ACTA BIOLOGICA CRAC0V1ENSIA
Series: Botanica Vol. XXlll, 1981
PL ISSN 0001-5296

Antipodals in *Phleum Boehmeri* Wib., Development and Structure

by

Andrzej JOACHIMIAK

presented the 7th May 1979 by Mrs E. Pogan and Mrs H. Wcislo

(Plate 6)

Abstract

The development of antipodals in *Phleum Boehmeri* Wib. is highly differentiated; it concerns the number of antipodal cells in the embryo sac, the number of nuclei in the antipodal cell and their levels of polyploidy.

In a considerable amount of mature embryo sacs three antipodals were observed. Additional mitotic divisions of antipodal nuclei, usually followed by cytokinesis lead to the increase of the number of antipodals as well as to the formation of antipodal tissue. Its cells are commonly uninucleate; sporadically, however, inhibited cytokinesis is followed by the formation bi- three- and fournucleate cells. Polyploidization takes a significant part in the differentiation of antipodals in *Phleum Boehmeri.* Successive restitution cycles and fusion of mitotic figures lead to the formation of high polyploid nuclei reaching 256 n as a maximum.

The antipodals in *Phleum Boehmeri* are long lasting cells. They were still observed in the stage of multicellular embryo with well developped cellular endosperm.

Introduction

Phleum Boehmeri Wib. is a diploid species with the sexual mode of reproduction. Apart of the normal chromosome complement (A-chromosomes) the occurrence of the accessory chromosomes (B-chromosomes) was stated in this species (Bocher 1950, Bosemark 1956, 1967, Joachimiak in Skaliriska et al. 1976, Joachimiak

1978). Microsporogenesis and the development of the male gametophyte in plants with B-chromosomes have been fairly well investigated. By contrast the development of the female gametophyte in this species has not yet been analysed. The respective studies performed by the present author revealed some interesting developmental processes in the antipodals of this species. Apart of the ovules having three antipodals in mature embryo sac, some others with a several-celled antipodal tissue have been observed. Inhibited mitoses as well as high volumes of nuclei suggested that the development of antipodals is combined with polyploidization.

The occurrence of disturbed mitoses as well as the fusion and restitution in the antipodal cells have been observed in different species of the *Angiospermae* (Grafl 1941, Hasitschka-Jenschke 1959, Trela 1963a, Titz 1965, Kubieri 1968).

Another process taking part in the differentiation of the antipodals of Angiosperms is endomitotic polyploidization. Most often it was observed in the representatives of *Ranunculaceae, Papaveraceae* and *Compositeae* (Tschermak-Woess 1956, 1957, Hasitschka-Jenschke 1959, Turata 1966). In *Gramineae* endomitosis occurs rarely. It has been observed in *Triticum* (Ivanovskaya 1973) and *Hordeum* (Odenbach 1965, cit from Ivanovskaya 1973, Erdelska 1966).

The aim of this work was to perform the detailed examination of developmental processes in the antipodals of *Phleum Boehmeri.* Special attention has been paid to cytological mechanisms leading to polyploidization of their nuclei.

Material and Methods

Inflorescences at various stages of development were used for investigations. They originated from plants cultivated in uniform conditions in the experimental field of the Department of Plant Cytology and Embryology of the Jagellonian University, at Modlnica near Cracow. The respective plants originated from natural populations in Poland (the exact data, Joachimiak 1978). All investigated plants were diploids (2n = 14); some of them had 1—3 B-chromosomes in addition to the normal chromosome set.

Inflorescences were collected in the period from 10th till 28th of June, 1977 and fixed directly in ethanol-acetic acid (3:1). After two months the material was transferred into the 96 % alcohol in which it was maintained for further cytological treatment.

The developmental stages of antipodals were studied on paraffin sections. Separate flowers with young ovules, or separate ovules in more advanced stages, were embeded in paraffin. The 12—18 μm thick microtome sections were stained with Heidenhains haematoxylin.

