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EFFECTS OF IRON AND PGPR ON ANTIOXIDANT STATUS AND SOME PHYSIOLOGICAL TRAITS OF TRITICALE UNDER DIFFERENT IRRIGATION LEVELS

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Abstract

A factorial experiment was conducted to study the effects of iron and plant growth promoting rhizobacteria (PGPR) on antioxidant status and some physiological traits of triticale under different irrigation levels. Experimental factors were included irrigation in three levels [(i) normal irrigation (I_0) as control; (ii) moderate water limitation (I_1) and (iii) severe water limitation (I_2)]. Three PGPR levels [(i) no PGPR (P_0), (ii) *Psedomunas putida* (P_1), (iii) *Azospirillum lipoferum* (P_2)] and three nano iron oxide levels [(i) without nano iron oxide (F_0) as control, (ii) application of 0.3 (F_1) and (iii) 0.6 (F_2) g/l)]. Results showed that water limitation decreased chlorophyll content, relative water content and grain yield of triticale. Whereas, electrical conductivity, proline content and the activity of catalase (CAT), peroxidase (POD), polyphenol oxidase (PPO) enzymes increased. However, inoculation of plants with PGPR and iron application improved these traits under water limitation condition and normal irrigation. Based on the results, it was concluded that the application of PGPR and iron can be a proper tool for increasing triticale yield under water limitation condition.

Introduction

Triticale is a man-made crop, being a hybrid by cross-fertilization of wheat (*Triticum* spp.) and rye (*Secale* spp.). Triticale, which is an interesting crop in the areas where environmental conditions limit the productivity of other cereals (Giunta *et al*. 1999).

Drought is prominent among the most important ecological factors that impact crop growth and productivity (Bagci *et al*. 2007). Many physiological processes in plants are impaired by drought stress, including photosynthesis, enzyme activity, membrane stability and ultimately growth (Valentovic *et al*. 2006). Growth reduction under drought stress has been studied in barley (Samarah 2005) and wheat (Rampino *et al*. 2006). De Ronde *et al*. (2004) demonstrated that proline accumulation in plants can enhance tolerance to abiotic stresses. Drought also induces free radicals affecting antioxidant defenses and reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals resulting in oxidative stress. At high concentrations ROS can cause damage to various levels of organization, like initiate lipid peroxidation, membrane deterioration and degrade proteins, lipids and nucleic acids in plants (Nair *et al*. 2008).

The role of microorganisms in plant growth, nutrient management and biocontrol activity is well established. These beneficial microorganisms colonize in the rhizosphere of plants and promote growth of the plants through various direct and indirect mechanisms (Grover *et al*. 2011). Sandhya *et al*. (2010) reported that *Pseudomonas* inoculated maize plants showed increased antioxidant enzymes activity under drought stress. Ghorbanpour *et al*. (2013) reported that inoculation of *Hyoscyamus niger* plants with *Pseudomonas* stimulated the activities of antioxidant

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enzymes and increased proline accumulation. Nadeem *et al.* (2006) reported that inoculation of maize with PGPR increased the chlorophyll content.

Mineral nutrients are essential for plant growth through their fundamental roles in plant metabolism (Bagci *et al.* 2007). Iron is the first rare element recognized as necessary for plants and animals, playing an important role in biochemical and physiological processes. It works as a key enzyme co-factor that plays a role in plant hormone synthesis and is engaged in many electron transportation reactions (Kerkeb and Connolly 2006). Iron is critical for chlorophyll formation and photosynthesis and is important in the enzyme systems and respiration of plants.

A better understanding of triticale antioxidant status and physiological responses may help the programs with the objectives to improve the grain yield under water limitation. Therefore, the aim of this study was to evaluate the effects of PGPR and iron on the physiological responses of triticale under water limitation.

