

MEASUREMENT OF THE DEVELOPMENT DEGREE OF THE SELECTED CROP ROOT SYSTEM BASED ON THE COMPUTER IMAGE ANALYSIS

Summary

Our paper describes a method of computer classification of roots of selected cereals using the digital images. The roots classification based on the digital images requires various processing algorithms and analysis to obtain the necessary data. The paper describes preparation methods of biological material and research samples as well as computer image processing and analysis, which was used in the practical part of the work. The discussed issues are the basis for the design and implementing an application for classification of selected cereal roots. The second part of the work concerns a description of the produced software that explains the basic functions that enable you to understand the program.

Key words: measurement of the roots, computer image analysis

POMIAR STOPNIA ROZWOJU SYSTEMU KORZENIOWEGO WYBRANYCH ROŚLIN UPRAWNYCH W OPARCIU O KOMPUTEROWĄ ANALIZĘ OBRAZU

Streszczenie

Praca dotyczy metody komputerowej klasyfikacji korzeni wybranych zbóż z wykorzystaniem obrazów cyfrowych. Klasyfikacja korzeni na podstawie obrazów cyfrowych wymaga zastosowania różnych algorytmów przetwarzania i analizy w taki sposób, aby uzyskać niezbędne dane. W pracy opisane zostały metody przygotowania materiału biologicznego i próbek badawczych oraz zagadnienia z dziedziny komputerowego przetwarzania i analizy obrazu, która wykorzystana została w praktycznej części pracy. Omówione zagadnienia były podstawą do zaprojektowania i wytworzenia aplikacji klasyfikującej korzenie wybranych zbóż. Druga część pracy to opis wytworzonego oprogramowania zawierający wyjaśnienie podstawowych funkcji programu, które umożliwiają zrozumienie działania programu.

Słowa kluczowe: pomiar korzeni, komputerowa analiza obrazu

1. Introduction

A laborious effort and technical difficulties related to the research of root systems make this subject rarely seen in papers concerning plant and soil sciences. Observations in vegetation study show a number of phenomena whose causes have not been explained yet. The lack of information about the root system makes it impossible to find a direct link between changes in the soil and the reaction of the crop [2]. Roots form a bridge between the substrate and the aboveground parts of plants. Through them, the plant is supplied with water and minerals extracted from the soil and maintains the vertical position. Thanks to numerous modifications roots perform many other functions: granary, shrink, support, respiratory, tacky. A lot of chemical compounds are created in roots, which are required throughout the plant. Moreover certain hormones, alkaloids or amino acids are produced in roots [8]. Most of the agricultural treatments is directly geared to ensure the roots of crops favorable conditions for the development and functioning. Soil tillage, fertilization, weed control, irrigation and other drainage treatments directly affect the root systems of plants and only indirectly influence the development of the aerial parts of plant. Scientific research, however, focus on the evaluation of the aerial parts omitting the root system reactions. Accurate knowledge of the roots reaction would explain many phenomena and

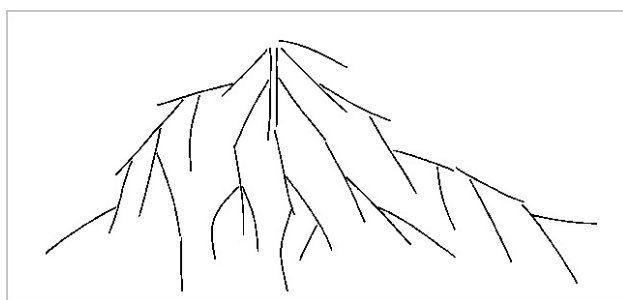
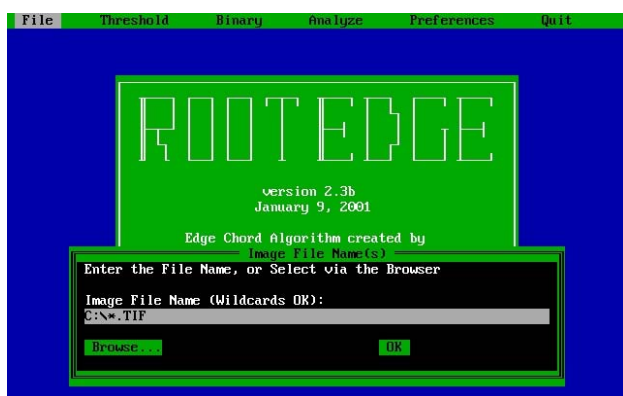
relationships that are observed but their causes are still a scientific mystery [4, 11].

