Characterization and evaluation of flax seeds (*Linum usitatissimum* L.) on selected genotypes

Charakteristika a hodnotenie semien ľanu siateho (*Linum usitatissimum* L.) na vybraných genotypoch

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Abstract

The aim of this work was to verify usefulness of image analyses software to determine genotype differences on quantitative linseed traits (*Linum usitatissimum* L.). Also the goal was to propose new quantitative characters of seeds suitable for determination infra-specific differences. The study was realized on seeds of 6 linseed genotypes from two harvested years – 2010 (5 genotypes), 2012 (5 genotypes). The 7 quantitative traits measured on whole seeds and 3 quantitative traits measured on longitudinal seed section were chosen. Seed samples were randomly selected from genetically and physically purified seeds. The experimental data were obtained by the software for image analysis with module for automatic measurement, from detailed digital images. The methodology for measuring parameters in micropyle region of flax seeds was proposed. According to results of descriptive statistics was found out that the smallest seeds had genotype Oural and the biggest seeds had genotypes Flanders and Recital. The three newly proposed characters from micropyle region of seed had shown higher variability than the traits measured on the whole seeds. Coefficient of variation had mostly values more than 10 %. The One-Way ANOVA confirmed statistically significant differences ($p < 0.05$) between all analysed samples in chosen quantitative traits. The results of the post hoc means comparison revealed that genotype Recital received the highest values of means and significantly differentiated from means of genotype Oural in the traits measured on whole seeds in both harvested years. Also there was found out that the Pearson correlation coefficient was mostly around $r = 0.50$ ($p < 0.001$), except two cases among traits - Area - Feret ratio and Diameter – Feret ratio. The correlations between parameters of micropyle seed region were found very weak ($r < 0.50$; $p < 0.001$).

Keywords: image analysis, linseed, *Linum usitatissimum* L., micropyle seed region, seeds

Abstrakt

Hlavným cieľom práce bolo overiť využitie softvéru pre obrazovú analýzu na determináciu genotypových rozdielov na vybraných kvantitatívnych znakoch semien ľanu siateho (*Linum usitatissimum* L.) olejného hospodárskeho typu. Tiež bolo čiastkovým cieľom navrhnúť nové kvantitatívne znaky semien, ktoré by boli vhodné pre determináciu vnútrodruhových rozdielov. Práca sa realizovala na semenách 6 genotypoch ľanu olejného, získaných z dvoch rokov – 2010 (5 genotypov), 2012 (5 genotypov). Vybrali sme 7 kvantitatívnych znakov pre celé semená a 3 kvantitatívne znaky hodnotené na pozdĺžnom reze semena. Vzorky semien boli náhodne vybrané z geneticky a fyzikálne čistených semien. Experimentálne údaje z hodnotenia detailných obrazových záznamov semien boli získané pomocou softvéru pre obrazovú analýzu s modulom pre automatické meranie. Bol navrhnutý metodický postup hodnotenia parametrov v mikropilárnej oblasti ľanového semena. Na základe výsledkov popisnej štatisticky sa zistilo, že najmenšie semená mal genotyp Oural a najväčšie genotyp Flanders a Recital. Tri novo navrhnuté znaky hodnotené v mikropilárnej oblasti semena vykazovali väčšiu variabilitu ako znaky merané na celých semenách. Variačný koeficient ma vo väčšine prípadov hodnoty väčšie ako 10 %. Jednofaktorovou analýzou (ANOVA) sa potvrdili štatisticky preukazné rozdiely medzi všetkými analyzovanými vzorkami vo vybraných kvantitatívnych znakoch. Testovaním kontrastov medzi priemermi sa ukázalo, že genotyp Recital dosiahol najvyššie hodnoty priemerov a tiež štatisticky preukazné rozdiely od priemerov genotypu Oural v znakoch hodnotených na celých semenách v oboch hodnotených rokoch. Okrem toho sa zistilo, že Pearsonov korelačný koeficient bol vo väčšine prípadov okolo $r = 0{,}50$ ($p < 0{,}001$), až na dva pripady, a to medzi znakmi plocha a feretov pomer a tiež priemer – feretov pomer. Korelačné vzťahy medzi znakmi meranými v mikropilárnej oblasti semien boli veľmi slabé ($r < 0{,}50$; $p < 0{,}001$).

