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EFFECTS OF IRON, PHOSPHORUS AND MICROBIAL INOCULUM ON YIELD AND QUALITY OF THE GRAIN OF WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

Effects of iron, phosphorus and microbial inoculums on yield and quality of the grain of wheat (*Triticum aestivum* L.) were studied during cropping seasons of 2019 and 2020 in Khorasan Razavi province, Mashhad following a split plot experiment based on Randomized Complete Block Design with three replications. Results showed that the application of phosphorus in the presence of iron increased the grain yield. The highest grain yield (516 g/m^2) was obtained from both iron foliar and bacterial application and the lowest grain yield (435 g/m^2) was obtained from no iron and phosphorus treatment. Foliar iron application and supply of microbial inoculum (bacteria and fungi) at rhizosphere resulted in the highest quantity and quality of grain yield.

Introduction

In Iran as a semi-arid area with alkaline soils, the activity of trace elements like iron, copper, zinc and manganese can be decreased and form sediment carbonate, and thus their uptake efficiency will be reduced. On the other hand, plants are usually poor in phosphate uptake because the phosphate anions ($H_2PO_4^{-2}$ and HPO_4^{-2}) in traditional fertilizers quickly form metal complexes with Ca^{+2}, Fe^{+2} and Al^{+3} in the soil and become inaccessible by the plant (Qureshi *et al*. 2012). Several bacterial species present in the soil rhizosphere by lowering the soil pH can dissolve insoluble mineral phosphate and convert it to soluble organic phosphorus (Li *et al*. 2015). Phosphorus is a limiting factor for plant growth in alkaline soil. Wheat (*Triticum aestivum* L.) is one of the main food grain crops in the world including Iran and its quantitative and qualitative yield increase is a great concern.

Therefore, due to the quantitative and qualitative importance of wheat production, high cost of supply of nutrients from chemical sources, lack of availability of micronutrients in alkaline soils of the country, finding an effective and low-cost method to meet wheat needs for these micronutrients and improve their absorption is quite important. Thus the aim of the present study was to determine the best source of phosphorus fertilizer and iron application method on yield, grain quality and some other characters of wheat.to determine the best source of phosphorus fertilizer and iron application method on yield, grain quality and antioxidants of immunity system of wheat.

Materials and Methods

The present research was carried out in two years (2019, 2020) in Khorasan Razavi province, Mashhad as a split plot based on Randomized Complete Block Design with three replications. The main factor included iron fertilizer at three levels of iron foliar application, soil application of iron and control (no-application) and four levels of phosphorus fertilizer including (1-inoculation with

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PSB bacteria, 2-inoculation with mycorrhizal fungus (AM) (*Glomus mosseae*), 3-fertilizer Triple superphosphate and 4-no phosphorus application), as subplots. The dimensions of the plots were 6×6 m and the between-rows distance was 25 cm. The distance between the plots was one meter and between the blocks was 2 m. Some climatic characteristics of the study site during the experimental seasons are presented in (Table 1). Land preparation operations including plowing, disc, leveling and harrowing were carried out before planting. Wheat seeds of Omid cultivar after disinfection with carboxin thiram fungicide (ratio of 2 per thousand) were planted on November 6 and November 2, in the first and second year of experiment, respectively with population of 350 plants per square meter. The employed cultivar is adapted for areas where the temperature in winter does not reach to less than -4 or -5 Celsius degrees. At planting time, 300 (kg/ha) nitrogen in form of urea (46% N (W/W) (CO (NH2) 2)) in a strip pattern at depth of 5-7 cm below the seedbed was applied. Some of soil characteristics of the study site were determined after random sampling from a depth of 0-30 cm of study field and the results are presented in (Table 2).

Table 1. Some climatic characteristics of the study site during the two cropping years 2019 and 2020.

		Oct	Nov	Dec	Jan	Feb	March	April	May	June
Evaporation mean (mm)	2018-2019	4.32	2.02	-	-	-	2.56	3.36	7	10.04
	2019-2020	4.47	1.96	0.3	-	-	2.5	4	8.64	12.50
Precipitation mean(mm)	2018-2019	1.32	0.66	0.01	1.44	0.55	2.30	2.75	1.41	0.77
	2019-2020	0.32	0.35	0.26	1.43	0.55	3.16	2.14	1.03	0.16
Temperature mean (°C)	2018-2019	14.52	8.40	7.35	2.27	7.32	10.24	13.77	21.53	26.7
	2019-2020	16.29	6.37	7.09	2.27	7.32	9.95	13.92	22.02	27.81
Sunshine hours mean	2018-2019	7.50	4.65	5.83	4.77	6.17	6.47	5.6	9.15	11.96
	2019-2020	7.98	5.65	5.47	4.76	6.18	6.07	5.86	10.36	12.14
Relative humidity mean	2018-2019	47.05	69.55	69.35	77.17	57.75	63.61	71.83	50.80	26.99
	2019-2020	41.36	61.08	67.65	77.17	57.76	63.97	69.81	43.42	20.06

