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Systematic Determination of The Ultrastructure of Local Faba Bean (Vicia faba L.) Seeds Using Light and Scanning Electron Microscopes

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A B S T R A C T

The present study was conducted to determine the seed morphologies and macromorphological characteristics of fifteen local faba bean (*Vicia faba* L.) genotypes collected from different region of Northern Cyprus. The characteristics in the seeds were evaluated using a light microscope (LM) and scanning electron microscope (SEM). SEM pictures were taken of the whole seed and its details. The scope of the research was examined comparatively using LM and SEM, and the seed shapes, surface ornamentation, and quantitative measurements were determined. Different characteristics for seed size and surface were found among the local faba beans accessions. Seed dimensions were between 12.44 and 24.26 mm long and between 9.49 and 17.45 mm wide, colors ranged from yellowish-brown to dark brown, and ornamentation of the seed coat varied. The local faba bean seeds were of subprolate and prolate types. We believe that using this technique to determine the differences among the seeds of different genotypes may play a role in helping to create different programs that can choose specific genotypes to improve cultivars based on the seed characteristics.

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Introduction

Faba beans (*Vicia faba* L.) belong to the Fabaceae family and are healthy in both human nutrition and for improving the soil as a good rotation plant, as is true of all leguminous plants. The faba bean is a product that can be used in many recipes and can be grown under different ecological conditions. It has high nutritional value and the ability to adapt to large areas, and it is an important rotation crop, especially within cereal-growing regions (Gasim et al., 2004; Torres et al., 2006; Karaköy et al., 2017). Local genotypes, which have been cultivated for centuries, are extremely important because of their high level of adaptation to the conditions within their location. Plant genetic resources consist of village populations characterized by local varieties, their wild relatives, and unused old varieties and lines. Genetic resources are an important and reasonable tool used to contribute the raw material for producing improved cultivars to meet the world's future food and ecological needs (Göl et al., 2016; Yılmaz 2020). It is imperative that genetic resources be conserved to ensure future agricultural production and the future of those who use the product (Sarı et al., 2016). The faba bean is a valuable vegetable high-protein and energy source that can be consumed both fresh and dry (Duc et al., 2010; Gnanasambandam et al., 2012; Al Barri and Shtaya, 2013; Ammar et al., 2015; Sözen and Karadavut, 2016; De Cillis et al., 2019). Morphological variations are of great importance in the development of a program to breed varieties of the plants. Determining variations in the cultivated species and the distribution of these variations are very important for effective implementation of breeding programs. Variation in the genetics of a quantitative feature is also of great importance for vegetable breeding. A comparison of these variations with each other helps to identify the genetic characteristics of the current population (Balkaya et al., 2010). The morphological characterizations of the varieties in terms of broad bean characteristics have been made by different researches on green bean species, and the morphological variations and their distribution have been revealed in detail (Madakbaş et al., 2006; Ekincialp and Şensoy, 2013; Kobal et al., 2019).

Cultivated broad bean varieties are systematically defined under three different groups—*V. faba* var. *equina*, *V. faba* var. *minor*, and *V. faba* var. *major*. There are great differences among these groups in terms of plant morphology and seed characteristics. Plant height is 40–200 cm, leaf number is 20–70/plant, fodder yield varies between 200 and 480 kg/da, and raw protein ratios are close to 25%. The plant can grow in warm and rainy climates, where the plant becomes quite high, green mass yield increases, and the dry matter ratio decreases (Manga et al., 1995).

The seed coat consists of five layers, which are, from outside to inside, the epidermis, hypodermis, mechanical layer, parenchyma, and chlorenchyma. The seed contains suberin, lignin, and cutin. The seed surface can be smooth, shiny, jagged, or hairy. They are mostly gray, brown, and black. Special structures on the seeds help them disperse and ensure that the embryo remains dormant (Toker, 2004). The outer surface of the seeds shows differences in ornamental structures, which are used to help classify the plants. In addition, the length and width of the seed and its color and shape are also used in plant classification. The formation of these characteristics are factors that allow the seed to be transported (Mosquero et al., 2002).

