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Foliar application of nano-Fe3O4 induced Iron Content and Phytase Activity in corn seed Single Cross 704

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Abstract. This study was conducted in an experimental field in a randomized complete block with three replications in Urmia, West-Azarbayjan province of Iran. Foliar application nano Fe3O4 was performed at 3 stages of corn growth, knee stage, vegetative growth and grain filling. Corn seeds after harvested, were soaked and then germinated and dried. Iron content and phytase activity were determined. The results of this study revealed that foliar application of nano Fe3O4 significantly induced iron concentration and the activity of phytase ($P<0.01$). The results of this study showed that nano iron oxide fertilizers enhances the quality of corn seeds by decreasing phytate content and increasing Iron concentrations.

Key words: foliar application, nano-Fe3O4, Iron, phytase, corn

1. INTRODUCTION

Changes in agricultural technology have been a major factor shaping modern agriculture. Among the latest technological innovations, nanotechnology occupies a prominent position in transforming agriculture and food production. The development of nano-devices and nano-materials could open up novel applications in plant biotechnology and agriculture [1]. The use of metal based nanoparticles like ZnO and Fe_3O_4 as foliar application for increasing intake of minerals by plant cell wall has paved the way for use of such nanoparticles for studying their interaction in plant cells [1]. Iron is an element that used by crops in small quantities [2], yet are essential to normal plant growth, development and play important roles in enzyme reactions, photosynthesis, improves the performance of photosystems, DNA transcription, RNA synthesis and auxin activity [3].

Maize (*Zea mays L.*) is the most important crop among all cereal grain crops [4]. It is an important plant that is used as human food, livestock and poultry feed and as raw material in industry [5]. It was reported that seed germination and root growth of corn can be affected by solution containing zinc and iron nanoparticles [1, 6]. In the other hand, many of crops

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particularly maize, have high quantity of phytate [7]. Phytate or Phytic acid is a major storage form of Phosphorus, ubiquitously distributed in plants and for which low-phytate alleles have been identified [8]. The effects of phytate in human and animal nutrition are related to the interaction of phytic acid with proteins, vitamins and several minerals, and thereby restrict their bioavailability. In viewing the anti-nutritional effects of phytate, many attempts were carried out to reduce it; other attempts were performed to reduce the phytate content such as fertilization [9]. These include activation of the indigenous enzyme phytase using nano fertilizers and addition of microbial phytase [9].

In northwest of Iran, iron deficiency is nutritional disorder in many plants grown on calcareous soils with pH>8.0 [10]. To the best of the authors' knowledge, the literature is poor regarding effects of nano-iron oxide on corn growth. Hence, the present field experiment was carried out to investigate the effect of foliar application of nano-iron oxide on grain yield production and seed set of corn. The aim of this study was to evaluate Fe content and Phytase activities in corn seed.

2. EXPERIMENTAL PROCEDURES:

2.1. Study design

The experiment was conducted as a randomized complete block (RCBD) and three replicates. This study was carried out in an experimental field at Osalo, Urmia, West Azarbayjan province of Iran, on the corn seed (*Zea mays* L.), cultivar "*Single Cross* 704" during 2011 cropping season. The experiment site was located at 25 km of Urmia on latitude 37° 43' 04" N and longitude 45° 13' 19.03" E and 1276.5 m altitude. Composite surface soil samples were collected from surface horizon (0−30 cm) of the soil before the experiment was initiated, air-dried, passed through a 2- mm sieve and analyzed for the following properties.

2.2. Soil analysis

Particle-size of the soil distribution was determined using hydrometer method [11]. Soil pH and ECe (electrical conductivity) were measured at a 1:2.5 soil/water ratio and saturated extract, respectively, organic matter (OM) content was determined by the Walkley-Black method [12]. Soil available K was determined by 1 M NH_4OAc extraction and K assessment in the extract by flame photometer (Thomas, 1982). Soil available P was measured by using Olsen method [13]. Available Fe, Zn, Mn and Cu in the soil were first extracted by DTPA (Diethylene triamene penta-acetate) and then were read by atomic absorption Schimadzu AA6800 (Japan) [4]. Soil available B was extracted by hot water and measured by Azomethine-H colorimetric method [14]. The experimental field soil had a loam texture, pH 8, 0.56 % organic matter, 9 $mg.kg^{-1}$ available P, 205 $mg.kg^{-1}$ available K, DTPA extractable Fe, Mn, Zn and Cu concentration were 6.2, 9.9, 0.63 and 1.4 $mg.kg^{-1}$ respectively and available B with hot water extractable was 0.87 $mg.kg^{-1}$ [10, 14]. Physical and chemical properties of soil in experimental field are presented in (*table 1)*.