About 600 ovules originating from 20 plants were investigated. Five of these plants had accessory chromosomes. No differences in the developmental cycles of antipodals have been observed in plants with and without B-chromosomes. Thus the results of the investigations are presented jointly for both cytotypes.

The volume of the nuclei has been calculated according to the formula $4/3\ r_1r_2r_3\ \pi$ (Tschermak-Woess and Hasitschka 1953). In the case of large, high polyploid nuclei the r_3 dimension represents the total sum of values from successive microtome sections. No investigations have been performed for the nuclei of highly irregular shapes as well as these showing signs of degeneration.

Results

The ovule of *Phleum Boehmeri* is crassinucellate; unicellular archesporium is of subepidermal origin. Macrosporogenesis has, on the whole, a regular course resulting in a linear or sometimes T-tetrad of macrospores. The embryo sac is monosporic. Three successive mitotic divisions lead to the eight-nucleate stage. In a mature embryo sac the egg apparatus consists of one egg cell and two synergids. Two polar nuclei lie somewhat below the egg apparatus. They fuse very late.

Initially, antipodals are situated on the basal pole of embryo sac. Then, they take lateral position, due to the asymmetrical growth of embryo sac of this species (Fig. 7).

In 38 % of mature embryo sacs three antipodals could be observed.

Young antipodals are bigger than other cells of an embryo sac already at the moment of its differentiation. They have dense, dark staining cytoplasm and spherical or somewhat ellipsoidal nuclei of slightly granular structure with scarce and vaguely marked chromocentres. In the initial stages the antipodals are either not vacuolated or they have small vacuoles situated by the cell wall.

In four 8-nucleate polarized (4-4) embryo sacs (Fig. 1) additional division of one nucleus at the chalazal pole could be observed. This division may result in the formation of two binucleate antipodals occasionally found in young fully developed embryo sacs. The nuclei of these antipodals were haploid (Fig. 26).

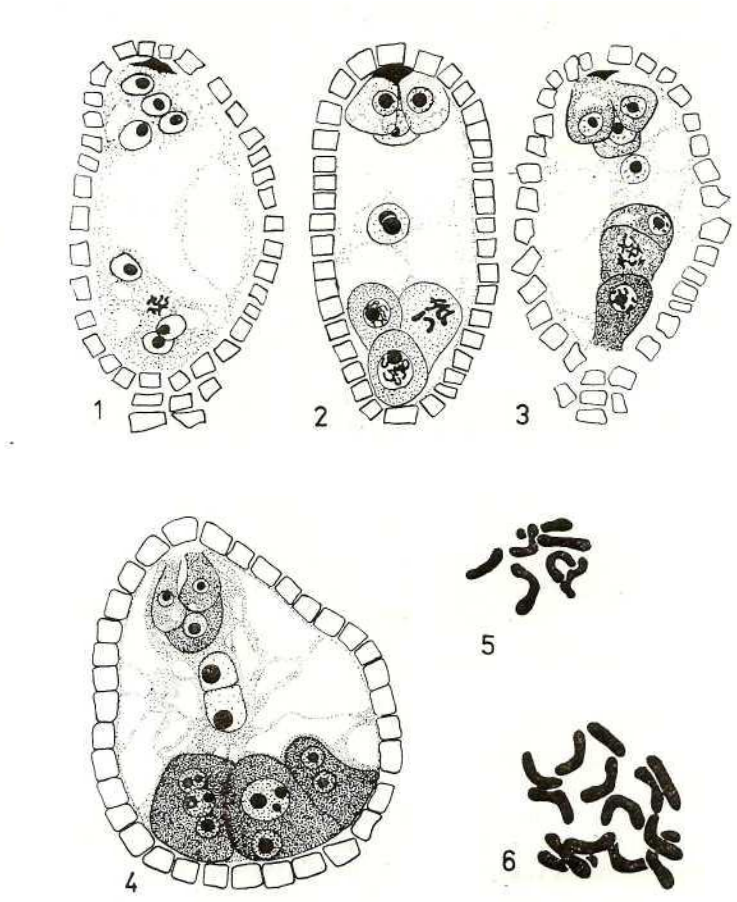
In some completely differentiated embryo sacs additional mitotic divisions of antipodals could occur (Fig. 2, 3, 5, 6). As their result, 4—10 antipodals could be formed. They are usually uninucleate, but sometimes also polynucleate antipodals up to fournucleate resulting from inhibited cytokinesis have been observed (Fig. 4, 25).

