

# Morphometrical signs of vicia faba I. for breading 

Yulia N. Kurkina ${ }^{1 *}$, Ngo T.Z. Kieu ${ }^{2}$, Irina V. Batlutskaya ${ }^{2}$, Konstantin S. Boyarshin ${ }^{2}$, Ludmila A. Tohtar ${ }^{2}$<br>${ }^{1}$ Department of Biotechnology and Microbiology, Belgorod State University, Belgorod, RUSSIA<br>${ }^{2}$ Belgorod State University, 308015, Belgorod, Pobeda Street, 85, RUSSIA<br>*Corresponding author: kurkina@bsu.edu.ru


#### Abstract

Most vegetable bean varieties have signs of xeromorphism, which testifies in favor of their high biological resistance to drought, for example Aquadul, Belarusian, White Large-Fruited, Bobchinsky, Velena, Children's Delight, Dachnik, Optics, Russian Black, Royal Harvest). For breeding, samples with amphestomatic vertically arranged leaves (varieties Windsor and Gourmet Dish) may be of interest. The most productive in the soil and climatic conditions of the Belgorod region (Russia) were the selections Belgorod-1 and Belgorod-2, as well as the varieties Royal Harvest, Bobchinsky, Batrom and Leader.


Keywords: broad beans, breeding, influence drought, anatomy of leaf, relationship of signs
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## INTRODUCTION

The ability of plants to endure unfavorable conditions with preservation of active life and the ability to reproduce (often with a decrease in productivity) reflects the biological, and without a decrease in yield, the agronomic sense of sustainability (Ulanova 1988, Agrawal et al. 2000, Novitskii et al. 2011, Lan et al. 2019, Mencuccini et al. 2019, Wu et al. 2019, Zhu et al. 2019). The ability of plants to respond to environmental factors is genetically determined and can be regulated by agrotechnical measures (Pasko 2000, Marty et al. 2010). Information about the reaction of the plant at different levels of tension factor, as well as about the genetic properties and the state of the plant at a given point in time, allow to take preventive measures to reduce adverse effects.

The problem of drought is significant for the majority of the territory of the Russian Federation, therefore, agriculture requires productive varieties that are adaptive to specific climatic conditions, which is one of the most urgent problems of breeding.

The seeds of broad beans (Vicia faba L.) contain up to $37 \%$ protein, the digestibility of which is $50-86 \%$, and is used in the food industry and medicine, and the culture itself is an indispensable component of crop rotation within the framework of ecological farming (Ivshin 2004, Sharifi 2014, Bulson et al. 1997). Our long-term studies of the broad bean collection in the soil and climatic conditions of the Belgorod region have shown that there are samples of both successfully adapted to the conditions of the Central Black Earth Region and poorly adapted to (Kurkina 2012).

Plants of broad bean are comprehensively studied all over the world, but so far the number of works devoted to the study of the anatomical and morphological characteristics of different varieties, including those related to the problems of drought resistance, remains insignificant. In this regard, morphometric and anatomical study of a collection of vegetable beans becomes timely.

## MATERIAL AND METHODS

Broad beans (20 varieties) were grown on the territory of the Botanical Garden of the Belgorod State University in 2014-2019, observing the requirements of zonal agrotechnology. Anatomical studies were performed according to G. Furst's method on living material in the laboratory of the Department of Biotechnology and Microbiology of the Belgorod State University (Furst 1979). For a comparative study of the features of the epidermis of the one-pair leaf, 5 samples of leaves of broad beans were examined at the beginning of budding. The number of stomata (per 1 $\mathrm{mm}^{2}$ ) on the upper (NSUpE) and lower (NSLE) epidermis of the lamina, the length of the stomata (guard cells) on the upper (LSUpE) and lower (LSLE) epidermis, the width of the stomata on the upper (WSUpE) and lower (WSLE) epidermis. The studies were carried out on permanent and temporary preparations using a microscope of the Micromed-2

