Original Paper

Zea mays L. hybrids kernels evaluated by image analysis tools

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The aim of this study was to distinguishing between kernels of maize hybrids by the use of image analysis tools. We analyzed 10 registered *Zea mays* L. hybrids (5 – dent, 2 – semi-flint to flint, and 3 – semi-flint to dent type). Different parameters on ventral, dorsal, corolla side, and lateral side cross section of kernel were measured. Sample per each hybrid comprised 50 maize kernels. Acquired bio-images were processed by software Zeiss AxioVision Rel. 4.8. We analyzed the segmented regions of interest on the kernels. The data for area (mm^2), height and width (mm) were gathered from these regions. The hybrid ZE EDOX significantly differed (p <0.05) from all other hybrids almost in all traits. It is the hybrid with the smallest area of the whole kernel, floury endosperm proportion, and depressed part on corolla. The new trait the area of the depressed part on the kernel corolla was measured. The hybrids with smaller proportion of floury endosperm had smaller area of depressed part, and vice versa. The image analysis methods can usefully contribute to selection of proper hybrids for different types of use.

Keywords: maize, *Zea mays* L., kernel, image analysis

1 Introduction

The maize (*Zea mays* L.) is one of the most important dietary staple food globally. Many different hybrids are producing by breeding and they are for different use. Appropriate type of use strongly depends on kernel characters, like size, shape, and color. The amount of different substances correlates with these bio-morphological kernel traits (Guelpa et al., 2015). The chemical content depends on convariety (group). They differ in content of starch, proteins, lipids, sugars, and other substances (Janda and Michalec, 1982).

The important way of maize kernel use is maize meal producing by dry-milling after de-germing (the germ and pericarp are removed). The quality of maize meal also depends on the amount of two types endosperm. The vitreous one is harder, of higher density, translucent and placed on outside part of kernel. The floury endosperm on the contrary is softer, of less density, and placed in the center of kernel (Watson, 1987). There were many ways of analyzing maize kernel hardness which mainly depends on the content of floury and vitreous endosperm (Fox and Manley, 2009). The macro imaging of morphological and anatomical parts of maize kernel can be also fast method to determine it.

Image analysis methods are successfully used in agriculture research. The number of papers about application of these methods is increasing (Glasbey and Horgan, 2001; Dell´Aquila, 2006; Rodríguez-Pulido et al., 2012; Wiwart et al., 2012; Blaschke et al., 2014). The advantage is that the numerous amount of numerical data can be extracted from acquired images. The color, size, shape characteristics of plant products can be quickly processed and then statistically elaborated.

The objective of this study was to use the methods of image analysis for distinguishing between kernels of maize hybrids. Different parameters on maize kernels were measured. This method can contribute to quality characterization of maize kernels utilized for different purposes.

2 Materials and methods

The experimental set consists of 10 registered maize hybrids (*Zea mays* L.) which were cultivated under the equal climatic and vegetation conditions of field experiment, with the uniform agrotechnics. The characterization of hybrids is documented in table 1. The hybrids were provided by company ZELSEED spol. s r.o Horná Potôň.

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Table 1 List of evaluated maize hybrids

Hybrid	Registration	FAO	Type of maize kernel	Color of kernel corolla	Use
ZE ADULAR	2009	350	dent	yellow	kernel
ZE EDOX	2008	280	flint to semi-flint	yellow	kernel and ensilage
ZE ELMO	2013	380	dent	yellowish-white	kernel
ZE HILDA	2015	350	semi-flint to dent	yellow	kernel and ensilage
ZE KARUZEL	2014	420	semi-flint to dent	yellow	kernel and ensilage
ZE OTIS	2010	300	dent	yellow	kernel
ZE ZEAMAX	2015	420	semi-flint to dent	yellow	kernel and ensilage
ZE ZELMA	2014	410	flint to semi-flint	yellow	kernel
ZE ZELSTAR	2015	330	dent	yellow	kernel and ensilage
ZE 4501	2005	450	dent	yellow	kernel and ensilage

The images processing and analyzing is always done in steps. The workflows how to automatically analyze bio-images have to be prepared (Uchida, 2013). In our experiment the image analysis was realized in four steps (Fig. 1). The first step was images acquiring. The kernels were imaged by fully automated macroscope Zeiss SteReo Discovery V20 with digital Camera AxioCam MRC5. For imaging were randomly selected 50 kernels for each hybrid. The sample images were prepared from whole kernels and cross-sections (Fig. 2). There were acquired 250 images for each hybrid. The kernels before cutting were placed to distilled water for 24 hours.

