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Research Article

Physiological, biochemical, and grain yield responses of wheat cultivars to *Azospirillum brasilense* under dryland conditions

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ABSTRACT- The combined use of improved wheat cultivars and growth-promoting bacteria offers a promising strategy for enhancing wheat (*Triticum aestivum* L.) performance under dryland conditions. This study evaluated the response of different wheat cultivars to inoculation with *Azospirillum brasilense* Sp7 in a field setting. The experiment was conducted during the 2021–2022 growing season at the Faculty of Agriculture, Ilam University, under rainfed conditions. A factorial arrangement within a randomized complete block design was used, with three replications. The treatments included *Azospirillum* inoculation (with and without) as the main factor, and four wheat cultivars (Ivan, Sardari, Homa, and Azar 2) as subplots. The bacterial inoculant was applied to seeds prior to sowing. Physiological and biochemical traits were measured at the late flowering stage (Zadoks stage 69), including proline content, leaf relative water content (LRWC), electrolyte leakage (EL), photosynthetic pigment levels, net CO_2 assimilation rate (Pn), leaf temperature (Lt), and transpiration rate (Tr). The results showed that seed inoculation did not significantly influence proline accumulation. However, chlorophyll content was the highest in the Ivan cultivar when inoculated. Inoculated plants also exhibited reduced electrolyte leakage compared to non-inoculated controls. LRWC, Pn, and Tr were significantly influenced by both cultivar and bacterial inoculation. Across cultivars, *Azospirillum* inoculation led to increases in LRWC (13.7%), Pn (16.1%), and Tr (24.3%). Additionally, grain nitrogen and protein content varied among cultivars and responded positively to *Azospirillum* inoculation.

INTRODUCTION

Iran ranks among the world's leading wheat producers, with cultivation spanning over 4.9 million hectares. This extensive wheat-growing area is divided into two distinct agro-ecological zones: approximately 1.58 million hectares of irrigated land located in arid regions, and around 3.48 million hectares of rain-fed land situated in semi-arid regions (Ministry of Agricultural Jihad, 2022). Drought remains the most critical challenge limiting crop productivity in semi-arid zones globally. Numerous studies have demonstrated that under drought or water-deficit conditions, wheat productivity can be enhanced through the application of plant-growth-promoting rhizobacteria (PGPR) (Zarea et al., 2019). PGPR have been shown to improve plant drought tolerance by increasing root length and volume, thereby enhancing access to soil moisture (Cohen et al., 2015; Kasim et al., 2021; Yaghoubian et al., 2022). Among PGPR, the genus *Azospirillum* is particularly notable for its growth-promoting capabilities. Bacteria from this genus can colonize a wide range of plant species—over one hundred have been reported (Pedrosa et al., 2020). *A. brasilense*, the most extensively studied species, has been shown to enhance plant nutrient uptake, solubilize otherwise unavailable phosphorus, and induce the accumulation of soluble sugars, amino acids, and proline (Boleta et al., 2020; Rosa et al., 2020). Inoculation with *A. brasilense* has been reported to increase grain yield in several major cereal crops, including maize (*Zea mays* L.) (Hungria et al., 2010; Coelho et al., 2020), wheat (Hungria et al., 2010; Filho et al., 2017; Zaheer et al., 2022), rice (*Oryza sativa*) (Isawa et al., 2010), and canola (*Brassica napus*) (Naderifar and Daneshi, 2012). *A. brasilense* enhances plant performance through both direct and indirect mechanisms. These include increased root growth (length and volume), improved nitrate reductase activity, enhanced nitrogen use efficiency, and more effective phosphate solubilization, all of which contribute to improved plant growth and yield (Galindo et al., 2022). Fukami et al. (2016) also observed increased chlorophyll content in maize leaves following *A. brasilense* inoculation. Similarly, Zaheer et al. (2022) found that wheat plants inoculated with *A. brasilense* RA-17 produced significantly higher grain yields than uninoculated controls, attributing this increase to improved yield components and assimilate accumulation. Hungria et al. (2010) further reported that