Materials and Methods

A factorial experiment based on RCBD with three replications was conducted under greenhouse condition in 2015. Experimental factors included irrigation in three levels [(i) normal irrigation (I_0) as control, (ii) moderate water limitation (I_1)-irrigation with holding at 50% of heading stage and (iii) severe water limitation (I_2) - irrigation with holding at 50% of booting stage]. Three PGPR levels [(i) no PGPR (P_0), (ii) *Psedomunas putida* strain 9 (P_1) and (iii) *Azospirillum lipoferum* strain OF (P_2)] and three nano iron oxide levels [(i) (without nano iron oxide (F_0) as control, (ii) application of 0.3 (F_1) and (iii) 0.6 (F_2) g/l)]. The soil was silty loam, with pH about 6.9. Air temperature ranged from 23 - 26°C during the day and 18 - 20°C during the night. Humidity ranged from 65 ±7 %. The triticale cultivar 'Joanilo' was used in the present experiment. Optimal density of cultivar 'Joanilo' is 400 seeds m^{-2}, so fifty seeds of triticale were sown in each prepared pot and filled approximately with 22 kg of above mentioned soil. The experiment was carried out on the soil with a texture of silty loam, with pH about 7.8, total organic C- 0.62 g/kg soil, Fe - 8.6 mg/kg. The pots were immediately irrigated after planting. *Psedomunas putida* strain 9 and *Azospirillum lipoferum* strain OF were isolated from the rhizospheres of wheat by Research Institute of Soil and Water, Tehran, Iran. The strains and cell densities of microorganisms used as PGPR in this experiment were 1×10^7 cfu. Foliar application with nano iron oxide was done in two stages of growth (3 - 4 leaf stage and before booting stage).

Samples were placed in aluminum foil and transported from the greenhouse on ice bath. CAT, POD and PPO activity was assayed according to Karo and Mishra (1976).

Relative water content was measured following the formula of Che Lah *et al.* (2011).

Electrical conductivity of flag leaves was calculated following the standard method of Jodeh *et al.* (2015) at room temperature of 23 ± 1°C using an electrical-conductivity meter.

A portable chlorophyll meter (SPAD-502; Konica Minolta Sensing, Inc., Japan) was used to measure the leaf greenness of the triticale plants. For each plant, measurements were taken at three locations of each leaf, two on each side of the midrib of flag leaves, and then averaged. Proline was measured following the method of Bates *et al.* (1973).

In order to measure grain yield per plant, 10 plants of each pot randomly harvested and then the grains were collected. Analysis of variance and mean comparisons were performed using SAS ver 9.1 computer software packages. The main effects and interactions were tested using the least significant difference (LSD) test at the 0.05 probability level.

Results and Discussion

Analysis of variance showed a significant interaction effect between irrigation and PGPR on chlorophyll index, electrical conductivity and PPO activity (Table 1). Interaction of irrigation levels and nano iron oxide significantly affected all traits (except PPO activity and grain yield) (Table 1). Also all traits (except RWC) were affected by the interaction of PGPR and nano iron oxide (Table 1). There were significant interactions between irrigation, PGPR and nano iron oxide on electrical conductivity, proline, POD activity and grain yield. The activity of CAT, POD and PPO enzymes increased as water limitation increased. The highest content of CAT (5.5, 6.06 and 6.53 OD µg protein/min) and POD (4.95, 5.41 and 6.01 OD µg protein/min) were observed in severe water limitation, application of *Azospirillum* and 0.6 g/l nano iron oxide, respectively (Table 1). The lowest of CAT (3.83, 2.85 and 3.4 OD µg protein/min) and POD activity (3.87, 2.48 and 3.03 OD µg protein/min) were obtained at I_0, P_0 and F_0 (Table 1). The maximum content of PPO (3.7, 4.27 and 4.39 OD µg protein/min) reached in severe water limitation, application of *Psedomunas* and 0.6 g/l nano iron oxide, respectively (Table 1). The lowest of PPO (3.07, 1.97

Table 1. Means comparison and variance analysis effects of PGPR and nano iron oxide on chlorophyll index, electrical conductivity, proline content, activity of CAT, POD and PPO enzymes, relative water content and grain yield of triticale under water limitation.

