# Variation of some seed mineral contents in open pollinated faba bean (Vicia faba L.) landraces from Turkey 

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#### Abstract

The first step towards the biofortification of edible portions of crop species with improved nutritional value is to understand the genetic diversity available to breeders in germplasm collections. A faba bean germplasm ( 129 landraces and 4 cultivars) from diverse geographic regions of Turkey was assessed for micro- and macroelement contents of seeds. The results showed high diversity in open-pollinated faba bean germplasm for contents of $\mathrm{N}\left(27.5-93.3 \mathrm{~g} \mathrm{~kg}^{-1}\right), \mathrm{P}\left(1.24-4.89 \mathrm{~g} \mathrm{~kg}^{-1}\right), \mathrm{K}\left(4.5-19.3 \mathrm{~g} \mathrm{~kg}^{-1}\right), \mathrm{Fe}(29.7-96.3 \mathrm{mg}$ $\left.\mathrm{kg}^{-1}\right), \mathrm{Mn}\left(15.5-29.2 \mathrm{mg} \mathrm{kg}{ }^{-1}\right), \mathrm{Cu}\left(10.3-33.0 \mathrm{mg} \mathrm{kg}^{-1}\right)$, and $\mathrm{Zn}\left(10.4-49.3 \mathrm{mg} \mathrm{kg}^{-1}\right)$. Meanwhile, the ranges of mineral elements in the landraces were significantly higher than those in the commercial cultivars. Concentrations of these mineral elements were significantly and positively correlated with each other. Principal component analysis clearly split the faba bean landraces into 2 groups and the first 2 principal components accounted around $70.91 \%$ of the total variations. These findings indicate a wide range of variations for the investigated minerals in the Turkish gene pool that can provide a good source of diversity to use in faba bean biofortification for increased levels of available mineral elements and better yield.


Key words: Biofortification, breeding, diversity, faba bean, landraces, mineral elements

## 1. Introduction

The importance of nutrients (micro and macro) for normal human growth is universally recognized (White and Broadley, 2005; Khan et al., 2008; Menkir, 2008; Chatzav et al., 2010). The mineral elements most frequently lacking in human diets are iron ( Fe ), zinc ( Zn ), and iodine ( I ), although other elements such as calcium (Ca), magnesium $(\mathrm{Mg})$, copper $(\mathrm{Cu})$, and selenium (Se) could be deficient in the diets of some populations in the developing world (Welch and Graham, 2002; White and Broadley, 2005). It has been estimated that over 3.7 billion people worldwide are facing Fe deficiency ( $60 \%$ ), and $54 \%$ of these 3.7 billion people are severely deficient (Yang et al., 1998). Similarly, one-third of the world's population is suffering from Zn malnutrition and its severity varies between $4 \%$ and $73 \%$ in people in developing countries, depending upon the living and economic conditions that ultimately affect their diets.

Interventions to address mineral malnutrition can be implemented through dietary diversification and mineral supplementation. However, none of these remedies are
universally successful. This has led to the suggestion of a complementary solution for mineral malnutrition though the development of food varieties with enhanced levels of bioavailable mineral micronutrients in the edible portion of crops. This would be accomplished through agronomic intervention or genetic selection that would ameliorate the incidence of these mineral deficiencies in humans. This phenomenon is called biofortification. Enrichment of food crops with mineral nutrients is currently a high-priority research area. To ensure healthy diets, increasing emphasis is being placed on the content of essential elements in seeds (Moraghan et al., 2006). Producing micronutrientenriched cultivars (biofortified), particularly those with either agronomically or genetically increased Zn and Fe , and improving the bioavailability of these nutrients are considered promising and cost-effective methods to manage micronutrient deficiencies (Duc et al., 2010)

Faba bean is one of the major food legume crops in Turkey as well in the Mediterranean region. Faba beans are an important dietary source of protein, starch, minerals, vitamins, and antioxidant compounds (Karaköy et al.,

[^0]2014). Legumes, including faba bean, are consumed by people throughout the world. Therefore, increasing the bioavailability of mineral elements in legume seeds could be used to decrease mineral deficiency.

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 exist for all the mineral elements most frequently lacking in human diets. This can be used in breeding programs to increase mineral concentrations in edible products (White and Broadley, 2005). A large number of faba bean landraces have arisen over time because of differences in traditional farming practices and taste preferences. These landraces are a valuable source of genetic variation. In the recent years, few faba bean varieties were released within Turkey; most of the faba bean cultivars were introduced from different countries and started to replace the landraces. These faba bean landraces are still local agroecotypes, usually named after their cultivation area. The Mediterranean region, particularly Turkey, with a concentration of large-seeded forms, is considered to be a secondary center (Muratova, 1931) with thousands of landraces in their natural habitat. Faba bean is one of the most neglected crops worldwide and is considered an "orphan crop". Although faba bean is also one of the most important legumes in Turkey and other Mediterranean countries, very limited genetic and breeding studies have been conducted. Therefore, there is a dire need to study and characterize the faba bean germplasm from its areas of origin and domestication for different traits of interest for breeders and consumers. We studied the morphological and quality traits in a set of 178 faba bean landraces (Karaköy et al., 2014) and we found that Turkish faba bean landraces harbored high phenotypic diversity compared with Chinese, Greek, and International Center of Agricultural Research in the Dry Areas (ICARDA) varieties. Therefore, we expected that these germplasms also harbored high variations for mineral elements. Here our objective was to investigate the available diversity of 7 mineral elements ( $\mathrm{N}, \mathrm{P}, \mathrm{K}, \mathrm{Fe}$, $\mathrm{Mn}, \mathrm{Cu}$, and Zn ) in seeds of 129 open-pollinated faba bean landraces and 4 commercial cultivars.

## 2. Materials and methods

### 2.1. Plant material and crop sowing

The research materials included 129 open-pollinated faba bean landraces and 4 commercial cultivars (Eresen87, Salkım, Filiz99, and Kıtık2003) released in the last few years. Cultivars Eresen87 and Salkım are grown in the Marmara region, and Filiz99 and Kıtık2003 are mostly grown in the Aegean and Mediterranean regions. During the last 2 decades, faba been landraces from Turkey were
collected and preserved at the gene bank of ICARDA. Therefore, all the seeds of the landraces were kindly provided by the ICARDA gene bank in Aleppo, Syria. Names of the landraces and their collection sites are shown in Table 1 and their collection sites are shown in Figure 1. All faba bean landraces and the 4 cultivars were sown in December 2010 on a well-prepared seedbed using a randomized block design with 3 replications at the research and implementation area of the Çukurova Agricultural Research Institute, Adana ( $37^{\circ} 00^{\prime} 56^{\prime \prime} \mathrm{N}, 35^{\circ} 21^{\prime} 29^{\prime \prime} \mathrm{E}$ ), Turkey, which has a typical Mediterranean climate with high precipitation in winter and spring and high temperatures and drought conditions in summer. All landraces and cultivars were grown in plots of 4 rows, each 4 m in length, with 10 cm between plants within a row and 50 cm between rows. All faba bean landraces were grown on homogeneous soil and were treated identically with standard local agricultural practices. Harvest was done in June 2011. Since V. faba is a partially allogamous species undergoing considerable outcrossing (Suso et al., 2001) and the field plots were not protected from the insect pollinators, some degree of hybridization might have occurred. Thus, the seeds used for the determination of the minerals were not the initial landraces but some unspecified hybrids resulting from open pollination among the several landraces. Therefore, the plant material used in this study for observing the mineral elements variations was referred to as open-pollinated landraces.

The upper $0-15 \mathrm{~cm}$ of the soil at the experimental site was classified as a clay loam structure that contained low organic matter contents with an average of $1.89 \%$, salt of 0.29 mmhos $\mathrm{cm}^{-1}$, lime of $11.6 \%$ with $\mathrm{pH} 7.6,1100 \mathrm{~kg}$ $\mathrm{ha}^{-1}$ and $29.30 \mathrm{~kg} \mathrm{ha}^{-1}$ available K and $\mathrm{P}, 0.10 \%$ total N , $0.45 \mathrm{mg} \mathrm{kg}^{-1} \mathrm{Zn}$, and $2.73 \mathrm{mg} \mathrm{kg}{ }^{-1} \mathrm{Fe}$. Total precipitation of the growing season (sowing to physiological maturity) was around 633.3 mm . After soil analysis, composite NP fertilizers were applied as a basal dose containing $40 \mathrm{~kg} \mathrm{ha}^{-1}$ N and $50 \mathrm{~kg} \mathrm{ha}{ }^{-1} \mathrm{P}_{2} \mathrm{O}_{5}$.

### 2.2. Micro- and macronutrient analysis

Some amount of seed samples was taken from every landrace with 3 replications and seeds were bulked. Seed samples ( 0.4 g ) were digested in a closed microwave digestion system (MARSxpress, CEM Corp.) in 5 mL of concentrated $\mathrm{HNO}_{3}$ and 2 mL of concentrated $\mathrm{H}_{2} \mathrm{O}$ and were then analyzed for mineral nutrients with an inductively coupled plasma optical emission spectrometer (ICP-OES; Vista-Pro Axial; Varian Pty Ltd., Australia). Nitrogen was measured using the method given by the AOAC (1984) on a Leco TruSpec CN3342 System (LECO Corp., USA) with 0.2 g of sample. Three readings for each mineral element were recorded from the seed lot.

