SHAPE DEVELOPMENT OF WHEAT SEEDS DURING GERMINATION

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Abstract. Image analysis is a useful tool for seed monitoring during germination. An important advantage of the image analysis consists in easy determination of the dimensional changes in time without any manipulation with the germinated seeds. Many researches focus on the final germination phase and the image analysis is used for automatic detection of the visible radicle protrusion from the seed coat. However, an interesting behavior can be found in the whole germination process. The aim of this paper consists in development of an automatic method for observation and detection the image processes connected with seed germination. The setup consists of a photographic camera, a stand, an illumination LED panel and a glass vessel for the germination test. The camera is controlled via a computer and images are stored in the hard disk. The area of the seeds and their shape parameters were determined by a special program. The development of image moments is presented in our work. It was found that image moments can be useful tools for automatic detection of the primary shoot during the final germination phase as well as during the imbibition process.

Keywords: imbibition, water movement, image moments, image analysis.

Introduction

The seed germination is a critical process for plant propagations. Germination starts by a quick water uptake and it is terminated with an elongation of the embryonic axis [1]. The final germination stage is usually represented by penetration of the radicle through the structures surrounding the embryo. This stage is often called visible germination [2].

Image analysis is a useful tool for seed monitoring during germination. Shouche et al. [3] tested several shape parameters of Indian wheat seeds as a tool for identification of different varieties. The authors stated that the use of over 45 different parameters enabled them to distinguish between 15 samples of Indian wheat varieties. The moment analysis played a key role in their work. Kuhl and Giardina [4] introduced in their work a method for shape analysis based on elliptic Fourier descriptors. This method was widely used for shape analysis of plant organs by many researchers [5-7]. Mebatsion et al. [8] developed an algorithm to classify cereal grains. The authors tested their algorithm with barley, oat, rye and two varieties of wheat. The results show that the method can be used for seed classification. The classification accuracies were almost 100 %.

Dell'Aquila [9] dealt with the application of the digital imaging information technology to seed germination testing. In his review article, he presents several parameters, which can be monitored during germination. The presented parameters are, e.g.: area, perimeter, length, width, roundness, calculated with the formula: $\text{perimeter}^2 / 4\pi$ Area, and aspect, calculated with the ratio between the longer axis and the shorter axis of the ellipse equivalent to the seed area. The author noted one particular example of roundness factor utilization: a seed with a circular shape has the roundness factor of 1 at the start of imbibition, a different shape of the seed, due to the changes occurring with radicle protrusion from the seed coat, should produce a roundness factor higher than 1 [10] and the inflection point of the related curve marks the start of visible germination.

Number of researchers dealt with development of methods for automated detection of seed germination [11-13]. Joosen et al. [13] state that a fast and reliable automated procedure would enable high-throughput screens and unlock the full potential of the seed science research. It is clear that the image analysis is well established in the seed science. However, it is often the final phase of germination that is subjected to the greatest attention. That is the reason why this paper focuses on the seed shape monitoring during the whole germination process. The aim of this research consists in development of an automatic method for observation and detection the image processes connected with seed germination.

Materials and methods

The laboratory setup used in this research is described in our previous paper [14]. The setup consists of a photographic camera (Canon 450D with lens Canon EFS 18-55 mm) on a stand, an illumination LED panel and a glass vessel for the germination test (see Fig. 1). The camera is controlled via a computer. The focal length was set to the top surface of the seeds. The LED panel is controlled via the computer and it illuminates the specimens only during taking pictures. The illumination period lasted for 4 seconds. The whole setup was located in a dark box, thus it is possible to assume that the germination process took place under dark conditions. The interval between each snap was 5 minutes.

