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Research Article

Exploring the Phenotypic Diversity for Seed Mineral Contents in Turkish Faba Bean Germplasm

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Abstract. Biofortification emerged as a sustainable approach for the improvement of nutritional contents of food crops through the utilization of plant breeding, transgenic techniques, or agronomic practices. Legumes are serving as the primary source of plant-based protein for millions of people all over the world. Faba bean is an important legume crop having high protein, mineral, and vitamin contents beneficial for human health. The present investigation involved the seed mineral profiling of faba bean germplasm collected from 20 provinces of Turkey. A good range of variations were observed for nitrogen (N) (5.19-7.52%), phosphorus (P) (0.102-0.668), potassium (K) (0.63-2.46), calcium (Ca) (0.50-0.64), magnesium (Mg) (0.230-0.363), iron (Fe) (57.047-145.63), zinc (Zn) (28.76-90.10), copper (Cu) (6.23-32.33) and manganese (Mn) (12.93-45.37) were investigated. Analysis of variance revealed highly significant variance for Fe, Zn and Cu. A highly significant and positive correlation was observed between Zn and Fe contents and should be considered as parents for the development of Fe and Zn enriched faba bean cultivars. Scatter plot analysis revealed Malatya3 and Izmir3 landraces rich in Fe and Zn contents. Principal component analysis (PCA) was performed and the first five PCs accounted for 75.80% variations. The constellation plot was constructed and the studied germplasm was divided into two populations based on their Fe contents. Landraces present in population B were found rich in Zn and Fe contents. Present investigation enlighted the seed minerals diversity in faba bean germplasm and it is recommended that studied germplasm should be used for the biofortification of faba bean to minimize the malnutrition problems.

Türk Bakla Genetik Kaynaklarının Tohum Mineral İçeriği için Fenotipik Çeşitliliğin Araştırılması

Anahtar kelimeler:
Bakla, yetersiz beslenme, biyofortifikasyon, germplazm karakterizasyonu, mineral çeşitliliği

Özet. Biyofortifikasyon; bitki ıslahı, transgenik teknikler veya agronomik uygulamalar yoluyla gıda ürünlerinin besin içeriklerinin iyileştirilmesi için sürdürülebilir bir yaklaşım olarak ortaya çıkmıştır. Baklagiller, tüm dünyada milyonlarca insan için bitki bazlı proteinin birincil kaynağı olarak hizmet vermektedir. Bakla, insan sağlığı için faydalı yüksek oranda protein, mineral ve vitamin içeriğine sahip önemli bir baklagil bitkisidir. Bu çalışma, Türkiye'nin 20 ilinden toplanan bakla genetik kaynaklarının tohum mineral profilini içermektedir. Araştırmada aynı şartlarda yetiştirilen bakla tohumlarının; azot (N) (% 5.19-7.52), fosfor (P) (0.102-0.668), potasyum (K) (0.63-2.46), kalsiyum (Ca) (0.50-0.64), magnezyum (Mg) (0.230-0.363), demir (Fe) (57.047-145.63), çinko (Zn) (28.76-90.10), bakır (Cu) (6.23-32.33) ve manganez (Mn) (12.93-45.37) bakımından önemli varyasyon gösterdiği belirlenmiştir. Fe ve Zn içerikleri bakımından anlamlı ve pozitif bir ilişki belirlenmiştir. Fe ve Zn bakımından zenginleştirilmiş bakla çeşitlerinin geliştirilmesinde ebeveyn olarak kullanılabilecek materyaller tespit edilmiştir. Scatter plot analizi, Malatya3 ve Izmir3 yerel türlerinin Fe ve Zn içeriği bakımından zengin olduğunu ortaya çıkarmıştır. Temel bileşen analizi (PCA) sonucunda ilk beş temel bileşen varyasyonun %75.80'nini açıklamıştır. Takımyıldız grafiği, incelenen genetik kaynakları Fe içeriklerine göre iki popülasyona bölmüş ve B popülasyonunda bulunan yerel türlerin Fe ve Zn içerikleri bakımından zengin olduğunu göstermiştir. Araştırma sonucunda, bakla genetik kaynaklarının tohum mineral çeşitliliği aydınlatılmıştır. Yeni geliştirilecek bakla çeşitlerinin bazı mineral içerikleri biyolojik olarak zenginleştirilermek için çalışmada yer alan genetik kaynakların kullanılabileceği ön görülmüştür.

INTRODUCTION

The world population is predicted to reach around 9.5 billion by 2050. Kush *et al.* (2012) stated that a huge number of the world population is going to bed hungry. Climate change is threatening adversely to the agriculture production system through huge losses of crops yield each year. Under such circumstances, the agriculture production system is under huge pressure to feed the rapidly increasing world population under changing climatic conditions. Previous reports suggested that there is a need to increase world food production by 60%–110% (Tilman ***et al.***, 2011). The human body needs a significant amount of various minerals and vitamins for its normal functioning. However, minerals malnutrition is also becoming a big threat to humankind by affecting millions of people globally (Khazaei and Vandenberg, 2020). Among these minerals, deficiency of micronutrients which is also known as "Hidden hunger" is becoming more and more severe (Yeken *et al*., 2018). It is reported that more than 2 billion people worldwide are suffering iron (Fe), zinc (Zn) deficiencies (Valença ***et al.***, 2017). Previously, scientists were largely involved to increase the production of crops and therefore the nutritional quality of crops remained less focussed (Nadeem *et al.,* 2021). Considering the importance of the above-discussed problems, it is very important to take measures sustainably to develop high-yielding crop varieties having higher nutritional concentrations for this and coming generations. These problems can be addressed through the biofortification of crops using various plant breeding and biotechnological approaches.