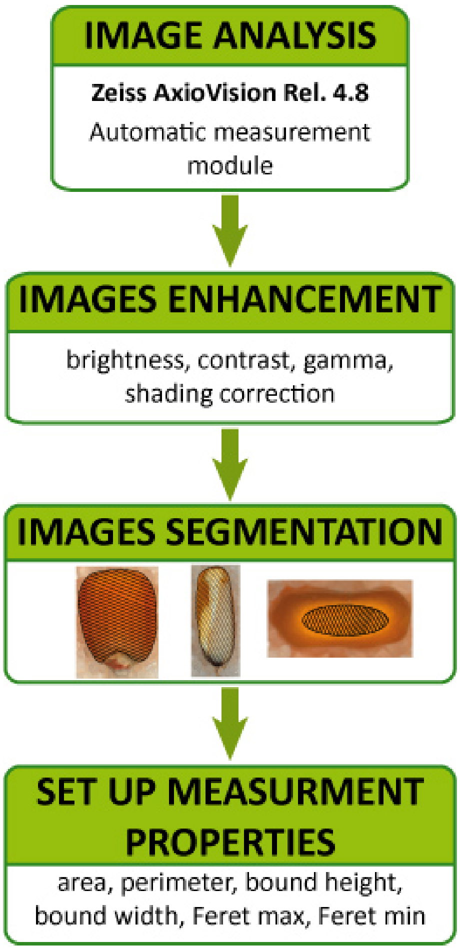

Figure 1 The workflow for image analysis of maize kernels

The images were then processed by software for image analysis Zeiss AxioVision Rel. 4.8 with module for automatic measurement. We used measurement program wizard. The images enhancement was the second step of analysis (Fig. 1). It was important to pre-process the images by several tools. We used brightness, contrast, gamma, shading correction.

The other essential step was the images segmentation. We segmented the objects of interest in the image. In some cases the images had to be processed to binary mode and then the concerning section was segmented.

The last step of image analysis was quantification of recognized regions of interest. The parameters of measurement were set up (Fig. 1). We measured the different parts of maize kernels in images. It depended on the kernel orientation. Figures (3 – 6) are documenting the measured regions.

The automatic measurement module was used for quantification of images with kernels oriented on the ventral side and lateral section (Fig. 3, 6). The concerning parts were automatically selected in images which were in binary mode.

All other images were not measured by automatic measurement module. In these cases, we used measuring tools like distance or outline.

For statistical analysis was used software SAS 9.3 Enterprise guide 5.1. Obtained data were subjected to basic statistics and parametric test (ANOVA). We used Ryan-Einot-Gabriel-Welsch Multiple Range Test for contrasts testing. The images used for article publication