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wheat and maize inoculated with *A. brasilense* and *A. lipoferum* exhibited superior grain yields and nutrient uptake compared to non-inoculated plants. While advances in wheat breeding have resulted in the development of high-performing genotypes, these newer cultivars may vary in their response to PGPR (Moradi and Zarea, 2021). It has been suggested that PGPR may exert a stronger growth-promoting effect under sub-optimal agronomic conditions than under optimal ones. The effectiveness of PGPR is influenced by both the wheat cultivar and the specific *Azospirillum* strain used (Veresoglou and Menexes, 2010; Zarea, 2017). Variations in root architecture and metabolic profiles among wheat genotypes can impact plant-PGPR interactions, including the establishment of successful associative symbiosis with *Azospirillum* (Millet et al., 1984). Genotype-dependent responses to PGPR have been widely reported (Åström and Gerhardson, 1988; Kazi et al., 2016; Salem et al., 2018). For instance, Salem (2018) observed a genotype-specific response of wheat to bacteria producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase, while Kazi et al. (2016) showed that wheat responses to *A. brasilense* Sp7 and its mutant strain Sp7-S varied by genotype. Despite the potential of PGPR, there remains limited information on the interactions between newly developed wheat cultivars for dryland agriculture and growth-promoting rhizobacteria, particularly *Azospirillum* spp.

Wheat holds the top position among cereal crops in Iran in terms of production. In recent years, the use of plant growth-promoting bacteria (PGPB), such as *Azospirillum* spp., has emerged as a promising approach to enhance cereal crop performance. Environmental stresses, particularly drought, can severely disrupt photosynthetic activity as well as physiological and biochemical processes in plants (Wahab et al., 2022). Although modern breeding programs have successfully improved crop yields and stress tolerance, they may inadvertently weaken the plant's natural associations with PGPR, potentially reducing the effectiveness of such symbiotic relationships (Valente et al., 2020). In light of this, the present study aimed to evaluate the effects of *A. brasilense* inoculation on the physiological and biochemical responses of four wheat cultivars (Sardari, Ivan, Homa, and Azar 2) grown under rainfed conditions.

MATERIALS AND METHODS

The experiment was conducted in late fall of the 2021–2022 growing season at the Experimental Farm of the Faculty of Agriculture, Ilam University, Ilam, Iran (45°28′N, 33°37′E; 1174 m above the sea level). The study aimed to evaluate and compare the biochemical, physiological, and yield-related performance of four dryland winter wheat cultivars, i.e., Ivan, Sardari, Homa, and Azar 2, under conditions with and without *Azospirillum brasilense* inoculation. These cultivars are widely cultivated under rainfed conditions and are considered commercial dryland wheat varieties adopted by Iranian wheat farmers. Key agronomic characteristics of the cultivars used in the study are presented in Table 1.

The experiment was conducted under dryland farming conditions from October 2021 to June 2022. Fig. 1 illustrates the long-term trends in rainfall and temperature in the study area. During the wheat growing season, total precipitation amounted to 370 mm (Fig. 2). The chemical and physical properties of the experimental site's soil are summarized in Table 2. Based on soil test results, phosphorus (as triple superphosphate) was applied at planting, while nitrogen (as urea) was applied one month after sowing to support the establishment of a cooperative interaction between the plants and the bacterial inoculant. The experiment was laid out as a 2 × 4 factorial arrangement in a randomized complete block design (RCBD) with three replications. The two main factors included *A. brasilense* inoculation (inoculated and uninoculated) and four Iranian dryland wheat cultivars (Ivan, Sardari, Homa, and Azar 2).

Azospiriilum inoculation procedure

Seed inoculation with *Azospirillum brasilense* Sp7 (prepared by Ilam University) was carried out prior to sowing. Seeds were first surface-sterilized by immersion in 70% ethanol for 3 minutes, followed by treatment with a 5% sodium hypochlorite solution for another 3 minutes. Subsequently, the seeds were thoroughly rinsed several times with distilled water to remove any residual sterilizing agents. The sterilized seeds were then divided into two groups. One group was inoculated with A. brasilense Sp7 (Tarrand, Krieg, and Döbereiner 1979 AL) (Tarrand et al., 1978) at a concentration of 2 × 10^6 colony-forming units (CFU) mL^{-1}, while the second group received an autoclaved (inactivated) version of the *A. brasilense* inoculant, serving as a control. The bacterial strain was cultured in a modified nitrogen-free medium as described by Karimi et al. (2018), and cells at the exponential growth phase were used for inoculation. Seeds were immersed in the bacterial suspension and gently agitated on a rotary shaker at 100 rpm for 30 minutes at room temperature. After treatment, the seeds were air-dried for approximately 30 minutes before planting. The selected bacterial concentration (2 × 10^6 CFU mL^{-1}) was determined based on preliminary trials that identified this level as optimal for promoting wheat growth and enhancing grain yield.