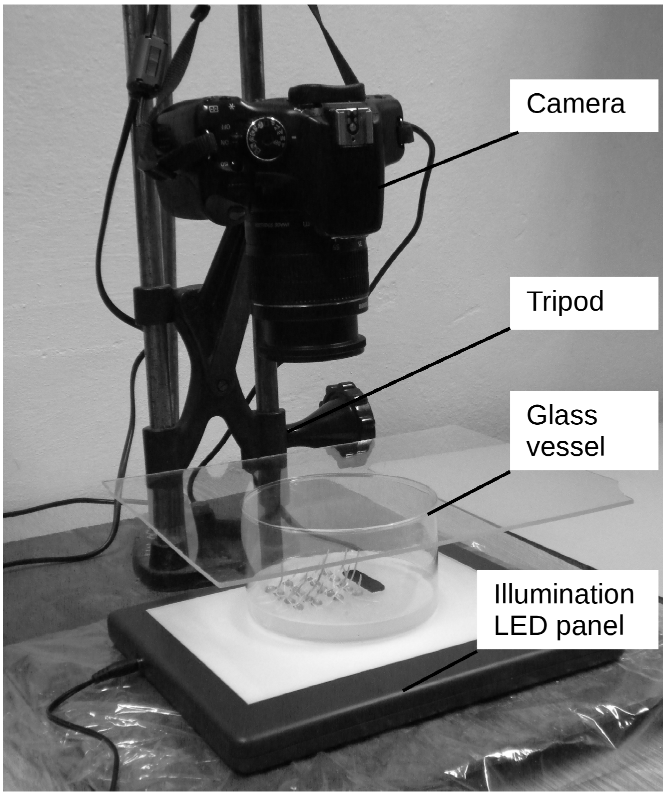

Fig. 1. **Laboratory setup for automatic detection in laboratory germination tests**

The experiment was done with winter wheat (variety: Tosca, harvested in 2016, supplier: Selgen a.s.). The grain moisture content (wet basic) was 8-9 %. The germination test was performed on agar 0.8 % in a glass vessel, and 20 seeds were monitored at the same time. All seeds were placed with the germ on the left side. A scale (small plastic plate with known sizes; its area: 318.5 mm^2) was placed next to the seeds for the purpose of determination of the true sizes of the seeds. The glass vessel was covered by a thin acrylic glass plate. The experiment was performed on 6th of January, 2017. The temperature in the laboratory was 20-21 ºC.

The image analysis and data processing were performed by Python 2.7 programming language and by its supporting libraries: OpenCV 2.4.8, NumPy 1.8.2, and Matplotlib 1.3.1. The image processing started with conversion to the gray scale (blue channel was used) and then conversion to the binary image (threshold method was used). Then an erosion-dilation filter was used for noise reduction. Outlines of the individual seeds as well as their cross areas were determined on the binary image. Each seed outline was rotated so that its major axis was parallel with the *x*-axis. The angle of orientation can be calculated by the following formula:

$$\theta = \frac{1}{2}\arctan\left(\frac{2\mu_{1,1}}{\mu_{2,0} - \mu_{0,2}}\right), \tag{1}$$

where θ – angle of the outline major axis, rad;
$\mu_{1,1}$, $\mu_{2,0}$, $\mu_{0,2}$ – central moments.

The central moments used in eq. (1) were described, for example, by Gonzalez and Woods [15] and can be calculated as follows:

$$\mu_{p,q} = \sum_x \sum_y (x - x_c)^p (y - y_c)^q f(x, y) \tag{2}$$

where p, q – natural numbers;
x_c, y_c – components of the centroid;
$f(x, y)$ – digital image.

The final step of the image processing was to determine the normalized central moments. That was performed after the normalized rotation of the seed outlines. The following equation was used:

$$\eta_{p,q} = \frac{\mu_{p,q}}{\mu_{0,0}^{\left(1+\frac{p+q}{2}\right)}} \tag{3}$$

where $\eta_{p,q}$ – normalized central moment.

For the purpose of evaluation of the seed shape development during germination the following normalized central moments were used: $\eta_{2,0}$, $\eta_{0,2}$, $\eta_{3,0}$, $\eta_{0,3}$.

