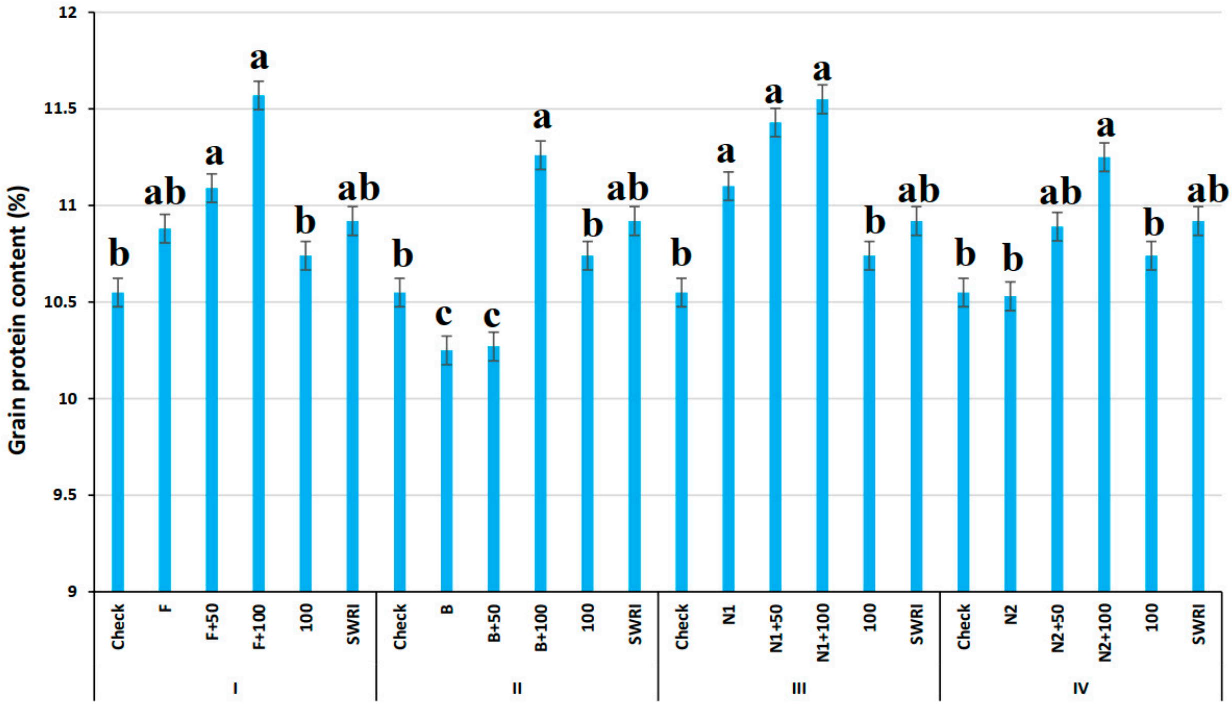

Figure 3. Changing trend of the grain protein content of the Azar-2 rainfed wheat cultivar under the effects of different applied biofertilizers and their combinations with N, P, and Zn soil test need-based chemical fertilizers (I = F, F + 50% ST, F + 100% ST, 100% ST, and SWRI; II = B, B + 50% ST, B + 100% ST, 100% ST, and SWRI; III = N1, N1 + 50% ST, N1 + 100% ST, 100% ST, and SWRI; IV = N2, N2 + 50% ST, N2 + 100% ST, 100% ST, and SWRI). Means followed by the same letters within columns are not significantly different at the 5% level.

4. Discussion

Increasing global demands for the consumption of fresh water, due to rapid socioeconomic development, is an important challenge for sustainable agriculture in the 21st century [23,24]. Rainfed cultivation is an alternative to reduce the global water demand for agriculture. However, the unpredictable and limited precipitation are two negative characteristics of rainfed cultivation. Water stress is the main limiting factor affecting plant production in this farming system [25]. These conditions were obvious in the present study as the effect of year on the investigated quantitative and qualitative characteristics of the Azar-2 wheat cultivar was significant, and the highest grain yield was obtained during the 2018–2019 growing season, which had the highest amount of total rainfall among the three investigated cropping seasons.

