



Genetic Variation for Biofortifying The Maize Grain

Gönül Cömertpay¹, Faheem Shehzad Baloch², Halil Erdem³

¹Eastern Mediterranean Agricultural Research Institute, 01330 Yüreğir/Adana, Turkey.

²Department of Field Crops, Faculty of Agricultural and Natural Science, Abant İzzet Baysal University, 14280 Bolu, Turkey

³Department of Soil Science and Plant Nutrition, Faculty of Agriculture, University of Gaziosmanpaşa 60240 Tokat, Turkey

ARTICLE INFO

Article history:

Received 24 April 2016

Accepted 23 June 2016

Available online, ISSN: 2148-127X

Keywords:

Mineral variation
Landraces
Maize
Turkey
Quality

ABSTRACT

The maize germplasm variation is valuable for breeders to develop elite hybrids with increased mineral contents in the maize grain to eliminate mineral malnutrition, which is referred as HIDDEN HUNGER. Therefore, we aimed to determine mineral element diversity of maize landraces collected from different geographical regions of Turkey. There was huge diversity for all mineral traits and other quality traits. Turkish maize landraces showed high variation for Zn (17-41.34 mg kg⁻¹), Fe (13.52-29.63 mg kg⁻¹), Cu (0.77-3.34 mg kg⁻¹), Mn (5.68-14.78 mg kg⁻¹), Protein (6.6-11.6%), starch content (73.3-80.0%), oil content (3.15-4.7%) and thousand grain weight (177.0-374.9g). There were significant positive and negative associations among mineral elements and quality traits. The principal component analysis differentiated some maize landraces from the rest, and these diverse landraces could be used in the maize breeding program with biofortification purpose.

* Corresponding Author:

E-mail: balochfaheem13@gmail.com

Introduction

A sufficient and balanced diet is possibly the most important contribution to human health and also animal feed. Mineral and vitamin deficiencies combine together effect the most population of the world more than does the protein-energy malnutrition. Micronutrient deficiency is a widespread critical problem in many developing and least developed countries where people rely upon cereal-based diets that are inherently deficient in micronutrients (Bouis and Welch, 2010; Pfeiffer and Mc Clafferty, 2007). According to report published by world health organization (WHO, 2002) more than half of the world's population is afflicted by iron (Fe) and zinc (Zn) deficiencies, these ranking fifth and sixth among the ten most important risk causes of illness and disease in low-income countries, it popularly phrased as "hidden hunger" (Khush et al., 2012; Stein, 2010). Micronutrients not only plays important role in the human's health but also for plant nutrition. Thus plant breeding hold a great promise for making major, low cost and sustainable contribution for reducing micronutrient malnutrition and may have important spin-off effects on increasing farm productivity of low income farmer communities in the developing world (Bouis, 2003).

Micronutrients play a critical role in cellular and humoral immune responses, cellular signaling and function, work capacity, reproductive health, learning and

cognitive functions (Guerrant et al., 2000; Kapil and Bhavna, 2008). Since human body cannot synthesize micronutrients, they must be made available through diet (Baloch et al., 2014). Traditional interference to address mineral deficiencies have focused on supplementation, food fortification and dietary diversification. For various reasons, none of these have been universally successful. Among strategies for enhancing iron and zinc levels in cereal grains, plant breeding strategy (biofortification) appears to be the most sustainable and cost-effective approach (e.g. Cakmak, 2008; Graham et al., 1999; Welch and Graham, 2002).

The development of an effective breeding program to improve mineral content in maize depends on the presence of genetic variation. Exploring natural biodiversity as a source of novel alleles to improve the productivity, adaptation, quality, and nutritional value of crops is of prime importance in 21st century breeding programs (Saha et al., 2009). Genetic variations have been reported in maize inbred lines, landraces and hybrids for all the mineral elements most frequently lacking in human diets. This can be used in breeding programs to increase mineral concentrations in maize grain (White and Broadley, 2005).

Maize is one of the most important crop in Turkish agriculture after wheat and barley (Comertpay et al.,

2012). It is extensively cultivated in Mediterranean (29.1%) and Southeast Anatolia (29%) regions and, followed by the Aegean (10.5%) regions (TUIK, 2015)). According to the Statistical database of Food and Agricultural organization of the world (FAOSTAT, 2015), Turkey produces 6.4 million tons of maize grains per year from 688.169 ha of land (about 3.33% of areas under cultivation in Turkey. In Turkey, 64% of maize is used for forage purposes and 36% for food and industrial products (Ege and Karahocağil, 2001). Maize alone is responsible for providing 15% of the protein and 20% of the calories in the human diet, and this crop covered a cultivated area of 159.5 million hectares in 2009 (FAOSTAT, 2009). The importance of this crop is demonstrated by the multiple ways it is exploited (Messias et al., 2013). Cereal grain is a good and easily accessible source of Fe and Zn for both feed and food. Although maize grain is low in some micronutrients, humans and animals can obtain at least part of their nutritional requirements from maize grain (Mason and D'Croz-Mason, 2002). It was proved that there is sufficient genetic variation and workable heritability to improve Fe and Zn levels in maize (Graham et al., 1999; Bänziger and Long, 2000)

Maize landraces have long been of socio-economic importance for family farming systems in Turkey and are still cultivated throughout different regions of Turkey. Maize landraces are open-pollinated varieties (OPVs), and therefore they underwent long-term natural and artificial selection in the past centuries. A large number of maize landraces have arisen over time, selected for their adaptation to local environmental conditions by farmers. Natural diversity detected in the maize germplasm, provides an opportunity for incorporating higher levels of iron, zinc, and beta-carotene into these grains (Hoisington, 2002).

