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PHOTOPERIOD INFLUENCE ON THE STEM STRUCTURE OF FIBRE FLAX

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Plants of 13 fibre flax varieties were tested for their photosensitivity and differences of stems structure while grown in long and short daylight. It was detected that seven varieties did not change time of flowering in short 12 hours daylight in comparison with long 19 hours daylight. The extended diversity of fibre flax reactions on photoperiod changes and its relative independence of photosensitivity degree were found. Also, weak but significant correlation was found between photosensitivity coefficient and the degree of leaves number increase (r = 0.57). At the same time photo insensitive variety Belosnezhka improved leaves number up to 34%. Numbers of fibre bundles, their individual and total area on the stem cross section were not changed or reduced. Width, height, perimeter and shape of fibre bundles on a cross sections changed multidirectional. The areas of stem, its wooden part and cavity on a cross section showed no significant differences between plants grown in long and short day conditions. Evaluated flax characters were not strictly correlated with each other except those indicating the structure of fibre bundles. For breeding it is important that their relationships are not strongly influenced by the environment.

Key words: flax, photosensitivity, stem microstructure, fibre

Species *Linum usitatissimum* L. is a cosmopolitan one. It means that plant is adapted to great diversity of climatic conditions including, particularly, different photoperiods. Despite of the evident importance for agriculture of flax physiological response to photoperiodic changes, this problem was not evaluated adequately. After the first description of photoperiod influence on plant´s flowering time (Garner & Allard 1920), *Linum usitatissimum* L. photosensitivity was intensively evaluated in Russia. It was confirmed that flax is a long day plant (Sizov 1955; Minkevitch 1957; Afonin 1969; etc.). Sizov (1952) have approved that at stage of photosensitivity all genotypes of cultivated fibre flax and linseed demand light and do not require darkness. But among wide scale of *Linum usitatissimum* L. accessions a broad variability of photosensitivity degree was found in different countries (Sinskaya 1951; Sizov 1952; Minkevitch 1957; Sorotchinskaya 1968 and 1975; Basu & Bose 1975; Sirohi & Wasnik 1978; Pavelek 1992; Darapuneni *et al.* 2014). But genotypes independent of photoperiod were not found.

Evaluation of flax photosensitivity in Russia was reactivated in 2004. In these experiments held in VIR (St. Petersburg region, 60° NL), the evaluation

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of photosensitivity among self-pollinated lines of different origin showed wide diversity of their photosensitivity coefficient in short 12 hours daylight (Brutch *et al.* 2008; Domantovich 2009; Domantovich *et al.* 2011 and 2012). Also, it was found that genotypes' low photosensitivity is not linked to their early flowering in long day conditions, as it was detected for other crops. All four marginal combinations of the degrees of flowering earliness and photosensitivity were found: early flowering – low photosensitivity; early flowering – high photosensitivity; late flowering – low photosensitivity; late flowering – high photosensitivity. Moreover, it was discovered that short day causes not only the delay of flowering, but also the increase of leaves number on the stem – character which is considered to be the indicator of different plants earliness (Obraztcov 1983), including flax (Brutch 2007). Evaluation of this character is important not only for theoretical research but also for breeding, because short internodes are considered to decrease fibre quality (Doronin & Tikhvinskiy 2003).

Following Sizov's (1955, etc.) investigations, for a long time the structure of different *Linum usitatissimum* L. plant types (fibre flax and linseed) was considered to be the outcome of the sawing place latitude, which is linked to photoperiod. But now in some countries linseed and fibre flax varieties are moved to new non-traditional territories. In this aspect it is essential to investigate the influence of photoperiod on plants agronomical characters. These data will help to develop new strategy for specialized varieties breeding.

In practice it is important to know how short day conditions influence fibre formation. Existing data on this topic is rather few and contradictory. Sizov (1963) notified that in short daylight flax forms less bast cells, fibre bundles become friable and fibre quality is deteriorated. But Afonin (1969) reported that such environment increases fibre yield. In our previous experiments the structure of stems cross sections of self-pollinated lines was evaluated in conditions of long (19 hours) and short (12 hours) daylight (Domantovich *et al.* 2011). It was discovered that different genotypes have different reactions on the shortening of daylight, which explains the discrepancy of different experiments results. We observed that number of bast fibre bundles on the stem cross section in short daylight in comparison with that of long day conditions mainly increased or did not change. Only in some lines this character decreased. Also, short daylight did not have definite influence on the length and width of the fibre bundles on a cross sections. The area of fibre bundles on a cross sections varied from year to year and had high variation within the genotypes. Short day light mainly led to the reduction of the average number of elementary fibres within the fibre bundle in genotypes with different photosensitivity. Only in several lines number of fibres permanently increased. In some of them also increased the number of fibre bundles. In these trials there was no definite effect of short daylight on the length and width of the elementary fibres in bundles. Evaluation of the stem diameter in different flax types showed that there was no definite system of their changes in short day conditions.

Individual reaction of genotypes on photoperiod changes opens good opportunities for selection of new breeding material adapted to different climatic conditions and aimed to different potentials of their utilization, which are actual nowadays. Also genotypes with contrast reaction on photoperiod open wide possibilities to be used as models for discovering the mechanisms of characters' formation. Thus, it is very important to evaluate maximum diversity of flax genetic resources to find genotypes useful for different purposes.

Nevertheless, evaluation of separate characters is not enough because plant is the entire organism and in the process of growth and development characters are not formed independently. One can better understand the mechanism of development by means of traits correlations analyses. The data presented on this topic in bibliography is also very contradictory, which is explained by the heterogeneity of the used plant material (Brutch 2007; Brutch *et al.* 2011). Evaluating the influence of photoperiod on flax stem characters, we also analysed their correlations (Brutch *et al.* 2015). During three years of research in conditions of both long and short daylight characters of fibre bundles on stems cross sections: area of fibre bundle, its width and height, and also width and height of elementary fibre were strongly correlated with each other and independent of photosensitivity coefficient.

Taking into account all the mentioned information there is still strong need to continue in evaluation of flax stem structure of different genetic resources, and monitor the influence of day length on stem characters. For that reason, we have also focused on these aims, and for the flax stem characterization we used the modern technics of image analysis to increase the precision of quantitative data.

MATERIAL AND METHODS

In 2011 in conditions of Leningrad region, Russia, 13 fibre flax varieties originating from Russia, Belorussia, The Netherlands and one inbred line from local Russian accession, from VIR Collection of Plant Genetic Resources, were evaluated for their photosensitivity and influence of short daylight on their stems structure. Seeds were sown at the end of May in pots on moved trolleys. 10 seeds of each genotype were sown in two variants. Experimental plants right after germination and up to flowering were exposed to short daylight (12 hours) which was created by replacing of trolleys in lightproof pavilion. Control plants for this time were removed to glass pavilion. Thus, they remained in natural illumination (17.5–19.0 hours), and influence of the other factors of environment were equalised. For each plant the date of the first flower opening was marked, and period from germination to flowering was calculated. The average duration of this phase in long (T1) and short (T2) daylight for each genotype was calculated. Coefficient of photoperiodic sensitivity (CPhPS) was calculated with the use of formula CPhPS = T2/T1. After ripening the number of leaves on each stem below inflorescence was calculated.