Within the scope of this study, the seed morphologies of 15 local faba bean (*V. faba* L.) genotypes collected from Northern Cyprus were analyzed comparatively using a stereo light microscope (LM) and a scanning electron microscope (SEM). Seed shapes, seed surface ornamentation, and quantitative measurements of the local faba bean genotypes were revealed in detail among the 15 genotypes studied.

Materials and Methods

The plant material for the present study was composed of local faba bean genotypes grown in different regions (Nicosia, Morphou, Kyrenia, Famagusta, Lefka) of Northern Cyprus. The study samples were collected from 15 local populations grown in Northern Cyprus (Table 1).

LM and SEM were used to determine the seed surfaces. SEM studies were conducted using the Zeiss GeminiSEM 500 (Carl Zeiss AG, Jena, Germany) computer-controlled digital SEM at Erciyes University Technology Research and Application Center, Turkey. Dry seeds of local faba bean genotypes were immersed in a 1:1 solution of chloroform and methanol for 48 h and dehydrated in a series of ethanol concentrations (70, 90, and 100%) to remove surface contaminants. They were then kept in xylene for 72 h, after which the seeds were placed on the sample holder (aluminum stub) with double-sided adhesive tape. Samples were coated with the gold/palladium and then observed by standard techniques using the Zeiss GeminiSEM 500. The macromorphological characteristics of the seeds, such as width, height, color, and shape, were determined using SEM (Mosquero et al., 2002). For each feature, 30–50 measurements were taken, and their averages were calculated.

Results

LM and SEM results of the macromorphological analyses conducted on the 15 local faba bean genotypes examined for their seed morphology are provided in Table 2. Among the studied genotypes, the seed shape was either prolate or subprolate. The subprolate seeds were from the Bak-05 and Bak-18 genotypes; the shape of the seeds of all the other genotypes were prolate. In addition, when the seeds were examined for surface ornamentation, it was determined that all the seeds had a Pusticulate seed surface. The colors of the different genotypes varied from yellowish-brown to dark brown (Figure 1), and seed surface luster varied from very bright to dull.

In angiosperms, seed morphologies, seed size, color, and seed surface ornamentation are among the important characteristics in taxonomic and evolutionary studies (Juan et al., 1994; Riahi and Zarre, 2009; Kaya et al., 2011; Fagundez and Izco, 2011). In the present study, seed morphological characteristics of 15 local faba bean genotypes cultivated intensively in Northern Cyprus were examined using LM and SEM. SEM was used to evaluate the seed properties and distinguish them for the 15 genotypes (Javadi and Yamaguchi, 2004; Özbek and Ekici, 2014; Kahraman et al., 2014; Mirzaei et al., 2015). The results of the present study showed that the macromorphological structures of the seeds of the 15 genotypes differed from each other (Tables 2), which was an important factor for evaluating the genotypes.

Table 1. Collected faba bean accessions used in the study and their collection location.

Collector	Species	Voucher information and location in Northern Cyprus
Yılmaz et al.	Vicia faba L.	Bak-02: Lefka prov.: Yedidalga village
Yılmaz et al.	Vicia faba L.	Bak-04: Lefka prov.: Yedidalga village
Yılmaz et al.	Vicia faba L.	Bak-05: Morphou prov.: Aydınköy village
Yılmaz et al.	Vicia faba L.	Bak-07: Lefka prov.: Bağlıköy village
Yılmaz et al.	Vicia faba L.	Bak-08: Morphou prov.: Bostancı village
Yılmaz et al.	Vicia faba L.	Bak-13: Morphou prov.: Doğancı village
Yılmaz et al.	Vicia faba L.	Bak-17: Morphou prov.: Zümrütköy village
Yılmaz et al.	Vicia faba L.	Bak-18: Kyrenia prov. Lapta village
Yılmaz et al.	Vicia faba L.	Bak-22: Famagusta prov.: Beyarmudu village
Yılmaz et al.	Vicia faba L.	Bak-23: Famagusta prov.: Vadili village
Yılmaz et al.	Vicia faba L.	Bak-24: Famagusta prov.: Akdoğan village
Yılmaz et al.	Vicia faba L.	Bak-28: Famagusta prov.: Beyarmudu village
Yılmaz et al.	Vicia faba L.	Bak-29: Kyrenia prov.: Lapta village
Yılmaz et al.	Vicia faba L.	Bak-33: Famagusta prov.: Tatlısu village
Yılmaz et al.	Vicia faba L.	Bak-38: Famagusta prov.: Çınarlı village

Table 2. Seed sizes and morphological characteristics of the *Vicia faba* L. accessions studied.