Table 1. Agronomic characteristics of the mentioned varieties

Agricultural characteristic	Homa	Sardari	Azar 2	Ivan
Growth habit	Winter	Winter	Winter	Interstitial
Yellow rust	Sensitive	Sensitive	Semi-sensitive	Resistant
Drought tolerance	Tolerable	Tolerable	Tolerable	Resistant
Lodging tolerance	Resistant	Resistant	Resistant	Semi-resistant
Plant height (cm)	81	84	89	84
Total kernel weight (g)	44	41	39	34

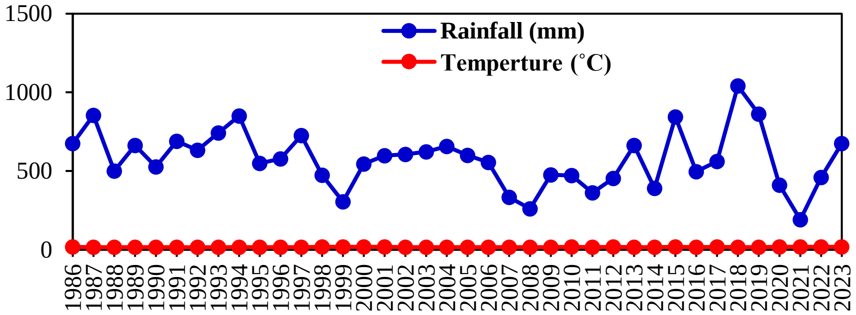

Fig. 1. Average rainfall (millimeter, mm) and temperature (degrees Celsius, °C) in the growing season (during 38 years) in Ilam.

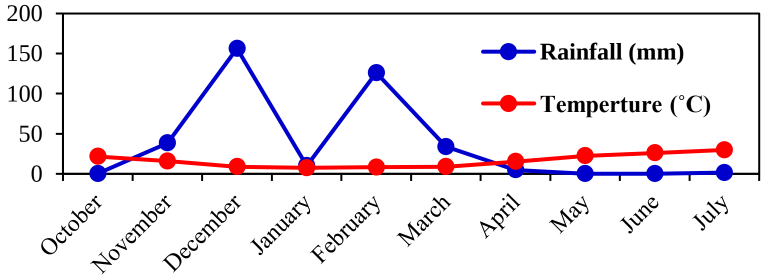

Fig. 2. Monthly mean rainfall (millimeter, mm) and temperatures (degrees Celsius, °C) during growing season in Ilam.

Table 2. Physical and chemical properties of experimental site

Soil texture	EC (dS/m)	pH (1:2 Soil: H_2O)	Organic C (%)	N (%)	K (mg kg^{-1})	P (mg kg^{-1})	S (mg kg^{-1})
Clay loam	0.3	7	1.2	0.12	420	8.5	12

Physiological and biochemical measurement

Biochemical and physiological measurements were conducted at the late flowering stage, corresponding to Zadoks growth stage GS 69. Leaf proline content, leaf relative water content (LRWC), and electrolyte leakage (EL) were assessed using flag leaf samples randomly collected from each plot. Each sample comprised three flag leaves. Proline accumulation was quantified using the ninhydrin-based colorimetric method described by Bates et al. (1973). Fresh leaf samples were extracted with 3% sulfosalicylic acid, and the absorbance of the resulting proline-toluene complex was measured by a spectrophotometer at 520 nm. LRWC and EL were determined following the protocols of Ritchie et al. (1990) and Lutts et al. (1996), respectively. For LRWC measurement, three flag leaves per plot were immediately weighed to record fresh weight (FW), then rehydrated in the dark at room temperature for 24 hours to obtain turgid weight (TW), and subsequently oven-dried at 75 °C for 24 hours to determine dry weight (DW). The following equation was used to determine the percentage of flag leaf relative water content (RWC): RWC (%) = (FW) / (TW - DW) × 100 Eq. (1)

To measure EL (%), 3 flag leaves from 3 different plants from each plot were collected and saturated by immersing them in water for 4 hours at room temperature in the dark. After immersion, the initial conductivity was determined using a conductivity meter. The leaf samples were then incubated in a water bath at 100 °C for 60 minutes, and the absolute conductivity was determined. The electrolyte leakage was calculated as follows:

Electrolyte leakage (%) = (initial conductivity / absolute conductivity) × 100 Eq. (2)

Net CO_2 assimilation rate (Pn), transpiration rate (Tr), and leaf temperature (Lt) were measured on ten randomly selected flag leaves per plot using a portable gas exchange system (Plant Photosynthesis Meter, Korea Tech). Chlorophyll pigment concentrations, including chlorophyll a, chlorophyll b, and total chlorophyll, were determined from flag leaves following the method described by Arnon (1949). The absorbance of pigment extracts was recorded at wavelengths of 645 nm, 663 nm, and 470 nm using a spectrophotometer, in accordance with the procedure outlined by Lichtenthaler (1983). Chlorophyll pigments were extracted with acetone (80%) and were calculated via the following calculations:

Chlorophyll a = [12.7 × (A663) - 2.69 × (A645)] × v / (100 × w) Eq. (3)

Chlorophyll b = [22.9 × (A645) - 4.68 × (A663)] × v / (100 × w) Eq. (4)

Equation 5 was used to calculated carotenoids concentration in samples:

Carotenoid (mg/mL) tissue = [(1000 A470 - 1.8 Chlorophyll a - 85.02 Chlorophyll b) / 198] v / 1000 w Eq. (5)

Where v is the final volume of chlorophyll extract in 80% acetone (20 mL), and w is the fresh weight of tissue extracted (0.5 g).

Grain protein measurement

The grain nitrogen and protein contents were measured at seed maturity according to the Kjeldahl method (Jackson, 1969). The grain protein content was then calculated from the nitrogen concentration of the grain using the following equation:

Protein content (%) = Nitrogen content × 5.7 Eq. (5)

Data analysis

The analysis of variance (ANOVA) of all data was performed using SAS, version 9.3 (SAS Institute 2012). Least significant difference (LSD) test at 5% level of significance was used for comparing among the mean values.

RESULTS AND DISCUSSION

Interactions between A. brasilense inoculation and cultivars on proline accumulation, EL, and photosynthetic parameters

Leaf proline content was significantly affected by cultivar, but not by *Azospirillum* inoculation (Table 3). The bacterial treatment had no statistically significant impact on proline accumulation in wheat leaves. Among the cultivars, Ivan and Sardari exhibited the highest proline concentrations (Table 4). LRWC was significantly influenced by both main factors, cultivar and *Azospirillum* inoculation (Table 3). Inoculation enhanced LRWC by 13.7% compared to non-inoculated plants (Table 4). Among the cultivars, Sardari showed the lowest LRWC value. EL was significantly affected by both cultivar and inoculation, as well as their interaction (Table 3). The lowest EL was recorded in *Azospirillum*-inoculated Azar 2 plants, while the effect was less pronounced in the Ivan cultivar (Fig. 3). These findings are consistent with the results reported by Creus et al. (1998), Creus et al. (2004) and Zarea (2024), who found that inoculation with *Azospirillum* improved water status in wheat seedlings compared to non-inoculated controls. The observed improvement in LRWC contributed to a corresponding reduction in EL.

The effect of *Azospirillum* inoculation on photosynthetic pigments was significant for all pigments, except for chlorophyll b (Table 3). Additionally, there was a significant interaction between cultivar and inoculation on the levels of photosynthetic pigments (Table 3). The highest concentrations of chlorophyll a, chlorophyll b, and total chlorophyll were observed in the Ivan cultivar following *Azospirillum* inoculation (Fig. 4a-c). The highest carotenoid content due to the inoculation was found in the Azar 2 cultivar (Fig. 4d). The Sardari cultivar showed no significant response to *Azospirillum* inoculation for either chlorophyll or carotenoid pigments (Fig. 4d). The significant increase in chlorophyll content due to *Azospirillum* inoculation supports the findings of Bashan et al. (2005) about higher chlorophyll levels in wheat after inoculation with *A. brasilense* CD. This result contrasts with Kazi et al. (2016) regarding a decrease in chlorophyll content after post-anthesis in wheat inoculated with *A. brasilense* Sp7.

Table 3. Two-way analysis of variance of the effect of wheat cultivars and *A. brasilense* inoculation, as well as their interaction, on some leaf properties and grain yield under rainfed conditions.

Property	Mean Square			Error mean square	C.V.
	Cultivar (Cv)	***Azospirillum* inoculation (*Az*)**	**Cv × *Az***		
Proline	26.1**	0.24^{ns}	0.09^{ns}	1.62	16.7
LRWC	247*	454*	25.8^{ns}	54.03	10.8
EL	51.5**	242.4**	21.3*	6.7	6.8
Chl a	0.49**	0.42*	0.48**	0.06	8.1
Chl b	0.37**	0.15^{ns}	0.22**	0.049	20.6
Total Chl	5.1**	1.4**	1.05**	0.17	10.02
Car	1.88**	0.65*	0.44*	0.12	8.6
Lt	8.99**	37.7**	6.7**	0.38	1.8
Pn	2.7*	6.3*	1.5^{ns}	0.76	14.3
Tr	0.58^{ns}	5.22**	0.55^{ns}	0.52	17.41
GN	0.018**	0.042**	0.006**	0.0006	3.72
Protein	0.61**	1.38**	0.19**	0.022	3.72
GY	1.6**	4.8**	0.42*	0.12	17.4

Pr, proline content; LRWC, relative water content; EL, electrolyte leakage; Chl a, chlorophyll a; Chl b, chlorophyll b; total Chl, total chlorophyll; Car, carotenoids; Pn; net CO_2 assimilation rate, Tr, transpiration rate; Lt, leaf temperature, and GN, grain nitrogen; GY, grain yield; C.V., coefficient of variance; ns, no significant; *, Significant at $p < 0.05$; **, Significant at $p < 0.01$.