Biofortification emerges as an important, feasible, and cost-effective approach for the improvement of nutritional contents of various crops (Ronoh *et al.,* 2017). Biofortification is an approach aiming to improve the minerals and vitamins contents in crops through the application of plant breeding, transgenic techniques, or agronomic practices. Significant advancement in the biofortification of crops comes through "HarvestPlus" in 2003. HarvestPlus program addressed the micronutrient deficiencies by involving various plant breeding approaches and more than 20 million people from all over the world are now growing biofortified crops (Bouis and Saltzman, 2017). Biofortified crops are getting the attention of consumers especially those living in the least developed and developing countries to address micronutrient malnutrition.

Legumes are an important pillar of the agriculture system and serving as a primary source of protein for millions of people all over the world (Nadeem *et al.,* 2021; Yeken *et al.,* 2019). Legumes are considered a rich source of various minerals and vitamins compared to cereals and have been found very beneficial for human health. Legumes can be a good target for the improvement of nutritional contents, especially for the developing countries where legumes and cereals are consumed as a major food source (Roriz *et al.,* 2020). Among various legumes, faba bean (*Vicia faba* L.) is one of the oldest cultivated crops famous for its high protein contents ranging from 24% to 35% with an average of 29% (Warsame *et al.,* 2018; Tufan and Erdogan, 2017). Moreover, the faba bean is considered a rich source of starch, dietary fiber, minerals, and vitamins (Vilarino *et al.,* 2009). Faba bean is a very old crop and its cultivation has been documented back to the 10th millennium BC (Tanno *et al.* 2006). The Near East region is considered the origin center of this crop and now it is cultivated in more than 70 countries of the world on an area of 2.2 million ha with an annual production of nearly 4 million tons (Warsame *et al.,* 2018). China is the major producer of faba bean by contributing 33% of the global output. Ethiopia, Australia the United Kingdom are other key producers of faba bean (Khazaei and Vandenberg, 2020; Warsame *et al.,* 2018).

Screening of genetic resources is considered starting point toward the breeding of crops as it facilitates novel variations that can be used for marker-assisted breeding (Nadeem *et al.,* 2020; Barut *et al.,* 2020; Karık *et al.,* 2019). Moreover, the collection and conservation of germplasm safeguard the species from their extinct. Currently, more than 43,500 faba bean accessions are conserved in nearly 37 genebanks. The maximum number of accessions (9000) are conserved by ICARDA gene bank house followed by the Chinese Academy of Agricultural Sciences (CAAS) in China having 5200 accessions (Duc *et al.,* 2010; Kaur *et al.,* 2014). This germplasm is a wealth of material for future breeding efforts. Previous report confirmed the historic presence of faba bean in northwest Syria and Turkey (Tanno and Willcox, 2006); therefore, this region is expected to house of highly diverse germplasm. During 2019, faba bean was cultivated in Turkey on an area of 4332 ha and its production was 12346 tonnes (FAO, 2021). Maximum faba bean production in Turkey comes from Marmara and Aegean regions are (Karbuz *et al.,* 2008). Keeping in view the importance of this crop, it is very important to collect, conserve and characterize the germplasm for the investigation of variations that can be used for the breeding of this crop. The current investigation aimed to explore the diversity of nine mineral elements in faba bean germplasm collected from 20 provinces of Turkey.

MATERIAL AND METHOD

A total of 105 faba bean landraces and three commercial cultivars (Salkım, Filiz99, and Kıtık2003) were used as plant material. International Center for Agricultural Research in the Dry Areas (ICARDA) collected a good number of landraces from various provinces of Turkey. Seeds for the plant material used in this study were kindly provided by the ICARDA gene bank in Aleppo, Syria. Information about plant material and collection provinces is provided in Fig. 1. Kıtık2003 and Filiz99 are the main cultivated cultivars in the Mediterranean and Aegean region, while Salkım is mainly grown in the Marmara region (Baloch *et al.,* 2014). Field experimentation was performed according to augmented block design and sowing was done on 10 December 2018. Chemical and physical properties of experimental area are provided in Table 1. Row to row distance was 75 cm and a total of 10 plants were maintained for each landaces. 40 kg ha^{-1} N and 50 kg ha^{-1} P_2O_5 were fertilizer rates applied as a basal dose during the study. Harvesting was performed at 90% of pods' maturity and seeds were stored before their mineral element analysis.

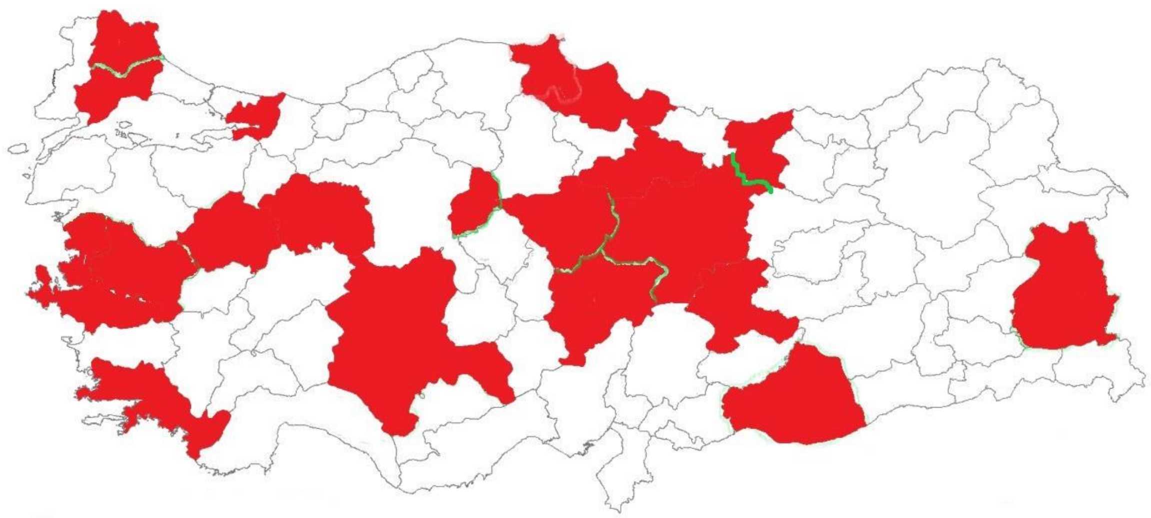

Figure 1. Collection province of faba bean germplasm used in this study.
Şekil 1. Çalışmada kullanılan bakla genetik kaynaklarının toplandığı bölgeler.