### 2.3. Statistical analysis

Standard one-way analysis of variance (ANOVA) was performed for each mineral element using the PROC GLM
Table 1. Name, collection sites, mean values, and standard deviations of 129 Turkish faba bean landraces and varieties used in this study.

| Number in Figures 1 and 2 | Name of landrace | Collection site | $\mathrm{N}\left(\mathrm{g} \mathrm{kg}^{-1}\right)$ | $\mathrm{P}\left(\mathrm{g} \mathrm{kg}^{-1}\right)$ | $K\left(\mathrm{~g} \mathrm{~kg}^{-1}\right)$ | $\mathrm{Fe}\left(\mathrm{mg} \mathrm{kg}^{-1}\right)$ | $\mathrm{Mn}\left(\mathrm{mg} \mathrm{kg}^{-1}\right)$ | $\mathrm{Cu}\left(\mathrm{mg} \mathrm{kg}^{-1}\right)$ | $\mathrm{Zn}\left(\mathrm{mg} \mathrm{kg}^{-1}\right)$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | Adana 1 | Adana | $33.9 \pm 0.24$ | $2.51 \pm 0.04$ | $16.2 \pm 0.32$ | $55.9 \pm 2.93$ | $21.7 \pm 1.66$ | $19.7 \pm 1.15$ | $23.6 \pm 6.34$ |
| 2 | Adana 2 | Adana | $32.8 \pm 0.16$ | $3.76 \pm 0.03$ | $6.8 \pm 0.07$ | $39.8 \pm 1.52$ | $19.4 \pm 0.73$ | $11.8 \pm 1.34$ | $13.8 \pm 0.84$ |
| 3 | Adana 3 | Adana | $37.1 \pm 0.44$ | $3.13 \pm 0.04$ | $15.5 \pm 0.16$ | $72.8 \pm 2.34$ | $22.8 \pm 2.42$ | $20.0 \pm 1.23$ | $33.3 \pm 1.96$ |
| 4 | Antakya 1 | Antakya | $36.9 \pm 0.13$ | $3.07 \pm 0.02$ | $14.4 \pm 0.18$ | $62.0 \pm 1.49$ | $24.6 \pm 1.03$ | $19.6 \pm 1.12$ | $29.7 \pm 1.06$ |
| 5 | Antakya 2 | Antakya - Kücüknehir | $36.1 \pm 0.37$ | $1.67 \pm 0.02$ | $6.1 \pm 0.08$ | $31.8 \pm 1.73$ | $17.2 \pm 1.53$ | $10.7 \pm 1.48$ | $14.3 \pm 1.10$ |
| 6 | Antakya 3 | Antakya - Serinyol | $34.2 \pm 0.31$ | $3.00 \pm 0.03$ | $15.1 \pm 0.23$ | $40.9 \pm 1.58$ | $22.9 \pm 2.61$ | $17.8 \pm 1.49$ | $28.5 \pm 0.79$ |
| 7 | Antalya 1 | Antalya - Taşağl | $34.9 \pm 0.32$ | $1.52 \pm 0.03$ | $5.8 \pm 0.17$ | $31.7 \pm 1.35$ | $18.3 \pm 1.05$ | $11.5 \pm 1.55$ | $15.9 \pm 1.41$ |
| 8 | Antalya 2 | Antalya - Çamyuva | $34.1 \pm 0.06$ | $2.71 \pm 0.04$ | $14.9 \pm 0.07$ | $52.2 \pm 2.56$ | $22.8 \pm 2.35$ | $18.3 \pm 1.07$ | $28.4 \pm 1.22$ |
| 9 | Antalya 3 | Antalya - Kestel | $31.8 \pm 0.15$ | $1.24 \pm 0.02$ | $6.4 \pm 0.11$ | $29.7 \pm 1.22$ | $17.2 \pm 1.72$ | $10.3 \pm 0.68$ | $12.6 \pm 1.38$ |
| 10 | Antalya 4 | Antalya | $31.1 \pm 0.57$ | $3.79 \pm 0.05$ | $5.1 \pm 0.06$ | $37.4 \pm 2.11$ | $17.1 \pm 1.83$ | $13.7 \pm 2.24$ | $10.9 \pm 1.41$ |
| 11 | Antalya 5 | Antalya | $35.9 \pm 0.18$ | $2.37 \pm 0.02$ | $13.3 \pm 0.11$ | $60.9 \pm 1.64$ | $20.9 \pm 1.79$ | $16.9 \pm 1.69$ | $25.5 \pm 2.09$ |
| 12 | Aydin 1 | Aydın - Gökçealan | $37.4 \pm 0.13$ | $1.66 \pm 0.03$ | $5.7 \pm 0.06$ | $41.1 \pm 1.13$ | $16.7 \pm 1.37$ | $13.3 \pm 1.15$ | $27.4 \pm 1.28$ |
| 13 | Aydin 2 | Aydın | $39.5 \pm 0.11$ | $3.84 \pm 0.03$ | $5.4 \pm 0.05$ | $64.6 \pm 1.63$ | $17.9 \pm 0.19$ | $13.2 \pm 0.98$ | $12.3 \pm 1.01$ |
| 14 | Aydı 3 | Aydın | $40.3 \pm 0.14$ | $3.61 \pm 0.04$ | $16.6 \pm 0.11$ | $62.1 \pm 1.37$ | $23.5 \pm 2.33$ | $21.3 \pm 1.63$ | $49.3 \pm 0.71$ |
| 15 | Aydin 4 | Aydın | $38.5 \pm 0.18$ | $2.12 \pm 0.02$ | $11.9 \pm 0.03$ | $54.7 \pm 2.19$ | $24.8 \pm 1.44$ | $20.7 \pm 1.03$ | $27.1 \pm 1.51$ |
| 16 | Balikesir 1 | Balkesir - Can | $37.1 \pm 0.22$ | $1.92 \pm 0.02$ | $6.9 \pm 0.07$ | $32.1 \pm 1.65$ | $18.8 \pm 0.97$ | $12.4 \pm 1.16$ | $16.3 \pm 0.88$ |
| 17 | Balikesir 2 | Balkesir | $33.8 \pm 0.48$ | $1.53 \pm 0.03$ | $6.5 \pm 0.07$ | $33.1 \pm 2.23$ | $16.3 \pm 2.10$ | $12.6 \pm 1.59$ | $11.6 \pm 1.52$ |
| 18 | Balikesir 3 | Balkesir | $32.4 \pm 0.31$ | $2.25 \pm 0.03$ | $12.2 \pm 0.08$ | $61.5 \pm 1.57$ | $23.0 \pm 2.75$ | $16.6 \pm 1.40$ | $20.5 \pm 1.96$ |
| 19 | Balkesir 4 | Balkesir | $37.3 \pm 0.24$ | $2.57 \pm 0.04$ | $11.3 \pm 0.11$ | $60.2 \pm 1.55$ | $24.0 \pm 1.31$ | $18.2 \pm 1.69$ | $23.4 \pm 2.19$ |
| 20 | Balkesir 5 | Balkesir | $34.7 \pm 0.32$ | $1.55 \pm 0.03$ | $5.0 \pm 0.08$ | $37.8 \pm 1.40$ | $17.3 \pm 1.68$ | $10.5 \pm 1.23$ | $12.2 \pm 1.77$ |
| 21 | Balkesir 6 | Balkesir | $33.1 \pm 0.23$ | $2.69 \pm 0.04$ | $13.5 \pm 0.13$ | $54.3 \pm 2.06$ | $22.6 \pm 2.21$ | $19.5 \pm 1.55$ | $22.1 \pm 1.54$ |
| 22 | Balkesir 7 | Balkesir | $34.4 \pm 0.45$ | $3.33 \pm 0.05$ | $13.1 \pm 0.25$ | $52.2 \pm 2.46$ | $25.6 \pm 1.73$ | $23.2 \pm 1.97$ | $35.2 \pm 2.06$ |
| 23 | Balkesir 8 | Balkesir | $38.9 \pm 1.13$ | $2.49 \pm 0.02$ | $13.6 \pm 0.11$ | $59.6 \pm 2.66$ | $23.1 \pm 2.47$ | $19.3 \pm 2.02$ | $23.5 \pm 2.45$ |
| 24 | Balikesir 9 | Balkesir | $35.1 \pm 0.27$ | $2.78 \pm 0.03$ | $14.5 \pm 0.21$ | $56.4 \pm 2.28$ | $27.4 \pm 1.22$ | $21.0 \pm 1.46$ | $29.4 \pm 2.09$ |
| 25 | Balkesir 10 | Balkesir | $36.1 \pm 0.19$ | $2.75 \pm 0.04$ | $14.6 \pm 0.13$ | $63.1 \pm 2.65$ | $24.3 \pm 2.18$ | $19.8 \pm 4.22$ | $25.6 \pm 2.20$ |
| 26 | Balkesir 11 | Balkesir | $33.4 \pm 0.11$ | $2.81 \pm 0.05$ | $16.4 \pm 0.23$ | $59.7 \pm 2.49$ | $25.3 \pm 2.80$ | $17.0 \pm 2.16$ | $24.6 \pm 1.61$ |
| 27 | Balkesir 12 | Balkesir | $42.8 \pm 0.53$ | $1.47 \pm 0.02$ | $5.4 \pm 0.11$ | $38.5 \pm 2.78$ | $17.9 \pm 1.63$ | $11.1 \pm 1.63$ | $20.2 \pm 1.52$ |
| 28 | Balikesir 13 | Balkesir | $33.1 \pm 0.17$ | $1.41 \pm 0.04$ | $4.5 \pm 0.09$ | $34.2 \pm 1.89$ | $15.5 \pm 2.10$ | $11.3 \pm 1.37$ | $16.2 \pm 1.85$ |
| 29 | Balikesir 14 | Balkesir | $43.1 \pm 0.35$ | $2.73 \pm 0.06$ | $14.5 \pm 0.23$ | $68.8 \pm 2.99$ | $25.8 \pm 2.44$ | $22.1 \pm 1.83$ | $29.8 \pm 1.64$ |
| 30 | Balikesir 15 | Balkesir | $34.7 \pm 0.37$ | $1.65 \pm 0.04$ | $5.7 \pm 0.05$ | $35.7 \pm 1.27$ | $17.4 \pm 2.03$ | $11.0 \pm 1.68$ | $14.9 \pm 2.70$ |
| 31 | Balkesir 16 | Balikesir - Kayapınar | $41.5 \pm 0.25$ | $2.66 \pm 0.03$ | $14.7 \pm 0.19$ | $68.6 \pm 3.16$ | $25.0 \pm 2.49$ | $22.5 \pm 2.95$ | $29.5 \pm 1.25$ |