Stems cross sections were evaluated at Slovak Agricultural University in Nitra. For each genotype stem cross sections of 10 plants were taken from the middle part of dried stem. The slices of cross sections were taken by blade and dyed by diamond green, because the individual anatomical parts of flax stem have very similar colours. The area of bast

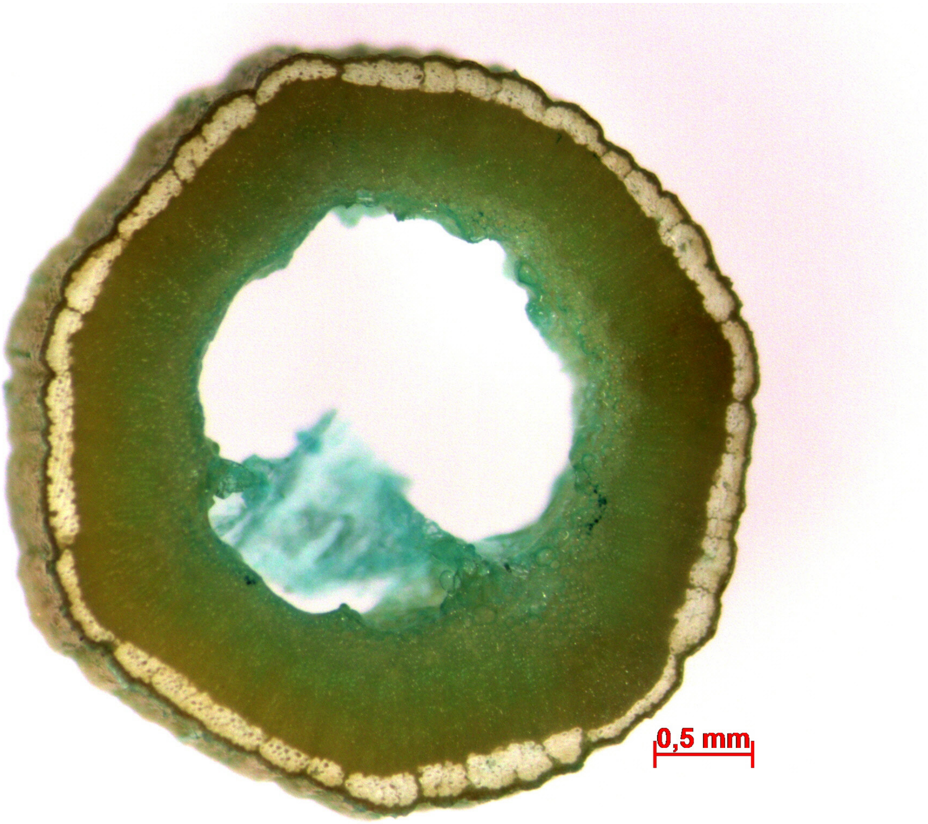


Figure 1. Image of flax stem cross section made by fully automatic macroscope Zeiss Discovery V20 with digital Camera AxioCam MRC5

fibre bundles was not coloured, and it helped to discriminate it from neighbour parts of stem. For evaluation of different parts of flax stem cross section, we used image analysis methods. These methods are nowadays widely used in different fields of research (Knott & Genoud 2013; Uchida 2013; Blaschke *et al.* 2014; Schindelin *et al.* 2015). They are finding their application also in agriculture research (Glasbey & Horgan 2001; Rodríguez-Pulido *et al.* 2012; Lidke & Lidke 2012; Sandeep *et al.* 2013; Walter *et al.* 2015).

Images of stem cross sections were acquired by fully automatic microscope Zeiss SteReo Discovery V20 with digital Camera AxioCam MRC5. Then images were analysed using software for image analysis AxioVision 4.8, with module for automatic measuring. The images were adjusted using various tools such as magnifying, thresholding, dynamic, events to get the most accurate results (Figure 1–5).

We selected 5 bast fibre bundles for plant. Each area of bast fibre bundle was outlined manually.

First of all, images were adjusted by the software tool – dynamics to get the binary mode of image and then by the module for automatic measurement we highlighted the total area of bast fibre bundles.

We selected 5 bast fibre bundles per plant to count the number of elementary fibres per bast fibre bundle. They were labelled manually by the software tool – events.

In the images we also measured the areas of whole cross section, wooden part and cavity [mm^2]. In the first step we adjusted images by the software tool – thresholding to get binary mode. Then we outlined the mentioned sections manually and finally measured their areas.

The last step of images analyses was quantification of recognized objects of interest. We set the measurements and selected these parameters – count [no], perimeter [mm], area [mm^2], bound width [mm] and bound height [mm]. We used the automatic measurement software tool to generate the tables in excel format. The full list of the evaluated characters is presented in Table 1.