Table 1. Chemical and physical properties of experimental area.
Çizelge 1. Deney alanının kimyasal ve fiziksel özellikleri.

Sand (%)	Silt (%)	Clay (%)	pH (1:2.5 H2O)	Salt (mmhos/cm)	P_2O_5 (kg/da)	K2O (kg/da)	Org. Matt. (%)	Fe (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)
14.1	47.6		36.3	0.28	3.1	91.79	1.9	3.86	0.44	4.50	1.14

Mineral Element Analysis

During this study, various mineral elements like nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) were investigated according to methodology described by *Baloch et al.* (2014). Seed samples were taken from each accession and grinding was performed. The resulted fine powder (0.4 g) from each accession was digested through closed microwave digestion system (MARSxpress, CEM Corp.). Digestion was performed using 5 mL of concentrated HNO_3 and 2 mL of concentrated H_2O. Later, analyzed with an inductively coupled plasma optical emission spectrometer (ICP-OES; Vista-Pro Axial; Varian Pty Ltd., Australia). Kjeldahl method was used for the investigation of total nitrogen (Bremner, 1965). Methodology described by Jackson (1962) was used to calculate P contents using spectrophotometer. Concentrations of potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) were investigated through atomic absorption spectrometry (Varian SpektrAA-300, Vienna, Austria) (Beaty and Kerber, 1978).

Statistical Analysis

The analysis of variance (ANOVA) was calculated for each mineral element using agricolae: an R package (De Mendiburu and Simon 2015).The mean, maximum and minimum contents for studied mineral elements were calculated using XLSTAT (www.xlstat.com) software. The Pearson's correlation, scatter plot and principal component analysis (PCA) were also using XLSTAT (www.xlstat.com) software. The cluster constellation plot for 108 faba bean accessions was performed using JMP 14.1.0 statistical software (2018, SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) was found non significant except Fe, Zn and Cu (Table 2).

Table 2. Chemical and physical properties of experimental area.
Çizelge 2. Deney alanının kimyasal ve fiziksel özellikleri.

Nitrogen	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Accessions	107	19.3879	0.18119	2.5934	0.1815
Control	2	0.1509	0.07543	1.0797	0.4217
Phosphorus	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Accessions	107	0.79655	0.007444	1.7837	0.3083
Control	2	0.03138	0.01569	3.7594	0.1206
Potassium	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Accessions	107	6.0361	0.056412	0.9984	0.5899
Control	2	0.0455	0.022756	0.4027	0.6929
Calcium	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Accessions	107	0.071766	0.0006707	0.3078	0.9852
Control	2	0.002091	0.0010457	0.4799	0.6504
Magnesium	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Accessions	107	0.039421	0.00036842	0.4556	0.9256
Control	2	0.004551	0.00227531	2.8137	0.1726
Iron	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Accessions	107	21354.8	199.58	21.6861	0.004066**
Control	2	40.6	20.28	2.2031	0.226419
Zinc	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Accessions	107	7931.5	74.126	16.8992	0.006577**
Control	2	75.5	37.747	8.6056	0.035562*
Copper	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Accessions	107	1742.96	16.289	5.7593	0.04851*
Control	2	14.05	7.023	2.4832	0.19901
Manganese	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Accessions	107	1685.43	15.7516	2.5562	0.1855
Control	2	7.17	3.5864	0.582	0.6