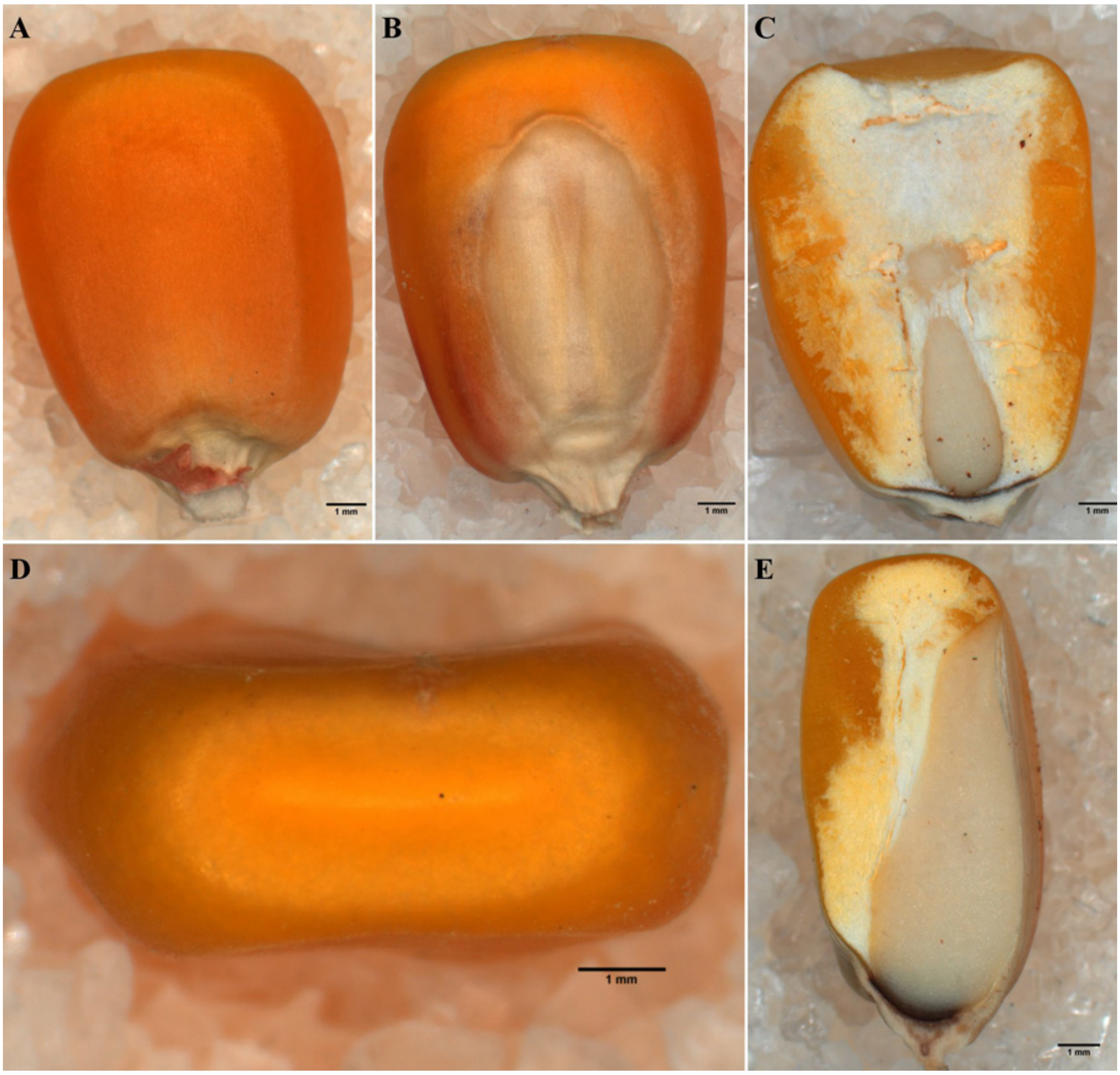

Figure 2 The images acquired from maize kernels: A – the ventral side of kernel; B – the dorsal side of kernel; C – the front side section; D – the corolla of kernel; E – the lateral side section

