Journal of Plant Physiology and Breeding

2021, 11(1): 137-156 ISSN: 2008-5168

**Research paper**

# Potential survey of Iranian hexaploid landraces and modern cultivated bread wheat for iron, zinc, phytate, and phytate/mineral molar ratio

**Mojgan Gholami Malekroudi[1], Ghasem Karimzadeh[1*], Tahmineh Lohrasebi[2], Mohammad Sadegh Sabet[1], and Serveh Fathi[1]**

Received: May 23, 2021 Accepted: June 29, 2021
[1]Department of Plant Genetics and Breeding, College of Agriculture, Tarbiat Modares University, Tehran P. O. Box 14115-336, Iran
[2]Department of Agricultural Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
[*]Corresponding author; Email: karimzadeh_g@modares.ac.ir

**Abstract**
Phytate, the highest inorganic phosphorus in cereal grains, is an anti-nutritional factor that reduces the bioavailability of iron (Fe) and zinc (Zn). The whole grains of 109 hexaploid landraces and modern bread wheat cultivars (*Triticum aestivum* L.) were used to determine Fe, Zn, and phytate concentrations and their bioavailability using phytate:mineral molar ratios. In addition, some morphological and physiological features were identified as contributory factors for the screening process. In the field experiment, the concentrations of Fe and Zn, ranged between 0.036-0.255 mg/g and 0.030-0.085 mg/g, respectively. There were no significant differences in Fe and Zn concentration between the bread wheat cultivars and landraces. Therefore, efforts made by breeders for developing high-yielding wheat didn't have detrimental effects on Fe and Zn concentrations. The modern bread wheat cultivars showed a significantly higher phytate concentration than landraces. Grain phytate ranged from 15.07-28.77 mg/g resulting in a variation in phytate:Fe of 6.24-58.14 and phytate:Zn of 32.20-77.22, indicating poor bioavailability of these minerals. The identified drawbacks were due to relatively high phytate concentration which in turn could be due to a high level of soil phosphorus concentration, suggesting increasing mineral bioavailability by the breeding methods to reduce the phytate and phytate:mineral ratio. In the solution culture experiment, the role of root length, root dry weight, and root nutrient concentration in distinguishing cultivars' Fe uptake was demonstrated. The study also revealed that lower values of root length, root dry weight, and leaf dry weight led to lesser leaf Fe which in turn caused reduced contents of photosynthetic pigments and chlorophyll fluorescence. Due to high Zn and Fe concentration, and low concentration of phytate and phytate:mineral molar ratio, some of the current landraces such as Khoram Abad (1), Sarouq (4), Eskan Arak (2), and Hoseinabad Arak could be exploited by breeding strategies in biofortification programs.

**Keywords:** Biofortification; Micronutrients; Molar ratio; Photosynthetic pigments; *Triticum aestivum*

**Citation:** Gholami Malekroudi M, Karimzadeh G, Lohrasebi T, Sabet MS, and Fathi S, 2021. Potential survey of Iranian hexaploid landraces and modern cultivated bread wheat for iron, zinc, phytate, and phytate/mineral molar ratio. Journal of Plant Physiology and Breeding 11(1): 137-156.

## Introduction

More than two billion people, especially women, infants, and children in developing countries, are suffering from micronutrient deficiency (Webb *et al*. 2018). It has been estimated that nearly one-third and one-half of the world's population is affected by the deficiency of iron (Fe) (Grimm *et al*. 2012) and Zinc (Zn), respectively (Cakmak 2008). Fe and Zn deficiency may lead to impairments in brain function and damage to the immune system (Cakmak 2008; Yilmaz *et al*. 2017). The strong reliance of developing countries on cereal grains which are low in essential minerals has resulted in health concerns in human beings (White and Broadley 2009). Among cereals, wheat is the most globally grown crop plant that provides food to millions of people all over the world. However, the lack of attention to grain micronutrients improvement during breeding programs had led to poor grain micronutrients content and loss of genetic variability for Fe and Zn among wheat cultivars (Gupta *et al*. 2020). Consequently, there is a great need for improving the Zn and Fe density of wheat grains to minimize micronutrient deficiency-related health problems in humans (Swaminathan 2012). The cause of mineral deficiency is due to the low bioavailability of minerals in the diet (Gharib *et al*. 2006).

One of the inhibitory factors in plant-based food is the presence of phytate (Frontela *et al*. 2011; Yilmaz *et al*. 2017). Phytate in cereals is usually regarded as an anti-nutritional factor (Lorenz *et al*. 2007). In the biofortification program, increasing the nutrient density of food crops by the conventional breeding strategy using candidate grains with proper mineral bioavailability has often been suggested (Bouis 2003). As previously reported, breeding just to increase mineral density in wheat may not be helpful in improving the human diet; rather the programs should concentrate on increasing the bioavailability of minerals through the reduction of the phytate:mineral ratio (Hussain *et al*. 2012a). To determine the mineral bioavailability in grains of wheat cultivars, the phytate:mineral molar ratio should be estimated (Frontela *et al*. 2011; Hussain *et al*. 2012a).

In the present study, the level of grain Fe, Zn, and phytate concentration in some hexaploid landraces and bread wheat cultivars grown in the field was determined to find suitable genotypes to be used in wheat fortifying breeding programs. On the other hand, Fe and Zn are essential micronutrients for plant growth involved in the synthesis of photosynthetic pigments in plants (Song *et al*. 2017; Roosta *et al*. 2018). Therefore, the study was followed in the greenhouse to examine chlorophyll (Chl), Chl fluorescence, concentrations of carotene and carotenoid, and concentrations of Fe and Zn in different plant parts. The parameters investigated could serve as physiological and morphological indicators to screen hexaploid landraces and bread wheat cultivars to determine their potential use in selection programs.

## Materials and Methods

### Plant material and planting conditions for the field screening experiment

For the current study, 109 winter wheat accessions, including 36 modern bread wheat cultivars and 73 hexaploid landraces belonging to different regions of Iran were obtained from the Seed and Plant Improvement Institute, Karaj, Iran (Table 1). The cultivars and landraces were grown at the research station of the Shahed University, Tehran, Iran (35°55′N, 51°34′E, 1032.6 m asl) in the 2016 growing season and were assessed for their grain mineral nutrient concentration of Fe, Zn, and phytate [Experiment (Exp) 1]. Before sowing, random soil samples (0-15 cm depth) were collected from the field. The physico-chemical properties of the soil samples were presented in Table 2.

**Fe, Zn, and phytate concentrations of wheat grains**

The grains of different landraces and cultivars were separated after the harvest (Exp 1). They were grounded immediately after drying at 60 °C, and the samples were put at 550 °C for 8 h. The produced ash was dissolved in 37% (v/v) HCl to determine Zn and Fe by an Atomic Absorption-Flame Emission Spectrophotometer (Aliehyaee and Behbahanizadeh 1993). Phytate was determined according to Lorenz *et al*. (2007). To determine the bioavailability of mineral, the phytate:mineral molar ratio were estimated. The calculation of phytate to Zn and Fe molar ratios was done based on the following equations (Gargari *et al*. 2007):

$$Phytate\ to\ Zn\ molar\ ratio = \frac{Phytate\ (mg/100g)/660}{Zn\ (mg/kg)/65.4}$$

$$Phytate\ to\ Fe\ molar\ ratio = \frac{Phytate\ (mg/100g)/660}{Fe\ (mg/kg)/55.85}$$

**Plant material and conditions for the solution culture experiment**

Wheat seeds of the selected hexaploid landraces from Exp 1 were vernalized for four weeks at 4 °C. Before transferring to pots, the seeds were put on the moist filter paper at room temperature to germinate. Seedlings were transferred to perlite culture and nourished using the nutrient solution of Johnson *et al*. (1957). This pot experiment was conducted using a randomized complete block design with three replicates. There were two pots [diameter across top (22 cm) and bottom (15 cm), vertical height (21 cm)] in each replication, and six plants per pot (Exp 2). Planting was done in mid-July 2017. Seedlings were grown in a growth chamber at 22/18 °C (day/night) with a 16/8 h light/dark cycle, relative humidity of 60/70% (light/dark), and photosynthetic flux density of 400 μmol/m$^2$/s. In the current study, plant growth characteristics, such as root length and dry weight, leaf dry weight, Chl fluorescence, chlorophyll a, chlorophyll b, chlorophyll (a+b), and concentration of carotene and carotenoids as well as Fe and Zn concentrations in roots and leaves were measured. The measurements were done twice, i.e., at 42 days after planting (DAP) and 70-DAP (Exp 2). To determine the grain Fe, Zn, and phytate concentrations in Exp 1 as well as Fe and Zn concentrations of root and leaf in Exp 2, all three replicates were pooled.

**Chl fluorescence**

At each stage (42-DAP and 70-DAP), one day before harvesting, Chl fluorescence was measured at midday (Exp 2) using a PAM Fluorometer (Walz, Effeltrich, Germany). Before the measurements, the leaves were kept in the dark for 30 min. Measurements were made on two seedlings per pot. From each pot, four mature leaves were selected. Readings were taken along the middle section of the leaf, and maximum fluorescence/variable fluorescence (Fm/Fv) was recorded.

