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Effect of I_2/KI water solution to wheat seeds imbibition assessed by image analysis

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Abstract. Water plays key role in a seed germination due to its participation in starting of many metabolic processes that accompany the seed germination. Rate of water uptake into seeds is a usual basis for determination of the three germination phases. The water uptake into seeds during their germination was investigated by many researchers who used various methods (e.g. magnetic resonance micro-imaging, near-infrared hyperspectral imaging and visualization with I_2/KI solution (Lugol's iodine)). The method of using I_2/KI water solution for this purpose is quite popular for its relatively applicability. In this paper we compared the seed surface area projection and shape development of the seeds imbibed in the I_2/KI solution and in the pure water via image analysis. It was found that the presence of the I_2/KI in water changes the increase of seeds volume during germination and the effect is different during the initial imbibition and during the next germination phases. The seed shape development is similar for both variants, pure water and I_2/KI solution.

Key words: imbibition, germination, image analysis, water diffusion.

INTRODUCTION

Seeds germination represents one of the key processes in a plant growth which affects its subsequent grain yield. The water plays important role in this process because it allows the start of the important metabolic activities in the seeds. The germination phases are defined by seed water uptake and it is generally accepted that the process consists of three phases (Bewley, 1997; Weitbrecht et al., 2011). The first phase (denote as the phase I), which is distinguished by the initial rapid absorption of water, is referred to as 'early imbibition'. For wheat, this takes about 5–7 h (Abenavoli et al., 2006; Dell'Aquila, 2006; Rathjen et al., 2009; Harb, 2013). The early imbibition is followed by a phase, termed phase II, in which water absorption is lowered; in this phase the previous seed increase of volume and mass is nearly stopped. Phase II is finished by a new increase in the seed's moisture content that indicates start of phase III that is usually termed 'proper germination' (Rathjen et al., 2009). The total increase in mass in phases I–III is about 100%, and slightly more than one half of this increase is achieved in the phase I.

Research works dealing with the water uptake in germinating seeds have been regularly appearing for a long time ago (King 1984; Becker 1960; Shull 1913). However, a significant amount of research works was published in the twenty-first century (Alvarado & Bradford, 2002; Wiwart et al., 2006; Finch-Savage et al., 2007; Ribeiro-Oliveira & Ranal, 2017). The water uptake is normally used as a chronological marker in molecular biology and physiological studies focusing on seeds germination (Harb 2013; Ribeiro-Oliveira & Ranal 2017). Dong et al. (2015) presented the dynamic proteome analysis of wheat seed germination. Authors revealed the dynamic changes in the proteome involved in wheat seed germination. Authors also showed a connection between the dynamic changes in the proteome and germination phase.

Many researchers used various methods for the water movement monitoring in the seeds (Kikuchi et al., 2006). One of them consists in monitoring of the morphological changes in the seed under microscope or electron microscope (Dell 1980; McDonald et al., 1988). Nakanishi & Matsubayashi (1997) dealt with nondestructive water imaging by the neutron beam analysis in living plants. Gruwel et al., (2001) used magnetic resonance technique for the water uptake monitoring in barley seeds. Authors were able distinguish two stages which correspond to the first and the second germination phase. Rathjen et al. (2009) and Kikuchi et al. (2006) used micro-magnetic resonance imaging for water movement monitoring during imbibition. This technique allowed precise monitoring of the water movement during whole imbibition process.

Manley et al. (2011) and Lancelot et al. (2017) used near infrared hyperspectral imaging for monitoring of water diffusion in wheat seeds. Lancelot et al. (2017) presented that the developed system allows real-time monitoring of the diffusion of water into different tissues of the wheat seeds and it can be fully automated.

In many cases a simple and time-saving method is required. These requirements could be complied with the I_2/KI solution (Lugol's iodine) (Kikuchi et al., 2006; Rathjen et al., 2009). Rathjen et al. (2009) presented that I_2/KI was found to be the most effective marker for visualizing water uptake into the endosperm, due to its small solute size and the ability to bind with and stain starch tissue.

