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Morpho-Physiological Responses of Wheat to Silicon and Bio-Fertilizer under Water-Deficit Conditions

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Recently, the use of silicon and bio-fertilizer has become important worldwide to improve the physicochemical characteristics of soil. Thus, they are used as an alternative approach to cope with the water-deficit stress on wheat plant. In this regard, a split-plot factorial experiment based on randomized complete block design with three replications was conducted during 2017 and 2018 at Sistan and Baluchestan Research Station, Iran. In the present study, treatments included water-deficit stress (irrigation after 60, 120, and 180 mm of evaporation from Class A pan) as the main plot, as well as the application of silicic acid (Si) foliar nutrition (0, 1, and 1.5 mM) and Nitro-Kara bio-fertilizer as sub-plots. The results represented the significant interaction effects of irrigation regimes, bio-fertilizer, and Si on 1000-grain weight and panicle number. In addition, the co-application of Nitro-Kara and Si fertilizers (1 mM) under irrigation after 120 mm evaporation led to the highest grain per panicle (44 number), which reflected a 21.36% increase compared to the control. The greatest 1000-grain weight (39.66 g) was obtained by inoculating seed with Nitro-Kara and without to apply Si fertilizer under 120 mm irrigation treatment in the first year. Further, inoculation under moderate stress (120 mm) in the second year demonstrated the highest grain yield (5,256.3 kg ha^{-1}). Furthermore, a high chlorophyll index (57.6) was achieved when consuming 1.5 mM Si fertilizer and without Nitro Kara under 120 mm irrigation regime during the first year, which had a 20.2% enhancement compared to the control treatment. Under low irrigation conditions, the use of Nitro-Kara bio-fertilizer and Si fertilizer resulted generally in improving physiological parameters and increasing the growth and yield indices of wheat.

Keywords: chlorophyll fluorescence, 1000-grain weight, limited irrigation, Nitro-Kara bio-fertilizer, water scarcity

Abbreviations: Fo—minimum fluorescence, Fm—maximum fluorescence, Fv—variable fluorescence, RCBD—randomized complete block design, PGPR—plant-growth-promoting rhizobacteria

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a staple food and principal protein source for approximately 35% of the global population. It originated in southwestern Asia and used as a chief food commodity since the ancient era. Additionally, wheat is called as the king of cereal crops and is considered as the second most cultivated staple food and consumed product (one-third population) in the world (Haider et al. 2020). Today, wheat is cultivated in six continents and occupies more than 220 million hectares, providing 20% of the protein and caloric intake of humans (El-Metwally et al. 2019; Aleliunas et al. 2020). On average, 100 g of wheat grains contains 69.1-75.5 g of carbohydrates, 1.2-2.5 g of fat, 1.8-2.3 g of fiber, and 9.4-13.9 g of protein (Liu et al. 2020).

Plants constantly confront undesirable environmental conditions throughout their life, which impairs their growth, development, and productivity. Considering the ongoing climate change, water scarcity is the primary abiotic stress, which affects crop growth and yield, reduces crop production worldwide, and is a severe problem for the arid and semi-arid regions in the world (Sil et al. 2018). Water deficit can lead to the maximum variability in grain protein and yield at the critical growth stages by disturbing morpho-physiological characteristics (Bonfil et al. 2004). Further, a 50% reduction in water consumption results in decreasing wheat grain yield by 40.7% (Ramezanpoor and Dastfal 2004). Thus, the development of such techniques and strategies can enhance soil water and nutrient holding capacity, yield crops, and productivity. Furthermore, the application of silicon (Si) and bio-fertilizers may improve the growth of cereals and other plants, and increase their production in arid or semi-arid areas under water scarcity (Kaya et al. 2006; Mahdavi Khorami et al. 2020).

Silicon is considered as the most abundant metalloid in the soil and a “functional” plant nutrient, the deficiency of which in crops was recognized since the 1970s (Islam et al. 2018). It is absorbed as uncharged silicic acid [$Si(OH)_4$] and precipitated as amorphous silica throughout plant (Sil et al. 2018). In addition, it is the second most abundant element in the Earth's crust, which is absorbed by plants in various amounts. Silicon is vital to develop several plant species, especially graminea, although it is not an essential element for plants (Siah et al. 2018). Further, it forms 0.1-10% of the aerial part dry weight of plant (Guntzer et al. 2012). Some of the beneficial effects of Si include enhancing plant tolerance to biotic and abiotic stresses in crops (Luyckx et al. 2017) and improving the stress resistance of some plant species, especially to drought (Maghsoudi et al. 2016). Furthermore, the consumption of Si fertilizer is an ecologically compatible and environmentally friendly technique for stimulating plant growth and alleviating various environmental stresses such as water stress in the plants (Rezakhani et al. 2019). According to Munjal and Dhanda (2016), the interaction of drought stress and Si is significant on grain number, spike weight, 1000-grain weight, and wheat cultivar yield.

Nitrogen element is most frequently applied in the cultivation of many crops, the principal sources of which are urea, ammonium nitrate, ammonium sulfate, calcium nitrate, and the like (Li et al. 2021). It is known as a major nutrient for all plants, and Nitro-Kara is a nitrogen-fixing bio-fertilizer. The bio-fertilizer contains the highly efficient nitrogen-fixing bacteria of *Azorhizobium caulinodans* isolated from nature. Additionally, *A. caulinodans* is considered as a beneficial soil and root bacterium, and an associative nitrogen-fixing one (Osorio Vega 2007). Azotobacter in Nitroxin or Nitro-Kara bio-fertilizers can produce and secrete active biological substances such as B vitamins, auxins, and gibberellins in plant roots, which play an essential role in increasing the growth of plant aerial parts (Charkhab and Mojaddam 2018). Biodiversity, as well as supplying nutrients in a manner fully compatible with the natural nutrition of plants, intensifying vital activities, improving quality, preserving environmental health, and generally maintaining and protecting national capital (soil, water, and non-renewable energy sources) are among the most critical benefits of bio-fertilizers (Verma et al. 2014). Due to the importance of bio-fertilizers in enhancing plant growth and yield and maintaining and sustaining the environment, many studies have been performed on the effects of the fertilizers on different plants, most of which recommended the application of bio-fertilizers because of their effectiveness. Some researchers found yield improvement by using bio-fertilizers in fennel (Salama et al. 2015) and basil (Smitha et al. 2019).