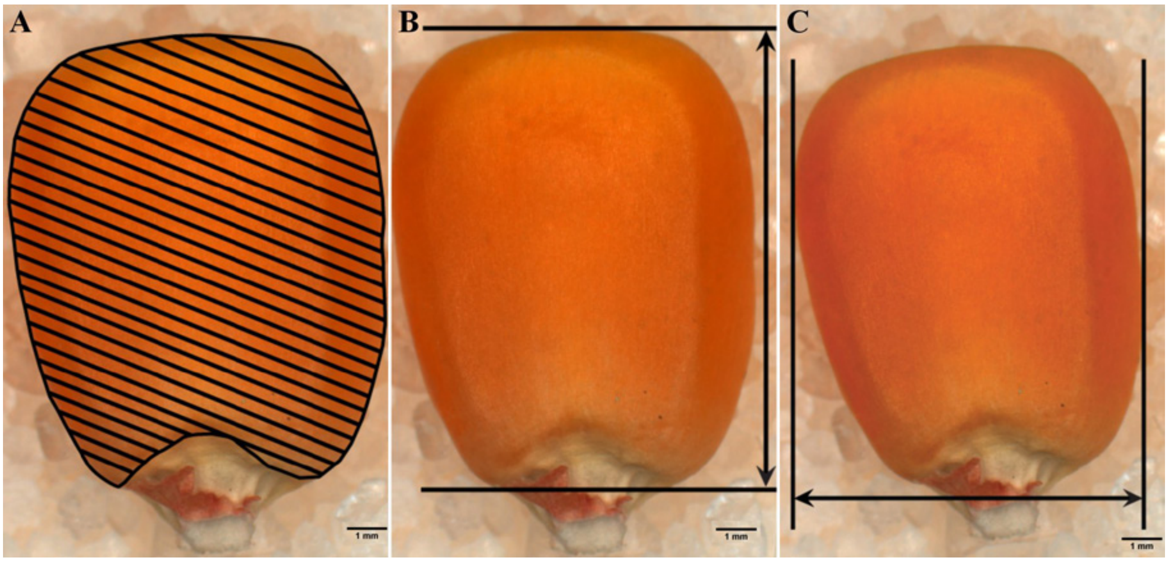

Figure 3 Parameters measured on the ventral side of kernel: A – area (mm^2), B – height (mm), C – width (mm)

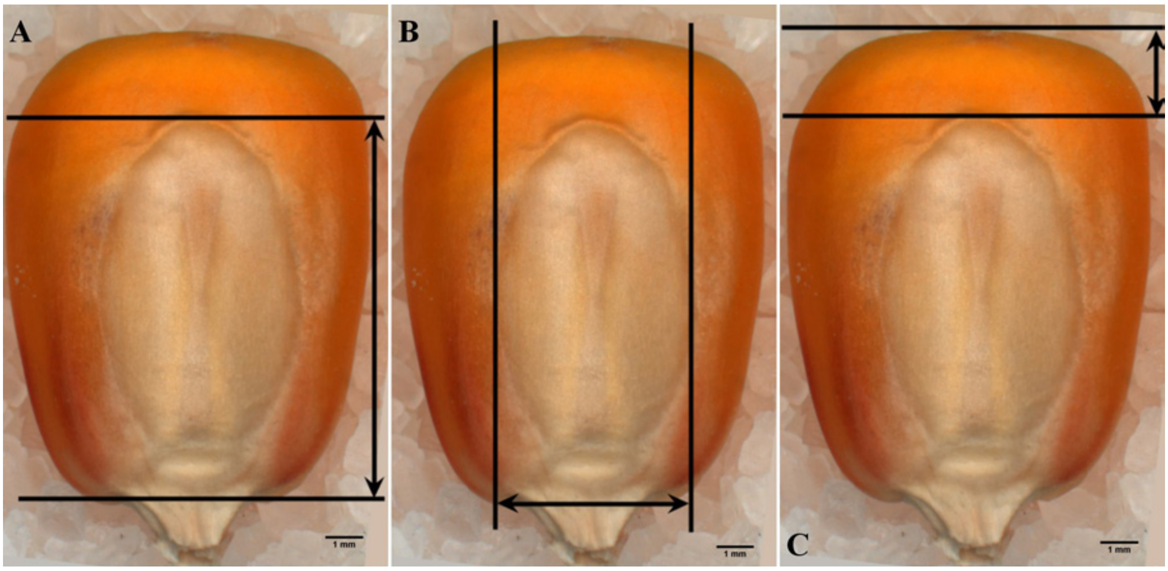

Figure 4 Parameters measured on the dorsal side of kernel by tool distance: A – length of embryo (mm), B – width of embryo (mm), C – the subtraction of embryo length from kernel length (mm)