	Chloro-phyll index	Electrical conductivity (µS/cm)	Proline (µg/g FW)	CAT (OD µg protein/ min)	POD (OD µg protein/ min)	PPO (OD µg protein/ min)	Relative water content (%)	Grain yield (g/plant)
Irrigation levels								
I_0 = Normal irrigation	53.5 a	48.95 c	6.37 c	3.83 c	3.87 c	3.07 c	82.33 a	1.77 a
I_1= Moderate water limitation	50.5 b	53.01 b	6.64 b	4.75 b	4.21 b	3.38 b	75.45 b	1.58 b
I_2= Severe water limitation	46 c	58.71 a	6.96 a	5.5 a	4.95 a	3.7 a	71.03 c	1.44 c
Nano iron oxide (g/l)								
F_0 = Without iron as control	45.3 c	59 a	6.43 b	3.4 c	3.03 c	2.26 c	71.13 c	1.32 c
F_1 = 0.3	49.4 b	52.14 b	6.44 b	4.15 b	3.99 b	3.5 b	74.71 b	1.47 b
F_2 = 0.6	55.4 a	49.54 c	7.1 a	6.53 a	6.01 a	4.39 a	82.96 a	2.02 a
PGPR								
P_0 = No inocula-tion as control	45.8 c	58.66 a	6.04 c	2.85 c	2.48 c	1.97 c	72.29 c	1.2 c
P_1 = *Psedomunas*	51 b	52.19 b	7.14 a	5.18 b	5.15 b	4.27 a	79.68 a	1.69 b
P_2 = *Azospirillum*	53.2 a	49.83 c	6.79 b	6.06 a	5.41 a	3.91 b	76.84 b	1.91 a
I * F	*	**	**	**	**	ns	**	ns
I * P	**	**	ns	ns	**	ns	ns	ns
F * P	*	**	**	**	**	**	ns	**
I * F * P	ns	**	**	ns	**	ns	ns	**
CV	2.8	2.76	1.92	9.07	9.07	8.52	3.67	6.01

The same letters in each column show non-significant difference at $p \leq 0.05$ by LSD test. (ns) and (*, **) show no significant and significant differences at 0.05, 0.01 probability level, respectively. CV: Coefficient of variation; CAT: Catalase; POD: Peroxidase and PPO: Polyphenol oxidase.

and 2.26 OD µg protein/min) were obtained at I_0, P_0 and F_0 (Table 1). Inoculation with the PGPR under water limitation, significantly increased CAT, POD and PPO enzymes activity of triticale and also the antioxidant enzymes activity increased when nano iron oxide was applied. Interaction effect between nano iron oxide and PGPR showed that the highest content of CAT (8.03 OD µg protein/min) and PPO (5.0 OD µg protein/min) were obtained in F_2P_2 and the lowest of the mentioned traits (2.12 and 1.19 OD µg protein/min, respectively) were observed in F_0P_0 (Table 2). The interaction effect between water limitation and nano iron oxide showed that the highest content of CAT (7.7 OD µg protein/min) was found in I_2F_2. The lowest of it (2.84 OD µg protein/min) was obtained in I_0F_0 (Table 3). Interaction effect between water limitation × PGPR × nano iron oxide showed that the highest content of POD (8.52 OD µg protein/min) was observed in $I_2P_1F_2$ (Table 4). While, the lowest of it (1.31 OD µg protein/min) was obtained at $I_0P_0F_0$ (Table 4). Exposure of plants to unfavorable environmental conditions such as drought, salinity, temperature extremes and nutrient deficiency can increase the production of ROS (e.g., O2$^{\cdot -}$, H_2O_2, $^{\cdot}OH$ and $^1O_2$) (Singh *et al.* 2008). ROS are highly reactive molecules and can damage cell structures such as carbohydrates, nucleic acids, lipids, and proteins and alter their functions. In order to overcome these effects, plants develop antioxidant defense systems comprising both enzymatic and non-enzymatic components that serve to prevent ROS accumulation and alleviate the oxidative damage occurring during drought stress (Miller *et al.* 2010).

Table 2. Effects of nano iron oxide × PGPR on CAT and PPO activity.

Treatments	CAT (OD µg protein/min)			PPO (OD µg protein/min)		
	F_0	F_1	F_2	F_0	F_1	F_2
P_0	2.12d	2.33d	4.1c	1.19e	1.4e	3.32c
P_1	3.95c	4.12c	7.46a	3.15c	4.64ab	4.85a
P_2	4.14c	5.99b	8.03a	2.44d	4.45b	5.0a
$LSD_{0.05}$		0.84			0.39	

Means with similar letters are not significantly different at $p \leq 0.05$ by LSD test.

Table 3. Effects of irrigation × nano iron oxide on CAT activity.

Treatments	CAT (OD µg protein/min)		
	F_0	F_1	F_2
I_0	2.84d	3.56cd	5.09b
I_1	3.31cd	4.15bcd	6.8a
I_2	4.06bcd	4.74bc	7.7a
$LSD_{0.05}$		1.48	

Means with similar letters are not significantly different at $p \leq 0.05$ by LSD test.