Very limited results have been published on the micronutrient contents of the maize grain. The natural genetic variation harbored by maize grain could be very important for biofortifying the maize grain for reducing the mineral malnutrition in the developing world. Therefore the objective of this study was to check the natural variation existed in the maize grain. We discussed here available genetic variation for Fe and Zn, relationship among micronutrients and pattern of variation through multivariate analysis. We examined 79 Turkish maize landraces for 3 quality parameters and four micro-elements. This will open ways for starting the biofortification of maize grain in Turkey

Material and Method

As part of a biofortification studies in maize at Eastern Mediterranean Research institute, we are trying to develop maize hybrids having increased mineral concentrations. For crossing, we need to identify the natural germplasm having increased mineral concentrations. Therefore here our main aim was to identify the landraces having high concentration Zn, Fe and other mineral elements. The research material consisted of 79 maize landraces collected from maize growing areas of various geographical provinces of Turkey. The seeds of the landraces were kindly obtained from Menemen gene bank of the Aegean Agricultural Research Institute, Izmir, Turkey. Identification numbers and collection locations are presented in Table 1. Field experiment was carried out in 2009 at the University of Çukurova, Adana (37°00'56"N,35°21'29"E), a location which experiences a typical Mediterranean climate of hot, dry summers

Table1 Origin, collection sites of 79 open pollinated Turkish maize populations used in this study

No	Genbank Identification Number	Geographical Province	Collection Site	Kernel Type
1	TR 51484	Adana	Kozan, Gaziköy	Flint / Dent
2	TR 51540	Adapazarı	Karasu	Flint / Dent
3	TR 37944	Adıyaman1	Kahta, Adalı vil.	Dent
4	TR 37985	Adıyaman2	Samsat, Balcılar vil.	Dent
5	TR 37998	Afyon	Dinar	Flint / Dent
6	TR 38147	Ağrı2	Tutak, Yoğunhisar vil.	Flint / Dent
7	TR 38150	Amasya1	Taşova	Flint / Dent
8	TR 38201	Amasya2	Evince	Flint / Dent
9	TR 38036	Amasya3	Göynücek	Flint / Dent
10	TR 38039	Artvin1	Erhavi	Flint / Dent
11	TR 38243	Artvin2	Borçka	Flint / Dent
12	TR 38272	Artvin3	4 Km E Orus, Şenköy vil.	Flint / Dent
13	TR 37484	Artvin4	Şavşat	Flint / Dent
14	TR 37490	Aydın1	Bozdoğan, Kılavuzlar vil.	Flint / Dent
15	TR 37499	Aydın2	Sultanhisar, Uzunlar vil.	Flint / Dent
16	TR 37500	Balıkesir1	Gönen, Tütüncüler vil.	Dent
17	TR 38375	Balıkesir2	Manyas, Süleymanlı vil.	Flint / Dent
18	TR 38411	Balıkesir3	Bigadiç, Kadıköy	Flint / Dent
19	TR 38437	Bolu	Düzce, Döngelli vil.	Flint / Dent
20	TR 37543	Burdur1	Yeşilova	Flint / Dent

