

## Comparison of reproductive organs structure in various genotypes of *Phaseolus vulgaris* grown under the same conditions in Mersin, Turkey

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### Abstract

The common bean (*Phaseolus vulgaris*) is a plant having hermaphroditic flowers, which are self-fertilizing. Common bean is an important commercial crop and one of Turkey's most valuable grain legumes for human consumption. Genetic variation exists within and between bean populations. These variations can have an impact on the plants morphology, including reproductive organs. Variations in size and length of reproductive organs may cause different cell numbers and various fertilization successes. In this study, the response of four determinate common bean genotypes (Akman, Bitlis 117, Göksun, and Karacaşehir) to reproductive organ structure was studied. Plants were grown at 21/16 °C (day/night) and 12 h photoperiod in a greenhouse in Mersin, Turkey. Besides focusing mainly on pollen development, fertilization success factors such as pod and seed set were also assessed. Pod and seed set were evaluated numerically for each genotype. For pollen morphology, flower buds of the equal growth stage of all four bean genotypes were collected, and these samples were analyzed by scanning electron microscopy. The species have distinct morphological characteristics concerning pollen size, ornamentation patterns of the exine, and endoaperture type. It is also crucial to examine pollen dispersion in the pollinated flowers, as it may influence reproductive success. However, pollen wall architecture was similar in all genotypes tested. Despite general morphological similarity, differences in pollen viability and pod and seed number were observed. Although Akman genotype had the largest flowers, the Karacaşehir genotype was found to be the most productive one. The findings presented here can be helpful for researchers studying bean breeding and those investigating the morphological variation in different *Phaseolus* genotypes.

**Keywords:** *Phaseolus vulgaris*, common bean, pollen, reproduction

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**Authors' contributions:** Conceived and designed the experiments: D. Tekdal. Performed the experiments: D. Tekdal, S. Shawuti Can, and A. Küçükrecep. Supervised the study: D. Tekdal. Wrote the paper: D. Tekdal. Critically revised the manuscript: D. Tekdal, S. Shawuti Can, and A. Küçükrecep.

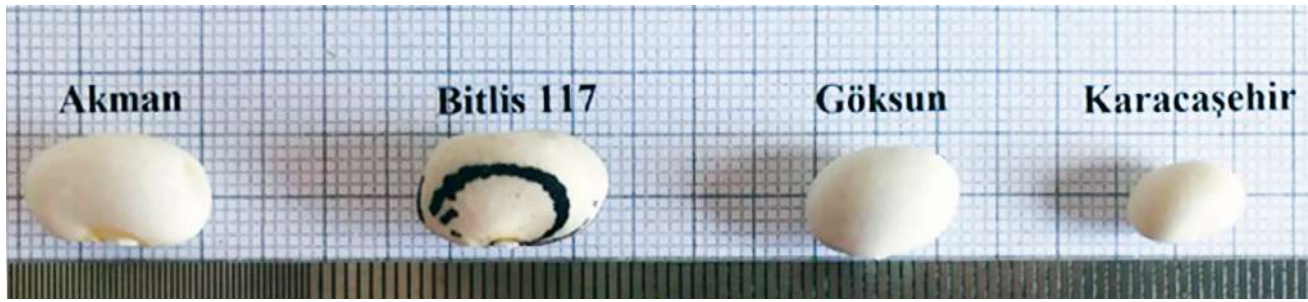
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### Introduction

Fabaceae Lindl. (Leguminosae Juss.) is one of the most prominent families of flowering plants, consisting of 630 genera and about

20,000 species (Croser et al., 2006; da Luz et al., 2013; Chase et al., 2016). It is the world's third-largest widely distributed angiosperm family, second in agricultural



**Figure 1.** Seeds of investigated *Phaseolus vulgaris* genotypes.

economic significance (da Luz et al., 2013). Fabaceae is divided into six subfamilies: Cercidoideae LPWG, Detarioideae Burmeist., Duparquetioideae LPWG, Dialioideae LPWG, Papilionoideae DC., and Caesalpinioideae DC. (Azani et al., 2017). Fabaceae, in general, have floral features that lead to a wide range of pollination mechanisms (Stirton, 1981). Although the papilionaceous flowers have a huge impact on pollen economy, the pollen morphology and development has been studied very little in this family.

Common bean (*Phaseolus vulgaris* L.) belongs to the tribe Phaseoleae DC., one of the Papilionoid clades (Croser et al., 2006). The common bean is specific in its morphology, root nodules, and pollination mechanism. These are diploid ( $2n=22$ ), herbaceous, autogamous annual plant, self-pollinating with a limited percentage of out-crossing.