Antipodals of *Phleum Boehmeri* have haploid nuclei (n = 7) only at their very early developmental stages. In the course of their further differentiation their nuclei undergo some cytological changes concerning their volume and shape, as well as the number and size of their nucleoli.

Nuclei of the differentiated antipodals in *Phleum Boehmeri* are of two structural types: 1) nuclei (spherical, ellipsoidal or more or less irregular, often dumbbell--shaped) with a great number of nucleoli, 2) nuclei (spherical or ellipsoidal) with one, two or at most with a few larger nucleoli. Measurements of the volume of antipodal nuclei presented in Table I have shown that the frequency of haploid nuclei volumes ranging from 70 to 81 μm is rather low. In the majority of nuclei their

volumes increase in the geometrical progression corresponding with the ploidy levels n, 2n, 4n, 8n, up to 256n. Nuclei of the highest level of polyploidy are of regular shape.

As to the polynucleate antipodals (Table II), sometimes the sum of the polyploid



Figs. 1—6. *Phleum Boehmeri* Wib. 1. Additional division of one of the chalazal nuclei in eight-nucleate polarized, but not yet differentiated embryo sac. 2, Additional division (metaphase, n = 7) of the antipodal nucleus in young E. S. 3. Additional division (metaphase, 2n = 14) of the antipodal nucleus in young E. S.; in the neighbouring antipodal cells inhibited prophases are visible. 4. The young, fully developped E. S., the polynucleate antipodals are visible. 5. A metaphase plate from Fig. 2. 6. The metaphase plate from Fig. 3. (Figs. 1—4. 650 X, Figs. 5, 6. 3100 x)

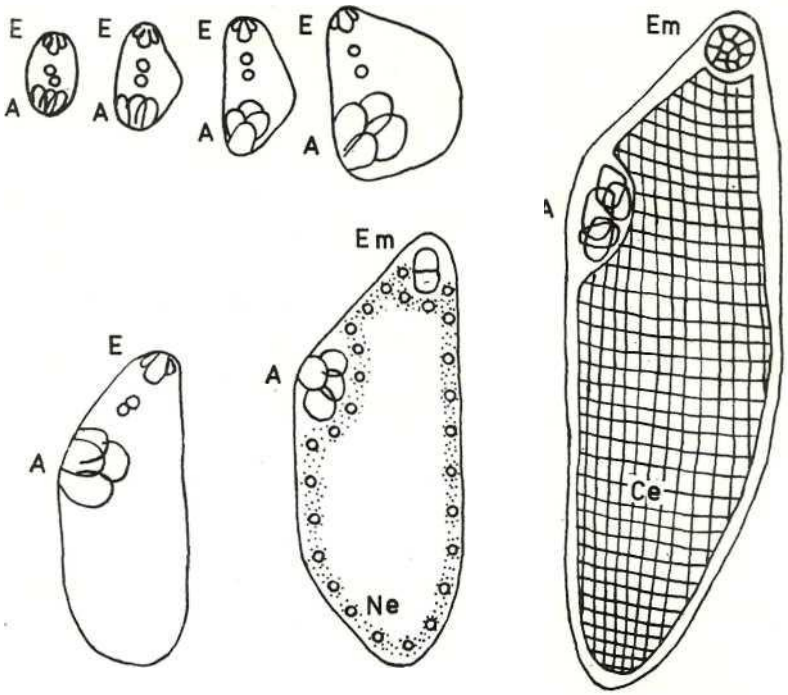


Fig. 7. *Phleum Boehmeri* Wib. The asymmetrical growth of the embryo sac, scheme. E — egg apparatus, A — antipodals, Em — embryo, Ne — nuclear endosperm, Ce — cellular endosperm. (220 x)

levels of particular nuclei occurring in a cell does not fit to the above series. It proves that the nuclei occurring within one cell may divide asynchronously.