Signif. codes: 0.001 '**' 0.01 '*' 0.05

Analysis of variance revealed highly significant variations for both accessions and controls for Zn. In case of Fe and Cu, only accessions were found highly siginificant. Mean maximum, minimum contents for studied mineral elements are reported in Table 3, while investigated minerals contents for each accession are provided in Table 4. During this study, N contents ranged 5.19-7.52% for Konya5 and Tekirdağ2 respectively, and mean N contents were 6.32%. Range and mean N contents found in this study were much higher than the reported by Lombardo *et al.* (2016). Phosphorus contents ranged 0.10-0.67% for Muğla12 and Izmir3, while mean P contents were 0.47. Range and mean P contents were found lower than the reported by Baloch *et al.* (2014). Similarly, Khan *et al.* (2015) also reported higher P contents than this study. Potassium contents ranged 0.63-2.47% for Izmir22 and Izmir23 respectively, while 1.79 were mean K contents. Similar to N, P, this study reported lower K contents compared to previous reports by Baloch *et al.* (2014) and Khan *et al.* (2015). Calcium contents ranged 0.50-0.64 mg kg^{-1} for Tokat4 and Izmir14 respectively, while mean Ca contents were 0.56 mg kg^{-1}. Khan *et al.* (2015) reported higher Ca contents than the reported by the current study. Magnesium contents ranged 0.23-0.36 mg kg^{-1} for Izmir23 and Sivas1 respectively, while mean Mg contents were 0.32 mg kg^{-1}. Khazaei and Vandenberg (2020) found higher Mg contents than reported in the present study. Iron contents ranged from 57.05-145.63 mg kg^{-1} for Eskişehir and Malatya3 respectively, while mean Fe contents were 85.62 mg kg^{-1}. Range and mean Fe contents found in this study were much higher than the reported by previous studies (Labba *et al.,* 2021; Baloch *et al.*, 2014; Khan *et al.* 2015; Cabrera *et al.* 2003). Zinc contents ranged from 28.76 to 90.10 mg kg^{-1} for Muğla2 and Izmir3, while 44.28 mg kg^{-1} was the mean Zn contents. Similar to Fe contents, the present investigation reported higher Zn contents as well than the previous studies (Labba *et al.,* 2021; Baloch *et al.,* 2014; Khan *et al.* 2015; Cabrera *et al.*, 2003). Copper contents ranged 6.23-32.33 mg kg^{-1} for Şanlıurfa and Giresun1 respectively, while mean Cu contents were 17.27 mg kg^{-1}. Range and mean Cu contents found in this study were in line with Baloch *et al.* (2014). Manganese contents ranged from 12.93-45.37 mg kg^{-1} Tekirdağ13 and Tokat4 respectively, while mean Mn contents were 23.77 mg kg^{-1}. Range and mean Mn contents reported in this study were found much higher than the previous reports (Khazaei and Vandenberg, 2020; Baloch *et al.,* 2014). The present investigation reported lower contents of N, P, K, Ca, and Mg from the previous studies. However, the present study reported higher concentrations of various micro-nutrients like Fe, Zn, Co, and Mn than the previous reports. Possible reasons behind the existence of these variations might be due to differences in germplasm, climatic conditions, experimental site coordinates, soil conditions, and mineral element detection methodology. As a huge number of the world population is facing Fe and Zn deficiency and this problem is becoming more and more adverse due to insufficient availability of these elements in routine food. This situation can be solved through the utilization of germplasm having sufficient diversity for these minerals. Present germplasm reported a variation of 2.55 -fold for the iron content and almost 3.14-fold for the zinc content among the analyzed faba bean germplasm. Therefore, it is strongly recommended that the present germplasm should be used for the development of Fe and Zn enriched faba bean cultivars to mitigate malnutrition problems.

Table 3. Mean, maximum, minimum values for investigated mineral elements for faba bean germplasm.
Çizelge 3. Bakla genetik kaynaklarında incelenen mineral elementlerin ortalama, maksimum ve minimum değerleri.

Variable	Minimum	Maximum	Mean	Std. deviation
Nitrogen (%)	5.190	7.520	6.320	0.435
Phosphorus (%)	0.102	0.668	0.468	0.092
Potassium (%)	0.633	2.467	1.789	0.242
Calcium (mg kg-1)	0.500	0.640	0.556	0.028
Magnesium (mg kg-1)	0.230	0.363	0.317	0.019
Iron (mg kg-1)	57.047	145.630	85.616	14.187
Zinc (mg kg-1)	28.760	90.100	44.279	8.665
Copper (mg kg-1)	6.230	32.330	17.267	4.060
Manganese (mg kg-1)	12.930	45.373	23.774	3.993

Table 4. Mineral elements diversity in faba bean germplasm used in this study.
Çizelge 4. Çalışmada kullanılan bakla genetik kaynaklarının mineral elementlerinin çeşitliliği.

Genotypes	N (%)	P (%)	K (%)	Ca (mg kg-1)	Mg (mg kg-1)	Fe (mg kg-1)	Zn (mg kg-1)	Cu (mg kg-1)	Mn (mg kg-1)
Eskişehir	5.84	0.62	1.74	0.55	0.31	57.05	48.75	21.76	25.51
Giresun1	6.88	0.49	2.16	0.57	0.35	87.81	36.88	32.33	25.90
Giresun2	5.90	0.60	1.99	0.57	0.32	73.35	32.88	22.91	23.25
Giresun3	6.29	0.57	1.87	0.57	0.32	79.59	43.13	18.37	22.96
Giresun4	6.09	0.58	1.82	0.54	0.32	81.63	40.57	17.00	21.38
Giresun5	7.06	0.46	1.80	0.58	0.33	93.54	46.47	13.06	24.63
İzmir1	6.12	0.53	1.59	0.59	0.32	92.11	49.96	24.79	23.35
İzmir2	6.47	0.46	1.62	0.55	0.32	91.10	52.12	17.29	24.88
İzmir3	6.74	0.67	2.23	0.56	0.35	129.61	90.10	15.43	21.16
İzmir4	6.52	0.50	1.75	0.57	0.33	107.47	51.86	16.15	27.92
İzmir5	6.91	0.53	1.74	0.56	0.33	80.81	46.59	20.24	25.17
İzmir6	6.83	0.45	1.73	0.55	0.32	86.72	36.68	18.89	22.15
İzmir7	6.41	0.50	1.78	0.59	0.34	77.38	45.60	20.48	24.79
İzmir8	6.23	0.47	1.67	0.57	0.34	84.48	51.00	20.14	27.80
İzmir9	6.08	0.47	1.63	0.58	0.31	90.00	46.30	20.23	24.53
İzmir10	6.86	0.53	1.55	0.56	0.31	93.39	57.35	16.71	30.73
İzmir11	6.46	0.54	1.83	0.59	0.32	98.25	59.20	24.59	25.25
İzmir12	6.41	0.58	1.56	0.58	0.30	87.66	52.49	24.37	20.42
İzmir13	7.06	0.59	1.83	0.58	0.33	69.47	36.96	11.82	24.72
İzmir14	6.71	0.49	1.69	0.64	0.32	80.68	42.65	19.55	29.12

Table 4. Continue.
Çizelge 4. Devamı.