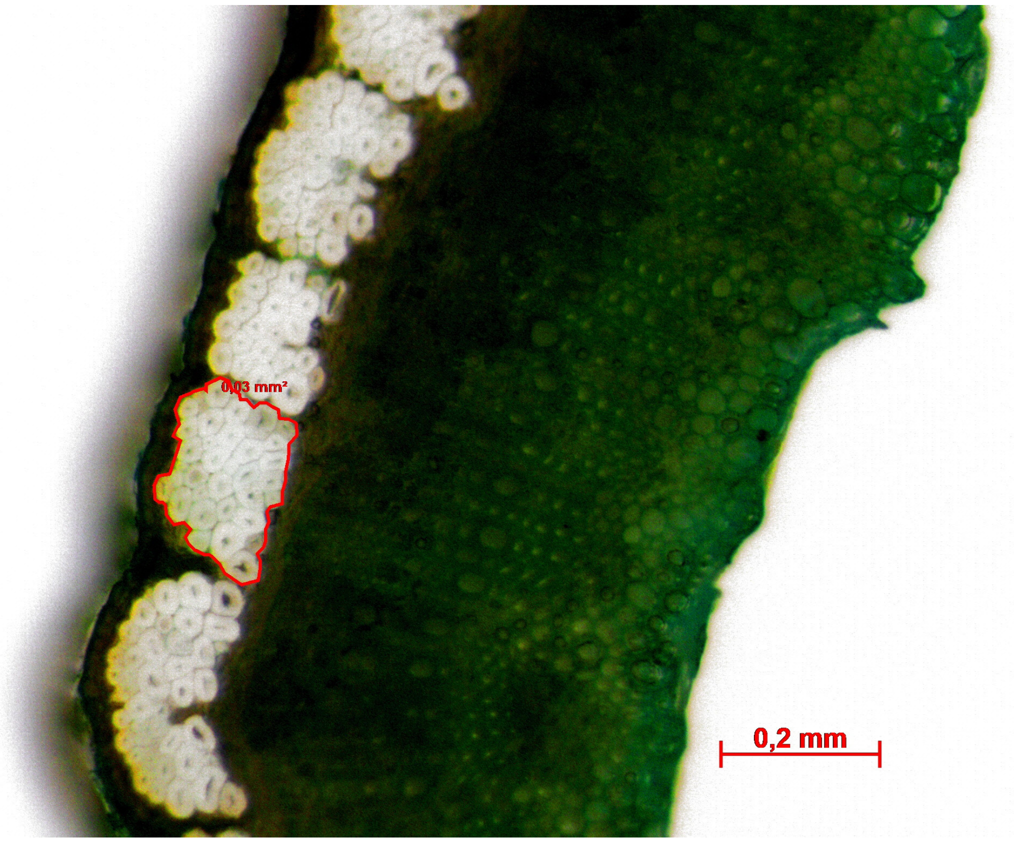


Figure 2. The magnified image of flax stem cross section for measuring of bast fibre bundle perimeter [mm] and area [mm^2]

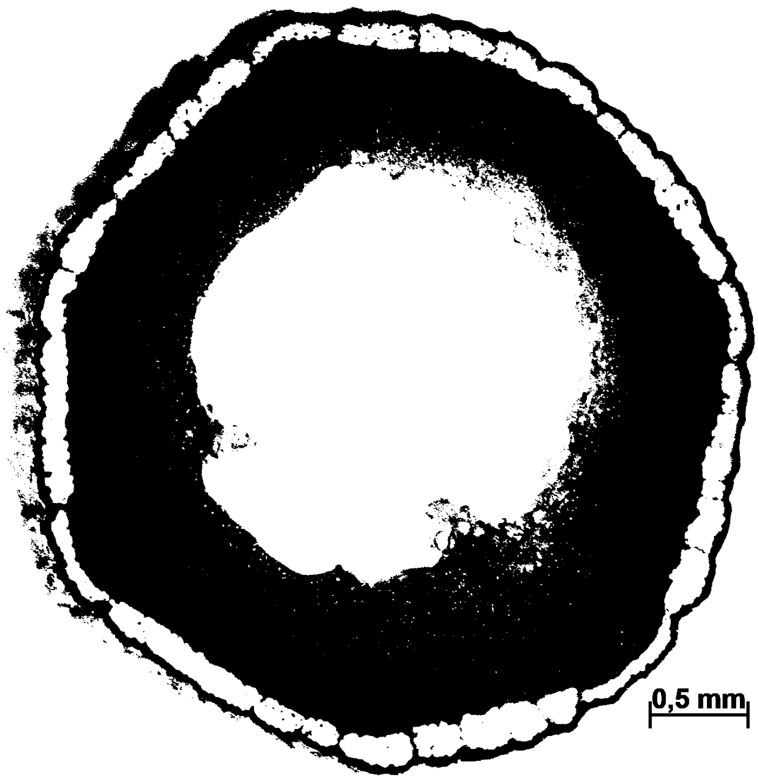


Figure 3. Images of flax stem cross section in binary mode and highlighted the total area of bast fibre bundles

T a b l e 1

The list of evaluated characters

Evaluated characters	Unit of measurement	Abbreviation
Photosensitivity coefficient	–	PhS
Period germination – flowering	days	G-Fl
Number of leaves	units	NL
Number of fibre bundles	units	NB
Number of elementary fibres in the bundle	units	NF
The parameters measured on fibre bundles on a cross section		
Area of bundle	mm^2	SB
Width of fibre bundle – the narrowest part (bound width)	mm	WB
Height of fibre bundle – the widest part (bound height)	mm	HB
Width / Height of fibre bundle	–	W/HB
Perimeter of bundle	mm	PB
The parameters measured on the whole stem cross section		
Total area of fibre bundles	mm^2	TSB
Area of stem cross section	mm^2	SSt
Area of wooden part	mm^2	SW
Area of cavity	mm^2	SC

For statistical analysis was used software SAS 9.3. Basic statistics of the obtained data showed wide variation in the values for all the genotypes and for all the traits in this study. Data were subjected to parametric (ANOVA) and non-parametric tests (Wilcoxon Two-Sample test) and significant differences ($p < 0.05$) were found among genotypes and different photoperiod conditions. Correlations between characters were calculated using excel statistical properties and presented by method of correlations pleiads (Rostova 1985).