Table 1. (Continued).

| 32 | Balıkesir 17 | Balıkesir - Gökçeağac | $41.7 \pm 0.16$ | $2.74 \pm 0.05$ | $15.3 \pm 0.04$ | $65.9 \pm 2.84$ | $23.5 \pm 2.29$ | $21.6 \pm 1.71$ | $30.9 \pm 2.06$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 33 | Balıkesir 18 | Balkesir - Tepeören | $42.4 \pm 0.73$ | $2.61 \pm 0.07$ | $12.8 \pm 0.24$ | $55.7 \pm 3.26$ | $23.6 \pm 1.51$ | $19.9 \pm 1.16$ | $29.7 \pm 1.33$ |
| 34 | Balıkesir 19 | Balkesir | $34.4 \pm 0.44$ | $3.37 \pm 0.04$ | $16.5 \pm 0.11$ | $65.1 \pm 1.67$ | $27.8 \pm 1.44$ | $21.5 \pm 1.70$ | $26.2 \pm 2.17$ |
| 35 | Balikesir 20 | Ballkesir - Geçitli | $33.2 \pm 0.47$ | $2.85 \pm 0.04$ | $15.9 \pm 0.25$ | $61.1 \pm 2.84$ | $25.8 \pm 1.56$ | $21.0 \pm 1.82$ | $31.6 \pm 1.90$ |
| 36 | Burdur | Burdur | $32.3 \pm 0.45$ | $2.78 \pm 0.05$ | $15.9 \pm 0.15$ | $54.8 \pm 2.15$ | $21.8 \pm 2.04$ | $21.1 \pm 2.07$ | $30.1 \pm 1.59$ |
| 37 | Bursa 1 | Bursa - Yenice | $34.3 \pm 0.39$ | $3.13 \pm 0.04$ | $15.7 \pm 0.19$ | $74.4 \pm 3.86$ | $21.9 \pm 2.26$ | $24.4 \pm 1.42$ | $24.2 \pm 1.69$ |
| 38 | Bursa 2 | Bursa - Yenice | $46.9 \pm 0.49$ | $2.42 \pm 0.05$ | $12.7 \pm 0.22$ | $96.3 \pm 2.84$ | $24.5 \pm 1.53$ | $21.5 \pm 1.60$ | $33.8 \pm 1.26$ |
| 39 | Bursa 3 | Bursa | $41.9 \pm 0.41$ | $3.40 \pm 0.04$ | $16.5 \pm 0.13$ | $66.3 \pm 4.26$ | $27.7 \pm 1.16$ | $33.0 \pm 1.68$ | $33.6 \pm 1.74$ |
| 40 | Bursa 4 | Bursa | $37.6 \pm 0.21$ | $2.84 \pm 0.03$ | $17.3 \pm 0.16$ | $62.9 \pm 1.76$ | $27.5 \pm 1.68$ | $22.5 \pm 1.66$ | $27.2 \pm 1.63$ |
| 41 | Bursa 5 | Bursa - Mürseller | $35.8 \pm 0.41$ | $2.56 \pm 0.03$ | $15.8 \pm 0.18$ | $61.9 \pm 2.16$ | $22.0 \pm 2.36$ | $18.2 \pm 1.51$ | $31.8 \pm 2.04$ |
| 42 | Bursa 6 | Bursa | $43.8 \pm 0.53$ | $4.17 \pm 0.03$ | $7.3 \pm 0.06$ | $41.3 \pm 2.05$ | $19.4 \pm 1.64$ | $13.8 \pm 1.74$ | $12.8 \pm 1.83$ |
| 43 | Bursa 7 | Bursa - Orhaneli | $34.9 \pm 0.48$ | $3.73 \pm 0.06$ | $18.4 \pm 0.15$ | $80.3 \pm 1.35$ | $25.5 \pm 2.18$ | $21.5 \pm 1.55$ | $36.1 \pm 1.77$ |
| 44 | Çanakkale 1 | Çanakkale | $39.8 \pm 0.55$ | $3.58 \pm 0.09$ | $16.5 \pm 0.31$ | $63.2 \pm 1.77$ | $25.5 \pm 2.43$ | $21.8 \pm 1.92$ | $29.7 \pm 1.85$ |
| 45 | Çanakkale 2 | Çanakkale | $40.6 \pm 0.13$ | $4.12 \pm 0.11$ | $15.3 \pm 0.22$ | $61.5 \pm 2.06$ | $23.0 \pm 1.61$ | $23.7 \pm 2.18$ | $27.8 \pm 1.55$ |
| 46 | Çanakkale 3 | Çanakkale | $35.4 \pm 0.33$ | $2.66 \pm 0.03$ | $13.4 \pm 0.12$ | $55.2 \pm 1.53$ | $21.9 \pm 1.62$ | $19.7 \pm 1.62$ | $26.6 \pm 1.68$ |
| 47 | Çanakkale 4 | Çanakkale - Karkın | $34.5 \pm 0.33$ | $3.00 \pm 0.04$ | $1.5 \pm 0.27$ | $72.2 \pm 2.07$ | $22.6 \pm 2.58$ | $18.9 \pm 2.53$ | $31.6 \pm 1.62$ |
| 48 | Çanakkale 5 | Çanakkale - Ezine | $33.5 \pm 0.43$ | $4.19 \pm 0.04$ | $6.3 \pm 0.11$ | $39.5 \pm 1.89$ | $19.4 \pm 1.77$ | $11.4 \pm 1.34$ | $10.4 \pm 1.29$ |
| 49 | Çanakkale 6 | Çanakkale - Biga | $40.4 \pm 0.24$ | $3.90 \pm 0.02$ | $5.7 \pm 0.07$ | $39.4 \pm 1.75$ | $19.1 \pm 1.21$ | $10.6 \pm 1.01$ | $17.8 \pm 1.73$ |
| 50 | Çanakkale 7 | Çanakkale - Adatepe | $37.5 \pm 0.28$ | $3.28 \pm 0.05$ | $13.4 \pm 0.11$ | $71.1 \pm 1.62$ | $23.3 \pm 2.07$ | $28.3 \pm 1.60$ | $29.8 \pm 1.42$ |
| 51 | Çanakkale 8 | Çanakkale - Göktepe | $36.4 \pm 0.24$ | $2.35 \pm 0.04$ | $13.1 \pm 0.11$ | $62.9 \pm 1.74$ | $26.1 \pm 1.55$ | $19.9 \pm 1.51$ | $30.2 \pm 1.86$ |
| 52 | Çanakkale 9 | Çanakkale - Şerbetli | $30.1 \pm 0.34$ | $4.89 \pm 0.03$ | $6.5 \pm 0.05$ | $56.9 \pm 1.53$ | $23.8 \pm 1.28$ | $31.6 \pm 1.52$ | $33.1 \pm 2.16$ |
| 53 | Çanakkale 10 | Çanakkale | $27.5 \pm 0.07$ | $3.05 \pm 0.03$ | $17.4 \pm 0.17$ | $63.8 \pm 1.32$ | $27.8 \pm 1.53$ | $17.8 \pm 1.34$ | $25.7 \pm 1.43$ |
| 54 | Çanakkale 11 | Çanakkale | $41.2 \pm 0.13$ | $3.42 \pm 0.05$ | $16.8 \pm 0.17$ | $62.4 \pm 3.13$ | $25.2 \pm 2.15$ | $21.8 \pm 1.49$ | $28.1 \pm 1.49$ |
| 55 | Çanakkale 12 | Çanakkale | $40.6 \pm 0.18$ | $2.92 \pm 0.06$ | $14.2 \pm 0.03$ | $57.9 \pm 1.56$ | $23.8 \pm 1.51$ | $19.7 \pm 1.65$ | $24.7 \pm 1.61$ |
| 56 | Çanakkale 13 | Çanakkale | $42.6 \pm 0.46$ | $2.27 \pm 0.02$ | $13.1 \pm 0.08$ | $71.4 \pm 1.89$ | $24.7 \pm 1.59$ | $22.4 \pm 0.92$ | $28.2 \pm 1.54$ |
| 57 | Çanakkale 14 | Çanakkale - Gelibolu | $41.9 \pm 0.36$ | $2.47 \pm 0.04$ | $11.9 \pm 0.15$ | $66.4 \pm 2.59$ | $24.6 \pm 1.80$ | $20.3 \pm 1.71$ | $24 \pm 10.5$ |
| 58 | Çanakkale 15 | Çanakkale | $31.5 \pm 0.24$ | $2.74 \pm 0.04$ | $15.9 \pm 0.16$ | $49.4 \pm 1.97$ | $21.7 \pm 1.67$ | $18.0 \pm 1.38$ | $20.7 \pm 2.44$ |
| 59 | Çanakkale 16 | Çanakkale - Bihramlı | $35.2 \pm 0.32$ | $3.22 \pm 0.03$ | $15.9 \pm 0.22$ | $68.4 \pm 2.03$ | $25.2 \pm 2.10$ | $20.5 \pm 1.92$ | $23.8 \pm 2.38$ |
| 60 | Çanakkale 17 | Çanakkale | $31.5 \pm 0.12$ | $2.51 \pm 0.04$ | $14.7 \pm 0.16$ | $54.1 \pm 1.78$ | $21.7 \pm 1.37$ | $19.4 \pm 1.13$ | $31.6 \pm 2.04$ |
| 61 | Çanakkale 18 | Çanakkale - Hurmaköy | $38.5 \pm 0.18$ | $4.69 \pm 0.02$ | $16.4 \pm 0.12$ | $93.6 \pm 2.54$ | $27.7 \pm 2.07$ | $21.6 \pm 2.73$ | $25.5 \pm 2.32$ |
| 62 | Çanakkale 19 | Çanakkale - Can | $36.7 \pm 0.13$ | $3.02 \pm 0.02$ | $14.1 \pm 0.04$ | $64.1 \pm 1.99$ | $26.3 \pm 1.90$ | $19.7 \pm 1.55$ | $28.6 \pm 1.14$ |
| 63 | Çanakkale 20 | Çanakkale - Guyemalan | $35.1 \pm 0.09$ | $3.11 \pm 0.03$ | $13.1 \pm 0.19$ | $49.5 \pm 1.88$ | $26.8 \pm 1.52$ | $21.7 \pm 1.54$ | $23.7 \pm 1.49$ |
| 64 | Çanakkale 21 | Çanakkale - Can | $37.5 \pm 0.12$ | $3.59 \pm 0.02$ | $16.4 \pm 0.11$ | $70.1 \pm 1.53$ | $27.5 \pm 1.09$ | $21.8 \pm 1.58$ | $30.5 \pm 0.97$ |
| 65 | Çanakkale 22 | Çanakkale - Guyemalan | $33.6 \pm 0.24$ | $3.14 \pm 0.03$ | $15.8 \pm 0.17$ | $65.3 \pm 1.99$ | $20.9 \pm 1.73$ | $21.3 \pm 1.83$ | $35.4 \pm 2.15$ |