Table 4. Effect of wheat cultivar (Cv) and *A.* inoculation (*Az*) on leaf proline content, relative water content (LRWC), net CO_2 assimilation rate (Pn), and transpiration rate (Tr)

Treatment		Proline content (μg g^{-1} flag leaf fresh weight)	LRWC (%)	Pn (μ mol CO_2 m^{-2} s^{-1})	Tr (mmol H_2O_2 m^{-2} s^{-1})
Az	Inoculation	7.4^{a}	72.2^{a}	6.5^{a}	4.6^{a}
	Control	7.6^{a}	63.5^{b}	5.6^{b}	3.7^{b}
	LSD	1.1	6.4	0.76	0.63
Cv	Sardari	8.7^{a}	59.1^{b}	5.4^{b}	3.83^{a}
	Ivan	9.8^{a}	74.4^{a}	6.8^{a}	4.41^{a}
	Homa	5.3^{b}	69.9^{a}	6.3^{ab}	4.43^{a}
	Azar 2	6.4^{b}	67.9^{ab}	5.6^{b}	3.95^{a}
	LSD	1.57	9.1	1.08	0.89
Interaction		ns	ns	ns	ns

In each column, means followed by similar letters have no significant differences (LSD at α = 0.05); ns = non-significant.

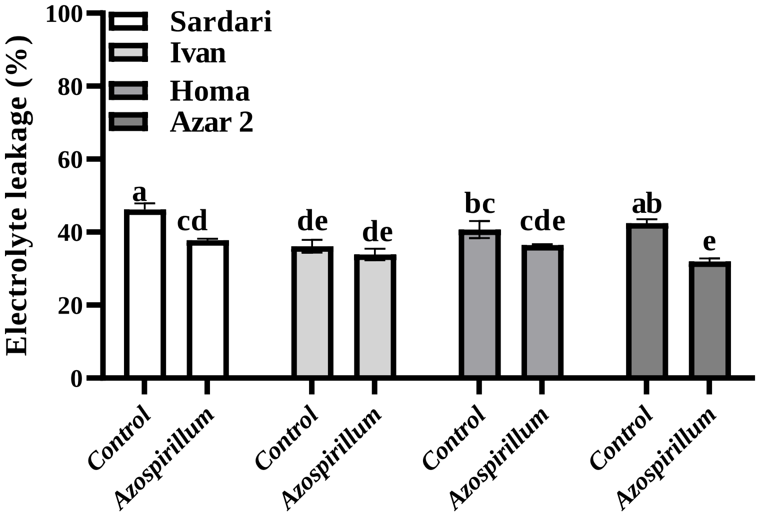

Fig. 3. Effect of *Azospirillum* inoculation on electrolyte leakage of four wheat cultivars under rainfed land conditions. Means with the different letter are significantly different according to LSD test at P < 0.05; Bar graphs are the mean values ± standard error.

