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Localization and morphology of the buckwheat embryo

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ABSTRACT

The position, shape and size of the embryo in buckwheat seed has not yet been resolved. This paper describes computer aided three-dimensional reconstruction of the buckwheat embryo using two-dimensional images obtained by progressively grinding the seeds. We found that cotyledons are double curved in the proximal part and that they reach the testa only with their margins. In the distal part, the two cotyledons extend tightly along the testa. In some seeds the cotyledons form a spiral in a clockwise direction and in others in an anticlockwise direction. The ratio among the two cotyledon orientations was 55 : 45. We found no correlation between kernel weight and the orientation. The length of the embryo proper was found to be in the range of 31–56 % of the seed length (in average 42%). The variability of the length of the embryo proper was much higher than the variability of the seed length.

INTRODUCTION

Products made from buckwheat seeds represent an important food in some countries. The seeds contain starch, proteins with a well-balanced amino acid composition (Eggum et al., 1981), dietary fibres (He et al., 1995), polyphenols with antioxidative properties (Watanabe et al., 1997) including rutin (Kitabayashi et al., 1995; Kreft et al., 1999), minerals such as zinc, copper and manganese (Ikeda and Yamashita, 1994) and some fat. The majority of all nutrients, except the starch, are located in the embryo (Kreft, 1999). Most of the starch is stored in the endosperm. The nutritional value of buckwheat seeds might be improved by selection for the cultivars with relatively larger embryo in relation to the total seed volume.

The embryo proper was already found to be located in the pointed (distal) part of the seed (Kreft and de Francisco, 1989) and the cotyledons were found to be spirally packed in the seed (Pomeranz and Sach, 1972; Kreft and de Francisco, 1989). The exact three-dimensional position of the cotyledons has not yet been determined and is the subject of this study. Preliminary results were published in a paper by Kreft and Kreft (1999).

MATERIALS AND METHODS

In the first set of experiments buckwheat seeds (cv. Siva, harvested in Slovenia) were manually dehulled. The groats were embedded into a polyester resin (Colpoly, Color Medvode, Slovenia) and 0.1 mm thick slices were progressively removed by grinding. Each newly exposed plane was treated with the $FeCl_3$ solution (0.2 g/ml in 70 % ethanol), dried and photographed with a 1 : 1 macro objective (Pentax 105 mm, Kodak 100 ASA film).

On the photographs, the embryo with cotyledons was manually emphasised, scanned and imported into a Silicon Graphics O_2 computer. Three-dimensional morphology of the embryo was reconstructed by Imaris software package (Bitplane AG, Zürich, Switzerland), based on UNIX operating system, transformed into an Open Inventor 2.0 file type and visualised by Scene Viewer software (Silicon Graphics, Inc., USA). In this set of experiments, 13 seeds were analysed. Three-dimensional reconstruction of the seed morphology shown in Fig. 3 was derived from 32 cross sections of one typical seed. Alternatively, autofluorescence was detected using a Zeiss Inverted Microscope, Axiovert 135 with objective lenses Plan-Neofluar 5x/0.15 and Plan-Neofluar 1.25/0.035. Excitation light from a mercury lamp was filtered with a band pass 450–490 nm (blue) filter. Emission and excitation light was separated using a dichroic longpass beamsplitter 510 nm and autofluorescent emission light was selected using a long pass 520 nm filter.

The second set of experiments was performed to determine the orientation of cotyledons in seven samples of common buckwheat and in two samples of Tartary buckwheat seeds, to determine the orientation of the cotyledons in seeds of different weight and to determine the orientation in seeds deriving from individual plants.

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In this set of experiments, one sample (Tartary 1) of Tartary buckwheat was grown in Luxembourg (the number of seeds used, n = 98) and the other (Tartary 2) was cultivated from the same seed material but grown in Slovenia (n = 70). The seeds of three individual plants from the Tartary 2 sample were analysed separately to determine if the orientation of cotyledons differed among the plants. The sample of common buckwheat cv. La Harpe (n = 85), cv. Darja (n = 97), cv. Podhomska (n = 99), and cv. Prekmurska (n = 98) were grown in Slovenia. The two samples of common buckwheat cv. Siva (Siva 1 (n = 100) and Siva 2 (n = 195)) were grown at different locations in Slovenia. A sample of common buckwheat from Shanxi (China) was kindly provided by Dr. Zhang Zheng. One part (n = 99) of the seeds from the Siva 2 sample were weighted (balance type: Europe 60; Gibertini, $d_d = 0.0001$ g) before the incorporation into the resin. All seeds were incorporated into the resin as described in the first set of experiments and the orientation of cotyledons was determined.