Kľúčové slová: *Linum usitatissimum* L., mikropilárna oblasť semena, obrazová analýza, olejný ľan, semená

Detailný abstrakt

Hlavným cieľom práce bolo overiť využitie softvéru pre obrazovú analýzu na determináciu genotypových rozdielov na vybraných kvantitatívnych znakoch semien ľanu siateho (*Linum usitatissimum* L.) olejného hospodárskeho typu. Z toho dôvodu, že znaky na semenách patria k stabilným znakom s nízkou variabilitou sa predpokladalo, že sa potvrdia vnútro-druhové rozdiely medzi vybranými 6 genotypmi. Z hľadiska šľachtiteľských cieľov, ktoré sú v prípade olejného hospodárskeho typu ľanu siateho, zamerané aj na znaky semien bolo cieľom navrhnúť nové kvantitatívne a kvalitatívne znaky, ktoré by boli vhodné tiež na determináciu vnútro-druhových rozdielov. Okrem uvedených cieľov sa overila a navrhla metodika hodnotenia vybraných parametrov v mikropilárnej oblasti semena ľanu.

Experimentálne práce sa realizovali na 6 genotypoch ľanu siateho (*Linum usitatissmum* L.) olejného hospodárskeho typu. Materiál poskytla firma Agritec, výzkum, šlechtění a služby s.r.o. z Českej republiky (tabuľka 1). Z každej vzorky sa náhodným spôsobom vybrali zrelé, nepoškodené semená. Na celých semenách sa hodnotilo 7 kvantitatívnych znakov a na pozdĺžnom reze semena sa hodnotili 3 kvantitatívne znaky. Makroskopické snímky semien boli pripravené pomocou plnoautomatickej makrolupy Zeiss Discovery V20 s digitálnou kamerou MRC5. Meranie kvantitatívnych znakov sa realizovalo pomocou softvéru pre obrazovú analýzu AxioVision 4.8.2.0 s modulom pre automatické meranie. Na celých semenách sa hodnotili – plocha (mm^2), hraničná výška (mm), hraničná šírka (mm), priemer (mm), feret minimum (mm), feret maximum (mm) a feretov pomer (feret min/feret max). Pozdĺžne rezy semien sa robili na semenách, ktoré boli na 24 hodín namočené do destilovanej vody. Na charakterizáciu mikropilárnej oblasti semena boli navrhnuté na hodnotenie tieto znaky – dĺžku embryonálnej osi (mm), vzdialenosť medzi okrajmi klíčnych listov (mm) a dĺžku embryonálnej osi od plumuly po miesto pretínania osí (mm).

Popisnou štatistikou sa charakterizoval experimentálny súbor údajov získaných z hodnotenia celých semien a pozdĺžnych rezov semien. V tabuľkách 2 a 3 sú uvedené priemerné hodnoty a variačné koeficienty všetkých hodnotených genotypov. Z výsledkov uvedených v tabuľke 2 vyplýva, že najmenšie semená mal genotyp Oural a najväčšie semená mali genotypy Flanders a Recital. Variačný koeficient bol vo väčšine prípadov veľmi nízky (menej ako 10 %), a to pri kvantitatívnych znakoch hodnotených na celých semenách. Pri hodnotení parametrov v mikropilárnej oblasti semena bol variačný koeficient väčší ako 10 % v znakoch – dĺžka embryonálnej osi a dĺžka embryonálnej osi od plumuly po miesto pretínania osí. V znaku vzdialenosť okrajov klíčnych listov bol opäť variačný koeficient nižší ako 10 %. Z uvedených výsledkov môžeme konštatovať, že znaky hodnotené na celých semenách sú menej variabilné.

Jednofaktorovou analýzou (ANOVA) sa potvrdil štatisticky preukazný rozdiel ($p < 0,05$) medzi analyzovanými genotypmi keď závislými premennými boli kvantitatívne znaky merané na celých semenách (tabuľka 4). Testovaním kontrastov priemerov medzi genotypmi sa zistilo, že genotyp Recital bol štatisticky preukazne ($p < 0,05$) rozdielny od genotyp Omega v 6 znakov, okrem znaku feretov pomer, a to voboch hodnotených rokoch. Medzi genotypmi Flanders a Recital sa nepotvrdil štatisticky preukazný rozdiel ($p > 0,05$) vo všetkých 7 znakoch. V prípade keď boli do modelu jednofaktorovej analýzy (ANOVA) zaradené závislé premenné hodnotené v mikropilárnej oblasti semena (tabuľka 5) sa zistilo, že F hodnoty boli vo všetkých prípadoch veľmi nízke, aj napriek tomu, že $p < 0,05$. Z toho vyplýva, že genotyp ako závislá premenná sa len nízkym percentom podieľal na genotypových rozdieloch v hodnotených znakoch.