Results and discussion

In the present paper two demonstrations of the seed image moment development during germination are presented. Fig. 2 shows a seed, which finished germination during the observation period (40 hours), while Fig. 3 shows a seed where the visible germination did not start during the observation period. Two graphs are displayed in each figure where the upper one shows the development of the moments $\eta_{2,0}$ and $\eta_{0,2}$ and the bottom one shows the development of the moments $\eta_{3,0}$ and $\eta_{0,3}$.

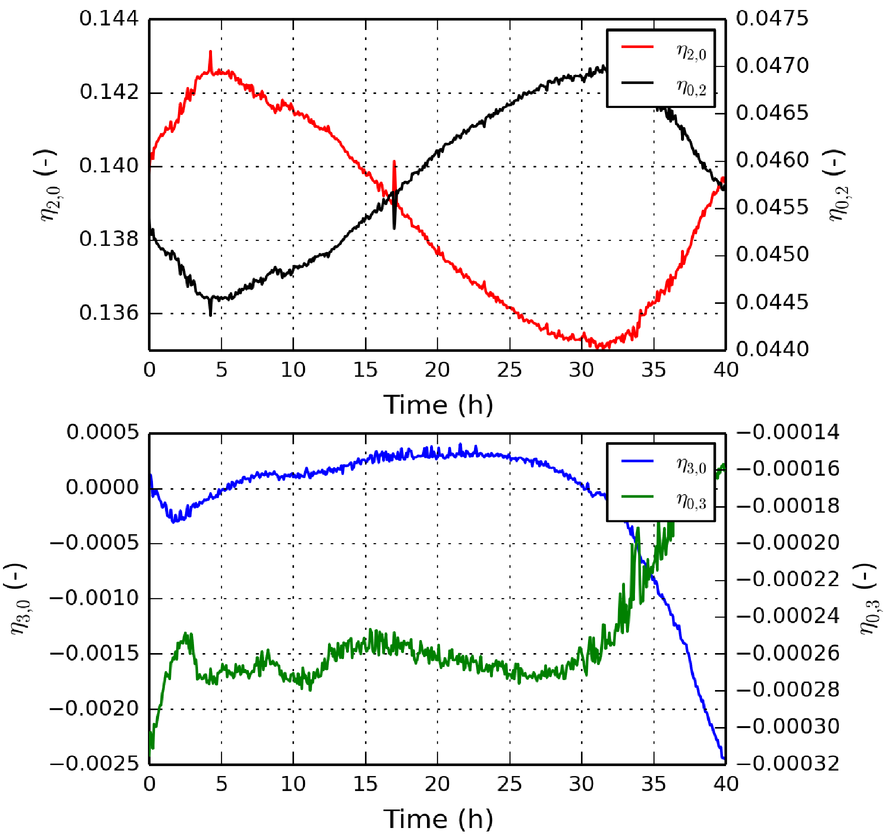

Fig. 2. **Image moment development of wheat seed during germination, visible germination started at 32 hours**

It is apparent that the seed shape is significantly changing during germination. The time courses of the moments $\eta_{2,0}$, $\eta_{0,2}$ are mainly influenced by the change of the width and length ratio and it can be stated that they are an alternative to the aspect parameter [9]. Both moments provide comparable information about the seed shape changes. Their time course can be divided into three phases. In the first phase the moments respond to a slight seed extension caused by the seed's swelling in areas close to the embryo and the brush. The trend is opposite in the second phase. In that phase the seed width increases more quickly than the seed length. Probably, it is connected with the water penetration into endosperm [16]. The third phase constitutes the visible germination and there is an evident significant increase of the seed length (growing radicle). From Fig. 2 it is apparent that the visible germination starts 32 hours after the start of imbibition. On the contrary, the seed which is represented by the moment development in Fig. 3 did not finish the germination process during the observation period and thus the moment development does not include the third phase.

