



Investigation of the growth bacteria and Nano iron on the chlorophyll and some nutrients triticale

Zohreh Sepehrzadegan¹

Omid Alizadeh²

Abstract

This study evaluated the effect of growth bacteria and nano iron fertilizer and ordinary iron fertilizer on the chlorophyll content and concentration of some nutrients. The experiment was conducted as 4×6 factorial in RCBD design with three replications in two years (2016-2017). Treatments included: use of plant growth-promoting bacteria in four levels (Non-inoculation, inoculation with *Azotobacter crococcoccus*, *Azospirillum methylpofrome*, and *Pseudomonas putida*) and nano iron fertilizer in five levels (0.0 %, 0.5 %, 1.0 %, 1.5 %, and 2.0 %) and ordinary iron fertilizer on two levels (2.0 % and 0.0 %). Based on the results, the application of biofertilizer and Fe fertilizer had significant effects on all traits at 1.0 % or 5.0 % level. The results of the comparison of the mean of treatments showed that the highest chlorophyll a (0.806) was obtained by *Azotobacter crococcoccus* + 0.5 % nano-Fe and the highest means of chlorophyll b and carotenoid with 0.275 mg g FW⁻¹ and 0.224 (mg g FW⁻¹) values, respectively, were observed by *Pseudomonas putida* + 0.5 % nano-Fe. The highest value of P (55.24), N (4.42) and Fe (84.43) were obtained by *Pseudomonas putida* + control Fe, *Azotobacter crococcoccus* + 1 % nano-Fe and *Pseudomonas putida* + 1 % nano-Fe, respectively.

Keywords: Elements. Inoculation. Nutrition. Pigments.

Introduction

Triticale, a man-made cereal from wheat and rye hybridization, is mainly used as animal feed. In recent years, there has been increasing interest in utilizing triticale for food production (ZHU, 2018). The need for sufficient and healthy food for an ever-increasing global population requires that biological methods be used along with chemical methods to increase the production of agricultural products. One of the biological methods for increasing the production of agricultural products is the use of terrestrial micro-organism. Bio-fertilizers are the best ecofriendly approach for plant and soil environments (RIAZ *et al.*, 2020). Inoculation of different plant species with growth bacteria stimulators has caused to stimulate the growth of the root or increase the formation of the secondary roots through secretion of the auxin hormones by these bacterias (ZHANG *et al.*, 2019) and following this, the effective level of root increased, which, in addition to increasing the absorption of water and nutrients by crops (KUMAR *et al.*, 2015; BASHAN *et al.*, 2017), increased the amount of pigment production (MARIUS *et al.*, 2005). *Azotobacter*, *Azospirillum*, and *Pseudomonas* are the most important bacteria in the growth-promoting plant (YOUSEFI *et al.*, 2017). Some microorganisms play a role in soil element cycles, such as phosphates, and dissolve low soluble organic and mineral (KHAVAZI; ASADI RAHMANI; MALAKOUTI, 2002). Many researchers pointed to the effect of these

1 Islamic Azad University, Firoozabad, Iran, Department of Agriculture. Ph.D. student. zohre.sepehrzadegan@gmail.com

2 Islamic Azad University, Shiraz, Iran. Department of Agriculture. Associate Professor. omid.alizade709@gmail.com

bacteria on the yield of the plant such as wheat (NAILI *et al.*, 2018), soybean (OMARA *et al.*, 2017), tomato and potato (LEDGER *et al.*, 2018). Also, it determined that the Plant Growth-Promoting Rhizobacteria (PGPR) are correlated with the physicochemical properties of the soil (FLORES-NÚÑEZ *et al.*, 2018). Iron is one of the essential elements in the production of agricultural products in the world, and plants need to be used continuously for optimal growth (KOBRAEE *et al.*, 2011). There is a lot of evidence that shows the shortage of low-energy elements, including iron, in limestone soils in arid and semi-arid regions, has limited growth, yield, and quality of the plant (EISA; TAHA; ABDALLA, 2011). Organic iron and mineral iron sources are used to correct the iron deficiency (BASHAN *et al.*, 2017). Nanotechnology is one of the new technologies that is now being developed and developed. One of the most important applications of nanotechnology in agriculture is plant nutrition (REZAEI; MOAVENI; MOZAFARI, 2015), at this order, the use of nano fertilizer leads to increased nutrient utilization, reducing soil toxicity, minimizing the negative effects of excessive fertilization, and reducing the frequency of application of fertilizers (NADERI; SHAHRAKI, 2013). There are many reports about nano Fe effects on the increase of physiological traits and yield of the plant, such as Yousefzadeh and Sabaghnia (2016), who mentioned that nano Fe fertilizer led to the increase of chlorophyll content and total flavonoid of *Dracocephalum moldavica*, and Afshar, Hadi, and Pirzad (2013) reported that nano iron led to increasing the yield and yield component of cowpea. Similar results have been reported on soybean (SHEYKHBAGLOU *et al.*, 2010), apple (AVESTAN; NASERI; NAJAFZADEH, 2018), barley (JANMOHAMMADI *et al.*, 2016). This study aimed to investigate the effect of growth bacteria, Nano iron fertilizer, and ordinary iron fertilizer treatments on the chlorophyll content and concentration of some nutrients in triticale.