Table 1. (Continued).

| 66 | Çanakkale 23 | Çanakkale - Burhaniye | $42.8 \pm 0.24$ | $2.32 \pm 0.04$ | $15.7 \pm 0.16$ | $89.5 \pm 1.20$ | $27.0 \pm 1.59$ | $19.6 \pm 1.40$ | $32.1 \pm 1.36$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 67 | Çanakkale 24 | Çanakkale | $37.1 \pm 0.09$ | $3.23 \pm 0.05$ | $14.1 \pm 0.04$ | $55.5 \pm 1.97$ | $23.9 \pm 1.67$ | $21.6 \pm 1.60$ | $25.7 \pm 2.44$ |
| 68 | Çanakkale 25 | Çanakkale | $36.4 \pm 0.21$ | $2.91 \pm 0.02$ | $14.4 \pm 0.05$ | $73.3 \pm 3.06$ | $27.1 \pm 1.62$ | $22.1 \pm 1.69$ | $28.8 \pm 0.83$ |
| 69 | Çanakkale 26 | Çanakkale - Seddulbahir | $41.4 \pm 0.37$ | $3.35 \pm 0.04$ | $14.4 \pm 0.12$ | $66.1 \pm 1.58$ | $23.0 \pm 2.68$ | $18.2 \pm 1.71$ | $25.4 \pm 2.01$ |
| 70 | Çanakkale 27 | Çanakkale - Eskipazar | $37.1 \pm 0.29$ | $3.57 \pm 0.03$ | $14.8 \pm 0.04$ | $63.3 \pm 2.47$ | $22.9 \pm 2.47$ | $19.1 \pm 1.87$ | $37.7 \pm 1.43$ |
| 71 | Çanakkale 28 | Cannakale | $34.7 \pm 0.27$ | $1.92 \pm 0.02$ | $6.2 \pm 0.03$ | $40.6 \pm 2.44$ | $21.3 \pm 1.30$ | $11.7 \pm 1.65$ | $14.2 \pm 1.81$ |
| 72 | Çanakkale 29 | Çanakkale - Altınoluk | $35.2 \pm 0.44$ | $3.26 \pm 0.04$ | $1.6 \pm 0.25$ | $50.1 \pm 1.09$ | $27.3 \pm 2.21$ | $21.1 \pm 1.48$ | $28.2 \pm 1.67$ |
| 73 | Çanakkale 30 | Çanakkale - Kepez | $35.8 \pm 0.19$ | $2.05 \pm 0.03$ | $5.5 \pm 0.02$ | $34.5 \pm 1.14$ | $17.8 \pm 1.54$ | $12.2 \pm 1.02$ | $16.2 \pm 2.31$ |
| 74 | Canakkale 31 | Çanakkale | $27.7 \pm 0.22$ | $3.64 \pm 0.02$ | $16.2 \pm 0.11$ | $49.8 \pm 1.32$ | $21.4 \pm 1.72$ | $12.9 \pm 1.41$ | $24.7 \pm 1.69$ |
| 75 | Çanakkale 32 | Çanakkale | $34.1 \pm 0.22$ | $3.04 \pm 0.02$ | $14.7 \pm 0.22$ | $70.8 \pm 1.36$ | $27.7 \pm 1.06$ | $20.8 \pm 2.10$ | $21.7 \pm 2.31$ |
| 76 | Çanakkale 33 | Çanakkale | $31.2 \pm 0.08$ | $2.9 \pm 0.02$ | $16.2 \pm 0.19$ | $70.0 \pm 1.46$ | $23.1 \pm 1.85$ | $18.3 \pm 1.51$ | $23.5 \pm 2.21$ |
| 77 | Çanakkale 34 | Çanakkale | $36.5 \pm 0.42$ | $2.47 \pm 0.02$ | $15.7 \pm 0.11$ | $57.8 \pm 1.80$ | $26.6 \pm 2.79$ | $18.9 \pm 2.40$ | $22.4 \pm 1.40$ |
| 78 | Çanakkale 35 | Çanakkale - Ezine | $29.9 \pm 0.14$ | $4.83 \pm 0.07$ | $5.1 \pm 0.03$ | $41.5 \pm 2.14$ | $18.8 \pm 1.29$ | $10.5 \pm 1.86$ | $13.1 \pm 1.64$ |
| 79 | Çanakkale 36 | Çanakkale - Ezine | $39.9 \pm 0.21$ | $3.61 \pm 0.02$ | $14.4 \pm 0.08$ | $62.2 \pm 1.44$ | $26.1 \pm 1.73$ | $21.3 \pm 1.90$ | $21.5 \pm 1.65$ |
| 80 | Edirne 1 | Edirne | $31.7 \pm 0.18$ | $3.02 \pm 0.02$ | $16.5 \pm 0.13$ | $50.4 \pm 1.85$ | $20.6 \pm 0.78$ | $16.5 \pm 1.01$ | $21.4 \pm 1.47$ |
| 81 | Edirne 2 | Edirne | $39.9 \pm 0.19$ | $3.29 \pm 0.05$ | $15.3 \pm 0.04$ | $64.6 \pm 1.66$ | $21.8 \pm 1.83$ | $19.0 \pm 1.87$ | $23.6 \pm 1.07$ |
| 82 | Edirne 3 | Edirne | $37.3 \pm 0.25$ | $3.23 \pm 0.03$ | $7.3 \pm 0.09$ | $38.9 \pm 1.70$ | $17.2 \pm 1.67$ | $13.4 \pm 2.13$ | $12.1 \pm 1.53$ |
| 83 | Elazığ | Elazığ | $39.5 \pm 0.08$ | $2.94 \pm 0.02$ | $15.6 \pm 0.15$ | $67.8 \pm 4.11$ | $25.5 \pm 1.34$ | $22.2 \pm 1.38$ | $24.5 \pm 2.32$ |
| 84 | İçel 1 | İçel | $40.3 \pm 0.17$ | $3.18 \pm 0.03$ | $16.7 \pm 0.11$ | $77.7 \pm 1.39$ | $23.2 \pm 1.98$ | $21.3 \pm 2.31$ | $24.8 \pm 1.16$ |
| 85 | İçel 2 | İçel | $36.4 \pm 0.22$ | $3.08 \pm 0.02$ | $12.8 \pm 0.05$ | $65.4 \pm 1.21$ | $24.6 \pm 1.45$ | $23.1 \pm 1.28$ | $22.3 \pm 2.12$ |
| 86 | İçel 3 | İçel | $35.1 \pm 0.08$ | $2.76 \pm 0.02$ | $14.3 \pm 0.07$ | $60.9 \pm 2.55$ | $28.2 \pm 1.89$ | $21.2 \pm 1.96$ | $24.3 \pm 0.97$ |
| 87 | İçel 4 | İçel | $41.4 \pm 0.33$ | $4.44 \pm 0.03$ | $6.1 \pm 0.03$ | $40.9 \pm 2.11$ | $18.4 \pm 1.00$ | $13.5 \pm 0.66$ | $12.2 \pm 1.62$ |
| 88 | İzmir 1 | İzmir - Karşıyaka | $39.2 \pm 0.17$ | $2.96 \pm 0.06$ | $13.6 \pm 0.07$ | $63.7 \pm 1.65$ | $23.6 \pm 2.37$ | $18.6 \pm 2.10$ | $19.6 \pm 1.57$ |
| 89 | İzmir 2 | İzmir - Karşıyaka | $39.4 \pm 0.09$ | $2.44 \pm 0.04$ | $13.2 \pm 0.04$ | $60.3 \pm 1.40$ | $23.1 \pm 2.66$ | $19.4 \pm 1.11$ | $20.8 \pm 2.42$ |
| 90 | İzmir 3 | İzmir - Karşıyaka | $33.5 \pm 0.05$ | $2.73 \pm 0.02$ | $13.7 \pm 0.05$ | $63.7 \pm 3.10$ | $22.4 \pm 2.11$ | $18.8 \pm 1.37$ | $25.5 \pm 2.01$ |
| 91 | İzmir 4 | İzmir - Kaşıiyaka | $41.2 \pm 0.28$ | $2.83 \pm 0.03$ | $14.2 \pm 0.08$ | $72.5 \pm 1.65$ | $25.2 \pm 1.53$ | $21.4 \pm 1.02$ | $24.2 \pm 2.42$ |
| 92 | İzmir 5 | İzmir | $37.5 \pm 0.16$ | $1.47 \pm 0.03$ | $5.6 \pm 0.07$ | $32.0 \pm 1.58$ | $18.2 \pm 1.10$ | $13.1 \pm 1.51$ | $12.2 \pm 1.16$ |
| 93 | İzmir 6 | İzmir | $36.9 \pm 0.22$ | $3.48 \pm 0.02$ | $16.9 \pm 0.17$ | $81.3 \pm 1.95$ | $29.2 \pm 7.14$ | $23.3 \pm 2.10$ | $23.5 \pm 0.75$ |
| 94 | İzmir 7 | İzmir | $36.1 \pm 0.29$ | $2.89 \pm 0.03$ | $17.4 \pm 0.16$ | $66.5 \pm 1.44$ | $28.3 \pm 1.65$ | $24.2 \pm 1.16$ | $26.2 \pm 1.45$ |
| 95 | İzmir 8 | İzmir | $35.9 \pm 0.14$ | $4.28 \pm 0.03$ | $6.4 \pm 0.04$ | $64.9 \pm 2.65$ | $18.8 \pm 1.19$ | $13.0 \pm 1.17$ | $18.2 \pm 1.53$ |
| 96 | İzmir 9 | İzmir - Karşıyaka | $41.9 \pm 0.32$ | $3.13 \pm 0.04$ | $13.3 \pm 0.06$ | $57.1 \pm 1.53$ | $21.2 \pm 2.28$ | $22.6 \pm 2.01$ | $28.7 \pm 1.42$ |
| 97 | İzmir 10 | İzmir | $93.3 \pm 0.82$ | $3.47 \pm 0.03$ | $14.5 \pm 0.05$ | $69.7 \pm 1.36$ | $26.3 \pm 1.30$ | $19.8 \pm 1.40$ | $23.4 \pm 0.91$ |
| 98 | İzmir 11 | İzmir - Karşıyaka | $42.5 \pm 0.19$ | $4.14 \pm 0.11$ | $13.7 \pm 0.09$ | $63.3 \pm 2.18$ | $26.3 \pm 1.52$ | $22.1 \pm 1.05$ | $26.6 \pm 1.85$ |
| 99 | İzmir 12 | Menemen | $34.6 \pm 0.34$ | $3.09 \pm 0.02$ | $14.4 \pm 0.11$ | $73.9 \pm 1.36$ | $27.3 \pm 1.87$ | $20.3 \pm 1.22$ | $25.6 \pm 1.86$ |

Table 1. (Continued).

