METHOD FOR THE MICROSCOPIC IMAGE COMPILATION OF THE POTATO TUBER'S CELLULAR STRUCTURE*

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The proposed method of image compilation is used to obtain a picture with a higher number of structural elements than in a single image. Flat images of plant tissue obtained by means of an optical confocal microscope with a controlled shift of the objects studied in the X-Y plane were used for the present study. The results of the pictures, consisting of 16 single images were presented; in total, 40% more cells for analysis were obtained than the count of cells obtained from single images. The number of cells obtained is sufficient to carry out statistical analysis of structure parameters. Examples of results obtained for four potato varieties taking into consideration the inner and outer core have been presented. The method can be applied to any microscopic image of good quality, which ensures the precise combination of the structural elements in the neighbouring images.

microscope image; parameters of cellular structure; potato tuber tissue

INTRODUCTION

Highly developed technologies require more and more knowledge of material properties. The above is also true of agricultural raw materials used both for direct consumption and industrial processing (Wilkinson et al., 2000). In both cases, requirements as to the raw material's quality and the quality of the end-product increase, as well as to the monitoring of the properties of the material during the whole production process. The potato, *Solanum tuberosum* L., is a common commercial plant, and potato products are a popular component of our diet. They are also widely used in industry.

The structure is one of the most important properties of the material directly associated with other properties of the material centre. Studies have shown that, among others, the microstructure influences the mechanical resistance of plant tissues (Pitt, Chen, 1983; Zdunek, Konstankiewicz, 2001), colour and taste (Reeve, 1968), which undergo changes during drying (Wang, Brennan, 1995), freezing (Da-Wen Sun, Bing Li, 2003) and also as a result of heating (Aguilera et al., 2001).

To show the complexity of plant tissue structures, microscopic images obtained by various techniques, are used. However, most often such structures are evaluated descriptively (Fσrnal, 2002), and it is only possible to utilise structural studies, when the structure is described numerically (Kalab et al., 1995; Rys, 1995).

In order to describe the structure of a plant's tissue, especially its changes as a result of all kinds of impacts, it is necessary to carry out observations preserving the most natural state possible of the object studied. Microscopic methods require complex procedures of preliminary sample preparation and should take into account any structural changes at this stage of the examination (Konstankiewicz, 2002; Petran et al., 1995).

The most frequent microscopic images of the structure are flat cross-sections of such a structure. Quantitative analysis of such images is limited to the determination of the geometrical parameters of the structural elements and their location in relation to one another. The lack of universal methods and computer procedures, which could be applied for various types of materials is a very difficult to this type of study (Cwajna et al., 1994; Konstankiewicz et al., 2002a).

In order to carry out a quantitative analysis of the structural parameters of plant tissue, it is necessary to have a high-quality microscopic image and a sufficient amount of structural elements; in the case of plant tissue, these elements are cells. Plant tissue is characterised by a heterogeneous structure, and very often there are only a few cells in the one microscopic image. It is therefore necessary to work out a method to obtain composite images (Czachor et al., 2000).

This study presents a method of compiling flat microscopic images of the parenchyma tissue structure of potato tubers in their natural state. An optical confocal microscope, equipped to precisely shift an object along an X-Y plane was used for the examination. Parameters of the cell structure for single images and images compiled of 16 single images were determined. The experiment was carried out on four potato varieties, taking into account two types of tissue: inner core and outer core.

MATERIALS AND METHODS

The study material consisted of potato tubers (*Solanum tuberosum* L.) of four Polish selected varieties, i.e.: Danusia, Kuba, Mila, Triada. All varieties were