RESULTS AND DISCUSSION

The incorporation of the groats into the resin was firm enough to enable grinding throughout the whole groat, without the groat falling out of the resin. Staining with $FeCl_3$, which is known to produce a dark blue colour in combination with tannins and other polyphenols (Stahl-Biskup and Reichling, 1998), coloured the embryo, including the cotyledons, and produced a good contrast against the endosperm (Fig. 1).

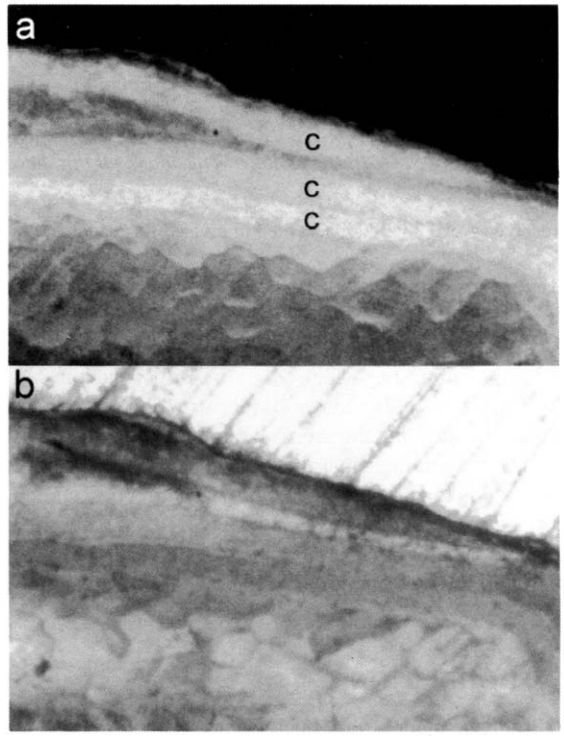

Fig. 1. Autofluorescent image (a) and transmitted light micrograph (b) of a thin section of buckwheat seed. The embryo emitted green fluorescent light when excited with blue light (488 nm). The embryo displayed a brown-blue colour under transmitted light microscopy when the section was stained with $FeCl_3$. Both methods of staining are appropriate to detect and discriminate the two cotyledons. The sign c denotes cotyledons.

We confirmed the location of the embryo proper in the pointed (distal) part of the seed as reported previously (Kreft and de Francisco, 1989). The length of the embryo proper was in average 42% of the seed length with a range of 31–56 % (Table 1). Relative standard deviation (RSD) of the proper length (22.8 %) was more than two fold higher than the RSD of the seed length (9.8 %). The wide range of the relative length of the embryo proper may be due to the fact that there is no simple method to measure embryo length or volume as a character in buckwheat improvement. Nevertheless, embryo size may be an important property of buckwheat seeds used for human nutrition. The two cotyledons were found to be in tight contact with each other. In the wider (proximal) part, they are double curved and reach the testa with their margins (Fig. 4 panels 5 and 6). In the pointed (distal) part, the two cotyledons extend tightly along the testa (Fig. 4, panels 1, 2, 3 and 4). The outer cotyledon extends further than the inner one. Observing from the distal towards the proximal part of the seed, the cotyledons formed a spiral in a clockwise (as in letter S) or anti-clockwise direction (as in letter Z or number 2).

To further study this phenomenon additional samples

Table 1. Seed and embryo proper lengths and their ratio, measured by the analysis of the pictures taken during progressive grinding of the seed (n=13).