Enzymatic components include CAT, POD, SOD and PPO (Kaushal and Wani 2016). An increase in POD activity was also observed by different authors during drought and salt stress (Wang *et al.* 2012). Wang *et al.* (2012) found that application of PGPR strains improved plant enzyme activity, which alleviates the oxidative damage induced by drought and salinity. Iron role in the activity of some enzymes such as catalase, peroxidase and cytochrome oxidase has been shown (Blakrishman 2000). Iron as a cofactor involved in the structure of many antioxidant enzymes and results indicate that lack of micro-nutrients elements, antioxidant enzyme activity decreased and therefore the sensitivity of plants to environmental stresses will increase (Kaviani *et al*. 2016).

Table 4. Effects of irrigation × PGPR × nano iron oxide on POD activity.

Treatments		POD (OD µg protein/min)		
		F_0	F_1	F_2
I_0	P_0	1.31 i	1.39 i	3.29 jk
	P_1	1.63 i	1.94 i	3.7 ijk
	P_2	1.5 i	1.73 i	3.85 hij
I_1	P_0	4.46 fgh	5.18 de	5.99 bc
	P_1	5.52 cd	4.97 def	6.21 b
	P_2	4.25 ghi	5.23 de	6.47 b
I_2	P_0	3.18 k	4.92 def	6.06 ghi
	P_1	4.14 ghi	5.03 def	8.52 a
	P_2	4.66 efg	5.49 cd	8.46 a
$LSD_{0.05}$		0.64		

Means with similar letters are not significantly different at $p \leq 0.05$ by LSD test.

The proline content significantly increased under water limitation condition. Inoculation with PGPR under water limitation increased proline of triticale. In addition, the proline content significantly increased when nano iron oxide was applied. Results showed that the highest content of proline (7.75 µg/g FW) was obtained in severe water limitation, application of *Psedomunas* and 0.6 g/l nano iron oxide (Table 5). The minimum of proline (5.30 µg/g FW) was obtained in $I_0P_0F_0$ (Table 5). There was an increase of 9.46% in content of proline in the $I_2P_1F_2$ in comparison with $I_2P_0F_0$ (Table 5). Proline has a function of osmotic adjustment in plants, but it also protects enzymes and membranes against oxidative stress (Agarwal and Pandey 2004). Indeed, proline has been demonstrated to confer drought stress tolerance to wheat plants by increasing the antioxidant system rather than increasing osmotic adjustment (Szabados and Savoure 2009). PGPRs consortia alleviated drought stress in rice plants by accumulation of proline in rice plants grown under drought there by improving the plant growth (Gusain *et al*. 2015). The electrical conductivity significantly increased under water limitation condition. Inoculation with PGPR under water limitation decreased electrical conductivity of triticale. In addition, the electrical conductivity content significantly decreased when nano iron oxide was applied. The highest content of electrical conductivity (76.09 µS/cm) was obtained in $I_2P_0F_0$ (Table 5) and the lowest of it (45.06 µS/cm) was obtained in $I_0P_2F_2$ (Table 5). There was a decrease of 26.14% in content of electrical conductivity in the $I_2P_2F_2$ in comparison with $I_2P_0F_0$ (Table 5). Cell membrane is one of the first targets of plant stresses and the ability of plants to maintain membrane integrity under drought is what determines tolerance towards drought. Under water deficit, cell membrane subjects to changes such as penetrability and decrease in sustainability (Blokina *et al*. 2003). Alexiva *et al*. (2001) reported that drought and ultra violet stress in pea and wheat plant through amplifying of reactive oxygen species production increased electrolyte leakage. Inoculation with PGPR decreased electrolyte leakage compared to un-inoculated seedlings under drought stress (Sandhya *et al*. 2010). Bacteria mediated changes in the elasticity of the root cell membranes is one of the first steps towards enhanced tolerance to water deficiency (Dimkpa *et al*. 2009). PGPR improves the stability of plant cell membranes by activating the antioxidant defense system, enhancing drought tolerance in plants (Gusain *et al*. 2015).

Table 5. Effects of irrigation × PGPR × nano iron oxide on proline and electrical conductivity of triticale.