The present work compares the morphology of certain reproductive structures in four *P. vulgaris* genotypes. The main goal of the study was to gain knowledge of pollen architecture, which provides indirect information about the breeding system. In addition, it was also targeted to understand the floral and reproductive biology of selected *P. vulgaris* genotypes, which could optimize their management and restoration.

## Material and methods

### Plant material

This research used seeds of four genotypes of *P. vulgaris* (i.e., Akman, Bitlis 117, Göksun, and Karacaşehir) (Fig. 1). The greenhouse experiments were conducted in Mersin, Turkey. Plants were grown individually in plastic pots and watered keeping adequate soil moisture. Plants were grown at the

temperature of 21/16 °C (day/night) with roughly 150  $\mu\text{E m}^{-2}\text{s}^{-1}$  light intensity, and a 12 h photoperiod. The relative humidity was preserved below 80 % by controlling the air exchange. Each experiment was performed in a fully randomized design with triplicates.

Seed germination in all tested varieties happened one week later, and plants have been grown for two months to achieve the required developmental stage of flower buds (Fig. 2). After that, the flower buds were harvested for further analysis.

### SEM analysis

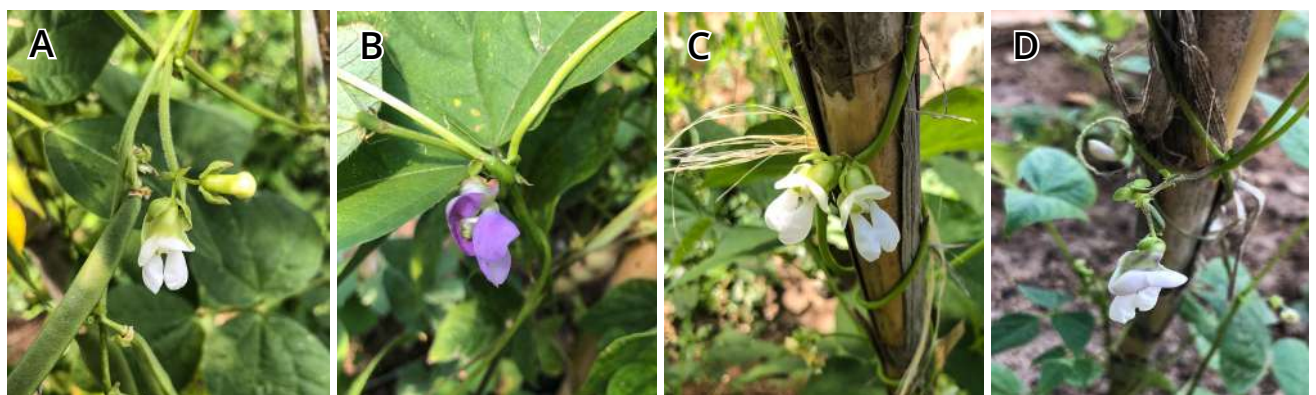
Flower buds were collected in May-June 2020. Anthers were dissected under a stereomicroscope (Olympus SZ61, Japan) and air-dried for scanning electron microscopy (SEM). The anther of each genotype was dried under a vacuum chamber for 48 h. The samples were gold-coated before being placed in the SEM vacuum chamber. The surface topography of the pollen grains and grain size distributions were observed by SEM with an energy dispersive x-ray spectrometer (EDS, FEG-SEM Leo Supra 35, and Bruker XFlash). For stomata observation, flowers were collected and fixed in 70 % ethanol immediately at the greenhouse, taken through a dehydration series (70 % ethanol – 80 % ethanol – 96 % ethanol – 96 % ethanol:acetone (1:1) – acetone), and observed under SEM. Stomata were observed and photographed for each genotype.

### Pollen viability, pollen fertility, and flower morphology

To examine the morphology of the anthers, flower buds were collected during the blooming season. Three flowers from different



**Figure 2.** One-month-old *Phaseolus vulgaris* plants grown in the greenhouse: A – Akman; B – Bitlis 117; C – Göksun; D – Karacaşehir.



**Figure 3.** One-month-old *Phaseolus vulgaris* plants grown in the greenhouse: A – Akman; B – Bitlis 117; C – Göksun; D – Karacaşehir.

branches were examined for pollen fertility; as a result – at least 150 pollen grains were counted per each flower.