Genotypes	N (%)	P (%)	K (%)	Ca (mg kg-1)	Mg (mg kg-1)	Fe (mg kg-1)	Zn (mg kg-1)	Cu (mg kg-1)	Mn (mg kg-1)
İzmir15	6.44	0.54	1.68	0.56	0.33	63.92	48.53	11.85	24.77
İzmir16	6.71	0.45	1.82	0.56	0.32	81.68	43.00	21.25	18.77
İzmir17	6.60	0.64	2.23	0.62	0.36	91.40	41.75	18.57	22.22
İzmir18	6.21	0.56	2.39	0.59	0.34	77.07	40.89	16.47	22.97
İzmir19	6.20	0.43	2.02	0.57	0.30	96.70	46.70	17.87	21.68
İzmir20	6.22	0.59	1.85	0.59	0.32	90.40	37.95	17.43	27.33
İzmir21	6.97	0.39	2.20	0.60	0.34	83.91	36.79	14.51	22.51
İzmir22	6.43	0.54	2.47	0.58	0.36	89.16	48.29	14.25	27.24
İzmir23	6.79	0.48	0.63	0.52	0.23	100.56	54.28	21.38	26.10
Kayseri1	6.63	0.50	1.94	0.57	0.31	115.68	42.12	13.74	23.60
Kayseri2	5.83	0.46	2.39	0.57	0.33	95.63	38.42	20.41	24.12
Kayseri3	6.08	0.49	2.21	0.55	0.31	87.88	36.51	16.54	20.45
Kırklareli1	6.04	0.36	1.74	0.56	0.31	84.46	48.72	18.78	19.42
Kırklareli2	5.49	0.45	1.70	0.59	0.32	71.26	51.24	19.57	25.36
Kırklareli3	6.21	0.45	1.63	0.57	0.32	68.47	47.53	12.91	25.99
Kırklareli4	5.96	0.64	1.87	0.55	0.31	66.10	43.40	14.25	35.51
Kırklareli5	6.76	0.41	1.64	0.55	0.32	87.42	39.53	15.36	23.12
Kırklareli6	6.83	0.34	1.85	0.56	0.30	79.53	40.11	16.88	26.42
Kırşehir1	6.23	0.44	1.62	0.54	0.31	69.85	38.74	18.41	19.87
Kırşehir2	6.18	0.51	1.69	0.60	0.33	79.22	49.23	21.21	20.41
Kocaeli	6.14	0.46	1.90	0.56	0.32	99.37	49.45	19.79	23.11
Konya1	6.29	0.28	1.77	0.57	0.31	112.08	61.03	17.65	25.41
Konya2	6.61	0.55	1.84	0.55	0.31	98.61	52.63	16.55	18.56
Konya3	6.68	0.43	1.76	0.57	0.31	113.83	54.36	18.58	33.05
Konya4	6.45	0.41	1.75	0.57	0.32	76.41	50.48	14.32	28.23
Konya5	7.52	0.47	1.87	0.58	0.32	85.54	51.94	17.88	23.22
Konya6	6.91	0.46	1.66	0.55	0.32	82.42	45.04	15.94	20.42
Konya7	6.25	0.45	1.74	0.57	0.33	93.21	42.12	18.41	19.45
Kütahya	7.17	0.44	1.74	0.55	0.31	95.87	40.36	16.23	26.23
Malatya1	6.90	0.30	1.73	0.55	0.32	89.75	52.45	15.18	24.36
Malatya2	6.38	0.26	2.17	0.58	0.34	96.42	63.21	20.63	22.33
Malatya3	6.78	0.36	1.94	0.54	0.32	145.63	59.36	23.47	25.09
Malatya4	6.33	0.45	1.86	0.55	0.33	110.23	55.42	19.48	18.74
Manisa1	6.18	0.43	1.74	0.57	0.34	74.20	48.77	13.35	16.84
Manisa2	6.33	0.26	1.87	0.58	0.32	77.38	39.52	15.99	20.09
Muğla1	5.83	0.20	2.23	0.57	0.34	94.30	39.31	11.91	22.82
Muğla2	5.97	0.47	1.74	0.55	0.31	83.92	28.76	13.26	23.30
Muğla3	6.22	0.46	1.97	0.60	0.36	88.18	29.51	12.26	22.02
Muğla4	6.22	0.48	1.55	0.59	0.31	89.30	42.73	14.28	23.54
Muğla5	6.40	0.51	1.63	0.56	0.31	79.25	37.14	15.87	20.70
Muğla6	6.41	0.42	1.83	0.55	0.31	78.94	33.10	14.03	24.37
Muğla7	5.93	0.47	1.54	0.59	0.31	81.42	39.43	13.12	20.11
Muğla8	6.46	0.50	1.96	0.59	0.34	90.28	50.21	19.20	23.06
Muğla9	5.30	0.58	1.64	0.57	0.31	76.56	45.56	14.24	23.81
Muğla10	6.07	0.40	1.69	0.55	0.31	93.40	46.25	16.65	22.46
Muğla11	5.96	0.50	1.77	0.57	0.32	94.95	39.48	17.16	20.51
Muğla12	6.45	0.10	1.57	0.56	0.31	88.16	29.49	12.40	25.61
Muğla13	6.13	0.48	1.74	0.57	0.33	95.43	33.20	18.18	19.59
Samsun1	6.65	0.45	1.66	0.57	0.32	72.41	39.78	15.40	21.44
Samsun2	6.83	0.48	1.60	0.59	0.32	94.14	41.28	19.87	27.69
Samsun3	6.24	0.51	1.65	0.58	0.33	115.94	40.26	16.39	24.73
Sinop	7.15	0.47	1.75	0.59	0.31	67.29	50.45	9.32	24.91
Sivas1	6.50	0.46	2.12	0.58	0.36	93.43	49.87	18.83	25.10
Sivas2	6.44	0.54	1.79	0.57	0.34	78.36	48.53	12.55	22.02
Sivas3	6.24	0.44	1.50	0.51	0.31	80.06	45.42	15.75	20.55
Sivas4	6.38	0.33	1.42	0.55	0.31	79.44	29.42	6.31	28.04
Sivas5	6.08	0.42	1.55	0.53	0.32	76.07	36.52	18.26	25.93

Table 4. Continue.
Çizelge 4. Devam.