The main cause of neglecting the root system in agro-technical research is considered to be a significant technical and methodological difficulties. Plant roots thrive in soil, so the direct observation is impossible. Sampling soil becomes necessary because from it samples of roots are extracted for further measurements. Plant roots occupy only a very small volume in the soil and at the same time develop a large absorbing surface and length of up to several kilometers for a single plant. They do not overgrow the soil uniformly, their number and diameter varies with depth and distance from the plant. All this for complete analysis requires sampling from many places. It is relatively easier to conduct tests on a laboratory scale. Growing of individual plant in a vase or no soil condition in hydroponics we can obtain full, intact root system of plant, on which measurement of mass, main root length, the number of first-order lateral roots, the number of embryonic roots can be carried out.

However, it is difficult to measure parameters, e.g. the total length of all the roots, their diameter, number or length of each size classes, the number of lateral branches that because of the enormous effort and technical difficulties which can be determined only in very small root systems, especially seedlings. Soil samples or separated roots can be preserved and stored for any length of time before

measurement. The entire process consists of four main phases: soil sampling, isolation and purification of roots, getting their clear picture and finally the measuring. The early methods of this group were based on carrying out all stages of the analysis manually. For measuring the density of roots different versions of the method of intersection were mostly used. The lattice of thin wires was put on the flat unfolded sample of the roots by hand and the number of intersections of the lattice and roots was counted.

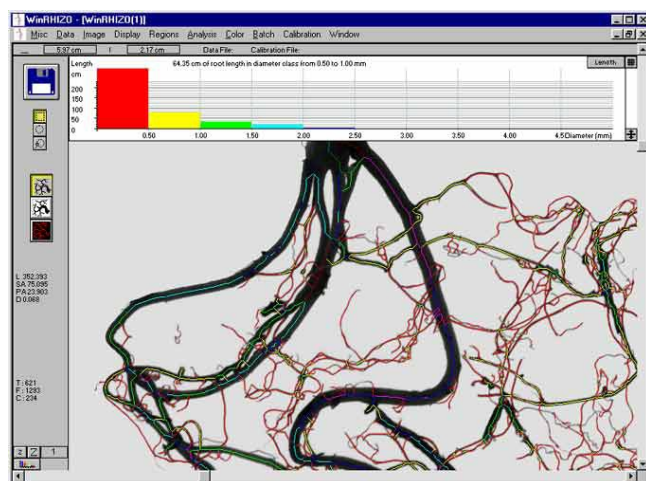
With the advent of the computer image analysis, the first programs to measure binary images of sampled roots were used. First versions gave the possibility of measuring the total root length of the sample, then came the ability to divide a sample into fractions depending on the diameter of the root. But these are simple programs (e.g. working in a DOS environment), created for specific research teams and adjusted for them specifically (Fig. 1).



Source: own work / Źródło: praca własna

Fig. 1. Program ROOTEDGE (left) and the appearance of the image file as required by the program

Rys. 1. Program ROOTEDGE (z lewej) i wygląd pliku graficznego w postaci wymaganej przez program



Source / Źródło: <http://www.regent.qc.ca>

Fig. 2. The program WinRHIZO of Regent Instruments Inc.
Rys. 2. Program WinRHIZO firmy Regent Instruments Inc.

The problem was in the need for extensive purification of samples and obtaining sufficiently contrasting image which required destaining the roots and the use of scanners with an additional light source. At the same time programs that allow you to make measurements on any image of the roots were produced, but they required manual tracking of the measured object with the mouse, e.g. WinRHIZO produced by Regent Instruments Inc. (Fig. 2) [3, 5, 6].

A common disadvantage of all the solutions consists in the lack of user-friendly work environment, which requires performing multiple manual tasks like the selection of a file for analysis, operating on the image and saving the results, which is particularly troublesome when analysing long series of measurements [7, 15, 16, 18].

