

Quantitative method for determining cell structural parameters of plant tissues**

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Received January 3, 2001; accepted April 19, 2001

Abstract. The present work focuses on a method for quantitative evaluation of cell structural parameters of plant tissues such as: surface area, perimeter, Feret's diameters, elongation. The method is based on analysis of tissue section images obtained by an optical confocal microscope (Tandem Scanning Reflected Light Microscope - TSRLM). Examples of structural parameters distribution for the inner core of potato tuber parenchyma of Danusia variety (*Solanum tuberosum* v. Danusia) are presented.

Key words: cell structural parameters, image analysis, parenchyma tissue of potato tuber

INTRODUCTION

Biological raw materials of plant origin produced by agriculture are heterogeneous with a discrete cell structure. Long-term studies showed that microstructure influences on the properties of plant media, and especially on their mechanical parameters (Gao and Pitt, 1991; Haman and Konstankiewicz, 2000; Haman *et al.*, 1999; Konstankiewicz and Zdunek, 2001; Pawlak and Król, 1999). To lessen the negative results of mechanical influences, it is necessary to get to know the structure and physical processes that accompany deformations. A dominant destructive process for such objects is cell structure cracking (Haman and Konstankiewicz, 2000; Konstankiewicz and Zdunek, 2000; Mohsenin, 1979). An initial physical status of the medium exerts a decisive influence on its creation and propagation, mainly the structural parameters related to variety or type of tissue (Fornal, 1998; Haman *et al.*, 1999; Konstankiewicz and Zdunek, 2001).

Similarly to other materials, mechanical properties of plant tissues depend on the parameters of its microstructural composition. This hypothesis is confirmed by theoretical models that describe mechanical properties of

plant tissue (Haman and Konstankiewicz, 2000; Pitt and Chen, 1983). A common feature of the proposed models is a special role of cell walls as basic elements of the construction skeleton. Whereas, cell sizes influence on tensile stresses in their walls and the cracking process in the whole structure (Haman and Konstankiewicz, 2000; Konstankiewicz and Zdunek, 2001). However, so far theoretical models were not verified experimentally, mainly due to the lack of quantitative methods for the description of cell structural parameters.

The problem of describing the structure, i.e., cell shape and cell sizes, is complex and difficult both from the technical and interpretation point of view. Obtaining of the plant tissue microstructure by the latest microscopic methods is easy. However, preparation of objects for observations is necessary in most cases (Fornal, 1998). It increases costs of such an experiment as a longer time period required for preparation as well as special reagents that must be applied according to individual methods of preparation. It is also necessary to take into consideration the influence of sample preparation on its structure at the initial stage of such an experiment, which poses an additional problem. There are also microscopes that are used to observe and obtain images of plant tissues in the natural state without any preparation. Such methods are quick and easy. A microscope proper for samples in natural - state is the optical confocal microscope (Petran *et al.*, 1995).

The aim of this work is to present a quantitative method for obtaining parameters of plant tissue cell structure. The method was applied for potato tuber tissue sections of Danusia variety. In the paper distributions of some chosen cell structural parameters of parenchyma inner core are presented as an example of the application of the presented method.

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**This work was supported by the Polish Committee of Scientific Research under Grant No. 5 P06F 01317.

MATERIALS AND METHODS

The inner core of potato tuber parenchyma variety Danusia (*Solanum tuberosum* v. Danusia) was chosen as an exemplary structure of a plant material. Potatoes came from the 1999 harvest carried out in the Department of Potato Storage and Processing of the Institute of Plant Breeding and Acclimatization in Jadwisin.

A slice 20 mm thick was cut off the inner part of the potato tuber, perpendicularly to the stolon-apex axis. Next, cylindrical samples of 7 mm in diameter and 20 mm in height were cut by core-borer from inner core of parenchyma tissue according to direction stolon-apex axis. After that, the cylinder was cut up by means of a special apparatus equipped with three parallel blades. It allows to obtain two samples: Sample 1 and Sample 2 (Fig. 1). The Sample 1 can be used for supplementing determinations (mechanical properties, turgor, etc.), whereas the Sample 2 was used for microscopic observations. In the present study, the Sample 2 had a cylindrical shape with diameter of 7 mm and height of 1 mm. Immediately after cutting off, Sample 2 was rinsed in distilled water in order to remove starch remains from its surface, and the excess water was delicately removed with filter paper.