Pollen was collected from each genotype during anthesis, distributed on a glass slide with a drop of acetocarmine, and covered with a coverslip. The pollen grains were observed through a fluorescent microscope (Olympus BX51, Japan) and subsequently classified as fertile or infertile.

Fifty fruits were collected at the fruiting stage from each plant to determine seed production. A total number of fully formed seeds and aborted ovules were counted. The ratio of the number of seeds to the number of ovules per pod (%) was calculated as an indicator of reproductive efficiency.

The perianth color of each genotype was determined during the visual examination of the fresh material.

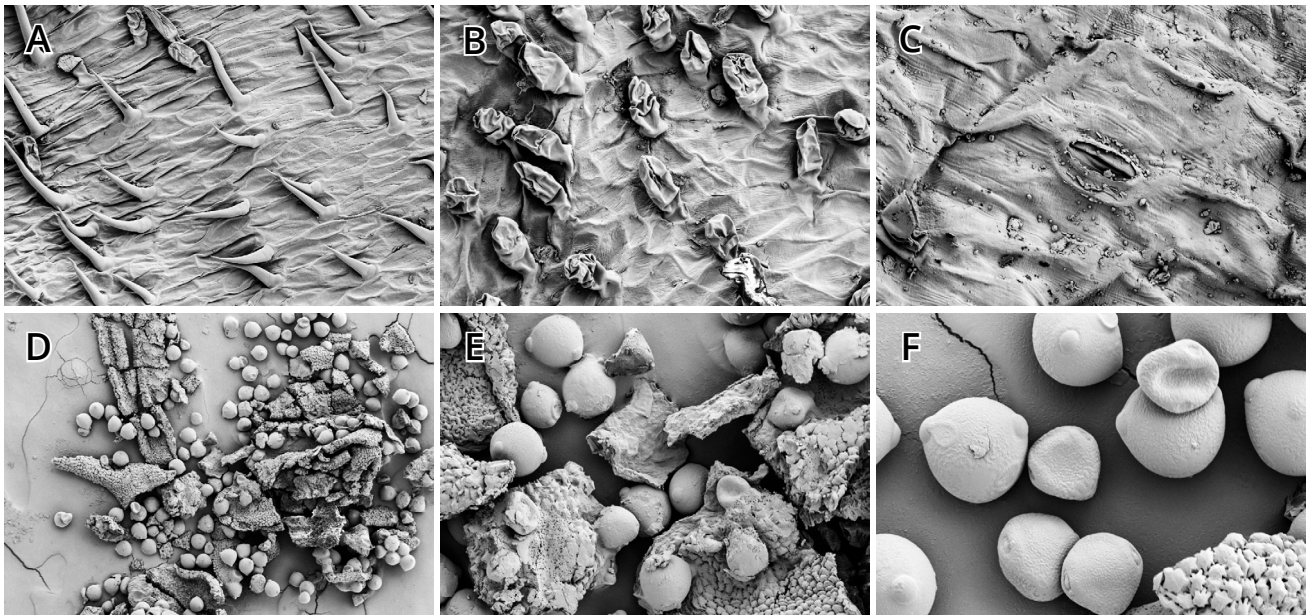
## Results and discussion

### Morphological observations

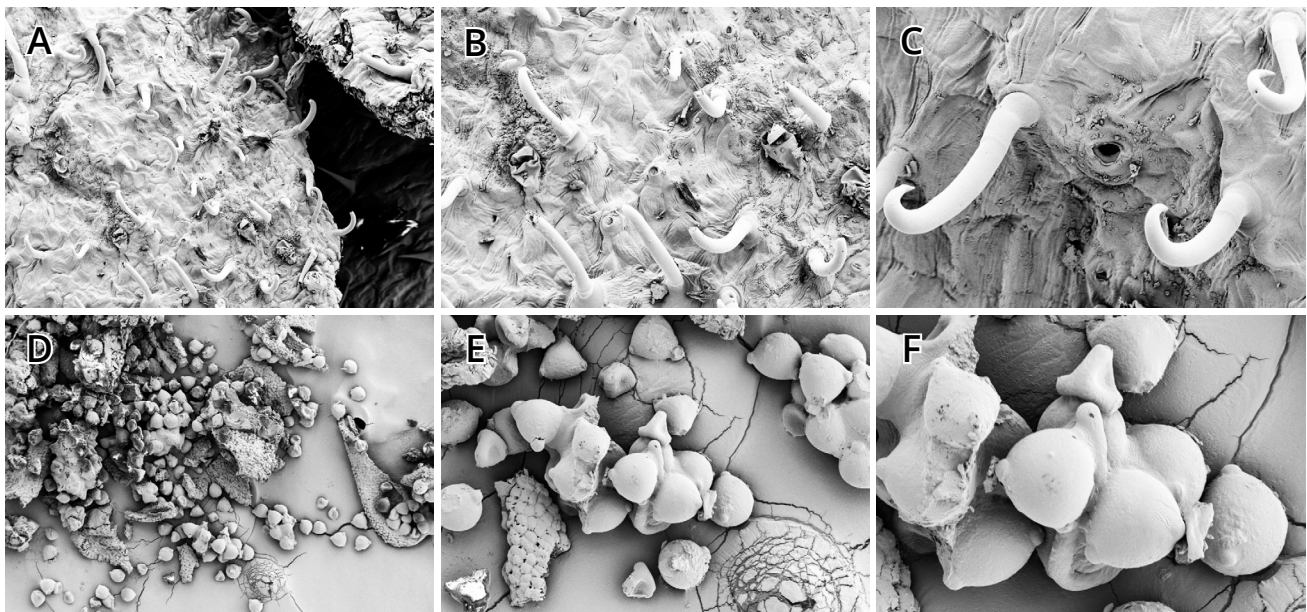
The appearance of Fabaceae flowers to the insects is complex, with the main variations between the genera and the species depending on the wing characteristics (López et al., 1999). In Mersin (Turkey) *P. vulgaris* usually blooms in April and May. Its flowering lasts for about 20 days on an individual plant. The petals of these plants are typically whitish, and less frequently, they can be violet-purple (Fig. 3). Some petals reflect UV radiation due to pigment variation or presence of reflective hairs.