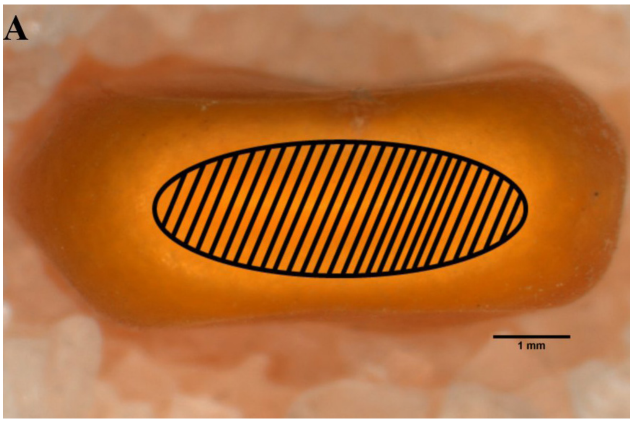

Figure 5 The area measured on the depressed part of corolla (mm^2)

were elaborated by open source software ImageJ and FigureJ (Schindelin et al., 2015; Mutterer and Zinck, 2013).

3 Results and discussion

In the article are presented the results obtained by measuring the traits on ventral and dorsal side of kernel, kernel corolla, and area of floury endosperm on kernel lateral section.

From the ventral side of kernel, we displayed the results of the following traits – area (mm^2), kernel width – measured by tool Bound height and kernel length – measured by tool Bound width (mm). Coefficient of variation was less than 10% for each trait (n = 50). It means that the maize kernels of each hybrid were even in evaluated traits.

The figure 7 illustrates the results gathered by measuring the area of the whole kernel (mm^2). The data show that the average values of area ranged from 69.67 mm^2 (ZE EDOX) to 89.06 mm^2 (ZE ELMO). The hybrid ZE ELMO and ZE KARUZEL differed significantly (P <0.05) from all others hybrids.

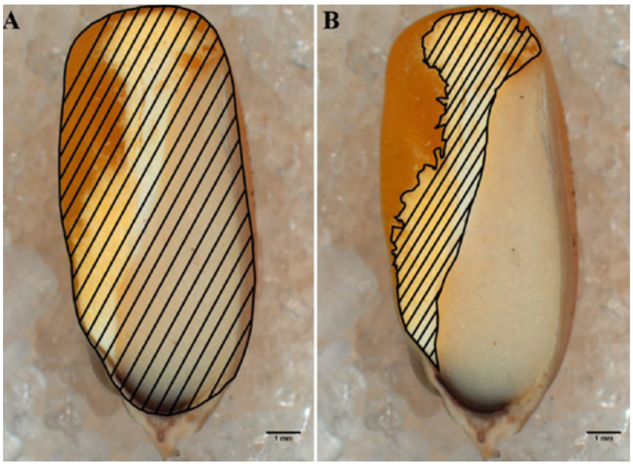

Figure 6 Parameters measured on the kernel lateral section: A – area of cross-section (mm^2), B – area of floury endosperm (mm^2)

The second chart (Fig. 8) demonstrates the width of maize kernel which was measured by tool Bound height (mm). The data show that the widest average values reached the hybrids – ZE ELMO (9.14 mm), ZE KARUZEL (9.15 mm), ZE ZELMA (8.93 mm), and ZE EDOX (8.87 mm). The hybrid ZE 4501 reached the smallest value (8.12 mm).

The chart displayed in the figure 9 shows the results obtained by measuring the length of maize kernel. It is evident that the hybrid ZE EDOX (10.15 mm) significantly differs from other hybrids. Hybrid ZE ELMO reached the highest average value (11.97 mm) and also is significantly different from all other samples.

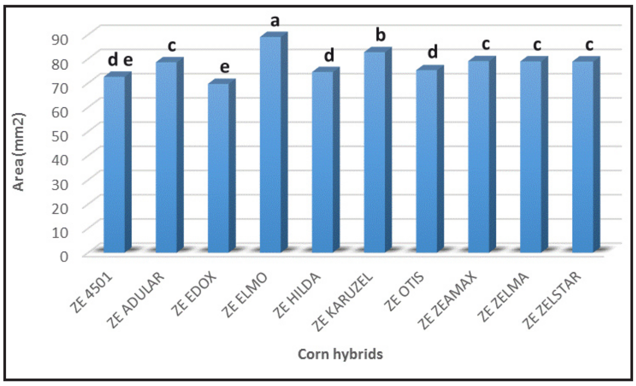

Figure 7 The area of kernel from ventral side (mm^2)
Legend: *n* = 50; different letters (a, b, c, d, e, f) point out significant difference (*p* <0.05)