V tabuľkách 6 až 9 sú zobrazené výsledky korelačnej analýzy za znaky hodnotené na celých semenách a aj v mikropilárnej oblasti semena. Korelačné vzťahy boli analyzované zvlášť za každý rok (2010, 2012). Štatisticky veľmi významný vzťah ($p < 0,001$) sa potvrdil medzi rovnakými znakmi v oboch testovaných rokoch (tabuľka 6 a 7),a to medzi znakmi hodnotenými na celých semenách. V rovnakých tabuľkách 6 a 7 medzi znakmi plocha feretov pomer, a tiež znakmi priemer a feretov pomer sa nepotvrdil štatisticky významný vzťah ($p > 0,05$). Výsledky korelačnej analýzy realizovanej na znakoch hodnotených v mikropilárnej oblasti semena (tabuľka 8, 9) ukázali, že medzi znakmi sú slabé vzťahy ($r < 0,50$), aj napriek tomu že $p < 0,001$ ($N > 150$ hodnotených vzoriek).

Experimentálna práca prináša originálne výsledky v oblasti hodnotenia nových morfologických znakov v mikropilárnej oblasti semien ľanu siateho. Na základe zistených údajov navrhujeme pokračovať v hľadaní vhodných znakov na semenách, ktoré pomôžu pri charakterizácii a identifikácii odrôd ľanu siateho. Nástroje obrazovej analýzy (modul pre automatické meranie) sa nám potvrdili ako dostatočný, objektívny, opakovateľný a spoľahlivý nástroj pre identifikáciu odrôd, ich klasifikáciu a charakterizáciu.

Introduction

The flax (*Linum usitatissimum* L.) is the technical crop with wide range of different uses in industrial areas. Nowadays it has become also interesting commodity for food industry (Nykter, et al., 2006; Jhala, Hall, 2010). Linseed has important nutritive value connected to unsaturated fatty acids composition, lignans and mucilage components in seeds (Hosseinian, et al., 2004; El-Beltagi, et al., 2007; Westcott, Muir, 2003; Schmidt, et al., 2012; Bjelková, et al., 2012). Due to mentioned valuable characters of flax the breeding and plant improvement processes are still the best way to look for new characters and features of genotypes. Many research collectives deal with flax collections characterization and evaluation (Diederichsen, 2001; Diederichsen, Raney, 2006). They use different traditional or modern methods, like molecular analyses (Žiarovská, et al., 2012; Fu, 2006; Everaert, et al., 2001) or image analyses (Wiesnerová, Wiesner, 2008; Smykalova, et al., 2013). The use of image analyses tools has increased in previous years. Together with mathematical and statistical methods they revealed new detailed characters and relationships. Image analyses can in details describe morphological parts what is impossible with traditional morpho-metric methods (Keefe, 1999; Iwata, et al., 2002; Yoshioka, 2004).

Seeds of different species and varieties within plant species have specific characters which are suitable for distinguishing of varieties differences. This fact has important place for DUS testing, and variety identification and verification (Keefe, 1999).

The size of flax seed ranged from 4 to 6 mm in length, and from 2.5 to 4 mm in width (Nôžková, et al., 2011). The weight of thousand seeds range from circa 4 to 13 g (Nôžková, et al., 2011) and variability in these characters is not very wide. The varieties are mostly stable. For this reason it would have practical impact to seek for new characters on flax seed.

The main aim was to verify the usefulness of image analyses software to determine genotype differences on selected quantitative traits of linseed (*L. usitatissimum* L.) seeds. On the ground of traits measured on seeds are stable with low variability, we assumed that infra-specific differences between selected genotypes will be confirmed. In term of breeding targets which in the case of linseed cultivars are mostly oriented to seed characters, the specific goal was to propose new quantitative characters, according to which would be possible to determine infra-specific differences.

Material and Methods

The experiments were realized on seeds of 6 genotypes. All genotypes belonged to oil economic type. The material was provided by Agritec Plant Research Ltd. (Czech Republic). The information about selected genotypes are mentioned in table 1.

Table 1. Selected genotypes of linseed

Sample	Country of origin (FAO)	Name	Economic type	Harvest year

1	FRA	Bajkal	Linseed	2010
2	FRA	Bajkal	Linseed	2012
3	CZE	Flanders	Linseed	2012
4	AUT	Omega	Linseed	2010
5	AUT	Omega	Linseed	2012
6	FRA	Oural	Linseed	2010
7	FRA	Oural	Linseed	2012
8	CZE	Raciol	Linseed	2010
9	FRA	Recital	Linseed	2010
10	FRA	Recital	Linseed	2012

The seed samples were selected according to genetic and physical purity standards (ÚKSÚP, 1999).