Increasing plant water uptake capacity and plant water availability are the two main avenues for upgrading rainfed agriculture. Soil (tillage, crop rotation, mulching, and manure fertilizers) and crop (selection of better crops, intercropping/crop rotation, timing of operations, weeds, and pest management) management are important practices to maximize the depth and density of roots and increase plant water uptake capacity. Among them, soil fertility management, through organic and inorganic fertilizers, is key to crop water uptake capacity and is a prerequisite to crop growth [26,27]. However, in rainfed cultivation, the response of a crop to fertilizer applications is heavily reliant on water availability [28]. PGPR are eco-friendly efficient biotechnological tools to improve plant water uptake and growth in various, especially stressful, conditions [18]. PGPR application is a sustainable strategy for increasing crop production under drought stress and replacing chemical fertilizers [29]. In the present study, the yield components of the Azar-2 rainfed cultivar, including biological yield, grain yield, straw yield, and 1000-seed weight, were significantly affected by the applied F, B, N1, and N2 PGPR as seed-inoculated plants showed higher means than noninoculated controls. Increased yield components, such as grain yield, straw yield, biological yield, above- and belowground biomasses, have been reported in wheat plants under the effect of *Agrobacterium fabrum* or *Bacillus amyloliquefaciens* PGPR [30]. Zia et al. [31] inoculated wheat seeds with the *Proteus mirabilis* R2, *Pseudomonas balearica* RF-2, and *Cronobacter sakazakii* RF-4 bacterial strains and reported significantly improved plant growth, leaf area, and biomass for primed seeds under water stress (PEG, −0.6 MPa) conditions. In a pot experiment, the *Pseudomonas azotoformans* FAP5 strain was applied as PGPR to alleviate drought stress in wheat plant, and significant improvement in growth attributes, photosynthetic pigment efficiency, and antioxidative enzymatic activities in FAP5-inoculated wheat plants compared to uninoculated plants was reported [32]. The coapplication of *B. amyloliquefaciens* PGPR with two biochar doses led to improved 100-grain weight, and grain N, P, and K up to 59%, 58%, 18%, and 23% in wheat plants under drought conditions [33]. Biofertilization with various types of PGPR is an agronomical strategy that has been applied to affect the growth pattern of other plants, such as tomato, maize, chickpea, pea, lentil, cabbage, canola, sunflower, strawberry, and sorghum [34].

The type of applied PGPR is another important factor that can affect the quantitative and qualitative characteristics of host plants. There are different bacteria genera in different PGPR, which modulate differential responses in their host plants [31]. In addition to the interaction between PGPR and the plant host's roots, there is also the interaction between these bacteria and environmental (soil–climate) factors [34]. There is a significant correlation between bacteria strain and physicochemical properties of the soil [35]. Depending on the host plant (species/genotype) and environmental conditions of the research site, different results can be obtained through the application of various types of PGPR. Therefore, optimization steps are required to find the best PGPR to increase the valuable characteristics of target plants in target environments. In the present study, five different types of PGPR were tested in a cool-rainfed cultivation of the Azar-2 wheat cultivar, and the better results, in terms of grain protein content, were obtained by the application of F and N1. In a study conducted in different regions of Iran, an applied Fla-Wheat biofertilizer increased the wheat grain yield by 15%, compared with noninoculated control [34]. Nitroxin (N1), harboring the airborne nitrogen-fixing bacteria *Pseudomonas* and *Enterobacter cloacae*, is a good choice to increase the protein content of cereals in cool-rainfed areas. Chaechian et al. [36] reported the positive effect of a Nitroxin biofertilizer on the yield and yield components of wheat as the grain yield of Nitroxin-inoculated wheat was increased by 7.12%, in comparison with no inoculation treatment. Jafari et al. [37] investigated the effects of Nitrokara and Barvar-2 biofertilizers and their combinations with 50% and 100% of need-based chemical fertilizers on the quantitative traits of a rainfed wheat cultivar and reported that the coapplication of Nitrokara and Barvar-2 along with 50% of need-based chemical fertilizers led to the highest means of dry weight, number of seeds per plant, 1000-grain weight, and grain yield. The authors reported that the integrated application of biological and chemical fertilizers could significantly reduce the use of chemical fertilizers [37]. The positive effects of a Nitrokara biofertilizer on the growth and morphophysiological traits of chicory (*Cichorium intybus* L.) have also been reported during aeroponic and soil culture experiments [38]. The authors reported that the applied *Azorhizobium* bacterium in Nitrokara affects plant hosts through nitrogen fixation and provides them growth regulators, such as auxins, cytokinins, abscisic acid, and growth-promoting materials, such as riboflavin and vitamins [38].

In the present study, all investigated PGPR showed a lower effect during the 2016–2017 growing season, which had the highest number of frosty days among the three studied cropping seasons. With these testimonials, it seems that the combination of different bacteria strains would be better to improve the quantitative and qualitative characteristics of crops in unpredictable conditions of rainfed systems [15,39]. Akhtar et al. [13] reported that the combination of *Bacillus* sp. *Azospirillum lipoferum* and *Azospirillum brasilense* PGPR was better than their sole application to increase the drought tolerance of wheat.