Recently, Etesami (2018) suggested Si and bio-fertilizer interaction in combination as a novel field. According to Mahmood et al. (2016), the combined use of *Bacillus drentensis* strain and Si fertilizer leads to the most significant intensification of growth, physiological traits, and mung bean yield under the salinity-affected conditions. Si and bio-fertilizers can increase plant resistance to environmental stresses through many mechanisms such as improving plant root system, increasing plant nutrient uptake, and inducing the plant synthesis of antioxidative enzymes (i.e., superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase) (Etesami and Maheshwari 2018; Rezakhani et al. 2019). Considering the importance and role of Si and bio-fertilizers in wheat under water scarcity conditions and lack of sufficient studies in this field, the present study aimed to assess the effect of Si and Nitro-Kara bio-fertilizer on some morpho-physiological parameters of wheat under water-deficit stress in the Sistan and Baluchestan regions of Iran.

MATERIALS AND METHODS

The present study was conducted at the Research Farm of Islamic Azad University, Zabol Branch, Sistan during two crop years (2018 and 2019). Sistan is located in the east of Iran and north of Sistan and Baluchestan province in a flat plain (30° 18' N, 60° 10' E, 480 meter above mean sea level). The area of Sistan plain is 15197 km^2, 5560 km^2 of which is covered by Hamoon Lake and overlooking lands. Fig. 1 depicts the temperature and rainfall rate of the experimental site during the two-year crop-growing periods.

The present split-plot factorial experiment was performed based on randomized complete block design (RCBD) with three replications. The treatments were water-deficit stress (irrigation after 60, 120, and 180 mm of evaporation from Class A pan) as the main plot as well as the foliar application of Si at three levels (0, 1, and 1.5 mM) as first factor (sub-plot) and Nitro-Kara bio-fertilizer (inoculation: one liter per hectare by seed soaking and non-inoculation) as second factor (sub-plot). In other words, the whole plot factor is the irrigation treatment; the split-plot factor is the bio-fertilizer inoculation; and the split-split-plot factor is the Si concentration.

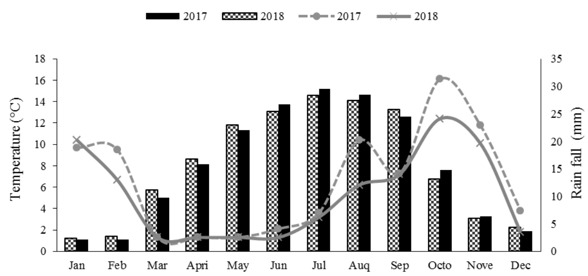

Fig. 1. Climate conditions (temperature and precipitation) during the growing season 2017 and 2018 (Zabol city, Sistan and Baluchestan province, Iran).

The soil of the experimental site had clay loam soil texture, 148 mg kg^{-1} potassium, 10.4 mg kg^{-1} phosphorus, 0.07% nitrogen, and 1.63% organic matter. Before planting, ammonium phosphate (rate of 200 kg ha^{-1}) and urea fertilizers (150 kg ha^{-1}) were applied in all stages of tillering and stalking to improve the soil fertility. The planting operations involved plowing with reversible plow, disc, and lever. After plotting in the dimensions of 3 × 2 m^2, the seeds were planted in rows at a distance of 30 cm and depth of 1 cm with a density of 400 plants per m^2. Wheat cultivation of 'Kavir' cultivar was planted in mid-December in both years.

The bio-fertilizer used for seed soaking was Nitro-Kara. The nitrogen-fixing bacterium in the formulation of Nitro-Kara was *A. caulinodans* from Kara Industrial Biotechnology Company Product, Iran and was grown at a rate of 109 CFU/g. One hundred g of bio-fertilizer was dissolved in 5 L of distilled water, followed by seed soaking for 6 h at a temperature of 15-20°C. Si fertilizer from Manvert, Spain was sprayed during stem elongation, booting, and heading stages (30, 41, and 51 BBCH) during the growing season. In addition, the sprays were performed in the afternoon at clear and mild weather for better effect. The irrigation after 60, 120, and 180 mm evaporation from Class A pan was considered as well-watered, moderate, and severe stress, respectively. Evaporation rate was calculated by installing a Class A pan in the field on a daily basis at 7 o'clock in the morning. Irrigation restrictions started at 15 BBCH-scale. Further, irrigation water required before irrigation (VN) is the amount of water (measured by using a water counter) needed during irrigation to replenish soil moisture deficit, and subsequently restore the soil to field capacity. The VN was calculated according to Rahimzadeh and Pirzad (2019). Over the whole growing season, the volume of the water consumed for three irrigation regimes was 5000, 3200, and 2000 m^3 ha^{-1} for 60, 120, and 180 mm evaporation from Class A pan, respectively.

Some physiological traits such as chlorophyll concentration and fluorescence and canopy temperature were measured at the end of flowering stages (69 BBCH-scale). To this end, chlorophyll concentration was manually determined by using a SPAD-502 Plus chlorophyll meter in young and terminal leaves (10 samples per plant). In order to obtain the quantum yield of photosystem II, fluorescence parameters were calculated from the surface of the youngest leaves in plants by placing clamps for 20 min under a dark condition at the end of flowering and then using a portable fluorometer (OS-30, Opti-sciences). Some of the fluorescence parameters under study included minimum (Fo), maximum (Fm), and variable fluorescence (Fv), and photosystem quantum yield (quantum yield of photosystem II: Fv/Fm). Furthermore, four middle rows were harvested from each plot after removing the marginal effects for final harvesting (at the harvest maturity, which was for the first and second year in late May). Finally, panicle, grain, and tiller number, plant height, panicle length, 1000-grain weight, and grain yield were measured.