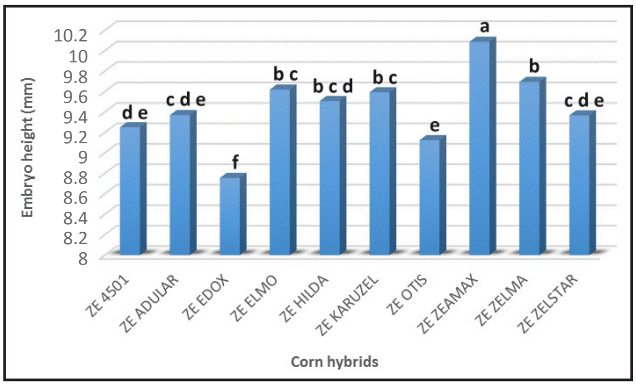

Figure 10 The length of embryo on dorsal side of kernel (mm)
The legend is the same as in Figure 7

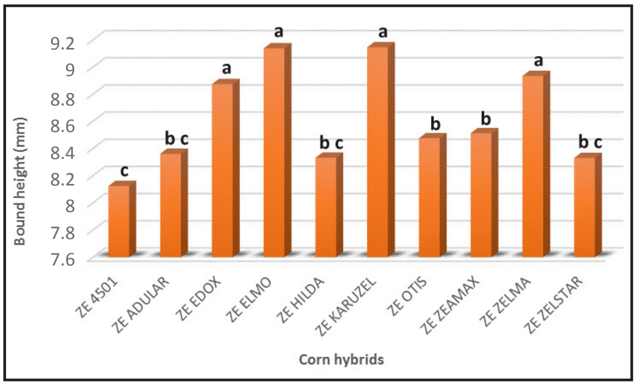

Figure 8 The width of kernel measured by Bound height (mm)
The legend is the same as in Figure 7

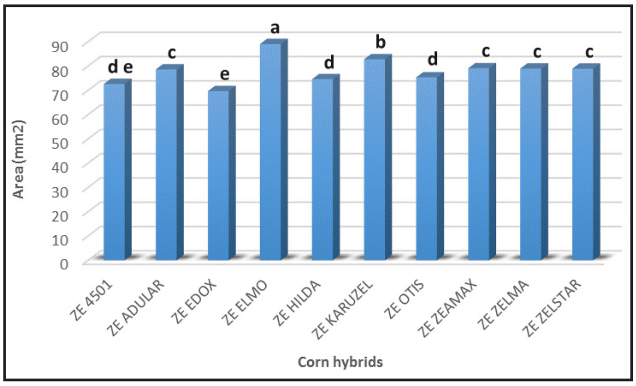

Figure 11 The area of depressed part on corolla (mm^2)
The legend is the same as in Figure 7

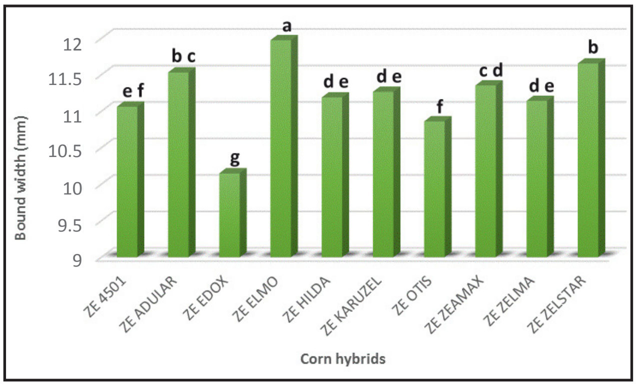

Figure 9 The length of kernel measured by Bound width (mm)
The legend is the same as in Figure 7