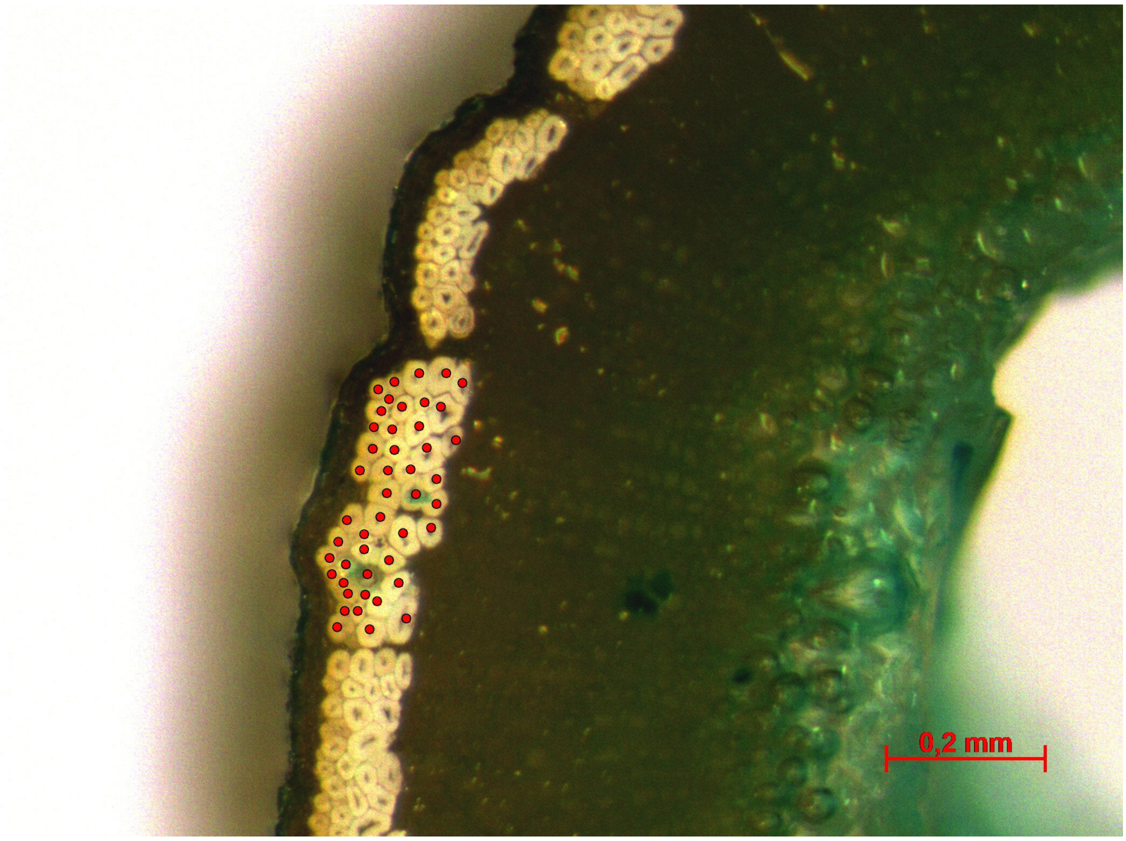


Figure 4. Labelling of elementary fibres in the magnified image of the flax stem cross section by software tool – events

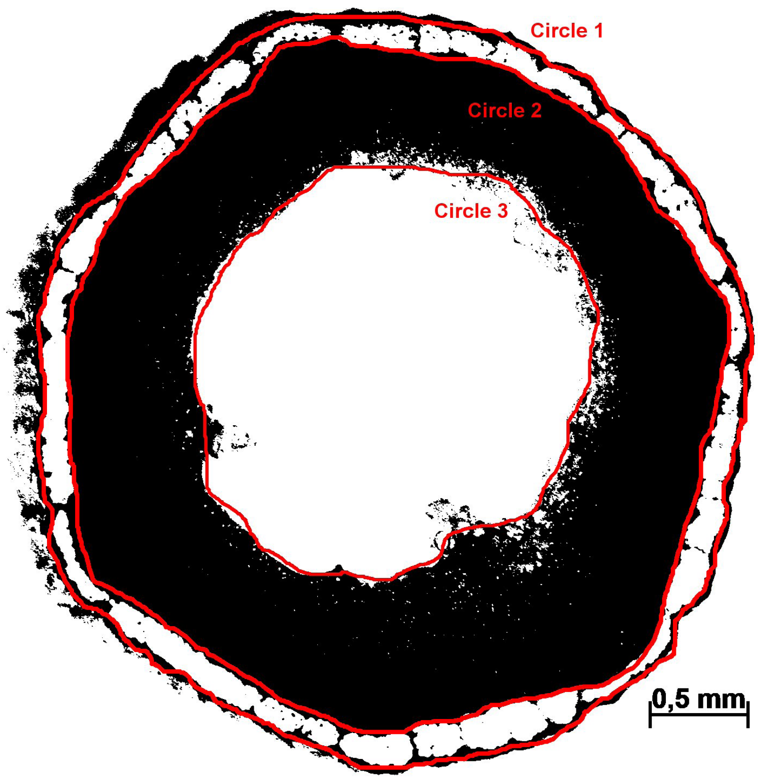


Figure 5. Image of flax stem cross section in binary mode with outlined areas of whole stem cross section, wooden part and cavity [mm^2]

RESULTS AND DISCUSSION

Evaluated fibre flax genotypes differed in their photosensitivity. Varieties Svetoch, Orshanskiy-2, Lenok, Merilin, Belosnezhka, Nord and Orion did not have significant difference between their flowering time in long and short day conditions (Table 2) and were identified as insensitive ones to short 12 hours daylight. Others increased period from germination to flowering in short daylight for 5–13%. All photosensitive varieties also significantly increased the number of leaves on the stem for 17–62%. There was a weak but significant correlation between photosensitivity coefficient and the degree of leaves number increase (r = 0.57). On the other hand, among insensitive genotypes which did not increase leaves number, variety Belosnezhka improved leaves number up to 34%. Summarizing these results with previous research ones (Domantovich *et al.* 2012) we can say, that process of leaves formation in flax is really linked to that of flowering, but probably it is more sensitive to 12 hours daylight than flowering earliness and their interaction is not equal in different genotypes.

Modification of fibre bundles structure in short daylight conditions was not so unambiguous and correlations of its characters with photosensitivity were insignificant. Varieties which were not sensitive to 12 hours daylight – Svetoch, Lenok and Merilin did not change the number of fibre bundles on the stem cross sections (Table 3). The same referred to photosensitive varieties Priziv-81, Tomskiy-16 and Zarianka. Other genotypes significantly reduced fibre bundles number. The major decrease was observed in photo insensitive variety Belosnezhka – 25%. It is important that genotypes increasing number of fibre bundles in short daylight, which were abundant in our previous experiments (Domantovich *et al.* 2011), were not found in present sampling. It means that commercial varieties are less variable for this character and useful breeding material can be found primarily among local accessions. This fact explains the stable belief that in south regions flax forms less fibre yield with poor quality (Sizov 1963).

Changes of elementary fibres average number per bundle in conditions of short daylight compared to that of long daylight ones were not uniform and did not correlate with photosensitivity (Table 3).

T a b l e 2

Photosensitivity, germination – flowering period and leaves number of flax varieties in long and short day conditions (Leningrad region, 2011)

VIR Accession number	Variety	Origin	Photo sensitivity	Germination -flowering LD*	Germination -flowering ShD**	Leaves number LD*	Leaves number ShD**
5333	Svetoch	Russia	1.03	40.8±1.1	41.9±0.7	98.1±6.3	90.0±2.9
6807	Orshanskiy-2	Byelorussia	1.02	45.7±0.6	46.8±0.6	109.5±3.6	105.6±5.4
7472	Priziv-81	Byelorussia	1.13	37.7±0.8	42.6±1.2^{+}	85.2±5.5	111.8±3.9^{+}
7694	Tomskiy-16	Russia	1.05	38.8±0.6	40.8±0.4^{+}	71.1±4.5	90.5±2.9^{+}
7804	Mogilovskiy-2	Byelorussia	1.05	44.4±0.7	46.5±0.9^{+}	98.9±2.7	115.8±6.3^{+}
7887	Krom	Russia	1.06	43.8±0.5	46.4±0.7^{+}	97.8±3.5	120.2±4.4^{+}
7940	Lenok	Russia	0.99	46.2±0.6	45.9±0.8	100.9±4.6	110.8±5.1
8241	Zarianka	Russia	1.11	38.4±1.0	42.6±1.4^{+}	78.3±4.7	94.7±4.1^{+}
8345	Merilin	The Netherlands	0.98	45.9±0.5	45.2±0.5	93.4±3.3	104.5±4.3
8351	Belosnezhka	Russia	1.01	46.1±0.5	46.4±0.6	100.0±5.1	134.3±7.9^{+}
8410	Nord	Russia	1.03	42.3±0.5	43.7±0.7	115.7±7.9	114.7±6.7
8411	Orion	Russia	0.98	43.1±0.4	42.3±0.5	102.5±1.9	104.3±5.7
gk-13	Line-5 k-489	Russia	1.10	35.7±0.5	39.3±0.9^{+}	62.1±3.9	100.6±5.2^{+}