Randomly selected mature seeds from each sample were evaluated in 10 quantitative traits. Seven parameters were evaluated on whole seed (15 seeds per sample) and three parameters were measured on inner parts of longitudinal seed section (20 seeds per sample). For evaluation of macroscopic parameters of inner parts, the seeds had to be prepared. Each seed sample was dipped to distilled water for 24 hours.

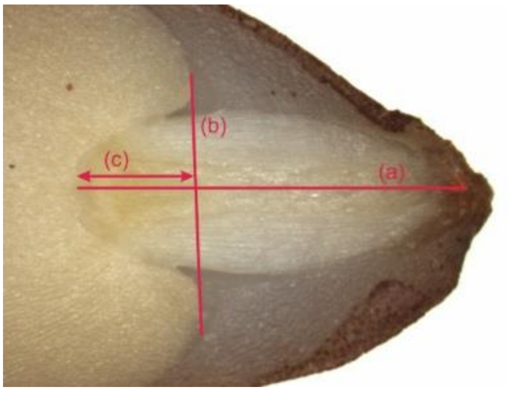
Macroscopic images of whole and longitudinal section of seeds were prepared by fully automatic macroscope Zeiss Discovery V20 with digital camera MRC5. The quantitative traits were evaluated by software for image analyses AxioVision 4.8.2.0 with module for automatic measure.

Evaluation of anatomic parts of seed

We proposed the methodology for measuring of chosen parts in micropyle region of seed (Figure 1). There were measured the length of embryonic axis (mm) (LEA), the distance between edges of cotyledons (mm) (DEC) and the length of embryonic axis from plumule to the point of axis intersection (mm) (LPAI).

Evaluation of whole seed

The images with whole seed were measured with module for automatic measure which was the part of software AxioVision 4.8.2.0. From all parameters were chosen – area (mm^2), bound height (mm), bound width (mm), diameter (mm), feret minimum (mm), feret maximum (mm) and feret ratio.



Notes: (a) the length of embryonic axis (mm); (b) the distance between edges of cotyledons (mm); (c) the length of embryonic axis from plumule to the point of axis intersection (mm)

Figure 1. Measured parts in micropyle region of linseed

Statistical analysis

Experimental data were evaluated by using descriptive statistics. The normality was tested by tests – Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, Anderson-Darling and was used the procedure UNIVARIETE. The Pearson correlation coefficient and one-way ANOVA were used to look for correlations and differences among analysed genotypes. All statistical methods were processed by using SAS Enterprise Guide 5.1.

Results and Discussion

Information about the characterization of flax seed is limited in literature sources. The morphological characterization of seed has important role to discriminate the flax varieties (Keefe, 1999; Wiesnerová, Wiesner, 2008; Smykalova, et al., 2013), what has also economic importance. Literature sources are mostly oriented to detailed analyses of embryo and flax seed development (Venglat, et al., 2011). In our work we are proposing the parameters characterization for mature seed evaluation. We chose 10 quantitative traits on whole seeds and on seed longitudinal section.

The descriptive statistics was done to characterize the experimental data sets. In table 2 and 3 are viewed selected results for all evaluated genotypes and quantitative traits.

Table 2. Descriptive statistics for evaluated whole seeds samples

Harvested year 2010											
Genotype		**Bajkal**		**Omega**		**Oural**		**Raciol**		**Recital**	
Variable	**N**	**Mean**	**CV**	**Mean**	**CV**	**Mean**	**CV**	**Mean**	**CV**	**Mean**	**CV**
1	15	8.76±0.12	5.23	8.33±0.15	6.77	8.42±0.17	7.89	8.35±0.15	7.02	9.41±0.10	4.24
2	15	2.56±0.02	2.86	2.44±0.02	3.51	2.41±0.02	3.33	2.39±0.02	4.04	2.57±0.02	2.58
3	15	4.52±0.03	2.98	4.64±0.05	4.05	4.63±0.06	4.91	4.66±0.05	4.07	4.93±0.03	2.05
4	15	2.55±0.02	2.80	2.43±0.02	3.44	2.39±0.02	3.44	2.37±0.02	3.80	2.55±0.02	2.58
5	15	4.55±0.04	3.02	4.67±0.05	4.04	4.65±0.06	4.98	4.68±0.05	4.16	4.95±0.03	2.04
6	15	3.34±0.02	2.59	3.26±0.03	3.43	3.27±0.03	3.99	3.26±0.03	3.52	3.46±0.02	2.12
7	15	0.56±0.00	1.64	0.52±0.00	2.18	0.51±0.00	3.62	0.51±0.00	2.41	0.52±0.00	2.31
Harvested year 2012											
Genotype		**Bajkal**		**Flanders**		**Omega**		**Oural**		**Recital**	
Variable	**N**	**Mean**	**CV**	**Mean**	**CV**	**Mean**	**CV**	**Mean**	**CV**	**Mean**	**CV**
1	15	8.67±0.08	3.62	9.13±0.30	12.52	8.35±0.14	6.38	8.02±0.08	3.89	9.44±0.13	5.48
2	15	2.53±0.02	3.34	2.49±0.05	7.33	2.42±0.02	3.38	2.29±0.02	2.85	2.53±0.03	4.04
3	15	4.51±0.02	1.84	4.86±0.07	5.64	4.66±0.04	3.14	4.60±0.04	3.66	4.99±0.03	2.52
4	15	2.51±0.02	3.09	2.49±0.05	7.25	2.41±0.02	3.69	2.28±0.02	2.84	2.51±0.03	4.19
5	15	4.53±0.02	1.65	4.88±0.07	5.61	4.68±0.04	3.24	4.61±0.04	3.63	5.01±0.03	2.44