The main mechanism involved in the polyploidization of antipodal nuclei in *Phleum Boehmeri* are the disturbed additional mitoses. Cytological pictures of such mitoses in the investigated material have been observed only occasionally. Nevertheless, the shape and volume of the nuclei as well as their structure are in favour of this opinion. Ellipsoidal nuclei as a rule are more or less narrowed in their central part; thus they could arise as restitution nuclei after the disturbed anaphase. They may undergo further inhibited divisions. Spiralized chromosomes are a proof for it (Fig. 10, 11, 30). They are more frequent at lower levels of polyploidy. Low frequency of such nuclei at higher levels of ploidy suggests that the further polyploidization is accomplished rather in the way of inhibited prophases or inhibited metaphases (Fig. 9, 10, 11, 27, 28, 29, 30).

In some antipodals the formation of restitution nuclei by inhibition of prophases or metaphases may occur from the very begining of their differentiation.

TABLE I

Phleum Boehmeri Wib. Volumes of antipodals nuclei

Degree of ploidy	Number of nuclei	Averages in μm^3	Extreme values μm^3	q
n	20	77	70—81	
2n	31	165	151—173	2·14
4n	32	355	315—376	2·15
8n	38	729	681—777	2·05
16n	111	1326	1121—1452	1·82
32n	35	2664	2073—3070	2·01
64n	9	5025	4820—5060	1·89
128n	11	11380	10685—12075	2·26
256n	2	20261	20093—20429	1·78
Nuclei with intermediate values	12			
Total	301			

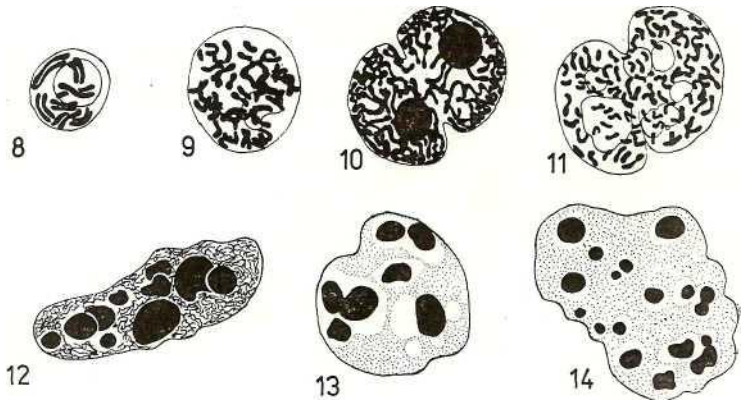
It can be inferred from a great percent (83 %) of regularly shaped nuclei at various levels of polyploidy. Cytological picture of a diploid nucleus with seven pairs of strongly spiralized chromosomes situated near the nuclear membrane (Fig. 8) is the confirmation of this opinion.

It should be added, that the presence of spherical polyploid nuclei with enlarged nucleoli and the rhythmical growth of the volume of the nuclei have been reported also as a sign of endomitotic polyploidization. In the studied material, however, no structural changes of chromatin, characteristic for endomitosis have been observed.