Genotypes	N	P	K	Ca	Mg	Fe	Zn	Cu	Mn
Şanlıurfa	6.01	0.61	1.57	0.53	0.33	91.15	42.15	6.23	25.17
Tekirdağ1	5.70	0.48	1.59	0.53	0.31	88.91	43.26	12.55	26.96
Tekirdağ2	5.19	0.48	2.06	0.54	0.36	77.81	50.45	14.11	24.45
Tekirdağ3	5.34	0.59	1.58	0.51	0.32	66.93	32.59	14.79	26.96
Tekirdağ4	5.84	0.47	1.77	0.50	0.30	78.13	30.09	16.06	20.87
Tekirdağ5	6.39	0.47	1.96	0.51	0.29	74.08	40.12	23.11	24.17
Tekirdağ6	6.72	0.49	1.85	0.52	0.32	82.24	41.29	26.05	21.87
Tekirdağ7	6.63	0.42	1.75	0.50	0.30	81.12	40.23	16.63	20.39
Tekirdağ8	5.56	0.47	1.49	0.51	0.30	71.44	39.46	18.05	19.23
Tekirdağ9	6.17	0.51	1.51	0.52	0.30	74.04	36.50	15.05	21.70
Tekirdağ10	5.20	0.39	1.92	0.54	0.30	78.42	43.83	18.65	20.15
Tekirdağ11	6.26	0.42	1.50	0.52	0.30	86.01	45.44	16.57	18.77
Tekirdağ12	6.00	0.54	1.91	0.53	0.33	92.26	49.76	18.35	21.18
Tekirdağ13	5.98	0.59	2.02	0.56	0.32	76.30	52.33	16.24	12.93
Tekirdağ14	6.49	0.29	2.04	0.55	0.31	109.12	44.78	21.88	21.08
Tekirdağ15	6.38	0.33	1.86	0.53	0.31	81.46	41.23	8.67	19.24
Tekirdağ16	6.19	0.46	1.51	0.52	0.29	75.74	40.78	22.38	19.52
Tekirdağ17	6.49	0.55	1.62	0.50	0.29	74.04	36.58	19.45	28.59
Tokat1	5.88	0.42	2.17	0.52	0.33	69.02	52.17	18.18	23.81
Takat2	6.00	0.53	1.78	0.52	0.30	74.36	45.76	14.84	28.47
Tokat3	7.03	0.50	1.79	0.52	0.30	77.79	49.83	19.85	22.96
Tokat4	6.87	0.51	1.76	0.50	0.31	78.09	52.23	13.45	45.37
Tokat5	5.96	0.39	1.74	0.54	0.30	74.40	29.75	19.76	31.75
Tokat6	6.44	0.46	1.54	0.53	0.30	125.05	36.54	16.75	24.54
Urfa	6.35	0.42	1.73	0.52	0.29	67.30	29.74	8.46	20.24
Van	5.55	0.40	1.52	0.51	0.29	80.80	39.47	20.30	25.38
Yozgat1	5.80	0.44	1.84	0.54	0.29	79.18	45.39	23.28	22.13
Yozgat2	5.76	0.50	1.84	0.51	0.30	79.06	49.90	17.44	23.75
Filiz-99*	6.33	0.38	1.90	0.53	0.31	69.87	36.47	19.45	22.76
Kıtık-2003*	6.40	0.45	1.62	0.53	0.31	75.38	39.27	20.32	24.58
Salkım*	6.17	0.63	2.13	0.51	0.30	72.43	41.62	21.50	27.72

*Control cultivar

Table 5. Correlation analysis exploring the association between studied mineral traits in faba bean germplasm.
Çizelge 5. Bakla genetik kaynaklarının mineral içeriklerinin korelasyon analizi.

Variables	N	P	K	Ca	Mg	Fe	Zn	Cu	Mn
N	**1**	-0.065	-0.008	0.224	0.031	**0.254****	0.180	0.027	0.146
P		**1**	0.053	0.049	0.116	-0.148	0.144	0.103	0.101
K			**1**	**0.268****	**0.620****	0.104	0.117	0.093	-0.086
Ca				**1**	**0.544****	0.194	0.147	0.019	-0.055
Mg					**1**	0.130	0.164	-0.066	-0.060
Fe						**1**	**0.406****	0.147	-0.015
Zn							**1**	0.177	0.043
Cu								**1**	-0.052
Mn									**1**

Values in bold are different from 0 with a significance level alpha=0.01, **=0.001

The Pearson's correlation coefficient was calculated that revealed a highly significant and positive correlation of Mg with K and Ca. Similarly, a highly significant and positive correlation was found between Fe and Zn contents. Correlation analysis is an important statistical methodology that enlightens the association between two or more traits (Nadeem *et al.,* 2021). Mudasir *et al*., (2012) stated that when traits are significantly associated with each other, selection of one trait will exert variations to the other significantly associated trait as well. A previous report revealed that two traits are significantly associated because of epistatic effects or genetic linkage among their genes (Ozer *et al*., 2010). Baloch *et al.* (2014) reported a highly significant and positive correlation between Zn and Fe in faba bean and these results are in line with the current findings.

A Scatter plot was constructed between Fe and Zn contents and maximum Zn and Fe contents were reflected by Izmir3 and Malatya3 (Fig. 2). As above mentioned landraces were found phenotypically rich for Fe and Zn contents. Therefore, it is recommended that these landraces should be considered for the development of Fe and Zn enriched faba bean cultivars.