Material and methods

This project was carried out in a farm located in Firoozabad, Fars province with a length of 52° and 33 ° East and a latitude of 28 ° 53' N and a mean altitude of 1,362 m from the sea level as a factorial in a randomized complete block design with three replications. Based on the results of soil analysis (TABLE 1), 150 kg ha⁻¹ nitrogen fertilizer from urea source and 75 kg ha⁻¹ potassium sulfate fertilizer was added to all experimental plots. Treatments included: use of growth-stimulating bacteria in four levels (Non-inoculation, inoculation with *Azotobacter crococcoccus*, *Azospirillum methylpofrome*, and *Pseudomonas putida*) and Nano iron Fertilizer in five levels (0.0 %, 0.5 %, 1.0 %, 1.5 %, and 2.0 %), and ordinary iron Fertilizer on Two Levels (2.0 % and 0.0 %). Nano iron Fertilizer used from a source of nano iron oxide with a purity of 99 and a particle diameter of less than 30 nm were used.

Table 1 – The properties of experiment soil included Organic carbon %, Phosphor, Potassium, Iron, Manganese, pH, Lime %.

Soil Patt ern	Organic Carbon %	P (ppm)	K (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	pH	Lime Percentage
Clay Loo mi	66 %	4.2	320	5.2	5.6	0.46	1	7.6	32.2

Source: Elaborated by the authors (2016).

On the farm, the plot size was 6 m × 1.6 m. Each plot consisted of 8 planting lines with a spacing of 20 cm apart and a total area of 9.6 square meters. For inoculation of seeds, seven grams of

inoculum per hectare of 107 live and active bacteria have been used. Fighting with pests and diseases was also done according to technical recommendations during the growth period. To estimate the amount of remobilization of materials from vegetative organs to the seed, from the time of filling to the physiological stage, 15 identical and uniform plants are marked in each plot, and from one week of filling the grain (step 65 from Zadoc's coding table) to the examination Physiological (stage 93 of the Zadoc syllabus) was performed every four days.

Chlorophyll (Chl) a and Chl b were calculated according to the following equations (IANCULOV *et al.* 2005):

$$\text{Chl a} = 12.21 \cdot A_{663} - 2.81 \cdot A_{646} \quad (1)$$

$$\text{Chl b} = 20.13 \cdot A_{646} - 5.03 \cdot A_{663} \quad (2)$$

In which:

A_{663} is the sample absorbance at 663 nm;

A_{646} is the sample absorbance at 646 nm.

Results and discussion

Based on the results, the application of biofertilizer on all traits was significant at 1.0 % statistical level. The interaction between different treatments on the amount of chlorophyll a, chlorophyll b, N, and Fe content in seeds was significant at 1.0 % level, and it was significant on carotenoid content and P content in seed at 5.0 % level, but on the amount of K was not significant in the seeds (TABLE 2).

Table 2 – Analysis of variances for Chlorophyll, Carotenoid, Potassium, Phosphor, Nitrogen, Iron traits in responses to treatments at 2017 year.

S.O. V	d.f	Chlorophyll a	Chlorophyll b	Carotenoids	K	P	N	Fe
Block	2	49.39ns	1.17ns	1701**	385078**	0.67ns	0.001 ^{ns}	0.3ns
Bacteria	3	9282**	2017**	5323**	26549**	332.4**	1.74**	1981**
Fe	5	2073**	3786**	139.6*	23.25ns	4.22*	0.05**	31.55**
Bacteria*Fe	15	125.8ns	34.72ns	12.37ns	6.5ns	1.05ns	0.02ns	2.39ns
Error	46	53.15	5.39	28.41	5592	1.42	0.005	1.62
CV%		13.2	11	24.3	15.7	22.5	17.1	16.4

ns, *, ** show no significant and significant at 5.0 % and 1.0 % statistically level, respectively.