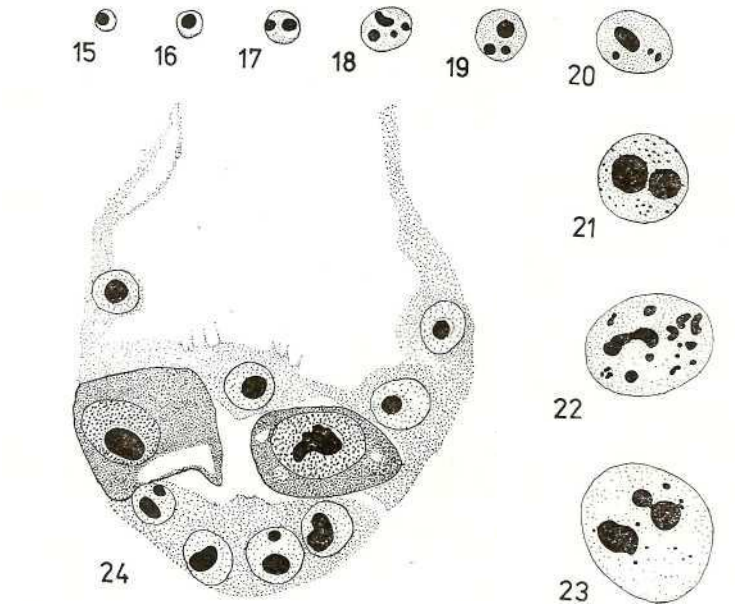
In many antipodals another type of mitotic disturbances have been observed. Strongly spiralized prophase and metaphase chromosomes stick together (Fig. 31). Polyploid nuclei of highly irregular shapes as well as those with many nucleoli have been probably formed as a result of fusion of mitotic figures of polynucleate cells (Fig. 12, 13, 14, 32).

Older antipodals increased their volumes consequently with a considerable increase of volumes of their nuclei. They differ also from the young ones by the looser, less stained and vacuolated cytoplasm and the thick cell wall which can be perfectly seen in a light microscope.

Antipodals *of Phleum Boehmeri* are viable for a very long time (Fig. 24). Their presence could be discerned in some ovules in which there was a well advanced



Figs. 8—14. *Phleum Boehmeri* Wi b., the nuclei from antipodal cells. 8. Inhibited prophase in the 2n nucleus ; 7 pairs of chromosomes are visible. 9. Inhibited prophase in the 8n nucleus (56 chromosomes, not all drawn). 10,11. Inhibited prophases in the irregular, narrowed in their central part nuclei. 12—14. Polyploid nuclei arisen after mitotic disturbances. (1500 x)



Figs. 15—24. *Phleum Boehmeri* Wib. 15—23. Representatives of all observable classes of the antipodal nuclei: 15 —haploid (n); 16—2n; 17—4n; 18—8n; 19—16n; 20—32n; 21—64n; 22—128n; 23—256n. 24. The antipodals in E. S. with the well developped, polynucleate endosperm. (650 x)

TABLE II

Phleum Boehmeri Wib. Degrees of ploidy of polinucleate antipodal cells

Degree of ploidy of cell	Degree of ploidy of cell nuclei	Number of cells
2n	2×n	4
4n	2×2n	6
6n	2n, 4n	2
8n	2×2n, 4n	2
	2×4n	1
10n	2n, 8n	1
20n	4n, 16n	2
24n	8n, 16n	1
32n	16n, 16n	1
34n	2n, 32n	1
320n *	256n, 32n, 2×16n	1
Total:		22

* — see Fig. 25.

