Research Reports, Biotechnical Faculty, University of Ljubljana, 73, 1999 p. 331-336

University of Ljubljana
School of Medicine
Institute of pathophysiology
Laboratory of neuroendocrinology - molecular cell physiology
Head: Prof. Dr. Robert Zorec

University of Ljubljana
Faculty of Pharmacy
Chair for pharmaceutical biology
Head: Ass. Prof. Dr. Andrej Umek

COMPUTER AIDED THREE-DIMENSIONAL RECONSTRUCTION OF THE BUCKWHEAT (*Fagopyrum esculentum* Moench) SEED MORPHOLOGY

Marko KREFT[1], Samo KREFT[2]

ABSTRACT

The position, shape and size of the embryo in buckwheat seed were not yet resolved, although they were studied by several authors. In this study we used computer aided three-dimensional reconstruction to analyse the two-dimensional images obtained by progressively grinding the seeds. We found out, that in the proximal part, the cotyledons are double curved and they reach the testa only with their margins. In the distal part, the two cotyledons extend tightly by the testa. In some seeds the cotyledons form a spiral in a clockwise direction and in the others in anticlockwise direction. The variability of the embryo proper length is much higher than the variability of the seed length. The embryo proper length was found to be in a range of 31 - 56 % of the seed length (in average 42%).

Key words: buckwheat, cotyledons, computer aided reconstruction, embryo, seed

IZVLEČEK

RAČUNALNIŠKA TRIDIMENZIONALNA REKONSTRUKCIJA SEMEN AJDE (*Fagopyrum esculentum* Moench)

Položaj, oblika in velikost kalčka v semenih ajde doslej še niso bili določeni, čeprav jih je raziskovalo že več avtorjev. V tej raziskavi smo s pomočjo računalniške tridimenzionalne rekonstrukcije obdelali podatke dvodimenzionalnih slik, ki smo jih posneli med zaporednim brušenjem semen. Ugotovili smo, da sta klična lista v proksimalnem delu semena dvakrat ukrivljena, z robovi pa se le dotakneta semenske ovojnice. V distalnem delu se klična lista razprostirata tesno ob semenski ovojnici. V nekaterih semenih tvorita klična lista spiralo v smeri urinega kazalca, pri drugih pa v nasprotni smeri. Variabilnost dolžine osi kalčka je mnogo večja od variabilnosti dolžine semena. Dolžina kalčka je od 31 % do 56 % dolžine semena (v povprečju 42%).

Ključne besede: ajda, kalček, klična lista, račinalniška rekonstrukcija, seme

[1] Ph. D., B. Sc. Biol., SI-1000 Ljubljana, Zaloška 4
[2] Ph. D., M. Pharm., SI-1000 Ljubljana, Aškerčeva 7

INTRODUCTION

Buckwheat seeds are an important component of human nutrition in some countries. They contain starch, proteins with a well balanced amino acid composition (Eggum et al., 1981), dietary fibres (He at al., 1995), polyphenols with antioxidative properties (Watanabe et al., 1997) including rutin (Kitabayashi et al., 1995; Kreft et al., 1999), minerals as zinc, copper and manganese (Ikeda and Yamashita, 1994) and some fat. The bulk of all nutrients except the starch is 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 the relatively larger embryo in respect to the seed volume.

The embryo proper was 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 was not determined before.

MATERIALS AND METHODS

Buckwheat grains (cv. Siva, harvested in Slovenia) were manually dehulled. The seeds were embedded into polyester resin (Colpoly, Color Medvode, Slovenia) and 100 μm 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 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 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 Open Inventor 2.0 file type and visualised by Scene Viewer software (Silicon Graphics, Inc., USA). 13 seeds were analysed. Three-dimensional reconstruction of the seed morphology shown in Fig. 2 is derived from 32 sections of one typical seed. Alternatively, autofluorescence was detected using Zeiss Invert Microscope Axiovert 135 with objective lenses Plan-Neofluar 5x/0.15 and Plan-Neofluar 1.25/0.035. Excitation light from mercury lamp was filtered with band pass 450-490 nm (blue) filter, emission and excitation light was separated using dichroic longpass beamsplitter 510 nm and autofluorescent emission light was selected using long pass 520 nm filter.