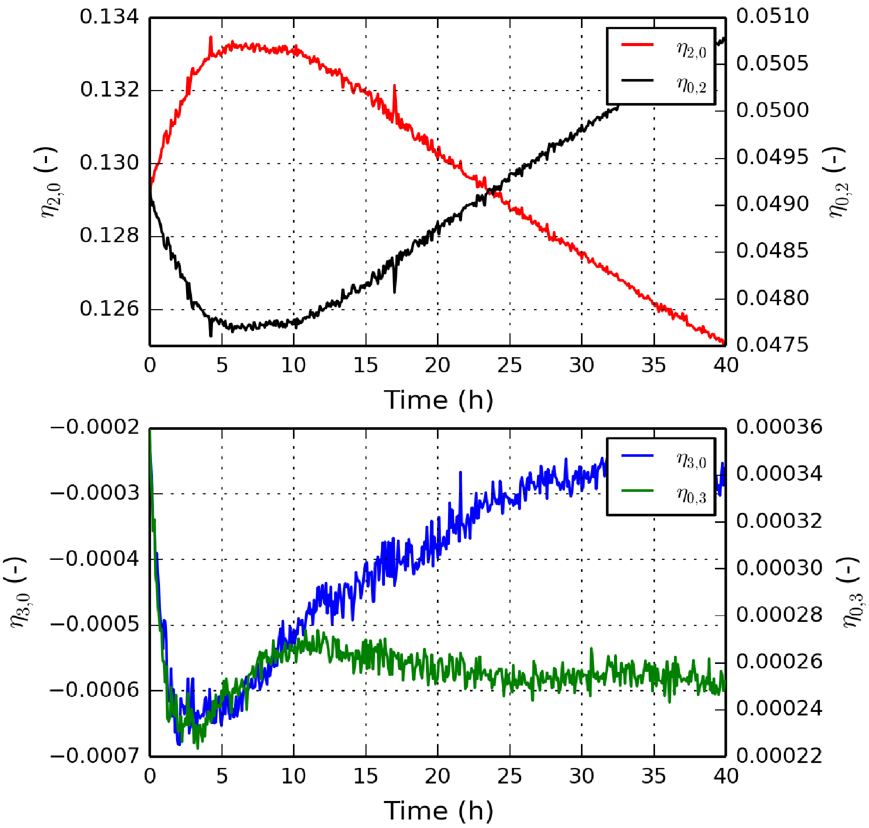

Fig. 3. **Image moment development of wheat seed during germination, visible germination started after 40 hours and it is not shown in this figure**

The moments $\eta_{3,0}$, $\eta_{0,3}$ represent the seed asymmetry ($\eta_{3,0}$ by the minor axis and $\eta_{0,3}$ by the major axis). Also the time course of the moment $\eta_{3,0}$ can be divided into three phases. The moment significantly decreases immediately after the start of the imbibition process and can be identified by the embryo swelling in the first two hours (Fig. 2 and 3). In the second phase the moment gradually increases with decreasing rate until the maximum value is reached. Then the third phase starts and the moment decreases with an increasing rate. This decrease starts before the visible germination, but the growing radicle significantly contributes to the moment decrease.

The moment $\eta_{0,3}$ is very variable for different seeds. Similarly to the moment $\eta_{3,0,}$ it responds to various events that are happening in the seed during germination. However, the interpretation of the time course is much more complicated because the seed development influences mainly the seed transverse asymmetry.

The presented results are part of a bigger research and this contribution aims to bring only our preliminary results.

Conclusions

The presented results show that the seed shape is significantly changing during the whole germination process. The moments $\eta_{2,0}$ and $\eta_{3,0}$ were selected as the most perspective. The moment $\eta_{2,0}$

is an alternative to the aspect parameter, which represents the ratio between the longer axis and the shorter axis, whereas the moment $\eta_{3,0}$ is connected with the seed transverse asymmetry.

The shape changes are the result of the development of the individual seed parts and it is highly probable that they relate with the water absorption in the seed.

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