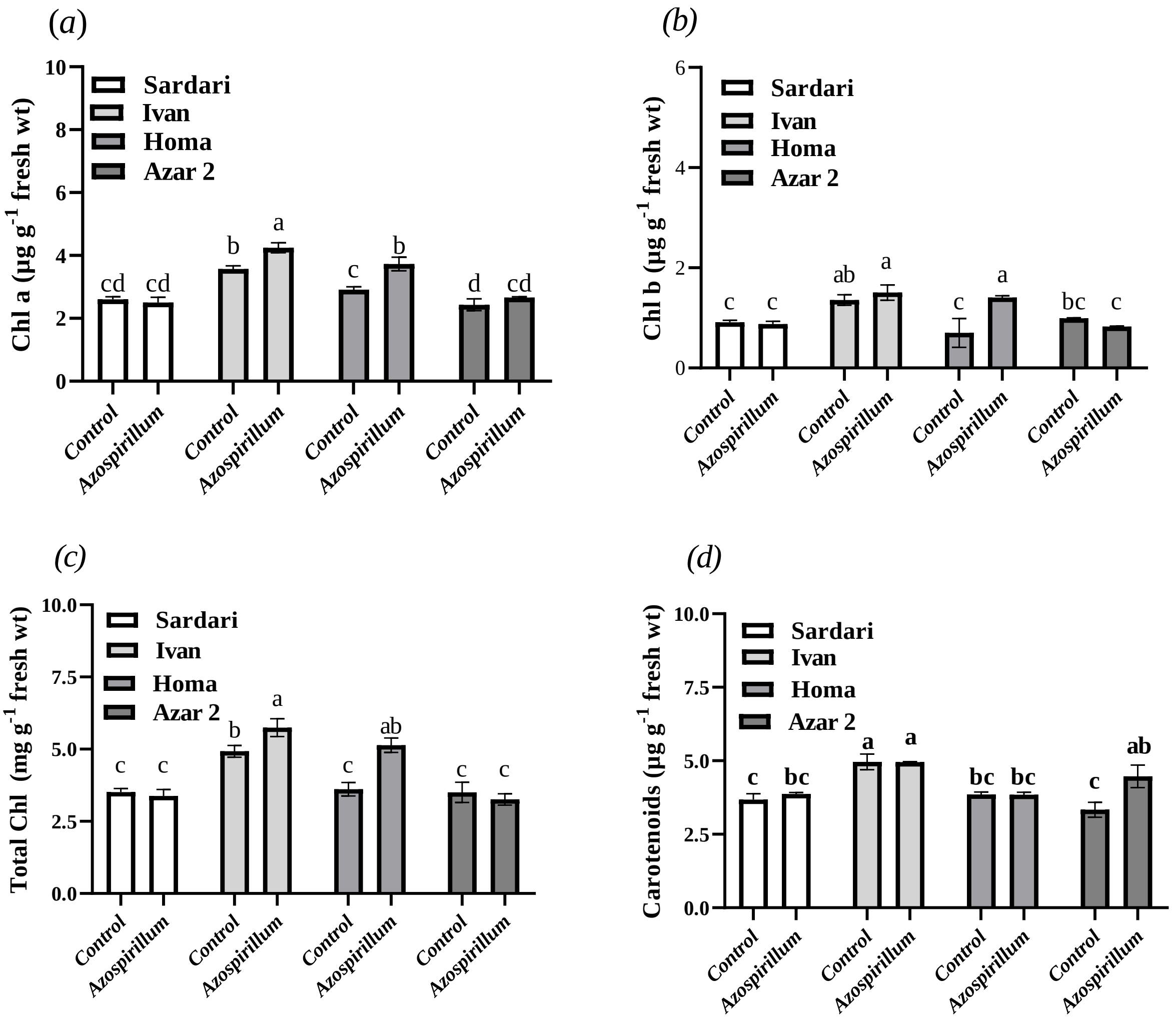

Fig. 4. Effect of *Azospirillum* inoculation on Chlorophyll pigment [chlorophyll (Chl) a, Chl b, total Chl, and carotenoids] contents of four wheat cultivars under rainfed conditions. Means with the different letter are significantly different according to LSD test at P < 0.05; Bar graphs are the mean values ± standard error.

Leaf temperature was significantly influenced by both main factors (cultivar and inoculation) and their interaction (Table 3). Notably, seed inoculation lowered leaf temperature in the Ivan, Homa, and Azar 2 cultivars (Fig. 5), while the Sardari cultivar did not exhibit a significant response to inoculation with regard to leaf temperature (Fig. 5). Leaf temperature is considered as a reliable indicator of crop water status. The reduction in leaf temperature following inoculation in the Ivan, Homa, and Azar 2 cultivars suggests an improvement in their water status, whereas inoculation had no effect on the water status of the Sardari cultivar. This suggests that *Azospirillum* inoculation may improve water content in plants, thereby lowering leaf temperature. Net CO_2 assimilation rate (Pn) was significantly influenced by both cultivar and inoculation (Table 3). Although there was no significant difference between cultivars in Pn response to inoculation (Table 3), but the Ivan cultivar showed the highest Pn among the four wheat cultivars (Table 4). Inoculation with Azospirillum increased Pn by 16.1% compared to non-inoculated plants (Table 4). Transpiration rate (Tr) was solely affected by seed inoculation (Table 3), with Azospirillum inoculation increasing Tr by 24.3% compared to non-inoculated plants (Table 4). No significant differences were observed among cultivars with respect to Tr (Table 4). Inoculation with Azospirillum significantly improved leaf relative water content (LRWC), net CO_2 assimilation rate (Pn), and transpiration rate (Tr), while also reducing leaf temperature. These findings align with the results of Sarig et al. (1988), who reported improvements in water status, lower canopy temperature, and higher transpiration rates in dryland sorghum following inoculation. In this study, inoculated plants exhibited a higher Pn than control plants, particularly under dryland conditions where drought stress is known to affect CO_2 assimilation. Zhao et al. (2020) and Ulfat et al. (2021) reported that drought stress reduces Pn, Tr, and stomatal conductance in wheat. In the present study, the enhanced LRWC in Azospirillum-inoculated plants indicates a positive effect of the bacteria on water uptake, potentially mitigating the impact of drought stress.