In recent years we have noted the very rapid progress in the field of image analysis, image processing and computing capabilities of computers, giving a chance to develop a new generation of software, allowing to simplify procedures for obtaining an image and its processing, as well as enabling the measurement of parameters that could not have been measured before.

Not many papers on the growth and development of the root system can be found in Polish literature in the field of cultivation and crops, and most of them still operates with a simple parameter of the amount of dry matter. For some issues it is sufficient, but also points to the lack of appropriate solutions for more advanced studies in this field.

2. Materials and Methods

2.1. Preparation of experimental plots and crops in order to obtain samples of roots

The roots of corn, wheat and canola were used to work on the model of the evaluation process. The collection of specimens were selected with diverse agricultural techniques: tillage cultivation, no-tillage cultivation, direct seeding, mineral fertilization, straw fertilization, intercrop fertilization, manure fertilization, cultivation in monoculture and crop rotations. This provided morphological differentiation of collected roots and the diversity present in the samples of pollutants.

Samples were taken from experimental fields of the Department of Agronomy at the Poznan University of Life Sciences. Obtained soil samples were stored in a refrigerator or a freezer after preliminary treatment, depending on the further treatment time. To acquire the whole, intact root systems, plants were grown in a greenhouse at the Department of Agronomy, with the use of vases of 10 liters capacity. We grown wheat, canola and corn, one plant per vase. The samples were obtained every two weeks consecutively from plant emergence to the time when the developing root system has become too large for the measurement intact.

Pot growing was carried out on different surfaces. In the first stage of the soil derived from experimental fields of the Department of Agronomy (loamy sands light and strong) and after the development of the first version of the methodology of obtaining samples also on mineral soil with a much heavier granulometric composition (clay medium and heavy) and organic gardening substrates.

2.2. Acquisition and preparation of test specimens

Samples were taken from the roots of corn, wheat and spring wheat in full development of their root system, all

plants were after flowering. Samples came from traditionally cultivated plots and zero cultivation plots, to obtain various quality of the results referring to pollution by organic parts in different phase of root development. For test we used soil cores collected by auger with diameter of 8 cm produced by the company Eijkelkamp. The cores were extracted from soil layers of 0-10, 10-20, 20-30, 30-45, 45-60 cm, then placed in a well-described bags, kept in cold storage at 5°C for up to a week. Some tests were previously mixed and washed several times by hand in a bucket of water. A solution was obtained by pouring through laboratory sieves having a diameter of 20 cm and a diameter of mesh sieve of 0.5 and 0.25 mm. The time required to wash a sample was measured, also the whole method was observed for possible difficulties [1].

The remaining samples were washed using hydropneumoelutriation apparatus with its camera developed at Michigan State University in the United States [14]. On the basis of the described apparatus a prototype adapted to Polish conditions such as the availability of PVC pipes, tubes of different diameter, and a nozzle of a different liquid flow rate were constructed. After gaining roots and separation of impurities by tweezers they were transferred to containers with a volume of 125 cm³. Samples were stored in the freezer at -18°C temperature.

Thawed samples were thoroughly cleaned by pouring clean water several times and decanting. The roots settled on the bottom, and larger particles floated and were poured out. In order to obtain the pure samples of roots they were transferred to a flat dish and the impurities were removed manually by tweezers. The small impurities were subsequently removed by decantation technology. The water with roots was stirred and heavy dirt (e.g. weed seeds) sank on the bottom and the roots were lifted up and poured on the pan. Some samples were intentionally not purified to an end, because this is a tedious task, moreover they were used to estimate the possibilities of the proposed algorithms.

In order to obtain the appropriate image for the program, we analysed the research material. For the research material we selected roots of:

- corn,
- rye,
- wheat.

All samples were supplied frozen in containers of different degrees of soiling. Degrees of soiling can be divided into three groups:

- roots completely dirty, unwashed after pulling out of the ground,
- roots after the initial cleaning, cleaned of loose dirt,
- roots cleaned, cleared of major and minor dirt.

To obtain a digital image of roots, samples had to be defrosted. Attempts were made to scan any kind of roots, at any stage of dirtiness.

