

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

RANA GHASEMI, RAOUF SEYED SHARIFI\* AND YOUNES KHEIRIZADEH AROUH

*Department of Agronomy and Plant Breeding, Faculty of Agriculture and Natural Resources,  
University of Mohaghegh Ardabili, Ardabil, Iran*

*Keywords:* Catalase, Electrical conductivity, Peroxidase, PGPR, Proline, Triticale, Water deficit

### 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) *Pseudomonas 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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\*Author for correspondence: <Raouf\_ssharifi@yahoo.com>.

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) *Pseudomonas 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<sup>-2</sup>, 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. *Pseudomonas 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<sup>7</sup> 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

**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-<br>phyll<br>index | Electrical<br>conductivity<br>( $\mu$ S/cm) | Proline<br>( $\mu$ g/g<br>FW) | CAT (OD<br>$\mu$ g protein/<br>min) | POD<br>(OD $\mu$ g<br>protein/<br>min) | PPO<br>(OD $\mu$ g<br>protein/<br>min) | Relative<br>water<br>content<br>(%) | Grain<br>yield<br>(g/plant) |
|-------------------------------------------------|---------------------------|---------------------------------------------|-------------------------------|-------------------------------------|----------------------------------------|----------------------------------------|-------------------------------------|-----------------------------|
| <b>Irrigation levels</b>                        |                           |                                             |                               |                                     |                                        |                                        |                                     |                             |
| I <sub>0</sub> = Normal<br>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 <sub>1</sub> = Moderate<br>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 <sub>2</sub> = Severe water<br>limitation     | 46 c                      | 58.71 a                                     | 6.96 a                        | 5.5 a                               | 4.95 a                                 | 3.7 a                                  | 71.03 c                             | 1.44 c                      |
| <b>Nano iron oxide (g/l)</b>                    |                           |                                             |                               |                                     |                                        |                                        |                                     |                             |
| F <sub>0</sub> = Without iron<br>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 <sub>1</sub> = 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 <sub>2</sub> = 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                      |
| <b>PGPR</b>                                     |                           |                                             |                               |                                     |                                        |                                        |                                     |                             |
| P <sub>0</sub> = No inocula-<br>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 <sub>1</sub> = <i>Pseudomonas</i>             | 51 b                      | 52.19 b                                     | 7.14 a                        | 5.18 b                              | 5.15 b                                 | 4.27 a                                 | 79.68 a                             | 1.69 b                      |
| P <sub>2</sub> = <i>Azospirillum</i>            | 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.

PPO enzymes increased as water limitation increased. The highest content of CAT (5.5, 6.06 and 6.53 OD  $\mu$ g protein/min) and POD (4.95, 5.41 and 6.01 OD  $\mu$ g 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  $\mu$ g protein/min) and POD activity (3.87, 2.48 and 3.03 OD  $\mu$ g protein/min) were obtained at I<sub>0</sub>, P<sub>0</sub> and F<sub>0</sub> (Table 1). The maximum content of PPO (3.7, 4.27 and 4.39 OD  $\mu$ g protein/min) reached in severe water limitation, application of *Pseudomonas* and 0.6 g/l nano iron oxide, respectively (Table 1). The lowest of PPO (3.07, 1.97

and 2.26 OD  $\mu\text{g protein/min}$ ) 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  $\mu\text{g protein/min}$ ) and PPO (5.0 OD  $\mu\text{g protein/min}$ ) were obtained in  $F_2P_2$  and the lowest of the mentioned traits (2.12 and 1.19 OD  $\mu\text{g protein/min}$ , 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  $\mu\text{g protein/min}$ ) was found in  $I_2F_2$ . The lowest of it (2.84 OD  $\mu\text{g protein/min}$ ) was obtained in  $I_0F_0$  (Table 3). Interaction effect between water limitation  $\times$  PGPR  $\times$  nano iron oxide showed that the highest content of POD (8.52 OD  $\mu\text{g protein/min}$ ) was observed in  $I_2P_1F_2$  (Table 4). While, the lowest of it (1.31 OD  $\mu\text{g protein/min}$ ) 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.,  $O_2^{\cdot-}$ ,  $H_2O_2$ ,  $\cdot OH$  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  $\times$  PGPR on CAT and PPO activity.**

| Treatments          | CAT (OD $\mu\text{g protein/min}$ ) |       |       | PPO (OD $\mu\text{g protein/min}$ ) |        |       |
|---------------------|-------------------------------------|-------|-------|-------------------------------------|--------|-------|
|                     | $F_0$                               | $F_1$ | $F_2$ | $F_0$                               | $F_1$  | $F_2$ |
| $P_0$               | 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 <sub>0.05</sub> | 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  $\times$  nano iron oxide on CAT activity.**