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Table 1. Ecological and morphological characteristics of varieties of broad beans (Vicia faba L.) for 2014-2019 ( $\mathrm{P}=0.05$ )

| Sample names | Signs of leaf, cm |  | Stomata |  |  | Plant length, cm | Loss of moisture,\% | Seed productivity, g/rast. |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | length | width | $\begin{aligned} & \hline \frac{\text { NSUPE }}{\text { NSLE } /} \\ & \mathrm{mm}^{2} \end{aligned}$ | $\begin{gathered} \frac{\text { LSUPE }}{\text { LSLE, }} \\ \mu \mathrm{m} \end{gathered}$ | $\begin{gathered} \hline \text { WSUPE } \\ \hline \text { WSLE, } \\ \mu \mathrm{m} \end{gathered}$ |  |  |  |
| Aquadul | $7.46 \pm 0.94$ | $2.93 \pm 0.47$ | $\frac{41.2}{43.8}$ | $\frac{39.9}{44.6}$ | $\begin{array}{r} 24.0 \\ \hline 27.2 \\ \hline \end{array}$ | 63 | 1 | 15 |
| Batrom | $6.98 \pm 0.63$ | $3.48 \pm 0.81$ | $\frac{36.0}{4.6}$ | $43.2$ | $\frac{28.2}{28.2}$ | 65 | 7 | 73 |
| Belgorod 2 | $8.40 \pm 1.03$ | $3.02 \pm 0.32$ | $47.4$ | $\overline{40.0}$ | $\frac{29.2}{30.2}$ | 70 | 6 | 87 |
| Belarusian | $7.33 \pm 0.64$ | $2.76 \pm 0.19$ | $\frac{32.0}{47.6}$ | $\frac{41.2}{41.2}$ | $\frac{27.1}{26.3}$ | 58 | 3 | 37 |
| White Large-Fruited | $8.01 \pm 1.48$ | $3.18 \pm 0.42$ | $\frac{27.2}{43.0}$ | $\frac{42.6}{42.9}$ | $\frac{30.9}{27.9}$ | 54 | 10 | 72 |
| Bobchinskiye | $7.11 \pm 0.47$ | $2.59 \pm 0.33$ | $\frac{38.2}{49.2}$ | $\frac{44.1}{47.0}$ | $\begin{aligned} & 29.7 \\ & \hline 29.6 \\ & \hline \end{aligned}$ | 63 | 7 | 53 |
| Velena | $7.53 \pm 0.70$ | 2,33 $\pm 0,17$ | $\frac{30.0}{37.2}$ | $\frac{44.7}{44.4}$ | $\frac{30.4}{31.1}$ | 60 | 12 | 88 |
| Vindzorskie | $8.80 \pm 0.92$ | $3.39 \pm 0.42$ | $\frac{44.4}{44.8}$ | $\frac{41.1}{45.7}$ | $\begin{aligned} & 25.0 \\ & \underline{27,8} \\ & \hline \end{aligned}$ | 73 | 8 | 36 |