In order to obtain the best results, the roots were scanned in three different environments:

- wet roots, taken from the sample container,
- partially dried roots,
- dry roots,
- roots dipped in water in a suitable cuvette.

This trial showed that dried roots cannot be scanned due to high rate of contraction resulting from roots losing their volume.

The wet roots, while not entirely soaked in water were heavily matted, which meant that many branches of the root were not visible on the scanned image. The best images were obtained from the samples soaked in water in a suitable cuvette made of transparent glass not reflecting light. We used two types of water for scanning, distilled water and normal water in order to get the smallest loss of the quality and the lack of light reflection. For measurements in the RootDetector program wheat roots were chosen because of their large size which allowed us to test the system performance. The roots of wheat have also proved to be the most difficult case to investigate.

The roots can be scanned in various degrees of contamination due to the possibility of subsequent removal of dirt in the RootDetector by editing the image. Data acquisition of digital images, obtained for the setting up the system, took place using a scanner EPSON Protection V750Pro. After many tests the image acquisition parameters giving the best results were set as follows:

- image size: 3209x2513 px,
- resolution: 600 dpi,
- color: black and white.

Scanning was performed with a lamp placed in the scanner cover. After image acquisition, they were stored on the hard drive, and then loaded into the program.

The mixture of roots was placed in the transparent plastic plates 13x13 cm size, filled with shallow layer of water. The plates were put directly on the scanner's glass. Different scan parameters were used while searching for the maximum contrast between roots and the background (black - white). We had started by scanning the pictures in full color, eventually we were scanning using the following parameters: document type - film, the type of film - black and white, resolution - 600 dpi, without compression, the best quality, file format - bmp.

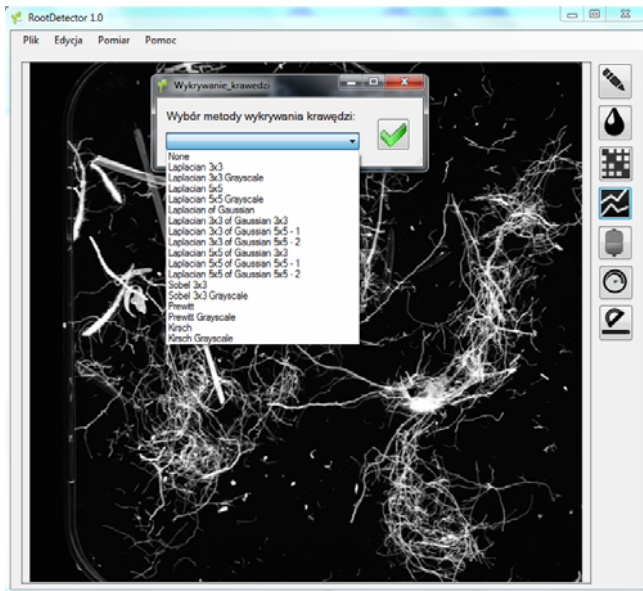
3. Processing and image analysis, testing methods adopted to obtain information about the roots

The "RootDetector" program and the algorithms that it uses were designed for the classification of the roots of cereals on digital images. The application is equipped with image thresholding function, roots edge detection, removal of impurities at the image. All functions available in the program and algorithms for implementing them are described below.

Prior to calculating the root perimeter, an edge detection pass is performed. The correct measurement requires detecting edges, algorithm returns edges of single pixel width. An additional limitation of the algorithm is occurrence of a single object, for multiple objects the result is a sum of their perimeters.

To make a correct perimeter measurement, image resolution must be specified in the dpi units (dots per inch - i.e. the number of dots per one inch) [9]. The algorithm works properly only on the binary image in which the edge detection is performed in such a way that all the edges are closed and one pixel wide [10].