Phosphate-releasing bacteria included two strains of (*Pseudomonas putida*) and (*Pantoea agglomerans*) at a rate of 500 cc per hectare with a population of 107 (CFU per milliliter) were applied through irrigation water at planting time. Hand spraying for foliar application of Fe EDTA iron with a ratio of 0.1% (w/v) was performed at the emergence of the second stem node (Zadoks stage 32). About 200 mg/l of Tween was used as a surfactant. Soil application of iron (chelated) at a rate of 3 kg/ha was also performed at the emergence of the second stem node (Zadoks stage 32). Phosphorus fertilizer treatment, 100 kg/ha of triple superphosphate, was applied at planting time. Mycorrhizal fungus treatment was also applied at planting and in a strip form under seedbeds (based on 20 g/m^2). The inoculum was used as a mixture of spores, filaments and isolated parts of infected roots at a depth of 2 cm below each seed.

Grain quality parameters including grain fiber (neutral detergent fiber [NDF]) and grain protein were measured by near-infrared reflectance (NIR) technique using analyzer Perten DA 7250 NIR analyzer instrument (Perten Instruments AB, Huddinge, Sweden). Determination of mineral nutrients was done by using inductively coupled plasma optical emission spectrometry (ICP-OES) (Vista-Pro Axial, Varian Pty Ltd, Mulgrave, Australia).

The protein concentration of the samples was determined based on Bradford (1976) method. Catalase, superoxide dismutase and Peroxidase activities were measured as cited from Beauchamp and Fridovich (1971), Aebi (1984) and Devi *et al.* (2002), respectively.

Data were subjected to analysis of variance (ANOVA) using SAS statistical program and the difference between treatment means was separated using Duncan's test.

Results and Discussion

Wheat grain quality parameters strongly depend on growth conditions, soil fertility, fertilizer application, water access, genotype, transport and grain storage conditions. One of the effective factors on grain quality traits is access to nutrients through fertilizers (Shahbazi *et al*. 2015). Results of analysis of variance showed that the simple effects of year, iron application and phosphorus fertilizer source as well as the interaction effect of iron application and phosphorus source on grain protein content were significant (Table 3). Significance of the effect of the year shows that this trait is affected by environmental factors such as temperature, rainfall and relative humidity, and climatic differences in the two years of the experiment. Comparison of the mean interaction between iron application and phosphorus source showed that in general, phosphorus and iron application increased the amount of grain protein compared to no-application. However, at different levels of iron, the rate of increase in grain protein was not the same across different sources of phosphorus (Table 4). The highest increase (10.4%) compared to the control (no phosphorus application) was observed due to the application of PSB bacteria in no-application iron. While in the treatment of triple superphosphate consumption and iron application in soil, the amount of grain protein decreased by 3.8% compared to the control (no phosphorus application) (Table 4).

The simple effects of iron application and phosphorus source on grain fiber were significant (Table 3). Foliar application of iron and soil application of iron reduced grain fiber content by 11.8 and 4.04%, respectively. Application of PSB bacteria, mycorrhiza fungus and triple superphosphate fertilizer also reduced the amount of grain fiber by 6.6, 5.3 and 2.8%, respectively (Table 5).

The interaction effect of iron application and phosphorus fertilizer source on the concentration of phosphorus, potassium, iron and zinc was significant (Table 3). Comparison of the mean interaction of iron application and phosphorus fertilizer source on grain phosphorus concentration showed that application of phosphorus sources increased grain phosphorus. However, the mean of this increase in no-application iron treatment was higher than foliar spraying and in soil application treatments (Table 4). The mean increase in grain iron concentration in phosphorus-free treatment was higher than other phosphorus treatments (Table 4). The amount of zinc in the grain decreased by application iron and phosphorus source however the mean reduction was not the same in all treatments (Table 4). The highest amount of zinc was observed in the treatment of no-application of iron and phosphorus and the lowest amount was observed in the treatment of foliar application of iron and consumption of triple superphosphate (Table 4). In the present study, grain iron concentration increased, especially in iron foliar spraying treatments. Comparison of the mean interaction of grain potassium showed that in general in iron application treatments (especially in the form of foliar application) the average grain potassium was higher than the lack of iron application (Table 4) but using phosphorus showed different responses. In iron-free treatment, phosphorus fertilizer from PSB and mycorrhizal fungi increased grain potassium, while in iron application treatments, phosphorus sources reduced grain average potassium (Table 4). The highest grain potassium was obtained in the treatment of no-application of phosphorus with iron spraying and the lowest grain potassium was obtained from the treatment of triple superphosphate application and no iron application (Table 4).