RESULTS AND DISCUSSION

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

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 embryo proper length was found to be in average 42% of the seed length in a range of 31-56 % (Fig. 4). Relative standard deviation (RSD) of the embryo proper length (22.8 %) is more than

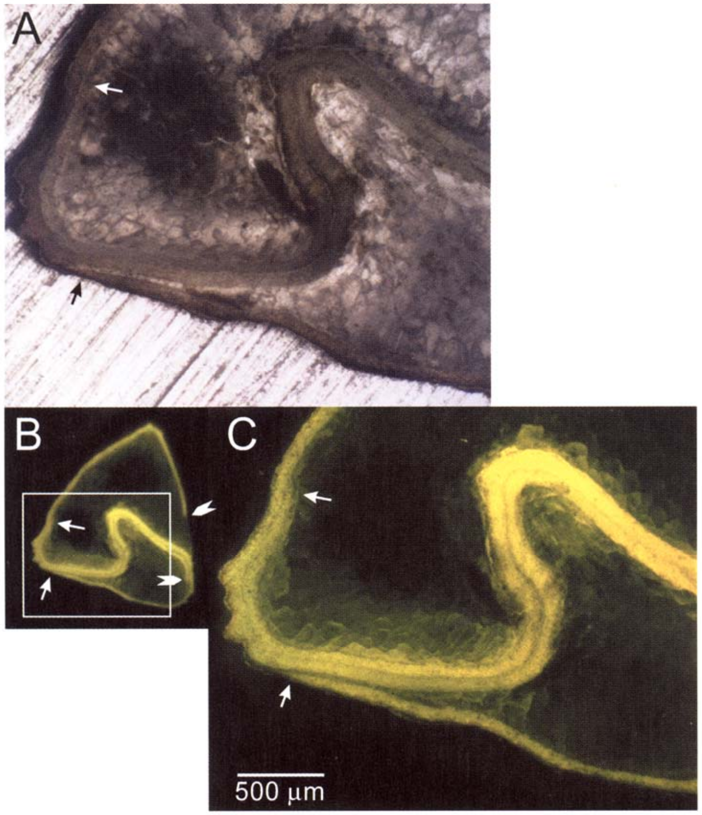

Figure 1: Autofluorescent image (B,C) and the transmitted light micrograph (A) of the thin section of the buckwheat seed. Embryo is emitting green fluorescent light when excited with blue light (488 nm). Embryo is displaying brown-blue colour in transmitted light microscopy, when the section was stained with $FeCl_3$. Two arrows and two arrowheads are pointing at two ends of each cotyledon. Both methods of staining are appropriate to detect and discriminate two cotyledons.

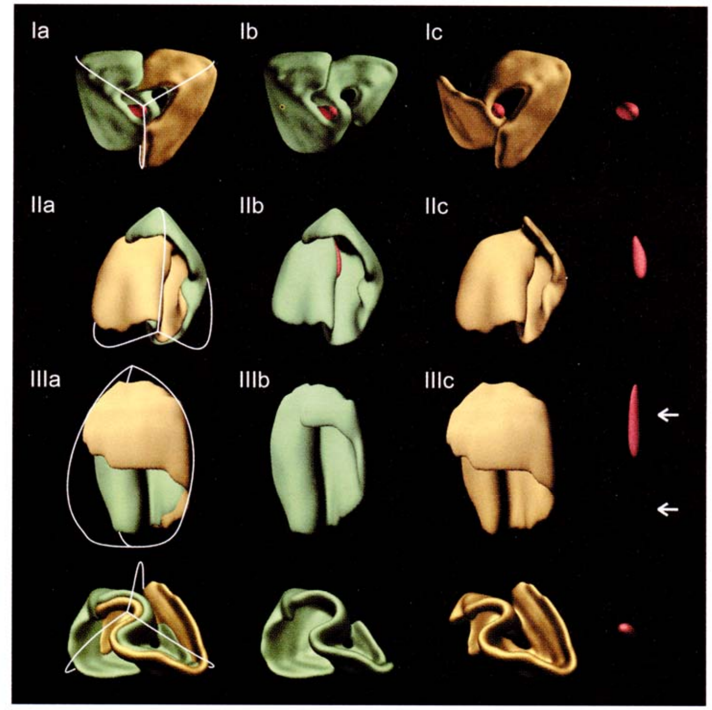

Figure 2: Computer generated three-dimensional model of the buckwheat embryo. White lines on the left panels (a) are representing buckwheat grain edges. Yellow and green are respective cotyledons, red is the embryo proper. At the panels "b" and "c" one cotyledon is removed, at the panel "d" both cotyledons are removed to expose the embryo proper. Arrows in the panel IIId are pointing at the sections which are shown on figure 3.