Statistical Analysis

The data were analyzed by using statistical analysis software 9.2 (SAS 9.2). A RCBD-based split-plot factorial experiment was performed to estimate the variance components of the effects of water-deficit, Si and bio-fertilizer, and their interactions. The differences among the treatments were evaluated through employing Tukey's test (HSD) only when ANOVA F-test represented the significance level of 0.05.

RESULTS AND DISCUSSION

Plant Height

The results of variance analysis indicated the significant effects of year, irrigation, Si, and irrigation × bio-fertilizer, as well as the interaction of year, irrigation, and bio-fertilizer on plant height (Table 1). The comparison of the interaction between year, irrigation regimes, and bio-fertilizer on height attribute demonstrated the highest plant height when inoculating seed with Nitro-Kara bio-fertilizer under 60 and 120 mm treatments in the first year (84.77 and 84.66 cm, respectively). Although, these values are not significantly different with inoculation with bio-fertilizer in severely stressed plants (Table 2). However, the greatest plant height in the second year was related to non-inoculation treatment under 60 mm irrigation regime (85.33 cm). The least plant height (71.44 cm) was observed in plants coming from seeds not inoculated with bio-fertilizer and subjected to severe

Table 1. Combined (2-year data) analysis of variance of morpho-physiological responses of wheat to silicon and bio-fertilizer under irrigation regimes (irrigation after 60, 120, and 180 mm of evaporation from Class A pan).

SOV	df	Plant Height	Number of Panicles	Number of Grains per Panicle	Panicle Length	Number of Tillers	1000-Grain Weight	Grain Yield	Chlorophyll Index	Chlorophyll Fluorescence	Canopy Temperature
		Mean Square (MS)									
Year (Y)	1	611.50**	7008.33*	14.81 ns	7400.33**	0.59 ns	237.00**	1111973.20 ns	11.40 ns	0.32**	24.08*
Replicate (Y)	4	17.64	595.57	126.32	18.22	0.26	6.60	622739.88	3.96	0.00003	2.57
Irrigation regime (I)	2	275.30**	14874.48**	66.17**	224.33**	0.86*	74.08**	1431162.65**	90.38 ns	0.002 ns	2.67 ns
Y × I	2	230.60**	10238.11**	9.93 ns	2.33 ns	0.39 ns	16.23*	159847.96 ns	29.80 ns	0.028 ns	52.75**
Rep (Y × I)	8	3.56	108.52	6.74	13.09	0.10	2.69	136583.87	25.99	0.024	1.86
Bio-fertilizer (B)	1	7.25 ns	249.03 ns	22.23 ns	78.37**	0.33 ns	0.33 ns	2863.40 ns	11.40**	0.26**	24.08**
Silicon (S)	2	10.84*	800.56 ns	18.34 ns	49.75**	1.36**	52.52**	10801.18 ns	206.71**	0.005**	7.00**
Y × B	1	133.3**	1.33 ns	7.78 ns	29.03 ns	0.0001 ns	35.59**	279369.91**	326.91**	0.32**	6.75*
I × B	2	38.84**	477.81 ns	97.11**	219.70**	0.194 ns	38.08**	394274.38**	5.17*	0.01**	14.25**
Y × S	2	1.84 ns	924.08 ns	10.07 ns	93.25**	0.39 ns	28.45**	69768.19 ns	3.30 ns	0.008**	25.08**
I × S	4	3.76 ns	1555.14**	82.52**	5.20 ns	0.22 ns	11.44 ns	46871.36 ns	15.31**	0.003**	5.80**
S × B	2	2.81 ns	180.67 ns	24.14 ns	9.50 ns	0.08 ns	12.69 ns	31924.99 ns	3.30 ns	0.004**	1.08 ns
Y × B × S	2	1.00 ns	8987.52**	27.50 ns	1.78 ns	0.19 ns	8.62 ns	20940.39 ns	70.34**	0.0008 ns	0.19 ns
Y × I × B	2	59.80*	2948.11**	10.50 ns	123.25**	2.02**	11.56 ns	211986.87**	0.20 ns	0.004**	2.02 ns
Y × I × S	4	1.35 ns	1809.27**	54.40**	4.45 ns	0.53 ns	6.73 ns	78590.19 *	2.87 ns	0.002*	7.70**
I × B × S	4	2.29 ns	2250.28**	17.42 ns	11.96 ns	0.61 ns	28.52**	24440.43 ns	3.26 ns	0.001 ns	1.62 ns
Y × I × B × S	4	4.89 ns	7119.13**	46.99**	9.71 ns	0.72*	40.92**	7750.81 ns	25.98**	0.001 ns	3.84*
Error	60	2.33	295.67	10.67	8.81	0.24	5.40	35264.82	1.47	0.0008	1.06
CV (%)	-	11.88	5.96	9.20	9.90	14.17	6.97	12.68	12.36	8.19	15.34

ns: non-significant; *: significant at the 5% probability level; **: significant at the 1% probability level

water stress (180 mm evaporation) in the second year. Inoculation with bio-fertilizer somehow improved the plant height of the severely stressed plants during the second year (Table 2).

Panicle Number

The interaction effects of year, irrigation regime, bio-fertilizer, and Si were significant on panicle number ($p \leq 0.01$) (Table 1). As shown in Fig. 2, panicle number is maximized by inoculating seed with Nitro-Kara bio-fertilizer and applying 1.5 mM Si under irrigation after 60 mm evaporation in the second year (359.6), which increases 10.56% compared to the control. During the second year, regardless of silicon treatment, plants inoculated with bio-fertilizer and subjected to severe water stress gave the lowest number of panicles.

