PRECISE AUTOMATIC DETECTION OF PLANT SEED GERMINATION

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Abstract
The paper deals with an automatic system for precise detection and evaluation of the most important stages in laboratory germination tests using photographic camera and special illumination procedure. The system makes possible to detect the main pre-plant stages of the germination process via image analysis including the detailed description of the shape changes. The relation between seed's area increase and the shape changes is shown in some special cases. More detailed evaluation of the obtained data is in progress and will be published later.

Key words: wheat, seeds, germination, imbibition, area, shape, evaluation.

INTRODUCTION

Movement of water into ripe and dry grains is on one side a critical step of germination and on the other side the important step of wetting, the undesirable process leading to the losses of both quality and quantity of the stored cereals. For seed germination, the wetting of seeds in moist conditions plays the crucial role (McGuinness et al., 2000). The initial part of this process is known with the complex term "imbibition" (Schopfer, 2006; Weitbrecht et al., 2011). During imbibition the dimensions of the seeds change following wetting. Variation in the rate or the pathway of water movement may be affected by the plant species including the dormancy phenotype (Finch-Savage and Leubner-Metzger, 2006; Rathjen et al., 2009) studied by Mares (Mares, 1983, 1999; Mares et al., 2005). Most of the biochemical and molecular changes are intensified during the first hours of imbibition (Harb, 2012; Mares et al., 2005) and also later during the proper germination (Shopfer and Plachy, 1984).

In germination, the imbibition process is followed by two more phases (Rathjen et al., 2009; Weitbrecht et al., 2011). Whereas in the imbibition the seed moisture content steeply increases up to a value representing about few tens percent increase of the imbibed seed mass, in germination (Blahovec and Lahodová, 2015a), there is a second phase where the process of wetting stops and then in a third phase, the process of wetting continues again (Weitbrecht et al., 2011). The prolongation of the first phase for wheat is about 5 hours and does not strongly depend on the seed state dormancy (Rathjen et al., 2009). In this phase, the grain wetting is limited to the seed coleoptile and to the surface of the seed (Rathjen et al., 2009). The second phase represents a set of metabolic processes (Weitbrecht et al., 2011; Manz et al., 2005; Shopfer and Plachy, 1984) necessary for the seed germination in the third phase. The second and the third phases are very variable depending on the different species and different conditions.

Seeds have different shapes, so that shape differences can be used for sorting them according to type, cultivar, quality etc. (Mebatsion et al., 2012; Dornez et al., 2011). The seed shape was studied with computer vision (Shouche et al., 2001) as a basis for automatic sorting. The volume of the seeds during their germination increases and also their shape changes even in cases of very symmetric shapes close to the spherical one (Robert et al., 2008). The process of wetting is an inhomogeneous process (Rathjen et al., 2009), so that the changes in shape during the seed wetting should be nontrivial (Waszkiewicz, 1988; Blahovec and Lahodová, 2015b,). There are several methods that can be used for a quantitative shape description. Among others, a method based on elliptic Fourier descriptors (Kuhl and Giardina, 1982) is the most commonly used. This method describes an overall shape mathematically by transforming coordinate information concerning its contours into Fourier coefficients (Yoshioka et al., 2004).

Previous paper (Blahovec and Lahodová, 2015b) brought information on mass and dimensions of cereal seeds (barley, wheat rye and oat) after 10 and 24 hours of wetting. It was found that variability of all tested parameters can be described by Gaussian distribution and the distribution differences can be expressed via CP (crossing point of the distributions) and RSD (ratio of standard deviations). The grain mass increase due to wetting under the same conditions was different for different crops and/or varieties but generally is in relation with changes of dimensions and shape that could be detected automatically using some forms of vision systems (DELL' AQUILLA, 2009). The relative change of dimension was irregular, the higher for length and lower for width and thickness of grains. The dimensional changes during wetting sensitively depended on the initial dimensions. The seed changes during its wetting are usually described by changes of its mass, more difficult studies of its volume, density and porosity are rather rare even for the seed characteristics in the base dry state (CHANG, 1988).

The aim of this paper consists in development of an automatic method for observation and detection the image processes connected with the seed germination. The potential of the detection of the seed area and the shape is studied in this paper.

