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STEM RESISTANCE IN TRADITIONAL AND SELF-COMPLETING FABA BEAN CULTIVAR (*Vicia faba* spp. *minor*) TO BENDING PART II. EFFECT OF ANATOMICAL TRAITS OF STEMS

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Abstract. Analysis of anatomical traits of faba bean stems, in traditional cultivar Neptun and self-completing one, Granit, was carried out in two stages of seed maturity, both physiological and technical one, on segments of the length of 40 mm, sampled from an internode right under the first pods. Studied faba bean cultivars differed significantly in terms of 8 out of 15 tested anatomical traits of stems. Stem in cultivar Neptun was characterized by a greater width of sclerenchyma, and thickness and width of its cells, as well as width of phloem and xylem. In cultivar Granit, a significantly greater thickness of parenchyma layer and of xylem cells was observed, as well as a higher proportion of tissues in the stem. Plant maturation was accompanied by a significant decrease in the width of epidermis cells, in the thickness of parenchyma layer and in the thickness of sclerenchyma and phloem cells. Only 6 morphological traits of plants were significantly correlated with the anatomical structure of faba bean stems, and they included: plant height and height of the first pod setting, length of the stem fruit-bearing, thickness of the stem wall and its internal diameter, as well as the dry weight of faba bean plant. Significant negative correlations between anatomical traits of stem, and its resistance to bending was observed for: width of epidermis cells, thickness of parenchyma layer and in phloem thickness. Stem section modulus in faba bean was positively correlated with the width of epidermis cells and phloem thickness.

Key words: anatomical traits of stems, cultivar type, faba bean, stem resistance to bending

INTRODUCTION

Leguminous plants belong to a group of monocarpic species, for whom health of vegetative parts is closely connected with transfer of metabolites from the whole plant to growing and developing pods and seeds, mainly with the use of phloem [Górecki *et al*. 1999]. According to Księżak [2002], at the stage of physiological maturity of seeds,

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a rapid increase in the dry weight of seeds and pods is observed as well as in faba bean stems (including the crude fiber). Plant aging is accompanied by the formation of vascular elements in xylem, and accumulation of lignin in cell walls of different tissues, mainly in lignified mechanical tissues such as sclerenchyma, and in lignified parts of vascular bundles – in wood fibers and bast fibers. Sclerenchyma occurs exclusively with bast-wood bundles, surrounding them completely or partly, while main weight of this mechanical tissue is located on the outer part of bundles, close to the stem surface [Górecki *et al*. 1999].

The decreasing degree of moisture content in tissues, along with plants' aging [Górecki *et al*. 1999, Haman and Kostankiewicz 1999, Skubisz 2002], is connected with changes in their morphological [Wang *et al*. 2006, Skubisz *et al*. 2007] and anatomical structure [Banniza *et al*. 2005]. From the few studies on the effect of anatomical structure on mechanical properties of stems in leguminous plants it follows that lodging of plants, e.g. peas, is negatively correlated with the proportion of parenchyma and wood [Banniza *et al*. 2005]. Sclerenchyma is a typical mechanical tissue formed from cells of thick, usually lignified, secondary walls. Xylem consists of dead cells of lignified secondary wall, parenchyma cells as well as fibers. Phloem in turn, is a tissue specialized in conducting nutritive substances, and consists of living phloem cells, parenchyma cells (companion cells), and quite often it also contains sclerenchyma.

From the study on resistance of cereals to lodging it follows that, among others, proportion of sclerenchyma significantly determines mechanical resistance of wheat stem [Wang *et al*. 2006]. In barley, on the other hand, area of the cell wall per unit of cell area was substantially positively correlated with maximum bending stress (r = 0.93 for epidermis, r = 0.90 for sclerenchyma and r = 0.84 for parenchyma) [Kokubo *et al*. 1989].

The aim of the research was characteristics of anatomical traits of traditional and self--completing faba bean cultivar, as well as evaluation of their correlation with mechanical properties of stems, determined at the stage of physiological and full seed maturity.