6	15	3.32±0.02	1.80	3.40±0.06	6.27	3.26±0.03	3.19	3.20±0.02	1.95	3.47±0.02	2.75
7	15	0.55±0.00	3.18	0.51±0.00	3.31	0.52±0.00	2.73	0.50±0.01	4.89	0.50±0.00	3.75

Notes: N - count of measured seeds; CV – coefficient of variation %; 1 – Area (mm); 2 - Bound height (mm); 3 - Bound width (mm); 4 - Feret min (mm); 5 - Feret max (mm); 6 – Diameter (mm); 7 - Feret ratio.

In the trait area, the average values ranged from 8.02 (Oural 2012) to 9.44 mm (Recital 2012). The variation coefficient was in all cases less than 10 % except the genotype Flanders (12.52 %). The average values in the trait Bound height (mm), ranged from 2.29 (Oural 2012) to 2.57 (Recital 2010). The variation coefficient was less than 10 % for each genotype. The minimal average value in the trait Bound width (mm) achieved the genotype Bajkal 2012 (4.51 mm) and maximal average value genotype Recital 2012 (4.99 mm). In the trait Feret min (mm) the average value ranged from 2.28 mm (Oural 2012) to 2.55 mm (Bajkal 2010 and Recital 2010). In the trait Feret max (mm) the average value ranged from 4.53 mm (Bajkal 2012) to 5.01 mm (Recital 2012). The minimal average value achieved the genotype Oural 2012 (0.49) and the maximal value achieved the genotype Bajkal 2010 (0.56) in the trait Feret ratio. The variation coefficient was again very low (less than 10 %) for the traits Feret min, Feret max and Feret ratio. The genotype with the smallest average value in diameter (mm) was Oural 2012 (3.20 mm) and the largest average value achieved the genotype Recital (3.47 mm). The coefficient of variation was very low (less than 10 %) also in this trait. According to results from table 2 is clear that the smallest seeds had the genotype Oural and the biggest seeds had genotypes Flanders and Recital.

Similar experiments on whole flax seed, analysed by image analysis, were done by collectives from Czech Republic (Smykalova, et al., 2011; Smykalova, et al., 2013; Wiesnerová, Wiesner, 2008). Smykalova, et al. (2011) analysed also parameters – area, diameter, Feret min and Feret max, Feret ratio. In comparison with our experiment they had oil and also fibre type varieties (Amon, Bonet, Jantar, Jitka, Lola, Marylin and Rita). The varieties from our experimental set reached the higher minimal and maximal values in the trait area. Average values for diameter were also higher. In their experiment the minimal value for Feret min was 2.267 mm (Bonet) and maximal was 2.522 mm (Lola). Minimal value for Feret max was 4.125 mm (Rina) and maximal value reached the same variety Rina (4.953 mm). In the paper Smykalova et al. (2013) included into experimental set also the variety Flanders. In comparison with our results for parameters area, diameter, Feret min, Feret max, Feret ratio we reached the higher values for Flanders. In both experiments they confirmed that computerised image analysis is reliable tool for identification flax varieties. Wiesnerova, Wiesner (2008) quantitatively analysed 53 flax genotypes both oil and fibre type. They also used seed parameters (shape and colour) for variability classification which is used in physiological and genetic studies.