However, the effect of the I_2/KI solution is not wholly assessed. The aim of this paper is to compare the effect on parameters of germination in pure water and standard solution of I_2/KI usually used for visualization of internal changes in the seeds.

MATERIALS AND METHODS

The imbibition process of two wheat varieties was monitored for 24 h. The seeds were imbibed in pure water and in I_2/KI solution. The I_2/KI solution contains 1 g I_2, 2.5 g KI and 96.5 mL distilled water. Winter wheat (varieties: Tosca and Steffi, supplier: Selgen Plc.) harvested in 2016 was used for the experiments. The moisture content (wet basic) of the seeds was 6–7% (ASABE Standard S358.2, 2006). Data was collected during four measurements, and they took place in December 2017. The imbibition monitoring was performed on three layers of a polyester cloth (white Novolin, area density: 100 g m^{-2}). The polyester cloth and seeds were placed into a glass vessel and the cloth was moisturized by 12 g of pure water or 12 g of I_2/KI solution. For the purpose of minimizing an evaporation the glass vessel was covered by a thin glass plate. The tests with pure water were performed with 49 seeds and the tests with I_2/KI solution were performed with 42 seeds.

The seeds were monitored with a photographic camera (Canon EOS 750D with lens Canon 18–55 mm) on a stand. The second part of the laboratory setup for seeds monitoring was an illumination LED panel that was used for illumination of the specimens while taking pictures. The LED panel was placed under the glass vessel. The whole laboratory setup was placed in a laboratory incubator (Friocell 111 – EVO, manufacturer: BMT Medical Technology Inc.) that ensure a constant temperature 21 °C during experiment. The camera and LED panel were controlled via computer using a program written in the Python programming language (version 2.7). Images from the camera were stored on a hard disk. The focal length was set to the top surface of the seeds. The interval between each photograph was 120 s. The moisture content of each seed was determined by the standard oven-dried method (ASABE Standard S358.2).

The image processing and the data analysis were partially mentioned in our previous paper (Lev & Blahovec, 2017). The image processing and data analysis 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 grey scale. The blue channel was used for experiments with pure water and the green channel for I_2/KI solution. The next step was conversion to the binary image using standard threshold method and application of erosion-dilation filter fir noise reduction (Gonzalez & Woods, 2002). Outlines of the individual seeds as well as their areas (surface area projections) 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 as follows:

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

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

The central image moments (Hu, 1962; Gonzalez &Woods, 2002) are defined by the following equation:

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

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

The last step is determining of the normalized central moment $\eta_{2,0}$:

$$\eta_{2,0} = \frac{\mu_{2,0}}{m_{0,0}^2} \tag{3}$$

where $m_{0,0}$ – the outline area.

For purpose of the $\eta_{2,0}$ comparison, the data were recalculated by the following formula:

$$n_{2,0} = \eta_{2,0} - \eta 0_{2,0} \tag{4}$$

where $n_{2,0}$ – the moved normalized central moment; $\eta 0_{2,0}$ – initial normalized central moment.

The time development of the $n_{2,0}$ contains a local maximum. For purpose of maximum detect, the data were fitted (using the least squares method) by polynomial of fourth degree. The desired maximum then corresponds to a local maximum of the

polynomial function. This procedure was applied for each seed. The increase of seed area (at all time steps), positions of the $n_{2,0}$ maxima and seeds' moisture contents were compared by the Mann–Whitney–Wilcoxon test (Mann & Whitney, 1947).

RESULTS AND DISCUSSION

The Fig. 1 shows the time development of the seed area. The Fig. 1 (a) depicts the Tosca variety and the Fig. 1 (b) depicts the Steffi variety. The variants with pure water and I_2/KI water solution are represented by black and grey colour, respectively. In both cases (Tosca and Steffi variety) the time development of the seed area is possible divide into two stages. The first stage is shown by mildly higher seed area increase for variant with I_2/KI solution. During the second stage the seed area increase is higher for the variant with pure water. For the Tosca variety the statistically significant differences (p-value < 0.01) were found between 0.2 and 0.8 h and from 5.8 h for the first and the second stages, respectively. For the Steffi variety the statistically significant differences (p-value < 0.01) were found between 0.2 and 1.1 h and from 8.6 h for the first and the second stages, respectively.