MATERIAL AND METHODS

The research included two faba bean cultivars: traditional one, Neptun, and the self--completing one, Granit. Detailed methodology of the field experiment, and strength studies, concerning effect of morphological traits on stem resistance to bending (Wy), as well as on maximum bending stress (σ), can be found in the first part of the article [Prusiński *et al*. 2011]. For anatomical tests, 6 plants of both cultivars were randomly collected in the years 2008-2010, 4 times in two developmental stages: at the beginning of their physiological maturity and at full seed maturity. In each plant, segments of 120 mm were cut out from a node part right under the first pod, of which 80 mm were intended for strength studies (part I), and 40 mm for anatomical tests. Stem sections were initially fixed for 24 hours in 30% ethanol, and then they were transferred for 48 hours into 50% alcohol, and they were stored in 75% ethanol. From thus fixed plant material, preparations were formed, which were cut with the use of the sliding microtome SLIDE 2003. In order to increase image contrast, lignified elements were coloured red with safranin (2,8-dimethyl-3,7-diamino-phenazine, $C_{20}H_{19}N_4^+$, Cl^-, 350.84 $g \cdot mol^{-1}$). Measurements of cells and tissues were taken in three different places on the preparation, with magnification 10x and 20x, with the use of a microscope MB-30, with a micrometer scale.

Obtained results were subjected to analysis of variance for a randomized block design, and Tukey's test was applied to evaluate significance of differences with P = 0.05. Package STATISTICA® was used to evaluate correlation between studied anatomical and morphological traits and stem resistance to bending.

RESULTS AND DISCUSSION

Plants' resistance to lodging stays in a close relation with resistance traits of stems, and has been the subject of research for many years, with the use of their morphological and anatomical traits and advanced measuring techniques [Skubisz 2008]. Physical properties of stems change along with a developmental stage of plants, and along with the tissue moisture [Haman and Kostankiewicz 1999, Skubisz 2002]. Interior of faba bean stem lacks parenchyma tissue – its walls having an average thickness (A) of 1.62 mm [Prusiński *et al*. 2011] retain a typical ring structure of vascular bundles (Fig. 1). Sclerenchyma (D) is built of dead cells of very hard walls, and mean width 450 μm (Table 1). Phloem (F), whose task is transport of organic substances formed in the process of photosynthesis, had a mean thickness of 112 μm, while wood (G) having mainly the function of support and storage, 635 μm.

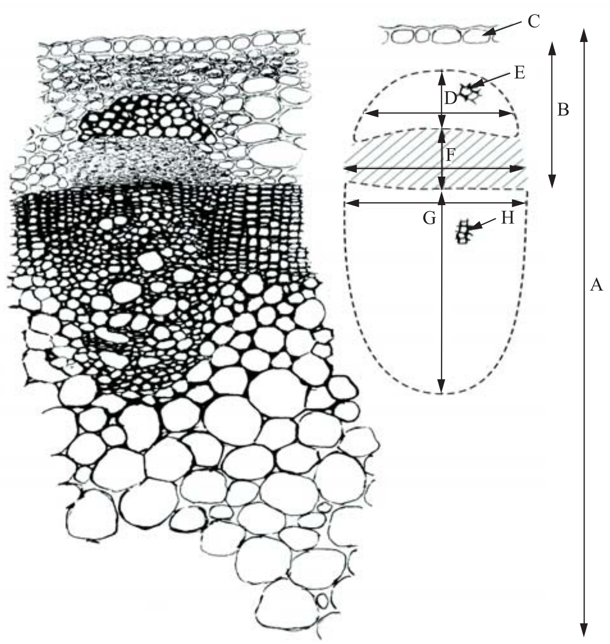

Fig. 1. Cross-section of the stem of faba bean cultivar Neptun: A – width of tissue layer, B – width of parenchyma layer, C – length and width of epidermis cells, D – thickness and width of sclerenchyma, E – thickness and width of sclerenchyma cells, F – thickness and width of phloem, G – thickness and width of xylem, H – thickness and width of xylem cells