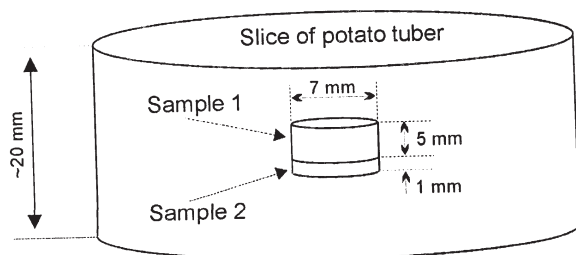


Fig. 1. Potato tuber sampling (un-prepared sample).

Next, the Samples 2 were subjected to observation by means of an optical confocal microscope (Tandem Scanning Reflected Light Microscope - TSRLM), (Petran *et al.*, 1995). A Plan 10/0.25 lens was used for the present observations, and the observations were carried out at the magnification of about x20. It allowed for observing from 10 to 15 full cells in one observation field (image).

TSRLM allows to obtain microscopic images of the plant tissue sections in their natural state similar to this one presented in Fig. 2A. It is easy to distinguish cell walls, visible in the form of thin lines. However, other elements are also visible, e.g., surfaces of cell films, that make direct application of computer analysis difficult. In order to distinguish the structural elements of interest, e.g., cell walls univocally, each image was subjected to manual processing. Each cell walls were drawn on the background of the original microscopic picture by means of the Corel Draw® software. That way a skeleton of the structure in the form of closed polynomials representing two-dimensional cells of plant tissues were received, as in Fig. 2B.

The images prepared that way fulfill basic conditions necessary to automatic computer analysis, i.e.:

1. Images are binary - values of individual pixels of the image take on only one of the two possible values (0 or 1), which is indispensable to qualify a given pixel either as the object of interest for us or its background;
2. Pixels with the value of 1 form the structure of cell walls of the studied tissue and determine its location and shape univocally.

Binarisation is one of the final stages of any quantitative image analysis. Following measurements were carried out by means of specially worked out computer procedures indispensable to find out the interiors of individual, separated cells on the basis of their skeletons made up of pixels with the value of 1 (Fig. 2C). Next, each cell in the picture was described by cell area, cell perimeter and other parameters related to the cell shape such as Feret's diameters and

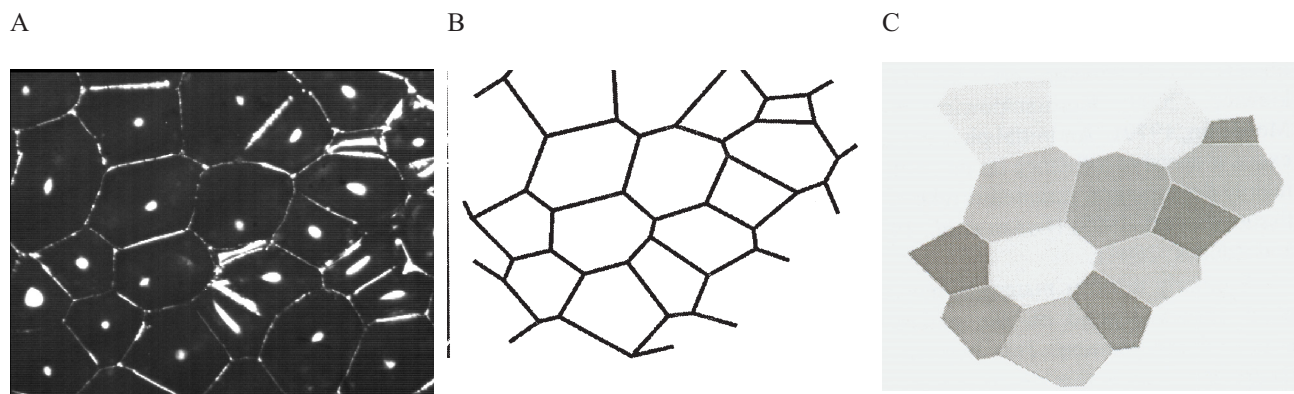


Fig. 2. Microscopic image of potato tuber tissue obtained by confocal microscope (Tandem Scanning Reflected Light Microscope - TSRLM) - A, the image prepared for analysis - B, and the image after analysis with the cells found - C.

elongation - e , that has be defined in a graphic form in Fig. 3. The results obtained in pixels can easily be calculated into the units of length by comparison with object of a known planar size.

