Studies on the imbibition of triticale kernels with a different degree of sprouting, using digital shape analysis

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ABSTRACT

The imbibition of triticale kernels with a different degree of sprouting and with no visible sprouting symptoms was studied using digital image analysis and the classical weight method. Measurements were performed at two-hour intervals for the first 14 hours of imbibition, and at 24 and 26 hours. The area, perimeter, length and width of kernel images, and ten different shape factors were determined along with the weight of imbibing kernels. The germination capacity was determined on the seeds used for measurements. Kernels showing the lowest degree of sprouting were characterized by the highest rate of water uptake in comparison with the control. Most of shape descriptors were significantly correlated with kernel weight, especially image area (r = 0.688) and two shape factors (calculated on the basis of image perimeter and area) of S_9 and S_{10} (r = 0.742 and 0.958, respectively), which makes them the best descriptors of image of imbibing seeds.

Keywords: image analysis; imbibition; triticale; sprouting

Imbibition is a process preceding seed germination that consists in water absorption and activation of nutritive substances contained in seeds. King (1984) and King and Richards (1984) found that water absorption by kernels of different wheat varieties is in 18% conditioned by spike and grain structure – arista and spike shape affect significantly both the degree of sprouting and water uptake. Kernels of different wheat varieties differ considerably in the imbibition rate in the first hours of the process, though sometimes these differences can be still visible after 30 hours. Germination is closely correlated with the amount of water absorbed during the first two hours of imbibition (King and Richards 1984). According to Clarke (1980), seeds coming from various environmental conditions may be characterized by a different imbibition rate. It was also demonstrated that fruit-seed coat damage caused by mechanical grain threshing might contribute to an increased imbibition rate, whereas various drying methods do not play a significant role in this process (Clarke and DePauw 1989). According to King (1984), differences in the rate of water uptake by kernels may be related to their susceptibility to sprouting. Sprouting is one of the major factors deteriorating the quality of triticale seeds (Moś and Wójtowicz 2002, Moś 2003).

The most widely used technique for imbibition rate determination is the weight method (ISTA 1985). However, this method is burdened with certain errors. In addition, it seems that a complex and reliable assessment of the rate of this process should comprise changes in the kernel shape caused by imbibition. Computer image analysis, having a wider application in agricultural research (Brosnan and Sun 2002), can be used in this type of study. It enables a fast and objective determination of kernel image parameters (shape, brightness, colour), which may be correlated with several properties considered important from the practical perspective, such as nutrient content, varietal features and quality (Sapirstein 1995, Shouche et al. 2001, Wiwart et al. 2001). McCormac and Keefe (1990) applied this technique to measure the imbibition of cauliflower seeds, and Dell'Aquila

(2003) and Dell'Aquila et al. (2000) used it while monitoring cabbage seed imbibition. Van der Heijden et al. (1999) applied image analysis to the development of an automated germination test. Digital image analysis may find a practical application while studying the imbibition rate and determining the duration of successive phases of this process (Bewley and Black 1994).

The objective of the present study was to determine the imbibition patterns of triticale kernels, characterized by a different degree of sprouting, using an image analysis technique, and to find the best descriptors of size and shape changes that occur during imbibition.

MATERIAL AND METHODS

Spring triticale (*Triticosecale* Wittm.) cv. Gabo seeds were harvested at the full ripeness stage and stored for eight months. Imbibition of kernels placed between two layers of moist filter paper in Petri dishes was carried out at a constant temperature of 20°C. Changes in kernel weight were also determined. The experiment was performed on untreated kernels (control) and kernels subjected to sprouting induction under laboratory conditions. Sprouting induction was performed according to the method described by Moś (2003). The kernels were classified as follows: 1°, kernels with the testa broken near the embryo; 2° kernels with visible primordial of coleoptiles and embryo roots; 3° kernels which developed coleoptiles and one primary root; 4° kernels which developed coleoptiles and three primary roots. The weight of kernels was determined before imbibition and at two-hour intervals over 14 h imbibition, and additionally at 24 and 26 h. When a tendency to kernel weight decrease was observed, imbibition measurements were stopped. The kernels were left on Petri dishes for eight days to determine their germination. All measurements were performed in four replicates of 50 kernels each.