Rys. 1. Przekrój poprzeczny przez łodygę bobiku odmiany Neptun: A – grubość warstwy tkanek, B – grubość warstwy miękiszowej, C – długość i szerokość komórek skórki, D – grubość i szerokość sklerenchymy, E – grubość i szerokość komórek sklerenchymy, F – grubość i szerokość łyka, G – grubość i szerokość drewna, H – grubość i szerokość komórek drewna

From among 13 determined and 6 listed traits of anatomical structure of faba bean stem, significant intercultivar differences were found in 8 of them. Stem in the traditional cultivar Neptun was characterized by a significantly greater width of sclerenchyma, as well as thickness and width of its cells, and width of phloem and wood. On the other hand, in the self-completing cultivar Granit, a significantly greater thickness of parenchyma layer and wood cells was observed, as well as a greater proportion of tissues in the stem (Table 1). Loosely arranged clusters of sclerenchyma occurring in it, make the stem more flexible to bending. In the research on peas, cultivars Brutus and Piast, the more resistant ones to lodging than cultivar Komandor, were characterized by a greater thickness of stem wall, as well as of thickness of primary fibers of phloem, and the smallest thickness of cell wall in parenchyma [Skubisz *et al*. 2007].

Table 1. Differentiation of stem morphological traits of faba bean cultivars studied
Tabela 1. Zróżnicowanie cech anatomicznych łodyg badanych odmian bobiku

Trait – Cecha	Cultivar – Odmiana		Mean Średnia
	Neptun	Granit	
Length of skin cells, µm Długość komórek skórki	59.4 a	57.6 a	58.5
Width of skin cells, µm Szerokość komórek skórki	46.4 a	43.4 a	44.9
Thickness of parenchyma, µm Grubość warstwy miękiszowej	418 b	538 a	478
Share of parenchyma, % Udział miękiszu	40.3 a	40.1 a	40.2
Thickness of sclerenchyma, µm Grubość sklerenchymy	252 a	242 a	247
Width of sclerenchyma, µm Szerokość sklerenchymy	472 a	428 b	450
Thickness of sclerenchyma cells, µm Grubość komórek sklerenchymy	38.6 a	37.6 b	38.2
Width of the sclerenchyma cells, µm Szerokość komórek sklerenchymy	42.1 a	40.1 b	41.1
Share of sclerenchyma, % Udział sklerenchymy	14.8 a	15.0 a	14.9
Thickness of phloem – Grubość łyka, µm	105 a	119 a	112
Width of phloem – Szerokość łyka, µm	518 a	448 b	483
Share of phloem – Udział łyka, %	7.6 a	5.7 a	6.7
Thickness of wood – Grubość drewna, µm	634 a	636 a	635
Width of wood – Szerokość drewna, µm	582 a	490 b	536
Thickness of wood cell, µm Grubość komórki drewna	23.5 b	25.1 a	24.3
Width of wood cell, µm Szerokość komórki drewna	19.7 a	20.5 a	20.1
Share of wood – Udział drewna, %	37.1 a	39.2 a	38.2
Thickness of tissue layer, µm Grubość warstwy tkanek	1606 a	1690 a	1648
Participation of tissues in the stem, % Udział tkanek w łodydze	14.2 b	17.3 a	15.7

means followed by the same lower letters did not differ significantly at P = 0.05 – średnie oznaczone tymi samymi małymi literami nie różniły się istotnie przy P = 0,05

Along with the progressing aging of plants and maturation of faba bean, a significant decrease in the width of epidermis cells (C) (Table 2, Fig. 1), in the thickness of parenchyma layer (B) and sclerenchyma cells (E) was observed as well as in the thickness of phloem (F). Other anatomical traits of faba bean stems did not undergo significant changes in the studied developmental stages.