**Chlorophyll and carotenoid concentrations**

The chlorophyll concentration was measured at 42 and 70 days after the beginning of the solution treatment (Exp 2). Chlorophyll a and

Table 1. Bread wheat cultivars and hexaploid landraces

| Modern bread wheat cultivars | | | |
|---|---|---|---|
| Mazrae No (1) | Bezostaya (10) | Dez (19) | Cross Shahi (28) |
| Moghan (2) | Azar 2 (11) | Shahriar (20) | Chenab (29) |
| Marvdasht (3) | Omid (12) | Shiraz (21) | Adl (30) |
| Hirmand (4) | Golestan (13) | Khazar 1 (22) | Maron (31) |
| Moghan 2 (5) | Azadi (14) | Niknejad (23) | Akbari (32) |
| Zarin (6) | Arvand (15) | Gaskojen (24) | Zagros (33) |
| Mahdavi (7) | Arta (16) | Falat (25) | Gaspard (34) |
| Shiroudi (8) | Sorkh Tokhm (17) | Varinak (26) | Inia (35) |
| Alamout (9) | Sistan (18) | Kave (27) | Bam (36) |
| **Hexaploid landraces** | | | |
| Orouji Hamseni (37) | Koure Save 2 (56) | Sarouq 4 (75) | Jolge Khalaj Qalavandi 1(94) |
| Mashhad 1 (38) | Arak 1 (57) | Qahqe Sarouq (76) | Jolge Khalaj Qalavandi 2 (95) |
| Mashhad 2 (39) | Arak 2 (58) | Sarouq 5 (77) | Chamanjir Khoramabad (96) |
| Mashhad 3 (40) | Borqan Farahan (59) | Holour Sarouq (78) | Shahabad Oshtoreh (97) |
| Mashhad 4 (41) | Azizabad Farahan (60) | Khoramabad 1 (79) | Nahavand (98) |
| Mashhad 5 (42) | Farmihan Farahan (61) | Khoramabad 2 (80) | Malayer 1 (99) |
| Mashhad 6 (43) | Keshe Farahan (62) | Halekhani (81) | Malayer 2 (100) |
| Mashhad 7 (44) | Ebrahimabad Arak 1(63) | Valadkhani 1 (82) | Touyserkan (101) |
| Mashhad 8 (45) | Ebrahimabad Arak 2 (64) | Valadkhani 2 (83) | Hasanabad Kermanshah (102) |
| Mashhad 9 (46) | Robat Arak 1 (65) | Malmir Ahvaz (84) | Elyasabad Sarepolezahab (103) |
| Mashhad 10 (47) | Robat Arak 2 (66) | Posht Tonk Pariyan 1 (85) | Kermanshah (104) |
| Mashhad 11 (48) | Hoseinabad Arak (67) | Posht Tonk Pariyan 2 (86) | Hajiabad Kermanshah (105) |
| Mashhad 12 (49) | Eskan Arak 1 (68) | Oshtareh (87) | Kordestan (106) |
| Mashhad 13 (50) | Eskan Arak 2 (69) | Khoramabad Khouzestan (88) | Sharafkhaneh (107) |
| Mashhad 14 (51) | Alraj Arak (70) | Malavi Khoramabad Khouzestan (89) | Khoy (108) |
| Mashhad 15 (52) | Laht Olia Arak Borujerd (71) | Malavi (90) | Korkhan Ardabil (109) |
| Molabad Save (53) | Sarouq 1 (72) | Olousbord Save (91) | |
| Parand Save (54) | Sarouq 2 (73) | Hasanabad Save (92) | |
| Koure Save 1 (55) | Sarouq 3 (74) | Malavi Khouzestan (93) | |

Figures within each parenthesis were used in the scatter plot

Table 2. Physico-chemical properties of the soil

| Soil texture | Sandy-loam |
|---|---|
| pH | 7.45 |
| Ec [ds/m] | 2.51 |
| T.N.V [%] | 12.5 |
| P [mg/kg] | 40.83 |
| K [mg/kg] | 533 |
| S [mg/kg] | 29 |
| Zn [mg/kg] | 0.7 |
| B [mg/kg] | 1.15 |
| Fe [mg/kg] | 7.57 |
| Cu [mg/kg] | 0.81 |
| Mn [mg/kg] | 1.73 |

chlorophyll b were determined by extracting the leaf material in 80% (v/v) acetone and then the absorbance was read at 663, 645, and 470 nm for chlorophyll a, chlorophyll b, and carotenoids, respectively, using Cecil CE9500 Super Aquarius UV/VIS Dual Beam Spectrophotometer (Birmingham, West Midlands, UK). Finally, total chlorophyll (a+b) was calculated using Arnon's (1949) equations (Tourian *et al*. 2013) as shown below:

$$Chla\,(mg/g) = [(12.7 \times Abs_{663}) - (2.6 \times Abs_{645})] \times ml\ Acctone/mg$$

$$Chlb\,(mg/g) = [(22.9 \times Abs_{645}) - (4.68 \times Abs_{663})] \times ml\ Acctone/mg$$

$$Chltotal\,(mg/g) = Chla + Chlb$$

$$Carotenoids = (100 \times (Abs_{470}) + 3.27 \times (mg\ Chla)) - (104 \times (mg\ Chlb)/227)$$

**Statistical analysis**

The standard curves to predict grain Fe, Zn, and phytate of 109 modern bread wheat cultivars and landraces according to the concentration of known standards versus the standard optical density (OD), were prepared on Microsoft Excel 2007®. The Fe, Zn, and phytate concentration data were subjected to student's t-test analysis. Analyses of variance (ANOVA) of the selected hexaploid landraces for eight traits were performed using SPSS software, version 19.0, and the means were compared by Duncan's new multiple range test at 5% probability level at 42 and 70-DAP. R studio software version 4.0.0 (R Core Team 2020) was used to construct a scatter plot for grain phytate concentration (mg/g) and phytate:mineral molar ratio to study the relationships among selected 109 hexaploid landraces and modern bread wheat cultivars. Cluster analysis was performed by dendextend package (Galili 2015), using Ward's method based on squared Euclidean distance. To calculate the Pearson correlation coefficients among traits, the ggcorrplot package (Kassambara 2019) was used.

**Results**

**The field screening experiment**

The relationship of the concentration of known standards versus OD was linear with the $R^2$ of 0.999, 0.995, and 0.984 for Fe, Zn, and phytate, respectively (Supplemental Figures S1, S2, and S3). The strong linear relationship of OD with the standard concentrations indicates that it is possible to accurately predict the relative concentration of Fe, Zn, and phytate by these standards.

**Grain Fe and Zn concentrations**

Among the 109 modern bread wheat cultivars and hexaploid landraces, the concentration of grain Fe ranged from 0.036 to 0.255 mg/g and grain Zn ranged from 0.030 to 0.085 mg/g (Table 3). Highly remarkable contents and significant differences among the experimental entries for Fe and Zn concentrations ($p \leq 0.01$) were observed (Supplemental Table S1). There were no significant differences in Fe and Zn concentration between the bread wheat cultivars and hexaploid landraces ($p = 0.486$, $p = 0.274$, respectively). The average Fe and Zn concentrations in hexaploid landraces were 0.083 and 0.047 mg/g, respectively, and the average of Fe and Zn concentration in the modern bread

Table 3. Means (±SE) and the range of Fe, Zn, phytate, phytate:Fe, and phytate:Zn of 109 wheat landraces and cultivars

| Wheat | Range and mean ± SE | Phytate [mg/100 g] | Fe [mg/100 g] | Zn [mg/100 g] | Phytate:Fe [molar ratio] | Phytate:Zn [molar ratio] |
|---|---|---|---|---|---|---|
| Modern bread wheat cultivars n = 36 | Min | 1844.3 | 5.16 | 3.51 | 15.78 | 32.24 |
| | Max | 2876.9 | 11.18 | 6.94 | 43.24 | 63.57 |
| | Mean ± SE | 2268.1 ± 45.5 | 7.90 ± 0.28 | 4.82 ± 0.15 | 25.43 ± 1.11 | 47.93 ± 1.58 |
| Hexaploid landraces n = 73 | Min | 1506.8 | 3.59 | 3.00 | 6.25 | 27.65 |
| | Max | 2748.5 | 25.53 | 8.5 | 58.14 | 72.22 |
| | Mean ± SE | 2101.3 ± 32.1 | 8.26 ± 0.34 | 4.67 ± 0.11 | 24.00 ± 1.01 | 46.15 ± 1.17 |
| *p*-value | | 0.009 | 0.483 | 0.274 | 0.465 | 0.689 |

wheat cultivars were 0.079 and 0.048 mg/g, respectively (Table 3).

**Phytate concentration and phytate:Fe and phytate:Zn molar ratios**

In the present study, the hexaploid landraces showed a significantly lower phytate concentration ($p = 0.009$) than the modern bread wheat cultivars. The phytate concentration averaged 21.01 mg/g, ranging from 15.07 mg/g to 27.48 mg/g in the hexaploid landraces, whereas in the modern bread wheat cultivars the average phytate concentration was 22.68 mg/g, ranging from 18.44 mg/g to 28.77 mg/g. There were no significant differences in phytate:Fe and phytate:Zn molar ratios between the modern bread wheat cultivars and hexaploid landraces, ($p = 0.465$, $p = 0.489$, respectively). The phytate:Fe and phytate:Zn molar ratios in the hexaploid landraces were 24 and 46.15, respectively, and the phytate:Fe and phytate:Zn molar ratios in the modern bread wheat cultivars were 25.43 and 48.22, respectively (Table 3). Using cluster analysis, the 109 bread wheat cultivars and hexaploid landraces (Exp 1) were grouped into three clusters (Supplemental Table S1). Multivariate analysis of variance based on Wilks' Lambda and Roy's Largest Root showed significant differences ($p \leq 0.001$) among the cluster means, which indicated the efficiency of the clustering method in differentiating the clusters. The largest difference was found between the means of the $G_1$ and the $G_3$ clusters for the Fe and phytate:Fe molar ratio (Supplemental Table S1). According to the results of cluster analysis and the scatter plot diagram (Supplemental Figure S4), 14 hexaploid landraces (Table 4) with high and low levels of Fe concentration, having low phytate:Fe molar ratio were selected for further analysis (Exp 2). As well, such a selection was performed for hexaploid landraces with high and low levels of Zn concentration, having low phytate:Zn molar ratio (Supplemental Figure S5; Table 5).