2.1. Synthesis and Characterization of Fe3O4 Nanoparticle

Fe_3O_4 was synthesized according to the method that reported by Lopez *et al.* (2010) [15]. Briefly the synthesis of Fe_3O_4 magnetic nanoparticles (MNP) was based on the mixture of $FeCl_3.6H_2O$ and $FeCl_2.4H_2O$ salts in the molar ratio 1:2. After mixing 1.0 mL of 2M $FeCl_2.4H_2O$ and 4.0 mL of 1M $FeCl_3.6H2O$ in the same solution, 25 mL of a 1.0 M NaOH solution was added drop wise, under vigorous stirring. Then, the precipitation process was

occurred immediately, promoting a color change in the solution to dark-black, characteristic of the magnetite. In order to neutralize the anionic charges on the nanoparticles surface, 0.5 mL of 2M hydrochloric acid (HCl) was used [15]. The pH of the solution was constantly monitored when the NaOH solution was added and stirred to produce the precipitation of the Fe_3O_4 nanoparticles. Then, the sample was dispersed in kerosene (1 mL) and deionized water and, stirred for 2 h at 30°C. At the end of the reaction, an amount of 5 mL of oleic acid was added to the solution as a surfactant and coating material. To evaporate the liquid it was brought to a reaction temperature of 80° C and then was stirred for 1 h and cooled to room temperature. The precipitate was washed with deionized and distilled water and then centrifuged for 15 minutes at 2500 g.

Table1. Soil physical and chemical analysis

Properties	Values
Depth of soil (cm)	0-30
Soil texture	Loam
pH	8.1
EC (ds m^{-1})	1.27
Organic matter (%)	0.51
Nutrients (mg.kg $^{-1}$)	
P	9.14
K	194
Fe	5.74
Mn	8.5
Zn	0.52
Cu	1.61
B	0.89

The supernatant liquid was decanted, and centrifuged until only thick black precipitate remained [15, 16]. The chemical composition of the synthesized materials was checked by FTIR spectroscopy with a Biorad FTS-40 spectrometer. The crystallinity was determined by XRD using a Bruker D8 Advance X rays Diffractometer equipped with a Cu Ka (k = 1.54 A°) source (applied voltage 40 kV, current 40 mA). About 0.5 g of the dried particles were deposited as a randomly oriented powder onto a Plexi glass sample container, and the XRD patterns were recorded at angles between 20 and 80, with a scan rate of 1.5/min [17] *(figure 1).*

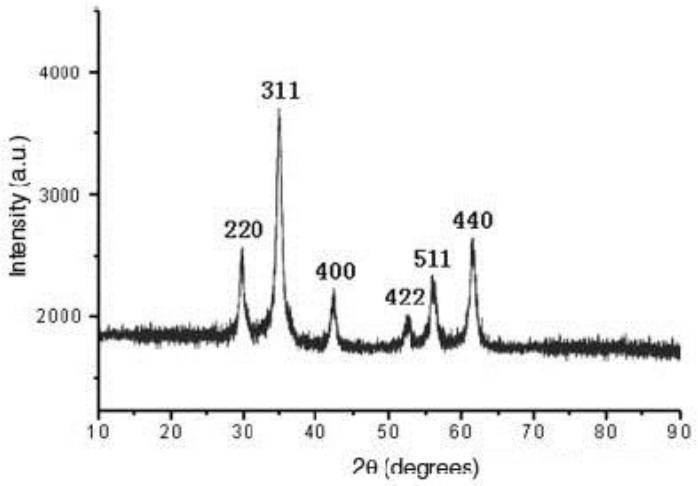

Figure 1. XRD pattern of Fe_3O_4 nanoparticles (36).