Table 3. Descriptive statistics for evaluated longitudinal seed sections samples

Harvested year 2010										
Genotype	**Bajkal**		**Omega**		**Oural**		**Raciol**		**Recital**	
Variable	**Mean**	**CV**	**Mean**	**CV**	**Mean**	**CV**	**Mean**	**CV**	**Mean**	**CV**

1	1.50±0.03	13.38	1.37±0.04	16.21	1.46±0.04	16.06	1.35±0.03	14.00	1.48±0.05	17.41
2	0.82±0.01	7.14	0.75±0.01	8.25	0.81±0.01	7.45	0.77±0.01	7.42	0.80±0.01	7.70
3	0.48±0.01	12.73	0.45±0.01	11.34	0.45±0.01	17.61	0.40±0.01	18.61	0.48±0.01	15.01
Harvested year 2012										
Genotype	**Bajkal**		**Flanders**		**Omega**		**Oural**		**Recital**	
Variable	**Mean**	**CV**	**Mean**	**CV**	**Mean**	**CV**	**Mean**	**CV**	**Mean**	**CV**
1	1.56±0.05	18.55	1.26±0.04	14.89	1.41±0.04	15.37	1.34±0.04	14.63	1.53±0.05	17.34
2	0.79±0.01	9.92	0.68±0.01	11.03	0.77±0.01	9.02	0.84±0.02	12.74	0.80±0.01	7.25
3	0.44±0.01	11.52	0.39±0.01	12.49	0.42±0.01	12.09	0.41±0.01	16.66	0.44±0.01	13.53

Notes: CV – coefficient of variation %; 1 – LEA the length of embryonic axis (mm); 2 – DEC the distance between edges of cotyledons (mm); 3 – LPAI the length of embryonic axis from plumule to the point of axis intersection (mm); N - count of measured seeds differed from variety to variety (2010 – n = 38, 32, 37, 35, 26 respectively in accordance with table; 2012 – n= 31, 25, 37, 29, 34 respectively in accordance with table)

The mean value ranged from 1.26 mm (Flanders) to 1.56 mm (Bajkal 2012) in the trait LEA. In the second trait DEC the minimal average value reached again the genotype Flanders (0.68 mm) and the maximal value had the genotype Oural 2012 (0.84 mm). In the trait LPAI the Flanders again obtained the minimal average value (0.39 mm) and maximal value obtained the genotypes Bajkal 2010 and Recital 2010 (0.48 mm). The coefficient of variation was more than 10 % in the traits LEA and LPAI. In the trait DEC the coefficient of variation was mostly less than 10 % with the exception of Flanders and Oural 2012 (> 10 %).

The analysed parameters in micropyle region of flax seed we cannot compare with any literature sources. The importance of characterization inner parts of seeds can bring new views for varieties identification and classification. Dellʼ Aquila (2004, 2009) mentioned that image analyses of quantitative parameters in seed germs can be useful addition to physiological, molecular and biochemical data.

The normal distribution of all experimental sets for 7 quantitative traits evaluated on whole seed and for 3 quantitative traits evaluated on micropyle region of seed, was statistically significant ($p > 0.05$).

One-way ANOVA was used to look for differences among analysed genotypes. The area (mm^2), bound height (mm), bound width (mm), diameter (mm), Feret min (mm), Feret max (mm) and feret ratio were set like dependent variables. Independent variable was Genotype and the results were grouped by year of harvest (2010, 2012). In all cases was the effect of independent variable (Genotype) statistically significant ($p < 0.05$). The results for the omnibus ANOVA procedure for all models are shown in table 4.

Table 4. Results for the omnibus one-way ANOVA analysis – dependent variables were traits evaluated on whole seed

Dependent variable	**Effect of independent variable “Genotype”**
Harvest year 2010	
Area	$F(4, 70) = 10.52$, $p < 0.05$, $\eta^2 = 0.375$
Bound height	$F(4, 70) = 16.75$, $p < 0.05$, $\eta^2 = 0.489$

Bound width	$F(4, 70) = 11.27$, $p < 0.05$, $\eta^2 = 0.392$
Feret Min	$F(4, 70) = 18.61$, $p < 0.05$, $\eta^2 = 0.515$
Feret Max	$F(4, 70) = 10.68$, $p < 0.05$, $\eta^2 = 0.379$
Diameter	$F(4, 70) = 10.14$, $p < 0.05$, $\eta^2 = 0.367$
Feret ratio	$F(4, 70) = 40.17$, $p < 0.05$, $\eta^2 = 0.697$
Harvest year 2012	
Area	$F(4, 70) = 12.07$, $p < 0.05$, $\eta^2 = 0.408$
Bound height	$F(4, 70) = 12.44$, $p < 0.05$, $\eta^2 = 0.416$
Bound width	$F(4, 70) = 19.69$, $p < 0.05$, $\eta^2 = 0.529$
Feret Min	$F(4, 70) = 11.73$, $p < 0.05$, $\eta^2 = 0.401$
Feret Max	$F(4, 70) = 20.35$, $p < 0.05$, $\eta^2 = 0.538$
Diameter	$F(4, 70) = 12.17$, $p < 0.05$, $\eta^2 = 0.410$
Feret ratio	$F(4, 70) = 23.60$, $p < 0.05$, $\eta^2 = 0.574$

Notes: F – degrees of freedom; η^2 – R-Square

Since the effect of independent variable was statistically significant, the post hoc means comparison was used by Ryan-Einot-Gabriel-Welsch test. This test is described in Gamst et al. (2008).