Treatments		Proline (µg/g FW)			Electrical conductivity (µS/cm)		
		F_0	F_1	F_2	F_0	F_1	F_2
I_0	P_0	5.3 m	5.38 m	6.47 hi	48.9 jkl	46.9 lmn	49.7 ijk
	P_1	6.6 gh	6.84 f	6.78 fg	49.17 ijkl	47.9 klm	45.9 mn
	P_2	6.35 ij	6.09 k	6.21 jk	47.3 klmn	45.2 n	45.06 n
I_1	P_0	5.74 i	5.69 i	6.68 fgh	66.7 b	54.03 fg	52.62 gh
	P_1	6.11 k	7.06 de	6.88 ef	58.7 d	51.4 hi	51.07 hij
	P_2	6.77 fg	6.25 jk	7.16 cd	50.7 hij	50.4 hij	47.2 lmn
I_2	P_0	7.08 de	7.1 d	7.22 cd	76.09 a	62.8 c	58.76 d
	P_1	7.14 cd	7.26 bcd	7.75 a	64.7 bc	52.46 gh	52.3 gh
	P_2	7.35 bc	7.12 d	7.45 b	58.36 de	55.7 f	56.2 ef
$LSD_{0.05}$			0.21			2.42	

Means with similar letters are not significantly different at $p \leq 0.05$ by LSD test.

Table 6. Effects of irrigation × nano iron oxide on RWC.

Treatments	Relative water content (RWC%)		
	F_0	F_1	F_2
I_0	80.13 b	80.63 b	86.78 a
I_1	69.28 d	79.59 b	81.98 b
I_2	63.5 e	69.46 d	75.1 c
$LSD_{0.05}$		3.92	

Means with similar letters are not significantly different at $p \leq 0.05$ by LSD test.

Zago and Oteiza (2001) stated that iron element by increasing the activity of antioxidant systems in plants decreased reactive oxygen species injuries and plays an important role in membrane stability. Interaction effect between nano iron oxide and water limitation showed that the highest content of relative water content (86.78%) was obtained in normal irrigation and 0.6 g/l nano iron oxide (Table 6). The minimum of its value (63.5%) was obtained in I_2F_0 (Table 6). There was an increase of 18.27% in content of relative water content in the I_2F_2 in comparison with I_2F_0 (Table 6). The decrease in leaf relative water content could be related to low water availability under stress conditions or to root systems, which are not able to compensate for water lost by transpiration through a reduction of the absorbing surface (Gadallah 2000). Sadeghipour and Aghaei (2012) showed that drought stress conditions significantly reduced the leaf relative water content. Shaharoona *et al.* (2006) reported that the inoculation treatment with PGPR isolates increased RWC from 5 - 16% under normal and 21.7 - 28.4% under stress conditions as compared to the un-inoculated control. The water limitation, PGPR and nano iron oxide significantly affected the chlorophyll index. Interaction effect between nano iron oxide and water limitation showed that the highest chlorophyll index (58.3) was obtained in I_0F_2 (Table 7). The lowest of it (41.4) was obtained in I_2F_0 (Table 7). There was an increase of 23.18% in chlorophyll index in the I_2F_2 in comparison with I_2F_0 (Table 7). Means comparison the effects of nano iron oxide and PGPR showed that the highest chlorophyll (58.2) was obtained in P_2F_2 (Table 8). The lowest of it (40.2) was obtained in P_0F_0 (Table 8). There was an increase of 26.80% in chlorophyll index in the P_2F_2 in comparison with P_2F_0 and increase of 13.23% in SPAD in the P_2F_2 in comparison with P_0F_2 (Table 8). Also, interaction effect between water limitation and PGPR showed that the highest chlorophyll index (56.0) was obtained in I_0P_2 (Table 9). The lowest of it (40.5) was obtained in I_2P_0 (Table 9). There was an increase of 15.30% in chlorophyll index in the I_2F_2 in comparison with I_2F_0 and decrease 16.60% in SPAD in the I_2P_2 in comparison with I_0P_2 (Table 9). Water limitation caused the reduction in chlorophyll index, while application of PGPR and nano iron oxide increased this trait values. Drought stress leads to increase reactive oxygen species production in plants resulted in decreasing of chlorophyll index, indicating the extent of the oxidative damages. This decrease may be also due to inhibition of chlorophyll biosynthesis pathway (Lalinia *et al.* 2012). Also, the reduction in chlorophyll content under drought stress has been considered as a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation (Oraki *et al.* 2012).