In the present study, both *Azospirillum* inoculation and wheat cultivar, as well as their interaction, significantly influenced wheat grain nitrogen and protein contents, as well as grain yield (Table 3). Grain nitrogen and protein contents were enhanced by *Azospirillum* inoculation across all wheat cultivars, except for the Sardari cultivar (Table 5). Inoculation did not significantly alter grain nitrogen content in Sardari (Table 5), but it was more effective in increasing both grain nitrogen and protein contents in the Homa cultivar compared to Azar 2 and Ivan cultivars (Table 5). The Sardari cultivar had the lowest average grain nitrogen and protein contents compared to the other wheat cultivars. *Azospirillum brasilense* can fix atmospheric nitrogen and convert it into ammonium, a form of nitrogen accessible to plants. This bacterium also promotes root growth through the production of hormones such as auxins and cytokinins, which can improve nutrient uptake (Zaheer et al., 2019a, b). In the present study, grain yield was also significantly influenced by both cultivar and inoculation, as well as their interaction (Table 3). As shown in Fig. 6, wheat cultivars responded differently to *Azospirillum* inoculation, with Ivan and Homa cultivars showing the best responses compared to Sardari. Several studies have similarly highlighted the interaction between plant genotype and *Azospirillum* inoculation in wheat (Åström and Gerhardson, 1988; Kazi et al., 2016; Salem et al., 2018). There is considerable evidence supporting the positive impact of *Azospirillum* on plant performance (Zarea et al., 2012; Karimi et al., 2018, 2020; Jafariyan et al., 2016; Kazi et al., 2016). A recent study confirmed that the degree of positive response to *Azospirillum* inoculation can vary with plant genotype (Pereira et al., 2020). Pereira et al. (2020) also reported that root exudates significantly influence *Azospirillum* growth and its nitrogen-fixing ability. The quantity and composition of root exudates differ among genotypes of the same plant species (Pereira et al., 2020). Additionally, the establishment of rhizosphere bacteria, which affect plant growth, can be influenced by the plant genotype (Åström and Gerhardson, 1988). Similarly, Salem et al. (2018) noted that wheat's response to ACC deaminase bacteria was genotype-dependent. In contrast, Boleta et al. (2020) found no significant interaction between *A. brasilense* and wheat genotypes for grain yield. They observed significant interactions between inoculation and cultivars for plant nutrient uptake, specifically potassium and phosphorus. This suggests that the interaction between plant genotype and *Azospirillum* inoculation may depend on environmental conditions. *Azospirillum* does not always enhance crop productivity (Tabassum et al., 2017), but it tends to have a greater growth-promoting effect under adverse conditions such as water deficit (Shakir et al., 2012).

CONCLUSION

This study aimed to examine the effects of *Azospirillum brasilense* on the physiological and biochemical performance of four wheat cultivars (Sardari, Ivan, Homa, and Azar 2) under rain-fed conditions. Seed inoculation with *A. brasilense* led to increased leaf relative water content, improved cell membrane integrity, and a reduction in leaf temperature. Additionally, the net CO_2 assimilation rate and transpiration rate were higher in inoculated plants. Inoculation also had a positive effect on seed nitrogen and protein content. The drought tolerance indicators assessed in this study were enhanced due to *Azospirillum* inoculation. By modulating the host plant's physiological traits, *A. brasilense* could potentially improve wheat productivity under rain-fed conditions, although the response to *A. brasilense* Sp7 was genotype-dependent. Considering the role of hormones in regulating cell structure and organelles, future research could explore the impact of sulfur spraying and bacterial inoculation on the production and effectiveness of plant hormones such as gibberellins, auxins, and cytokinins.