Grain Number per Panicle

As summarized in Table 1, the significant interaction effects are found for year, irrigation regimes, and bio- and Si fertilizer on grain number per panicle ($p \leq 0.01$). The co-application of Nitro-Kara and Si fertilizers (1 mM)

Table 2. Comparison of wheat traits by irrigation regimes (irrigation after 60, 120, and 180 mm of evaporation from Class A pan) and bio-fertilizer under different years of experiments.

Years	Irrigation Regimes (mm)	Bio-Fertilizer	Plant Height (cm)	Panicle Length (mm)	Grain Yield (kg ha^{-1})
First	60	Inoculation	84.77 ± 8.48 a	89.55 ± 11.12 a	2967.1 ± 114.2 cd
		Non-inoculation	82.88 ± 7.25 b	82.88 ± 9.50 cd	2967.1 ± 100.8 cd
	120	Inoculation	84.66 ± 8.88 a	81.66 ± 10.71 d	3198.3 ± 148.5 c
		Non-inoculation	82.55 ± 6.15 b	88.77 ± 8.44 a	3026.5 ± 124.6 cd
	180	Inoculation	83.88 ± 7.05 b	85.55 ± 6.93 b	2704.0 ± 119.3 d
		Non-inoculation	82.77 ± 9.14 b	76.88 ± 12.25 e	2114.0 ± 142.0 f
Second	60	Inoculation	79.50 ± 10.20 c	70.88 ± 7.46 f	2882.6 ± 155.9 cd
		Non-inoculation	85.33 ± 7.36 a	68.66 ± 8.69 g	3892.6 ± 137.4 b
	120	Inoculation	78.83 ± 8.88 c	67.66 ± 11.90 g	4256.3 ± 122.9 a
		Non-inoculation	83.83 ± 6.27 b	68.55 ± 10.10 g	3779.6 ± 143.9 b
	180	Inoculation	74.05 ± 9.05 d	65.44 ± 9.72 h	2249.2 ± 94.72 ef
		Non-inoculation	71.44 ± 6.22 e	64.77 ± 12.73 h	2316.7 ± 107.4 e

The same letter in each column (Mean ± SD, n = 3), is not significantly different according to Tukey's test (HSD) at 5% probability level.

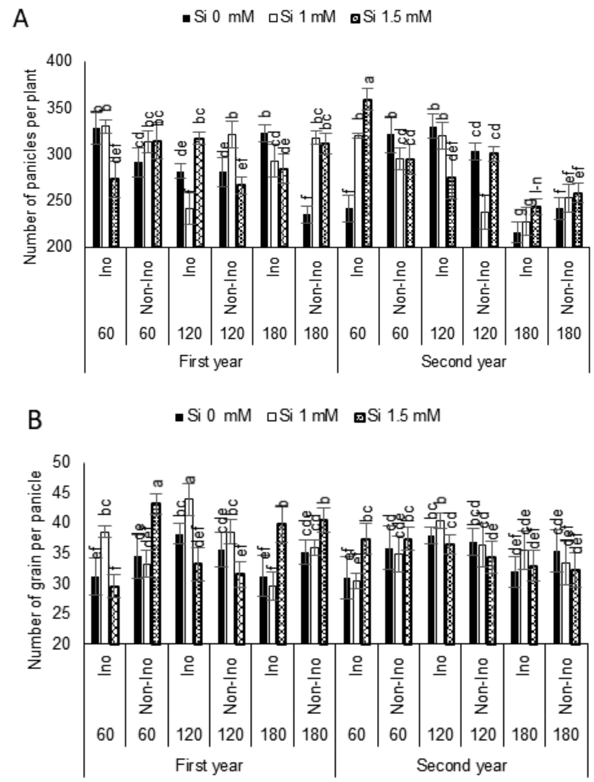

Fig. 2. The interaction effects of the year (first and second), irrigation regimes (irrigation after 60, 120, and 180 mm of evaporation from Class A pan), bio-fertilizer (non-inoculation: None-Ino; and inoculation with 'Nitro-Kara': Ino), and silicon (Si) fertilizer (0, 1, and 1.5 mM) on number of panicles (A) and number of grain per panicles (B) of wheat. The same letter in each figure (mean, n=3), is not significantly different according to the Tukey's test (HSD) at 5% probability level.

under irrigation after 120 mm evaporation led to the greatest grain number per panicle (44) during the first year, which increased by 21.36% compared to the control (non-inoculation of bio-fertilizers under irrigation after 60 mm evaporation) (Fig. 2). This did not differ significantly with the well-watered plants co-applied with Nitro-Kara and 1.0 mM Si, and the non-inoculated plants applied with 1.5 mM Si as well as the severely water-stressed plants, inoculated and co-applied with 1.5 mM Si. During the second year, the Si treatment did not give any significant effect in the moderately and severely stressed plants.

Panicle Length

Year, irrigation regimes, bio-fertilizer, and Si, as well as the interactions of year × irrigation regime × bio-fertilizer affected panicle length significantly (Table 1). Based on the results in Table 2, limited irrigation water resulted in decreasing panicle length (in the first year), while the mean length enhanced by applying the fertilizer. The panicle length was respectively maximized (89.55 mm) and minimized (64.77 mm) when seed was inoculated with Nitro-Kara under normal irrigation (irrigation after 60 mm evaporation) in the first year and non-inoculated with Nitro-Kara under severe water stress (irrigation after 180 mm evaporation) during the second year.