| 100 | İzmir 13 | İzmir - Kurfalliyi | $37.5 \pm 0.36$ | $2.62 \pm 0.05$ | $15.6 \pm 0.11$ | $67.7 \pm 2.17$ | $25.0 \pm 1.44$ | $19.5 \pm 1.64$ | $22.3 \pm 2.26$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 101 | İzmir 14 | İzmir - Karşıyaka | $34.9 \pm 0.24$ | $2.92 \pm 0.02$ | $16.6 \pm 0.11$ | $68.5 \pm 1.87$ | $23.7 \pm 1.76$ | $21.9 \pm 1.23$ | $26.2 \pm 1.81$ |
| 102 | İzmir 15 | İzmir - Kızılcaayas | $40.2 \pm 0.23$ | $1.68 \pm 0.03$ | $7.4 \pm 0.12$ | $37.4 \pm 2.07$ | $18.3 \pm 1.27$ | $13.7 \pm 1.60$ | $15.3 \pm 2.01$ |
| 103 | İzmir 16 | İzmir - Karşıyaka | $38.1 \pm 0.21$ | $2.78 \pm 0.04$ | $14.1 \pm 0.06$ | $64.9 \pm 1.26$ | $27.4 \pm 1.01$ | $21.8 \pm 1.23$ | $24.4 \pm 1.26$ |
| 104 | İstanbul | İstanbul | $32.7 \pm 0.26$ | $3.10 \pm 0.04$ | $13.4 \pm 0.09$ | $66.8 \pm 1.54$ | $25.3 \pm 1.99$ | $21.5 \pm 1.75$ | $22.2 \pm 1.33$ |
| 105 | Kars | Kars | $39.1 \pm 0.43$ | $3.02 \pm 0.03$ | $16.5 \pm 0.11$ | $68.7 \pm 2.57$ | $25.6 \pm 1.81$ | $23.1 \pm 2.04$ | $23.3 \pm 1.42$ |
| 106 | Kırklareli | Kırklareli | $37.5 \pm 0.25$ | $3.63 \pm 0.06$ | $13.8 \pm 0.09$ | $73.4 \pm 2.02$ | $26.9 \pm 1.36$ | $21.3 \pm 1.73$ | $26.7 \pm 1.39$ |
| 107 | Konya 1 | Konya | $42.3 \pm 0.31$ | $3.94 \pm 0.07$ | $7.3 \pm 0.05$ | $91.3 \pm 3.79$ | $17.4 \pm 1.05$ | $14.6 \pm 1.73$ | $13.8 \pm 1.51$ |
| 108 | Konya 2 | Konya | $40.6 \pm 0.22$ | $3.08 \pm 0.02$ | $15.8 \pm 0.15$ | $82.8 \pm 1.68$ | $22.9 \pm 1.65$ | $20.3 \pm 1.67$ | $23.8 \pm 1.64$ |
| 109 | Konya 3 | Konya | $27.6 \pm 0.28$ | $3.02 \pm 0.03$ | $14.2 \pm 0.10$ | $70.1 \pm 1.18$ | $23.8 \pm 1.68$ | $18.6 \pm 2.45$ | $24.5 \pm 2.38$ |
| 110 | Konya 4 | Konya | $38.1 \pm 0.31$ | $2.88 \pm 0.07$ | $14.6 \pm 0.08$ | $78.4 \pm 3.59$ | $27.2 \pm 2.10$ | $21.8 \pm 1.52$ | $24.5 \pm 2.39$ |
| 111 | Manisa 1 | Manisa | $39.2 \pm 0.24$ | $3.23 \pm 0.03$ | $14.3 \pm 0.11$ | $58.1 \pm 4.26$ | $24.3 \pm 1.90$ | $18.8 \pm 2.10$ | $23.7 \pm 1.61$ |
| 112 | Manisa 2 | Manisa | $37.3 \pm 0.26$ | $3.04 \pm 0.03$ | $14.6 \pm 0.09$ | $57.4 \pm 1.69$ | $24.9 \pm 1.50$ | $23.3 \pm 2.01$ | $24.4 \pm 2.18$ |
| 113 | Manisa 3 | Manisa - Kınık | $39.3 \pm 0.16$ | $3.12 \pm 0.04$ | $15.5 \pm 0.13$ | $65.5 \pm 0.66$ | $27.1 \pm 1.53$ | $21.2 \pm 2.99$ | $23.4 \pm 1.98$ |
| 114 | Manisa 4 | Manisa | $42.4 \pm 0.04$ | $3.43 \pm 0.07$ | $19.3 \pm 0.13$ | $78.1 \pm 1.86$ | $23.9 \pm 1.32$ | $22.4 \pm 2.19$ | $25.1 \pm 1.52$ |
| 115 | Manisa 5 | Manisa | $37.7 \pm 0.18$ | $3.83 \pm 0.04$ | $15.1 \pm 0.08$ | $64.0 \pm 1.63$ | $27.1 \pm 2.31$ | $19.4 \pm 0.73$ | $27.4 \pm 1.93$ |
| 116 | Manisa 6 | Manisa | $33.6 \pm 0.35$ | $3.34 \pm 0.04$ | $6.2 \pm 0.05$ | $34.7 \pm 1.66$ | $19.5 \pm 1.79$ | $10.4 \pm 0.75$ | $11.9 \pm 1.55$ |
| 117 | Muğla 1 | Muğla | $37.7 \pm 0.11$ | $3.15 \pm 0.03$ | $15.4 \pm 0.27$ | $58.7 \pm 1.67$ | $25.5 \pm 2.05$ | $17.3 \pm 2.09$ | $24.1 \pm 2.03$ |
| 118 | Muğla 2 | Muğla | $39.5 \pm 0.09$ | $3.78 \pm 0.05$ | $18.9 \pm 0.11$ | $67.0 \pm 2.31$ | $27.9 \pm 1.66$ | $22.5 \pm 1.93$ | $26.2 \pm 2.18$ |
| 119 | Muğla 3 | Muğla | $37.9 \pm 0.28$ | $2.23 \pm 0.02$ | $14.9 \pm 0.12$ | $71.3 \pm 2.31$ | $26.3 \pm 1.13$ | $19.8 \pm 1.21$ | $29.2 \pm 2.07$ |
| 120 | Muğla 4 | Muğla - Fethiye | $34.1 \pm 0.17$ | $2.02 \pm 0.02$ | $15.8 \pm 0.13$ | $65.3 \pm 2.48$ | $25.9 \pm 1.64$ | $19.9 \pm 1.52$ | $25.3 \pm 1.99$ |
| 121 | Muğla 5 | Muğla | $42.2 \pm 0.31$ | $3.45 \pm 0.07$ | $16.6 \pm 0.11$ | $62.5 \pm 2.10$ | $25.7 \pm 3.32$ | $21.4 \pm 1.81$ | $24.4 \pm 2.06$ |
| 122 | Tekirdağ 1 | Tekirdağ - Uçmakdere | $39.9 \pm 0.12$ | $3.10 \pm 0.03$ | $14.2 \pm 0.12$ | $69.5 \pm 2.12$ | $25.2 \pm 2.65$ | $23.3 \pm 1.98$ | $26.8 \pm 1.70$ |
| 123 | Tekirdağ 2 | Tekirdağ - Naip | $37.4 \pm 0.47$ | $2.90 \pm 0.04$ | $15.2 \pm 0.24$ | $65.6 \pm 3.12$ | $24.8 \pm 1.67$ | $20.3 \pm 0.71$ | $22.1 \pm 3.43$ |