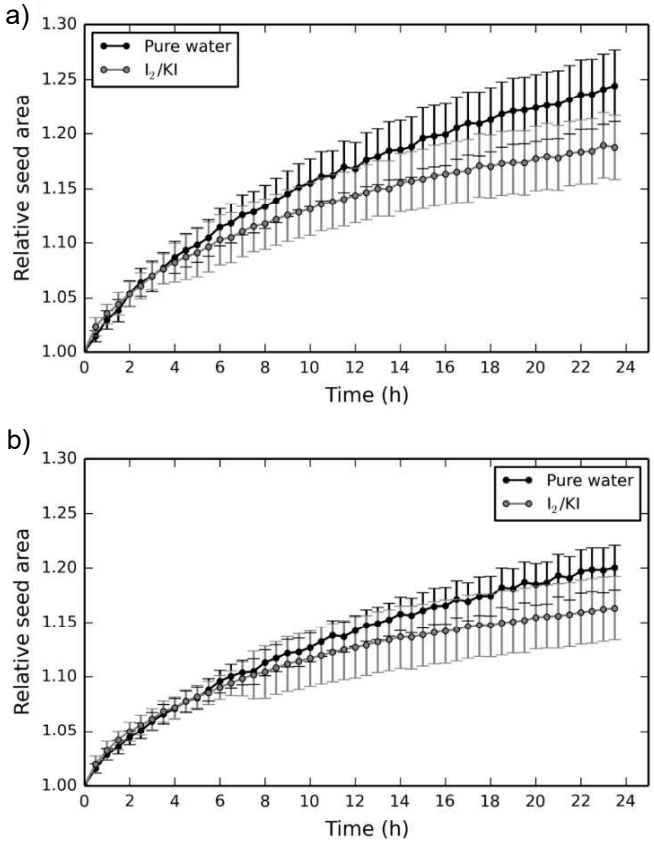

Figure 1. Time development of the relative seed area. The variants with pure water and I_2/KIs water solution are represented by black and grey colour, respectively. (a) Tosca variety, (b) Steffi variety.

The water enters the wheat seeds through the micropyle and it is known that the water is located mainly in the embryo part during the initial state of the imbibition (Gruwel et al., 2001; Rathjen et al., 2009). We reported in our previous paper (Lev & Blahovec, 2017) that the embryo part where the micropyle is located significantly influences the seed cross area increase during the first hour of imbibition. Then the water enters the other seed parts and around 7 h after the start of the imbibition it enters the seed endosperm (Gruwel et al., 2001; Rathjen et al., 2009). Results in the Fig. 1 show that the embryo and the other seed parts respond differently to the attendance of the I_2/KI solution. It seems that the endosperm plays important role in this process. This idea is supported by the fact that at the moment when the water or I_2/KI solution enters into endosperm, the other parts of the seed are almost fully saturated by water and/or I2/KI water solution (Rathjen et al., 2009). Also this is supported by our experiments that will be published later.

The Fig. 2 depicts the time development of the normalized image moment $n_{2,0}$. This parameter corresponds with the ratio of the seed length and width so the increase of the $n_{2,0}$ corresponds with the relative seed elongation. The time development of the $n_{2,0}$ is similar for both varieties and for both test variants. For variants with I_2/KI solution, mildly higher increase during the initial phase of the imbibition is noticeable (primarily for Steffi variety). This is probably due to the higher volume increase of the embryo part in this phase.

