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VARIABILITY OF BARLEY ALEURONE LAYER INDUCED BY X-RAYS

Romuald Kosina

Abstract. A series of *Hordeum vulgare* cultivars was irradiated by X-rays to induce mutations in endosperm. Many structural defects of endosperm were revealed in plants irradiated 8 DAF. Change of a cell cycle was especially frequent and this was visible in the form of clones of small or large cells in the aleurone layer. X-irradiation appeared as a successful tool in the study of development.

Key words: *Hordeum vulgare* cultivars, X-rays, mutations, aleurone layer

Plant Speciation Group, Institute of Environmental Biology, University of Wroclaw, Przybyszewskiego 63-77, 51-148 Wroclaw, Poland; kosina@biol.uni.wroc.pl

Introduction

Application of X-rays is still used for the quality improvement in cereals (Mlčochová *et al.* 2004). Achievements of Swedish mutation breeding in barley are commonly known and, between many others, waxy mutants of endosperm were also obtained (Eriksson 1965). In grass embryo, environmental stress can induce a mosaic of the presence *versus* absence of epiblast and also lead to the development of starchy cells in the aleurone layer instead of the proteinaceous ones. Such events were observed in *Triticum timopheevii* (Zhuk.) Zhuk. as well as in some amphiploids of Triticeae Dumort. (Kosina 1989a, 1989b). Disorders of the chalazal region of caryopsis change the development of endosperm in barley mutants (Felker *et al.* 1985) and in wheat tetraploids (Kosina 1988). Development of parts of the body from the cell segments is a characteristic of plants (see development of meristemoids in epidermis or cell complexes in *Aristolochia* L., Puławska 1982). Somatic mutation of the mother cell of the segment gives the mutated clone of cells, for instance in endosperm of maize (Fedoroff 1988). Earlier studies by Sawicki (1951) revealed large differentiation of the barley aleurone layer structure. The endospermal mosaics were also documented in *Triticum/Aegilps* amphiploids (Kosina & Zając 2010) and in Triticeae amphiploids (Kosina & Tomaszewska 2010).

Material and methods

The following five old cultivars of *Hordeum vulgare* L. from the collection of the Plant Breeding and Acclimatization Institute, Radzików, Poland, were used in the study: *Laschkego Tybetański, Michalovicky Nahy, Abyssinian, Perbete* and *Dornburger Heils Franken*. Ten plants of each cultivar were cultivated in experimental pots. Plants were irradiated with a dose of 0.03 Gy with the use of an X-rays medical equipment. A series of plants not irradiated served as a control. Irradiation was applied at 8 DAF (days after flowering), at a stage of still existing cell divisions in "endosperm cambium" of main ears. Ripe caryopses were treated by FAA fixative and cross-sections of a central part of caryopsis were stained with bromophenol blue to detect proteins and studied under an Amplival microscope. The aleurone layers were manually isolated and observed as a flat structure under the polarising Amplival microscope.

Results and discussion

The following morphological changes were observed on cross-sections of caryopsis, on the surface of isolated parts of the aleurone layer and in dissected embryos:

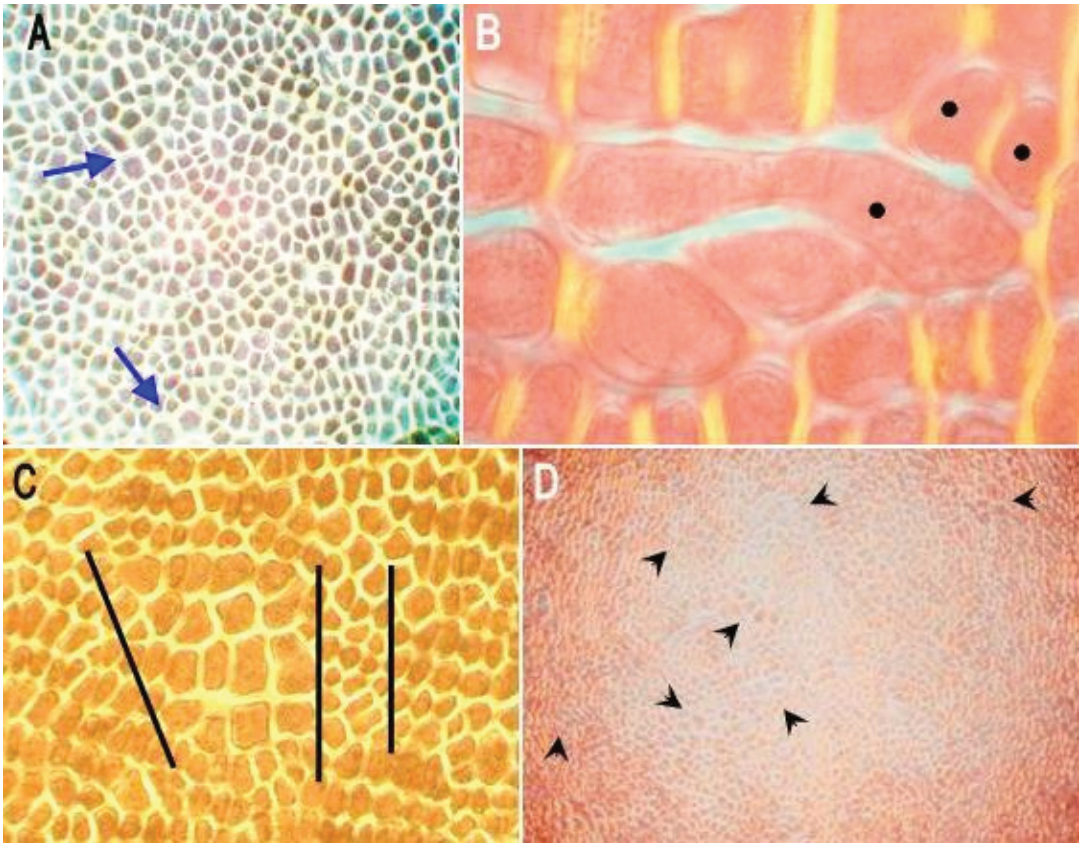

Fig. 1. Developmental mosaics of aleurone layer (surface views) in irradiated caryopses of barley: **A** – an aleurone layer expressing lighter and darker patches of cells with less or more protein, respectively (stained by bromophenol blue). Two clones of larger cells are marked by arrows; **B** – three cells (black dots) expressing changed cell cycles – a large cell is developed as omitting at least two cytokineses; two small cells are created after one additional cytokinesis, in excess when compared to normal aleurone cells; **C** – a mosaic of aleurone layer with two adjacent clones of large and small cells, marked by black lines; **D** – a large fragment of aleurone layer with increased frequency of mutated cell clones, some are marked by arrows. Pictures **B, C** and **D** are taken under a polarising microscope.

1. Appearance of cells within the aleurone layer with a changed rate of protein synthesis (see Fig. 1 A).
2. Development of cells synthesising starch instead of aleurone protein. Phenotypes with starch granules of different diameter, small, medium or large, were observed.
3. Abnormal development of aleurone layer in the form of clones of smaller or larger cells (see Fig. 1 A-D).
4. In the crease region the aleurone cells were often not developed.

The distinct mosaic in the aleurone layer was noted in cv. *Abyssinian*. One day after irradiation, the anaphase bridges were observed exceptionally in endosperm syncythium stained with acridine orange. It is known that groups of aleurone cells differentiate in separate areas around young starchy endosperm and form some kind of mosaic (O.-A. OLSEN, personal communication). A similar mosaic in the aleurone layer development was observed in the F_1 generation of *Triticum* × *Elymus* hybrids (IVANOVSKAYA 1983).

The development of mosaics was noted in various organs of grasses, in ears, embryos but especially frequently in the endosperm (KOSINA 2007). Mosaics are a very natural phenomenon in the endosperm however, the

frequency of mosaics is distinctly increased in hybrids and mutants. The X-irradiation of plants appeared to be as a successful tool in the research of development of grass endosperm. Such studies interpreted together with data on the development of endosperm in grass hybrids can improve our understanding of this cytogenetically complex tissue.

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