Table 2. Effect of seed maturity on the differentiation of anatomical traits of faba bean stem
Tabela 2. Wpływ stopnia dojrzałości nasion na zróżnicowanie cech anatomicznych łodyg bobiku

Trait – Cecha	Seed maturation stage Stopień dojrzałości nasion		Średnia Mean
	beginning of physiological maturity początek dojrzałości fizjologicznej	full maturity dojrzałość pełna	
Width of skin cells, µm Szerokość komórek skórki	48.7 a	41.1 b	44.9
Thickness of parenchyma, µm Grubość warstwy miękiszowej	552 a	404 b	478
Thickness of sclerenchyma cells, µm Grubość komórek sklerenchymy	39.9 a	36.3 b	38.1
Thickness of phloem, µm Grubość łyka	131 a	93 b	112

means followed by the same lower letters did not differ significantly at P = 0.05 – średnie oznaczone tymi samymi małymi literami nie różniły się istotnie przy P = 0,05

Only some anatomical traits were significantly correlated with morphological traits of faba bean stems (Table 3). Higher plants, more susceptible to lodging were characterized by a greater width of epidermis cells, thickness of parenchyma and phloem. In case of soybean [Wilcox and Sediyama 1981], but also in case of other legumes [Jasińska and Kotecki 1993], potentially the highest seed yields are obtained from long-stemmed cultivars which are more susceptible to lodging. Thus, breaking this negative correlation requires shortening of the stem and increasing resistance to lodging, among others through decrease in the thickness of parenchyma and phloem, which was proved in author's research. On the other hand, increase in the thickness of stem wall and its inner diameter was accompanied by increasing width of sclerenchyma. Thus, results of earlier research were confirmed [Haman and Kostankiewicz 1999, Prusiński *et al*. 2011], which indicated that strength traits of stems, including resistance to bending, are determined by their thickness and internal diameter.

Most of the studied anatomical traits of faba bean stems were not significantly correlated with their resistance traits to bending (Table 4). Increase in the resistance of stem section to bending (Wy) was determined substantially by phloem thickness ($r = 0.568$) and width of epidermis cells ($r = 0.552$). On the other hand, maximum value of bending stress (σ) was significantly negatively correlated with the phloem thickness ($r = -0.757$), width of epidermis cells ($r = -0.718$) and thickness of parenchyma ($r = -0.602$). In case of other anatomical traits of faba bean stems, no significant dependences were found with their resistance to bending.

Table 3. Significant correlation between morphological and anatomical faba bean stem traits
Tabela 3. Istotne korelacje cech morfologicznych i anatomicznych łodyg bobiku

Plant morphological traits Cechy morfologiczne roślin	Width of epiderma cells Szerokość komórek skórki	Thickness of parenchyma Grubość parenchymy	Thickness of sclerenchyma Grubość sklerenchymy	Width of sclerenchyma Szerokość sklerenchymy	Width of phloem Grubość łyka
Plant height Wysokość roślin	0.634 **	0.635**			0.731**
Height of 1st pod setting – Wysokość osadzenia 1. strąka					0.701**
Length of fruit-bearing stem – Długość owocostanu	0.568*	0.543*	0.541*		0.578*
Thickness of stem wall – Grubość ściany łodygi				0.582*	
Inner stem diameter Średnica wewnętrzna łodygi				0.603**	
Plant dry matter Sucha masa rośliny	-0.664**				

Table 4. Correlation coefficients of faba bean anatomical plant traits and values of indexes of resistance to bending of the stem cross-section (Wy) and the stem bending stress (σ)
Tabela 4. Współczynniki korelacji badanych cech anatomicznych łodyg bobiku i ich wytrzymałości przekroju na zginanie (Wy) i maksymalnej odporności na zginanie (σ)

Anatomical plant traits – Cechy anatomiczne roślin	Wy	σ
Length of epidermis cells – Długość komórek skórki	-0.315	0.213
Width of cells – Szerokość komórek skórki	0.552*	-0.718**
Thickness of parenchyma – Grubość warstwy miękiszowej	0.361	-0.602*
Thickness of sclerenchyma – Grubość sklerenchymy	0.310	-0.415
Width of sclerenchyma – Szerokość sklerenchymy	0.283	-0.262
Thickness of sclerenchyma cells – Grubość komórek sklerenchymy	0.391	-0.441
Width of sclerenchyma cells – Szerokość komórek sklerenchymy	-0.323	0.185
Thickness of phloem – Grubość łyka	0.568*	-0.757**
Width of phloem – Szerokość łyka	0.239	-0.157
Thickness of wood – Grubość drewna	0.052	-0.101
Width of wood – Szerokość drewna	0.230	-0.091
Thickness of wood cells – Grubość komórek drewna	-0.323	0.147
Width of wood cells – Szerokość komórek drewna	0.123	-0.035
Thickness of tissue layer – Grubość całej warstwy tkanek	0.188	-0.353
Share of tissue in the stem – % udział tkanek w łodydze	-0.194	-0.073