2.2. Treatment Foliar Application

Treatment was consisted of 3 groups: Nano-Fe3O4 (10 mg.L^{-1}, as foliar application), Fe^{2+} ions (equivalent to 83.4 g of $FeSO_4$.7 H_2O was dissolved in 6 liters of pure water (the final Fe concentration was 2.8 g.l^{-1}) and control group (no treatment). Plants were grown in five-row plots with 5 m length and 0.75 m spacing between rows. The plant density was 66000 plant.ha^{-1}. Foliar application of fertilizers was performed at 3 stages of corn growth: knee stage, vegetative growth and grain filling.Each plot was harvested at maturity for seed analysis.

2.3. Soaking of grains

Corn seeds were soaked in distilled water for 20 hours with 1:5 w.v.1^{-1} ratios and the soaked water was changed twice. At the end of soaking period, the soaked water was discarded. The seeds were rinsed twice in distilled water and the grains were dried at 45±5^o C. The grains were ground in a Laboratory mill to obtain fine flour and kept at -20^o C until analysis.

2.4. Chemical analysis
2.4.1. Iron determination

Total Iron contents were determined according to the method outlined in A.O.A.C [18] using the Shimadzu (Model AA6800, Japan) Atomic Absorption Spectrophotometer. Approximately 2 g sample was weighed and heated at 550^o C. Then the ashes were dissolved with hydrochloric acid 1 M [19].

2.4.2. Phytase activity assay
2.4.2.1. Extraction of phytase.

Phytase activity was assayed according to the procedure described by Barrientos et al. [20] and modified by Jog et al. [21]. In summary 2 g sample was added to ice cold Buffer (16 mL of 10 mM Tris–HCl, pH 7.0, containing reduced glutathione, 0.5 mM). The suspension was stirred with a glass rod. 80 mg Solid cetylpyridinium bromide (final concentration 0.5% w.v^{-1}) was added to the suspension. The suspension was homogenized with homogenizer at 27,000 rpm for 2 min. with a 1 min delay in-between. The resulting crude homogenate was centrifuged at 10,000 g for 30 min. The supernatant containing phytase activity was collected [19, 22].

2.4.2.2. Phytase assay (EC 3.1.3.72).

Alkaline phytase activity was assayed measuring the inorganic phosphorus (Pi) released by the enzyme. The assay mixture contained Tris–HCl buffer (100 mM, pH 8.0), NaCl (0.5 M), $CaCl_2$ (1 mM), sodium phytate (1 mM), NaF (10 mM), and an aliquot of enzyme solution in a total volume of 250 µl. The assay mixture was incubated at 37ºC for 1 h and the reaction was stopped by the addition of 50 mL of 50% TCA. In brief, ammonium molybdate solution (700 mL of a 1:6 solution of 10% w.v^{-1} ascorbic acid and 0.42% ammonium molybdate (w.v^{-1}) in 0.5 M H_2SO_4) was added and the solution was incubated at 37^o C for 1 h. Absorbance at 820 nm was measured and the inorganic phosphorus concentration was determined from a calibration curve using KH_2PO_4 as the standard [19, 22]. Soluble protein was determined according to Lowry et al [23] and specific activities of these two enzymes were defined as unit per milligram protein [19, 22].

2.5. Statistical analysis.

For the analytical data, mean values and standard deviation are reported. Data were subjected to analysis of variance (ANOVA) and the treatment means were compared using Duncan's multiple range test (alpha = 5%). The analysis was done by MSTATC and SAS (Ver. 9.1) soft wares.

3. RESULTS

2.6. Evaluation of iron concentration

The results of the determination of iron in corn seeds are mentioned in *(table 2)*. It could be noticed that the Fe content of corn seeds in control group ranged between 23.85-30.15 $mg.kg^{-1}$ and after foliar application of nano-iron-oxide and Fe ionized, its concentration increased significantly ($P<0.01$).