| Treatments          | CAT (OD $\mu\text{g protein/min}$ ) |         |       |
|---------------------|-------------------------------------|---------|-------|
|                     | $F_0$                               | $F_1$   | $F_2$ |
| $I_0$               | 2.84d                               | 3.56cd  | 5.09b |
| $I_1$               | 3.31cd                              | 4.15bcd | 6.8a  |
| $I_2$               | 4.06bcd                             | 4.74bc  | 7.7a  |
| LSD <sub>0.05</sub> | 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 (Blakrishnan 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 <sub>0</sub>          | F <sub>1</sub> | F <sub>2</sub> |
| I <sub>0</sub>      | P <sub>0</sub> | 1.31 i                  | 1.39 i         | 3.29 jk        |
|                     | P <sub>1</sub> | 1.63 i                  | 1.94 i         | 3.7 ijk        |
|                     | P <sub>2</sub> | 1.5 i                   | 1.73 i         | 3.85 hij       |
| I <sub>1</sub>      | P <sub>0</sub> | 4.46 fgh                | 5.18 de        | 5.99 bc        |
|                     | P <sub>1</sub> | 5.52 cd                 | 4.97 def       | 6.21 b         |
|                     | P <sub>2</sub> | 4.25 ghi                | 5.23 de        | 6.47 b         |
| I <sub>2</sub>      | P <sub>0</sub> | 3.18 k                  | 4.92 def       | 6.06 ghi       |
|                     | P <sub>1</sub> | 4.14 ghi                | 5.03 def       | 8.52 a         |
|                     | P <sub>2</sub> | 4.66 efg                | 5.49 cd        | 8.46 a         |
| LSD <sub>0.05</sub> |                | 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 *Pseudomonas* and 0.6 g/l nano iron oxide (Table 5). The minimum of proline (5.30 µg/g FW) was obtained in I<sub>0</sub>P<sub>0</sub>F<sub>0</sub> (Table 5). There was an increase of 9.46% in content of proline in the I<sub>2</sub>P<sub>1</sub>F<sub>2</sub> in comparison with I<sub>2</sub>P<sub>0</sub>F<sub>0</sub> (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<sub>2</sub>P<sub>0</sub>F<sub>0</sub> (Table 5) and the lowest of it (45.06 µS/cm) was obtained in I<sub>0</sub>P<sub>2</sub>F<sub>2</sub> (Table 5). There was a decrease of 26.14% in content of electrical conductivity in the I<sub>2</sub>P<sub>2</sub>F<sub>2</sub> in comparison with I<sub>2</sub>P<sub>0</sub>F<sub>0</sub> (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 <sub>0</sub>    | F <sub>1</sub> | F <sub>2</sub> | F <sub>0</sub>                  | F <sub>1</sub> | F <sub>2</sub> |           |
| I <sub>0</sub>      | P <sub>0</sub>    | 5.3 m          | 5.38 m         | 6.47 hi                         | 48.9 jkl       | 46.9 lmn       | 49.7 ijk  |
|                     | P <sub>1</sub>    | 6.6 gh         | 6.84 f         | 6.78 fg                         | 49.17 ijkl     | 47.9 klm       | 45.9 mn   |
|                     | P <sub>2</sub>    | 6.35 ij        | 6.09 k         | 6.21 jk                         | 47.3 klmn      | 45.2 n         | 45.06 n   |
| I <sub>1</sub>      | P <sub>0</sub>    | 5.74 i         | 5.69 i         | 6.68 fgh                        | 66.7 b         | 54.03 fg       | 52.62 gh  |
|                     | P <sub>1</sub>    | 6.11 k         | 7.06 de        | 6.88 ef                         | 58.7 d         | 51.4 hi        | 51.07 hij |
|                     | P <sub>2</sub>    | 6.77 fg        | 6.25 jk        | 7.16 cd                         | 50.7 hij       | 50.4 hij       | 47.2 lmn  |
| I <sub>2</sub>      | P <sub>0</sub>    | 7.08 de        | 7.1 d          | 7.22 cd                         | 76.09 a        | 62.8 c         | 58.76 d   |
|                     | P <sub>1</sub>    | 7.14 cd        | 7.26 bcd       | 7.75 a                          | 64.7 bc        | 52.46 gh       | 52.3 gh   |
|                     | P <sub>2</sub>    | 7.35 bc        | 7.12 d         | 7.45 b                          | 58.36 de       | 55.7 f         | 56.2 ef   |
| LSD <sub>0.05</sub> |                   | 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 <sub>0</sub>                | F <sub>1</sub> | F <sub>2</sub> |  |
| I <sub>0</sub>      | 80.13 b                       | 80.63 b        | 86.78 a        |  |
| I <sub>1</sub>      | 69.28 d                       | 79.59 b        | 81.98 b        |  |
| I <sub>2</sub>      | 63.5 e                        | 69.46 d        | 75.1 c         |  |
| LSD <sub>0.05</sub> |                               | 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<sub>2</sub>F<sub>0</sub> (Table 6). There was an increase of 18.27% in content of relative water content in the I<sub>2</sub>F<sub>2</sub> in comparison with I<sub>2</sub>F<sub>0</sub> (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. Shaharoon *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<sub>0</sub>F<sub>2</sub> (Table 7). The lowest of it (41.4) was obtained in I<sub>2</sub>F<sub>0</sub> (Table 7). There was an increase of 23.18% in chlorophyll index in the I<sub>2</sub>F<sub>2</sub> in comparison with I<sub>2</sub>F<sub>0</sub> (Table 7). Means comparison the effects of nano iron oxide and PGPR showed that the highest chlorophyll (58.2) was obtained in P<sub>2</sub>F<sub>2</sub> (Table 8). The lowest of it (40.2) was obtained in P<sub>0</sub>F<sub>0</sub> (Table 8). There was an increase of 26.80% in chlorophyll index in the P<sub>2</sub>F<sub>2</sub> in comparison with P<sub>2</sub>F<sub>0</sub> and increase of 13.23% in SPAD in the P<sub>2</sub>F<sub>2</sub> in comparison with P<sub>0</sub>F<sub>2</sub> (Table 8). Also, interaction effect between water limitation and PGPR showed that the highest chlorophyll index (56.0) was obtained in I<sub>0</sub>P<sub>2</sub> (Table 9). The lowest of it (40.5) was obtained in I<sub>2</sub>P<sub>0</sub> (Table 9). There was an increase of 15.30% in chlorophyll index in the I<sub>2</sub>F<sub>2</sub> in comparison with I<sub>2</sub>F<sub>0</sub> and decrease 16.60% in SPAD in the I<sub>2</sub>P<sub>2</sub> in comparison with I<sub>0</sub>P<sub>2</sub> (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.**