| 124 | Tekirdağ 3 | Tekirdağ - Yaci | $39.9 \pm 0.08$ | $3.78 \pm 0.02$ | $6.5 \pm 0.12$ | $39.5 \pm 1.73$ | $19.4 \pm 1.90$ | $10.7 \pm 1.31$ | $13.6 \pm 1.63$ |
| 125 | Tekirdağ 4 | Tekirdağ | $37.9 \pm 0.22$ | $2.32 \pm 0.02$ | $13.9 \pm 0.08$ | $53.5 \pm 1.88$ | $23.8 \pm 1.65$ | $18.7 \pm 1.27$ | $17.5 \pm 2.12$ |
| 126 | Tekirdağ 5 | Tekirdağ - Ortaca | $40.2 \pm 0.24$ | $3.03 \pm 0.02$ | $14.7 \pm 0.17$ | $58.2 \pm 1.81$ | $22.3 \pm 2.15$ | $18.0 \pm 2.30$ | $23.2 \pm 1.62$ |
| 127 | Tekirdağ 6 | Tekirdağ - Hayranbolu | $35.7 \pm 0.37$ | $2.60 \pm 0.03$ | $13.5 \pm 0.12$ | $62.3 \pm 2.01$ | $26.9 \pm 1.67$ | $15.4 \pm 2.03$ | $18.3 \pm 1.06$ |
| 128 | Tekirdağ 7 | Tekirdağ | $35.1 \pm 0.34$ | $3.14 \pm 0.06$ | $13.7 \pm 0.07$ | $60.9 \pm 2.55$ | $24.7 \pm 2.56$ | $15.7 \pm 1.81$ | $21.4 \pm 1.46$ |
| 129 | Urfa | Ezgil | $41.8 \pm 0.34$ | $2.79 \pm 0.04$ | $13.5 \pm 0.08$ | $75.0 \pm 2.29$ | $24.0 \pm 2.48$ | $21.3 \pm 2.75$ | $24.3 \pm 1.85$ |
| Varieties |  |  |  |  |  |  |  |  |  |
| 130 | Kıtik2003 |  | $39.4 \pm 0.22$ | $2.92 \pm 0.03$ | $14.4 \pm 0.05$ | $64.6 \pm 1.67$ | $23.4 \pm 2.25$ | $17.9 \pm 1.53$ | $17.4 \pm 1.61$ |
| 131 | Filiz99 |  | $34.5 \pm 0.28$ | $2.74 \pm 0.04$ | $13.9 \pm 0.06$ | $58.7 \pm 2.50$ | $25.2 \pm 1.13$ | $20.2 \pm 1.03$ | $19.6 \pm 0.85$ |
| 132 | Eresen87 |  | $39.6 \pm 0.11$ | $1.55 \pm 0.03$ | $5.9 \pm 0.04$ | $39.5 \pm 1.59$ | $18.0 \pm 1.64$ | $14.0 \pm 1.55$ | $16.7 \pm 1.12$ |
| 133 | Salkım |  | $39.1 \pm 0.12$ | $2.85 \pm 0.03$ | $12.9 \pm 0.07$ | $73.6 \pm 3.04$ | $26.5 \pm 1.52$ | $20.5 \pm 0.88$ | $20.1 \pm 1.32$ |



Figure 1. Geographic distribution of 129 faba bean landraces collected from different provinces of Turkey. Adana: 1-3, Antakya: 4-6, Antalya: 7-11, Aydın: 12-15, Balıkesir: 16-35, Burdur: 36, Bursa: 37-43, Çanakkale: 44-79, Edirne: 80-82, Elazig: 83, İçel (now Mersin): 84-87, İzmir: 88-103, İstanbul: 104, Kars: 105, Kırklareli: 106, Konya: 107-110, Manisa: 111-116, Muğla: 117-121, Tekirdağ: 122-128, Urfa: 129.
procedure of the SAS computer program. Significant differences between accessions ( $\mathrm{P} \leq 0.05$ ) were detected for all studied mineral traits. Standard deviations (SDs) were calculated for each landrace for different studied mineral characteristics. Principal component analysis (PCA) based on 7 mineral elements was used to identify the patterns of variation within the set of 129 open-pollinated landraces and 4 cultivars. 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 the PROC CORR procedure of SAS program.

## 3. Results

Based on initial ANOVA analysis (data not shown), the 129 open-pollinated faba bean landraces and 4 cultivars
differed significantly for all studied mineral traits. All landraces harbored high diversity for most of the studied mineral elements. Due to the outcrossing of the landraces, resulting from insect pollination, the seeds used for the mineral determinations were hybrids among the landraces and no accurate data could be presented for any specific landrace. However, we give the mean values and standard deviations for all landraces and cultivars in Table 1. The maximum, minimum, and mean and SD values for landraces as well as cultivars are presented in Table 2. The mean nitrogen content of all landraces and cultivars was $37.3 \mathrm{~g} \mathrm{~kg}^{-1}$; values ranged from $27.5 \mathrm{~g} \mathrm{~kg}^{-1}$ to 93.3 g $\mathrm{kg}^{-1}$. The potassium contents of the studied faba bean germplasm varied between $4.5 \mathrm{~g} \mathrm{~kg}^{-1}$ and $19.3 \mathrm{~g} \mathrm{~kg}^{-1}$ with an average of $13.0 \mathrm{~g} \mathrm{~kg}^{-1}$. The highest value of $P$ content was $4.89 \mathrm{~g} \mathrm{~kg}^{-1}$ while the lowest value was $1.24 \mathrm{~g} \mathrm{~kg}^{-1}$, with an average value of $2.97 \mathrm{~g} \mathrm{~kg}^{-1}$.