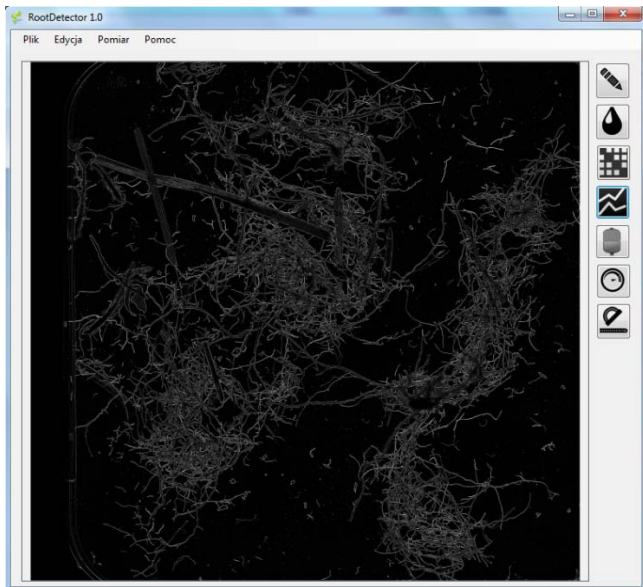
The algorithm used for calculating the perimeter explores the image by each pixel and counts all the white pixels that define the object's contour. Subsequently it divides the number of pixels found by the number of pixels per one centimeter. The algorithm returns the value of the perimeter in centimeters.



Source: own work / Źródło: praca własna

Figure 3. Drop-down list from where we can select the method of edge detection

Rys. 3. Lista rozwijana, z której możemy wybrać metodę wykrywania krawędzi



Source: own work / Źródło: praca własna

Figure 4. The effect of the algorithm for detecting edges

Rys. 4. Efekt działania algorytmu wykrywającego krawędzie

Diameter measurement algorithm moves the 3x3 mask through the image, first along the X axis and the Y axis within the mask, if there is a white pixel edge, the counter starts the algorithm for calculating the root diameter. The algorithm starts with the first encountered white pixel. Then checks the pixels in the X axis - five pixels towards the right and five pixels to the left. If five consecutive pixels are white we are dealing with a section of the root set horizontally. In this case, the diameter measurement is performed at a 90° angle. If five consecutive pixels are not white, we assume that we are dealing with a vertical section of the root and the diameter measurement is done by looking at a 180° angle. Each encountered black pixel increases the diameter by one. After encountering a white pixel, meaning the opposite edge of the root, the algorithm stores the number of pixels representing the diameter of the

root. Data are stored in a relational database for easy handling and analysis of the results. Before running the algorithm the user can specify the maximum root diameter. After exceeding the entered threshold value, the algorithm does not save diameter's length.

After measurement and storing the diameter value to the database the algorithm returns to the pixel from which the diameter was derived, the counter is reset to zero. The algorithm moves the 3x3 mask further until the density counter reaches the value specified by the user. After analyzing the image along the X axis, the algorithm tests the Y axis, in order to increase the accuracy of measurement.

4. System operation

Operation of the system is based on the actions the user must perform to achieve the desired effect. To start working with the program, load the images using the function menu "File" and then "Upload picture". Photos used in the program must have the following characteristics:

- can not be at a resolution lower than 600 dpi,
- the image must be previously subjected to binary thresholding
- it must be in a .bmp, .png, or .jpg image format.

Edge detection starts the operations on the analyzed image. The application allows for choosing from a number of methods. Methods of edge detection, which can be used in the application are described in previous chapters. The most effective method of detecting edges in measuring the diameter of the root is a method based on the Laplacian operator using 3x3 mask.

To use edge detection, select the button indicated in Figure 3 from the side menu. When we click the button, drop-down list opens where we can select the method of edge detection. We can also use edge detection choosing "Edit" from the main menu and then "Edge detection".

In the PictureBox we can see the edge detection results of the RootDetector program. Result of the operation depends on the chosen edge detection algorithm. On Figure 4 we can see the effect of the Laplacian algorithm 3x3.

4.1. Image correction

To eliminate pollution of the pictures (i.e. areas that are not the root), image correction module was introduced to the program. Selecting the appropriate button from the side menu launches the image correction algorithm. This module displays a new window with the original, unmodified image and an image with detected edges. Using a brush, we can paint over the areas that are not to be taken into account during analysis, the areas that can be painted over on an image with detected edges.

The thickness of the brush tool can be adjusted from 1px to 100px using the slider function.

Figure 22 shows a general view of the "Image adjustment" form. Two views are available for the user: the view of the original color image and the view of the processed image. In the lower left corner there is a preview, which simultaneously allows you to move around the image. The movement of the image causes the movement of the both loaded pictures.