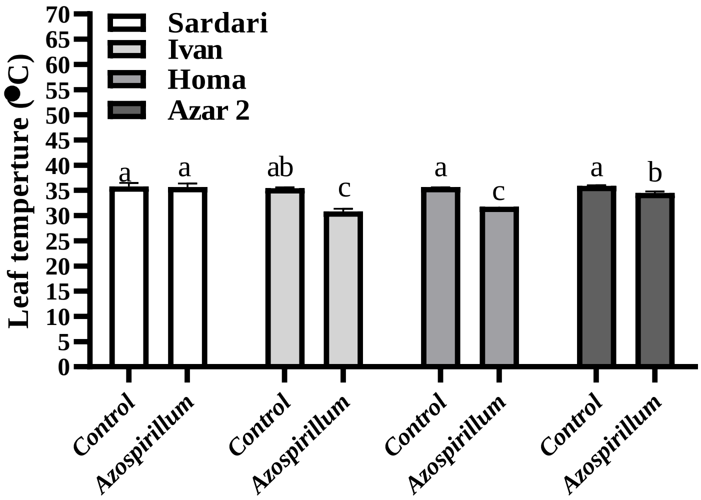

Fig. 5. Effect of *Azospirillum* seed inoculation on leaf temperature of four wheat cultivars under rainfed conditions. Means with the different letter are significantly different according to LSD test at P < 0.05; Bar graphs are the mean values ± standard error.

Table 5. Effect of wheat cultivar and *Azospirillum* seed inoculation on grain nitrogen and protein contents under dryland conditions in Ilam

Treatment	Wheat cultivar	Nitrogen (%)	Protein (%)
Inoculation	Sardari	0.62^{d}	3.6^{d}
	Ivan	0.79^{ab}	4.5^{ab}
	Homa	0.82^{a}	4.67^{a}
	Azar 2	0.75^{b}	4.3^{b}
Control	Sardari	0.62^{d}	3.5^{d}
	Ivan	0.65^{c}	3.7^{cd}
	Homa	0.68^{c}	3.9^{c}
	Azar 2	0.69^{c}	3.9^{c}
LSD (0.05%)		0.046	0.26

In each column, means followed by similar letters have no significant differences ((LSD at α = 0.05)

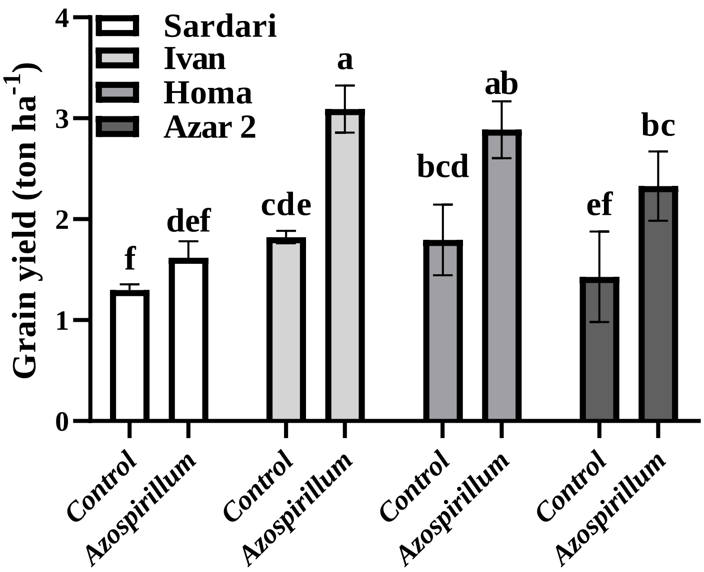

Fig. 6. Response of wheat cultivars to *Azospirillum* inoculation. Means with the different letter are significantly different according to LSD test at P < 0.05; Bar graphs are the mean values ± standard error.

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CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization: Zohreh Karimi and Mohammad Javad Zarea, Arash Fazeli and Batool Zarei; Methodology: Zohreh Karimi; Software: Mohammad Javad Zarea; Validation: Mohammad Javad Zarea; Formal analysis: Zohreh Karimi and Mohammad Javad Zarea; Resources: Zohreh Karimi; Data curation: Mohammad Javad Zarea, Arash Fazeli and Batool Zarei; Writing—original draft preparation: Mohammad Javad Zarea; Writing—review and editing: Zohreh Karimi and Mohammad Javad Zarea; Visualization: Zohreh Karimi and Mohammad Javad Zarea; Supervision: Mohammad Javad Zarea, Arash Fazeli and Batool Zarei; Project administration: Mohammad Javad Zarea, Arash Fazeli and Batool Zarei;

DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author on request.

ETHICAL STATEMENT

The conducted research is not related to either human or animals use. Author is aware of the content of the manuscript and consented to submit it to *Iran Agricultural Research* journal.

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