The most considerable floral lengths were found in the genotypes Akman (13.0 mm) and Göksun (14.0 mm), and the smallest ones – in the genotypes Bitlis 117 (7.5 mm) and Karacaşehir (4.5 mm). López et al. (1999) stated that floral size is correlated directly with the number of ovules. However, in our study, Karacaşehir genotype having the shortest





**Figure 4.** Generative structures of Akman genotype of *Phaseolus vulgaris* (SEM). **A** – hairs on the anther surface ( $\times 500$ ); **B** – anther epidermis ( $\times 1K$ ); **C** – stomata on the bottom of the calyx ( $\times 2K$ ); **D-F** – pollen grains at different magnifications ( $\times 300$ ,  $\times 1K$ , and  $\times 2K$ , respectively).



**Figure 5.** Generative structures of Bitlis 117 genotype of *Phaseolus vulgaris* (SEM). **A** – anther epidermis ( $\times 500$ ); **B** – hairs on the anther surface ( $\times 1K$ ); **C** – stomata on the bottom of the calyx ( $\times 2K$ ); **D-F** – pollen grains at different magnifications ( $\times 300$ ,  $\times 1K$ , and  $\times 2K$ , respectively).

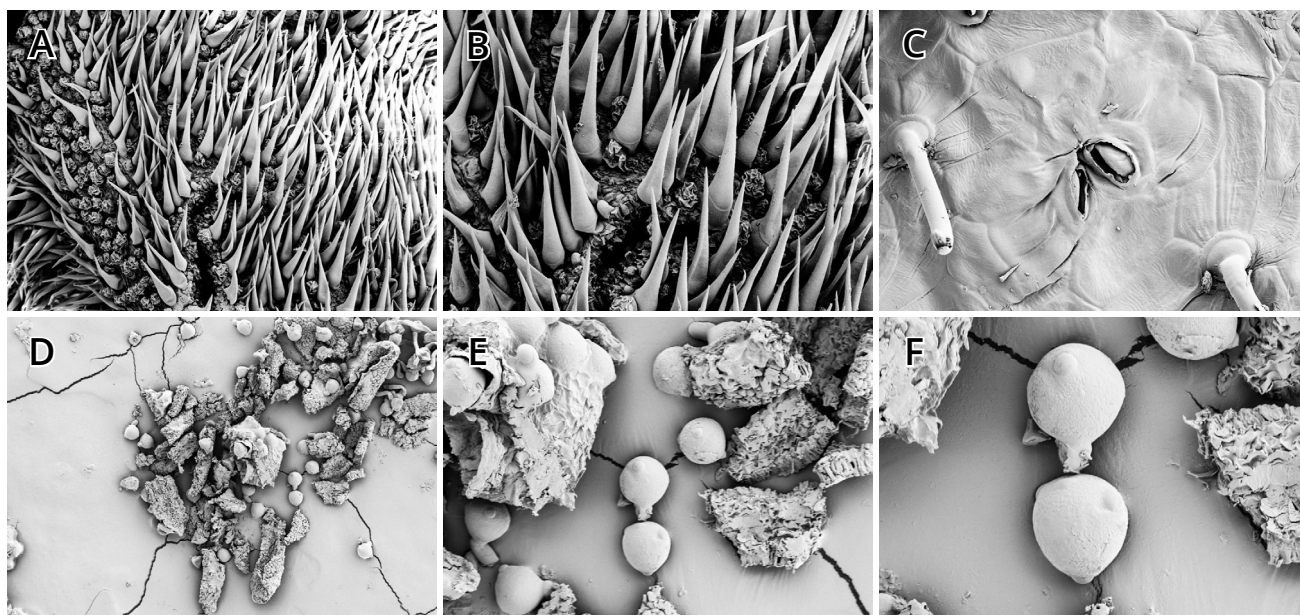
flower length was the most productive with six ovules. Akman genotype was in second place with five ovules. The least productive genotypes were Bitlis 117 and Gökşun with four ovules per pod.