Table 2. The physico-chemical soil characters of study site.

Soil texture	Acidity pH	Salinity ECe (dS/m)	Saturation Sp %	Neutralizing material T.N.V%	Organic carbon O.C%	Nitrogen N%	Phosphate uptake P (mg/Kg)	Potassium uptake K (mg/Kg)	Ca^{2+} (meg/ l)	Mg^{2+} (meg/ l)	Na^{+} (meg/ l)	SAR
Loamy	7.9	14.6	34	21	0.097	0.009	4.9	160	13	20	114	28

Table 3. Combined analysis of variance of the effect of iron application and phosphorus fertilizer source on the studied traits of wheat.

Source of variable	df	Grain protein	Grain fiber	Grain Phosphorus	Grain potassium	Grain iron	Grain zinc	Peroxidase enzyme	Catalase enzyme	Superoxide dismutase Enzyme	Grain yield
Year	1	1.24^{**}	0.02^{ns}	0.023^{ns}	0.0006^{ns}	529.9^{*}	0.41^{ns}	3.84^{ns}	5.91^{ns}	3.46^{ns}	0.24^{ns}
Error (Year × Rep)	4	0.05	1.66	0.056	0.0002	66.9	5.19	707.88	98.26	1.04	1270.59
Iron	2	15.97^{**}	15.46^{**}	2.77^{**}	0.0628^{**}	9336.5^{**}	1451.24^{**}	262.67^{*}	18.4^{ns}	168.84^{**}	8087.49^{**}
Iron × Year	2	0.01^{ns}	0.0001^{ns}	0.0001^{ns}	0.000^{ns}	1.6^{ns}	2.05^{ns}	21.74^{ns}	142.55^{**}	0.06^{ns}	5.81^{ns}
Error	8	0.15	1.68	0.006	0.0001	55.6	11.28	107.96	4.48	2	262.8^{ns}
Phosphorus fertilizer	3	2.21^{**}	2.61^{**}	2.93^{**}	0.0035^{**}	897.4^{**}	2353.02^{**}	248.76^{*}	105.32^{**}	21.31^{**}	6309.62^{**}
Phosphorus fertilizer × Year	3	0.01^{ns}	0.00002^{ns}	0.0002^{ns}	0.0000^{ns}	0.3^{ns}	0.53^{ns}	53.79^{ns}	59.21^{**}	0.03^{ns}	151.32^{ns}
Phosphorus fertilizer × Iron	6	0.26^{*}	0.12^{ns}	0.36^{**}	0.0017^{**}	371.4^{**}	94.45^{*}	14.69^{ns}	8.28^{ns}	1.55^{ns}	1092.16^{*}
Phosphorus fertilizer × Year× Iron	6	0.01^{ns}	0.000^{ns}	0.00005^{ns}	0.00004^{ns}	0.1^{ns}	4.23^{ns}	8.49^{ns}	17.36^{*}	0.004^{ns}	98.71^{ns}
Error	36	0.11	0.534	0.035	0.0003	74.54	34.20	75.13	5.79	0.89	457.09
Coefficient of variation		2.77	5.78	5.44	3.95	8.42	11.05	8.88	6.46	10.03	10.03

ns, * and **: non-significant and significant in the level of 5% and 1%, respectively.

Table 4. Comparison of the mean interaction of iron fertilizer application and phosphorus source on the studied traits.