Tiller Number

The interactions of year, irrigation regime, Si and bio-fertilizers were significant on tiller number ($p \leq 0.05$) (Table 1). As displayed in Fig. 3, the highest number of tillers (4.33 per plant) is found when inoculating seed and using Si fertilizer (1 mM) under irrigation after 120 mm evaporation during the second year, which exhibits 7.62% increase compared to the control treatment. Moreover, this is however, not significantly different as the 16 other combination treatments. In addition, it is worthwhile to note that in severely stressed plants during the first year, the inoculation of Nitro-Kara together with the application of 1.0 and 1.5 mM Si helped increase the number of tillers.

1000-Grain Weight

Based on the results of variance analysis (Table 1), year, irrigation regimes, and Si-fertilizer, as well as the interaction effects of year × irrigation regime × bio-fertilizer × Si-fertilizer were significant on 1000-grain weight ($p \leq 0.01$). The inoculation of seed with Nitro-Kara bio-fertilizer and non-application of Si fertilizer under irrigation after 120 mm evaporation in the first year led to the greatest 1000-grain weight (39.66 g), which led to a 4.18% improvement compared to the control. Also, this treatment is not statistically different as eight of the other treatments. However, the minimum of mean 1000-grain weight (28 g) was obtained when seed was inoculated with Nitro-Kara and Si fertilizer was consumed (1 and 1.5 mM, respectively) under 60 and 120 mm irrigation treatments (Fig. 3).

Grain Yield

The grain yield was significantly affected by year, irrigation regimes, and bio-fertilizer, as well as year, irrigation regimes, and Si fertilizer interactions (Table 1). As presented in Table 2, the yield is maximized with inoculation under moderate stress (irrigation after 120 mm evaporation) and non-inoculation under non-stress (irrigation after 60 mm evaporation) conditions during the second year (4892.6 and 5256.3 kg ha^{-1}). However, the non-inoculation treatment under severe water-deficit stress (irrigation after 180 mm evaporation) has the least grain yield (2114.0 kg ha^{-1}) and regardless of the

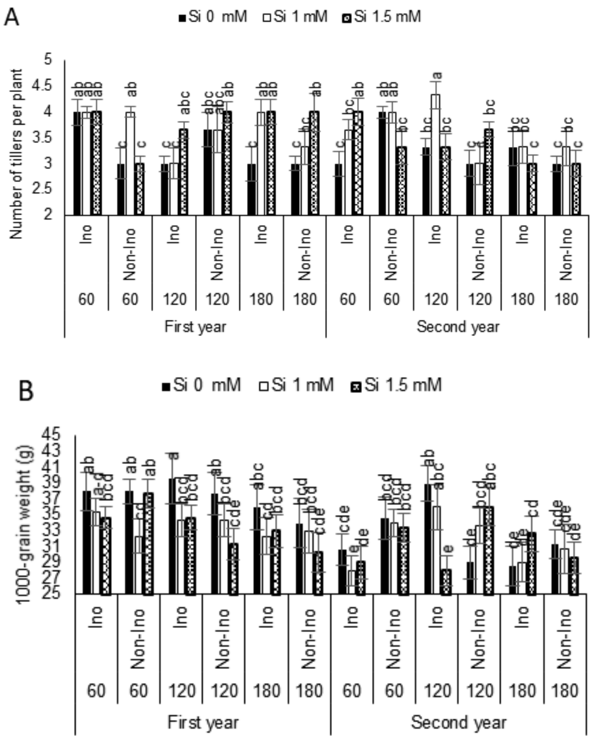

Fig. 3. The interaction effects of year (first and second), irrigation regimes (irrigation after 60, 120, and 180 mm of evaporation from Class A pan), bio-(non-inoculation: None-Ino; and inoculation with 'Nitro-Kara': Ino), and silicon (Si) fertilizer (0, 1, and 1.5 mM) on number of tillers (A) and 1000-grain weight (B) of wheat. The same letter in each figure (mean, n=3), is not significantly different according to the Tukey's test (HSD) at 5% probability level.

application of bio-fertilizer in the first and second years, the grain yield was still low. Additionally, the application of Si fertilizer (especially 1.5 mM) under moderate stress conditions in the second year exhibited the highest grain yield (2927.5 kg ha^{-1}), which had a 9.68% increase compared to the control. However, all levels of Si fertilizer under the severe water-deficit stress resulted in minimizing the yield during the first year (Table 3). Thus, Si foliar application under severe water stress conditions did not represent high efficiency in modulating the adverse effects of stress on grain yield. However, during the second year, Si application at 1.0 mM and 1.5 mM showed promise in maintaining a relatively high grain yield of plants subjected to moderate water stress.

Chlorophyll Index

The results of variance analysis indicated that bio- and Si-fertilizer treatments influenced chlorophyll index significantly (Table 1). Further, the interaction effects of year × bio-fertilizer, irrigation × bio-fertilizer, irrigation × Si fertilizer, year × bio-fertilizer × Si fertilizer, and year × irrigation × bio-fertilizer × Si fertilizer significantly affected chlorophyll content. The Si fertilizer improved the amount of leaf chlorophyll in the first year. Furthermore, Si fertilizer application (1.5 mM) and non-inoculation treatment under irrigation after 120 mm evaporation during the first year led to a large chlorophyll index (57.6), which enhanced 20.2% compared to the control. However, the minimum of mean chlorophyll index was related to the non-inoculation and inoculation of seed during the first and second years, respectively, under irrigation after 60 and 120 mm evaporation, with no Si application (Fig. 4).

Chlorophyll Fluorescence

As shown in Table 1, the effects of year, and bio- and Si-fertilizer, as well as the interaction effects of year × irrigation regime × Si-fertilizer on chlorophyll fluorescence were significant. Under severe water stress conditions (irrigation after 180 mm evaporation), all Si fertilizer treatments exhibited the highest chlorophyll fluorescence during the second year, which increased 6.66, 6.66, and 7.89% compared to the control. The least mean of the trait was achieved by non-application of Si fertilizer under irrigation after 180 mm evaporation during the first year (0.56) (Table 3).