| Dachnik | $8.54 \pm 0.76$ | $3.98 \pm 0.44$ | $\frac{27.8}{30.6}$ | $\frac{44.6}{49.4}$ | $\frac{30.8}{27.7}$ | 48 | 19 | 65 |
| Children's Delight | $6.26 \pm 0.59$ | $2.55 \pm 0.25$ | $\frac{30.6}{49.6}$ | $\frac{45.0}{45.7}$ | $\begin{array}{r} 25.8 \\ 26.7 \\ \hline \end{array}$ | 63 | 34 | 48 |
| Green Jack | $7.53 \pm 0.53$ | $3.58 \pm 0.47$ | $\frac{47.4}{57.8}$ | $\frac{38.2}{41.9}$ | $\begin{array}{r} 23.9 \\ 25.6 \\ \hline \end{array}$ | 65 | 11 | 36 |
| Gourmet Dish | $6.02 \pm 0.65$ | $2.13 \pm 0.29$ | $\frac{31.8}{33.8}$ | $\frac{44.2}{49.1}$ | $\begin{aligned} & 28.6 \\ & 28,9 \\ & \hline \end{aligned}$ | 46 | 24 | 28 |
| Karmazin | $5.69 \pm 0.54$ | $2.57 \pm 0.30$ | $\begin{array}{r} 57.4 \\ 76.8 \\ \hline \end{array}$ | $\frac{40.5}{42.8}$ | $\begin{array}{r} 24.5 \\ 25.1 \\ \hline \end{array}$ | 62 | 11 | 34 |
| Lider | $6.69 \pm 0.83$ | $3.37 \pm 0.38$ | $\frac{39.2}{47.0}$ | $\frac{40.7}{41.0}$ | $\frac{25,1}{24.4}$ | 80 | 6 | 36 |
| Optics | $8.70 \pm 0.94$ | $4.03 \pm 0.38$ | $\frac{31.6}{37.0}$ | $\frac{41.7}{44.1}$ | $\begin{array}{r} 25.8 \\ 25.3 \\ \hline \end{array}$ | 48 | 22 | 48 |
| Black Russian | $6.53 \pm 0.79$ | $2.71 \pm 0.35$ | $\frac{25.4}{39.2}$ | $\frac{42.1}{45.8}$ | $\frac{30.3}{33.3}$ | 50 | 12 | 13 |
| Pink Flamingo | $6.42 \pm 0.54$ | $3.14 \pm 0.38$ | $\frac{46.0}{56.8}$ | $\frac{40.7}{46.1}$ | $\frac{30.7}{31.3}$ | 68 | 13 | 25 |
| Royal Harvest | $7.11 \pm 0.37$ | $2.86 \pm 0.29$ | $\begin{aligned} & 28.4 \\ & 38.2 \\ & \hline \end{aligned}$ | $\frac{45.5}{49.3}$ | $\begin{array}{r} 33.3 \\ \hline 23.3 \\ \hline \end{array}$ | 66 | 1 | 34 |
| Yankel Byala | $7.52 \pm 0.71$ | $3.24 \pm 0.21$ | $\frac{39.8}{46.2}$ | $\frac{42.0}{42.6}$ | $\begin{aligned} & \frac{21.1}{24.2} \\ & \hline \end{aligned}$ | 67 | 29 | 43 |