21	TR 38471	Burdur2	Tefenni, Çaylıkköyü vil.	Flint / Dent
22	TR 37605	Bursa1	Orhangazi, Çeltikli vil.	Flint / Dent
23	TR 37630	Bursa2	Demirtaş vil.	Flint / Dent
24	TR 37780	Çanakkale	Çan	Flint / Dent
25	TR 55545	Çorum1	Ortaköy	Flint / Dent
26	TR 55463	Çorum2	Sungurlu	Flint / Dent
27	TR 55469	Denizli1	Acıpayam, Gölcük vil.	Flint / Dent
28	TR 49312	Denizli2	Kayhan vil.	Flint / Dent
29	TR 57657	Denizli3	Tavas, Solmaz vil.	Dent
30	TR 57661	Diyarbakır	Çermik, Pamuklu vil.	Flint / Dent
31	TR 44446	Edirne1	Havsa	Dent
32	TR 44469	Edirne2	Karaağaç	Flint / Dent
33	TR 44519	Edirne 4	Keşan	Dent
34	TR 36977	Erzurum1	Horasan, Esence vil.	Flint / Dent
35	TR 37006	Erzurum2	Tortum, Pehlivanlı vil.	Flint / Dent
36	TR 37010	Eskişehir1	Sivrihisar	Flint / Dent
37	TR 37013	Eskişehir2	Sivrihisar	Flint / Dent
38	TR 37056	Gaziantep1	Nizip, Belkız, Kavunlu vil.	Flint / Dent
39	TR 37105	Gaziantep2	Nizip, Aşağıçardaklı Fındıklı mezra	Flint / Dent
40	TR 50558	Giresun1	3 Km S Doğakent, Demirci vil.	Flint / Dent
41	TR 50550	Giresun2	Barça vil.	Flint / Dent
42	TR 50548	Isparta1	Keçiboru, Aydoğmuş vil.	Flint / Dent
43	TR 50537	Isparta2	Keçiborlu, Gümüşgün vil.	Flint / Dent
44	TR 50527	İstanbul	Çatalca, Karaca köy vil.	Flint / Dent
45	TR 50511	İzmir1	Bozdağ	Flint / Dent
46	TR 50565	İzmir2	Torbalı, Karaot vil.	Dent
47	TR 50563	K.maraş1	Andırın	Flint / Dent
48	TR 50564	K.maraş2	Türkoğlu	Flint / Dent
49	TR 50654	Kars	Kötek	Flint / Dent
50	TR 50667	Kastamonu1	Araç, Yeniceköy vil., Kösel mah.	Flint / Dent
51	TR 50674	Kastamonu2	Emirler vil.	Flint / Dent
52	TR 53245	Kırklareli	Çakıllı	Flint / Dent
53	TR 50643	Kocaeli	Kandıra, Akçaova	Flint / Dent
54	TR 47889	Konya	Beyşehir, Damlapınar vil.	Flint / Dent
55	TR 39563	Kütahya	Saphane, Gaipler vil.	Flint / Dent
56	TR 54214	Manisa	Yurtdağı	Flint / Dent
57	TR 54191	Muğla1	Köyceğiz, Beyobası vil.	Dent
58	TR 54199	Muğla2	Köyceğiz, Beyobası vil.	Flint / Dent
59	TR 48470	Ordu	Mesudiye, Güzle vil.	Flint / Dent
60	TR 48479	Rize1	Çayeli	Flint / Dent
61	TR 50136	Rize2	33 Km S İkizdere yolu, İskender vil	Dent
62	TR 50161	Sakarya1	Küçükhatatlı vil.	Flint / Dent
63	TR 48452	Sakarya2	Adapazarı-Hendek, Kazımiye vil.	Flint / Dent
64	TR 48454	Samsun1	Bafra, Altınkaya Dam	Flint / Dent
65	TR 42703	Samsun2	19 Mayıs, Karaköy vil.	Dent
66	TR 42719	Sinop	Gerze, Çalboğaz vil.	Flint / Dent
67	TR 42725	Ş.urfa	Hilvan, Uğra vil.	Dent
68	TR 42750	Tekirdağ1	Güngörmez	Flint / Dent
69	TR 42803	Tekirdağ2	Saray	Flint / Dent
70	TR 42856	Tokat1	Reşadiye, Soğukpınar vil.	Flint / Dent
71	TR 42949	Tokat2	Niksar, Kıraç vil.	Flint / Dent
72	TR 42958	Trabzon1	Tonya	Flint / Dent
73	TR 42985	Trabzon2	2 Km N Atatürk köşkü, Soğuksu vil.	Dent
74	TR 42614	Trabzon3	Akyaz vil.	Flint / Dent
75	TR 49202	Trabzon4	Akçaabat, Düzköy vil.	Flint / Dent
76	TR 49214	Uşak1	Dumlupınar	Flint / Dent
77	TR 49234	Uşak2	Banaz, Güllüçam vil.	Flint / Dent
78	TR 49309	Zonguldak1	Ereğli	Flint / Dent
79	TR 45513	Zonguldak2	Bartın	Flint / Dent

Quality and Micronutrient Analysis

Some amount of seed samples was taken from every landrace with 3 replications and seeds were bulked both for quality and micronutrient analysis. The quality parameters (protein, starch and oil content) were determined by using Fourier transform near infrared spectroscopy (FT-NIR). The micronutrient analysis was implemented following procedure. Seed samples (0.4 g) were digested in a closed microwave digestion system (MARSxpress, CEM Corp.) in 5 mL of concentrated HNO₃ and 2 mL of concentrated H₂O and were then analyzed for mineral nutrients with an inductively coupled plasma optical emission spectrometer (ICP-OES; Vista-Pro Axial; Varian Pty Ltd., Australia).

Statistical Analysis

Standard one-way analysis of variance (ANOVA) was performed for each mineral element using JUMP statistical computer software program. Significant differences between accessions ($P \leq 0.05$) were detected for all studied mineral traits. Principal component analysis (PCA) based on 8 characters was used to identify the patterns of variation within the set of 79 landraces. The PCA was done using JMP statistical software. The eigenvalue-one criterion was used to retain the principal components that contributed considerable variability. Correlation among studied traits was calculated using the Pearson correlation using JUMP statistical computer software program.

Results

There were high variations for studied mineral traits in 79 maize landraces. Means of the all 79 landraces greatly varied for all 8 traits (Table 2). The mean, maximum, minimum, standard deviation of all traits all are given in the Table 3.