About 10-15 random and non-overlapping microscopic images were obtained for each Sample 2. The above procedure was carried out for 30 Samples 2. As a result, the cell structure of inner core of potato tuber was described by 3720 full cells. It allowed to carry out statistical analysis and to obtain distributions of basic structural parameters of plant tissue.

RESULTS AND DISCUSSION

Statistical analysis for 3720 cells allowed to obtain distributions of the following structure parameters: cell area, cell perimeter, cell minimum and maximum Feret's diameter, cell elongation (Fig. 4).

The data was then subjected to the Kolmogorow - Smirnow's normality test. The tests showed that at the level

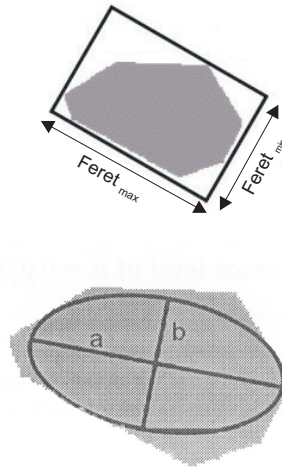


Fig. 3. Method for determining cell shape parameters, i.e.: Feret's diameters ($Feret_{max}$, $Feret_{min}$) and elongation ($e = (a-b)/(a+b)$).

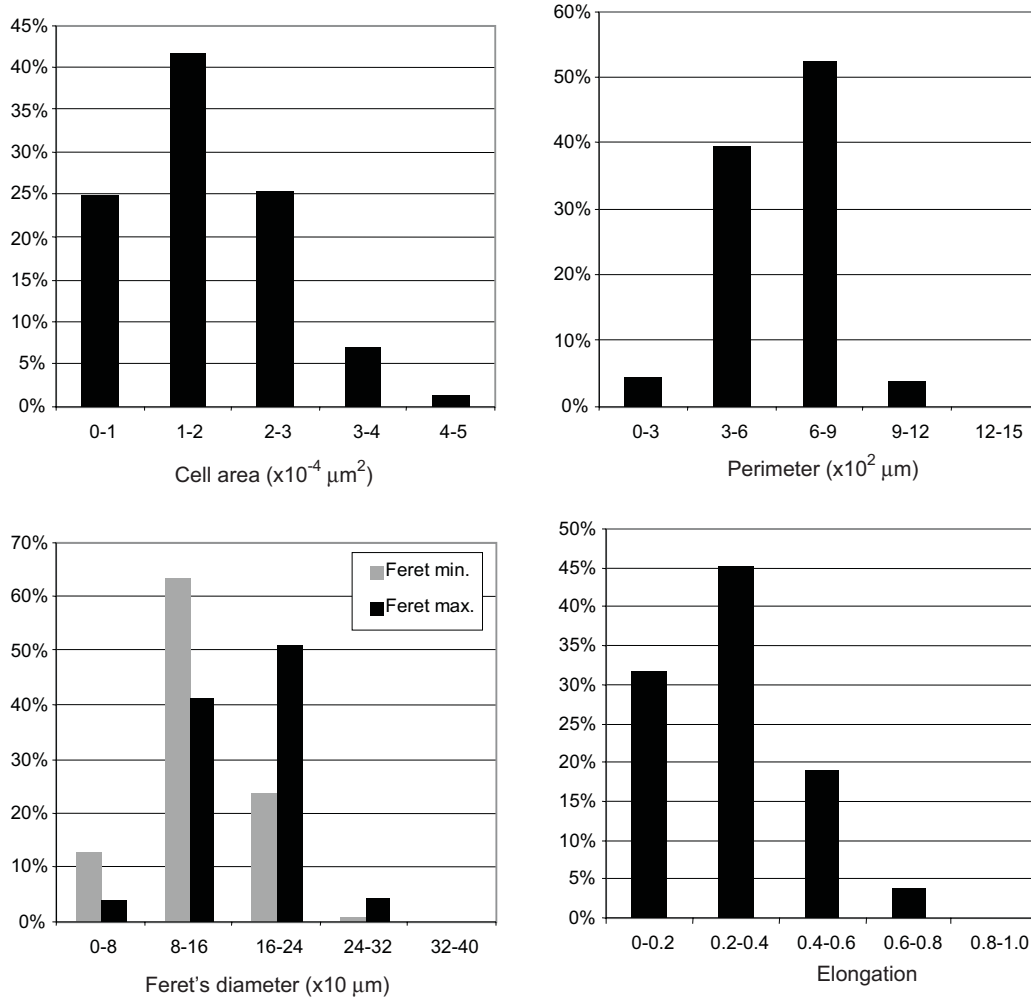


Fig. 4. Examples of distribution of some chosen cell structure parameters of the potato tuber tissue, Danusia variety, 1999 harvest (3720 cells).

of significance of $\alpha=0.05$, the D_{max} statistics was not significant only in the case of cell perimeter. It means that the remaining parameters: cell area, cell minimal (Feret_{min}) and maximal (Feret_{max}) Feret's diameter and cell elongation (e) were not subjected to the normal distribution. However, at such a high number of trials (cell number $n \gg 100$), evaluation of likelihood of mean values can be carried out by means of confidence intervals. Mean structural parameters of inner core cells of Danusia variety with confidence intervals (significance level of $\alpha = 0.05$) are presented in Table 1.

Table 1. Mean values of cell areas, cell perimeter, cell maximal and minimal Feret's diameters and cell elongation for the cells (3720) of the inner core of potato tuber Danusia variety, with standard deviation and confidence intervals at a significance level of $\alpha = 0.05$

Statistics	Area (μm^2)	Perimeter (μm)	Feret _{max} (μm)	Feret _{min} (μm)	Elongation
Mean	16946	616	157	125	0.292
Stand. dev.	9037	168	43	39	0.154
Confidence int.	290	5	1	1	0.004

It should be stressed that the results of the presented method are not related to cells as three-dimensional objects with no closely specified shapes but to their cross-sections.