Table 4. Hexaploid landraces with high and low levels of Fe, having low phytate concentration and phytate:Fe molar ratio

| Selected wheat groups | Hexaploid landraces | Phytate [mg/100 g] | Fe [mg/100 g] | Phytate:Fe [molar ratio] |
|---|---|---|---|---|
| Low phytate concentration, high Fe, and low phytate:Fe molar ratio | Halekhani | 1883.12 | 25.53 | 6.24 |
| | Sarouq 4 | 1859.58 | 15.49 | 10.16 |
| | Malayer 1 | 2021.87 | 15.45 | 11.08 |
| | Eskan Arak 2 | 1644.56 | 11.93 | 11.67 |
| | Hoseinabad Arak | 1789.62 | 10.88 | 13.93 |
| | Mashhad 3 | 1596.20 | 10.03 | 13.48 |
| Low phytate concentration, low Fe and low phytate:Fe molar ratio | Khoramabad Khuzestan | 1832.93 | 3.59 | 43.24 |
| | Qahqahe Sarouq | 1906.58 | 3.96 | 27.09 |
| | Valadkhani 2 | 1816.98 | 5.10 | 30.17 |
| | Posht Tonok Pariyan 2 | 1664.62 | 6.19 | 22.78 |
| | Koure Save 1 | 1858.24 | 6.89 | 22.84 |
| | Mashhad 6 | 1529.66 | 7.37 | 17.58 |
| | Hajiabad Kermanshah | 1506.74 | 7.47 | 17.08 |
| | Mashhad 12 | 1606.31 | 7.62 | 17.85 |

Table 5. Hexaploid landraces with high and low levels of Zn, having low phytate concentration and phytate:Zn molar ratio

| Selected wheat groups | Hexaploid landraces | Phytate [mg/100 g] | Zn [mg/100 g] | Phytate: Zn [molar ratio] |
|---|---|---|---|---|
| Low phytate concentration, high Zn, and low phytate:Zn molar ratio | Khoramabad 1 | 2372.19 | 8.50 | 27.65 |
| | Shahriar | 2258.10 | 6.94 | 32.24 |
| | Laht Olia Arak Boroujerd | 2165.70 | 6.55 | 32.76 |
| | Jolge Khalaj Qalavandi 1 | 2034.39 | 6.26 | 32.20 |
| | Mashhad 12 | 1606.31 | 5.21 | 30.55 |
| | Eskan Arak 2 | 2053.09 | 4.91 | 33.18 |
| | Ebrahimabad Arak 1 | 2383.42 | 4.66 | 33.22 |
| Low phytate concentration, low Zn and low phytate:Zn molar ratio | Mashhad 15 | 1529.66 | 3.05 | 49.69 |
| | Posht Tonok Pariyan 2 | 1664.62 | 3.50 | 47.12 |
| | Koure Save 2 | 1647.83 | 3.81 | 42.85 |
| | Hajiabad Kermanshah | 1506.75 | 3.83 | 38.98 |
| | Hirmand | 1934.31 | 3.85 | 49.78 |

**The solution culture experiment**

ANOVA revealed a significant difference ($p \leq 0.01$) among the selected genotypes in root length at 42-DAP, and in root length, root dry weight, chlorophyll, carotene, and carotenoid concentrations at 70-DAP (Supplemental Table S2). Therefore, 70-DAP was a preferable stage to differentiate among cultivars and landraces. The lowest root length and root dry weight among the 14 selected hexaploid landraces was noted for Khoramabad Khouzestan at 70-DAP (Tables 6 and 7). Khoramabad Khouzestan with the low root Fe concentration and the lowest leaf Fe concentration had the lowest leaf dry weight, chlorophyll, carotene, and carotenoid concentrations at 70-DAP among 14 selected hexaploid landraces (Tables 6, 7, and Supplemental Table S3). Hoseinabad Arak, Valadkhani 2, and Mashhad 12 with the high leaf Fe concentration had high values of leaf dry weight, chlorophyll, carotene, carotenoid, and Fv:Fm at 70-DAP (Tables 6 and 7).

**Cluster analysis and cluster properties (42-DAP)**

Using cluster analysis (Figure 1) based on the eight studied traits, the selected 14 hexaploid landraces were grouped into three clusters (Supplemental Table S4). The largest difference was found between the means of the $G_1$ and the $G_3$ clusters for root length, root Fe, and the leaf Fe and the differences were significant at $p \leq 0.05$. There were no significant differences between the means of these two groups regarding other examined traits.

**Cluster analysis and cluster properties (70-DAP)**

Three clusters were formed through cluster analysis (Figure 2). The G1 cluster had a higher amount of root and leaf dry weight, chlorophyll (a+b), leaf Fe concentration, and Fv:Fm than the G3 cluster (Supplemental Table S5). The difference among the groups of the hexaploid landraces in leaf Fe, root and leaf dry weight, chlorophyll, and Fv:Fm was significant ($p \leq 0.01$). Hoseinabad Arak showed the largest differences with Khoramabad Khouzestan (Table 7).

**Correlation coefficients at 42-DAP and 70-DAP**

There were significant correlations between root dry weight and leaf dry weight ($r$ = 0.77$^{**}$), root dry weight and stem dry weight ($r$ = 0.82$^{**}$), and between stem dry weight and leaf dry weight ($r$ = 0.58$^{*}$) at 42-DAP (Figure 3). Also, a highly positive correlation between chlorophyll and carotenoids was observed. In contrast, there was no significant correlation between Fv:Fm and the content of the pigments. At 72-DAP (Figure 4) leaf Fe had high and positive correlations with Fv:Fm ($r$ = 0.686$^{**}$), leaf dry weight ($r$ = 0.809$^{**}$), root dry weight ($r$ = 669$^{**}$), chlorophyll a ($r$ = 0.539$^{*}$), and chlorophyll (a+b) ($r$ = 0.540). Leaf dry weight had a significant correlation with root dry weight (0.890**) and root length (0.577*). Moreover, a highly significant correlation was obtained between Fv:Fm and pigments content.

**Discussion**

**The field screening experiment**

**Grain micronutrient concentrations**

Regarding several reports, the average Zn concentration in the wheat whole grain of various countries was between 0.020 to 0.035 mg/g (Cakmak *et al*. 2008) and the average Fe concentration in cereal grains was reported 0.01 to 0.05 mg/g (Frontela *et al*. 2011). As reported previously, grain Zn was significantly correlated to the soil Zn concentration (Karami *et al*. 2009; Hussain *et al*. 2012b), and the more Zn-deficient soil, the lower grain Zn concentrations occurred

Table 6. Differences in mean values for 10 traits in 14 selected hexaploid landraces (8 low-Fe and 6 high-Fe) at 42 days after planting

| Hexaploid Landraces | RL [m] | RDW [g] | SDW [g] | LDW [g] | Chla [mg/g] | Chlb [mg/g] | Chl(a+b) [mg/g] | Carotene [mg/g] | Carotenoid [mg/g] | Fv:Fm *100 |
|---|---|---|---|---|---|---|---|---|---|---|
| Mashhad 6 | 0.445$^{ab}$ | 0.026$^{ab}$ | 6.66$^{bc}$ | 0.051$^{ab}$ | 0.640$^{c}$ | 0.225$^{c}$ | 0.952$^{c}$ | 2.183$^{b}$ | 2.164$^{b}$ | 71.49$^{c}$ |
| Khoramabad Khuzestan | 0.279$^{c}$ | 0.020$^{abc}$ | 7.46b | 0.051$^{ab}$ | 0.794$^{bc}$ | 0.287$^{bc}$ | 1.049$^{bc}$ | 2.198$^{b}$ | 2.179$^{b}$ | 72.32$^{bc}$ |
| Posht Tonok Pariyan 2 | 0.448$^{ab}$ | 0.013$^{c}$ | 6.20$^{bcd}$ | 0.033$^{bc}$ | 0.824$^{bc}$ | 0.304$^{bc}$ | 1.241$^{bc}$ | 2.672$^{b}$ | 2.649$^{b}$ | 73.98$^{ab}$ |
| Sarouq 4 | 0.422$^{ab}$ | 0.016$^{abc}$ | 4.56$^{cd}$ | 0.033$^{bc}$ | 0.850$^{bc}$ | 0.303$^{bc}$ | 1.119$^{bc}$ | 2.196$^{b}$ | 2.177$^{b}$ | 72.66$^{abc}$ |
| Hoseinabad Arak | 0.409$^{ab}$ | 0.022$^{abc}$ | 6.70$^{bc}$ | 0.053$^{ab}$ | 0.877$^{abc}$ | 0.321$^{bc}$ | 1.163$^{bc}$ | 2.350$^{b}$ | 2.329$^{b}$ | 71.94$^{bc}$ |
| Koure Save 1 | 0.392$^{b}$ | 0.020$^{abc}$ | 5.79$^{bcd}$ | 0.040$^{abc}$ | 0.957$^{abc}$ | 0.335$^{abc}$ | 1.253$^{bc}$ | 2.638$^{b}$ | 2.615$^{b}$ | 73.27$^{abc}$ |
| Mashhad 3 | 0.425$^{ab}$ | 0.019$^{abc}$ | 5.75$^{bcd}$ | 0.040$^{abc}$ | 0.963$^{abc}$ | 0.342$^{abc}$ | 1.267$^{bc}$ | 2.530$^{b}$ | 2.508$^{b}$ | 72.63$^{bc}$ |
| Hajiabad Kermanshah | 0.282$^{c}$ | 0.025$^{abc}$ | 6.03$^{bcd}$ | 0.044$^{abc}$ | 0.963$^{abc}$ | 0.359$^{abc}$ | 1.455$^{abc}$ | 3.109$^{ab}$ | 3.082$^{ab}$ | 73.00a$^{bc}$ |
| Halekhani | 0.420$^{ab}$ | 0.034$^{a}$ | 9.49$^{a}$ | 0.043$^{abc}$ | 0.976$^{abc}$ | 0.331$^{abc}$ | 1.269$^{bc}$ | 2.668$^{b}$ | 2.645$^{b}$ | 73.45$^{abc}$ |
| Malayer 1 | 0.437$^{ab}$ | 0.014$^{c}$ | 4.44$^{d}$ | 0.027$^{bc}$ | 1.016$^{abc}$ | 0.362$^{abc}$ | 1.517$^{abc}$ | 3.193$^{ab}$ | 3.165$^{ab}$ | 72.12$^{bc}$ |
| Eskan Arak 2 | 0.392$^{b}$ | 0.009$^{c}$ | 4.79$^{cd}$ | 0.022$^{c}$ | 1.022$^{abc}$ | 0.388$^{ab}$ | 1.368$^{abc}$ | 2.722$^{b}$ | 2.698$^{b}$ | 71.85$^{bc}$ |
| Mashhad 12 | 0.495$^{a}$ | 0.031$^{a}$ | 7.13$^{b}$ | 0.063$^{a}$ | 1.195$^{ab}$ | 0.420$^{ab}$ | 1.568$^{ab}$ | 3.001$^{ab}$ | 2.975$^{ab}$ | 75.83$^{a}$ |
| Valadkhani 2 | 0.432$^{ab}$ | 0.019$^{abc}$ | 6.04$^{bcd}$ | 0.045$^{abc}$ | 1.199$^{ab}$ | 0.438$^{ab}$ | 1.589$^{ab}$ | 3.186$^{ab}$ | 3.158$^{ab}$ | 74.78$^{ab}$ |
| Qahqahe Sarouq | 0.408$^{ab}$ | 0.020$^{abc}$ | 5.91$^{bcd}$ | 0.040$^{abc}$ | 1.280$^{a}$ | 0.477$^{a}$ | 1.933$^{a}$ | 3.939$^{a}$ | 3.905$^{a}$ | 72.04$^{bc}$ |

Means followed by the same letters in columns are not different significantly at $p \leq 0.05$ according to the Duncan's multiple range test.
Abbreviations: RL = root length; RDW = root dry weight; SDW = stem dry weight, LDW = leaf dry weight; Chl = chlorophyll.