Table 2. Mean and range of some mineral element contents of 129 Turkish faba bean landraces and 4 cultivars.

| Parameters | Landraces |  |  |  | Cultivars |  |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :---: | :---: |
|  | Abbreviation | Min | Max | Mean | SD | Min | Max | Mean | SD |  |  |
|  | N | 27.5 | 93.3 | 37.3 | 0.662 | 34.5 | 39.9 | 38.1 | 0.241 |  |  |
| Phosphorous $\left(\mathrm{g} \mathrm{kg}^{-1}\right)$ | P | 1.24 | 4.89 | 2.97 | 0.072 | 1.55 | 2.93 | 2.52 | 0.065 |  |  |
| Potassium $\left(\mathrm{g} \mathrm{kg}^{-1}\right)$ | K | 4.5 | 19.3 | 13.0 | 0.391 | 5.9 | 14.3 | 11.8 | 0.395 |  |  |
| Iron $\left(\mathrm{mg} \mathrm{kg}^{-1}\right)$ | Fe | 29.7 | 96.3 | 59.6 | 13.913 | 39.4 | 73.5 | 59.1 | 14.45 |  |  |
| Manganese $\left(\mathrm{mg} \mathrm{kg}^{-1}\right)$ | Mn | 15.5 | 29.2 | 23.3 | 3.291 | 18.0 | 26.4 | 23.2 | 3.708 |  |  |
| Copper $\left(\mathrm{mg} \mathrm{kg}^{-1}\right)$ | Cu | 10.3 | 33.0 | 18.9 | 4.292 | 13.9 | 20.4 | 18.2 | 3.003 |  |  |
| Zinc $\left(\mathrm{mg} \mathrm{kg}^{-1}\right)$ | Zn | 10.4 | 49.3 | 24.2 | 6.691 | 16.6 | 20.1 | 18.5 | 1.649 |  |  |

There was high diversity of microelements in the studied faba bean germplasm collection. The amount of iron in the seeds of the studied faba bean germplasm varied highly from $29.7 \mathrm{mg} \mathrm{kg}^{-1}$ to $96.3 \mathrm{mg} \mathrm{kg}^{-1}$, and the mean value was $59.6 \mathrm{mg} \mathrm{kg}^{-1}$. Mn contents in Turkish faba bean germplasm ranged from $15.5 \mathrm{mg} \mathrm{kg}^{-1}$ to $29.2 \mathrm{mg} \mathrm{kg}^{-1}$ with a mean of $23.3 \mathrm{mg} \mathrm{kg}^{-1}$. The mean Cu concentration of the landraces and cultivars was $18.9 \mathrm{mg} \mathrm{kg}^{-1}$ and it varied between $10.3 \mathrm{mg} \mathrm{kg}^{-1}$ and $33.0 \mathrm{mg} \mathrm{kg}{ }^{-1}$. For Zn contents, substantial variation was observed among open-pollinated faba bean landraces (Table 1). The overall average value of Zn contents among all landraces was $24.2 \mathrm{mg} \mathrm{kg}^{-1}$, ranging from $10.4 \mathrm{mg} \mathrm{kg}^{-1}$ to $49.3 \mathrm{mg} \mathrm{kg}^{-1}$.

For all of the studied mineral element traits, with the exception of N , mean values and range for landraces were higher than those for cultivars. The maximum and minimum values for seed $\mathrm{Zn}, \mathrm{Fe}$, and other macro- and microminerals were within a narrow range for cultivars when compared with the landraces, which exhibited wide ranges for these traits.

Correlations among 7 mineral elements in 129 openpollinated faba bean landraces and 4 cultivars are presented in Table 3. There were significant and positive correlations among different mineral elements. Most of the minerals harbored 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. K harbored significant and positive associations with all 4 microelements. Fe had positive and significant correlations with $\mathrm{Mn}(\mathrm{r}=0.679 ; \mathrm{P}<0.01), \mathrm{Cu}(\mathrm{r}=0.680 ; \mathrm{P}$ $<0.01$ ), and $\mathrm{Zn}(\mathrm{r}=0.586 ; \mathrm{P}<0.01)$. Cu had positive and significant correlations with all 4 microelements. Similarly, Zn also exhibited positive and significant associations with $\mathrm{K}(\mathrm{r}=709 ; \mathrm{P}<0.01), \mathrm{Fe}(\mathrm{r}=0.586 ; \mathrm{P}<0.01), \mathrm{Mn}(\mathrm{r}=0.593$; $\mathrm{P}<0.01$ ), $\mathrm{Cu}(\mathrm{r}=0.718 ; \mathrm{P}<0.01)$, and macroelements (except N ).

Finally, PCA, based on 7 mineral traits, was used to assess the patterns of variation within a set of 129 open-
pollinated faba bean landraces and 4 cultivars. Using PCA based on the correlation matrix, we calculated eigenvalues, percentages of variation, and load coefficients of the first 5 components for all 7 mineral traits. In this study, the results of PCA among accessions showed consistent and large diversity in investigated element contents (Table 4). Using PCA based on the correlation coefficient, it appeared that the first 5 principal components accounted for $94.22 \%$ of the total variance (Table 4). The first principal component (PC1) was important and accounted for more than half of the total variation (with 56.28\%). The mineral elements with the highest contribution in PC1 were Cu and K , closely followed by $\mathrm{Mn}, \mathrm{Fe}$, and Zn . The second principal component (PC2) accounted for $14.63 \%$ of the variability and was highly dependent on N. The third principal component was built mostly on P content and accounted for $12.54 \%$ of total variability. The first 2 principal components were very important and accounted for approximately $70.91 \%$ of the total available variability; hence, they were plotted graphically to demonstrate the relationship among open-pollinated Turkish faba bean germplasm collection (Figure 2).

## 4. Discussion

Landraces are defined as geographically or ecologically distinct crop populations developed under the influence of the local and cultural environment (Camacho Villa et al., 2005). Landraces constitute important genetic resources for faba bean breeding schemes and can be maintained as inbred lines (Terzopoulos and Bebeli, 2008). To fully and effectively utilize the genetic variability of the Turkish faba bean germplasm for the enrichment of faba bean seeds with bioavailable mineral elements, it is necessary to study and evaluate the variations for mineral traits of faba bean germplasms from different origins and to identify germplasm groups from which elite inbred lines with high mineral elements could be created (Cakmak, 2008).

Considering the great value of plant germplasm collections, it is very important to characterize the local

Table 3. Correlation coefficients among the concentrations of seed mineral elements of 133 faba bean landraces and cultivars.

|  | P | K | Fe | Mn | Cu | Zn |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| N | 0.095 | 0.052 | $0.232^{* *}$ | 0.135 | 0.161 | 0.074 |
| P |  | $0.209^{*}$ | $0.326^{* *}$ | $0.254^{* *}$ | $0.289^{* *}$ | $0.192^{*}$ |
| K |  |  | $0.697^{* *}$ | $0.793^{* *}$ | $0.736^{* *}$ | $0.709^{* *}$ |
| Fe |  |  |  | $0.679^{* *}$ | $0.680^{* *}$ | $0.586^{* *}$ |
| Mn |  |  |  |  | $0.759^{* *}$ | $0.593^{* *}$ |
| Cu |  |  |  |  |  | $0.718^{* *}$ |

${ }^{*}: \mathrm{P} \leq 0.05,{ }^{* *}: \mathrm{P} \leq 0.01$.

Table 4. Eigenvectors, eigenvalues, and individual and cumulative percentages of variation explained by the first 5 principal components (PCs) of 129 open-pollinated Turkish faba bean landraces for contents of some mineral elements.