$\overline{\text { Note: the number of stomata on the upper (NSUpE) and lower (NSLE) epidermis of the lamina, the length of stomata on the upper (LSUpE) and lower (DULE) }}$ epidermis, the width of the stomata on the upper (WSUpE) and lower (WSLE) epidermis
optical microscope with a DCM 310 SCOPE video eyepiece. Evaluation of the water-holding capacity of the leaves of all varieties was carried out by the generally accepted weight method with some additions for the beans (Kurkina 2007, Saliu et al. 2016).

## RESULTS AND DISCUSSION

The leaf length ranged from 5.69 to 8.80 cm , and their width ranged from 2.13 to 4.03 cm . These characters had an average degree of variability ( $\mathrm{V}=$ $12 \%, V=15 \%$, respectively). The largest leaves were found in large-seeded varietal specimens White LargeFruited, Optics, Dachnik, Windsor and Belgorod 2. Plants with the smallest leaves are characteristic of the Karmazin variety (see Table 1).

Leaf area varied greatly among different representatives of the collection. So, plants of varieties White Large-Fruited, Aquadul, and sample Belgorod 1 possessed the maximum leaf area. The smallest leaf area was noted in plants of the varieties Karmazin, Gourmet Dish and Children's Delight.

In all studied faba bean samples, the average number of leaflets in a leaf ranged from 5 to 7 pcs. The
number of leaves in one sheet varied from 4 to 7 pcs. depending on the position (tier), this feature was also characterized by an average degree of variability in the samples ( $\mathrm{V}=22 \%$ ).

An analysis of the correlations showed that the length of the leaflets was closely related to their width ( $r$ $=+0.69$ ). The length, width, and number of leaves in one sheet positively correlated with the leaf area ( $r=+$ $0.6, r=+0.3$, and $r=+0.6$, respectively). No close relationship was found between leaf area and stomata characteristics.

All of the studied vegetable bean varieties have complex, paired-pinified leaves, of a bluish-green color. The apical tendril of the bean is reduced to a pointed. The stomata are anomocytic type and are found on both sides of the leaflets (see Fig. 1). The upper epidermis cells are distinguished by more winding walls than the walls of the cells of the lower epidermis. On both sides of the leaf are appendages of the epidermis - covering (single-celled) and glandular hairs.

The leaves of some varieties of beans in their structure have features of xeromorphism. Thus, the varieties Aquadul, Belarusian, Bobchinsky, Velena, Royal Harvest are distinguished by a relatively low plant


Fig. 1. Anatomical characteristics of Vicia faba leaf. The upper (A) and lower (B) epidermis of the leaf of the 8th tier of the adult plant on the example of the Belarusian variety: 1 - stomata, 2 - gland. A temporary preparation under the microscope. Increased x100
height and narrow, albeit long, leaves, combined with the highest water holding capacity (see Table 1).

The smallest leaf size and the average for the collection of the degree of moisture loss, distinguish the variety Karmazin.

For varieties with high stem and large leaves (mesophytes) include varieties Leader and Pink Flamingo. The largest leaves in the collection are characterized by low-growing varieties Optics and Dachnik, characterized by high moisture losses.

The rest of the variety samples stomata had more on the underside of the leaves (see table), which is typical for most dicotyledonous plants.

The largest number of stomata on both sides of the leaf is characteristic only for the Karmazin variety. The smallest number of stomata was recorded on the upper epidermis of the varieties Russian Black, White LargeFruited, Dachnik, Royal Harvest, Velena, Children's Delight and Optics. The smallest number of stomata on the lower epidermis was determined in the Dachnik and Gourmet Dishes varieties. The greatest difference between the number of stomata on the upper and lower epidermis is typical for the varieties Karmazin and Children's Delight.

For the majority ( $61 \%$ ) of varietal samples, larger stomata are characteristic on the lower side of the leaf. The largest difference in the length of the upper and lower epidermis stomata was observed in samples Belgorod 2 and varieties Pink Flamingo, Dachnik, Windsor, Gourmet Dish, Aquadul. All of the listed samples ( $35 \%$ of their total number) are characterized by statistically significant (at 5\% significance level) longer stomata on the lower epidermis.

A clearer difference between the upper and lower sides of the sheet can be traced along the stoma width. Thus, in $22 \%$ of the samples, the stomata of the upper epidermis were wider than the lower. The maximum difference was observed in the varieties Royal Harvest and Dachnik. The remaining specimens have wider stomata on the lower side of the leaf, although statistically significant differences were recorded only for the Russian Black variety and the Hybrid sample.

According to the ratio of the length of stomata to their width, the variety Yankel Byala differed on the upper epidermis, and on the lower one - the Royal Harvest, in which this indicator is equal to 2.

A strong positive correlation was noted between the number of stomata of the upper and lower epidermis ( $r$ $=+0.8$ ), as well as their length ( $r=+0.7$ ) and width ( $r=$ $+0.5)$. A negative relationship indicates that the more stomata on the upper epidermis, the smaller they are, i.e. shorter ( $r=-0.7$ ) and already ( $r=-0.5$ ). It is noteworthy that the relationship between the number and size of stomata for the lower epidermis is less pronounced (respectively, $r=-0.5$ and $r=-0.2$ ). The length and width of the stomata are positively interconnected, moreover, both from the top ( $r=+0.5$ ) and from the bottom ( $r=+0.3$ ) of the leaf.