Table 7. Differences in mean values for 10 traits in 14 selected hexaploid landraces (8 low-Fe and 6 high-Fe) at 70 days after planting

| Hexaploid Landraces | RL [m] | RDW [g] | SDW [g] | LDW [g] | Chla [mg/g] | Chlb [mg/g] | Chl(a+b) [mg/g] | Carotene [mg/g] | Carotenoid [mg/g] | Fv:Fm *100 |
|---|---|---|---|---|---|---|---|---|---|---|
| Mashhad 6 | 0.401$^{abc}$ | 0.095$^{ab}$ | 11.48$^{ab}$ | 0.091$^{abc}$ | 0.381$^{f}$ | 0.157$^{cdef}$ | 0.538$^{de}$ | 1.27$^{d}$ | 1.26$^{d}$ | 71.7$^{b}$ |
| Khoramabad Khuzestan | 0.253$^{e}$ | 0.028$^{e}$ | 8.47$^{bcd}$ | 0.056$^{c}$ | 0.373$^{f}$ | 0.129$^{f}$ | 0.503$^{e}$ | 1.26$^{d}$ | 1.25$^{d}$ | 72.3$^{ab}$ |
| Posht Tonok Pariyan 2 | 0.400$^{abc}$ | 0.048$^{de}$ | 7.15$^{d}$ | 0.076$^{bc}$ | 0.834$^{ab}$ | 0.292$^{ab}$ | 1.127$^{abc}$ | 2.49$^{ab}$ | 2.47$^{ab}$ | 74.0$^{ab}$ |
| Sarouq 4 | 0.418$^{abc}$ | 0.058$^{bcde}$ | 8.90$^{bcd}$ | 0.083$^{bc}$ | 0.538$^{def}$ | 0.173$^{cdef}$ | 0.711$^{de}$ | 1.58$^{cd}$ | 1.57$^{cd}$ | 72.7$^{ab}$ |
| Hoseinabad Arak | 0.406$^{abc}$ | 0.113$^{a}$ | 11.02$^{abc}$ | 0.150$^{a}$ | 0.824$^{abc}$ | 0.295$^{ab}$ | 1.163$^{ab}$ | 2.50$^{ab}$ | 2.48$^{ab}$ | 72.0$^{ab}$ |
| Koure Save 1 | 0.335$^{bcde}$ | 0.045$^{de}$ | 8.75$^{bcd}$ | 0.085$^{bc}$ | 0.634$^{bcde}$ | 0.232$^{bcd}$ | 0.866$^{bcde}$ | 1.93$^{bcd}$ | 1.92$^{bcd}$ | 73.3$^{ab}$ |
| Mashhad 3 | 0.435$^{ab}$ | 0.091$^{abc}$ | 10.77$^{abc}$ | 0.118$^{ab}$ | 0.656$^{bcde}$ | 0.225$^{bcde}$ | 0.882$^{bcd}$ | 2.03$^{bc}$ | 2.01$^{bc}$ | 72.6$^{ab}$ |
| Hajiabad Kermanshah | 0.262$^{de}$ | 0.051$^{cde}$ | 7.59$^{cd}$ | 0.090$^{bc}$ | 0.652$^{bcde}$ | 0.208$^{bcdef}$ | 0.860$^{bcde}$ | 1.97$^{bcd}$ | 1.95$^{bcd}$ | 73.0$^{ab}$ |
| Halekhani | 0.353$^{abcde}$ | 0.038$^{de}$ | 12.83$^{a}$ | 0.076$^{bc}$ | 0.574$^{cdef}$ | 0.192$^{cdef}$ | 0.766$^{cde}$ | 1.83$^{bcd}$ | 1.82$^{bcd}$ | 73.5$^{ab}$ |
| Malayer 1 | 0.408$^{abc}$ | 0.073$^{abcd}$ | 8.71$^{bcd}$ | 0.113$^{abc}$ | 0.587$^{bcdef}$ | 0.224$^{bcde}$ | 0.811$^{bcde}$ | 1.81$^{bcd}$ | 1.80$^{bcd}$ | 72.1$^{ab}$ |
| Eskan Arak 2 | 0.320$^{cde}$ | 0.045$^{de}$ | 7.77$^{cd}$ | 0.066$^{bc}$ | 0.663$^{bcde}$ | 0.134$^{ef}$ | 0.826$^{bcde}$ | 1.61$^{cd}$ | 1.60$^{cd}$ | 71.9$^{ab}$ |
| Mashhad 12 | 0.361$^{abcd}$ | 0.078$^{abcd}$ | 9.81$^{abcd}$ | 0.111$^{abc}$ | 0.775$^{abcd}$ | 0.247$^{bc}$ | 1.089$^{abc}$ | 2.42$^{ab}$ | 2.40$^{ab}$ | 75.8$^{a}$ |
| Valadkhani 2 | 0.452$^{a}$ | 0.073$^{abcd}$ | 9.07$^{bcd}$ | 0.110$^{abc}$ | 0.933$^{a}$ | 0.341$^{a}$ | 1.325$^{a}$ | 2.83$^{a}$ | 2.81$^{a}$ | 74.8$^{ab}$ |
| Qahqahe Sarouq | 0.330$^{bcde}$ | 0.063$^{bcde}$ | 9.02$^{bcd}$ | 0.096$^{abc}$ | 0.417$^{ef}$ | 0.144$^{def}$ | 0.561$^{de}$ | 1.26$^{d}$ | 1.25$^{d}$ | 72.0$^{ab}$ |

Means followed by the same letters in columns are not different significantly at $p \leq 0.05$ according to the Duncan's multiple range test.
Abbreviations: RL = root length; RDW = root dry weight; SDW = stem dry weight, LDW = leaf dry weight; Chl = chlorophyll

in wheat cultivars (Velu *et al*. 2016; Liu *et al*. 2019). High soil pH, especially above 6.5, decreases the plant accessibility of soil Zn. Also, accessibility of soil Zn is negatively related to soil phosphate and calcium carbonate content (Rengel 2015). All these issues led Iran to be considered a region with Zn-deficient soils (Cakmak *et al*. 2008). However, higher content of Fe and Zn concentration in some genotypes indicated the potential of improving the Iranian bread wheat cultivars for grain mineral content. A recent study by Khokhar *et al.* (2018) showed that the concentration of grain Fe varied from 0.028 to 0.042 mg/g and the grain Zn concentration from 0.026 to 0.032 mg/g among 36 Indian wheat genotypes. According to Hussain *et al.* (2012a), grain Fe concentration ranged from 0.032 to 0.046 mg/g and for Zn from 0.024 to 0.036 mg/g in 40 Pakistani bread wheat cultivars. Karami *et al*. (2009) also reported that the grain Fe concentration ranged from 0.021 to 0.096 mg/g and grain Zn from 0.012 to 0.064 mg/g among the wheat varieties grown in Central Iran. Morgounov *et al.* (2007) reported that the concentration of