| Variables | Eigenvectors |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- |
|  | PC1 | PC2 | PC3 | PC4 | PC5 |
| Nitrogen $\left(\mathrm{g} \mathrm{kg}^{-1}\right)$ | 0.1099 | 0.8537 | -0.4683 | 0.1208 | 0.1038 |
| Phosphorous $\left.\left(\mathrm{g} \mathrm{kg}^{-1}\right)^{111111111111111}\right)$ | 0.1924 | 0.4142 | 0.8725 | 0.1011 | 0.0909 |
| Potassium $\left(\mathrm{g} \mathrm{kg}^{-1}\right)$ | 0.4493 | -0.1993 | -0.0772 | -0.1395 | 0.0564 |
| Iron $\left(\mathrm{mg} \mathrm{kg}^{-1}\right)$ | 0.4256 | 0.1170 | -0.0034 | -0.3289 | -0.8090 |
| Manganese $\left(\mathrm{mg} \mathrm{kg}^{-1}\right)$ | 0.4425 | -0.0612 | -0.0655 | -0.4600 | 0.4630 |
| Copper $\left(\mathrm{mg} \mathrm{kg}^{-1}\right)$ | 0.4525 | -0.0571 | -0.0331 | 0.1120 | 0.3040 |
| Zinc $\left(\mathrm{mg} \mathrm{kg}^{-1}\right)$ | 0.4089 | -0.1978 | -0.0886 | 0.7894 | -0.1280 |
| Eigenvalue | 3.93 | 1.02 | 0.87 | 0.42 | 0.33 |
| Percent | 56.28 | 14.63 | 12.54 | 6.03 | 4.73 |
| Cum. percent | 56.28 | 70.91 | 83.46 | 89.49 | 94.22 |

populations with respect to their nutritional value. Deficiencies in essential mineral cations affect large populations in several parts of the world, as is well-known for Fe and Zn . The importance of $\mathrm{P}, \mathrm{Cu}, \mathrm{Ca}$, and Mn in the human diet, in addition to Zn and Fe , should also be taken into consideration (Pinheiro et al., 2010). Nutritionally enhanced grain legumes can not only contribute to health improvement directly by enhancing micronutrient availability, but also indirectly through improved agronomic performance (Welch, 1999; Gomez-Galera
et al., 2010). Mudryja et al. (2011) reported that pulse consumption had influenced and increased the mineral nutrient uptakes in Canadian adults of all ages, and pulses had been recommended as alternative food groups in the Canadian food guide. The amount of minerals in legumes is higher than that in cereal crops. Thus, identification of the nutritional characteristics of different landraces is an important prerequisite for their effective utilization in breeding programs to improve the mineral status of faba bean cultivars. A large number of faba bean landraces


Figure 2. Multivariate principal component analysis of 129 open-pollinated Turkish faba bean landraces and 4 commercial cultivars. The number given to each landrace can be seen in Table 1.
have been collected from different parts of Turkey, the most important center of diversity. However, information on the extent and pattern of genetic diversity for mineral elements in these landraces is not systematically available.

The current study presents a comprehensive analysis of micronutrient ( $\mathrm{Zn}, \mathrm{Fe}, \mathrm{Cu}$, and Mn ) and macronutrient $(\mathrm{N}$, K , and P ) concentrations for a large germplasm collection. The open-pollinated faba bean landraces exhibited a wide variation in the seed concentrations of all nutrients (Table 2). Moreover, traditional landraces showed significantly higher concentrations of seed mineral nutrients relative to commercial faba bean cultivars. For example, the highest values for Zn in the landraces were more than 2 -fold greater than in the cultivars. The other previous studies also demonstrated that chickpea and faba bean landraces exhibited much higher diversity for nutrient traits compared with other cultivars (Özer et al., 2011; Karaköy et al., 2014). There appears to be considerable genetic variation in the mineral concentrations present in edible portions of most crop species, such as in the grains of maize, rice, and wheat (Graham et al., 1999; Welch and Graham, 2004; Garvin et al., 2006; Jiang et al., 2008; Menkir, 2008) and in legumes such as lentil (Karaköy et al., 2012) and bean (Talukder et al., 2010). The physiological basis for the varying rates of mineral accumulation in the seeds of different genotypes is yet to be clearly elucidated (Welch and Graham, 2002). In their report, Bonfil and Kafkafi (2000) described that when any nutrient is naturally deficient in the soil, genotypes that store a higher concentration of that nutrient in the seed will have an extra advantage under such conditions. The amount of minerals in the seed depends on a plethora of processes, including absorption from the soil, uptake by the roots, translocation and redistribution within the plant tissues, and remobilization to the seed (Grusak and Cakmak, 2005; Cakmak et al., 2010). However, this is the first report about the mineral variations among large sets of open-pollinated faba bean germplasms.

This wide diversity for mineral elements, such as Zn and Fe and other elements present in the Turkish openpollinated faba bean landraces, demonstrates huge potential and should complement the ongoing efforts for faba bean improvement programs. For example, the wide variation in Zn content ( $10.4-49.3 \mathrm{mg} \mathrm{kg}^{-1}$ ) in these landraces indicates a wealth of allelic variation for the development of cultivars with high bioavailable Zn concentration. In addition, the landraces were sampled from a very variable set of environmental and geographical locations (Figure 1), which also tends to increase the level of diversity. Variation in chemical composition depends upon genotype, seed characteristics, seed composition, agronomic practices, climatic factors, soil type, pollination type, and other factors.

Correlation coefficients for the mineral elements were examined to determine whether selection for stability in one mineral trait might concurrently affect the stability in other mineral parameters. Correlation coefficients among studied grain concentrations of different mineral nutrients may indicate 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). Seed Zn and Fe concentration were significantly and positively correlated ( $\mathrm{r}=0.586 ; \mathrm{P}<0.01$ ) with each other in this study. Significant associations of Fe and Zn have been reported in numerous crops, such as in lentil (Karaköy et al., 2012), wheat (Garvin et al., 2006; Chatvaz et al., 2010), and Phaseolus vulgaris (Pinheiro et al., 2010). Similarly, Zn was positively correlated with $\mathrm{P}, \mathrm{K}, \mathrm{Cu}$, and Mn , similar to the findings of Karaköy et al. (2012) in lentil and Jiang et al. (2008) in rice. Positive associations among different traits showed that improvement of one character might simultaneously improve another desired trait (Yücel et al., 2009; Cömertpay et al., 2012). Selection of the right character is also important because of correlation among different traits (Yücel et al., 2009). For example, the significant and positive correlations between Fe and Zn concentrations also indicate the possibility of increasing the 2 minerals simultaneously.

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 (Andeden et al., 2013; Karaköy et al., 2014). To analyze the structure of the genetic diversity among a set of 129 open-pollinated faba bean landraces and 4 cultivars, we performed PCA based on mean values. Using PCA, it appeared that there were 2 important underlying components for accessions ( $70.91 \%$ of total variation; Table 4), which can be defined as mineral elements in the faba bean germplasm. PC1 was the most important, accounting for more than half of the total variability (around 56\%) in accessions, and the traits responsible for this high variation were $\mathrm{Cu}, \mathrm{K}, \mathrm{Mn}, \mathrm{Fe}$, and Zn among accessions. The interrelationships described by PC1 entailed a very important point of practical significance for an attempt to breed for high seed $\mathrm{Cu}, \mathrm{Mn}, \mathrm{Fe}, \mathrm{K}$, and Zn contents in the faba bean. PC2 was responsible for $14.63 \%$ of the total variability. Only N was the biggest contributor of this variation. The principal component graph showed that open-pollinated faba bean landraces were clearly split into 2 groups. The main variants of this grouping were Cu , $\mathrm{Mg}, \mathrm{Fe}$, and K in PC 1 and N in PC2. Figure 2 shows that
some of the landraces were clearly different from the rest of landraces for the above-mentioned mineral traits and that these mineral traits were the main source of variations in the germplasm. However, the data presented here are from unspecified hybrid samples resulting from 129 openpollinated faba bean landraces and 4 cultivars. It is desirable that future work on the landraces, taking precautions against faba bean outcrossing, be performed in order to precisely characterize each specific Turkish landrace, or characterization should be done for the selfed genotypes. One plant from each landraces was selfed 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 faba bean cultivars with high mineral elements.

In spite of the availability of rich genetic resources and the heavy consumption of legumes, including faba bean, in the Turkish diet, legume breeding in Turkey and other countries falls far behind cereals and other industrial crops (Baloch et al., 2010; Özer et al., 2011; Karaköy et al., 2012; Özer et al., 2012). The yield and the quality of cereals, soybean, cotton, and other crops in Turkey have shown tremendous improvements due to breeding activities. During the recent decade, scientists at several consultative groups have been investigating the genetic potential to increase bioavailable Fe and Zn and other minerals and vitamins in edible portions of staple food crops such as rice, wheat, maize, and legumes through breeding. However, breeding activities for faba beans in Turkey, as well as in the world, are negligible. That is why faba bean is referred to as an "orphan crop". The genetic resources of faba beans with high diversity have not yet been evaluated for their

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mineral elements. This is the initial step: we are reporting the diversity of faba bean genetic resources, which will be important for the success of breeding programs in Turkey. Our results demonstrate the importance of landraces as the base material for developing superior faba bean cultivars with high mineral concentrations in breeding projects

Lev-Yadun et al. (2000) proposed a "core area" for the origins of agriculture within the Fertile Crescent. This was based on the proposition that wild einkorn and wild emmer from this area are genetically more closely related to domesticated crop plants than elsewhere; legume crops such as chickpea and lentil are believed to have most probably originated in southeastern Turkey. Earlier studies have proposed the Near East and southeastern Turkey as the postulated area of faba bean domestication. Therefore, it is very important to study and characterize landraces from its area of diversity. In our previous study, we studied the genetic variation among faba bean landraces for 15 morphological and 6 quality traits (Karaköy et al., 2014). Karaköy et al. (2014) also observed that Turkish faba bean landraces harbored high phenotypic diversity compared with the landraces from China and Greece (Duc et al., 2010). 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 investigation by conducting field trials at multiple locations to verify the results and to study genotype $\times$ environment interactions and precautions should be done to study the mineral contents in selfed faba bean lines. These landraces and associated information are useful to researchers and breeders from all over the world who are interested in Turkish faba bean genetic resources.

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