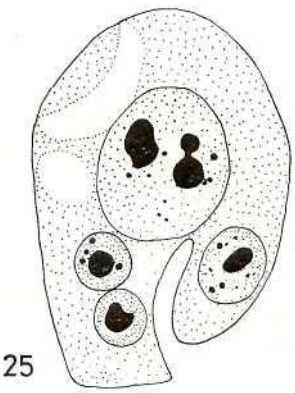
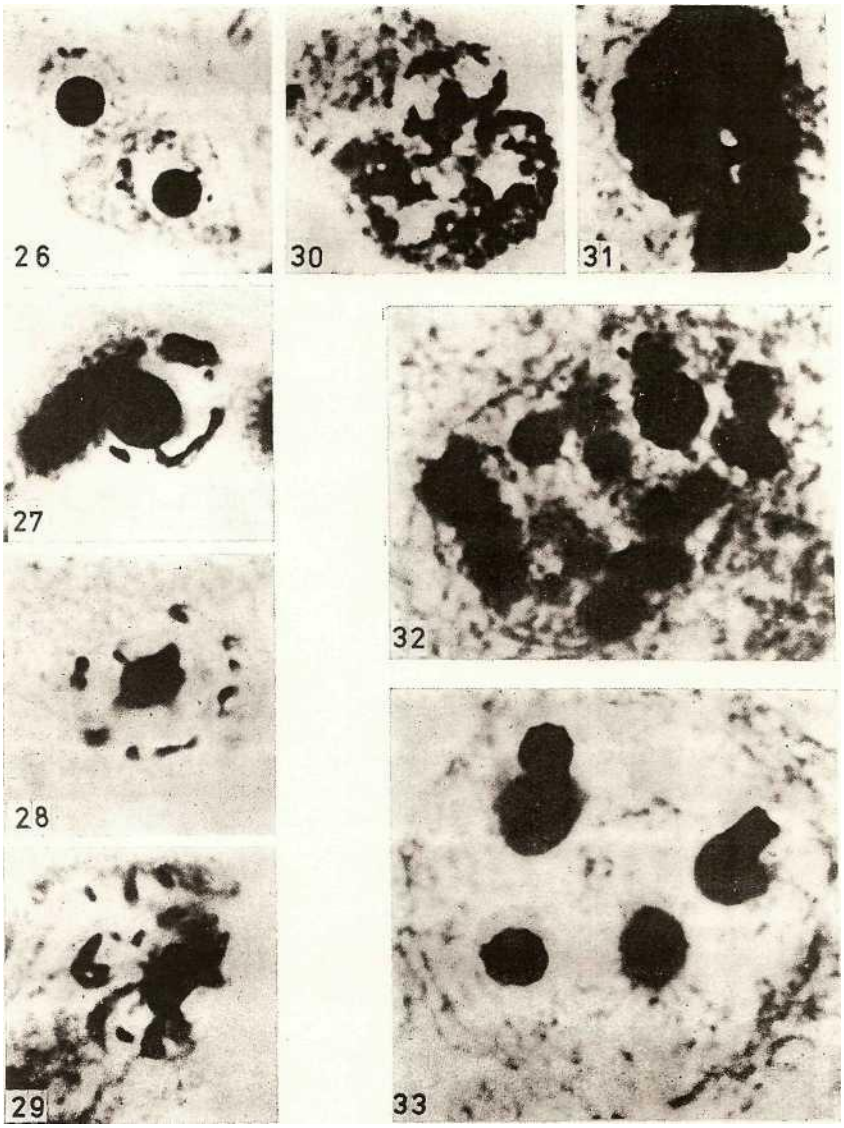


Fig. 25. High polyploid, fournucleate antipodal cell; two nuclei 16n, one nucleus 32n and one 256n. (650 x)



A. Joachimiak

embryo and a few hundred-cells of the endosperm. Most often, however, they degenerate at the stage of a few or several-celled embryo and nuclear endosperm. The characteristic feature of the initial stages of antipodals degeneration is the strong vacuolization of cytoplasm, collaps of cell walls and desintegration of nuclei. The nuclei of degenerating antipodals are usually dark stained. There also can be observed the aglutination of chromatin.

Discussion

In many representatives of *Gramineae* the increase of the number of antipodals and the development of the so called "antipodal tissue" have been observed. In *Phleum Boehmeri* the number of cells in this tissue is 3—10, in *Molinia coerulea* up till 43 (Shadovsky 1926), in *Hordeum* 40—60, but 84 as a maximum (Erdelska 1966) and in *Ammophila arenaria* 30 (Kubieri 1968).

The development of antipodals in some representatives of Angiosperms proceeds with the part of polyploidization. In *Phleum Boehmeri* the mechanism of polyploidization of antipodals is connected with successive restitution cycles and fusion of mitotic figures. As their result the antipodal cells may reach high polyploid levels — 256 as a maximum. Similar phenomena have been first observed in antipodals of *Caltha palustris* by Grafl (1941). In this species the fusion of mitotic spindles in three successive mitoses lead to the formation of octoploid nuclei. Restitution of nuclei and the fusion of mitotic figures lead to the formation of 16n and 32n antipodal nuclei in *Chrysanthemum alpinum,* and 8n and 16n antipodal nuclei in embryo sacs of *Achillea millefolium* (Titz 1965). The fusion connected with restitution is also a mechanism playing role in polyploidization of antipodals in *Anemone nemorosa* (Trela 1963a). The nuclei of antipodals in *Ammophila arenaria* (Kubien 1968) reach in the same way high polyploid levels, 128 or 256 as a maximum. In the latter case polyploidization is preceded by certain number of mitoses occuring with or without cytokinesis. As their result the antipodal tissue is formed.