Legend: $^{+}$– The difference is significant ($P \leq 0.05$); *LD – Long day; **ShD – Short day

Insensitive to short daylight varieties Svetoch, Orshanskiy-2, Lenok, Merilin, Orion and also sensitive one Priziv-81 did not change this character. Reduction of elementary fibres amount in bundles was observed only in photosensitive varieties – Tomskiy-16, Krom and Zarianka for 20–36%. The rest genotypes, both photosensitive and insensitive ones, significantly increased the number of fibres for 18–41%.

The majority of evaluated varieties in conditions of short day also reduced the average area of bast fibre bundle on a cross sections (Table 4). Only Svetoch, Orshanskiy-2, Tomskiy-16, Mogilovskiy-2 and Belosnezhka did not change this character. Others significantly decreased fibre cross sections area for 20–41%. As the result of the fibre bundles number and area changes, the total area of bast fibre bundles on the stem cross sections was also reduced in the majority of varieties. Only for Svetoch and Nord these data was not obtained because of the low images' quality. Line-5 from k-489 also reduced the resulting character, though it was not significant statistically because of trait high variability (50% and 45% in long and short daylight consequently).

Modifications of width, height and shape of fibre bundles also were not influenced by photosensitivity level of the genotypes (Tables 5, 6). Three varieties Orshanskiy-2, Priziv-81 and Tomskiy-16 did not change their size and shape of fibre bundles in short day conditions, though Priziv-81 slightly, but significantly reduced the area of bundle on a cross section. Svetoch enlarged all the parameters – width, height and perimeter, as the result, but the shape remained the same.

Fibre bundles on a cross sections perimeters modifications were also not linked to varieties' photosensitivity (Table 6). Only Svetoch significantly increased the average fibre bundles' perimeter for 19%. It is remarkable that the average area of fibre bundle on a cross section of this variety was also increased up to 19%, but statistically it was insignificant. Photosensitive varieties Mogilovskiy-2, Krom, Zarianka together with insensitive ones Lenokand Merilin diminished the perimeter of fibre bundles up to 13–26%. Other genotypes did not change the character.

The areas of stem, its wooden part and cavity on a cross sections (Figure 5) showed no significant

T a b l e 3

The number of fibre bundles and elementary fibres in bundles on a cross section of flax varieties in long and short day conditions (Leningrad region, 2011)

VIR Accession number	Variety	Origin	Photo sensitivity	Fibre bundles number LD*	Fibre bundles number ShD**	Number of fibres in the bundle LD*	Number of fibres in the bundle ShD**
5333	Svetoch	Russia	1.03	38.9±1.6	37.7±0.7	20.1±1.2	20.6±1.2
6807	Orshanskiy-2	Byelorussia	1.02	40.2±1.1	35.7±0.9$^+$	19.8±0.6	19.8±1.0
7472	Priziv-81	Byelorussia	1.13	33.0±0.9	35.3±1.5	23.1±0.7	22.1±0.7
7694	Tomskiy-16	Russia	1.05	32.3±0.8	29.7±1.1	25.0±1.2	18.5±1.0$^+$
7804	Mogilovskiy-2	Byelorussia	1.05	36.6±1.4	32.5±1.2	24.9±1.7	23.0±1.7
7887	Krom	Russia	1.06	36.6±0.2	32.3±0.6$^+$	26.2±1.6	20.9±1.2$^+$
7940	Lenok	Russia	0.99	34.4±0.7	32.9±1.3	24.9±1.7	23.7±1.5
8241	Zarianka	Russia	1.11	31.7±0.8	31.7±1.2	29.3±1.1	18.7±1.5$^+$
8345	Merilin	The Netherlands	0.98	33.4±0.9	30.0±0.9	28.5±1.9	26.8±2.4
8351	Belosnezhka	Russia	1.01	37.2±1.1	28.0±2.7$^+$	16.7±1.2	19.7±1.0$^+$
8410	Nord	Russia	1.03	36.6±1.0	30.7±0.6$^+$	23.9±1.8	28.9±2.5$^+$
8411	Orion	Russia	0.98	36.0±0.8	31.9±1.0$^+$	24.4±1.4	22.6±1.2
gk-13	Line-5 k-489	Russia	1.10	36.3±0.4	31.0±1.3$^+$	19.0±1.3	26.8±1.9$^+$

Legend: $^+$– The difference is significant ($P \leq 0.05$); *LD – Long day; **ShD – Short day

T a b l e 4

The area of fibre bundles on a cross section of flax varieties in long and short day conditions (Leningrad region, 2011)

VIR Accession number	Variety	Origin	Photo sensitivity	Area of bast fibre bundle [mm^2] LD*	Area of bast fibre bundle [mm^2] ShD**	Total area of bast fibre bundles per plant [mm^2] LD*	Total area of bast fibre bundles per plant [mm^2] ShD**
5333	Svetoch	Russia	1.03	0.015±0.001	0.018±0.001	–	–
6807	Orshanskiy-2	Byelorussia	1.02	0.014±0.001	0.014±0.001	0.482±0.034	0.244±0.040$^+$
7472	Priziv-81	Byelorussia	1.13	0.020±0.001	0.016±0.001$^+$	0.496±0.022	0.319±0.032$^+$
7694	Tomskiy-16	Russia	1.05	0.021±0.001	0.020±0.001	0.493±0.014	0.550±0.052
7804	Mogilovskiy-2	Byelorussia	1.05	0.017±0.001	0.014±0.001	0.508±0.057	0.558±0.048
7887	Krom	Russia	1.06	0.024±0.001	0.016±0.001$^+$	0.701±0.034	0.516±0.045$^+$
7940	Lenok	Russia	0.99	0.026±0.001	0.016±0.001$^+$	0.847±0.023	0.316±0.053$^+$
8241	Zarianka	Russia	1.11	0.021±0.001	0.013±0.001$^+$	0.476±0.054	0.266±0.054$^+$
8345	Merilin	The Netherlands	0.98	0.024±0.001	0.015±0.001$^+$	0.756±0.058	0.499±0.061$^+$
8351	Belosnezhka	Russia	1.01	0.017±0.001	0.016±0.001	0.463±0.012	0.206±0.054$^+$
8410	Nord	Russia	1.03	0.022±0.001	0.017±0.001$^+$	–	–
8411	Orion	Russia	0.98	0.018±0.001	0.013±0.001$^+$	0.464±0.049	0.293±0.044$^+$
gk-13	Line-5 k-489	Russia	1.10	0.013±0.001	0.010±0.001$^+$	0.243±0.046	0.190±0.035