2.7. Phytases activities

The activity of phytase enzyme in different treatment groups are showed in *(table 2)*. The results of this study revealed that nano-iron and iron ionized induced activity of phytase ($P<0.01$), however, nano iron fertilizer was more effective than ionized iron on the phytase activity in corn seeds.

Table 2. Concentration of iron ($mg.kg^{-1}$ dry weight) and phytase activity ($unit.mg^{-1}$ protein) after foliar application of Nano-Iron oxide & Fe ionized in corn seeds. The values were given as mean ± SD (standard deviation) of triplicate samples with 10 seeds each.

Treatments	Iron ($mg.kg^{-1}$DM)	Phytase (unit.mg-1 protein)
Control	27.1 ± 4.40^{a}	0.169 ± 0.016^{a}
Nano-Fe_3O_4	35.1 ± 9.43^{b}	0.318 ± 0.053^{b}
Fe ionized	32.4 ± 3.74^{b}	0.197 ± 0.022^{c}
P value	0.01	0.01

*Number in the same column followed by the different letter are significantly different at

$p<0.05$.

4. DISCUSSION

Corn is a strategic crop in many countries agriculture; therefore increase in its yield and production has received a great attention in recent years [24] and its nutritional quality is dictated mainly by chemical composition and the presence of anti-nutritional factors such as phytate [25]. Iron is an essential trace element in human nutrition and its deficiency is the major public health threats worldwide. In this study Fe contents of corn seed were determined after application of nano-iron fertilizer and iron ionized fertilizer.

According to the results of this study, Fe content of corn seeds in the control group were completely in agreement with findings of Hambidge et al. (2004) [8] but our results slightly were lower than those reported by Cabrera et al. (2003) who reported that Fe content ranged between 42.8–55.4 $mg.kg^{-1}$ [26]. After foliar application of nano-iron oxide and iron ionized, iron content of corn seed was increased significantly in comparison with control group ($P<0.01$). In relation with iron content, there was significant difference in iron concentration in

corn seeds between nano-iron oxide and iron ionized groups. In spite of this fact, that cereals high in phytate tend to have higher iron content [19], however, application of nano-iron oxide can positively affect the available iron concentration of corn seed.

Also the data showed significant differences between phytase activity after using nano fertilizer and significant increase in phytase activity after foliar application of nano-iron fertilizer. Phytase enzymes will be activated during drying in equal form in seeds [22]. Therefore, the main distinct point is the change of phytase activity as well as specific activity during different treatment which showed significant increase in phytase activities after application of nano-iron oxide. Iron as a cofactor, is found in a large number of enzymes and other proteins, where it plays an important structural role [27].

The highest phytate-degrading activities of these enzymes have been reported in cereals. In general, legumes and oilseeds exhibit a 10-fold lower phytate-degrading activity. Optimal pH value for phytate degradation was estimated to be about 5.0 (acid phytase). In addition, a second optimum at about pH 8.0 (alkaline phytase) was reported in legumes. In general, legumes exhibit a lower activity at pH 8.0 compared to pH 5.0 [28]. It means acid phytase has the major role in phytate metabolism in corn and its activity was correlated to some minerals like as zinc and iron content [7].

Maize is an example of a cereal grain that has very high phytate content and many attempts were carried out to reduce it [29]. Phytate degrading enzymes have been studied intensively in recent years because of the great interest in such enzymes for reducing phytate content in animal and human food consumption. Phytate can act as an anti-nutrient by chelating minerals, such as zinc, iron, calcium and magnesium. Hence, addition or activation of phytate-degrading enzymes can improve the nutritional value of plant-based foods by enhancing protein digestibility and mineral availability through phytate hydrolysis during digestion in the stomach or during food and feed processing. Phytate-degrading enzymes are widespread in nature, occurring in plants like as barley, maize, rice and wheat, micro-organisms and some animal tissues [30]. Beside of these attempts, use of nano fertilizers and activation of the indigenous enzyme phytase and or addition of microbial phytase can be more useful [19].

5. CONCLUSION

In conclusion, foliar application of nano-iron oxide fertilizer significantly increased phytase activity and iron concentrations in corn seed. These changes enhanced quality of corn seeds. In corn growth, nano-fertilizers are more effective than mineral salts.

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