The method of image obtaining by TSRLM is very quick. Microscopic images are recorded at once. Time necessary to obtain one image together with its writing into the computer memory is no longer than a few seconds, so one sample (of up to 20 images) is viewed for no longer than a few minutes. It does not let to drying the sample in the stable room temperature. On the other hand, following stages of presented method (preparing images to computer analysis) is very labour-consuming: an experienced observer needs a few minutes for one image.

Hence, it is very valuable as it is possible to observe plant tissues in their natural state without any preliminary preparations. An additional advantage of this method is a possibility of analyzing also images with inferior quality in which there is a higher number of element inclusions. The method can also be used for various plant tissues if it is possible to obtain an image with cell walls clearly visible by the optical confocal microscope (TSRLM). Computer image analysis is independent of the tissue type, univocal and automatic. At the moment there is no other method that would allow to obtain similar results.

A special advantage of the presented method is the possibility of its application in the mass studies that require multiple repetitions and statistical elaboration. It is also suitable for characterizing plant tissues in respect to their structural parameters such as: variety properties. It can also be used when looking for correlation with other physical properties (strength, elasticity, etc.).

CONCLUSIONS

The method presented in this research can be used for the quantitative analysis of structural parameters on the basis of images of plant tissues obtained by means of a confocal microscope that allows for the observations of objects in their natural state. The presented procedure for image analysis is universal and can be applied for any plant tissue type, and when supported by computer software, it is univocal and automatic. It is especially useful for carrying out studies on a large number of samples that require statistical analysis. The presented results obtained for the potato tuber parenchyma of

Danusia variety, show the potential for obtaining distribution of such structural parameters as cell area, cell perimeter, cell Feret's diameters and cell elongation.

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