Legend: $^+$– The difference is significant ($P \leq 0.05$); *LD – Long day; **ShD – Short day

T a b l e 5

The width and height of fibre bundles on a cross section of flax varieties in long and short day conditions (Leningrad region, 2011)

VIR Accession number	Variety	Origin	Photo sensitivity	Width of fibre bundle on a cross section [mm] LD*	Width of fibre bundle on a cross section [mm] ShD**	Height of fibre bundle on a cross section [mm] LD*	Height of fibre bundle on a cross section [mm] ShD**
5333	Svetoch	Russia	1.03	0.18±0.03	0.22±0.02$^+$	0.18±0.01	0.25±0.02$^+$
6807	Orshanskiy-2	Byelorussia	1.02	0.17±0.01	0.19±0.02	0.18±0.01	0.19±0.01
7472	Priziv-81	Byelorussia	1.13	0.22±0.01	0.19±0.01	0.20±0.01	0.21±0.01
7694	Tomskiy-16	Russia	1.05	0.21±0.01	0.21±0.01	0.20±0.01	0.22±0.01
7804	Mogilovskiy-2	Byelorussia	1.05	0.19±0.01	0.19±0.02	0.20±0.01	0.17±0.01$^+$
7887	Krom	Russia	1.06	0.21±0.01	0.19±0.01	0.21±0.01	0.17±0.02$^+$
7940	Lenok	Russia	0.99	0.22±0.01	0.21±0.01	0.21±0.01	0.16±0.01$^+$
8241	Zarianka	Russia	1.11	0.20±0.01	0.19±0.02	0.22±0.01	0.14±0.01$^+$
8345	Merilin	The Netherlands	0.98	0.22±0.01	0.21±0.01	0.21±0.01	0.16±0.01$^+$
8351	Belosnezhka	Russia	1.01	0.18±0.01	0.23±0.01$^+$	0.18±0.01	0.16±0.01
8410	Nord	Russia	1.03	0.20±0.01	0.22±0.01	0.22±0.01	0.18±0.01$^+$
8411	Orion	Russia	0.98	0.17±0.01	0.20±0.01$^+$	0.21±0.01	0.15±0.01$^+$
gk-13	Line-5 k-489	Russia	1.10	0.15±0.01	0.20±0.01$^+$	0.17±0.01	0.13±0.01$^+$

Legend: $^+$– The difference is significant ($P \leq 0.05$); *LD – Long day; **ShD – Short day

T a b l e 6

Width/height ratio and perimeter of fibre bundles on a cross section of flax varieties in long and short day conditions (Leningrad region, 2011)

Accession number	Variety	Origin	Photo sensitivity	Width/height of fibre bundle on a cross section LD*	Width/height of fibre bundle on a cross section ShD**	Perimeter of fibre bundle on a cross section [mm] LD*	Perimeter of fibre bundle on a cross section [mm] ShD**
5333	Svetoch	Russia	1.03	1.14±0.07	1.15±0.13	0.64±0.02	0.76±0.03^{+}
6807	Orshanskiy-2	Byelorussia	1.02	1.08±0.07	1.19±0.16	0.58±0.02	0.62±0.03
7472	Priziv-81	Byelorussia	1.13	1.20±0.08	1.11±0.12	0.70±0.02	0.64±0.03
7694	Tomskiy-16	Russia	1.05	1.18±0.11	1.08±0.09	0.67±0.02	0.68±0.03
7804	Mogilovskiy-2	Byelorussia	1.05	1.08±0.11	1.28±0.13	0.65±0.02	0.56±0.03^{+}
7887	Krom	Russia	1.06	1.03±0.06	1.42±0.11^{+}	0.70±0.02	0.56±0.02^{+}
7940	Lenok	Russia	0.99	1.13±0.06	1.52±0.12^{+}	0.76±0.02	0.58±0.02^{+}
8241	Zarianka	Russia	1.11	1.02±0.07	1.58±0.20^{+}	0.71±0.03	0.52±0.04^{+}
8345	Merilin	The Netherlands	0.98	1.15±0.07	1.40±0.09^{+}	0.73±0.02	0.59±0.02^{+}
8351	Belosnezhka	Russia	1.01	1.11±0.11	1.73±0.21^{+}	0.59±0.02	0.61±0.02
8410	Nord	Russia	1.03	1.03±0.08	1.39±0.11^{+}	0.69±0.02	0.63±0.02
8411	Orion	Russia	0.98	0.87±0.05	1.44±0.08^{+}	0.62±0.02	0.54±0.03
gk-13	Line-5 k-489	Russia	1.10	0.95±0.08	1.77±0.15^{+}	0.52±0.02	0.50±0.03