Immediately after weighing, colour images of kernels for each trial were captured with a scanner (HP scanjet 4500c) at a resolution of 600 dpi, and subjected to a shape analysis using the MultiScan 8.08 (Computer Scanning Systems, Warsaw) software. The following procedure was used: at the first stage a 24-bit colour image was subjected to blue filtration (B) (Figure 1a), then a median filter (3 × 3 in one replication) was applied to the image in 256 grayscale to reduce background noise. After binarization at the threshold 180, 1-bit images were subjected to shape analysis (Figure 1b). The area (mm^2), perimeter (mm), length (mm) and width (mm) for the image of each kernel, were determined, and the shape factors $S_1 \div S_{10}$ were

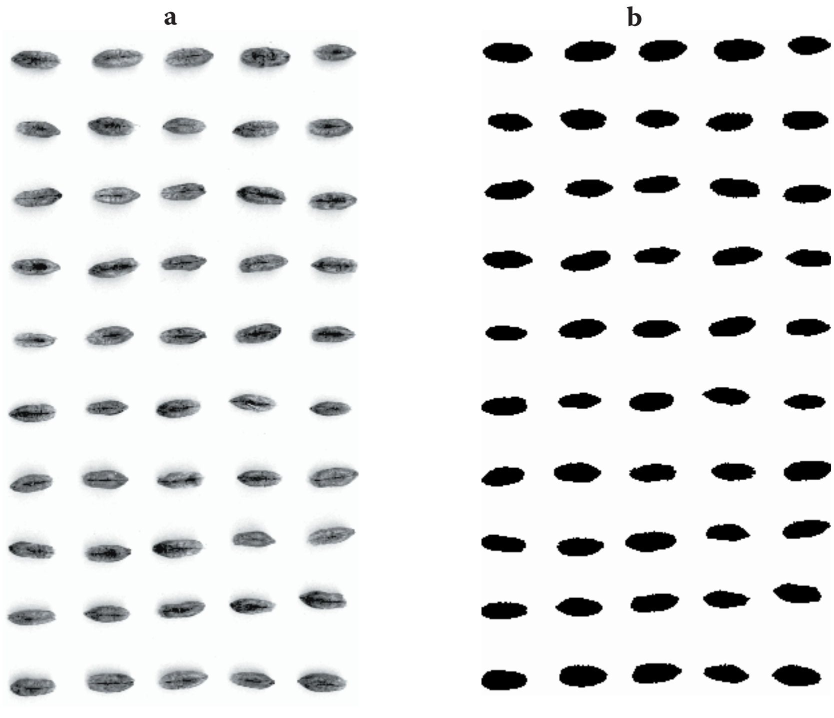

Figure 1. An example of a triticale kernel image prepared for shape analysis (control kernels after 4 h of imbibition; a – 8-bit image after blue filtration; b – 1-bit image after threshold at 180)

calculated according to the following formulas (Costa and Cesar 2001, Shouche et al. 2001):

$$S_1 = 2\sqrt{\frac{A}{\pi}}$$

$$S_2 = \frac{P}{2\sqrt{\pi A}}$$

$$S_3 = \frac{P}{D_{\max}}$$

$$S_4 = \frac{4\pi + A}{P^2}$$

$$S_5 = \frac{L}{A}$$

$$S_6 = \frac{A}{L^3}$$

$$S_7 = \frac{A}{\pi(0.5L)^2}$$

$$S_8 = \frac{A}{\pi(0.5L)(0.5W)}$$

$$S_9 = \frac{P - \sqrt{P^2 - 4\pi A}}{P + \sqrt{P^2 - 4\pi A}}$$

$$S_{10} = \frac{P^2}{A}$$

where: A – blob area (mm^2), P – blob perimeter (mm), L – blob length (mm), W – blob width (mm), D_{max} – maximal blob dimension (mm^2 or mm) of one of the four basic descriptors: A, P, L or W).

The results were analyzed by a two-factor analysis of variance and significance of differences between means was estimated by the multiple SNK test. The values of the Pearson's correlation coefficient between kernel weight and all descriptors of kernel image were also calculated.

RESULTS AND DISCUSSION

The weight of triticale kernels increased over 24 h of imbibition. This tendency was observed in both control kernels and in those with a different degree of sprouting, and the dynamics of the process was similar in all treatments (Table 1). The highest mean weight was recorded in kernels 1°, and the lowest – in the control kernels, not subjected to sprouting induction. The germination capacity of the control kernels and sprouted kernels 1° and 2° was 88.1, 95.5 and 82.4%, respectively, and did not differ significantly. A significant decrease in the germination capacity was observed in sprouted kernels 3° and 4° (42.3, 32.5%).