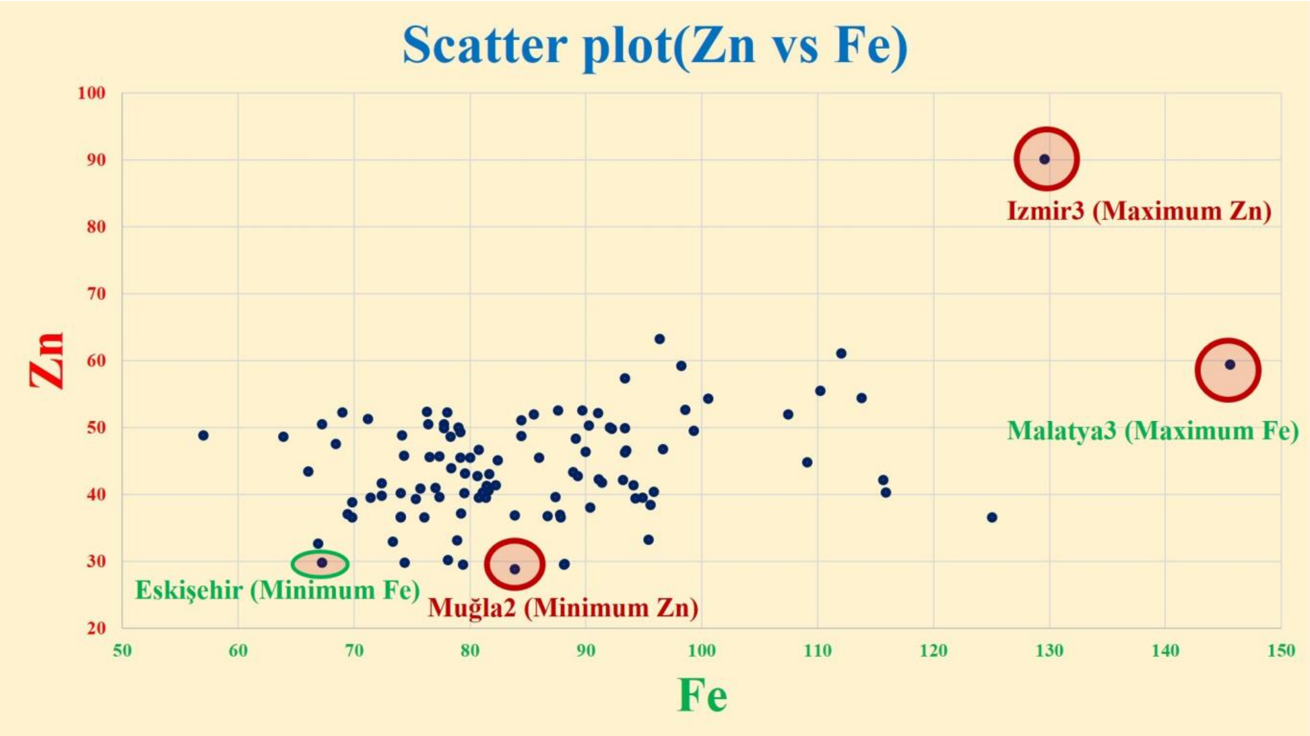

Figure 2. Scatter plot between Fe and Zn contents in faba bean germplasm.
Şekil 2. Bakla genetik kaynaklarının Fe ve Zn içerikleri bakımından Scatter plot analizi.

Principal Component Analysis (PCA) was performed and the first five PCs accounted for a total of 75.80% variation. Maximum variations (24.65%) were accounted for by PC1, while Mg was key variations contributing trait in this PC. A total of 16.38% variations were resulted by 2nd PC, while Fe was key variations contributing trait in this PC. A total of 13.16% variations were accounted for 3rd PC, while P was key variations contributing trait. A total of 12.57% and 9.035% variations were resulted by PC4 and PC5 respectively, while Mn and Cu were key variations contributing traits in these PCs respectively. Baloch et al., (2014) stated that multivariate analyses are effective tools to understand and explore the relative contribution added by various traits in the total variability of studied germplasm. Previous reports suggested that PCA analysis is very helpful in the grouping of germplasm having similar traits (Andeden et al., 2013; Karaköy et al., 2014). Maximum variations were present in PC1 and a key contributor to these variations were Mg, Ca, K and Zn. The inter-relationship between these key traits contributing variations in PC1 is very important for an attempt to develop faba bean cultivars with higher seed for high seed Mg, Ca, K, and Zn contents.

Table 6. Principal component analysis for faba bean germplasm.
Çizelge 6 Bakla genetik kaynakları için temel bileşen analizi.

	PC1	PC2	PC3	PC4	PC5
N	0.312	0.541	-0.261	0.377	0.432
P	0.125	-0.145	0.819	0.240	0.010
K	0.669	-0.420	0.044	-0.108	-0.048
Ca	0.707	-0.133	-0.175	0.181	0.302
Mg	0.791	-0.446	-0.043	0.119	-0.070
Fe	0.494	0.584	-0.210	-0.220	-0.274
Zn	0.504	0.503	0.286	-0.102	-0.436
Cu	0.172	0.313	0.480	-0.517	0.506
Mn	-0.066	0.276	0.235	0.739	-0.081
Eigenvalue	2.219	1.475	1.185	1.131	0.813
Variability (%)	24.656	16.388	13.162	12.570	9.035
Cumulative %	24.656	41.043	54.206	66.776	75.810