		Grain protein (%)	Grain phosphorus (mg/kg)	Grain potassium (%)	Grain iron (mg/kg)	Grain zinc (mg/kg)	Grain yield (g/m^2)
Iron foliar application	Bacterial inoculation (PSB)	12.6[a]	3.3[de]	0.49[b]	98.3[a]	43.4[fg]	515.9[a]
	Mycorrhizal fungus inoculation	12.6[a]	3.4[d]	0.53[a]	90.3[ab]	42[g]	500.1[ab]
	Triple superphosphate application	12.2[bc]	3.5[cd]	0.51[b]	62.7[cd]	38.1[g]	479.5[bcd]
	No-phosphorus	12.1[c]	2.69[g]	0.55[a]	84.3[b]	60.1[cd]	465.1[def]
Iron application in soil	Bacterial inoculation (PSB)	12.4[abc]	3.08[ef]	0.44[cd]	55.8[cde]	43[g]	495.6[abc]
	Mycorrhizal fungus inoculation	12.5[ab]	3.28[de]	0.43[def]	65.4[c]	54.2[de]	470.5[de]
	Triple superphosphate application	11.6[d]	3.69[c]	0.41[fg]	53.9[de]	39.3[g]	453[efg]
	No-phosphorus	12.1[c]	3.01[f]	0.46[c]	60.7[cd]	69.6[b]	444.5[fg]
No-iron application	Bacterial inoculation (PSB)	11.4[d]	4.1[ab]	0.44[de]	43.8[f]	61.4[c]	470.7[cde]
	Mycorrhizal fungus inoculation	10.9[e]	3.99[b]	0.44[cd]	48.8[ef]	55.7[cde]	438.2[g]
	Triple superphosphate application	10.6e[f]	4.32[a]	0.41[g]	40.4[f]	50.1[ef]	472.5[cde]
	No-phosphorus	10.4[f]	2.92[f]	0.42[efg]	46.8[ef]	77.9[a]	435.3[g]

Means with the same letter are not significantly different by Duncan test ($P< 0.05$).

The simple effects of iron and phosphorus fertilizer application on the activity of peroxidase and superoxide dismutase enzymes were significant (Table 3). Also, according to the results of analysis of variance, the simple effect of phosphorus fertilizer source and interactions of year and method of iron consumption, as well as the interaction of year and source of phosphorus fertilizer and triple interaction on catalase activity were significant (Table 3). According to the comparison results, the average foliar application of iron and soil iron application increased the activity of peroxidase enzyme activity by 8.2 and 4.5%, respectively. On the other hand, application of PSB bacteria, mycorrhiza fungus and triple superphosphate fertilizer increased the activity of peroxidase enzyme activity by 8.3, 2.2 and 7.2%, respectively (Table 5).

Comparison of the mean of the triple interactions of the year, iron application and phosphorus fertilizer source on catalase activity (Fig. 1) showed that although iron and phosphorus application treatments did not have a positive effect on catalase activity, but the rate of reduction of this enzyme activity under different treatments in two years was not the same. In the first year of the experiment, the highest amount of catalase activity was obtained from the treatment of no-application of iron and no-application of phosphorus, and in the second year from the treatment of foliar application of iron and no-application of phosphorus (Fig. 1). The lowest level of catalase activity was obtained in the first year from iron foliar application and triple superphosphate fertilizer application and in the second year from PSB bacterial treatment with iron foliar application (Fig. 1). This difference in enzyme activity in the two years of the experiment was a function of environmental and climatic conditions because no stress was imposed in this study. The activity of superoxide dismutase increased with application of iron and phosphorus source. Comparison of the mean of the simple effect of iron application showed that the highest activity of superoxide dismutase enzyme was in iron foliar treatment (Table 5). Among the phosphorus treatments, the highest activity of this enzyme was related to the treatment with PSB bacteria (Table 5). However, sometimes the high level of iron may be one of the sources of ROS production and induction of stress in plants (Gao *et al*. 2016). In addition, iron is a major component of CAT and SOD enzymes.

Table 5. Comparison of the average simple effects of the year, iron application and phosphorus source on the studied traits of wheat.

	Treatment	Grain protein (%)	Grain fiber (%)	Grain iron (mg/kg)	Peroxidase (OD µg protein. min^{-2})	Superoxide dismutase (OD mg protein. min^{-2})
Year	2018- 2019	11.65[b]		59.89b	-	-
	2019- 2020	11.91[a]		65.3a	-	-
Iron fertilizer	Iron foliar application	12.36[a]	11.77[b]	83.9[a]	100.97[a]	12.47[a]
	Iron application in soil	12.13[a]	12.81[a]	58.9[b]	97.57[b]	8.34[b]
	No-application of iron	10.85[b]	13.35[a]	44.9[c]	93.32[c]	7.52[b]
Phosphorus fertilizer	Bacterial inoculation (PSB)	12.16[a]	12.26[b]	65.9[a]	101.26[a]	10.93[a]
	Mycorrhizal fungus inoculation	11.99[a]	12.43[b]	68.2[a]	95.53[ab]	9.57[b]
	Triple superphosphate application	11.50[b]	12.76[ab]	52.3[b]	100.24[a]	8.66[c]
	No-phosphorus	11.46[b]	13.13[a]	63.9[a]	93.50[b]	8.61[c]

Means with the same letter are not significantly different by Duncan test ($P< 0.05$).