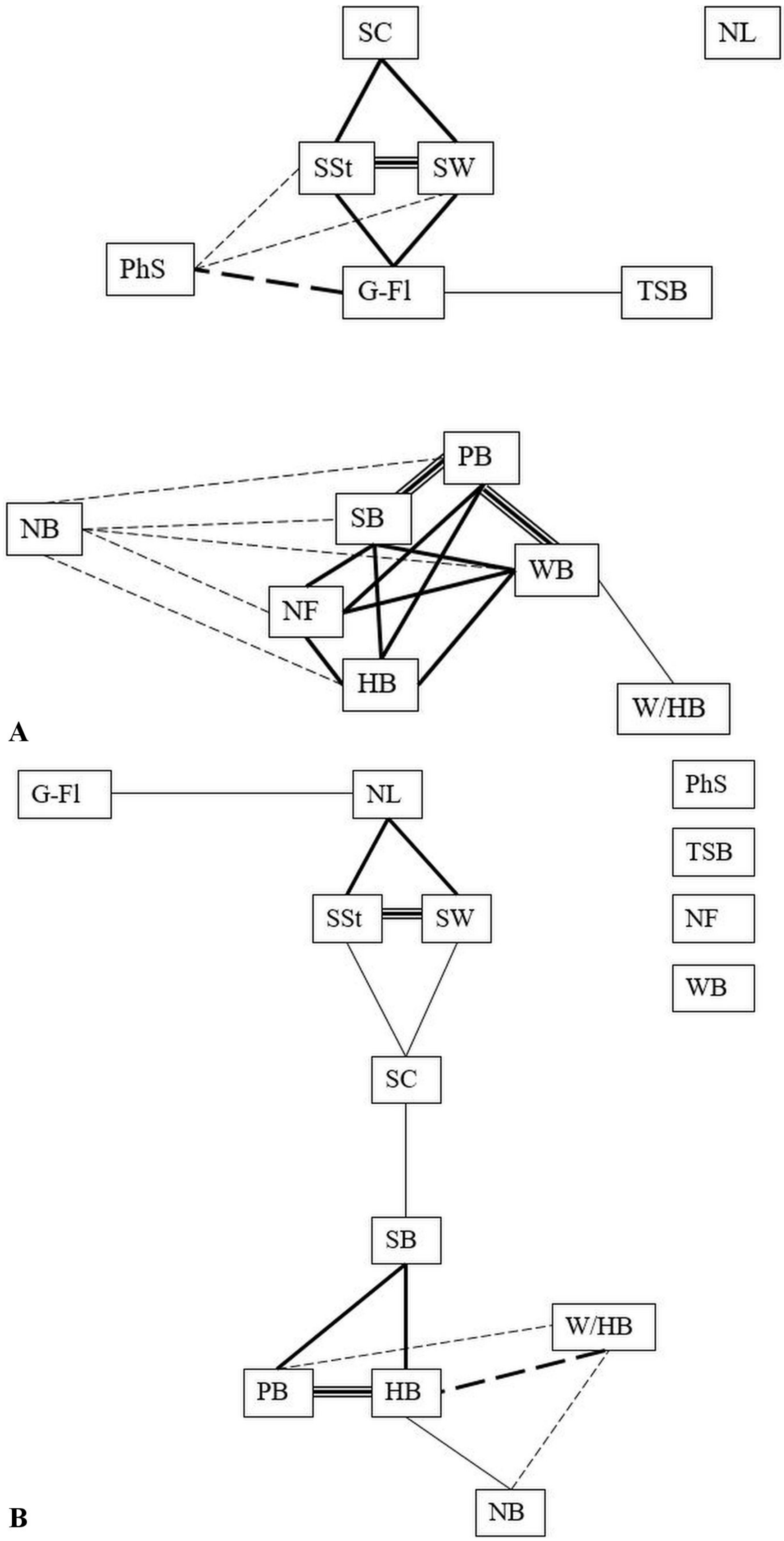
Legend: $^{+}$– The difference is significant ($P \leq 0.05$); *LD – Long day; **ShD – Short day

differences between plants grown in long and short day conditions (the data is not shown). So, they are stable enough while grown in different environments.

Evaluated characters in long daylight conditions formed two separate layers and the number of leaves was independent (Figure 6 A). The first layer consisted of the traits characterising the whole plant. Area of whole stem cross section had very strong correlation with the area of its wooden part. Area of stem cavity and duration of germination – flowering period had strong correlations with this group. The degree of photosensitivity strongly negatively correlated with the time of flowering and weakly negatively correlated with the areas of stem and its wooden part. Total area of fibre bundles was linked to this layer by weak correlation with the period from germination to flowering. The second layer include the group of characters strongly correlated with each other: width, height, perimeter, area of fibre bundles and number of elementary fibres in them, correlations of fibre bundles perimeter with their width and area being the strongest ones. All these characters were weakly negatively linked to the number of fibre bundles on the stem cross section. The ratio between width and height of fibre bundles on a cross section weakly correlated only with its width.

In short day conditions the structure of correlations system changed (Figure 6 B). Photosensitivity, total area of fibre bundles, width of fibre bundles and number of fibres in them were independent. The first layer had the same core of very strongly correlated areas of whole stem and its wooden part. They were strongly linked to leaves number and weakly correlated with the area of stem cavity. Period from germination to flowering weakly correlated with the number of leaves. Through the weak correlation between fibre bundle area and that of cavity, two layers were connected with each other. The second layer included very strongly correlated perimeter and height of bundle, which were strongly linked to the area of fibre bundle and negatively correlated with the ratio between width and height of fibre bundles on a cross section. Fibre bundles height also was weakly linked to number of fibre bundles, which in its turn weakly negatively correlated with the ratio between width and height of fibre bundles on a cross section.

In our previous trials both in long and short day conditions the degree of photosensitivity usually



Legend: The characters' abbreviations are described in the Table 1
——— $0.55 < r < 0.7$; ——— $0.7 \leq r < 0.9$; ═══ $r \geq 0.9$; ----------- $r < 0$

Figure 6. Correlations between characters in long (A) and short (B) day conditions

did not correlate with other characters (Brutch *et al.* 2015). In present experiment this character negatively correlated with period from germination to flowering, and also with area of stem and its wooden part cross sections. The reason of this could be the small size of sampling, which included three relatively early varieties with rather high photosensitivity. Also, in our previous investigations the number of leaves had a strong correlation with the period from germination to flowering both in long and short day conditions (Brutch *et al.* 2015), but in present trial this link was detected only in short day conditions. Area of stem, its wooden part and cavity on a cross section (characters which were not evaluated before) correlated with each other in long and short day conditions. The same could be said about traits characterising the structure of fibre bundles. The last statement is confirmed by previous experiments (Brutch *et al.* 2015).

Presented results show that evaluated flax characters are not strictly correlated with each other except those indicating the structure of fibre bundles. For breeding it is important that their relationships are not strongly influenced by the environment.

CONCLUSIONS

Presented results indicate the extended diversity of fibre flax reactions on photoperiod changes and its relative independence of photosensitivity degree. New genotypes insensitive to short 12 hours daylight with stable number of leaves were selected. It was confirmed that characters of fibre bundles structure are strictly correlated with each other independently of photoperiod conditions.

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REFERENCES

AFONIN, M.I. 1969. Influence of day light longevity and air temperature on growth, development and yield of fibre flax in its ontogenesis. In *Agriculture and plant cultivation in BSSR* : *proceedings of scientific works*. vol. 13, pp. 196–201 (in Russian).

BASU, N.C. – BOSE, S. 1975. Preliminary studies on some botanical, anatomical, and agronomical aspects of Flax (*Linum usitatissimum* L.). In *Science and culture*, vol. *41*, no. 7, pp. 323–325.

BLASCHKE, T. – HAY, G.J. – KELLY, M. – LANG, S. – HOFMANN, P. – ADDINK, E. – FEITOSA, R.Q. – MEER, F. van der – WERFF, H. van der – COILLIE, F. van – TIEDE, D. 2014. Geographic object-based image analysis – Towards a new paradigm. In *ISPRS Journal of Photogrammetry and Remote Sensing*, vol. *87*, pp. 180–191. https://doi.org/10.1016/j.isprsjprs.2013.09.014

BRUTCH, N.B. 2007. *Intraspecific diversity of flax* (*Linum usitatissimum* L.) *and its utilization in genetic investigations and breeding*. Abstract of doctor dissertation. Saint Petersburg (in Russian).