MATERIALS AND METHODS

A simple laboratory setup was developed for the purpose of precise automatic detection of a seed area and a shape in laboratory germination tests. The setup consists of photographic camera (Canon 450D with lens Canon EFS 18-55 mm), tripod, illumination LED panel and glass vessel for germination test (see Fig. 1). The camera was controlled via a computer using software DSLR Remote Pro for windows ver. 2.7.2 (Breeze Systems, United Kingdom). Images from camera were stored in a hard disk of the computer in RAW format. A focal length was set to the top surface of the seeds. The LED panel was controlled via a computer and it illuminates the specimens only during taking pictures. The illumination period was 3 seconds. Whole setup is placed in a dark box thus the germination process is under dark conditions.

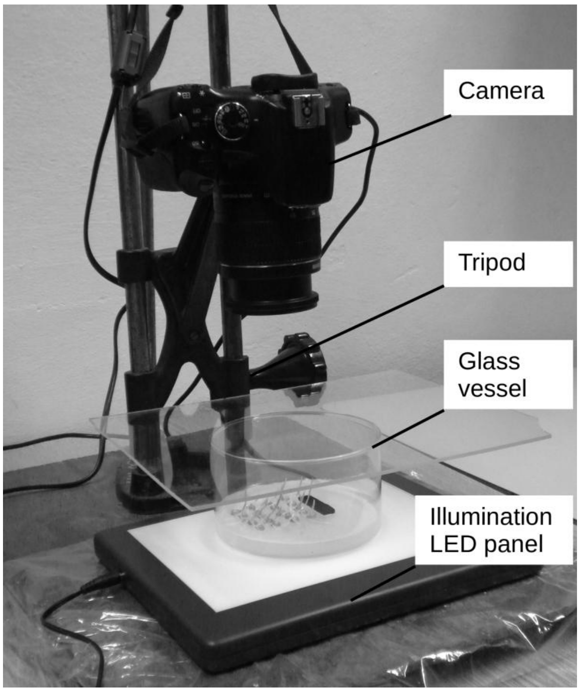

Fig. 1. – The laboratory setup for precise automatic detection in laboratory germination tests.

The area of seeds and their shape parameters were determined by a special program (programmed in language Python 2.7). The supporting libraries for image processing and data analysis were OpenCV 2.4.8, NumPy 1.8.2 and Matplotlib 1.3.1. The shape of seeds was characterized by elliptic Fourier descriptors (EFD; KUHL AND GIARDINA, 1982). In this work a modified python-implementation available at Github repository (https://github.com/alessandroferrari/ elliptic-fourier-descriptors) was used. This method describes an overall shape mathematically by transforming coordinate information concerning its contours into Fourier coefficients (YOSHIOKA ET AL., 2004). Each harmonic contains four coefficients a_n, b_n, c_n and d_n, where n is number of harmonic. In this work the shapes were approximated by the first 20 harmonics and others were omitted. It means that 80 coefficients were obtained for each seed.

The program procedure can be described in the following steps.

1. Loading of images.
2. Conversion to grey scale (it is possible to use red, green or blue channel).
3. Conversion to binary image. A threshold value used in this step is user defined.
4. Application of the erosion-dilation filter for noise reduction.
5. Outline definition and determination of seeds areas. The seed area in pixels is determined.
6. An object with known area (scale) is identified and the seed areas in square millimetres are determined.
7. Calculation of EFDs from outlines.

The laboratory setup and program were tested during germination test of winter wheat (variety Tosca, supplier: Selgen a.s.) harvested in 2014. Moisture content (wet basic) of the seeds was 8.7%. The germination test was performed on agar 0.8 % in a glass vessel. 20 seeds were evenly placed on the agar and next to them a scale with area 318.5 mm^2 was added. The vessel was covered by a thin acrylic glass plate. The experiment was started on the 7th March and terminated on the 11th March. Total length of the experiment was 90 hours and 25 minutes. Temperature in the laboratory during experiment was 20.1 ± 1.0 °C.

Pictures were taken in 5 minutes intervals during germination test.

The seed area development was evaluated using seed area rate (SAR). SAR is time derivative of the seed area and it can be defined by the following formula:

$$SAR = \frac{\mathrm{d}SA}{\mathrm{d}t}, \ (\mathrm{mm^2\ h^{-1}}) \quad (1)$$

where SA is the seed area (mm^2) and t is time (h). The time derivative of the seed area was calculated numerically. Experimental data were sequentially fitted by straight lines in order to estimate the course of the first derivative. The straight lines parameters were determined by application of a least squares method always in an interval containing twelve measured values. The interval was moving after measured value and the estimate of the derivative in the middle of the interval expressed the slope of the straight line found.