Canopy Temperature

Based on the results in Table 1, a significant effect was observed for year, and bio- and Si-fertilizer treatments on canopy temperature. The temperature was significantly influenced by the interaction effects of year × irrigation × bio-fertilizer × Si-fertilizer. In addition, the greatest canopy temperature was found when 1.5 mM Si-fertilizer was applied with and without inoculation treatments under irrigation after 60 mm evaporation in the first year and seed was inoculated with Nitokara fertilizer under irrigation after 180 mm evaporation during the second year (22.66, 22.46, and 22.36°C). However, these treatments are not statistically significant as two other treatments in the well-watered and moderately water-stressed irrigation regimes. The treatments reflected 2.91, 2.04, and 19.49% increase compared to the control. However, the temperature was minimized by non-application of bio- and Si fertilizer under severe water stress (14.66°C) during the first year, as well as the co-application of bio-fertilizer and Si at 1.5 mM under non-stress conditions during the second year (Fig. 4).

Correlation Coefficients

Significant negative and positive correlations were observed among grain yield and morpho-physiological traits (Table 4). For example, grain yield was significantly and positively correlated with plant height (0.33*), panicle (0.65**) and grain number (0.52**), panicle length (0.42*), 1000-grain weight (0.89**), and chlorophyll index (0.57**). However, a significant negative correlation was observed between grain yield with chlorophyll fluorescence (-0.37*) and canopy temperature (-0.30*).

Morpho-Physiological Responses to Irrigation Regime

The morphological parameters of wheat such as plant height, panicle length, and panicle, grain, and tiller number significantly decreased under severe water-deficit stress conditions (irrigation after 180 mm evaporation), which are consistent with the results obtained by some researchers. For instance, Haider et al. (2020) reported a reduction in plant growth parameters after imposing water-deficit stress at critical growth stages of wheat. They found that plant-cell dehydration and lower turgidity leads to disturbed protoplasmic functions, which decline cell division and poor plant height. Also, an increased chlorophyll degradation and reduced photosynthesis rate caused by stomatal closure significantly decrease leaf chlorophyll content and stomatal conductance under water-deficit conditions (Aown et al. 2012; Aslam et al. 2020). Rebey et al. (2012) suggested photosynthesis as the main reason for storing dry matter in seed. Therefore, the number of stem cells decreases due to water deficit stress, leading to fewer seeds. Mahdavi Khorami et al. (2020) reported a significant decline in the grain yield of wheat under drought stress during vegetative and reproductive developmental stages. This issue may result in manufacturing less photo-assimilates for grain filling or decreasing the sink power to absorb photo-assimilates and diminishing the grain-filling period. Further, grain yield reduces likely due to the premature cessation of sucrose activity, as well as a decline after anthesis photosynthesis and assimilate remobilization (Mahdavi Khorami et al. 2020). Furthermore, it has been proposed as the major reason for below drought stress situations (Saeidi et al. 2017).

Table 3. Comparison of wheat traits by irrigation regimes (irrigation after 60, 120, and 180 mm of evaporation from Class A pan) and silicon under different years of experiments.

Years	Irrigation Regimes (mm)	Silicon (mM)	Grain Yield (kg ha^{-1})	Chlorophyll Fluorescence
First	60	0	2464.8 ± 188.0 ^{cd}	0.60 ± 0.09 ^{d}
		1	2351.2 ± 152.4 ^{cde}	0.67 ± 0.08 ^{c}
		1.5	2468.5 ± 127.7 ^{cd}	0.67 ± 0.07 ^{c}
	120	0	2530.8 ± 133.4 ^{bc}	0.60 ± 0.07 ^{d}
		1	2585.8 ± 117.3 ^{bc}	0.60 ± 0.05 ^{d}
		1.5	2324.8 ± 124.4 ^{cde}	0.62 ± 0.09 ^{d}
	180	0	2236.6 ± 162.8 ^{e}	0.56 ± 0.09 ^{e}
		1	2214.6 ± 114.2 ^{e}	0.62 ± 0.07 ^{d}
		1.5	2230.5 ± 104.8 ^{e}	0.61 ± 0.05 ^{d}
Second	60	0	2644.0 ± 99.42 ^{b}	0.70 ± 0.08^{b}
		1	2550.6 ± 148.7 ^{bc}	0.73 ± 0.05 ^{ab}
		1.5	2754.3 ± 172.3 ^{b}	0.66 ± 0.05 ^{c}
	120	0	2703.3 ± 195.7 ^{b}	0.73 ± 0.07 ^{ab}
		1	2788.6 ± 133.3 ^{b}	0.71 ± 0.05 ^{ab}
		1.5	2927.5 ± 108.3^{a}	0.72 ± 0.06 ^{ab}
	180	0	2337.5 ± 134.2 ^{cde}	0.75 ± 0.09 ^{a}
		1	2272.5 ± 87.86 ^{de}	0.75 ± 0.07 ^{a}
		1.5	2255.8 ± 118.7 ^{de}	0.76 ± 0.08 ^{a}

The same letter in each column (Mean ± SD, n = 3), is not significantly different according to the Tukey's test (HSD) at 5% probability level.