	Seed lenght (μm)	Proper lenght (μm)	Ratio of the proper and seed lenghts (%)
average	3984	1692	42
standard deviation (SD)	391	387	8
relative SD (%)	9.8	22.8	19.7
minimum	3276	1248	31
maximum	4680	2340	56

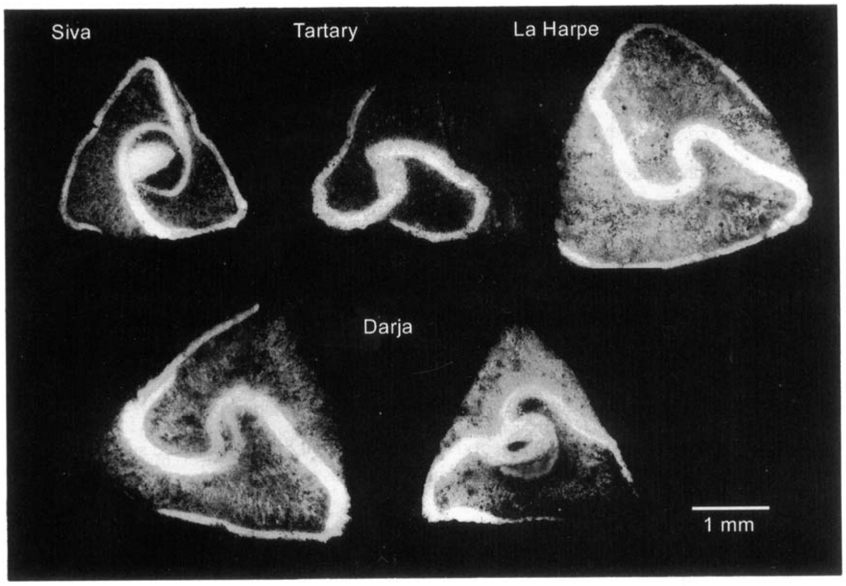

Fig. 2. Autofluorescent images of the ground buckwheat seeds of three cultivars of common buckwheat and of Tartary buckwheat. In the right hand seed of cv. Darja, the two cotyledons have different orientation. About 1% of such seeds were found in most samples.

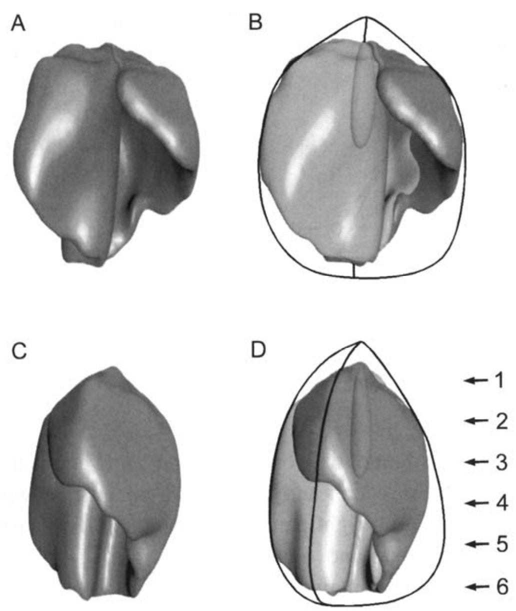

Fig. 3. An equatorial view of the three-dimensional model of the buckwheat embryo generated with a Silicon Graphics O_2 computer. Top two panels represent one view (from the bottom at Fig. 4) and the bottom two panels represent the view perpendicular to the first one (from the left at Fig. 4). The black lines on the panels (B and D) represent the buckwheat groat edges. The light grey and dark grey are the respective cotyledons; the embryo proper is visual in the center at the distal end of the cotyledons. On the panels A and C one cotyledon and the embryo proper has been removed. Arrows in panel D are pointing at the sections which are shown on Fig. 4.

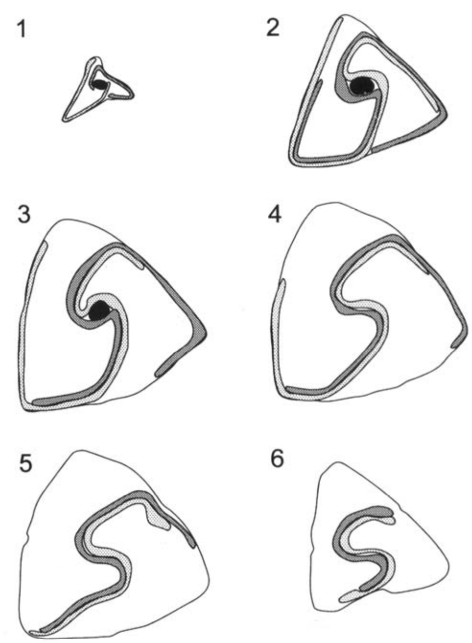

Fig. 4. Cross sections of a computer generated three-dimensional model of a buckwheat embryo. The black line represents the testa. The light grey and dark grey areas are the respective cotyledons; the black spot in the center is the embryo proper.