Source: Elaborated by the authors (2017).

Chlorophyll a, b, and carotenoid

Regarding the amount of chlorophyll, a and b, and carotenoid, the results of the data analysis indicated that bacterial treatment was significant at a 1.0 % level. The effect of different levels of nano iron and ordinary iron fertilizer on the amount of chlorophyll a and b at 1.0 % level, and carotenoid content was significant at 5.0 % level, however the interaction was not significant (TABLE 2). The results of the mean comparison for bacterial treatments showed that the treatment with *Azospirillum* had the highest effect and control treatment had the least effect on the amount of chlorophyll a and b and carotenoid.

The highest means for Chlorophyll a, b and carotenoid with 0.806, 0.275, and 0.225 value, respectively, were obtained by *Azotobacter crococcoccus* + 0.5 % nano-Fe, *Pseudomonas putida* + 1.0 % nano-Fe, *Azospirillum methylpofrome* + control of Fe (TABLE 3), in this regard, it can be said that the bacterium provides more water and nutrients to the plant, which, as a result, increases the production of pigments and facilitates the transfer of water and photosynthetic materials in the plant (MARIUS *et al.*, 2005).

Table 3 – Mean comparison of Chlorophyll, Carotenoid, Potassium, Phosphor, Nitrogen, Iron traits in responses to treatments at 2017 year.

	Chl a		Chl b		Car		K		P		N		Fe	
	mg.g FW ⁻¹													
	ppm													
az0fe0	0.7815	g	0.2623	e	0.2226	e	463.1	e	50.326	e	3.754	e	61.26	c
azfe0	0.7943	d	0.2696	d	0.2244	ab	464.0	d	54.652	ab	4.297	bc	77.66	b
azfen.5	0.8063	a	0.2709	cd	0.2237	c	464.3	c	53.965	b	4.386	a	80.64	ab
azfe2	0.8037	b	0.2719	c	0.2243	ab	464.5	c	52.632	c	4.337	b	79.19	ab
azfen1	0.8054	ab	0.2731	b	0.2241	b	464.7	bc	53.621	b	4.427	a	83.00	a
azfen1.5	0.7886	e	0.2725	b	0.2244	ab	464.3	c	52.947	bc	4.359	ab	78.37	ab
azfen2	0.7883	e	0.2714	c	0.2236	c	464.6	c	52.36	c	4.296	cb	75.36	b
Psfe0	0.7957	d	0.2702	d	0.2237	c	464.2	cd	55.241	a	4.248	c	78.67	ab
Psfen.5	0.8020	cb	0.2731	b	0.2242	b	464.9	b	53.681	b	4.307	cb	83.63	a
Psfe2	0.8046	b	0.2727	b	0.2232	d	464.3	cd	53.951	b	4.340	b	79.65	ab
Psfen1	0.8043	b	0.2757	a	0.2246	a	464.5	c	53.412	b	4.352	b	84.43	a
Psfen1.5	0.7927	de	0.2726	b	0.2245	a	465.0	a	52.741	bc	4.257	c	78.64	ab
Psfen2	0.7890	e	0.2722	b	0.2241	b	464.1	cd	51.224	d	4.226	cd	77.48	ab
azofe0	0.7986	cb	0.2714	c	0.2246	a	464.6	c	55.021	a	4.254	c	76.33	b
azofen.5	0.8035	b	0.2727	b	0.2231	d	464.8	b	54.112	b	4.314	cb	80.66	ab
azofe2	0.8020	cb	0.2730	b	0.2241	b	464.2	cd	54.101	b	4.287	cb	78.95	ab
azofen1	0.8014	cb	0.2745	ab	0.2241	b	464.4	c	52.342	c	4.335	cb	82.00	ab
azofen1.5	0.7883	ef	0.2727	b	0.2235	c	464.5	c	53.624	b	4.276	cb	77.21	b
azofen2	0.7867	f	0.2717	c	0.1	ab	463.9	d	51.627	d	4.164	d	73.21	b

Means followed by the same letter in the same column do not differ statistically at 5.0 % by Duncan test, Az: *Azo tobacter crococcoccus*, Azo: *Azospirillum methylpofrome*, Ps: *Pseudomonas putida*, Fen: nano iron fertilizer, Fe: ordinary iron fertilizer.