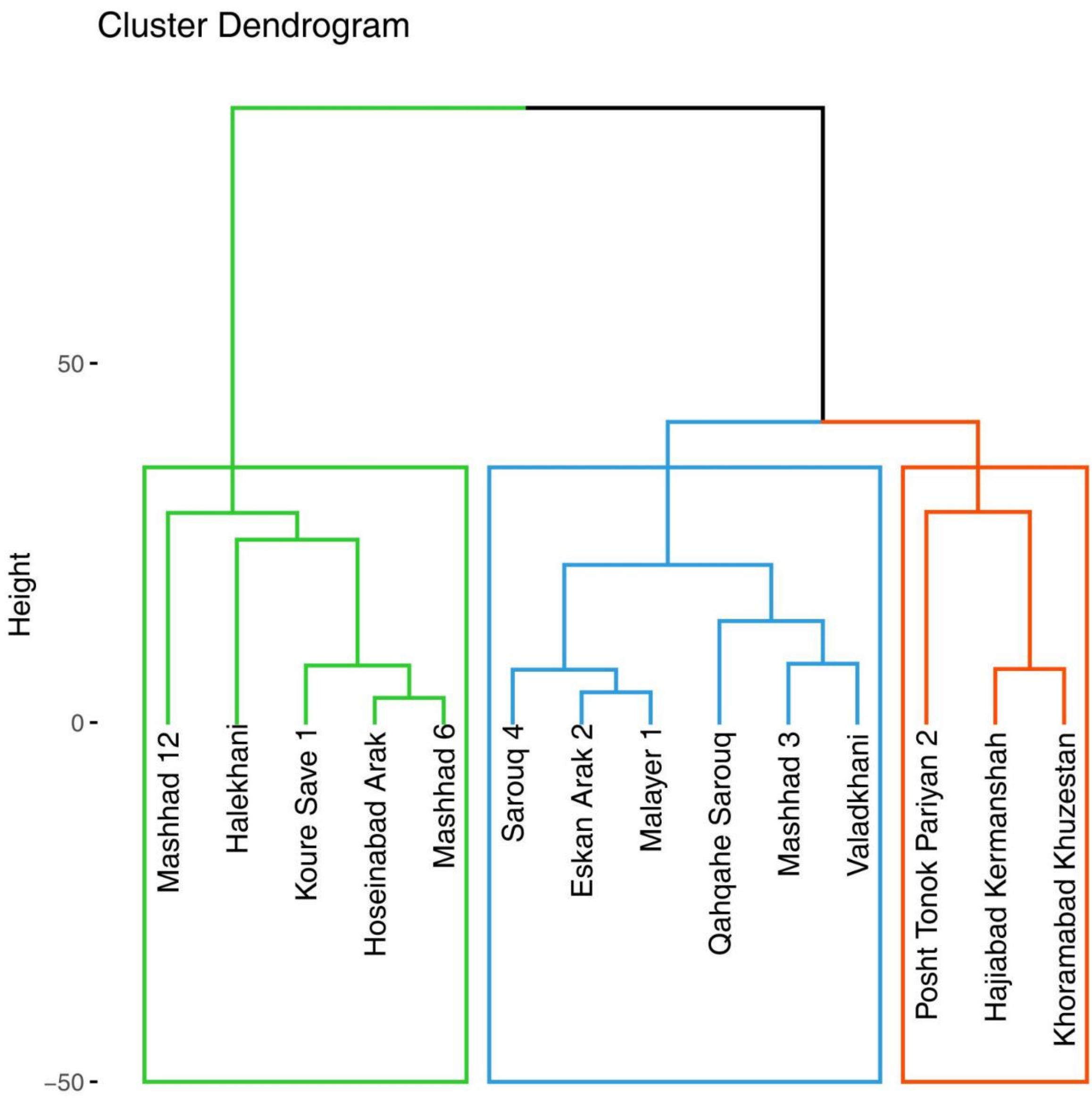


Figure 1. Dendrogram showing the relationships among the selected 14 hexaploid landraces at 42-DAP in terms of eight studied traits using Ward method and squared Euclidean distance; $G_1$ (GREEN): members of the 1st cluster, $G_2$ (BLUE): members of the 2nd cluster, $G_3$: (RED): members of the 3rd cluster

grain Fe varied from 0.025 to 0.056 mg/g and grain Zn from 0.020 to 0.039 mg/g among the 66 wheat genotypes of Central Asia. In the present study, among the 109 modern bread wheat cultivars and hexaploid landraces of Iran, the concentration of grain Fe ranged from 0.036 to 0.255 mg/g and grain Zn from 0.030 to 0.085 mg/g. This is while the soil Zn concentration in the study of Hussain *et al.* (2012a), with relatively similar soil characteristics (soil texture, pH, EC) to the present study, was higher (3.6 mg per kg soil dry weight) than the soil Zn

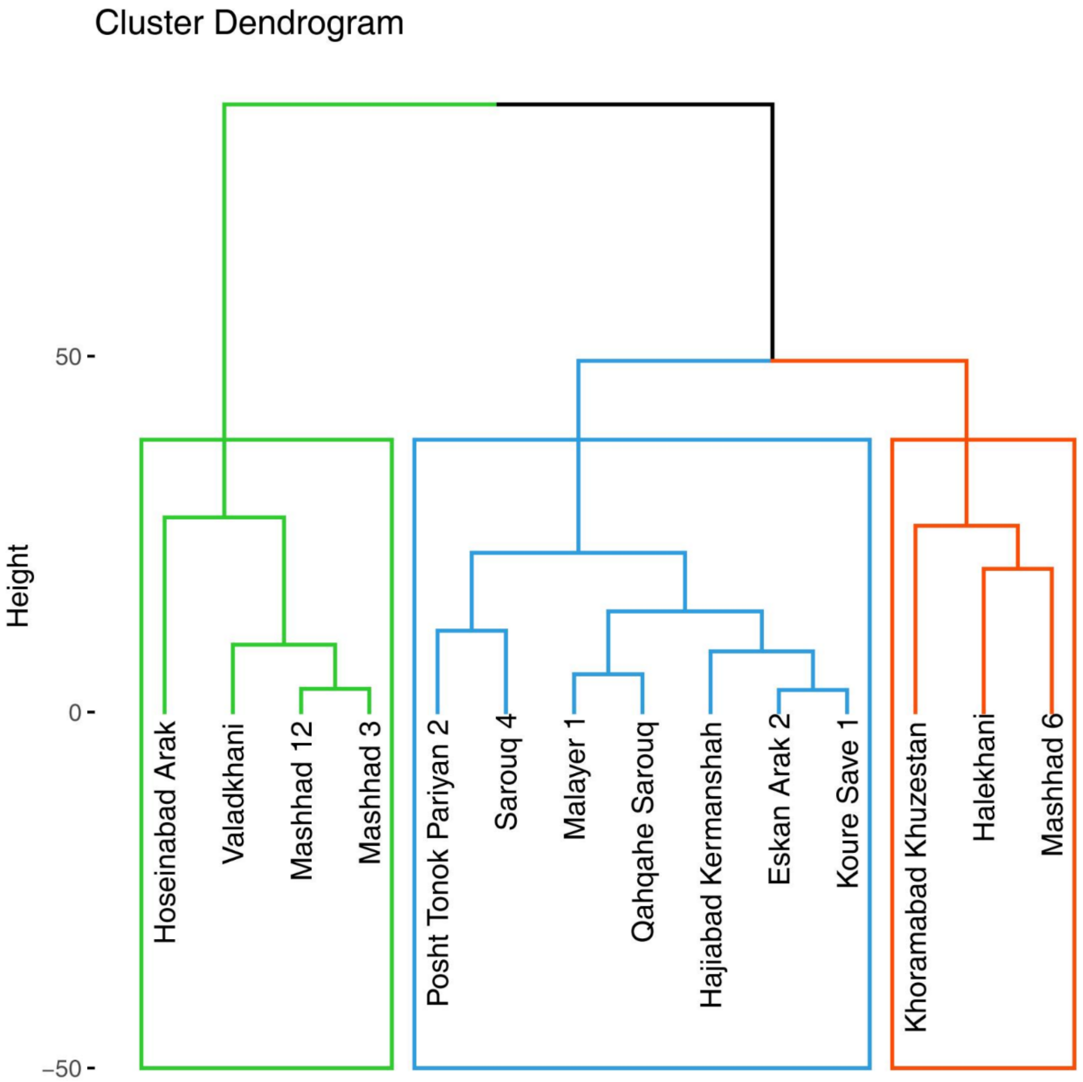


Figure 2. Dendrogram showing the relationships among the selected 14 hexaploid landraces at 70-DAP in terms of eight studied traits using Ward method and squared Euclidean distance; (G1 (GREEN): members of the 1$^{st}$ cluster, G2 (BLUE): members of the 2$^{nd}$ cluster, G3: (RED): members of the 3$^{rd}$ cluster

concentration in the present study (0.7 mg per kg soil dry weight). Similarly, the soil Zn concentration in the study of Karami *et al.* (2009) (2.33 mg per kg soil dry weight), with almost similar soil phosphorus concentration (42.10 mg per kg soil dry weight) to the present study (40.83 mg per kg soil dry weight), was higher than the soil Zn concentration in the current study. Given the soil pH of 8.62, phosphorus concentration of 40.83 mg P per Kg soil dry weight, and

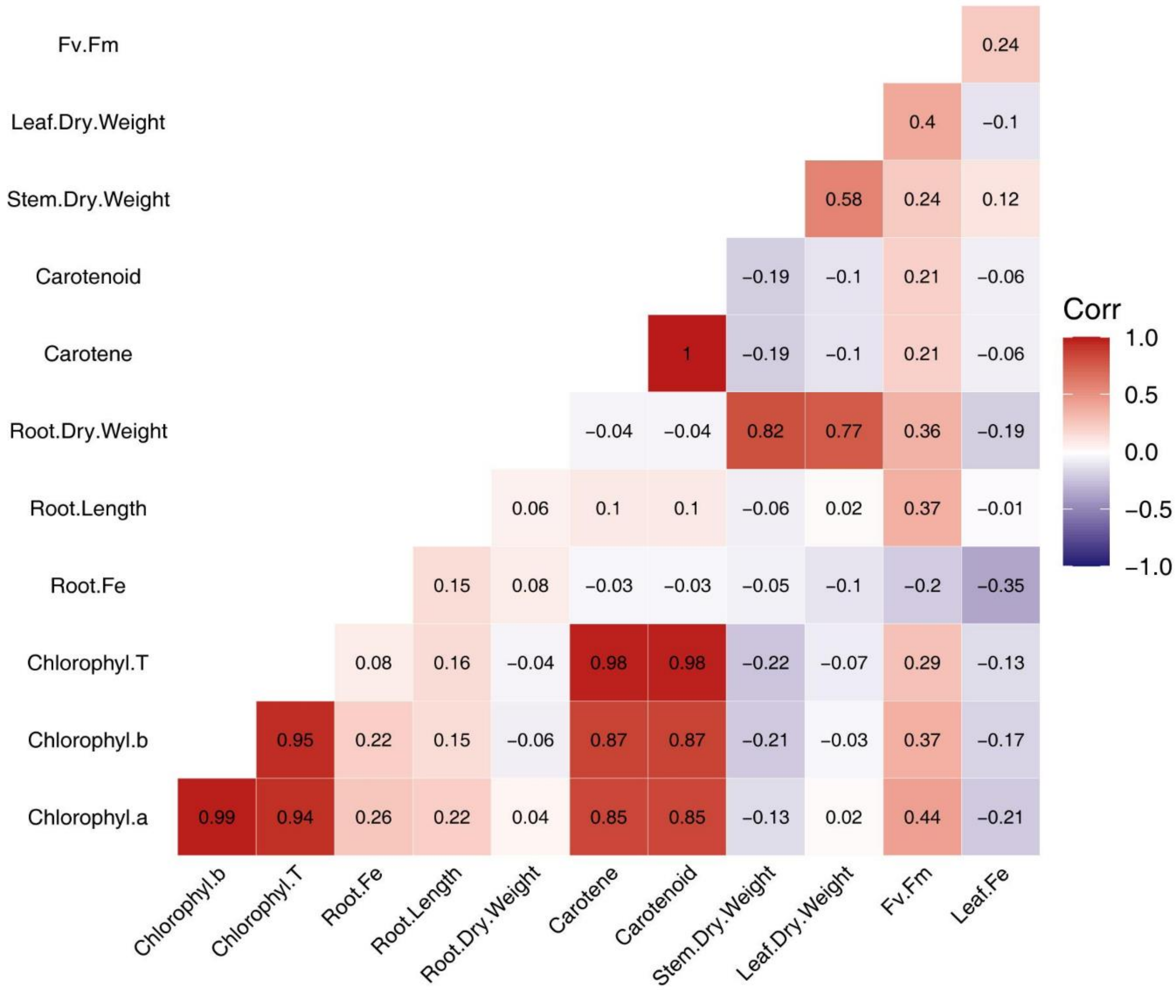


Figure 3. The heatmap of Pearson correlation coefficients among traits for 14 selected hexaploid landraces at 42 days after planting