were analysed (Fig. 5). Clockwise oriented cotyledons were predominantly or equally present in all the various samples. In all analysed seeds (n = 930) 55% of the cotyledons were clockwise oriented (when observed from the distal towards the proximal part of the seed),

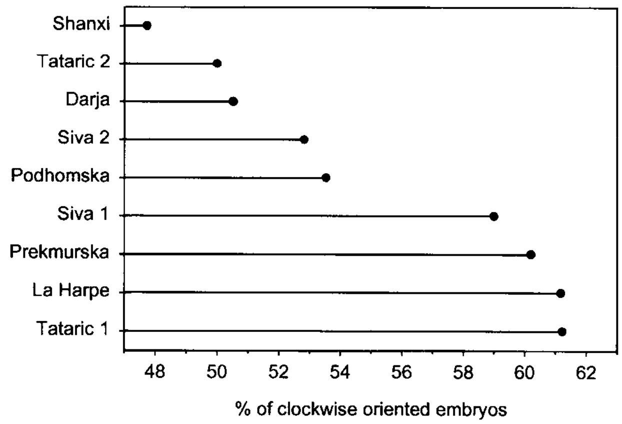

Fig. 5. Ratio of clockwise vs. anti-clockwise oriented cotyledons in different buckwheat seed samples. It can be seen that clockwise oriented cotyledons are predominant and equally present in the various samples.

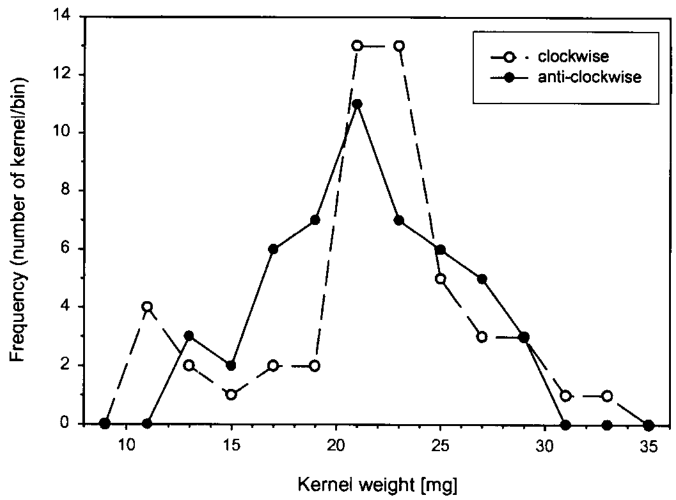

Fig. 6. Frequency distribution of the seeds with respective orientations of cotyledons as a function of their weight. No significant correlation can be observed between the weight of the seed and the clockwise or anti-clockwise oriented cotyledons.

which was significantly different from 1 : 1 ratio (chi-square test, $p = 0.002$). The differences among the samples are not significant. We observed that in seeds from a single plant of Tartary buckwheat both orientations of the cotyledons were present in a ratio, which does not differ significantly from the other plants.

The seeds of cv. Siva were weighted before being embedded into the resin and before determining the orientation of the cotyledons. No correlation was found between cotyledon orientation and seed weight (Fig. 6). The average weight of the seeds (including hull) with clockwise ($n = 50$) or anti-clockwise ($n = 49$) oriented cotyledons was 21.6 mg and 21.4 mg respectively ($SD = 5.1$ mg and $SD = 4.1$ mg, respectively). The difference was not significant ($p = 0.7$)

In some seeds (about 1%) a different shape of the cotyledons was found, with one cotyledon being oriented in a clockwise direction and the other in an anticlockwise direction (Fig. 2, right panel at the bottom). Two seeds with four edges and one seed with five edges were found. They all contained two cotyledons with the similar position and shape as in the three edged seeds.

On the three dimensional reconstruction of cotyledons (Fig. 3), it can be observed, that the wider part of each cotyledon is packed in the pointed (distal) part of the seed and only the cotyledon tips are located in the wider (proximal) part of the seed.

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