Source: own work / Źródło: praca własna

Figure 5. Image correction module with the original image and visible image of the detected edges

Rys. 5. Moduł korekcji obrazu z widocznym obrazem oryginalnym oraz obrazem z wykrytymi krawędziami



Source: own work / Źródło: praca własna

Fig. 6. Image enhancement using a brush with different diameter

Rys. 6. Korekcja obrazu za pomocą pędzla różnej średnicy

The effect of the brush is shown in Figure 6. Brush by default replaces the erased color with yellow, because during the diameter measurement items that have not been recognized as the root are not taken into account.

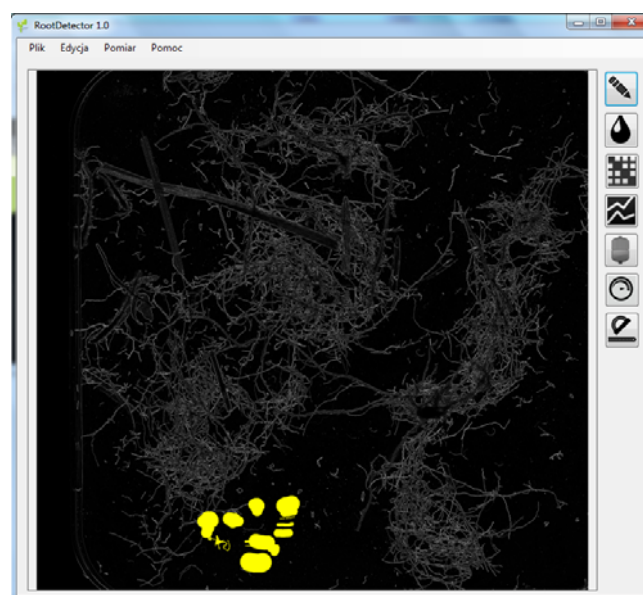
Having updated all the corrections, the new corrected image is loaded into the main form of the program, and there further operations are made.

4.2. Merging images

Merging images is a step preceding the measurement of the diameter of the root. This operation is necessary to measure the diameter properly.

The image subject to analysis is transferred to the original image, according to the following principle: each pixel that was white on the original image is transferred in exactly the same position (X, Y) in the processed image.

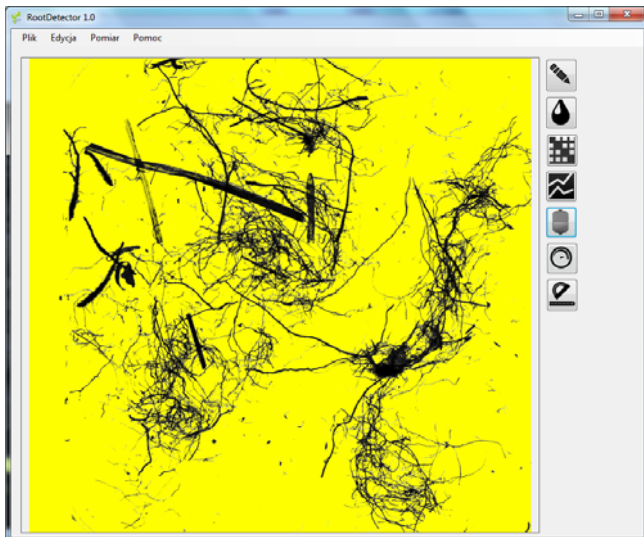
All the black pixels in the image (background) are changed to yellow. With this operation on the resulting image the roots are black, closed white edges and the background is yellow. The algorithm follower between one edge and the other on the black pixel indicates the diameter of the root, not going on the background image.