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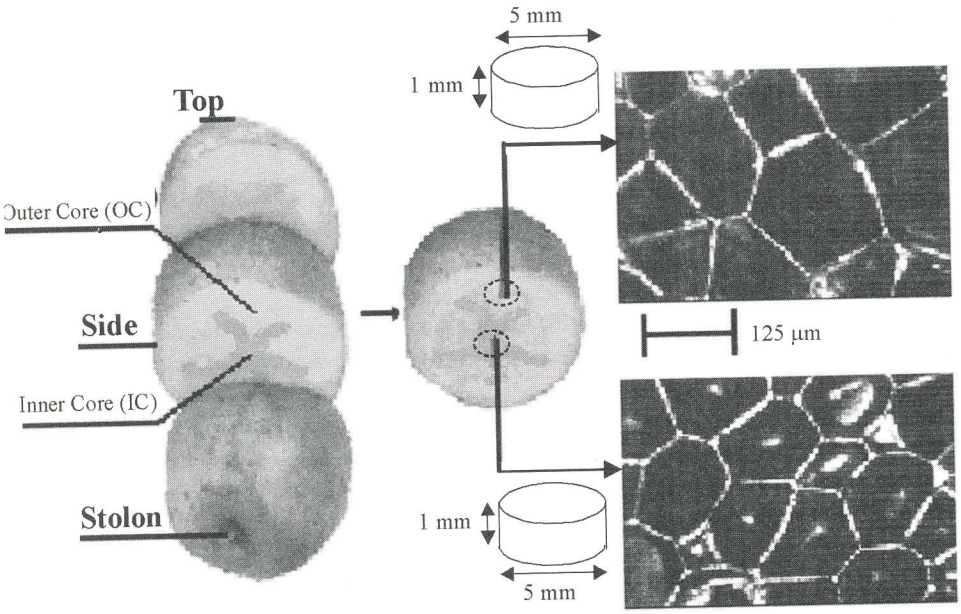

Fig. 1. Layout of sample collection for observation in the optical confocal microscope with an example of images of the parenchyma tissue structure of potato tubers taking into consideration the inner and outer cores

grown under the same conditions, and harvesting was carried out at full maturity. The highest content of dry mass and starch was found in the var. Kuba, and the lowest in tubers of the var. Danusia, whereas varieties Mila and Triada had a similar value of both components in the inner core and outer core.

Studies were carried out on tubers of medium size and without any outer damage, directly after the harvest. The present experiment was carried out at a constant room temperature (~20 °C) and relative air humidity of (50–60%).

Samples for the microscopic examination were collected from the central part of the tuber at two places, the inner core (IC) and the outer core (OC) according to the experimental scheme presented in Fig. 1. Samples with a cylindrical shape were cut out with a screw-drill with a diameter of 5 mm, and then 1 mm-thick slices were cut out by means of two parallel blades of the guillotine type. To enable better cell differentiation, starch was removed from the slice surface by quickly rinsing them in distilled water. Then the slices were placed on glass micro slides under the microscope for the observation.

An optical confocal microscope "CONFOCAL 2002" equipped with 20/0.4 object lens and a precise, continuous object shift along the X-Y plane was used for the preparation of the microscopic image of the tissue studied. Studies were carried out for the tissue in the natural state without any preliminary sample preparation. The whole observation lasted about 1 minute, what ensured that there was no drying out of the samples.

The controlled manner of the microscope table shift enabled an original method for compiling individual images during one observation of a series to be worked out. The precise combination of the adjacent structural elements of single images resulted in an image of a bigger surface area of the object studied, and hence in the higher number of visible cells. A quantitative analysis of a cell's structural parameters was carried out on the basis of a single flat image and a composite image consisting of 16 single images according to the method elaborated earlier (Konstankiewicz et al., 2001).

Ten images were made of each variety (5 images for each core) and each image consisted of 16 single images. Both for the composite image and single images, the number of whole cells visible for which the mean values of the geometrical parameters of their structure, was determined.

RESULTS AND DISCUSSION

The images of the microscopic structure of the parenchyma of potato tubers obtained from the varieties studied showed different cell structures and differ in relation to the place of the collection of the sample within the specific tuber. Some examples of results taking into consideration the outer and inner core for the four varieties studied, were in Fig. 2. In all cases, the cell of the outer core was bigger than the cells of the inner core. For example, in the case of var. Danusia, there is one whole cell in the image and for the var. Kuba, there are several whole cells in one image.

To obtain a higher number of cells in one image, a method of combining several single images was elaborated. An optic confocal microscope with a precise shift of the object observed in the X-Y plane was used for this purpose. The images of the parenchyma tissue of potato tubers observed were of very good quality with clearly visible cell walls, making the precise fitting of the cell walls of the adjacent images possible. Individual phases of the creation of a composite image have been presented in Fig. 3. Composite images consisting of 16 single images were used for the quantitative analysis of the structural parameters. One technical limitation is the providing and ensuring of the appropriate moisture level for the samples. In our study conditions, at fixed room temperature and humidity level, the time period for the picture