Accessions	Seed length (mm)	Seed width (mm)	Seed shape	Surface ornamentation	Seed L/W
Bak-02	19.76	13.46	Prolate	Pusticulate	1.47
Bak-04	20.01	14.49	Prolate	Pusticulate	1.38
Bak-05	12.44	9.49	Subprolate	Pusticulate	1.31
Bak-07	18.35	13.44	Prolate	Pusticulate	1.37
Bak-08	21.68	15.77	Prolate	Pusticulate	1.38
Bak-13	24.26	16.03	Prolate	Pusticulate	1.51
Bak-17	24.19	17.45	Prolate	Pusticulate	1.39
Bak-18	19.47	14.82	Subprolate	Pusticulate	1.32
Bak-22	20.89	15.28	Prolate	Pusticulate	1.37
Bak-23	22.20	15.55	Prolate	Pusticulate	1.43
Bak-24	21.14	14.95	Prolate	Pusticulate	1.41
Bak-28	22.56	15.70	Prolate	Pusticulate	1.44
Bak-29	17.17	12.12	Prolate	Pusticulate	1.42
Bak-33	19.41	13.91	Prolate	Pusticulate	1.40
Bak-38	20.99	14.94	Prolate	Pusticulate	1.40

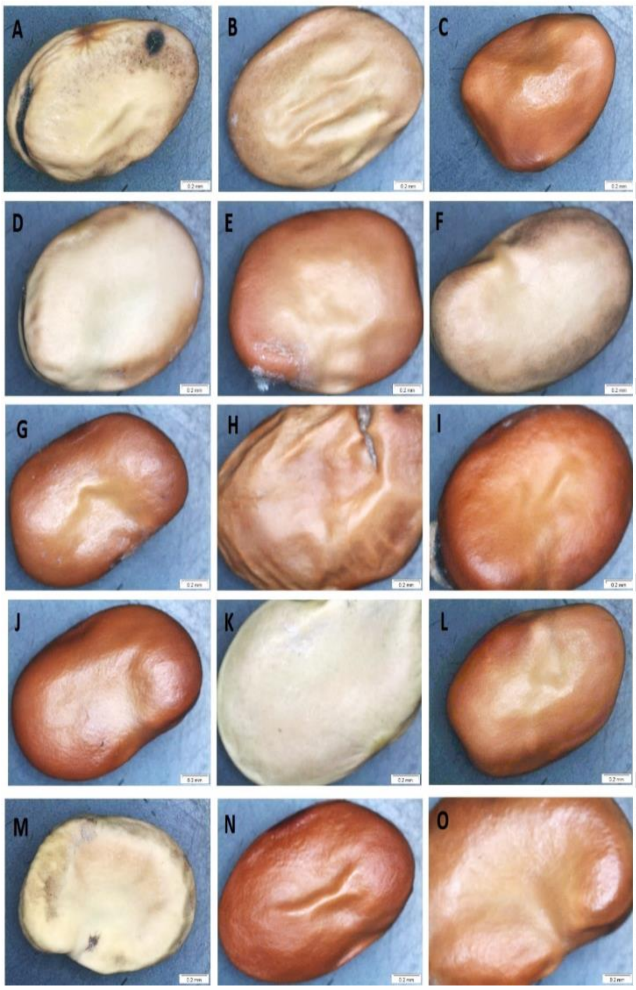

Figure 1. Stereo light microscope photographs of faba bean genotypes. A) Bak-02, B) Bak-4, C) Bak-5, D) Bak-7, E) Bak-8, F) Bak-13, G) Bak-17, H) Bak-18, I) Bak-22, J) Bak-23, K) Bak-24, L) Bak-28, M) Bak-29, N) Bak-33, and O) Bak-38.