percentage of $CaCO_3$ (12.5%) in our soil, the response of modern bread wheat cultivars in Iran to low plant availability of Zn in the calcareous soils of Iran is remarkable. As compared with Hussain *et al.* (2012a), the soil Fe concentration was higher in the present study, and Iranian modern bread wheat cultivars showed also a greater grain Fe concentration. Significant differences among the Iranian bread wheat cultivars in Fe and Zn concentrations indicated the high potential for grain mineral content improvement. On the other hand, studies have reported that breeding efforts have mainly increased yield and disease resistance without improving the concentration of micronutrients in the grain (Gomez-Coronado *et al*. 2016; Yilmaz *et al*. 2017). As a result, modern wheat cultivars possess lower concentrations of Fe and Zn in grains as compared to old varieties (Velu *et al*. 2017). In the present study, no significant differences in Fe and Zn concentration between the bread wheat cultivars and hexaploid landraces

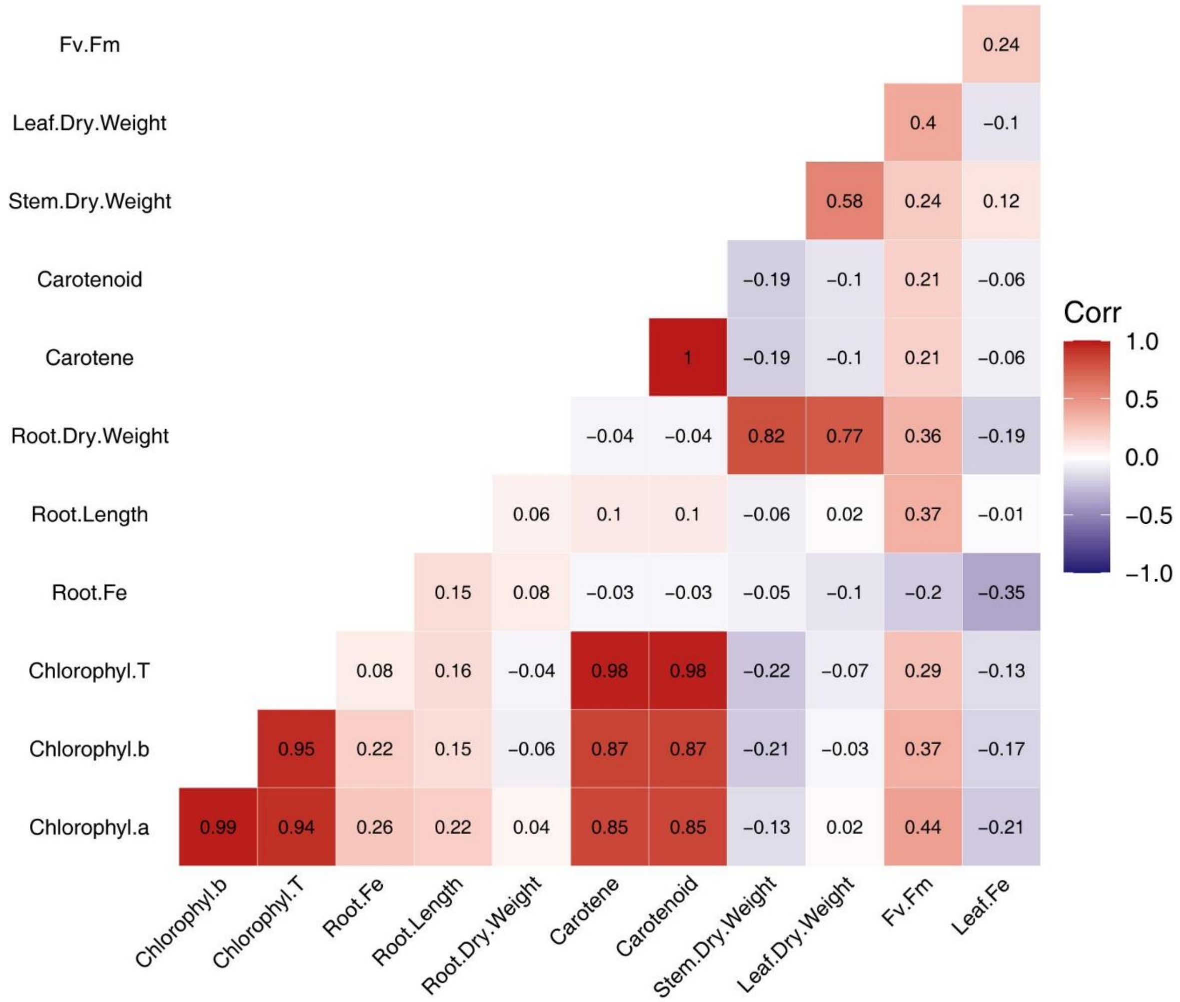


Figure 4. The heatmap of Pearson correlation coefficients among traits for 14 selected hexaploid landraces at 70 days after planting

were observed. Therefore, it seems that the efforts made by breeders for developing high-yielding disease-resistant wheat didn't have detrimental effects on Fe and Zn concentrations of the Iranian modern bread wheat cultivars. Therefore, it is possible to integrate high yield and high Fe and Zn concentrations in the breeding programs.

**Phytate concentration**

It has been shown that the phytate concentration affects the Fe and Zn bioavailability and therefore, it is essential to breed crops with minimum phytate concentration to improve the nutritional value of the foods. Liu *et al.* (2006) showed that the mean phytate concentration was 7.80 mg/g, with a range of 5.16 to 9.87 mg/g among 186 wheat genotypes from different areas of China. Hussain *et al.* (2012a) reported that the mean phytate concentration of 9 mg/g in the grains of 40 Pakistani bread wheat cultivars with the range of 7 to 11 mg/g. In the present study, the figures obtained (15.07 to 28.77 mg/g) were almost up to two times higher than in the aforementioned studies. This range was almost similar to that found by Gabaza *et al.* (2018) (7.03 to 21.97 mg/g) in a set of maize, red and white sorghum, and pearl and red finger millet, and the one reported by Kumar *et al.* (2017) (8.2 to 26.2 mg/g) in 32 rice cultivars cultivated mainly in the eastern part of India. Also, Lee *et al.* (2015) reported a similar range of phytate (18.27 to 32.36 mg/g) in 30 Bario rice cultivars. As reported previously, the phytate concentration in grains is highly dependent on the proportion of the root uptake of phosphorus (Erdal *et al*. 2002). Regarding 40.83 mg P per kg soil dry weight, it is possible therefore that the observed excess of phytate concentration in grains of wheat cultivars in the current study may arise from the phosphorus absorbed during grain development. This difference may also be attributable to the different methods and conditions applied: solvents, pH, time used to extract phytate (Saad *et al*. 2011), and the effect of environmental and varietal differences on phytate concentration (Lee *et al*. 2014; Bilal *et al*. 2018). Based on the result obtained in the current study, the mean phytate concentration in the hexaploid landraces was somewhat lower than the modern cultivars (21.01 mg/g against 22.68 mg/g, respectively). Bilal *et al.* (2018) evaluated the phytate and phosphorus concentrations of 57 wheat genotypes and showed that old wheat varieties had lower phytate concentrations than the latest varieties released. A likely explanation for this is that breeders applied strong selection to achieve increased grain phosphorus and did not consider the phytate concentration (Bilal *et al*. 2018). As a result, breeding schemes should be designed to select the cultivars with low phytate concentration in grains (Kumar *et al*. 2017).