Table 7. Effects of Irrigation × nano iron oxide on chlorophyll index.

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Treatments	Chlorophyll index		
	F_0	F_1	F_2
I_0	49.5b	52.8b	58.3a
I_1	45.7c	49.7b	56.8a
I_2	41.4d	44.9c	51b
$LSD_{0.05}$		3.5	

Means with similar letters are not significantly different at $p \leq 0.05$ by LSD test.

Table 8. Effects of nano iron oxide × PGPR on chlorophyll index.

Treatments	Chlorophyll content		
	F_0	F_1	F_2
P_0	40.2d	49.4bc	51.4b
P_1	46.3c	50.1b	56.6a
P_2	45.9c	52.2b	58.2a
$LSD_{0.05}$		3.48	

Means with similar letters are not significantly different at $p \leq 0.05$ by LSD test.

Several studies reported that chlorophyll content is higher in plants treated with biofertilizer (Belimov *et al.* 2009). It is known that a low Fe supply negatively affects the chlorophyll content and other components of chloroplasts, which reduces growth capacity (De La Guardia and

Alcantara 2002). Iron increased SPAD values. This is because iron functions as a component of proteins in significant cellular events such as respiration and cell division; moreover, it has a role in the reduction steps of important biological events, such as transpiration and photosynthesis, and also in chlorophyll biosynthesis (Zocchi *et al.* 2007).

Grain yield decreased as a result of moderate and severe water limitaion. Grain yield increased as result of application of PGPR and nano iron oxide under normal irrigation and water limitation. Means comparison showed that maximum of grain yield (2.341 g per plant) was observed in normal irrigation and application of *Azospirillum* and 0.6 g/l nano iron oxide (Table 10). The lowest of yield (0.823 g per plant) was obtained in $I_2P_0F_0$ (Table 10).

Table 9. Effects of irrigation × PGPR on chlorophyll index of triticale.

Treatments	Chlorophyll index		
	P_0	P_1	P_2
I_0	50.3bc	54.3 ab	56.0 a
I_1	50bc	51 bc	53.8 ab
I_2	40.5d	47.7 c	46.7 c
$LSD_{0.05}$		4.44	

Means with similar letters are not significantly different at $p \leq 0.05$ by LSD test.

Table 10. Effects of PGPR × nano iron oxide on grain yield of triticale under water limitation.

Treatments		Grain yield (g per plant)		
		F_0	F_1	F_2
I_0	P_0	1.892 def	2.045 bcd	2.115 bc
	P_1	2.04 bcd	2.05 bcd	2.157 b
	P_2	2.124 bc	2.08 bc	2.341 a
I_1	P_0	0.869 j	0.911 j	1.857 ef
	P_1	1.235 i	1.26 i	1.74 f
	P_2	1.49 gh	1.87 ef	1.997 cde
I_2	P_0	0.823 j	0.894 j	1.57 g
	P_1	0.914 j	0.927 j	1.457 gh
	P_2	1.35 hi	1.5 gh	1.82 f
$LSD_{0.05}$		0.158		

Means with similar letters are not significantly different at $p \leq 0.05$ by LSD test.

According to the report of Babaeian *et al.* (2011) drought stress by reducing plant growth and damage to flowering and grain filling, reduces the grain yield. Significant increases in growth and yield of agronomical important crops in response to inoculation with PGPR have been reported (Sandhya *et al.* 2010). Rengel and Romheld (2000) reported an increase in wheat grain yields with Fe application. Ghafari and Razmjoo (2013) showed that the foliar application of nano-iron oxide increased antioxidant enzymes activities, chlorophylls content, yield and yield components of wheat.

The results showed that water limitation reduced yield, chlorophyll index and relative water content of the plants. But the activity of CAT, POD and PPO enzymes, proline content and electrical conductivity increased. Application of PGPR and nano iron oxide also increased grain yield, chlorophyll index, activity of antioxidant enzymes, proline content and relative water content under water limitation conditions, while electrical conductivity decreased. The present results suggested that plants use defensive mechanisms, such as synthesis of antioxidant enzymes and proline to reduce the effects of stress. It seems that the application of PGPR and nano iron oxide can be recommended for profitable yield of triticale under water limitation condition.

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