The constellation plot was constructed and studied germplasm was divided into two populations based on their Fe contents. Population A was found smaller than population B. A total of 43 accessions were clustered in population A, while 65 accessions were clustered in population B. Population A was further divided into subpopulations A1 and A2. A total of 20 and 23 accessions were present in subpopulations A1 and A2 respectively. Subpopulation A2 was found more diverse by clustering accessions having Fe contents in a range of 68-80, while Fe contents ranged 70-80 for the subpopulation A1. Population B was also further divided into B1 and B2 subpopulations. Subpopulation B2 was larger than B1 and clustered a total of 42 accessions, while 23 accessions in the B1 subpopulation. Subpopulation B1 contains accessions having Fe contents ranging 67-90. Subpopulation B2 was found most diverse and riched Fe contents. Accessions present in this subpopulation were high in Fe contents and Malaysta3 having maximum Fe contents during this study was also present in this subpopulation. Moreover, the Izmir3 landrace having maximum Zn contents was also present in subpopulation B2. It is understandable from the scatter plot that maximum Fe and Zn contents are showed by Malaysta3 and Izmir3 landraces and in the constellation plot both landraces were present in the same population B. Therefore, it can be assumed that landraces present in population B have higher Zn and Fe contents and can be used for the biofortification of faba bean.

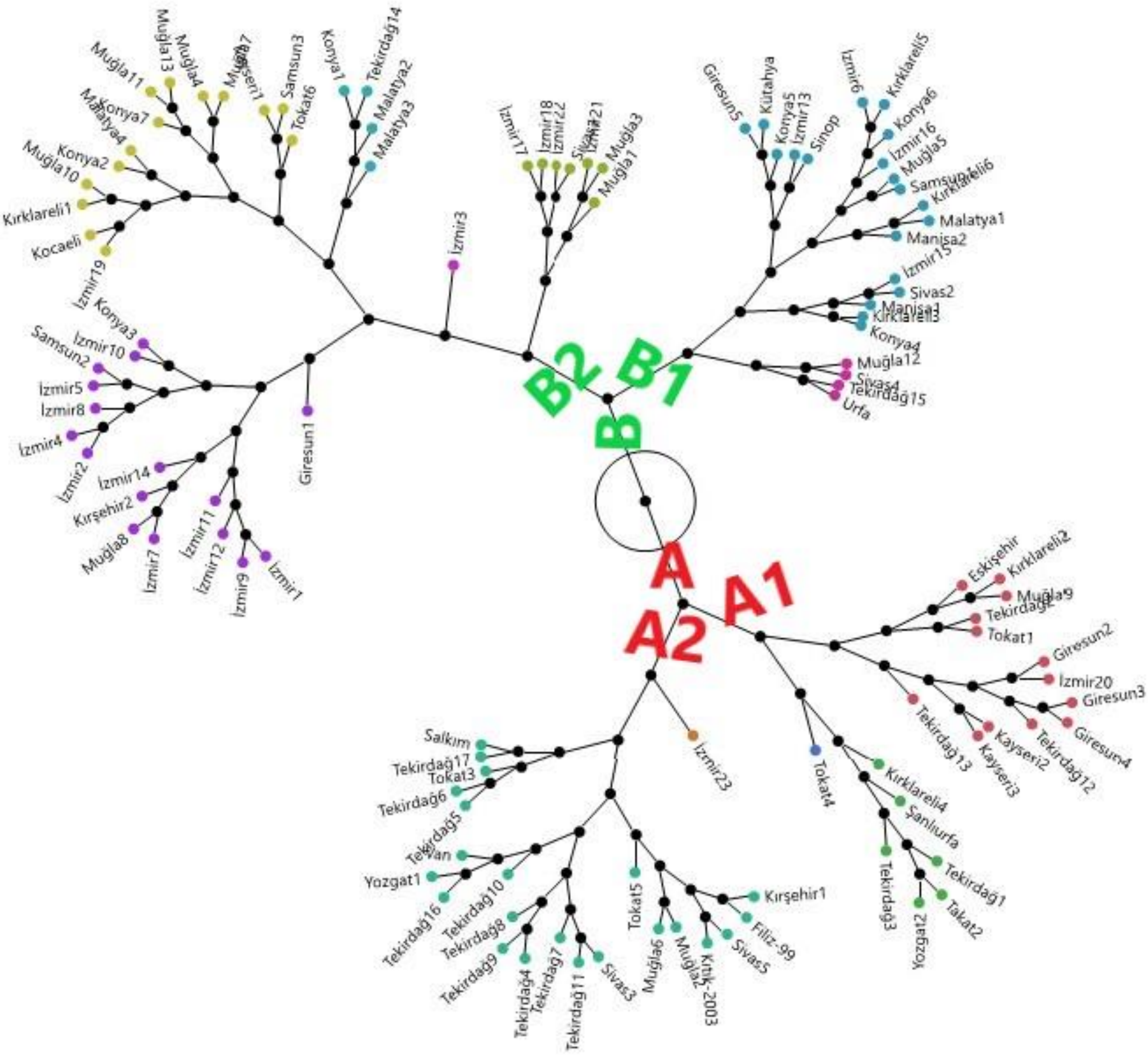

Figure 3. Constellation plot for mineral traits of faba bean germplasm.
Şekil. Bakla genetik kaynaklarının mineral özellikleri için takımyıldızı grafiği.

CONCLUSION

The present study comprehensively enlightened the phenotypic variations of mineral elements in a faba bean germplasm. A highly significant and positive correlation was observed between Fe and Zn. Izmir3 and Malatya3 landraces showed maximum Zn and Fe contents respectively and should be considered as candidate parents for the development of faba bean cultivars having higher Fe and Zn contents.

CONFLICT OF INTEREST

The author report that there are no conflicts of interest.

DECLARATION OF AUTHOR CONTRIBUTION

The planning, preparation, experimentation and writing of the manuscript was done by M.A.N.

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