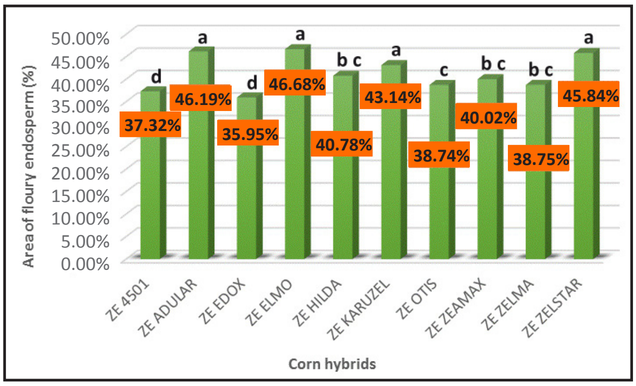

Figure 12 The proportion of floury endosperm measured on kernel lateral section (%)
The legend is the same as in Figure 7

On the dorsal side of kernel, we measured the embryo length (mm) and the results are documented in the Fig. 10. The smallest average value obtained hybrid ZE EDOX (8.75 mm) which is again significantly different from other hybrids. The highest average value had ZE ZEAMAX (10.09 mm), also differs from all other hybrids.

Another interesting morphological trait, which was measured, was the size of depressed part on kernel corolla. We assumed that the larger size of this area will cause the higher proportion of floury endosperm. This assumption was built on information that depressed part of corolla is the result of cracks in floury endosperm (Robutti et al., 1974; Erasmus, 2003, Guelpa et al., 2015), so the larger depressed part means the more cracks and higher proportion of floury endosperm. The cracks can be the result of the endosperm dehydration (De Carvalho et al., 1999; Guelpa et al., 2015). So we expected that there will be significant differences between different types of maize hybrids.

The smallest depressed area of corolla was measured on the hybrid ZE EDOX (8.11 mm^2) significantly different from all other hybrids. The largest area was reached by hybrid ZE KARUZEL (23.98 mm^2) also significantly different from other hybrids (Fig. 11).

According to literature sources we know that the floury endosperm is placed into the center of the kernel (De Carvalho et al., 1999; Guelpa et al., 2015). Due to this we measured the area of the floury endosperm on the kernel lateral section. The results displayed in the Fig. 12 show that the smallest area was reached by hybrid ZE EDOX (18.6 mm^2 – 35.95%), which was together with ZE 4501 (18.04 mm^2 – 37.32%) significantly different from other hybrids.

4 Conclusions

From the mentioned results we can conclude the following findings:

- According to obtained results we can confirm that the chosen parameters evaluated on kernels by the use of image analyses software, can be good tools for identifying the maize hybrids. The hybrid ZE ELMO differed from all other hybrids almost in all cases. The hybrids ZE ADULAR, ZE ZELSTAR were significantly different only in one trait.
- The coefficient of variation was less than 10% in the cases of traits measured on ventral side of kernel, and on embryo. But the traits measured on depressed part of corolla and on floury endosperm reached higher than 20% the values of coefficient of variation. In this case the tools for image segmentation should be improved.
- The hybrid ZE ELMO (dent type, kernel use hybrid) had the largest kernels (trait area), the second largest the area of depressed part on corolla, and also the highest proportion of floury endosperm. On the other hand, the hybrid ZE EDOX (flint to semi-flint, kernel and ensilage use hybrid) had the smallest kernels (trait area), the smallest area of depressed part on corolla, and also the smallest proportion of floury endosperm. This can prove our assumption that the higher proportion of floury endosperm in the kernels can cause larger area of depressed part on corolla, and as a consequence to this more cracks in the floury endosperm.
- In this experiment the new trait – area of depressed part on corolla was used. We suppose that this trait has connection with the proportion of floury endosperm in the maize kernel. The larger area of this part of corolla can cause the larger proportion of floury endosperm. This statement was confirmed by hybrids ZE ELMO, ZE KARUZEL, ZE ADULAR, and also by hybrids ZE EDOX, ZE 4501 (small area of depressed part, small proportion of floury endosperm). This trait can be used non-destructive way.
- The image analysis method for evaluation of size and different parts of maize kernel can be used for determination of kernel types, and proportion of vitreous and floury endosperm. This is proved by statistically significant differences between examined hybrids.

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