Source: Elaborated by the authors (2017).

Compared to the average of treatment with different levels of nano and ordinary iron, it was also found that the highest effect on chlorophyll a and b and carotenoids was on nano iron at 1.0 % level and the lowest was related to control. The reason for this increase can be the effect of iron on the manufacture of chlorophyll synthesis precursors. It seems that iron nano-silicates increase nutritional status by improving chlorophyll a and b content and carotenoid content in the leaf. The iron element does not play a direct role in the chlorophyll structure; however, the presence of sufficient iron causes the production of chlorophyll a and b in the plant, and the chlorophyll status of the plant can affect the amount of photosynthesis (RUBAN, 2016). Carotenoids are auxiliary pigments that affect the

absorption and transmission of light, and chlorophyll protectors are considered during the oxidation processes (LAZAR, 2015). The fermentation of iron micronutrients had a significant effect on the increase of carotenoid in the red bean plant (ARANGO *et al.*, 2014).

Evaluated elements

Regarding the value of K, the results of the data analysis indicate that the effect of bacterial treatment on potassium concentration in grain was significant at 1.0 % level, however the effect of nano-iron and ordinary iron was not significant (TABLE 2). The comparison of bacterial treatments showed that the highest amount of potassium was obtained using *Pseudomonas* and the lowest amount was related to the control Growth stimulating bacteria, and especially *Pseudomonas*, which often increase the intrusion of insoluble mineral elements in the soil and thus improve the absorption of these elements by the plant (PATTEN; GLICK 2002). In a study on corn, it was found that application of growth-stimulating bacteria had a significant difference in Fe, K, Zn, Mn concentrations in corn grain. Typically, growth-promoting bacteria affect the formation and horizontal development of roots (BASHAN *et al.*, 2017). Particularly, the application of iron fertilizers should be said, although the use of this fertilizer may lead to a slight increase in the potassium content of the seed. But this effect is not meaningful. The results of this study are consistent with the research by Taha, Omar, and Hadeer (2016). The results of Elazab (2014) also confirm this topic. Regarding the value of P, the results of the data analysis indicate that bacterial treatment was significant at 1.0 % level and iron treatment at 5.0 % level and no interaction was significant (TABLE 2). The result of the mean comparison for bacterial treatment shows that the highest amount of P is in the treatment with *Pseudomonas* and the lowest in the control treatment. *Pseudomonas* bacteria are plant growth promoting bacteria that can increase plant growth by increasing the number of elements such as phosphorus (dissolving dissolved phosphates) as well as the production of various types of herbal hormones. Studies have shown that PGPR bacteria can dissolve insoluble phosphate by using methods such as acidification, chlorate formation, and various types of exchange reactions (GOSAL *et al.*, 2012). It was also found that the highest effect on the P content was observed for the control and the lowest value was 1.5%. The highest means for P, N, and Fe content with 55.0, 244.42, and 84.43 value, respectively, were obtained by *Pseudomonas putida* + control of Fe, *Azotobacter crococcoccus* + 1.0 % nano-Fe, *Pseudomonas putida* + 1.0 % nano-Fe. Nair *et al.* (2010) concluded that with increasing iron levels, the average concentration of phosphorus in soybeans decreased significantly. Rabieyan, Yarnia, and Kazemi-e-Arbat (2011) found that increased iron levels have caused to reduce the concentration of phosphorus in chickpea, probably due to the formation of iron phosphate or phosphate-iron hydroxide. Regarding the value of N, the results of the data analysis indicate that the treatment of the bacteria and the treatment of iron was significant at 1.0 % level and the interaction treatment was not significant (TABLE 2). The result of the mean comparison of bacterial treatment showed that the highest amount of N was related to *Azotobacter* treatment and the lowest amount was related to control. In three field experiments and using different bacterial treatments, the highest amount of nitrogen in aerial parts and seeds was obtained from inoculation of wheat with *Azotobacter* bacteria. Some researchers believe that the increase in nitrogen and grain yields is due to three main causes, including the secretion of various hormones that increase root growth and absorb nitrogen from the soil (JAMAATI SOMARIH *et al.*, 2009). The effect on nitrate adsorption is due to the reduction of this compound by bacteria in the root zone (UL HASSAN; BANO 2015), and finally through the fixation of nitrogen (ALIZADEH *et al.*, 2007). Comparing the average treatment with different levels

