ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. 52, nr 3-4: 197-200
1983

Ultrastructural aspects of chromatin elimination in hybrid embryos of *Hordeum*

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(Received: February 7, 1983. Revision accepted: May 11, 1983)

Abstract

Chromatin elimination was observed in hybrid embryos of *Hordeum* as incorporation of single chromosomes into tiny vacuoles-lysomes and as fragmentation of interphase nuclei. The latter process led mostly to the formation of micronuclei which underwent gradual degradation.

Key words: Hordeum hybrids, chromatin elimination, embryo ultrastructure.

INTRODUCTION

The production of interspecific hybrid embryos of *Hordeum vulgare* (2n=2x=14) and *Hordeum bulbosum* (2n=2x=14) is followed by selective elimination of the chromosomes of *H. bulbosum* (Kasha and Kao 1970, Lange 1971, Subrahmanyam and Kasha 1973). The loss of chromosomes occurs mainly within the first few days of embryonal development (Bennett et al. 1976), but also in different meristematic regions of plants (Noda and Kasha 1981). Various hypotheses account for the mechanism of chromatin elimination in hybrid embryos. They include asynchrony in the mitotic cycle of parental nuclei, functional abnormality of the karyokinetic spindle, and modifications in the activities of restrictive endonucleases (Davies 1974, Kasha 1974).

When viewed with the light microscope, hybrid embryos of *Hordeum* contain a variety of abnormal structures such as chromatin bridges, non-congressed chromosomes at metaphase (Bennett et al. 1976), lagging chromosomes, chromosome fragments, micronuclei, microcells and degraded chromatin (Lange 1971, Subrahmanyam and Ka-

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sha 1973, Bennett et al. 1976, Orton and Tai 1977, Noda and Kasha 1981).

MATERIAL AND METHODS

Crosses were carried out in the field during June. Florets of *Hordeum vulgare cv.* Aramir were emasculated and one day later, freshly collected pollen from *Hordeum bulbosum* L. was applied with a brush to the receptive stigmas. After 3 and 5 days of pollination, the ovules were prepared for electron microscope examination. They were fixed in 6% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 6.8 for 18 hours at 4°C and post-fixed in 2% OsO_4. After dehydrated they were immersed in Epon 812, cut on LKB ultramicrotome and contrasted with uranyl acetate and lead citrate (Reynolds 1963, Venable and Coggeshall 1965). A JEOLCO model 7A electron microscope was used in examining the sections.

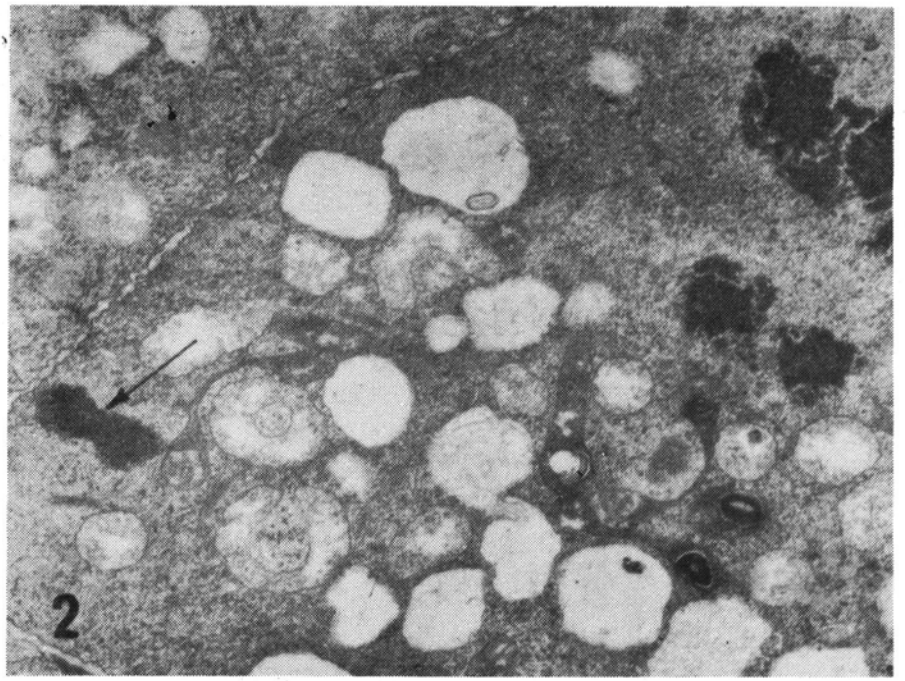
RESULTS

Observations were made of the ultrastructure of embryos of *Hordeum vulgare* x *Hordeum bulbosum* after 3 (Fig. 1) and 5 days following pollination. It was found that during these two embryonal stages chromatin is eliminated as single chromosomes which at the beginning lie freely in the ground cytoplasm (Fig. 2) and then may be incorporated into vacuoles (lysosomes) in which they undergo digestion (Fig. 3). Another way of chromatin elimination is the fragmentation of nuclei (Figs. 4 and 5), which results in the formation of micronuclei which are encased in an envelope consisting of two membranes and the ultrastructure of which is similar to that of the maternal nucleus (Figs. 4 and 6). Chromatin from micronuclei becomes highly condensed and is then digested (Fig. 6). A diversity of membranous structures are frequently observed throughout a degenerating micronucleus (Fig. 7). They resemble typical secondary vacuoles known for their lytic functions.