The mean protein content of all maize landraces was 8.7%, and it ranged from 6.6% for landraces of Adana to

11.6% for landraces of Diyarbakır. The highest protein content among Turkish maize landraces was approximately double from its minimum value. The mean oil content was 3.9 and varied between 3.15 and 4.8%. In case of starch contents, the highest starch content was 80% in landrace of Adana and minimum starch content was 73.5% in landrace of Çanakkale and mean value was 78.3%. For thousand grain weight, landrace of Adana showed minimum value of 177g and landrace of K.Maraş1 showed highest value of 1000 grain weight of 368.7, this value was more than 2 fold than the minimum.

When we look at the variation of microelement content in Turkish germplasm, we can easily see that there was much variation among Turkish maize germplasm for micro-element contents in the maize grain. Zinc concentration in the maize grain varied two and half times between maximum and minimum values and it varied between 17.0-41.3 mg kg⁻¹ with mean value of 26.0 mg kg⁻¹. The highest Zn value was found in landrace of Balıkesir2 and minimum in the landrace of Diyarbakır. The maximum value of the iron content in the Turkish maize germplasm was fold higher than minimum value. The amount of iron in the grain of Turkish maize germplasm varied between 13.5-29.6 mg kg⁻¹ with an average of 20.5 mg kg⁻¹. Lowest Fe content was depicted in the Giresun1 and highest seen in Artvin1. In case of copper content, maximum value was 5 times more than the minimum value and it ranged from 0.77 to 3.84 mg kg⁻¹ with an average of 2.2 mg kg⁻¹. Landraces of Mugla1 and Kastamonu2 showed highest and lowest copper contents respectively. When we see the variation of manganese content in the grain of maize, there was high variation in Mn content. The maximum value of Mn was 3 fold greater than the minimum value showing high diversity. The grain of maize landrace from Izmir harbored highest Mn content (14.7.2) and landrace of Trabzon3 with minimum Mn content (5.68) with a mean value of 10.0.

Table 2 Maximum, minimum, mean and standard deviation values of mineral element contents and quality traits of 79 maize landraces

Traits	Maximum	Minimum	Mean	Stdev
TGW (g)	374.9	177.0	282.6	±44.8
Protein (%)	11.6	6.6	8.7	±0.98
Oil (%)	4.7	3.15	3.9	±0.26
Starch (%)	80.0	73.3	76.3	±1.10
Zn (mg kg ⁻¹)	41.34	17.0	26.0	±4.80
Fe (mg kg ⁻¹)	29.63	13.52	20.5	±4.10
Cu (mg kg ⁻¹)	3.84	0.77	2.2	±0.70
Mn (mg kg ⁻¹)	14.78	5.68	10.0	±1.82

Table 3 Correlation coefficients among different quality and mineral traits

Traits	TGW	Protein	Oil	Starch	Zn	Fe	Cu
Protein	0.249	-					
Oil	0.385*	-0.482**	-				
Starch	-0.170	-0.553**	0.199	-			
Zn	-0.343**	0.123	0.285*	-0.150	-		
Fe	-0.405**	0.095	0.278*	-0.157	0.488**	-	
Cu	-0.147	0.060	0.156	-0.163	0.428**	0.244*	-
Mn	-0.302**	0.005	0.326**	-0.105	0.482**	0.470**	0.362**

Table 5 Eigenvectors, eigenvalues, individual and cumulative percentages of variation explained by the first six principal components (PC) after assessing quality and mineral nutrient traits in 79 Turkish maize landraces

Variables	Eigen vectors					
	PC1	PC2	PC3	PC4	PC5	PC6
TGW (g)	-0.38396	0.23183	0.41648	0.46887	0.38048	0.36483
Protein (%)	-0.07357	0.62379	-0.20089	-0.14362	0.07421	0.11143
Oil (%)	0.35903	-0.36960	0.09094	0.57630	-0.31839	0.21533
Starch (%)	-0.03520	-0.57021	0.06649	-0.45243	0.51635	0.21524
Zn (mg kg ⁻¹)	0.45974	0.17813	0.05715	-0.19149	0.03739	0.73652
Fe (mg kg ⁻¹)	0.44035	0.13574	-0.44483	0.03240	0.09804	-0.00093
Cu (mg kg ⁻¹)	0.33202	0.19022	0.75262	-0.32284	-0.20958	-0.23206
Mn (mg kg ⁻¹)	0.44889	0.09513	0.07696	0.28422	0.65330	-0.40678
Eigenvalue	2.7482	1.9593	0.8322	0.6449	0.5652	0.4863
Percent	34.353	24.491	10.402	8.061	7.065	6.079
Cum Percent	34.353	58.843	69.246	77.306	84.372	90.450

Correlation for 3 quality and 4 mineral trait parameters are shown in the Table 3. There were significant and positive correlations among the contents of different mineral elements. Thousand grain weight has significant but negative correlation with all mineral traits (Zn, $r=0.343$; $P<0.01$; Fe, $r=0.405$; $P<0.01$; Mn, $r=0.302$; $P<0.01$) except copper content. Oil content possessed significant and positive correlation with contents of Zn ($r=0.285$; $P<0.05$), Fe ($r=0.278$; $P<0.05$) and Mn ($r=0.326$; $P<0.01$) except Cu content. Oil content has also significant and negative correlation with protein content ($r=0.482$; $P<0.01$) and also significant positive correlation with thousand kernel weight ($r=0.385$; $P<0.05$). Starch content of the grain did not harbour any association with other traits except protein content ($r=0.553$; $P<0.01$). Most of the mineral contents harboured significant and positive relationships with each other; however, the large number of observations increased the test power, resulting in significance for most of the correlations. Hence, only values of 0.5 or above are discussed. When we observe the correlation pattern of micro elements with each other and with quality traits, it was noted that Zn and other mineral element contents have significant and positive correlations with each other. Cu content did not have any correlation with all three quality traits.