According to the results of analysis of variance, the simple and interaction effects of iron application and phosphorus fertilizer source on grain yield were significant (Table 3). Comparison of the average interaction between iron application and phosphorus fertilizer source showed that the application of phosphorus fertilizer sources in different iron treatments increased grain yield, but the rate of increase was different at different levels (Table 4). The highest increase in grain yield (11.5%) compared to the control (no phosphorus application) was obtained in application of iron in soil along with using PSB bacteria and the lowest increase in grain yield (1%) was obtained in no-iron treatment and mycorrhizal fungus treatment (Table 4). The highest grain yield (516 g/m^2) was obtained from iron foliar application and application of PSB bacteria, and the lowest grain yield (435 g/m^2) was obtained from no-iron and phosphorus treatment (Table 4). However, due to the increasing need for phosphorus fertilizers, it should be noted that the optimal management of the source and amount of phosphorus fertilizers is very important because of its effect on phosphorus use efficiency, grain yield and the effect of zinc and iron concentrations. Increased grain yield due to iron application has also been reported in many studies (Wissuwa *et al*. 2005). Therefore, it is normal that with the increase of leaf iron, the amount of chlorophyll increases and finally the production of dry matter and grain yield increase. Studies have shown that small amounts of nutrients, especially zinc and iron, in the form of sprays on the leaves cause a significant increase in yield. However, some researchers believe that many plants in the cereal family, such as wheat, release Fe-mobilizing compounds (phytosiderophores) that satisfy the plant's need for iron.

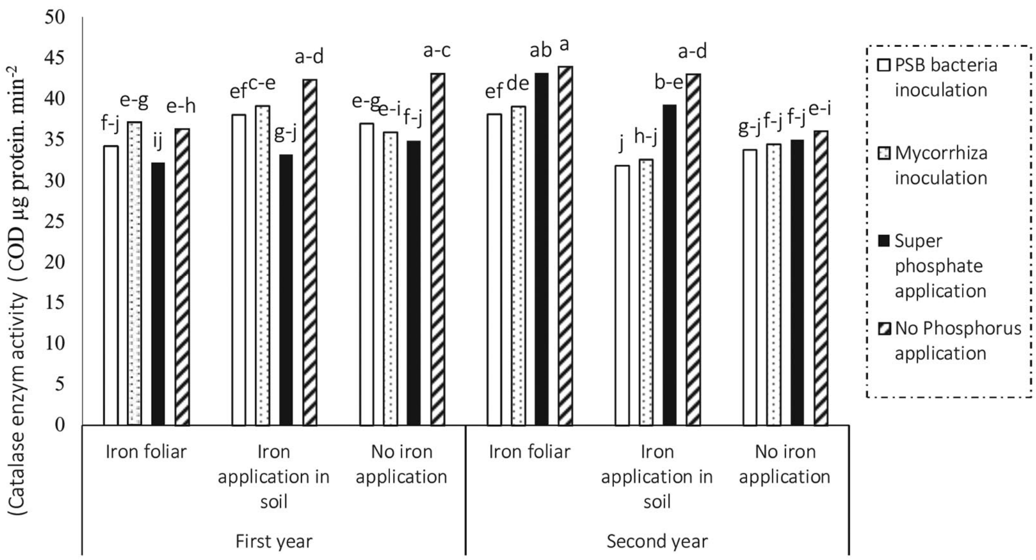

Fig. 1. Comparison of the average triple interaction of the year, iron intake and phosphorus source on catalase activity. Column means with the same letter are not significantly different by Duncan test ($P < 0.05$).

Results of this study demonstrated that iron application, especially in spraying form, significantly increased the wheat grain yield compared to control condition. Combination of foliar iron application with biological sources of phosphorus (PSB bacteria and mycorrhizal fungi) had a substantial effect in improving grain protein content and yield. Furthermore, antioxidant enzymes activity was significantly stimulated by iron spraying and PSB bacteria application in comparison with other studied treatments.

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