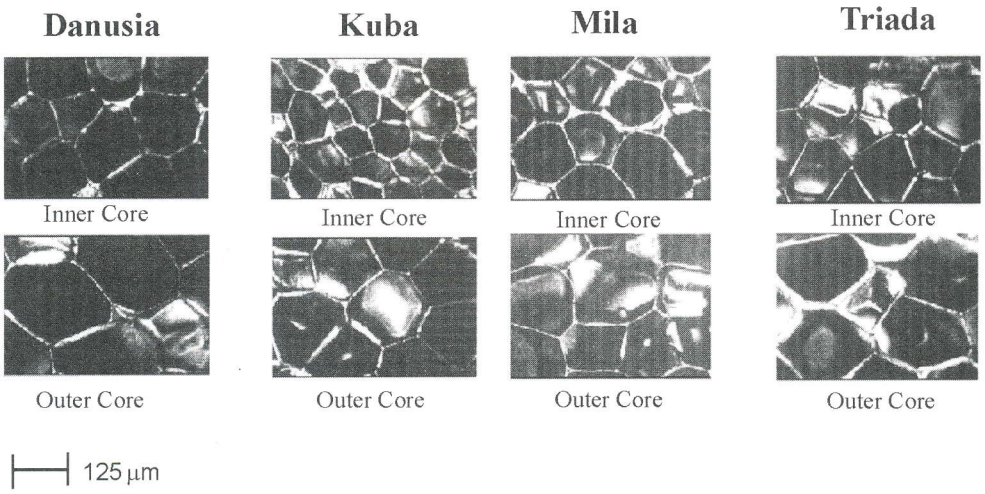

Fig. 2. Examples of microscopic images of the cell structure of the parenchyma tissue of potato tubers from the four varieties studied, taking into account the inner and outer cores

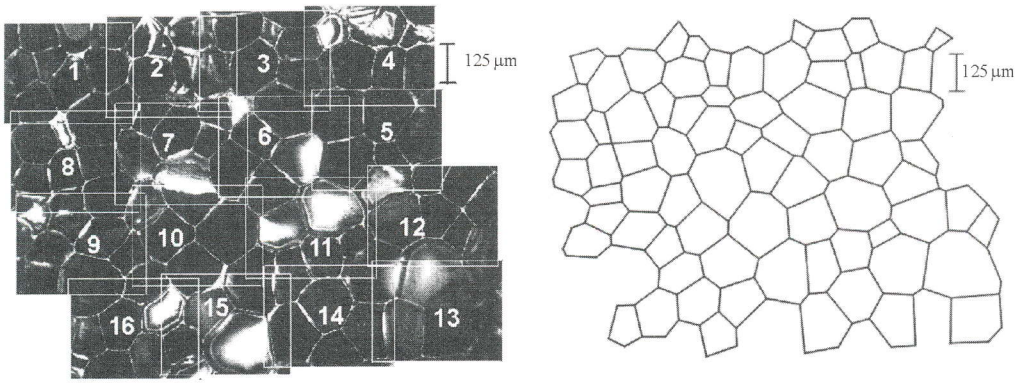

Fig. 3. A layout of the compilation of a composite image consisting of 16 single images and a respective imaged with cells prepared for the analysis of the structural parameters

composition was about 1 minute. Any longer observation period would not be possible due to the worsening of the image quality. In the case of potato tuber tissue, 16 individual images enabled scores of the whole cells to be obtained in one image. The count of cells obtained from the analysis of single images and composite images for the four potato varieties was presented in Table 1. With such a high number of cells it is possible to compare the mean values of the parameters measured (Konstankiewicz et al., 2002b).

The mean values of structural geometrical parameters, such as: surface area, circumference, Feret's diameter, elongation and compactness, together with standard deviations, are presented in Table 2. In order to compare parameters for the two core types, i.e. the outer and the inner, the 'Kolmogorov–Smirnov λ Compatibility Test' for all results obtained was carried out on the level of significance of 0,05; cases for which the values determined did not significantly differ were marked with the symbol *). Obviously, other comparisons of the parameters values are also possible, i.e. among varieties, harvest terms, storage conditions, etc.

Table 1. Number of cells of the inner (IC) and outer (OC) core for all varieties obtained for the single microscopic images of potato tissue and for the composition images

Variety	Danusia		Kuba		Mila		Triada	
Core	IC	OC	IC	OC	IC	OC	IC	OC
Single images	277	280	846	436	310	342	326	348
Composition images	454	474	1376	742	528	599	501	564

CONCLUSIONS

The method of image compilation presented here makes the increase of the number of structural elements for one sample possible. In the present study, flat images of plant tissue obtained in the optic confocal microscope were used.