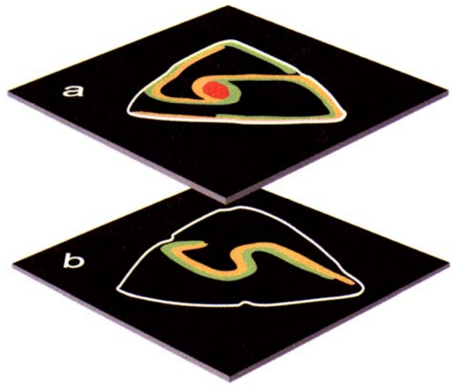

Figure 3: Crossections of the computer generated three-dimensional model of the buckwheat embryo. White line is representing testa.

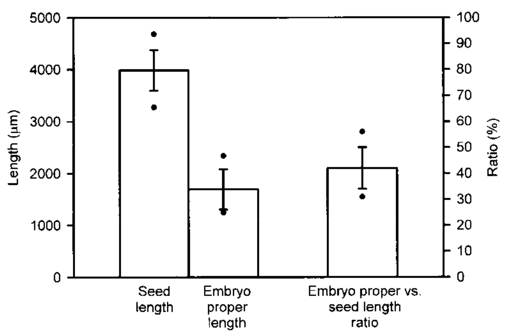

Figure 4: Seed and embryo proper lengths and their ratio, measured by the analysis of progressive grinding of the seed. Error bars are representing standard deviation of the mean, dots are representing minimum and maximum value.

two fold higher than RSD of the seed length (9.8 %). Wide range of the relative embryo proper length may be due to the fact, that there is no simple method to measure embryo proper length or volume as a property for buckwheat breeding. Although embryo proper size may be an important property of buckwheat grains for human nutrition. The two cotyledons are in a tight contact to each other. In the wider (proximal) part of the seed, they are double curved and they reach the testa with their margins (Fig. 3b). In the pointed (distal) part, the two cotyledons extend tightly by the testa (Fig. 1, Fig. 3a). The outer cotyledon extends further than the inner one. Observing from the proximal towards the distal part of the seed, the cotyledons form a

spiral in clockwise (n=6) or anticlockwise direction (n=7). The ratio of the two directions is not significantly different from 1 : 1.

On the three dimensional model of cotyledons (Fig. 2) can be observed, that the wider part of each cotyledon is packed in the pointed part of the seed, and only the cotyledon tips are located in the wider (proximal) part of the seed.

REFERENCES

Eggum, B.O., Kreft, I., Javornik, B., (1981): Chemical composition and protein quality of buckwheat (*Fagopyrum esculentum* Moench). Plant Foods for Human Nutrition, 30: 175-179.

He, J., Klag, M.J., Whelton, M.J., Mo, J.-P., Chen, J.-Y., Qian, M.-C., Mo, P.-S., He, G.-S., (1995): Oats and buckwheat intakes and cardiovascular disease risk factors in an ethnic minority in China. The American Journal of Clinical Nutrition, 61: 366-372.

Ikeda, S., Yamashita, Y., (1994): Buckwheat as a dietary source of zinc, copper and manganese. Fagopyrum, 14: 29-34.

Kitabayashi, H.; Ujihara, A.; Hirose, T.; Minami, M., (1995): Varietal differences and heritability for rutin content in common buckwheat, *Fagopyrum esculentum* Moench. Breeding Science, 45: 75-79.

Kreft S., Knapp M., Kreft I., (1999): Extraction of rutin from buckwheat (*Fagopyrum esculentum* Moench) seeds and determination by capillary electrophoresis. J. Agric. Food Chem., in printing.

Kreft I., de Francisco A., (1989): Morphological studies on the location and size of the buckwheat embryo. Fagopyrum, 9: 47-48.

Kreft I., (1999): Nährwert des Buchweizens - Kerne - Mehl - Brot - Gemüse - Tee - Essig - Honig. In: Das Buchweizenbuch, Islek ohne Grenzen EWIV, Arzfeld, 13-21.

Pomeranz Y., Sachs I.B., (1972): Scanning electron microscopy of the buckwheat kernel. Cereal Chem., 49: 23-25.

Stahl-Biskup E., Reichling J., (1998): Anatomie und Histologie der Samenpflanzen, Deutscher Apotheker Verlag Stuttgart, 15-18.

Watanabe, M., (1998): Catechins as antioxidants from buckwheat (*Fagopyrum esculentum* Moench) groats. J. Agric. Food Chem., 46: 839-845.