The increase in kernel weight due to imbibition was accompanied by an increase in volume, modifying the geometric features of their image (Figure 2). Kernel image area increased significantly during water absorption, varying from 22.5 mm^2 (dry kernels) to 27.7 mm^2 at 26 h imbibition. The largest image area and width were recorded in kernels with the slightest sprouting symptoms. The lowest image length and perimeter were observed in the control kernels.

Table 1. Average weight of 100 triticale kernels with by a different degree of sprouting during imbibition

Hour	C	1°	2°	3°	4°	Mean
0	3.654	4.286	3.692	3.690	3.456	3.756^{a}
2	4.014	4.662	4.070	4.066	4.840	4.330^{b}
4	4.106	4.808	4.270	4.264	4.100	4.310^{bc}
6	4.290	4.974	4.448	4.464	4.278	4.490^{cd}
8	4.402	5.084	4.548	4.590	4.396	4.604^{de}
10	4.550	5.246	4.734	4.790	4.610	4.786^{ef}
12	4.620	5.300	4.818	4.880	4.690	4.862^{f}
14	4.694	5.362	4.928	4.922	4.786	4.938^{f}
24	5.308	5.948	5.730	5.662	5.474	5.624^{g}
26	5.184	5.956	5.594	5.604	5.440	5.556^{g}
Mean	4.482^{a}	5.162^{c}	4.684^{b}	4.694^{b}	4.607^{b}	

Mean values followed by the same letter are not significantly different at $P = 0.01$ (separately for the sprouting degree and date of observation); C – kernels with no visible sprouting symptoms; 1°, 2°, 3°, 4° – kernels with a different sprouting degree