The results indicated that the means of genotype Recital significantly differentiated (p < 0.05) from means of Oural, Omega and Raciol genotypes in 6 traits (except Feret ratio) in harvest year 2010. The genotype Recital also gained the highest means in 5 traits (except Feret min and Feret ratio) in comparison with Raciol, Omega, Oural and Bajkal. In the harvest year 2012 again the means of genotype Recital were significantly different (p < 0.05) from means of Oural genotype in 6 traits (except Feret ratio) and from means of genotype Omega in 4 traits (except Bound height, Feret min, Feret ratio). Between means of genotypes Recital and Flanders were not found significant differences in all 7 traits. The highest means gained Recital in 5 traits (except Bound height and Feret ratio), and the smallest means were got by genotype Oural in 5 traits in harvest year 2012.

The one-way ANOVA procedure was applied also for depended variables evaluated in micropyle region of flax seed (LEA, DEC, LPAI). Independent variable was Genotype and the results were also grouped by year of harvest (2010, 2012). In all cases was the effect of independent variable (Genotype) statistically significant (p < 0.05). The results for the omnibus ANOVA procedure for all models are shown in table 5.

Table 5. Results for the omnibus one-way ANOVA analysis – dependent variables were traits evaluated in the micropyle region of seed

Dependent variable	**Effect of independent variable "Genotype"**
Harvest year 2010	
LEA	$F(4, 163) = 3.37$, $p < 0.05$, $\eta^2 = 0.076$
DEC	$F(4, 163) = 8.48$, $p < 0.05$, $\eta^2 = 0.172$

LPAI	$F(4, 163) = 7.84$, $p < 0.05$, $\eta^2 = 0.161$
Harvest year 2012	
LEA	$F(4, 151) = 8.20$, $p < 0.05$, $\eta^2 = 0.178$
DEC	$F(4, 151) = 15.73$, $p < 0.05$, $\eta^2 = 0.294$
LPAI	$F(4, 151) = 3.42$, $p < 0.05$, $\eta^2 = 0.083$

Notes: F – degrees of freedom; η^2 – R-Square; LEA - the length of embryonic axis (mm); DEC - the distance between edges of cotyledons (mm); LPAI - the length of embryonic axis from plumule to the point of axis intersection (mm).

From the above table 5 is highlighted that the F values are in all cases very low, so also eta-square values (η^2) are telling that only low percentages of the variance are explained by Genotype.

For the post hoc means comparison was used Fisher's least significant-differences test (LSD test). Results indicated that the genotype Raciol had lowest means in traits LEA and DEC and significantly differentiated (p < 0.05) from Bajkal, Recital and Oural in all 3 traits in 2010 harvest year. There was not found significant differences among genotypes Bajkal, Recital and Oural (year 2010). The means of genotype Flanders significantly differentiated from genotypes Bajkal, Recital in all 3 traits in the harvest year 2012. This genotype Flanders had the lowest means in all three traits. In this harvest year between means of genotypes Bajkal and Recital was not found significant differences in all 3 traits.

The results viewed in the tables 6 - 9 present the correlation analysis (Pearson correlation coefficient and p value). The statistically significant correlation was tested among parameters evaluated on the whole seeds and also in the micropyle region of seed. The correlations were tested separately in each harvest year (2010, 2012). Since the linear correlation was found out the Pearson correlation coefficient was used to display results.

Comparing the tables 6 and 7, there are visible similar results. With bold are highlighted the very high statistically significant relations (p > 0.001) with correlation coefficients r > 0.80 which represent very strong positive correlations. Insignificant relations (r < 0.60) are between the same pairs in both compared tables (table 6, 7) even the p values are < 0.001. This is caused because of big value of N - number. In tables 6 and 7 between traits Area and Feret ratio, and traits Diameter and Feret ratio is not found any relation (p > 0.05).