#### SEM analysis and anther morphology

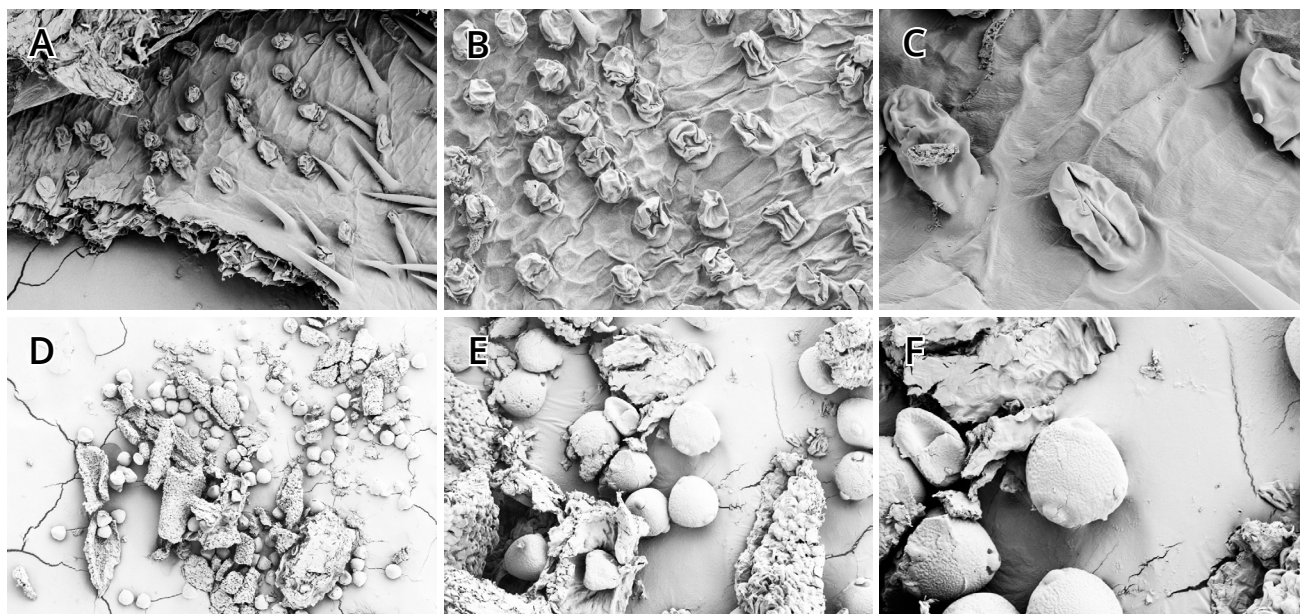
The surface morphologies of each sample obtained from SEM studies are illustrated in

**Figs. 4-7.** Anthers of the studied genotypes are uniform. They have comparable epidermal characteristics. Some stomata have been detected on the dorsal end of the conjunctive. There are staminal filaments, but they are devoid of stomata. Pollen grains are medium-sized, isopolar, circular, and polar-view. The pollen grains' exine is resistant to strong chemicals, allowing long-term preservation





**Figure 6.** Generative structures of Göksun genotype of *Phaseolus vulgaris* (SEM). **A** – anther epidermis ( $\times 500$ ); **B** – hairs on the anther surface ( $\times 1K$ ); **C** – stomata on the bottom of the calyx ( $\times 2K$ ); **D-F** – pollen grains at different magnifications ( $\times 300$ ,  $\times 1K$ , and  $\times 2K$ , respectively).