DISCUSSION

Besides the interphase nucleus, chromatin lumps called micronuclei are visible under the light microscope as one of the effects of chromosomal elimination in hybrid embryos of *Hordeum*. Particularly high frequencies of cells with micronuclei were observed in different meristematic regions spike primordia, root and leaf meristems of *Hordeum*

Fig. 1. Interspecific hybrid embryo of *Hordeum vulgare* × *H. bulbosum* 3 days following pollination. × 2700



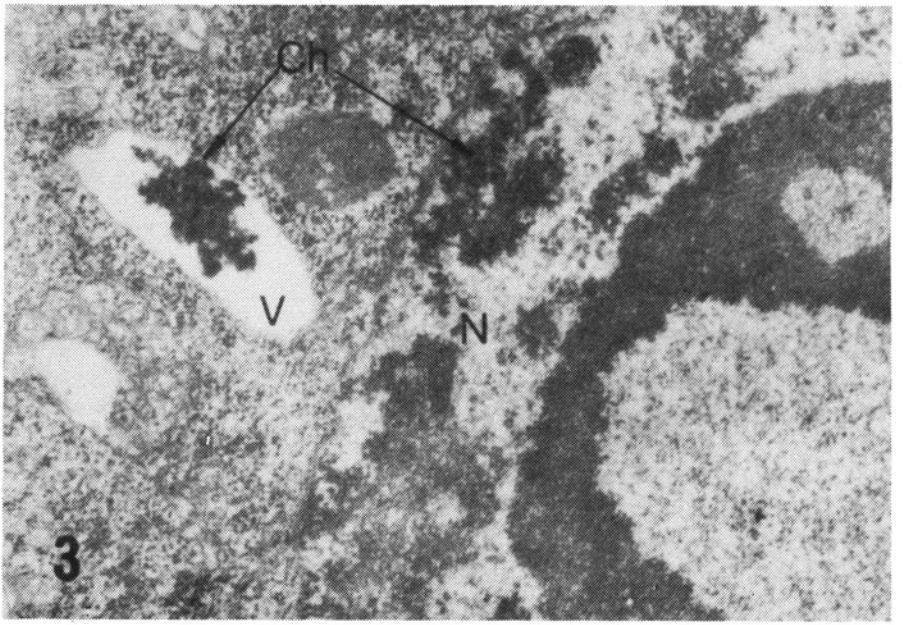
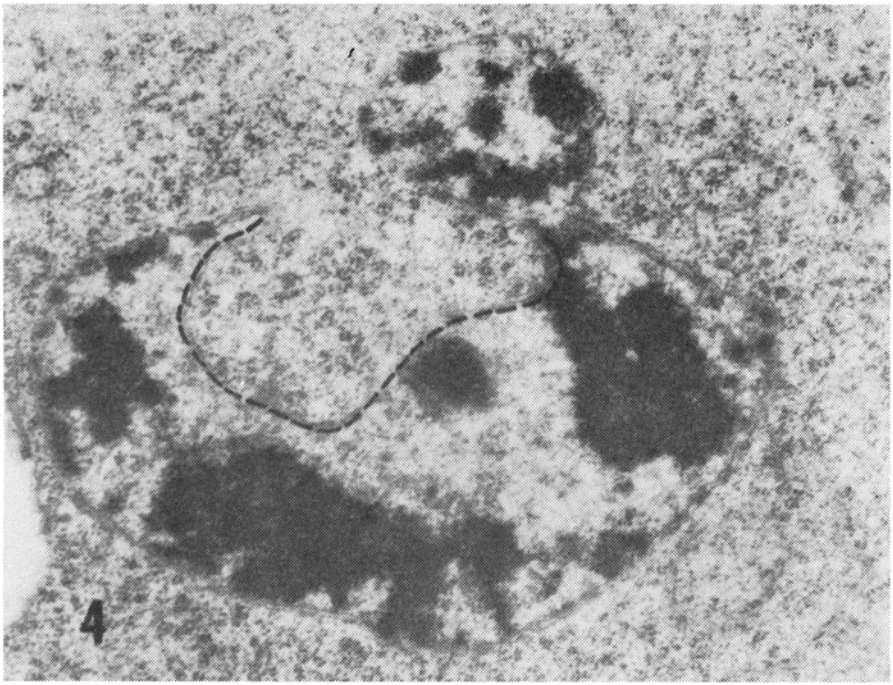