Finally, PCA, based on 3 qualities, and contents of 4 minerals was used to assess the patterns of diversity within a set of 79 Turkish maize landraces. Using PCA based on the correlation matrix, we calculated eigenvalues, percentages of variation, and load coefficients of the first 5 components for all traits (Table 4). PCA yielded six principal components (PC1, PC2, PC3, PC4, PC5, PC6), explaining 90,450% of total variance in data. First principal component was the most component of variation explaining 34.35% of the total variation. The contents of three mineral elements were the main treasure of variation. Zn content explained 45.9% of the total variation exhibited followed by Mn and Fe contents explaining around 44% of the total variation. Quality traits did not get any place in the PC1 and get identity in PC2. The second principal component (PC2) accounted for 58.84% of the variability and was highly dependent on protein and starch contents. In PC2, Protein content was

the main partner of the variation explaining 62% of the total variation of 79 Turkish landraces. Similarly starch content explained 57% of the total variations in PC2. Oil contents played important role in principle component four and explained 57% of the total variations. First two principle components were very important and explained more than half of the total variations hence, they were plotted graphically to demonstrate the relationship among Turkish maize germplasm collection (Figure 2).

Discussions

Biofortification is the development of micro nutrient dense crop varieties through conventional plant breeding. The main aim of the Harvest Plus is to reduce micronutrient malnutrition among poor populations in Africa, Asia, and Latin America, thereby improving food security and enhancing the quality of life. Harvest Plus currently focuses on three micronutrients that are recognized by the World Health Organization (WHO) as limiting: Fe, Zn, and provitamin A, but other nutrients may be added in the future.

In defining breeding strategies and target levels of micronutrient enhancement, it is important to look beyond the total amount of micronutrients present in the grain. Breeding strategies must aim to generate micronutrient-enhanced maize cultivars without compromising tolerance to abiotic and biotic stress, crop productivity, and acceptable end-use quality, thereby increasing the likelihood that farmers will adopt the cultivars and consumers will accept foods made from them.

To fully and effectively utilize the genetic variability of the Turkish maize germplasm for the enrichment of maize seeds with bioavailable mineral elements, it is necessary to study and evaluate the variations for mineral traits of maize germplasms from different origins and to identify germplasm groups from which elite inbred lines with high mineral elements could be created (Cakmak, 2008).

The current study presents a comprehensive analysis of quality traits (protein, oil, starch content) and micronutrient (Zn, Fe, Cu, and Mn) concentrations for a large germplasm collection. There was impressive

variation among Turkish maize landraces for contents of micro-elements and quality traits. For example, Zn and Fe contents showed high and impressive variation in grain of Maize which could be used in developing hybrids for biofortification purpose. Similarly landraces showing higher 1000 grain weight also possess high micro nutrient contents illustrating that Zn and Fe fortified grain might also possessed high grain yield. Therefore Turkish maize landraces could be used in breeding program with specific objectives.

Quality protein maize (QPM) genotype reported to have higher concentration of Fe and Zn provides opportunity to develop multi nutrient rich maize through a systematic breeding approach.

Correlations among different traits are generally due to the presence of linked genes and the epistatic effect of different genes. Environment plays an important role in correlations. In some cases, environment affects both the traits simultaneously in the same direction or sometimes in different directions (Yücel et al., 2009). Several studies have reported significant positive correlation between Fe and Zn contents in maize and other crops (Baxter et al., 2013; Chakraborti et al., 2009; Lungaho et al., 2011; Baloch et al., 2014). This could be possibly due to linkage between the genes affecting the accumulations or pleiotropic effects of the genes governing the accumulation of micronutrients and the existence of one or more common genetic-physiological mechanisms involved in mineral absorption or uptake by the root system, translocation and redistribution within the plant tissues, remobilization to the grain, or accumulation in the developing grain (Çakmak, 2008; Peleg et al., 2008; Chatvaz et al., 2010). Positive correlation was observed between kernel Fe and Zn contents by Qin et al. (2012), and it might be due to the colocalization of QTLs for both the traits which was also reported in the review of Gupta et al., (2015), there by suggesting the feasibility of simultaneous improvement of the both. Negative correlation of protein content with starch and oil contents showed that which trait should be selected in the breeding

program. From the results of the correlation analysis, it could be concluded that selection for right character is also important because of correlation among different traits. For example Zn content has positive and significant correlation with the contents of other three micronutrients. Similarly thousand grain weight is one of the most important traits determining the final yield. Therefore the quality and yield of maize kernel can be improved by selecting parents with either higher Zn concentration or higher grain weight. However, results require careful verification by testing the germplasm under different agro-climatic conditions.