**Figure 7.** Generative structures of Karacaşehir genotype of *Phaseolus vulgaris* (SEM). **A** – anther epidermis ( $\times 500$ ); **B** – hairs on the anther surface ( $\times 1K$ ); **C** – stomata on the bottom of the calyx ( $\times 2K$ ); **D-F** – pollen grains at different magnifications ( $\times 300$ ,  $\times 1K$ , and  $\times 2K$ , respectively).

in an airless environment (Erdtman, 1969). At the same time, male sterility is often caused by irregular exine creation (Heslop-Harrison, 1972; Radice et al., 2008).

The anthers of some Fabaceae have tufts of hairs that can aid pollen transmission to pollinators or serve as a filter to keep pollen grains apart from their stigmas (Schrire, 1989). *Phaseolus* is considered a self-compatible

genus since out-crossing rates are meager and depend on the season, pollinator density, and genotype. Graham & Ranalli (1997) demonstrated that *P. vulgaris* pollen could fertilize the ovules of the same flower. In our research, all four genotypes were able to produce seeds successfully. Pollen grains with light staining, abnormal morphology, and decreased or nonexistent protoplasm were

deemed sterile, but those with complete cell walls and intensely stained protoplasm were considered fertile. High seed production in all genotypes indicated that pollen was highly fertile.

It was reported that pollen fertility decreased under high temperatures and pollen was more heat-sensitive than the ovules (Nakano & Asada, 1981). During high-temperature conditions, the tapetum, which is the innermost layer of the anther, may degenerate, and this may cause structural abnormalities of microspores (Suzuki et al., 2001). In our study, we investigated anther tissues under optimum conditions. Therefore, degenerated pollen grains were not observed.

## Conclusions

Not so many surveys on anther structure of members of the Papilionoid clade have been available to date. No comprehensive observations on the anther structure and development of Akman, Bitlis 117, Göksun, and Karacaşehir genotypes of *P. vulgaris* have been performed to date. Here we present the results of the first detailed study on the structure of some generative organs of *P. vulgaris*. It is suggested that anther wall structure may affect pollen productivity and subsequently on seed set. Findings on the structure of the male organs of this species can be interesting for further breeding and seed productivity investigations.

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## Порівняльна будова репродуктивних структур різних генотипів *Phaseolus vulgaris*, вирощених в однакових умовах м. Мерсін, Туреччина

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Квасоля звичайна (*Phaseolus vulgaris*) – це рослина з двостатевими квітками, які піддаються самозапиленню. Це важлива комерційна культура, що є однією з найцінніших для спожитку серед бобових Туреччини. При цьому, генетичні варіації спостерігаються як в межах так і між окремими популяціями цих рослин. Ці варіації можуть впливати на будову органів рослин, включаючи репродуктивні структури. Відповідно, різниця у формі і розмірі репродуктивних структур може обумовлювати різний ступень якості запліднення. В цій праці представлено результати дослідження морфології деяких репродуктивних структур чотирьох генотипів квасолі звичайної (Акман, Bitlis 117, Göksun та Karacaşehir). Рослини вирощували при 21 / 16 °C (день / ніч) і 12-годинному фотоперіоді у теплицях м. Мерсін, Туреччина. Основна увага була приділена морфології пилку, однак деякі фактори, що визначають успішність запліднення (зокрема, будова бобів і набори насінин) було проаналізовано також. Було полічено кількість бобів та насінини у кожному з них. Для дослідження морфології пилку, було відібрано бутони усіх чотирьох генотипів однакового етапу розвитку і проаналізовано з використанням сканувального електронного мікроскопу. Зазвичай різні види відрізняються за розміром пилкових зерен, орнаментациєю екзини і типом ендоапerture. Також важливо звертати увагу на розподіл пилку у запилених квітках, адже це може впливати на результативність запліднення. Однак, у всіх вивчених генотипів архітектура пилкової стінки була значною мірою подібною. Не зважаючи на загальну морфологічну подібність, спостерігались відмінності у життєздатності пилку, а також кількості бобів та насінин у досліджених генотипів. Хоча у генотипу Акман були найбільші квітки, генотип Karacaşehir виявився найбільш продуктивним. Отримані результати можуть бути корисними для дослідників, які вивчають розмноження бобових та варіабельність генотипів квасолі.

**Ключові слова:** *Phaseolus vulgaris*, квасоля звичайна, пилкок, відтворення