Source: own work / Źródło: praca własna

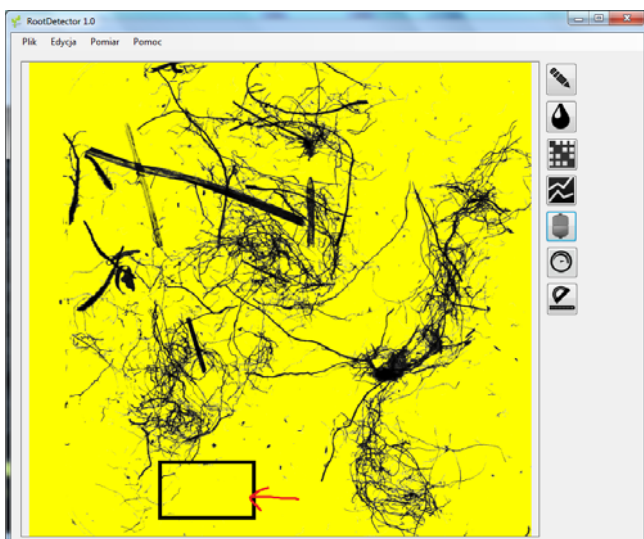
Fig. 7. The image in the main window with the excluded area that will not be analyzed in subsequent stages

Rys. 7. Obraz w głównym oknie programu z wykluczonym obszarem, który nie będzie poddawany analizie w kolejnych etapach



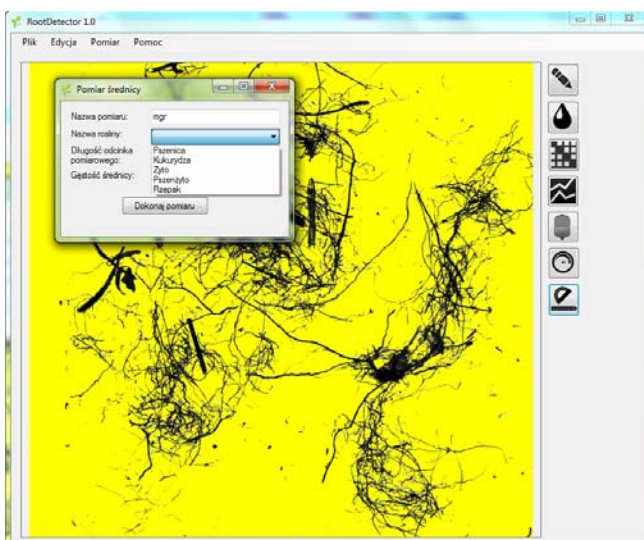
Source: own work / Źródło: praca własna

Fig. 8. The effect of the merging images algorithm
Rys. 8. Efekt działania algorytmu łączącego obrazy



Source: own work / Źródło: praca własna

Fig. 9. The area turned off by the user using the image correction module
Rys. 9. Obszar wyłączony przez użytkownika za pomocą modułu korekcji obrazu



Source: own work / Źródło: praca własna

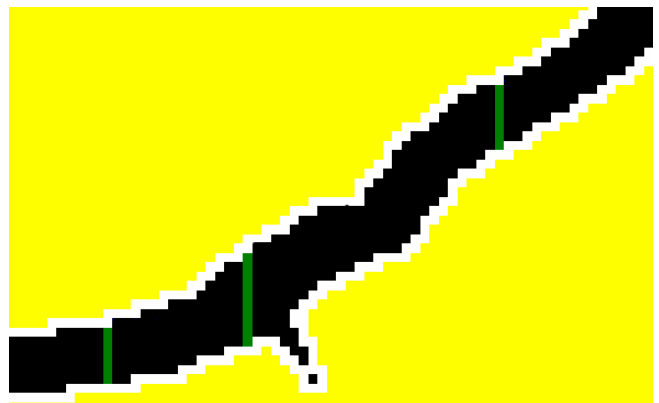
Fig. 10. The form in which the user sets the diameter measurement
Rys. 10. Formata, w której użytkownik ustawia parametry pomiaru średnicy

4.3. Diameter measurement

Diameter measurement is carried out by an algorithm, which follows the white pixels constituting the edge of the root. Each stem of this movement takes place at a user-defined distance measured in number of pixels.