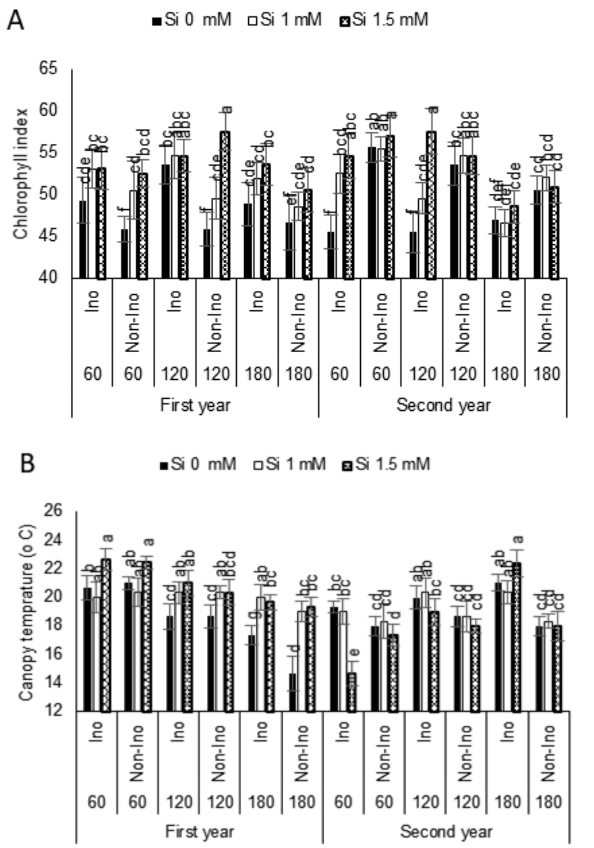

Fig. 4. The interaction effects of year (first and second), irrigation regimes (irrigation after 60, 120, and 180 mm of evaporation from Class A pan), bio-(non-inoculation: None-Ino; and inoculation with 'Nitro-Kara': Ino), and silicon (Si) fertilizer (0, 1, and 1.5 mM) on number of tillers (A) and 1000-grain weight (B) of wheat. The same letter in each figure (mean, n=3), is not significantly different according to the Tukey's test (HSD) at 5% probability level.

Water-deficit stress declines the number of endosperm cells in the base and head of the spike, leading to a diminution in seed weight. After flowering stage, the stress reduces the seed-filling period and decreases seed weight (Li et al. 2018). However, it leads to lower mean seed weight, and ultimately less yield by accelerating leaf aging, declining the seed-filling speed, and reducing the growth period during the seed-filling stage (Gonzalez et al. 2010). According to Iqbal et al. (2019), fertile tiller number, spike length, spikelets per spike, grain number per spike, 1000-grain weight, and grain yield in wheat plant decline significantly under water-deficit stress because of depriving assimilation and partitioning photosynthates. Iqbal et al. (2019) and Aslam et al. (2020) found a significant reduction in the spike length, 1000-grain weight, and grain yield of the water-stressed wheat plants. Finally, the water-deficit stress diminishes photosynthesis rate, which results in producing less biomass, and ultimately affecting the yield and yield-contributing attributes (Rostamza et al. 2011).

Morpho-Physiological Responses to Bio-Fertilizer Under Different Irrigation Regimes

The combined application of Nitro-Kara bio-fertilizer and a chemical fertilizer affect the yield and yield components of wheat such as biological yield positively (Amiri et al. 2013), which is in agreement with the results of the present study. The plant-growth-promoting rhizobacteria (PGPR) possess the tremendous potential for modulating the physiological response to water deprivation, ensuring plant survival under such stressful conditions (Mahdavi Khorami et al. 2020). In addition, the PGPR-inoculated plants exhibit a significant enhancement in growth and yield, and increase in drought tolerance in arid and semi-arid areas (Marulanda et al. 2007). The use of organic amendments plays an essential role in declining the water-deficit stress in plants through reducing diffusion and improving sorption. Bio-fertilizers result in increasing yield through biological nitrogen fixation, as well as producing solvent phosphate, siderophore, and growth hormones such as

Table 4. Correlation coefficients among morpho-physiological characteristics of wheat under irrigation regimes and fertilizers application.

	Plant Height	Number of Panicles	Number of Grains	Panicle Length	Number of Tillers	1000-Grain Weight	Grain Yield	Chlorophyll Index	Chlorophyll Fluorescence
Number of panicles	0.10 ns								
Number of grains	0.19 ns	0.27ns							
Panicle length	0.22 ns	0.17ns	0.43*						
Number of tillers	0.30*	0.33*	-0.09ns	0.36*					
1000-grain weight	0.31*	-0.15ns	-0.30*	-0.38*	-0.21ns				
Grain yield	0.33*	0.65**	0.52**	0.42*	0.20 ns	0.89**			
Chlorophyll index	0.13 ns	-0.05ns	0.38*	0.21ns	0.34*	0.58**	0.57**		
Chlorophyll fluorescence	-0.13ns	0.18ns	-0.34*	-0.31*	-0.23ns	-0.49*	-0.37*	-0.60**	
Canopy temperature	0.09 ns	0.09ns	-0.25ns	-0.24ns	0.12ns	-0.43*	-0.30*	-0.52**	0.65**

ns: non-significant; *: significant at 5% probability level; **: significant at 1% probability level.

indole, acetic acid, and gibberellin (Kızılkaya 2008). The consumption of the bio-fertilizers such as the nitrogen-fixing bacteria and mycorrhizae fungi leads to a decrease in chemical fertilizers, as well as increases tolerance to environmental stresses (Hoseini-Mazinani and Hadipour 2016). The use of bio-fertilizers improves the yield and yield components of wheat by enhancing soil physical characteristics, its fertility, and nutrient availability for plant uptake under normal and stressful conditions (Ahmed et al. 2011; Mahdavi Khorami et al. 2020).

Under drought stress conditions, stomatal closure diminishes the amount of photosynthesis and that of the material required to fill the seeds for less water consumption, and subsequently decreases the mean weight of each seed (Chai et al. 2016). However, the application of bio-fertilizer under drought stress conditions, by increasing the content of photosynthetic pigments (Mahdavi Khorami et al. 2020), water available for plant growth in the soil (Ebrahimian et al. 2019) and also improving the physico-chemical properties of the soil (Askari et al. 2019) leads to increased growth, yield, and yield components. As a result of Kamali and Mehraban (2020), grain yield of sorghum decreased under severe drought stress conditions but co-inoculation with bio-fertilizers under severe drought stress conditions increased grain yield and yield components, compared to non-application of these bio-fertilizers. Thus, bio-fertlizer can be recommended for profitable sorghum production under drought stress conditions.