Table 6. Correlation analyses (Pearson correlation coefficient) for parameters measured on the whole seeds in the year 2010 (n = 75; H0 = 0)

	Area	Bound height	Bound width	Feret min	Feret max	Diameter	Feret ratio
Area	1	**0.87** <.0001	**0.85** <.0001	**0.87** <.0001	**0.86** <.0001	**1.00** <.0001	-0.02 0.87
Bound height		1	0.53 <.0001	**0.98** <.0001	0.55 <.0001	**0.87** <.0001	0.42 0.0002
Bound width			1	0.53 <.0001	**1.00** <.0001	**0.85** <.0001	-0.51 <.0001

Feret min				1	0.55 <.0001	**0.87** <.0001	0.45 <.0001
Feret max					1	**0.86** <.0001	-0.50 <.0001
Diameter						1	-0.02 0.88
Feret ratio							1

Table 7. Correlation analyses (Pearson correlation coefficient) for parameters measured on the whole seeds in the year 2012 (n = 75; H0 = 0)

	Area	Bound height	Bound width	Feret min	Feret max	Diameter	Feret ratio
Area	1	**0.86** <.0001	**0.83** <.0001	**0.88** <.0001	**0.84** <.0001	**1.00** <.0001	0.13 0.25
Bound height		1	0.49 <.0001	**0.99** <.0001	0.51 <.0001	**0.87** <.0001	0.57 <.0001
Bound width			1	0.51 <.0001	**1.00** <.0001	**0.83** <.0001	-0.42 0.0002
Feret min				1	0.53 <.0001	**0.88** <.0001	0.56 <.0001
Feret max					1	**0.84** <.0001	-0.40 0.0003
Diameter						1	0.14 0.24
Feret ratio							1

In the tables 8 and 9 are showed the results from correlation analysis of traits measured in micropyle region of seed. There was not found out any significant correlation among chosen traits (r < 0.50) even p value was more than 0.001 in many cases (caused by big value of N).

Table 8. Correlation analyses (Pearson correlation coefficient) for parameters measured on the longitudinal seed sections in the year 2010 (n = 168; H0 = 0)

Trait	LEA (mm)	DEC (mm)	LPAI (mm)
LEA (mm)	1	0.47 <0.0001	0.40 <0.0001
DEC (mm)		1	0.24 0.0017
LPAI (mm)			1

Notes: LEA - the length of embryonic axis (mm); DEC - the distance between edges of cotyledons (mm); LPAI - the length of embryonic axis from plumule to the point of axis intersection (mm).

Table 9. Correlation analyses (Pearson correlation coefficient) for parameters measured on the longitudinal seed sections in the year 2012 (n = 156; H0 = 0)

Trait	LEA (mm)	DEC (mm)	LPAI (mm)
LEA (mm)	1	0.37 <0.0001	0.14 0.08
DEC (mm)		1	0.18 0.02
LPAI (mm)			1

Notes: LEA - the length of embryonic axis (mm); DEC - the distance between edges of cotyledons (mm); LPAI - the length of embryonic axis from plumule to the point of axis intersection (mm).

Conclusions

From all results presented in this study are displayed following conclusions.

1. The results of descriptive statistics obtained in our experiments are comparable with results of discussed literature sources.
2. In short, the analysis of variance (ANOVA) showed following findings:
   - The means of genotype Recital significantly differentiated from means of genotype Oural in same traits (evaluated on the whole seeds) in both harvest years (2010, 2012).
   - The genotype Recital gained the highest values of means in 5 traits (evaluated on the whole seeds) in both harvested years, except the Feret min (2010), Bound height (2012) and the trait Feret ratio which was the exception in both harvest years.
   - Comparing the results obtained for traits measured on the whole seed and in micropyle region was found out, that on one side the means of genotypes Recital and Flanders was not differentiated in any traits (evaluated on the whole seed) and on other side there were significant differences in all traits evaluated in micropyle region of seed.
   - The genotype Flanders had the smallest values of means in all 3 traits measured in micropyle region of seed.
   - Even ANOVA procedure results showed that the genotype had only low effect on differences among means of the traits evaluated in micropyle region of flax seed, there were found some significant differences between means.
3. The suitability of automatic measurement module for describing the varieties was also confirmed by results of correlation analysis:
   - Very high statistically significant strong positive correlations ($r > 0.80$; $p > 0.001$) were found between the same traits measured on the whole seeds from both harvested years.
4. In this study we found out that the module for automatic measurement supposed to be the suitable, sufficient, objective, repeatable and reliable tool for describing whole flax seeds in the quantitative traits and can be used for varieties identification, classification and characterization.

5. Even the results from analysing of traits evaluated in micropyle region of seed noticed low effect or insignificant correlations, the findings of this study are significant for the continuing of looking for the new morphological characters which would be suitable for variety description.
6. The work brought the original results in the field of morphological characterization of new quantitative parameters in micropyle region of flax seed. The methodology for evaluation of 3 new parameters in this region of flax seed was proposed. Further investigation should be performed to search for new parameters from this seed region.

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