The mechanism of antipodals polyploidization in *Phleum Boehmeri,* the occurence of both disturbed and inhibited mitoses, shows also great similarity to the mechanism of polyploidization of tapetal nuclei in *Solanum* (Turala and Urbariska-Worytkiewicz 1964) and to the differentiation of endosperm nuclei in *Anemone nemorosa* (Trela 1963b).

Endomitosis, however, is most frequent mechanism leading to the polyploidization of antipodals. In this way the nuclei of antipodals can reach 256 n in *Hordeum* (Erdelska 1966), 128n in *Aconitum variegatum* (Tschermak-Woess 1956), 64n in *Papaver rhoeas* (Hasitschka 1956), *Eranthis hiemalis* arid *Corydalis cava* (Hasitschka-Jenschke 1959), 32n in *Clivia miniata* (Tschermak-Woess 1957), *Hyacinthus orientalis* (Turata 1966), 16n in *Dicentra spectabilis, Othonna crassifolia* and 8n in *Helleborus niger* (Hasitschka-Jenschke 1959).

The endomitotic nuclei show a characteristic structure. The most interesting structural phenomenon observed in antipodals is the occurrence of giant chromo-

somes. So far they were found in antipodals of *Papaver rhoeas* (Hasitschka 1956), *Aconitum variegatum* (Tschermak-Woess 1956), in wheat (Ivanovskaya 1973) and *Hordeum* (Odenbach 1965, cit. from Ivanovskaya 1973).

Polyploidization of nuclei has been also frequently observed in other plant tissues, especially in endosperm and tapetum of many species. Only four cases are known till now in which both endomitoses and disturbed mitoses were involved in polyploidization of the same tissue viz. of the endosperm. They are in *Delphinium Kotulae* (Jankun 1970), *Ecbalium elaterium* (Turala 1971), *Echhim vulgare* (Malecka 1975) and *Lithospermum arvense* (Malecka 1977). Similar phenomena are unknown in the differentiation of the antipodals.

The present studies have been carried out in the Department of Plant Cytology and Embryology, Institute of Botany of the Jagellonian University. I wisch to express my deepest gratitude to Professor Dr E. Pogan for valuable advice and suggestions, as well as for hearty and never failing interest in the course of my investigations.

Department of Plant Cytology and Embryology
Jagellonian University
Krakow, Poland, Grodzka 52

References

Bocher T. 1950. Chromosome behaviour and syncyte formation in *Phleum phleoides* (L.) Karst. Bot. Not. 1950, 353—368.

Bosemark N. O. 1956. Cytogenetics of accessory chromosomes *in Phleum phleoides.* Hereditas 42, 443-466.

Bosemark N. O. 1967. Edaphic factors and the geographical distribution of accessory chromosomes in *Phleum phleoides.* Hereditas 57, 239—262.

Erdelska O. 1966. Einfiuss der Befruchtung auf die Entwicklung der Antipodenkerne der Gerste. Biologia 21, 857—864.

Grafl I. 1941. Uber das Wachstum der Antipodenkerne von *Caltha palustris.* Chromosoma 2, 1-11.

Hasitschka G. 1956. Bildung von Chromosomebundeln nach Art der Speicheldriisenchromosomen, spiralisierte Ruhekernchromosomen und andere Struktur-Eigentumlichkeiten in den endopolyploiden Riesenkernen der Antipoden von *Papaver rhoeas.* Chromosoma 8, 87—113.

Hasitschka-Jenschke G. 1959. Vergleichende karyologische Untersuchungen an Antipoden. Chromosoma 10, 229—267.