| Treatments          | Chlorophyll index |                |                |
|---------------------|-------------------|----------------|----------------|
|                     | F <sub>0</sub>    | F <sub>1</sub> | F <sub>2</sub> |
| I <sub>0</sub>      | 49.5b             | 52.8b          | 58.3a          |
| I <sub>1</sub>      | 45.7c             | 49.7b          | 56.8a          |
| I <sub>2</sub>      | 41.4d             | 44.9c          | 51b            |
| LSD <sub>0.05</sub> | 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 <sub>0</sub>      | F <sub>1</sub> | F <sub>2</sub> |
| P <sub>0</sub>      | 40.2d               | 49.4bc         | 51.4b          |
| P <sub>1</sub>      | 46.3c               | 50.1b          | 56.6a          |
| P <sub>2</sub>      | 45.9c               | 52.2b          | 58.2a          |
| LSD <sub>0.05</sub> | 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 limitation. 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<sub>2</sub>P<sub>0</sub>F<sub>0</sub> (Table 10).

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

| Treatments          | Chlorophyll index |                |                |
|---------------------|-------------------|----------------|----------------|
|                     | P <sub>0</sub>    | P <sub>1</sub> | P <sub>2</sub> |
| I <sub>0</sub>      | 50.3bc            | 54.3 ab        | 56.0 a         |
| I <sub>1</sub>      | 50bc              | 51 bc          | 53.8 ab        |
| I <sub>2</sub>      | 40.5d             | 47.7 c         | 46.7 c         |
| LSD <sub>0.05</sub> | 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 <sub>0</sub>            | F <sub>1</sub> | F <sub>2</sub> |           |
| I <sub>0</sub>      | P <sub>0</sub>            | 1.892 def      | 2.045 bcd      | 2.115 bc  |
|                     | P <sub>1</sub>            | 2.04 bcd       | 2.05 bcd       | 2.157 b   |
|                     | P <sub>2</sub>            | 2.124 bc       | 2.08 bc        | 2.341 a   |
| I <sub>1</sub>      | P <sub>0</sub>            | 0.869 j        | 0.911 j        | 1.857 ef  |
|                     | P <sub>1</sub>            | 1.235 i        | 1.26 i         | 1.74 f    |
|                     | P <sub>2</sub>            | 1.49 gh        | 1.87 ef        | 1.997 cde |
| I <sub>2</sub>      | P <sub>0</sub>            | 0.823 j        | 0.894 j        | 1.57 g    |
|                     | P <sub>1</sub>            | 0.914 j        | 0.927 j        | 1.457 gh  |
|                     | P <sub>2</sub>            | 1.35 hi        | 1.5 gh         | 1.82 f    |
| LSD <sub>0.05</sub> | 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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*(Manuscript received on 31 December, 2018; revised on 10 April, 2019)*