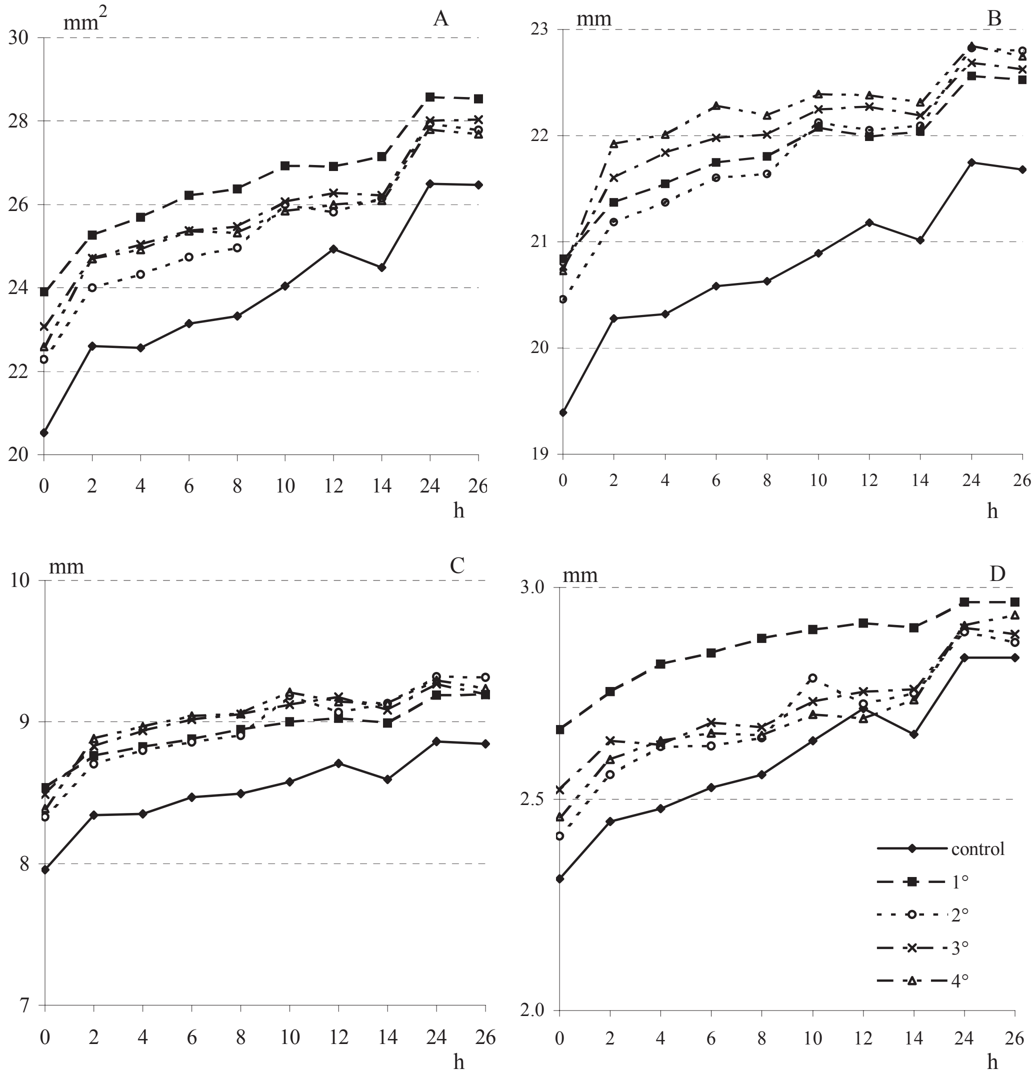

Figure 2. Dynamics of changes in the area (A), perimeter (B), length (C) and width (D) of images of triticale kernels with a varying degree of sprouting (1°–4°), compared with the control kernels

The shape factors used in the study differently described changes occurring in imbibing kernels. Significant differences between the mean values for kernels with a different degree of sprouting were found in all cases (Table 2). No significant interactions were recorded, which indicates a similar direction of changes in the image shape of imbibing kernels, regardless of the degree of sprouting and date of observation.

High activity of α-amylase in triticale grain causes starch granule damage and changes in the physicochemical properties of starch. The results obtained show that this modifies the rate of water uptake by kernels, especially those characterized by distinct sprouting changes. Furthermore, it was found that kernels with only initial macroscopic sprouting symptoms (fruit-seed coat damage near the embryo) showed the highest water absorption as well as germination capacity. At the initial stage of sprouting the damage to starch granules and embryos, affecting the imbibition rate and germination capacity, is slighter. In our experiment the kernels showing sprouting symptoms and the control kernels exhibited a similar imbibition dynamics, but the imbibition rate was significantly slower in the control ones, which probably results from changes occurring in starch granules under the influence of α-amylase. Damaged starch granules absorb water much more easily (Hoseney 1994). The results of investigations on wheat starch granules showing high α-amylase activity, based on electron scanning microscopy (Evers and McDermott 1970, Dronzek et al. 1972), demonstrated that large (A type) starch granules contain funnel-like openings, probably facilitating water penetration and accelerating imbibition. Very high amylolytic activity leads to complete degrada-

Table 2. Mean values of shape descriptors of the images of triticale kernels with a different sprouting degree

Descriptor	C	1°	2°	3°	4°
Area (mm^2)	23.463^c	26.544^a	25.235^b	25.820^b	25.624^b
Perimeter (mm)	20.77^c	21.85^b	21.81^b	22.02^{ab}	22.18^a
Length (mm)	8.52^b	8.93^a	8.96^a	9.02^a	9.04^a
Width (mm)	2.60^c	2.86^a	2.69^b	2.72^b	2.70^b
S_1	5.49^c	5.80^a	5.67^b	5.72^b	5.69^b
S_2	1.205^c	1.198^d	1.226^b	1.226^b	1.241^a
S_3	0.879^a	0.828^d	0.868^b	0.861^c	0.872^a
S_4	0.085^a	0.082^a	0.080^b	0.079^b	0.078^b
S_5	0.364^a	0.339^c	0.358^b	0.354^b	0.357^{ab}
S_6	0.039^a	0.038^a	0.036^b	0.036^b	0.036^b
S_7	0.418^b	0.424^a	0.403^c	0.405^c	0.400^c
S_8	1.402^a	1.338^c	1.372^b	1.366^b	1.370^b
S_9	20.415^d	21.496^c	21.451^c	21.657^b	21.812^a
S_{10}	27.450^d	32.299^a	29.604^c	30.319^b	29.644^{bc}

The values followed by the same letter are not significantly different at $P = 0.01$ within the same descriptor; C – kernels with no visible sprouting symptoms; 1°, 2°, 3°, 4° – kernels with a different sprouting degree

tion of starch granules, which can explain the lower imbibition rate of kernels 3° and 4°, compared with kernels 2°. Considerable fluctuations in the weight of kernels 4° after two and four hours of imbibition were not accompanied by visible changes in the values of shape descriptors. This may suggest that at the initial stage of imbibition water penetrates very quickly into kernels characterized by advanced starch degradation, and equally quickly gets out of them. These processes are not accompanied by changes in kernel volume.