Ivanovskaya H. V. 1973. Functional morphology of polytene chromosomes of wheat antipodal cells. Citologja 15, 1445—1452.

Jankun A. 1970. Studies in endosperm development of *Delphinium Kotulae* Pawi. Acta Biol. Crac. Ser. Bot. 13, 51—64.

Joachimiak A. 1978. Karyological studies in *Phleum Boehmeri* Wib. from Poland. Acta Biol. Crac. Ser. Bot. 21, 65—73.

Kubieri E. 1968. Cytological processes during the development of antipodals in *Ammophila arenaria* Link. Acta Biol. Crac. Ser. Bot. 11, 21—29.

Malecka J. 1975. The course of differentiation of the endosperm of *Echium rulgare* L. Acta Biol. Crac. Ser. Bot. 18, 13—26.

Malecka J. 1977. The course of differentiation of the endosperm of *Lithospermum arvense.* Acta Biol. Crac. Ser. Bot. 20, 33—40.

Odenbach W. 1965. Histologische und cytologische Untersuchungen der Entwicklungsvorgange nach der Bestfiubung von Gerste mit Roggen. Zs. Pflanzenziicht. 53, 1—52.
Shadovsky A. E. 1926. Der antipodale Apparat bei Gramineen. Flora, 342—379.
Skalinska M., Jankun A., Wcislo H., et al. 1976. Further studies in chromosome numbers of Polish Angiosperms. Eleventh contribution. Acta Biol. Crac. Ser. Bot. 19, 107—148.
Titz W. 1965. Vergleichende Untersuchungen iiber den Grad der somatischen Poliploidie an nahe verwandten diploiden und poliploiden Sippen einschliesslich der Cytologie von Antipoden. Ost. Bot. Zeit. 112, 101—172.
Trela Z. 1963 a. Embryological studies in *Anemone nemorosa* L. Acta Biol. Crac. Ser. Bot. 6, 1—14.
Trela Z. 1963 b. Cytological studies in the differentiation of the endosperm in *Anemone nemorosa* L. Acta Biol. Crac. Ser. Bot. 6, 177—183.
Tschermak-Woess E. 1956. Notizen iiber die Riesenkerne und "Riesenchromosomen" in den Antipoden von *Aconitum.* Chromosoma 8, 114—134.
Tschermak-Woess E. 1957. Ober Kernstrukturen in den endopolyploiden Antipoden von *Clivia miniata.*Chromosoma 8, 637—649.
Tschermak-Woess E. und Hasitschka G. 1953. Veranderungen der Kernstruktur wahrend der Endomitose, rhytmisches Kernwachstum un verschiedenes Heterochromatin bei Angiospermen. Chromosoma 5, 574—614.
Turala K. 1966. Strukturen endopolyploider Kerne im Bereich der Samenanlage einiger Monocotylen. Osterr. Bot. Zeit. 113, 529—541.
Turala K. 1971. Disturbed mitotic divisions and endomitoses during the differentiation of the endosperm in *Ecbalium elaterium (Cucurbitaceae}.* Acta Biol. Crac. Ser. Bot. 16, 27—35.
Turala K. and Urbariska-Worytkiewicz K. 1964. Cytological processes during the differentiation of the tapetal layer in *Solanum dulcamara* L. and *S. nigrum* L. Acta Biol. Crac. Ser. Bot. 7, 171—183.

Explanation of Plate 6

Figs. 26—33. *Phleum Boehmeri* Wib., structure of the antipodal nuclei. Fig. 26. binucleate antipodal cell, two haploid nuclei are visible. Figs. 27, 28, 29. inhibited prophases in 8n, 16n and 32n antipodal nuclei. Fig. 30. inhibited prophase in the irregular shaped nucleus. Fig. 31. antipodal nucleus, the chromosomes stick together. Fig. 32. polyploid nucleus after mitotic disturbances. Fig. 33. high polyploid, regular shaped nucleus — 256n. (2500 X).

Author's address:
mgr A. JOACHIMIAK
31-044 Krakow, Poland ul. Grodzka 52