The studies carried out showed that the number of cells which can be obtained from the same image was different when the single image was analysed as compared to a composite image made of such single images. The study presents results for composite images consisting of 16 single images.

The method presented can be applied to each microscopic image of good quality which ensures the precise compilation of structural elements on the adjoining images.

Studies carried out on four potato varieties taking into account the outer and inner cores, enabled the determination of the geometrical parameters of the cell structure for a composite image and the formulation of the following conclusions:

– The method of image compilation enables 40% more cells to be obtained than the number of cells obtained from single images.

Table 2. Mean values of the structural parameters measured with standard deviation for all varieties and types of core

Parameter	Variety							
	Danusia		Kuba		Mila		Triada	
	OC (s.d.)	IC (s.d.)	OC (s.d.)	IC (s.d.)	OC (s.d.)	IC (s.d.)	OC (s.d.)	IC (s.d.)
A	13.1* (8.2)	13.8* (7.4)	9.1 (6.4)	5.5 (3.3)	11.2 (8.4)	13.3 (6.7)	11.9 (8.0)	11.5 (6.4)
P	5.59* (1.7)	5.52* (1.5)	4.47 (166.5)	356.1 (1.0)	4.9 (1.9)	5.48 (1.5)	5.17 (1.7)	5.06 (1.4)
F_{max}	148.0 (47.3)	146.6 (40.8)	122.7 (47.0)	95.7 (29.2)	133.7 (54.0)	145.0 (39.7)	138.8 (46.5)	134.3 (38.1)
F_{min}	112.0* (39.3)	117.5* (37.7)	90.1 (36.7)	73.4 (24.0)	99.7 (42.4)	115.1 (36.4)	105.6 (39.9)	107.3 (35.2)
F_{min}/F_{max}	0.76 (0.15)	0.79 (0.13)	0.74 (0.16)	0.77 (0.15)	0.75 (0.15)	0.79 (0.13)	0.76 (0.15)	0.79 (0.13)
E	0.33 (0.16)	0.28 (0.15)	0.35 (0.18)	0.32 (0.16)	0.33 (0.17)	0.29 (0.15)	0.32* (0.16)	0.27* (0.15)
C	0.60 (0.08)	0.66 (0.08)	0.62 (0.08)	0.63 (0.08)	0.63* (0.08)	0.64* (0.08)	0.62 (0.09)	0.65 (0.07)

A – area (10^3 μm^2), P – perimeter (10^2 μm), F_{max} – Feret's maximum diameter (μm), F_{min} – Feret's minimal diameter (μm), E – elongation, C – compactness, s.d. – standard deviation, OC, IC – outer and inner core, respectively, * – mark values not differ significantly

- The method allows observations to be undertaken of a larger surface of the tissue studied; the number of images combined depends on protecting the sample against drying.
- The number of cells obtained is sufficient to undertake statistical analysis of cell structure parameters.
- The inner core is characterised by smaller cells as compared to the outer core for the three varieties. Only in the var. Mila the above relation was reversed and the cell shape similar, irrespective of the site of sample collection or varieties.

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GANCARZ, M. – KONSTANKIEWICZ, K. – KRÓL, A. – PAWLAK, K. (Institute of Agrophysics, Polish Academy of Science, Lublin, Poland):

Metoda sestavování mikroskopického obrazu molekulární struktury bramborové hlízy.

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Tato metoda sestavování obrázku slouží k získání obrazu s větším počtem strukturálních prvků ve srovnání s jedním obrázkem. Pro výzkum byly použity ploché obrázky rostlinné tkáně získané z optického konfokálního mikroskopu s regulovaným posunem předmětů sledovaných na rovině X-Y. Jsou zde uvedeny výsledky snímků sestávajících ze 16 jednotlivých obrázků; celkově se pro analýzu získalo o 40 % buněk víc, než je počet buněk získaných z jednotlivých obrázků. Získaný počet buněk je dostatečný pro statistickou analýzu ukazatelů. V práci jsou uvedeny příklady výsledků získaných z odrůd brambor vzhledem k vnitřnímu a vnějšímu jádru. Tuto metodu lze použít na jakýkoli mikroskopický obrázek dobré kvality, který zajišťuje přesnou kombinaci strukturálních prvků u sousedících obrázků.

mikroskopický obraz; ukazatele buněčné struktury; tkáň bramborové hlízy

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