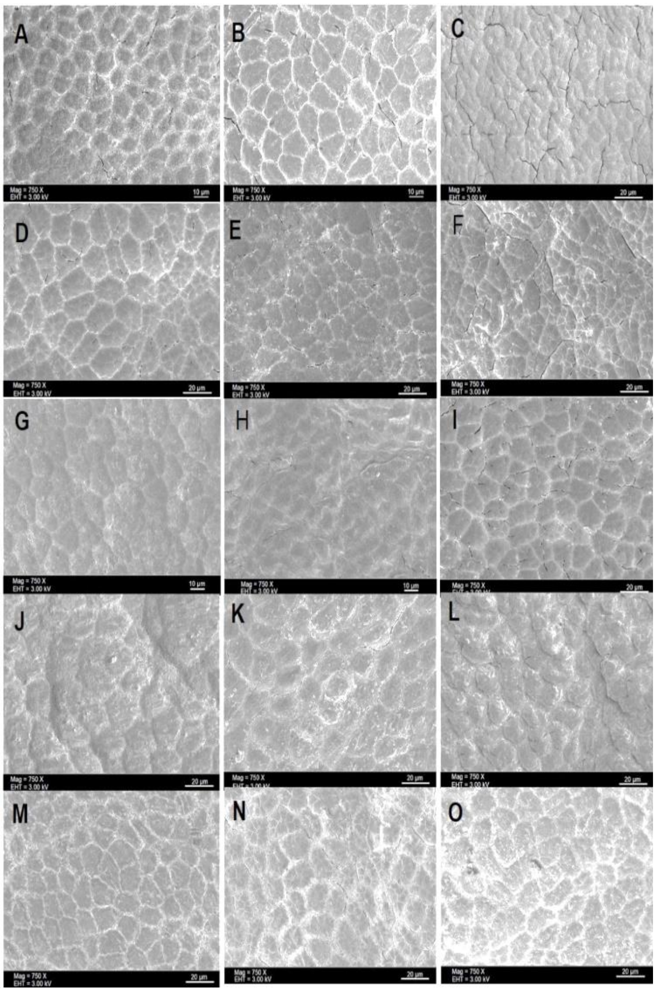

Figure 2. Scanning electron micrographs of faba bean genotypes. A) Bak-02, B) Bak-4, C) Bak-5, D) Bak-7, E) Bak-8, F) Bak-13, G) Bak-17, H) Bak-18, I) Bak-22, J) Bak-23, K) Bak-24, L) Bak-28, M) Bak-29, N) Bak-33, and O) Bak-38.

One study on the seed morphology of faba beans has included the ultrasculpture of pollen grains and the surface and macro- and microstructures of the seeds of broad beans, peas, and common beans (Kurkina et al., 2020).

In a study carried out by Kurkina et al. (2020) has stressed that seed characteristics are determined by ecological factors that have an important effect on the diversity of pod seed genotypes and that the growth and genotypes of plants may differ in their development because of this diversity. In addition, the results of their study suggest that it is predicted that these seed differences belonging to different genotypes can be used in breeding studies and will make important contributions to the programs that breed these plants for specific morphological features.

We determined that there is a homogeneity among the genotypes in terms of seed length and width. Accordingly, Bak-17 had the largest seed size, while Bak-05 had the smallest. In general, the shape of the seeds in all but two genotypes were prolate, the seeds from the remaining two were subprolate. Seed surface ornamentation was also examined. The results showed that the seed surface of the local faba bean genotypes has pusticulate ornamentation (Figure 2 and Table 2).

Conclusions

The present study provides the first detailed information on the morphological traits of the seeds of local faba beans grown in Northern Cyprus. It has been determined that the seed characteristics of the studied genotypes were mostly prolate shape and differ from each other. These characteristics can be easily determined using SEM. Consequently, results based on LM and SEM analyses, have shown that seed sizes and surface patterns have important morphological features that distinguish the studied genotypes from each other. Therefore, the fact that there was no previous systematic determination of the macro and micro-morphology of local seeds study in Northern Cyprus has increased the importance of this study and it was considered that seed morphological characterization studies alone are insufficient. It is believed that additional studies using LM and SEM for morphological characterizations and analyses may help to support information that distinguishes genotypes in breeding studies.

Conflict of Interest

The authors declare that there is no conflict of interest.

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