Digital image analysis permitted precise determination of changes in the image of imbibing kernels. Dell'Aquilla (2003) and Dell'Aquilla et al. (2000) applied this technique to estimate differences in the area, length, width and perimeter of images of cabbage seeds imbibing both under conditions of NaCl salinity and without additional stress factors modifying imbibition. Analyzing changes in seed shape image, the above authors demonstrated that the first phase of imbibition lasted six hours and was followed by the second phase, with no significant changes in the geometric parameters of the image. In the case of triticale an increase in image area, perimeter and length was recorded for the first ten hours of the experiment, and no significant changes were found in these parameters during the next four hours. Between the 14th and 24th hour of imbibition, the values of the four basic geometric parameters increased again and then decreased, which could be related to the initiation of germination. These parameters

Table 3. Values of the Pearson's correlation coefficient for kernel weight and studied shape descriptors

	Area	Perimeter	Length	Width	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}
C	0.986**	0.979**	0.856**	0.812**	0.984**	0.979**	–0.888**	–0.984**	–0.889**	–0.955**	–0.351	0.394	0.981**	0.987**
1°	0.840**	0.989**	0.981**	0.881**	0.842**	0.989**	–0.132	–0.625	–0.433	–0.651*	–0.332	0.221	0.986**	0.997**
2°	0.757**	0.603	0.664*	0.820**	0.753**	0.603	–0.104	–0.903**	–0.350	–0.850**	–0.505	0.187	0.983**	0.992**
3°	0.579	0.574	0.471	0.407	0.565	0.574	–0.397	–0.473	–0.478	–0.600	–0.207	0.565	0.946**	0.990**
4°	0.401	0.378	0.424	0.390	0.386	0.378	–0.269	–0.405	–0.312	–0.310	–0.393	0.162	0.893**	0.987**

*significant at $P = 0.05$, **significant at $P = 0.01$

Table 4. Parameters of linear regression equations ($y = a + bx$) and values of R^2 calculated for the weights of imbibing triticale kernels and four shape descriptors at successive dates of observation

		Control	1	2	3	4	In all
Area	b	3.564***	2.684***	2.687***	2.353***	2.277***	2.661***
	a	7.880	12.701	12.817	14.783	15.365	12.928
	R^2	0.973	0.989	0.987	0.981	0.955	0.855
Perimeter	b	1.351***	0.970***	1.124***	0.828***	0.812***	0.927***
	a	14.758	16.841	16.549	18.137	18.522	17.365
	R^2	0.962	0.972	0.964	0.883	0.793	0.540
Length	b	0.507***	0.368***	0.456***	0.324***	0.352**	0.370***
	a	6.243	7.036	6.826	7.499	7.449	7.151
	R^2	0.924	0.963	0.886	0.806	0.758	0.540
Width	b	0.326***	0.171***	0.224***	0.187***	0.215***	0.237***
	a	1.137	1.979	1.639	1.842	1.727	1.598
	R^2	0.972	0.901	0.934	0.980	0.957	0.878

are at the same time varietal features, so it would be difficult to determine the imbibition dynamics of triticale kernels based on their absolute values. Thus it seems that it is necessary to use and calculate shape factors, reflecting the relationships between these four variables.

The highest correlation between kernel weight and all image descriptors was found between kernel weight and factors S_9 and S_{10}: r varied from 0.893 to 0.997 (Table 3). Only these two descriptors were so closely correlated with the weight of kernels in all sprouting groups (none of the other shape factors was significantly correlated with the weight of kernels 3° and 4°). This indicates that they best describe changes in the images of imbibing kernels, in contrast to S_7, S_8, S_3 and S_5, which turned out to be the least reliable.

A regression analysis performed for the weights of imbibing kernels and four main image shape descriptors showed a significant linear relationship between the parameters studied (Table 4). The linear regression model well described the detected relationships, which was confirmed by high values of the determination coefficient R^2.

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