of nano iron and ordinary iron was found to have the highest effect on the content of nano iron at 1.0 % level and the lowest was related to control treatment. Iron is in combination with nitrate and nitrite reductase enzymes (in cytoplasm and chloroplasts, respectively), which in addition to nitrogen fixation directly affects the amount of nitrogen in the plant and increases the amount of nitrogen in the seed (HAGHIGHI; YARMAHMODI; ALIZADEH, 2010). The results obtained in this experiment are consistent with the result presented by Kobraee *et al.* (2011). Regarding the amount of Fe in grain, the results of the data analysis indicated that bacterial treatment and iron treatment were significant at 1.0% level, but the interaction was not significant (TABLE 2). Concerning Fe content, the result of the mean comparison for bacterial treatment shows that the maximum amount of Fe in the seed belongs to the *Pseudomonas* plant and the lowest amount is related to the control treatment. In this regard, as with *Pseudomonas*, the cause of increasing phosphorus concentration in the plant, *Pseudomonas* is one of the growth-promoting bacteria that can increase plant growth by increasing the amount of phosphorus (solubility of phosphate Insoluble) and iron (siderophore production). Siderophore and Chalatai or organic compounds with low molecular weight and with a strong combined desire for complexation with certain cations such as iron. Plants can use the siderophore produced by bacteria as an agent to supply the required iron (AHMAD; AHMAD; KHAN, 2006) and this iron plays an incremental role in plant growth and elevated iron element in the grain. Comparing the average treatment with different levels of nano and ordinary iron, it was also found that the maximum effect on Fe content in the seeds was related to nano iron at the 1.0% level and the lowest in the control level. Increasing iron concentrations could result from improving the production of assimilates caused by current photosynthesis, as well as the remanufacturing and desirable materials to be seeded. Khan, Hassan, and Maitlo (2006) showed that the amount of copper, iron, manganese, and zinc in straw and seeds increased due to the nutrition of wheat plants with mineral fertilizers.

Conclusions

Regarding the role of bacteria that enhance plant growth and the role of nano fertilizers in improving physiological characteristics and yield of plants, this study investigated them on triticales plants and determined that the application of these treatments would improve the amount of plant pigment and element content. Also, it determined that interaction of plant growth-promoting bacteria, *Azotobacter crococcocus*, and *Azospirillum methylpofrome* especially, with nano-Fe fertilized (1.0% and 1.5%) led to the improvement of agronomy and physiological properties of triticales and the use of *Azotobacter crococcocus* and *Azospirillum methylpofrome* with nano-Fe fertilization (1.0% and 1.5%) can be an alternative to improve to the sustainability of triticales cultivation, once it has the potential to reduce the use of conventional synthetic fertilizers.

Investigação das bactérias de crescimento e Nano ferro na clorofila e alguns nutrientes triticales

Resumo

Este estudo avaliou o efeito das bactérias de crescimento e fertilizantes de nano ferro e fertilizantes de ferro comum sobre o conteúdo de clorofila e a concentração de alguns nutrientes. O experimento foi conduzido em esquema fatorial 4×6 , Delineamento de Blocos Completos Casualizados (RCBD),

com 3 repetições em 2 anos (2016-2017). Os tratamentos incluíram: uso de bactérias promotoras de crescimento de plantas em quatro níveis (não inoculação, inoculação com *Azotobacter crococcus*, *Azospirillum methylpofrome* e *Pseudomonas putida*), fertilizante de nano ferro em cinco níveis (0,0%, 0,5%, 1,0 %, 1,5% e 2,0%) e fertilizante de ferro comum em dois níveis (2,0 % e 0,0%). Com base nos resultados, a aplicação de biofertilizante e fertilizante de Fe teve efeitos significativos em todas as características ao nível de 1,0 % ou 5,0%. Os resultados da comparação das médias dos tratamentos mostraram que a maior clorofila a (0,806) foi obtida por *Azotobacter crococcus* + 0,5% nano-Fe e as maiores médias de clorofila b e carotenóide com 0,275 mg g FW-1 e 0,224 (mg os valores de g FW-1), respectivamente, foram observados por *Pseudomonas putida* + 0,5% nano-Fe. Os maiores valores de P (55,24), N (4,42) e Fe (84,43) foram obtidos por *Pseudomonas putida* + Fe controle, *Azotobacter crococcus* + 1,0% nano-Fe e *Pseudomonas putida* + 1,0% nano-Fe, respectivamente.

Palavras-chave: Elementos. Inoculação. Nutrição. Pigmentos.

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