BRUTCH, N. – KOSHKIN, V. – MATVIENKO, I. – POROKHOVINOVA, E. – TAVARES DE SOUSA, M. – DOMANTOVICH, A. 2008. Influence of low temperatures and short photoperiod on the time of flowering in flax. In *Fiber foundations – transportation, clothing and shelter in the bioeconomy* : *proceedings of the 2008 international conference on flax and other bast plants*. Saskatoon Saskatchewan : Canada, 21.–23. July, pp. 81–91.

BRUTCH, N.B. – KOSHKIN, V.A. – DOMANTOVICH, A.V. – MATVIENKO, I.I. 2015. Influence of photoperiod on characters correlations in flax. In *Achievements of Science and Technology AIC*, vol. *29*, no. 7, pp. 43–46 (in Russian).

BRUTCH, N.B. – SHAROV, I.Y. – PAVLOV, A.V. – POROKHOVINOVA, E.A. 2011. Diversity of flax characters associated with fibre formation and environmental influence on their formation Russian. In *Russian Journal of Genetics: Applied Research*, vol. *1*, no. 5, pp. 361–370.

DARAPUNENI, M.K. – MORGAN, G.D. – IBRAHIM, A.M.H. – DUNCAN, R.W. 2014. Effect of vernalization and photoperiod on flax flowering time. In *Euphytica*, vol. *195*, pp. 279–285.

DOMANTOVICH, A.V. – BRUTCH, N.B. – KOSHKIN, V.A. – NOSEVITCH, M.A. 2011. Using of anatomical method for selection of initial material in breeding for the increase of fibre content and quality in flax with different photosencitivity. In *News of Saint-Petersburg State Agricultural University*, no. 23, pp. 11–18 (in Russian).

DOMANTOVICH, A.V. – KOSHKIN, V.A. – BRUTCH, N. – MATVIENKO, I.I. 2012. Investigation of photoperiod sensitivity of *Linum usitatissimum* L. lines and effect of short-day conditions on their economically valuable traits. In *Russian Agricultural Sciences*, vol. *38*, no. 3, pp. 173–177.

DOMANTOVICH, A.V. 2009. Effect of short-day conditions on economically valuable traits of flax lines with different photosensitivity. In *Proceedings of V International Conference of Young Scientists and Specialists*. VNIIMK, February 3–6, pp.74–78.

DORONIN, S.V. – TIKHVINSKIY, S.F. 2003. *Fibre flax*. *Technology of cultivation and breeding*. Kirov : VGSHA (in Russian).

GARNER, W.W. – ALLARD, H.A. 1920. Effect of length of day on plant growth. In *Journal of Agricultural Research*, vol. *18*, pp. 553–606.

GLASBEY, C.A. – HORGAN, G.W. 2001. Image analysis in

agriculture research. In *Quantitative Approaches* in *System Analysis*, special issue, vol. *23*, pp. 43–54.
KNOTT, G. – GENOUD, C. 2013. Is EM dead? In *Journal of Cell Science*, vol. *126*, no. 20, pp. 4545–4552. DOI: 10.1242/jcs. 124123 PMID: 24124192
LIDKE, D.S. – LIDKE, K.A. 2012. Advances in high-resolution imaging––techniques for three-dimensional imaging of cellular structures. In *Journal* of *Cell Science*, vol. *125*, no. 11, pp. 2571–2580. DOI: 10.1242/jcs.090027 PMID: 22685332; PubMed Central PMCID: PMC3706075
MINKEVITCH, I.A. 1957. *Linseed*. Moscow : Selhozgiz (in Russian).
OBRAZCOV, A.S. 1983. About some biological aspects of breeding for earliness problem. In *Agricultural Biology*, vol. *18*, no. 10, pp. 3–11 (in Russian).
PAVELEK, M. 1992. Response of cultivars and new selections of flax (*Linum usitatissimum* L.) to photoperiod. In *Plant Production*, vol. *38*, no. 2, pp. 133–141 (in Czech).
RODRÍGUEZ-PULIDO, F. et al. 2012. Ripeness estimation of grape berries and seeds by image analysis. In *Computers and Electronics in Agriculture*, vol. *82*, pp. 128–133.
ROSTOVA, N.S. 1985. Comparative analyses of correlations structures. Evaluation of biological systems by mathematical methods. In *Proceedings of Biological SRI LSU*, no. 37, pp. 35–50.
SANDEEP, V.V. – KANAKA, D.K. – KESHAVULU, K. 2013. Seed image analysis: its application in seed science research. In *International Research Journal of Agricultural Sciences*, vol. *1*, no. 2, pp. 30–36.
SCHINDELIN, J. – RUEDEN, C.T. – HINER, M.C. – ELICEIRI, K.W. 2015. The ImageJ ecosystem : An open platform for biomedical image analysis. In *Molecular Reproduction and Development*, vol. *82*, pp. 518–529. DOI:10.1002/mrd.22489
SINSKAYA, E.N. 1951. About the types of interactions between growth and development in “long and short day” plants. In *Brief Report on Work in 1950*. Krasnodar : VNIIMK, pp. 154–162 (in Russian).
SIROHI, G.S. – WASNIK, K.G. 1978. Photosensitivity at varietal level in linseed. In *Indian Journal of Plant Physiology*, vol. *21*, no. 2, pp. 27–32.
SIZOV, I.A. 1952. Features of passing through stage changes in various flax varieties and forms. In *Proceedings on Applied Botany, Genetics and Breeding*, vol. *29*, no. 2, pp. 69–123 (in Russian).
SIZOV, I.A. 1955. *Flax*. Moscow : Selhozgiz (in Russian).
SIZOV, I.A. 1963. Regularity of growth and development of flax under the influence of external factors. In *Proceedings on Applied Botany, Genetics and Breeding*, vol. *35*, no. 3, pp. 5–20 (in Russian).
SOROTCHINSKAYA, M.A. 1968. The value of summer sowings in breeding of linseed for earliness. In *Bulletin of Scientific-technical Information on Oil Ccrops*, pp. 11–15 (in Russian).
SOROTCHINSKAYA, M.A. 1975. Short day as the background for the linseed populations decomposition according to productivity. In *Proceedings of Ruban department of VOGIS*, vol. *1*, pp. 122–126 (in Russian).
UCHIDA, S. 2013. Image processing and recognition for biological images. In *Development, Growth and Differentiation*, vol. *55*, pp. 523–549. DOI: 10.1111/dgd.12054
WALTER, A. – LIEBISCH, F. – HUND, A. 2015. Plant phenotyping: from bean weighing to image analysis. In *Plant Methods*, vol. *11*, no. 14, pp. 1–11. DOI 10.1186/s13007-015-0056-8

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