The use of chemical fertilizers is inevitable; however, the required amount of these harmful fertilizers can be reduced with different tactics, such as their combination with biofertilizers. PGPR are important in this regard as they can increase the root growth and nutrient uptake of plants [40]. Through a 5-year field study of wheat, Varinderpal-Singh et al. [39] reported that the integrated nutrient management with a biofertilizer and need-based N led to the highest grain yield with 16.7% and 25% less use of the fertilizers N and P, respectively. In the present study, the combinations of F, B, N1, and N2 PGPR with soil test need-based chemical fertilizers was more effective than their sole applications to enhance the grain yield and grain protein content of the Azar-2 rainfed wheat cultivar. The grain yield and grain protein content of PGPR-chemical-fertilizer-treated plants were more than those of noninoculated controls. Grain yield was increased by 28%, 28%, 37%, and 33% with the integrated application of F, B, N1, and N2 PGPR and 50% of need-based N, P, and Zn chemical fertilizers, respectively. In comparison with the noninoculated treatment, the grain yield of the rainfed wheat cultivar was increased by 52%, 48%, 56%, and 56% when F, B, N1, and N2 treatments were integrated with 100% of need-based chemical fertilizers, respectively. Nitrokara (N2), containing the growth-promoting bacteria *Azorhizobium caulinodans*, was the best PGPR to increase the GY of the Azar-2 wheat cultivar. The grain protein content of the Azar-2 rainfed wheat cultivar was increased by 1.02%, 0.71%, 1.00%, and 0.70% by the integrated application of F, B, N1, and N2 PGPR with 100% of need-based chemical fertilizers, respectively. The grain protein content was increased by 0.54%, 0.88%, and 0.34% through the integrated application of F, N1, and N2 PGPR plus 50% of need-based chemical fertilizers, respectively. Nitroxin (N1) was the best treatment to increase the grain yield and grain protein content of the Azar-2 rainfed wheat cultivar while reducing the required N, P, and Zn fertilizers by 50%. The applied airborne nitrogen-fixing bacteria *Pseudomonas* and *Enterobacter cloacae* in a Nitroxin biofertilizer is a reasonable reason for its positive effect on the grain protein content of Azar-2 rainfed wheat. The application of the PGPR can affect the content of essential metal elements (e.g., Fe, Zn) in grains. In fact, the rhizosphere's microbes may regulate multiple biological processes and help in plant growth promotion and nutrient acquisition or be associated with plant responses against biotic and abiotic stresses, owing to the fact that they assist plants in producing hormones, siderophores, and other inhibitory chemicals [41,42].

The obtained results indicate the positive effects of the coapplication of the investigated PGPR with 100% of need-based chemical fertilizers on the quantitative and qualitative characteristics of the Azar-2 rainfed cultivar. This is very important in terms of preserving soil biodiversity. Low-input crop management (PGPR + 50% of need-based chemical fertilizers) can also be considered from both economic and environmental aspects as it causes a significant increase in the grain yield and protein content of the Azar-2 rainfed wheat cultivar. An extra control treatment (50% ST) could be useful to show the applicability of the integrated application of PGPR-50% chemical fertilizers in reducing N, P, and Zn fertilizers by 50% in wheat rainfed cultivation.

5. Conclusions

A 3-year open-field study was conducted to assess the effects of different types of PGPR and their combinations with soil test need-based N, P, and Zn chemical fertilizers on the yield components and qualitative characteristics of an Iranian rainfed wheat cultivar, Azar-2. Based on the obtained results, the integration of applied PGPR with 100% of need-based chemical fertilizers was more effective than their sole application. Combinations of Nitrokara + 100% of soil test need-based chemical fertilizers and Nitroxin + 100% of soil test need-based chemical fertilizers were the best treatments to increase the grain yield and grain protein content of the Azar-2 wheat cultivar, respectively. The integration of Nitroxin PGPR with 50% of required chemical fertilizers (30 kg/ha urea + 37.5 kg/ha triple superphosphate + 10 kg/ha zinc sulfate) can also be considered as an appropriate alternative to decrease chemical fertilization, while increasing grain yield and protein content, and help in the sustainable production of cereals in cool-rainfed cultivation systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12071524/s1, Table S1: Combined analysis of variance of the effects applied plant growth-promoting rhizobacteria and their combinations with need-based chemical fertilizers on yield components of Azar-2 rainfed wheat cultivar in open field experiments during three cropping seasons; Table S2: Means comparison analysis of yield components of Azar-2 rainfed wheat cultivar under the effects of PGPR and PGPR + chemical fertilizers during 2016–2017, 2017–2018, and 2018–2019 growing seasons; Table S3: Combined analysis of variance of the effects applied plant growth-promoting rhizobacteria and their combinations with soil test need-based chemical fertilizers on qualitative characteristics of Azar-2 rainfed wheat cultivar in open field experiments during three cropping seasons.

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