Morpho-Physiological Responses to Si-fertilizer Under Different Irrigation Regimes

Silicon modulates many plant biological functions, especially under stress conditions such as drought and salt stresses (Javaid et al. 2019). However, the mechanisms of the Si-mediated improvement in water-deficit stress tolerance have not been analyzed in detail. The present study evaluated the effect of Si nutrition on minimizing water-deficit stress and improving wheat growth by altering the distribution and compartmentalization of mineral ions within plant tissues under drought stress conditions. The results represented that Si fertilizer application, especially 1.5 mM, enhanced the plant height, 1000-grain weight, and grain number and yield. Additionally, it increased the growth and yield parameters, and moderated the harmful effects of water-deficit on wheat. The results are consistent with those of many studies regarding the effect of the fertilizer on raising plant biomass (Wang et al. 2015; Asgari et al. 2018). According to Ali et al. (2019), the Si fertilizer improved the plant biomass under stress, which may be related to plant nutritional status under stress conditions. Javaid et al. (2019) suggested that the inhibition of Na^+ uptake by Si fertilizer maintains nutrient homeostasis and improves physiological parameters for contributing to wheat growth enhancement under salt stress. However, the well-documented benefits of Si fertilizer are generally in the form of an increase in plant tolerance to biotic and abiotic stresses (Farooq and Dietz 2015). An enhanced silica deposits in root and leaf tissues have been long known to improve structural characteristics, and consequently physical defense against pathogen and herbivore attacks although the mechanisms underlying the beneficial effects of Si have mostly remained unknown (Javaid et al. 2019).

Silicon is considered as a beneficial element for plants, the positive effects of which on promoting crop growth were reported long ago (Huang et al. 2019). The exogenous application of Si fertilizer results in rising rice growth index, and root and shoot dry weight (Huang et al. 2018). Similarly, under the conditions of the present study, the exogenous application of Si fertilizer significantly increased the plant height and grain yield of wheat, the results of which are in line with the previous research on corn (Huang et al. 2018), which may be attributed to an enhancement in the absorption and utilization of nutrients by Si (Yong-chun et al. 2015). Silicon can precipitate (silicification) in plant cell walls and form specific cells (e.g., siliceous cells), and consequently improve plant disease and stress resistance, and nutrient absorption (Ma et al. 2015). Silicon fertilizers effectively alleviate the adverse effect of wheat under water-deficit stress mainly through promoting wheat growth and raising biomass in the various parts of wheat, increasing photosynthetic chlorophyll content, and reducing oxidative damage (MDA, H_2O_2) (Huang et al. 2019). Rezakhani et al. (2019) pointed out that roots provide essential functions such as the uptake of water and plant growth nutrients. An enhancement in root surface area introduces extra exposed sites for absorbing diffusible ions. Furthermore, Si fertilizer improves plant shoot biomass, along with root growth (morphological traits such as diameter, area, volume, dry root bulk, and total and main root lengths) (Etesami 2018). The motivation of root growth by Si may be related to the enhanced root elongation caused by raising cell wall extensibility in the growth region (Etesami and Jeong 2018).

Studies have shown that the Si application under stress conditions, despite the reduction of hydrogen peroxide production, increases the amount of chlorophyll, carboxylate biophosphate activity, and photosynthesis. On the other hand, Si, being located in the apoplasm of the outer walls of epidermal cells, in addition to leaf strength, produces uneven bumps on two leaf surfaces, which, while delaying leaf death, increases chlorophyll content and reduces stomatal transpiration (Merwad et al. 2018). Researchers also believe that one of the reasons for the effect of growth-promoting bacteria on leaf chlorophyll content under stress conditions, is to increase the plant's access to nitrogen through its stabilization. In these case, chlorophyll content is one of the important physiological parameters under stress conditions in wheat and other plants (Mohammadparst et al. 2019). The results of this study and other studies on wheat, the combined application of bio-fertilizers and Si by increasing biochemical properties such as photosynthetic pigments, activity of antioxidant enzymes, etc. can increase wheat grain yield under water restriction conditions (Ahmadi Nouraldinvand et al. 2021).

Results indicated that the effects of Si and Nitro-Kara bio-fertilizer were not consistent and these varied during the first year and the second year of the study. This difference can be related to climatic parameters. According to climatic data (Fig. 1), the favorable temperature as well as the amount of rainfall during the first year (leading to an increase in high soil moisture reserves) in this year, has led to an increase in the efficiency of bio-fertilizers and Si application. On the other hand, the unfavorable climatic parameters on the second year have reduced the positive effects and efficiency of these fertilizers. In this regard, Shahverdi et al. (2018) reported that the fertilizer efficiency is strongly dependent on climatic parameters such as temperature and rainfall. In other words, fertilizer efficiency will increase in favorable climatic conditions.

CONCLUSION

Water-deficit stress is considered as the main abiotic stress severely reduces crop yield, especially wheat. Soil amendments such as bio- and Si fertilizer are helpful to enhance soil fertility and maintain soil moisture. Seed inoculation with Nitro-Kara bio-fertilizer significantly improved the morphological and yield (plant height, and tiller and panicle number), and physiological (chlorophyll index and fluorescence) and yield traits, along with its contributing traits (grain number, 1000-grain weight, and grain yield) of wheat both under non-stress and water-deficit conditions. The difference in the effects of bio-fertilizer during the two years is an essential point so that the bio-fertilizer was more efficient in the second year due to favorable climatic and soil conditions. The physiological reactions such as the activity of antioxidant enzymes and other metabolites should be assessed after applying silicon and bio-fertilizer under water-deficit stress conditions to continue the present study.

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