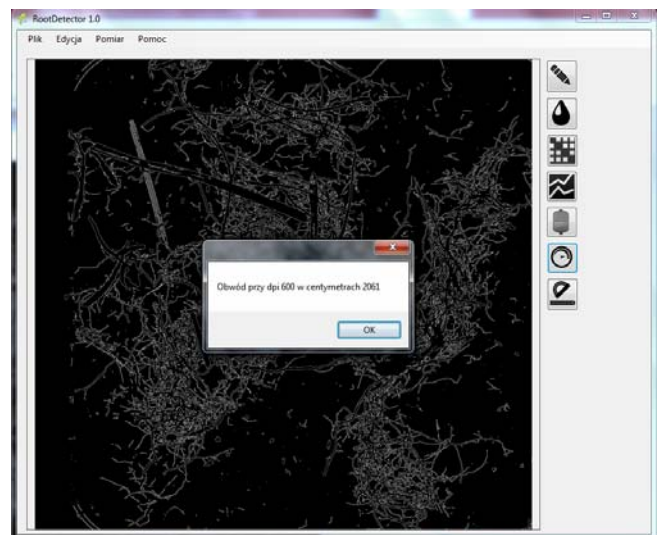
The length of the measurement section is controlled by the user. While hitting to the opposite edge of the root the algorithm goes back to the starting point. The number of pixels that the algorithm crossed on the way to the opposite edge, is recorded as the diameter of the root to the relational database.

Automatically updated Excel file is supplied with the RootDetector program, it allows real-time trace of the measurements and performing mathematical operations on the results.



Source: own work / Źródło: praca własna

Fig. 11. Part of the image showing the effect of the algorithm for measuring diameters
Rys. 11. Fragment obrazu prezentujący efekt działania algorytmu pomiaru średnic



Source: own work / Źródło: praca własna

Fig. 12. Presentation of the results of measuring the perimeter of the root
Rys. 12. Prezentacja wyników pomiaru obwodu korzenia

To start measuring the diameter select the appropriate button from the side menu. This button launches form, in which we can select measurement parameters such as:

- the name of the measurement,
- name of the plant,
- the length of the measurement section,
- density of diameters.

4.4. The perimeter measurement

The perimeter measurement is required to correctly classify the root sample. The algorithm counts all the white pixels in the image of detected edges. It converts the number of pixels on the basis of the image resolution expressed in dpi and gives the result in centimeters.

To run the algorithm for measuring the perimeter select the appropriate option from the side menu. Perimeter measurement should be made at the image of detected edges before merging images.

5. Conclusions and discussion

The purpose of this paper was to create practical software to support the classification of the roots of plants on digital images using the proposed methodologies and algorithms. Using suitable image processing methods, it was possible to classify the selected roots.

The project included:

- acquisition of digital images from analog or digital images and saving onto digital storage,
- image preprocessing or isolating the area of interest and filtering image to remove noise, which may affect subsequent processing steps.
- image segmentation, that is - division of pixels into separate, consistent components characterized by homogeneity into the corresponding real objects,
- acquiring features of separate objects.
- objects identification, that is assigning objects to the appropriate class.

Implementing the project allowed us to test the performance of the RootDetector program, in particular for measuring the diameter and perimeter. Three basic requirements for RootDetector were as follows

- the program allows measuring the diameter of the root,
- the program allows measuring the perimeter of the root,
- the program allows measuring the surface area of the roots.

The first two requirements were achieved, but because of high complexity of an algorithm for calculating the surface area and its low accuracy, we abandoned this functionality. Classification of the roots is not made automatically, but thanks to the availability of reporting module, user can manually perform this classification, using appropriate methods in resulting Excel file.

The scientific goal of the project was to elaborate an original methodology for measuring the degree of development of the selected crops root systems.

The proposed methodology was used to build the model of the measurement process. Implementing the model in the original computer system allowed automation and increasing the efficiency of measuring the degree of plants' root system development.

Prerequisites for project completion:

- establishing the method of root system sampling,
- choosing a set of parameters to identify the degree of development of the root system,
- development of measuring methods of selected parameters,
- creating a model of measuring process of the degree of development of the root system,

- designing and implementing the system.

The final result of the project is the description of the technology to assess the root system development of some indicated crops based on the computer image analysis and implementing assessment system. Description of the technology includes: specification of methodology for obtaining plant material and its preparation for the assessment, description of the technology of image acquisition for the evaluation, the algorithms necessary to carry out the assessment, a set of materials to verify the functioning of the developed methods (source images, the results of the evaluation, indirect data, etc.). The development of such models has both cognitive character, expanding the current knowledge regarding the evaluation of roots, and practical character, providing new methods for the implementation of such an assessment.

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