* r significant at P = 0.05 – r istotne przy P = 0,05
** r significant at P = 0.01 – r istotne przy P = 0,01

In search of traits which condition resistance of legume stems, not only physical traits of the stem should be taken into consideration (the most commonly studied include thickness of the stem and of the stem wall). From among anatomical traits, the most useful ones seem to be thickness of the phloem and parenchyma.

CONCLUSIONS

1. Studied faba bean cultivars, despite lack of differences in the degree of lodging, differed significantly in terms of 8 out of 13 studied anatomical traits of stems.

2. Stem in the traditional cultivar Neptun was characterized by a greater width of sclerenchyma and thickness and width of its cells, as well as width of phloem and wood, while cultivar Granit by a significantly greater width of parenchyma layer and wood cells, as well as a higher proportion of tissues in the stem.

3. Along with aging of plants, a significant decrease was observed in the width of epidermis cells, thickness of parenchyma layer, and thickness of sclerenchyma and phloem cells.

4. Only 6 morphological traits of plants were significantly correlated with the anatomical structure of faba bean stem. They included: height of plants and height of the first pod setting, length of the stem fruit-bearing, thickness of the stem wall and its internal diameter, as well as dry weight of the plant.

5. Significant negative correlation between anatomical traits of the stem, and its resistance to bending, was found for: the width of epidermis cells, thickness of parenchyma and phloem.

6. Stem section modulus of faba bean was positively correlated with width of epidermis cells and thickness of the phloem. On the other hand, maximum bending stress was significantly negatively affected also by the width and thickness of parenchyma.

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ODPORNOŚĆ ŁODYG TRADYCYJNEJ I SAMOKOŃCZĄCEJ ODMIANY BOBIKU (*Vicia faba* SPP. *minor*) NA ZGINANIE CZ. II. WPŁYW CECH ANATOMICZNYCH ŁODYG

Streszczenie. Analizę cech anatomicznych łodyg bobiku odmian: tradycyjnej Neptun i samokończącej Granit wykonano w dwóch fazach dojrzałości nasion – fizjologicznej i technicznej na odcinkach o długości 40 mm pobranych z międzywęźla tuż pod pierwszymi strąkami. Badane odmiany bobiku różniły się istotnie pod względem 8 z 15 badanych cech anatomicznych łodyg. Łodyga odmiany Neptun charakteryzowała się większą szerokością sklerenchymy oraz grubością i szerokością jej komórek, a także szerokością łyka i drewna. U odmiany Granit stwierdzono natomiast istotnie większą grubość warstwy miękiszowej i komórek drewna, a także większy udział tkanek w łodydze. Dojrzewaniu roślin towarzyszyło istotne zmniejszanie szerokości komórek skórki, grubości warstwy miękiszowej oraz grubości komórek sklerenchymy i łyka. Tylko 6 cech morfologicznych roślin było istotnie skorelowanych z budową anatomiczną łodyg bobiku, należały do nich: wysokość roślin i wysokość osadzenia pierwszego strąka, długość owocostanu, grubość ściany łodygi i jej średnica wewnętrzna oraz sucha masa rośliny bobiku. Istotne ujemne korelacje między cechami anatomicznymi łodygi a jej wytrzymałością na zginanie stwierdzono dla: szerokości komórek skórki, grubości warstwy miękiszowej i grubości łyka. Wskaźnik wytrzymałości przekroju łodygi bobiku na zginanie był dodatnio skorelowany z szerokością komórek skórki i grubością łyka.

Słowa kluczowe: bobik, cechy anatomiczne łodygi, typ odmiany, wytrzymałość łodyg na zginanie

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