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ANTIPODAL SYMMETRY AND ASYMMETRY IN THE EMBRYO SAC OF *AVENA SATIVA* L.

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Abstract. Inter- and intra-antipodal variation is presented for a free-nucleolar developmental stadium in the *Avena sativa* endosperm. Antipodals of common oat are uni- or multinuclear. Multinuclear antipodals, in a single cell, show nuclei of the same mitotic stage, but this is different for various antipodals. Examples of DNA amplification and anomalies occurring during mitosis are provided.

Key words: *Avena sativa*, antipodals, DNA amplification, karyokineses, variation

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Introduction

In various grasses, antipodals are polyploidised up to different level and can be active in the form of an antipodal tissue even in the cellular endosperm (Batygina 1987). In a common wheat × triticale cross, antipodal nuclei can reach 512*n* within three days after pollination (Wędzony 1992-1993). Some data points that nuclei in a multinuclear antipodal cell are mitotically synchronous, *e.g.* in *Triticum durum* Desf. (Ivanovskaya 1983). However, Chaban *et al.* (2011) observed synchronous and asynchronous karyo- and cytokinesis in antipodals of common wheat. As a rule, giant chromosomes are formed in the uninuclear antipodals, and following this, the number of rDNA loci does not increase, *e.g.* in a *Triticum durum* × *Aegilops tauschii* Coss. amphiploid (Kosina 1994). Less is known on the behaviour of antipodals in the genus *Avena* L. Some results have already been presented by Krawczyk (2008).

Material and methods

Young fruits of *Avena sativa* var. *grisea* Körn. were dissected from spikelets in a stadium of free-nuclear endosperm. Fruits were fixed in a Carnoy's solution and stored in a freezer at -20 °C. Embryo sacs were isolated from the fruits and washed in distilled water and then three times for 5 min in a 0.01 M citrate buffer. Material was enzymatically digested in a mixture of pectinase and cellulase in a hybridisation oven at 37 °C. The digested material was centrifuged three times for 3 min at 800 g, each time in fresh citrate buffer. After centrifugation, the supernatant was discarded. The material was prepared by squash or dropping methods according to Schwarzacher *et al.* (1980) and Ambros *et al.* (1986). For chromosome staining, 100 µl/slide of 0.5 µg/ml DAPI and 0.025 µg/ml propidium iodide were used, respectively. Slides were washed in PBS buffer and mounted in a medium that prevented the fading of fluorescence. Slides were stored in a refrigerator at 5 °C. Cytogenetic material