Fig. 2. A chromosome situated beyond the limits of the mitotic figure (arrow). × 11 700

Fig. 3. Chromatin-like substance inside a vacuole (V). Ch — chromatin, N — nucleus. × 22 500



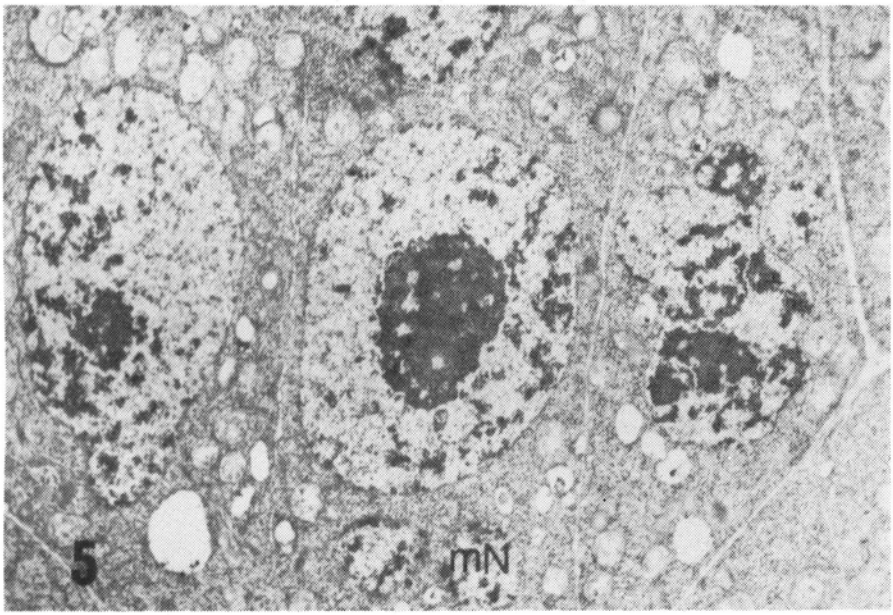
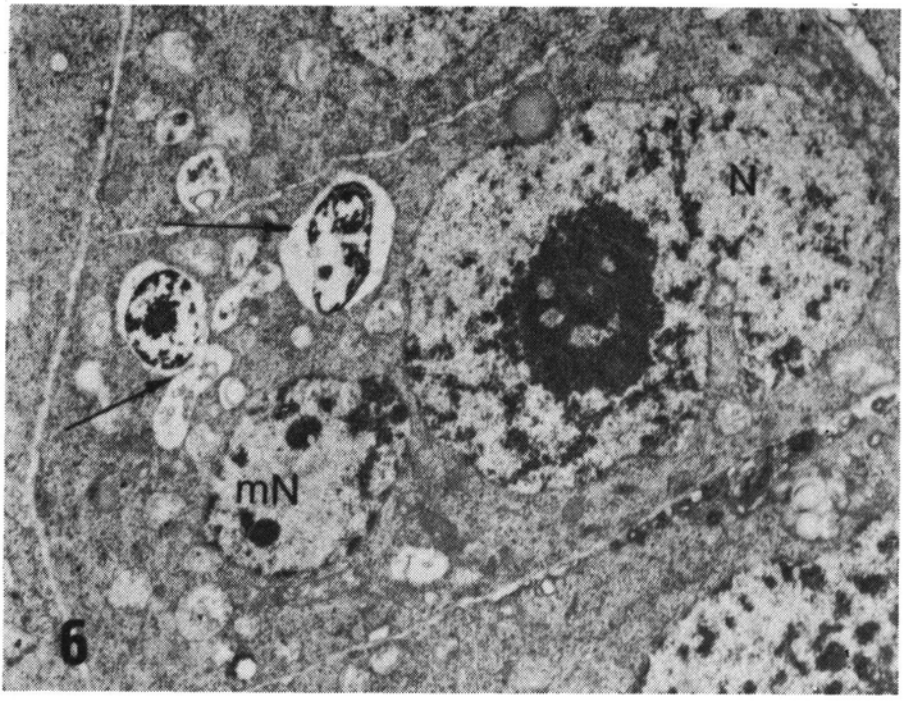