An analysis of morphometry correlations with the parameters of the stomatal apparatus showed the following. The area of the sheet is more dependent on its length $(r=+0.6)$ than on the width $(r=+0.4)$. The greater the length of the leaf and its area, the smaller the stomata on its lower epidermis ( $r=-0.3$ and -0.2 , respectively). The number of stomata on the upper ( $r=$ $+0.4)$ and lower epidermis $(r=+0.4)$ and their width on
the upper side of the leaf $(r=-0.4)$ are related to the height of the plant.

Leaf moisture loss negatively correlates with the stomata width on the upper epidermis $(r=-0.4)$ and leaf area ( $r=-0.3$ ), and positively with the leaf width ( $r=+$ 0.3).

The individual productivity of seeds of vegetable beans in the whole collection positively correlated with the height of the plants $(r=+0.6)$.

Thus, the Karmazin variety, both on the lower and on the upper epidermis, contains the maximum number of stomata per $\mathrm{mm}^{2}$ ( 76.8 units and 57.4 units, respectively). The smallest number of stomata ( 25.4 pcs.) On the upper epidermis is observed in the Russian Black variety, and on the lower epidermis, in the Dachnik variety. The stomata length varies from 38.2 to 49.7 microns, and the width - 21.1-33.3 microns.

The density of stomata on leaves increases in plants with an increase in xeromorphism (Kushnirenko, 1991; Vasiliev, 1988). The smallest (compared with the average value for the experiment) number of stomata was distinguished by samples of the Batrom, Belorussian, Velena, Dachnik, Children's Delight, Optics, Russian Blacks, Royal Harvest, Gourmet Dishes (see table). Stomata were observed in plants of the varieties Aquadul, Green Jack, Karmazin, Pink Flamingo and sample Belgorod 2.

It is known that, with an increase in xeromorphic characters in the structure of the leaf blade, stomata size decreases (Maimistov 2000). The maximum stomata length was found on the lower epidermis in the varieties

Dachnik, Royal Harvest, Gourmet Dish, the smallest on the upper epidermis in the variety Green Jack.

The number and size of stomata varied according to samples, which is consistent with published data (Ricciardi 1989). It was noted that the average variability is characteristic of the stomata density of the upper and lower leaf epidermis (coefficient of variation of $22 \%$ ). The stomata length and width slightly varied on the upper ( $V=5 \%$ and $V=11 \%$, respectively) and on the lower surface of the leaf ( $\mathrm{V}=6 \%$ and $\mathrm{V}=9 \%$, respectively).

## CONCLUSION

Comparative studying of one-pair leaf epidermis at the 20 samples of broad beans showed of perspective varieties for breeding (for example Vindzorskie) and hybrids with amphystomatical lives with vertical orientation. The variety Karmazin showed the maximum number of stomata per $\mathrm{mm}^{2}$ ( $76 / 8$ per $\mathrm{mm}^{2}$ on the lower and 57.4 per $\mathrm{mm}^{2}$ on the upper epidermis leaflets respectively). The variety Black Russian showed lowest number of stomata was found on the upper epidermis of the ( 25.4 per $\mathrm{mm}^{2}$ ) and the variety Dachnik - on the lower epidermis of ( 30.6 per $\mathrm{mm}^{2}$ ). Stomata length ranged from 38.2 to $49.7 \mu \mathrm{~m}$, stomata width - from 21.1 to $33,3 \mu \mathrm{~m}$. The high of plant has positive relationship with number of stomata on upper ( $r=+0.4$ ) and lower epidermis ( $r=+0.4$ ), and negative relationship with width of stomata on upper surface. The varieties Royal Harvest, Bobchinskiye, Batrom and Lider had maximum seed productivity in the Belgorod region.

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