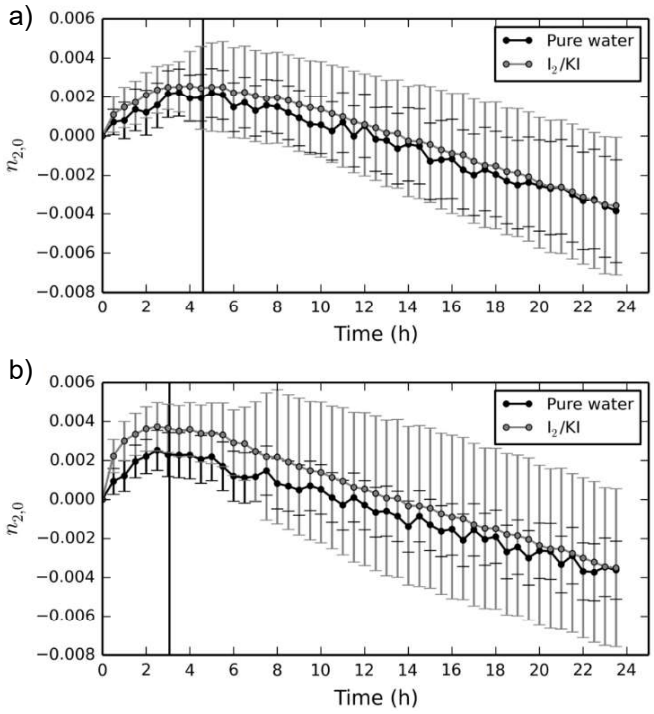

Figure 2. Time development of the moved normalized central moment $n_{2,0}$. The variants with pure water and I_2/KI water solution are represented by black and grey colour, respectively. (a) Tosca variety, (b) Steffi variety. Vertical line in the plots denotes the mean values of the both $n_{2,0}$ maxima (the time differences between them is approximately 1 min. for all cases).

The time development of the $n_{2,0}$ contains a characteristic point (maximum) and the position of the point is with high probability connected with the water movement in the seed (Lev et al., 2017). A comparison of the $n_{2,0}$ maxima positions is depicted in the Fig. 3. The $n_{2,0}$ maximum for Tosca variety was approximately 4.5 h and any statistical significant difference was not found (p-value: 0.30) between the variant with pure water and the variant with I_2/KI solution. The $n_{2,0}$ maximum for Steffi variety was approximately at 3 h and any statistical significant difference between the variant with pure water and the variant with I_2/KI solution was also not found (p-value: 0.26).

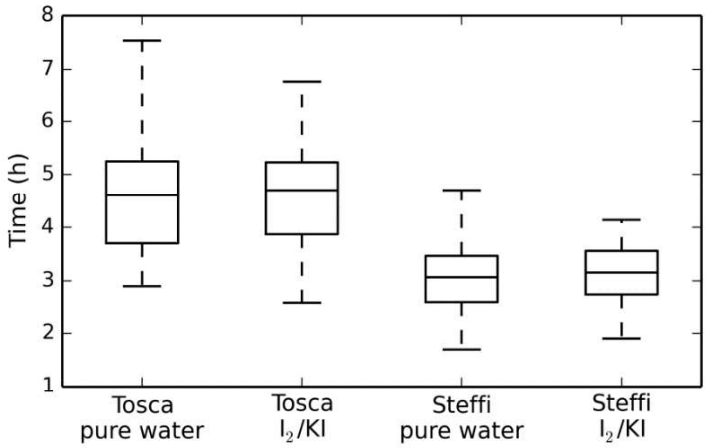

Figure 3. Comparison of the $n_{2,0}$ maximums detected during imbibition. Mean values, quartiles, outside values are depicted in the box-plot.

The behaviour described above could be significantly influenced by amount of water absorbed during imbibition. The seeds moisture content after experiment was assessed for this reason. The average moisture contents for Tosca variety were 29.17% and 28.56% (standard deviations: 1.85% and 2.26%) for the variants with pure water and the I_2/KI solution, respectively. For Steffi variety they were 27.62% and 27.58% (standard deviations: 1.27% and 1.79%) for the variants with pure water and the I_2/KI solution, respectively. No statistically significant differences between the variants with pure water and I_2/KI solution were found. It confirms that the behaviour described above is connected with I_2/KI solution attendance.

CONCLUSIONS

The I_2/KI solution influences the seed swelling during imbibition. The influence is different during initial part of imbibition (appr. 1 h) and again during the next germination phases at 5 and 8 h for Tosca and Steffi, respectively. The changes of the shape and mass during seed germination in both liquids (pure water and I_2/KI solution) exhibits similar time development.

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