Multivariate analyses were utilized to measure the variation in germplasm collections and to evaluate the relative contributions that various traits add to the total variability in a crop germplasm collection. These analyses permit germplasm entries to be classified into groups with similar traits (Baloch et al., 2014; Karaköy et al., 2014). To analyse the structure of the genetic diversity among a set of 79 Turkish maize landraces, we performed PCA based on mean values. First two principle components were most important explaining more than half of the total variation harboured by the Turkish maize landraces. Contents of Zn, Fe, Mn, protein and starch were the traits responsible for this variation. Graph drawn by first two principle component (Figure 3) showed that some landraces were differentiated from the rest of the landraces. For example, landraces 17, 10 69 and 47 were collected from Balıkesir2, Artvin1, Tekirdag2 and K.Maras1 possessed high Zn, Fe, Mn contents and TGW respectively. Similarly from PCA, many other landraces could be selected based on their diversity and could be used as parents in pre-breeding programs. One plant from each landraces should be selfed to produce inbred lines and future research should be focused on association mapping of the mineral elements by using these selfed genotypes. This will help to identify the locus responsible for increased mineral element in the faba bean, which will also help to develop the maize hybrids with increased mineral elements.

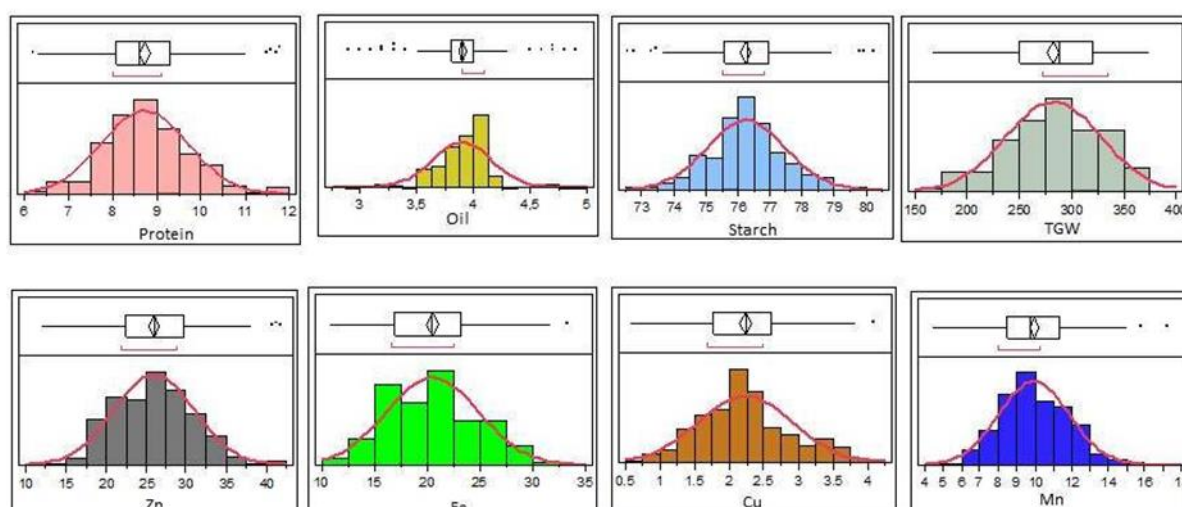


Figure 1 Frequency distribution of different quality and mineral traits in maize landraces