Fig. 4. Evagination (fragmentation) of nucleus. × 22 000

Fig. 5. Different stages of micronucleus formation. mN — micronucleus. × 6700



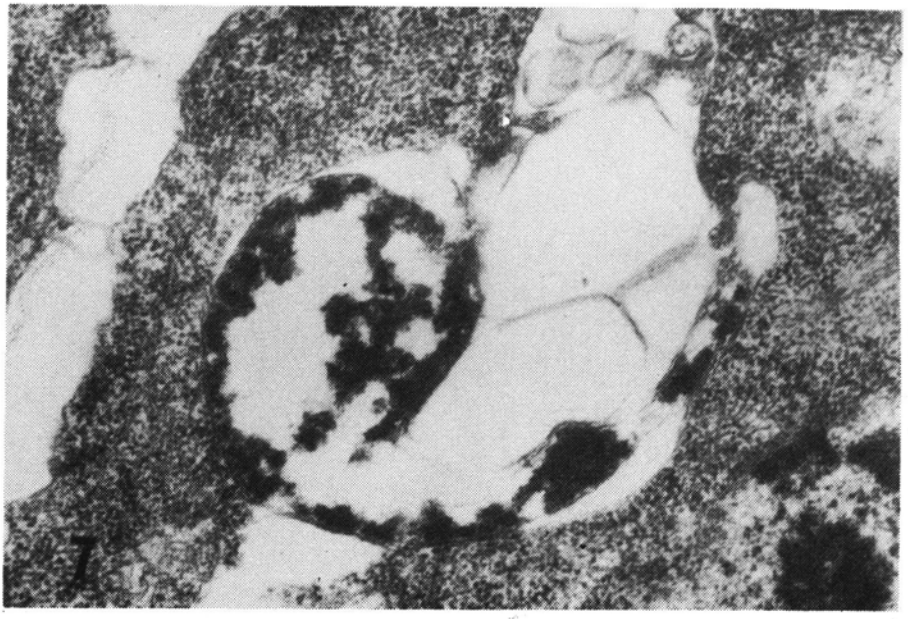


Fig. 6. Lobate nucleus (N), micronucleus (mN) and degenerating two micronuclei (arrows). One of the arrows indicates the site of vacuole fusion with the degenerating micronucleus. × 8200

Fig. 7. Remnants of chromatin in degenerating micronucleus (secondary vacuole?). × 35 000

hybrids (Noda and Kasha 1981). These authors as well as earlier Bennett et al. (1976) indicated that non-congressed chromosomes at metaphase, lagging chromosomes at anaphase and micronuclei at interphase were the main mitotic abnormalities associated with chromatin elimination. In addition, Noda and Kasha (1981) found some relationship between the frequencies of these abnormalities. These frequencies lead to the hypothesis that non-congressed chromosomes at metaphase become lagging chromosomes at anaphase and subsequently micronuclei at interphase.

The application of electronmicroscopic techniques permitted first of all to distinquish between real micronuclei, i.e. bodies enclosed in a double membrane within which there is chromatin showing a varying degree of condensation, and single chromosomes first lying freely in the cytoplasm and then lined with a vacuole membrane. According to our observation, micronuclei formation may be linked to the fragmentation of interphase nuclei. As evidenced already, the latter is a rather frequent phenomenon under experimental conditions, for the most part, and can lead to haploidy (D' Amato et al. 1980). The lobate nuclear profiles found in hybrid embryos (Fig. 5) support this view. When control embryos (*H. vulgare* x *H. vulgare*) were examined in the microscope, such images were not seen. Orton and Tai (1977) also recognized by ligh-microscopic observation, nuclear appendages as a result of active chromatin extrusion in *Hordeum* hybrid embryos and endosperm. If the above explanation for the mechanism of chromatin elimination is accepted the principles of selective elimination still have to be accounted for. Evidence generally suggests that *H. bulbosum* chromatin is eliminated from hybrid embryos of *Hordeum* (Subrahmanyam and Kasha 1973). Neither is there any solid line of evidence for the origin of real micronuclei, whether resulting from amitosis only, or originating from single chromosomes and their fragments. This potentiality of single chromosomes to re-create the nucleus and its envelope (double membranes) was also mentioned in the literature (Orton and Tai 1977). However, we incline rather to the opinion, that in hybrid embryos of *Hordeum*, the eliminated single chromosomes did not form micronuclei but were immediately digested in vacuoles.

Acknowledgment

We are most thankful to Professor Maciej Zenkteler for encouraging us to conduct research in experimental embryology. Our thanks are also due to Dr. Aleksandra Ponitka for contributing the material for study.

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Ultrastrukturalne aspekty eliminacji chromatyny w mieszańcowych zarodkach Hordeum

Streszczenie

W mieszańcowych zarodkach *Hordeum* obserwowano eliminację chromatyny w postaci pojedynczych chromosomów włączonych do drobnych wakuol-lizosomów oraz fragmentację jąder interfazalnych. Głównie w czasie tego ostatniego procesu tworzyły się mikrojądra, które ulegały stopniowej degeneracji.