**Phytate:Fe and phytate:Zn molar ratios**

According to Sakai *et al.* (2015), given that Fe might be bound to phytate even in low-phytate grains, having only small amounts of phytate in grains could not greatly increase Fe utilization. Hence, breeders need to consider the phytate:mineral ratio in wheat grains as well. The relationship between phytate and Zn bioavailability has also been reported in other studies (Hussain *et al*. 2012a; Gomez-Coronado *et al*. 2016). Therefore, to carry out a breeding process with the view of improving human nutrition, it is necessary to know the phytate:mineral ratio in wheat grains. In general, screening of cultivars with low phytate rates and high mineral bioavailability is a suitable approach to serving people suffering from micronutrient malnutrition (Kumar *et al*. 2017). Erdal *et al.* (2002) found the phytate:Zn molar ratio of 56 to 126 for 20 wheat cultivars grown in Central Anatolia and from 29 to 178 for four wheat cultivars grown in 55 different locations in Turkey. Hussain *et al.* (2012b) reported variation of phytate:Zn molar ratio from 24 to 41 for 65 bread wheat varieties of Pakistan. Gomez-Coronado *et al.* (2016) found that the phytate:Zn molar ratio for bread wheat grown under Mediterranean conditions ranged from 17 to 31 in the first year of the study and between 35 and 58 in the second year. Generally, it has been reported that phytate:Fe ratio > 1 and phytate:Zn ratio > 15 impair the bioavailability of minerals to the body (Hussain *et al*. 2012a; Gomez-Coronado *et al*. 2016). Brown *et al.* (2004) found a phytate:Zn molar ratio of 24.6 and Gharib *et al*. (2006) reported this ratio as 26 in the Iranian diets. Therefore, Iran is considered to have a medium risk of Zn deficiency. Cakmak *et al.* (2008) also reported Iran as one of the regions with the Zn deficiency problem. In our study, the phytate:Fe and phytate:Zn ratios in the grains of 109 wheat cultivars and landraces were higher than the above-mentioned critical values, which shows that these minerals have poor bioavailability in the bread wheat grains. The identified drawbacks in the wheat cultivars and landraces were due to relatively high phytate concentration which in turn could be due to a high level of soil phosphorus concentration. Previous studies reported that phosphorus fertilization greatly accelerates the accumulation of phytate (Lee *et al*. 2014). The phytate concentration had the greater impact on iron bioaccessibility, but the inhibition of zinc bioaccessibility by phytate was less impaired, which highlighted phytate as a predominant inhibitor of iron bioaccessibility. These findings show that Fe or Zn levels should be improved and phytate concentration in wheat grain be simultaneously reduced. The results of the present study showed that although the analyzed bread wheat cultivars had high levels of mineral concentration, they also had high levels of phytate concentration, which impair the bioavailability of minerals to the body. Hence, increasing the mineral density solely, may not considerably improve the human diet; instead, the breeding programs should focus on improving the mineral bioavailability through the reduction of phytate:mineral ratio in wheat grains. According to the results obtained in the present study, some hexaploid landraces had lower phytate:mineral ratio. Halekhani, Sarouq 4, Malayer 1, Eskan Arak 2, Hoseinabad Arak, and Mashhad 3 with the high concentration of Fe and low concentration of phytate and phytate:Fe molar ratio could be utilized in future crossbreeding programs. Furthermore, Khoramabad 1, Shahriar, Laht Olia Arak Boroujerd, Jolge Khalaj Qalavandi 1, Mashhad 12, Eskan Arak 2, and Ebrahimabad Arak 1 with the high Zn concentration and low concentration of phytate and phytate:Zn molar ratio could be exploited in breeding for biofortification. Also, concerning a major amount of phytate in the aleuron layer that could be removed through the milling process and cooking (Kumar *et al*. 2017), the grain phytate and the bioavailability of Fe and Zn, and finally the proper rate of flour extraction and the bakery value could be further investigated in these recommended hexaploid landraces.

**The solution culture experiment**

Taking into account that the nutritional status of the plant gives rise to morphological changes throughout the root system (Kiba and Krapp 2016) and physiological changes in a plant (van Maarschalkerweerd and Husted 2015), morphological and physiological traits might be used as appropriate parameters for the screening process in breeding programs. It was found that root length and root dry weight are essential traits to distinguish efficient cultivars from inefficient ones in Zn uptake (Fathi *et al*. 2016). The present study showed that tested bread wheat hexaploid landraces improved their nutrient uptake by adjusting root growth. According to the results of the present study, root length, root dry weight, and root nutrient concentration could determine the nutrient uptake rate and efficiency or inefficiency of investigated cultivars. There were significant differences among cluster means of the hexaploid landraces for root Fe, root length, and root dry weight at 42-DAP, and root dry weight at 70-DAP. The Mashhad 12 landrace with the high root Fe concentration had the highest root length and root dry weight at 42-DAP. Also, Hoseinabad Arak with high root Fe had the highest root dry weight at 70-DAP. In contrast, Khoramabad Khouzestan with low root Fe concentration had the lowest root length and root dry weight at 70-DAP which demonstrated the crucial role of root length, root dry weight, and root nutrient concentration in distinguishing efficient and inefficient cultivars. This agrees with the report of Liu *et al*. (2019) that nutrient uptake can be increased through the increase in the root nutrient concentration and root dry weight. They found that Zn uptake was influenced by root growth. Furthermore, the present study demonstrated the importance of the physiological responses of plants to the nutrient status. In our study, there was a significant correlation between leaf Fe and Fv:Fm ($r$ = 0.686$^{**}$) indicating that nutrient deficiency could lead to decreases in PSII photochemical efficiency and changes in the chlorophyll fluorescence values. Given the significant difference among the clusters of the investigated hexaploid landraces for leaf Fe, leaf and root dry weight, chlorophyll a, chlorophyll (a+b), and Fv:Fm at 70-DAP and according to the significant correlation of leaf Fe with leaf dry weight ($r$ = 0.809$^{**}$), root dry weight ($r$ = 669$^{**}$), Fv:Fm ($r$ = 0.686$^{**}$), chlorophyll a ($r$ = 0.539$^{*}$), and chlorophyll (a+b) ($r$ = 0.540$^{*}$), the lowest level of root and leaf dry weight may have led to the lowest level of Fe concentration in leaves which in turn caused lesser production of chlorophyll a, chlorophyll (a+b), and carotene and carotenoid concentrations for Khoramabad Khouzestan at 70-DAP. Also, Hoseinabad Arak and Valadkhani 2 with the highest root and leaf Fe showed the highest leaf dry weight, chlorophyll a, chlorophyll (a+b), carotene and carotenoids concentrations, and Fv:Fm at 70-DAP. The significant and positive correlation of the photosynthetic capacity and the nutrient content of the leaves has been indicated in other studies (van Maarschalkerweerd and Husted 2015; Kalaji *et al*. 2016). Roosta *et al*. (2018) reported that Fe, Zn, and Mn deficiency significantly declined the contents of photosynthetic pigments, chlorophyll fluorescence parameter (Fv/Fm), and dry mass in lettuce. In the present study, the significant correlation of leaf Fe with leaf dry weight and root dry wright indicated that growth retardation could occur by the Fe deficiency. They reported a lesser production of dry mass due to Fe, Zn, and Mn deficiency. All these findings suggest the possibility of being able to use the morphological and physiological traits as indicators for the early selection of plants with potentially high mineral content rather than waiting until harvest for grain analysis.

**Acknowledgments**

The authors acknowledge Tarbiat Modares University for financial supporting of this work. We are also thankful to Dr. Mohammad Amir Karimi Torshizi, Department of Poultry Science, Faculty of Agriculture, Tarbiat Modares University, Iran, for his assistance in the phytate analysis. We also appreciate the help of Dr. Nariman Zainali, Department of Agronomy and Plant Breeding, Agricultural and Natural Resources College, University of Tehran, Karaj, Iran in data analysis, preparing and review of the manuscript.

**Financial Support**

This research was supported by the Tarbiat Modares University, Iran, and by a grant from the National Institute of Genetic Engineering and Biotechnology, Iran (Wheat Mission-driven Project No. 451M).

**Conflict of Interest**

The authors declare that they have no conflict of interest with any people or organization concerning the subject of the manuscript.

**References**

Aliehyaee M and Behbehanizadeh AS, 1993. Description of methods of chemical analysis of soil. Technical Bulletin of Soil and Water Research Institute. Vol. 893. Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran [In Persian].

Bilal HM, Aziz T, Maqsood MA, and Farooq M, 2018. Grain phosphorus and phytate contents of wheat genotypes released during last 6 decades and categorization of selected genotypes for phosphorus use efficiency. Archives of Agronomy and Soil Science 65(6): 727-740.

Bouis HE, 2003. Micronutrient fortification of plants through plant breeding: can it improve nutrition in man at low cost? Proceedings of Nutrition Society 62: 403-411.

Brown KH, Rivera JA, Bhutta Z, Gibson RS, King JC, Lonnerdal B, Ruel MT, Sandtrom B, Wasantwisut E, and Hotz C, 2004. Assessment of the risk of zinc deficiency in populations and options for its control. Food and Nutrition Bulletin 25: S99-S203.

Cakmak I, 2008. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant and Soil 302: 1-17.

Erdal I, Yilmaz A, Taban S, Eker S, Torun B, and Cakmak I, 2002. Phytic acid and phosphorus concentrations in seeds of wheat cultivars grown with and without zinc fertilization. Journal of Plant Nutrition 25: 113-127.

Fathi S, Sabet MS, Lohrasebi T, Razavi Kh, Karimzadeh G, and Gholami Malekroudi M, 2016. Effect of root morphological traits on zinc efficiency in Iranian bread wheat genotypes. Acta Agriculturae Scandinavica, Section B - Soil and Plant Science 66: 575-582.

Frontela C, Ros G, and Martinez C, 2011. Phytic acid content and "*in vitro*" iron, calcium, and zinc bioavailability in bakery products: the effect of processing. Journal of Cereal Science 54: 173-179.

Gabaza M, Shumoy H, Muchuweti M, Vandamme P, and Raes K, 2018. Enzymatic degradation of mineral binders in cereals: impact on iron and zinc bioaccessibility. Journal of Cereal Science 82: 223-229.

Galili T, 2015. dendextend: an R package for visualizing, adjusting and comparing trees of hierarchical clustering. Bioinformatics 31: 3718-3720.

Gargari BP, Mahboob S, and Razavieh SV, 2007. Content of phytic acid and its mole ratio to zinc in flour and breads consumed in Tabriz, Iran. Food Chemistry 100: 1115-1119.

Gharib AG, Mohseni SG, Mohajer M, and Gharib M, 2006. Bioavailability of essential trace elements in the presence of phytate, fiber, and calcium. Journal of Radioanalytical and Nuclear Chemistry 270: 209-215.

Gomez-Coronado F, Poblaciones MJ, Almeida AS, and Cakmak I, 2016. Zinc (Zn) concentration of bread wheat grown under Mediterranean conditions as affected by genotype and soil/foliar Zn application. Plant and Soil 401: 331-346.

Grimm KA, Sullivan KM, Alasfoor D, Parvanta I, Suleiman AJM, Kaur M, Al-Hatmi FO, and Ruth LJ. 2012. Iron-fortified wheat flour and iron deficiency among women. Food and Nutrition Bulletin 33: 180-185.

Gupta PK, Balyan HS, Sharma S, and Kumar R, 2020. Genetics of yield, abiotic stress tolerance and biofortification in wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 133: 1569-1602.

Hussain S, Maqsood MA, Rengel Z, and Khan MK, 2012a. Mineral bioavailability in grains of Pakistani bread wheat declines from old to current cultivars. Euphytica 186: 153-163.

Hussain S, Maqsood MA, and Miller LV, 2012b. Bioavailable zinc in grains of bread wheat varieties of Pakistan. Cereal Research Communications 40: 62-73.

Johnson CM, Stout PR, Broyer TC, and Carlton AB, 1957. Comparative chlorine requirements of different plant species. Plant and Soil 8: 337-353.