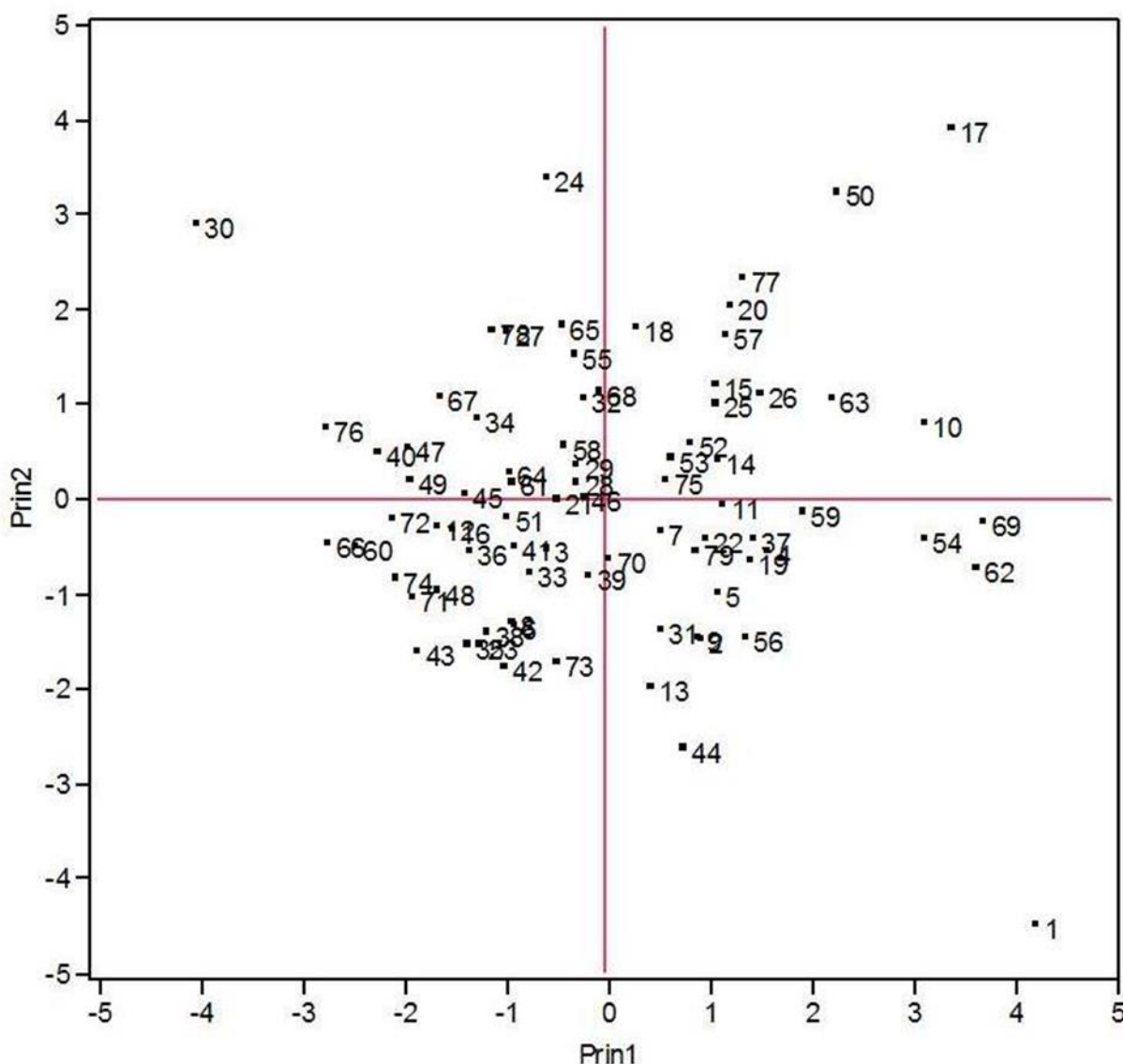


Figure 2 Principal component analysis of Turkish maize landraces for mineral and quality traits

In our previous study, we performed morphological characterization of Turkish maize landraces for nineteen agro-morphological traits (Comertpay et al., 2012) and genotypic diversity using SSR markers. Comertpay et al. (2012), also observed that Turkish maize landraces harbored high phenotypic and genotypic diversity. Identification of genetic variation is essential for achieving improvements in the mineral content of crops. Such variation can also be used to identify quantitative trait loci associated with mineral uptake and transport. Further detailed investigations by conducting field trials at multiple locations to verify the results and to study genotype x environment interactions and precautions should be done to study the mineral contents in selfed maize inbred lines. These landraces and associated information are useful to researchers and breeders from all over the world who are interested in biofortifying the maize grain. A promising genotype with stable trait expression can effectively be utilized as common donor or directly used as parent in the crossing program, across

environments. Maintaining locally well adapted landraces would be an asset for our future and may contribute to Turkish maize breeding programs as well as other breeders worldwide interested in Turkish maize genetic resource.

References

- Baloch FS, Karaköy T, Demirbaş A, Toklu F, Özkan H, Hatipoğlu R. 2014. Variation of some seed mineral contents in open pollinated faba bean (*Vicia faba* L.) landraces from Turkey. *Turk J Agric For* 38: 591–601.
- Bänziger, M, Long J. 2000. The potential for increasing the iron and zinc density of maize through plant breeding. *Food and Nutrition Bulletin* 20:397-400.
- Baxter IR, Gustin JL, Settles AM, Hoekenga OA. 2013. Ionic characterization of maize kernels in the intermated B73 × Mo17 population, *Crop Sci.*, 53: 208-220 <http://dx.doi.org/10.2135/cropsci2012.02.0135>
- Bouis HE, Welch RM. 2010. Biofortification—A Sustainable Agricultural Strategy for Reducing Micronutrient Malnutrition in the Global South *Crop Science* 50:1, 20-32 [doi:10.2135/cropsci2009.09.0531](https://doi.org/10.2135/cropsci2009.09.0531).