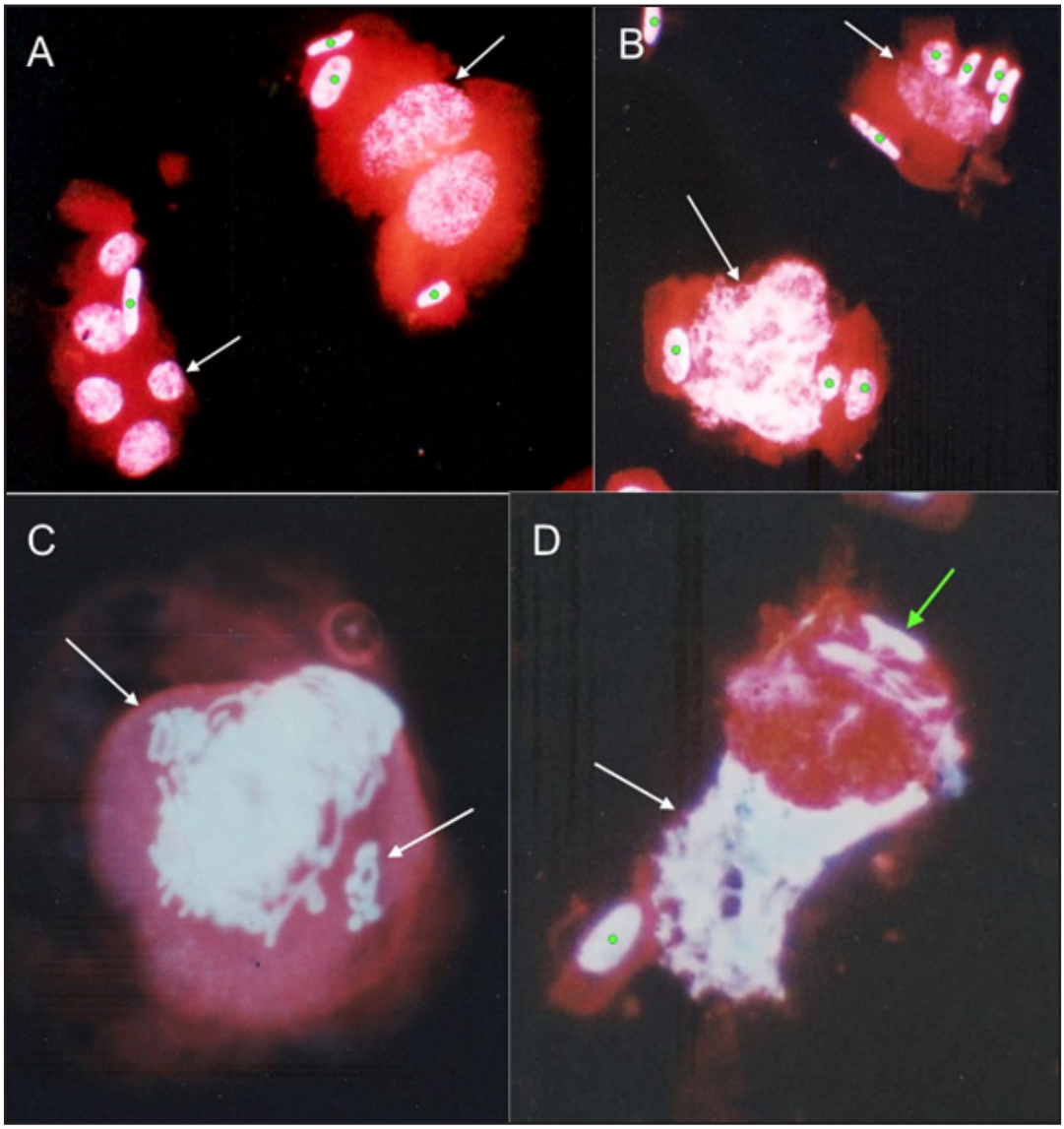

Fig. 1. Nuclear antipodal morphology at the free-nuclear endosperm stage in *Avena sativa*: **A** – two multinuclear (2 and 5 nuclei) antipodals (**arrows**) showing the different levels of ploidy and phase of the cell cycle; **B** – two uninuclear antipodals (**arrows**) with various DNA amplification and, probably, at a different stage of prophase; **C** – a highly polyploidised antipodal cell at prophase stage with two groups of laggard chromosomes (**arrows**); **D** – a partly apoptotised antipodal nucleus (**white arrow**) and semi-telophase of a small nucleus (remnant DNA) perhaps was formed from the laggard chromosomes (see **green arrow**). Other nuclei, not from antipodals, are marked by **green dots. A-D** – DAPI and propidium iodide sequential staining.

was documented under an Olympus BX60 epifluorescence microscope with a triple filter (DAPI, TRITC, FITC) and pictures were taken with Zenith TTL camera and Fuji 400 film.

Results and discussion

Amplification of DNA in an antipodal cell can occur by karyokineses or by the replication of DNA, without the separation of chromosomes. Karyokineses give cells

with the different numbers of nuclei and such a picture is presented in Fig. 1 A. In the red cytoplasm of two cells, five smaller nuclei in one cell *versus* two larger nuclei in another are compared. In both the cases, the nuclei are in prophase stage, but the larger nuclei are at a higher level of ploidy. This emphasizes the asynchrony between the two antipodals and these differences relate to the number of nuclei and their level of ploidy. However, synchrony exists among nuclei within each of cells. In Fig. 1 B, asynchrony is evident at the level of DNA amplification in the two uninuclear cells. No information is presented regarding the laggards in antipodals. Fig. 1 C shows that a highly polyploid prophase nucleus can be formed by an earlier non-disjunction of chromosomes. Some chromosomes are not included in the nucleus, providing micronuclei and creating intracellular nuclear asynchrony. The morphology of a nucleus presented in Fig. 1 D is not easy to interpret. A large light mass of highly condensed DNA is separated from some DNA remnants by a large fine-grained RNA nucleolar body stained by propidium iodide. These DNA remnants seem to be a micronucleus during a condensed telophase. Amplification of rDNA signals on polytene chromosomes, but not their number, has been detected by Kosina (1994) in a *Triticum* L. × *Aegilops* L. amphiploid.

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