RESULTS AND DISCUSSION

First visible signs of the third phase of germination (embryo evolution) were observed already 30 hours after the experiment started and 19 seeds successfully germinated during the whole germination test. Total 1,083 pictures were obtained during the experiment but only the first 446 pictures were used for the precise analysis. These pictures represent approximately the first 37 hours.

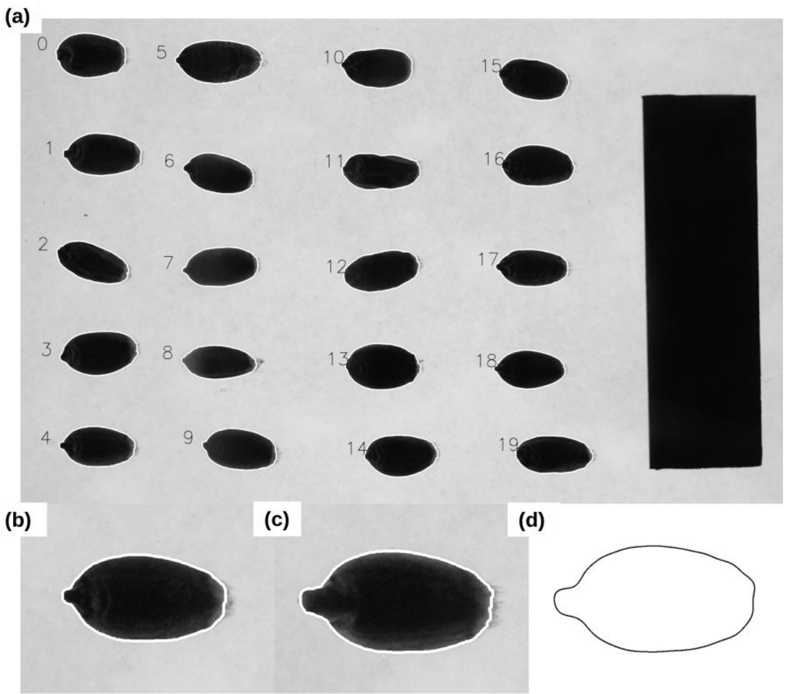

Fig. 2. – (a) The initial frame after conversion to grey scale (blue channel); (b) Detail of the seed 4 in the beginning of the experiment; (c) detail of the seed 4 after 37 hours; (d) the approximated shape of the seed 4 (after 37 hours) by the first 20 harmonics

The Fig. 2 (a) shows the initial frame. In the figure there are located 20 seeds and the seeds are labelled by identification number (numbers 0 – 19). The detected outlines of all seeds are highlighted by white colour. The scale is located on the right side of the picture. It is apparent that seed outlines were successfully recognized in all cases.

Fig. (b) and (c) represent details of the seed 4. The part (b) shows seed 4 in the beginning of the experiment and the part (c) shows the same seed after 37 hours. It is possible to observe the significant seed area change. The area increase is approximately 35 %. In the Fig. 2 (c) the beginning of the radicle penetration is visible as well. Fig. 2 (d) shows approximated shape of the seed 4 (after 37 hours) by the first 20 harmonics. It is possible to state that approximated shape is very close to original outline.

One example (seed 4) of the time courses of the SA and the SAR depicted in Fig. 3 (a). The time course of the SA can be divided into three stage. The first stage terminates at the moment when the SAR has maximum magnitude and the curve (SA) is convex in this stage. In this short stage (approximately 40 minutes), probably, the moisture transports through the dry seed coat. The second stage is characteristic with a decreasing SAR and thus the time course of the SA is concave. The end of the second stage comes approximately after 25 hours when the SAR has minimum magnitude. Then the time course continues in the third stage. In this stage the SA is increasing more quickly. The increase is caused by embryo evolution. The seed behaviour in the second and third stage is in agreement with observations of DELL' AQUILLA (2004).