- Bouis HE. 2003. Micronutrient fortification of plants through plant breeding: can it improve nutrition in man at low cost? Proc. Nutr. Soc. 62: 403-411.
- Çakmak I. 2008. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? Plant and soil, 302:1, pp 1-17. 10.1007/s11104-007-9466-3.
- Chakraborti M, Prasanna BM, Hossain F, Singh AM, Guleria SK. 2009. Genetic evaluation of kernel Fe and Zn concentrations and yield performance of selected maize (*Zea mays* L.) genotypes, Range Mgmt. and Agroforestry, 30: 109-114.
- Chatzav M, Peleg Z, Öztürk L, Yazici A, Fahima T, Çakmak I, Saranga Y. 2010. Genetic diversity for grain nutrients in wild emmer wheat: potential for wheat improvement. Ann Bot 105: 1211-1220.
- Cömertpay G, Baloch FS, Kilian B, Ülger AC, Özkan H. 2012. Diversity assessment of Turkish maize landraces based on fluorescent labelled SSR markers. Plant Mol Biol Rep 30: 261-274.
- Ege H, Karahocağıl P. 2001. Yemlik Tahıllar Arpa, Mısır durum ve tahmin 2001/2002 TEAE Yayını No 82, Ankara.
- FAOSTAT. 2009. Food And Agriculture Organization of the United Nations. <http://www.faostat.fao.org>.
- FAOSTAT. 2015 Food And Agriculture Organization of the United Nations. <http://www.faostat.fao.org>
- Graham RD, Senadhira D, Beebe S, Iglesias C, Monasterio I. 1999. Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. Field Crops Res 60: 57-80.
- Guerrant RL, Lima AA, Davidson F. 2000. Micronutrients and infection: interactions and implications with enteric and other infections and future priorities. J Infect Dis 182, S134-S138.
- Gupta HS, Hossain F, Nepolean T, Vignesh M, Mallikarjuna MG. 2015. Understanding genetic and molecular bases of Fe and Zn accumulation towards development of micronutrient enriched maize, In: Nutrient Use Efficiency: From Basics to Advances, Rakshit A., Singh H.B., Sen A., (eds.), Springer Publications, pp. 255-282, doi. 10.1007/978-81-322-2169-2-17
- Hoisington D. 2002. Opportunities for nutritionally enhanced maize and wheat varieties to combat protein and micronutrient malnutrition. Food Nutr Bull. 23(4):376-777.
- Kapil U, Bhavna A. 2008. Adverse effects of poor micronutrient status during childhood and adolescence. Nutr Rev;60:S84-S90.
- Karaköy T, Baloch FS, Toklu F, Özkan H. 2014. Variation for selected morphological and quality-related traits among 178 faba bean landraces collected from Turkey. Plant Genetic Resources: Characterization and Utilization 12: 5-13.
- Karaköy T, Erdem H, Baloch FS, Toklu F, Eker S, Kilian B, Özkan H. 2012. Diversity of macro-and micronutrients in the seeds of lentil landraces. The Scientific World Journal, 2012: 1-9. doi:10.1100/2012/710412
- Khush G, Lee S, Cho JI, Jeon JS. 2012. Biofortification of crops for reducing malnutrition. Plant Biotechnol Rep 6:195-202
- Lungaho MG, Mwaniki AM, Szalma SJ, Hart J, Rutzke MA, Kochian LV, Glahn R, and Hoekenga OA. 2011. Genetic and physiological analysis of iron biofortification in maize kernels, PLOS One, 6: e20429 <http://dx.doi.org/10.1371/journal.pone.0020429>
- Mason SC, D'Croz-Mason NE. 2002. Agronomic practices influence maize grain quality. Journal of Crop Production. Vol. 5: 75-91
- Messias, RS, Galli, V, Silva, SDA, Schirmer, MA, Rombaldi, CV. 2013. Micronutrient and functional compounds biofortification of maize grains. Critical Reviews in Food Science and Nutrition, in Press. <http://dx.doi.org/10.1080/10408398.2011.649314>
- Peleg Z, Saranga Y, Yazici A, Fahima T, Öztürk L, Çakmak I. 2008. Grain zinc, iron and protein concentrations and zinc-efficiency in wild emmer wheat under contrasting irrigation regimes. Plant Soil 306: 57-67.
- Pfeiffer WH, McClafferty B. 2007. HarvestPlus: breeding crops for better nutrition. Crop Sci 47:S88-S105
- Qin H, Cai Y, Liu Z, Wang G, Wang J, Guo Y, Wang H. 2012. Identification of QTL for zinc and iron concentration in maize kernel and cob. Euphytica 187(3):345-358
- Saha S, Singh G, Mahajan V, Gupta HS 2009. Variability of nutritional and cooking quality in bean (*Phaseolus vulgaris* L) as a function of genotype. Plant Foods Hum Nutr 64: 174-180.
- Stein AJ, 2010. Global impacts of human malnutrition. Plant Soil 335: 133-154
- TUIK. 2015. Türkiye İstatistik kurumu. (<https://biruni.tuik.gov.tr/bitkiselapp/bitkisel.zul>)
- Welch RM, Graham RD. 2002. Breeding crops for enhanced micronutrient content. Plant Soil 245: 205-214.
- White PJ, Broadley MR. 2005. Biofortifying crops with essential mineral elements. Trends Plant Sci 10: 586-593.
- World Health Organization. 2002. The World Health Report. Reducing Risks, Promoting Healthy Life (World Health Organization, Geneva).
- Yücel C, Baloch FS, Ozkan H. 2009. Genetic analysis of some physical properties of bread wheat grain (*Triticum aestivum* L. em Thell). Turk J Agric For 33: 525-535.