Kalaji H, Zivcak M, Goltsev V, Jajoo A, Samborska IA, Ladle RJ, Oukarroum V, Cetner MD, Brestic M, and Lukasik I, 2016. Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. Acta Physiologiae Plantarum 38: 102.

Karami M, Afyuni M, Khoshgoftarmanesh AH, Papritz A, and Schulin R, 2009. Grain zinc, iron, and copper concentrations of wheat grown in central Iran and their relationships with soil and climate variables. Journal of Agricultural and Food Chemistry 5: 10876-10882.

Kassambara A, 2019. ggcorrplot: visualization of a correlation matrix using 'ggplot2'. R package version 0.1.3. https://CRAN.R-project.org/package=ggcorrplot.

Khokhar JS, Sareen S, Tyagi BS, Singh G, Wilson L, King LP, Young SD, and Broadley MR, 2018. Variation in grain Zn concentration, and the grain ionome, in field-grown Indian wheat. PLoS ONE 13: e0192026.

Kiba T and Krapp A, 2016. Plant nitrogen acquisition under low availability: regulation of uptake and root architecture. Plant and Cell Physiology 57: 707-714.

Kumar A, Lal MK, Kar SS, Nayak L, Ngangkham U, and Samantaray S, 2017. Bioavailability of iron and zinc as affected by phytic acid content in rice grain. Journal of Food Biochemistry 41: e12413.

Lee HH, Bong CFJ, Loh SP, Sarbini SR, and Yiu PH, 2014. Genotypic, grain morphological and locality variation in rice phytate content and phytase activity**.** Emirates Journal of Food and Agriculture 26 (10): 844-852.

Lee HH, Loh SP, Bong CFJ, Sarbini SR, and Yiu PH, 2015. Impact of phytic acid on nutrient bioaccessibility and antioxidant properties of dehusked rice. Journal of Food Science and Technology 52: 7806-7819.

Liu DY, Liu YM, Zhang W, Chen XP, and Zou CQ, 2019. Zinc uptake, translocation, and remobilization in winter wheat as affected by soil application of Zn fertilizer. Frontiers in Plant Science 426: 10.

Liu ZH, Wanga HY, Wanga XE, Zhangb GP, Chen PD, and Liu DJ, 2006. Genotypic and spike positional difference in grain phytase activity, phytate, inorganic phosphorus, iron, and zinc contents in wheat (*Triticum aestivum* L.). Journal of Cereal Science 44: 212-219.

Lorenz AJ, Scott PM, and Lamkey KR, 2007. Quantitative determination of phytate and inorganic phosphorus for maize breeding. Crop Science 47: 600-606.

Morgounov A, Gomez-Becerra HF, Abugalieva A, Dzhunusova M, Yessimbekova M, Muminjanov H, Zelenskiy Y, Ozturk L, and Cakmak I, 2007. Iron and zinc grain density in common wheat grown in Central Asia. Euphytica 155: 193-203.

R Core Team, 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rengel Z, 2015. Availability of Mn, Zn and Fe in the rhizosphere. Journal of Soil Science and Plant Nutrition 15: 397-409.

Roosta HR, Estaji A, and Niknam F, 2018. Effect of iron, zinc and manganese shortage-induced change on photosynthetic pigments, some osmoregulators and chlorophyll fluorescence parameters in lettuce. Photosynthetica 56: 606-615.

Saad N, Esa NM, Ithnin H, and Shafei NH, 2011. Optimization of optimum condition for phytic acid extraction from rice bran. African Journal of Plant Science 5: 168-176.

Sakai H, Iwai T, Matsubara Ch, Usui Y, Okamura M, Yatou O, Terada Y, Aoki N, Nishida Sh, and Yoshida KT, 2015. A decrease in phytic acid content substantially affects the distribution of mineral elements within rice seeds. Plant Science 238: 170-177.

Soltanpour PN, Khan A, and Lindsay WL, 1976. Factors affecting DTPA-extractable Zn, Fe, Mn, and Cu from soils. Communications in Soil Science and Plant Analysis 7: 797-821.

Song YL, Dong YJ, Tian XY, Wang WW, and He ZL, 2017. Effects of nitric oxide and Fe supply on recovery of Fe deficiency induced chlorosis in peanut plants. Biologia Plantarum 61: 155-168.

Swaminathan MS, 2012. Combating hunger. Science 338: 1009.

Tourian N, Sinaki JM, Hasani N, and Madani H, 2013. Change in photosynthetic pigment concentration of wheat grass (*Agropyron repens*) cultivars response to drought stress and foliar application with Chitosan. International Journal of Agronomy and Plant Production 4: 1084-1091.

Yilmaz O, Altintas Kazar G, Cakmak I, and Ozturk L, 2017. Differences in grain zinc are not correlated with root uptake and grain translocation of zinc in wild emmer and durum wheat genotypes. Plant and Soil 411: 69-79.

van Maarschalkerweerd M and Husted S, 2015. Recent developments in fast spectroscopy for plant mineral analysis. Frontiers in Plant Science 6: 169.

Velu G, Crossa J, Singh RP, Hao YF, Dreisigacker S, Perez-Rodriguez P, Joshi AK, Chatrath R, Gupta V, Balasubramaniam A, Tiwari Ch, Mishra VK, Sohu VS, and Mavi GS, 2016. Genomic prediction for grain zinc and iron concentrations in spring wheat. Theoretical and Applied Genetics 129(8): 1595-1605.

Velu G, Tutus Y, Gomez-Becerra HF, Hao Y, Demir L, Kara R, Crespo-Herrera LA, Orhan S, Yazici A, Singh RP, and Cakmak I, 2017. QTL mapping for grain zinc and iron concentrations and zinc efficiency in a tetraploid and hexaploid wheat mapping populations. Plant and Soil 411: 81-99.

Webb P, Stordalen GA, Singh S, Wijesinha-Bettoni R, Shetty P, and Lartey A, 2018. Hunger and malnutrition in the 21st century. British Medical Journal 361: k2238.

White PJ and Broadley MR, 2009. Biofortification of crops with seven mineral elements often lacking in human diets-iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytologist 182: 49-84.

# ارزیابی پتانسیل ارقام بومی هگزاپلوئید و ارقام زراعی نوین گندم نان از نظر آهن، روی، فیتات و نسبت‌های مولی فیتات به عناصر معدنی

**مژگان غلامی ملکرودی[1]، قاسم کریم زاده[1*]، تهمینه لهراسبی[2]، محمد صادق ثابت[1] و سروه فتحی[1]**

۱- گروه ژنتیک و به‌نژادی گیاهی، پردیس کشاورزی، دانشگاه تربیت مدرس، تهران، صندوق پستی ۳۳۶-۱۴۱۱۵
۲- گروه زیست‌فناوری کشاورزی، موسسه ملی مهندسی ژنتیک و زیست‌فناوری، تهران
*مسئول مکاتبه؛ Email: karimzadeh_g@modares.ac.ir

**چکیده**

فیتات که عمده ترین شکل فسفر غیرآلی در غلات است، یک عامل ضد-تغذیه‌ای است که زیست‌فراهمی آهن و روی را کاهش می‌دهد. مجموع ۱۰۹ رقم بومی و زراعی نوین گندم نان (*Triticum aestivum* L.) جهت تعیین میزان آهن، روی، فیتات و نسبت‌های مولی فیتات به عناصر معدنی مورد بررسی قرار گرفتند. علاوه براین، تعدادی از ویژگی‌های مورفولوژیکی و فیزیولوژیکی تأثیرگذار بر فرآیند غربالگری نیز شناسایی شدند. در آزمایش مزرعه‌ای میزان غلظت آهن و روی به ترتیب بین ۰/۲۵۵-۰/۰۳۶ و ۰/۰۸۵-۰/۰۳۰ میلی‌گرم بر گرم متغیر بودند. تفاوت معنی‌داری بین محتوای غلظت آهن و روی بین ارقام بومی و ارقام زراعی مشاهده نشد. بنابراین، تلاش‌هایی که توسط به‌نژادگران برای تولید ارقام گندم با عملکرد بالا صورت گرفته است تأثیر تعیین کننده‌ای در کاهش محتوای آهن و روی نداشته است. از طرف دیگر ارقام زراعی نوین به طور معنی‌داری دارای مقادیر بالاتری از فیتات در مقایسه با ارقام بومی بودند. محتوای فیتات دانه دارای دامنه‌ای بین ۲۸/۷۷-۱۵/۰۷ میلی‌گرم بر گرم بود که منجر به ایجاد دامنه ۵۸/۱۴-۶/۲۴ برای نسبت فیتات به آهن و ۷۷/۲۲-۳۲/۲۰ در نسبت فیتات به روی شد که خود نشان‌دهنده زیست‌فراهمی ضعیف این دو عنصر است. این معایب ناشی از بالا بودن غلظت فیتات است که آن هم به نوبه خود می‌تواند ناشی از بالا بودن مقادیر فسفر در خاک باشد. در نتیجه به‌نژادی برای کاهش فیتات و نسبت فیتات به عناصر معدنی می‌تواند منجر به افزایش زیست‌فراهمی عناصر معدنی شود. در آزمایش محلول غذایی، نقش حیاتی طول و وزن خشک ریشه و غلظت عناصر ریشه در توانایی جذب آهن در ارقام مشخص شد. همچنین، مشخص گردید که مقادیر کم طول و وزن خشک ریشه و برگ منجر به کاهش آهن برگ شد و آن هم به نوبه خود منجر به کاهش محتوای رنگدانه‌های فتوسنتزی و کلروفیل فلوروسانس گردید. برخی از ارقام بومی مانند خرم آباد ۱، ساروق ۴، اسکان اراک ۲ و حسین آباد اراک به دلیل دارا بودن غلظت‌های بالای آهن و روی و همچنین مقادیر پایین فیتات و نسبت‌های مولی فیتات به عناصر معدنی می‌توانند در برنامه‌های به‌نژادی برای غنی‌سازی زیستی مورد بهره‌برداری قرار گیرند.

**واژه‌های کلیدی:** رنگدانه‌های فتوسنتزی؛ ریزمغذی‌ها؛ غنی‌سازی زیستی؛ نسبت مولی؛ *Triticum aestivum*