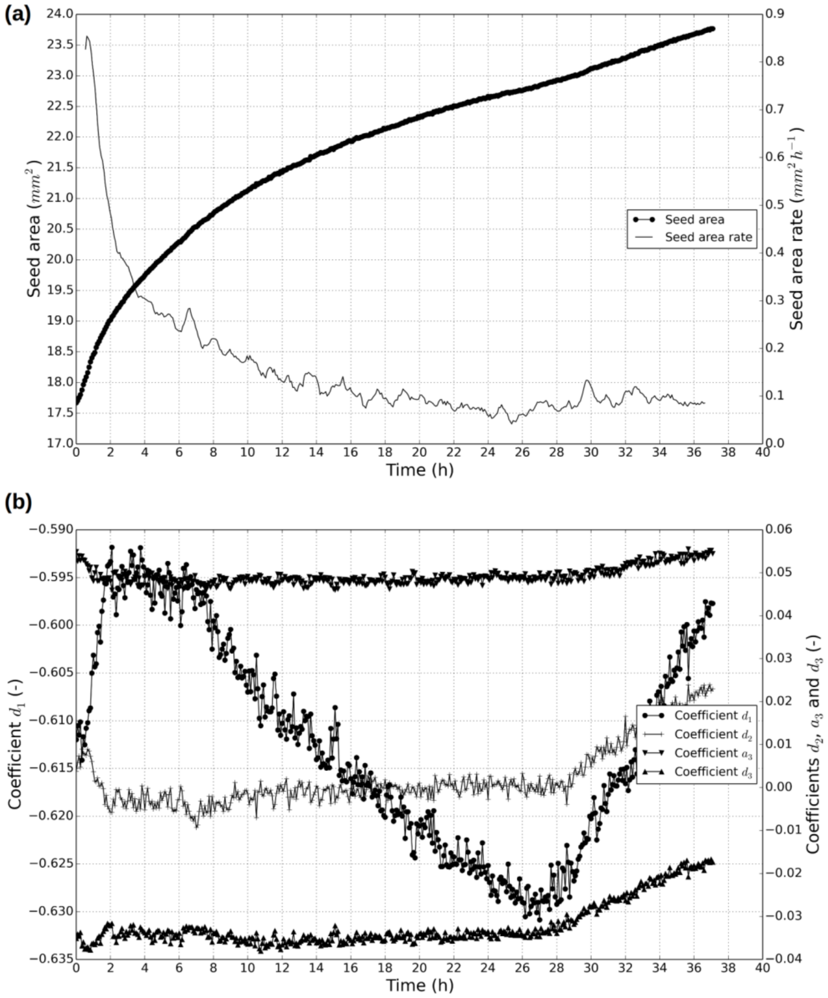

Fig. 3. – (a) the time courses of the seed area (SA) and the seed area rate (SAR); (b) the time courses of four elliptic Fourier coefficients (d_1, d_2, a_3 and d_3)

The time courses of four elliptic Fourier coefficients (d_1, d_2, a_3 and d_3) are depicted in Fig. 3 (b). These coefficients were chosen because they significantly influences the shape approximation. The coefficient d_1 plays the key role of the process. If the shape is approximated by the first harmonic only, the result is simple ellipse and d_1 represents a negative ration of the minor axis to the major axis. Thus it is possible to assume that d_1 corresponds approximately or with the negative ratio of the seed width to the length.

The situation in the Fig. 3 (b) can be divided into three time stages again. The first stage is terminated approximately after 2 hours. All monitored coefficients are significantly changing in this period. The end of this stage is very close to the time where the time course of the SAR should has an inflexion point. The second stage is relatively long and it is terminated at around 27 hours. The coefficients d_2, a_3 and d_3 are more or less constant but the absolute value of the coefficient d_1 is significantly increasing. This behaviour can be caused by an internal processes in the seed and its structure (DONG ET AL., 2015). In the last third stage all coefficients responds to the embryo evolution and the final germination phase because the shape is significantly changing.

The behaviour of only one seed is presented in this paper. However, very similar behaviour was observed for all the tested seeds. The detailed analysis of the whole seed set will be subject of the future paper.

CONCLUSIONS

Setup for precise automatic detection of seed germination in laboratory conditions was developed including the methods of testing and evaluation of the obtained data. It is possible to precisely detect seed area and shape parameters during the germination test. Preliminary results shows that there is nontrivial relations between the area and shape data. It is evident that there is a big challenge for future research. More precise evaluation of the data will be given later.

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