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Further observations on the activity of Y chromosome heterochromatin in *Rumex thyrsiflorus*

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Abstract:

Observation of premeiotic nuclei of plants containing different numbers (from one to five) of Y chromosomes reveal that is such plants prior to meiosis all Y chromosomes are in fuzzy state, while in nuclei of tapetal cells or nuclei from anther stalk the number of big chromocentres is directly correlated with the number of Y chromosomes in given plants. This finding provides a further indication of genetic activity of Y chromosomes in this particular stage of the life cycle (premeiosis). Another indication of genetical activity of Y chromosomes was obtained from analysis of the rate of RNA synthesis in premeiosis. It was found that PMC's in the last premeioitc interphase exhibit a high rate of ^{3}H-uridine incorporation. Thus in PMC's the fuzzy appearance of Y chromosomes coincides with intensive RNA synthesis.

INTRODUCTION

Diploid male plants of *R. thyrsiflorus* contain in their karyotypes two Y chromosomes (X+YY+12A). It is well established that these Y chromosomes are heterochromatic and have no influence on sex determination (Żuk, 1963, 1969a, 1969b). However, cytological and genetical data (Żuk, 1969a, 1970a, 1970b) suggest that Y chromosomes in this species carry some factors responsible for pollen fertility. In the interphase of mitotic cells, Y chromosomes are visible as two large chromocentres. This picture is in contrast to that observed in the premeiotic interphase, as in this stage all chromatin (including Y chromosomes) has a fuzzy appearance. The fuzzy state of Y chromosomes in premeiosis has been interpreted as an indication of their activity in RNA synthesis.

The present paper reports observations on premeiotic nuclei of plants containing different numbers (from one to five) of Y chromosomes. The finding that in such plants all Y chromosomes are in fuzzy state prior to meiosis provides a further

Table 1

^{3}H-uridine incorporation by PMC's and tapetal cells during various stages of PMC's development

Stage	Mean number of grains over 25 μ^2	
	PMC's	T.C.
Premeiotic cells	5.1±0.33	—
Last premeiotic interphase	14.2±0.30	14.8±0.39
Meiotic prophase	1.2±0.17	19.3±0.28

indication of their genetical activity in this particular stage of the life cycle. Another indication of genetical activity of Y chromosomes was obtained from analysis of the rate of RNA synthesis in premeiosis. It was found that PMC's in the last premeiotic interphase exhibit a high rate of ^{3}H-uridine incorporation. Thus in PMC's the fuzzy appearance of Y chromosomes coincides with intensive RNA synthesis.

MATERIAL AND METHODS

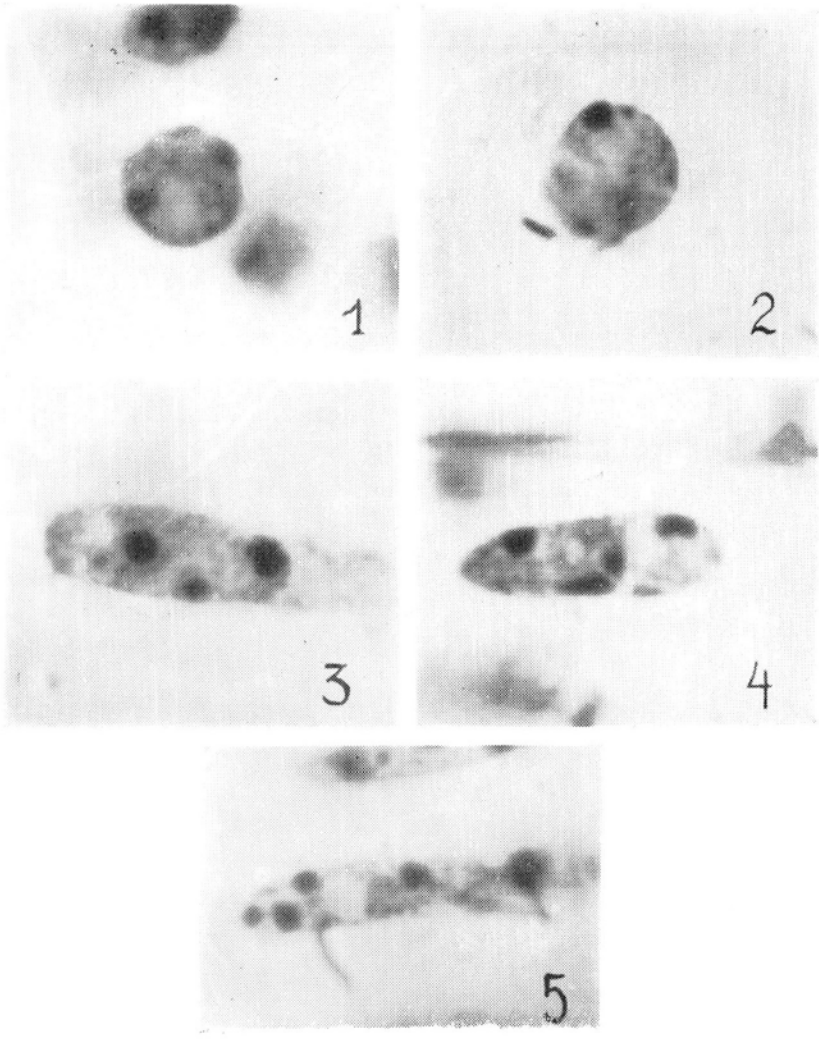
The origin of the male plants with different number of Y chromosomes has been previously described (Żuk, 1970 a). For cytological observations the flower buds were fixed in Navashin fixative or acetic alcohol (3:1), embedded in paraffin, sectioned at 15 microns and stained with Feulgen and light green or with methyl-green-pyronine. For investigation of RNA synthesis during meiosis parts of flower shoots up to 5 cm long were incubated in a solution of radioactive ^{3}H-uridine (5 μC/ml. s.a. 20 C/mM) for 48 hrs. Flower buds were fixed in acetic alcohol (3:1), embedded in paraffin and sectioned at 5 microns. Slides were dipped in Kodak NTB-2 liquid emulsion and exposed in the refrigerator for 3 months. After developing in Kodak D 19 developer the slides were stained with methyl green pyronine (Unna-Pappenheim) received form Edward Gurr Ltd., London. The number of grains was counted on an area of 25 μ^2 (5μ×5μ) over the tissue. For each stage 50 squares were counted, and mean values and standard errors were calculated.

RESULTS

1. Cytological observation on interphase nuclei of plants differing by the number of Y chromosomes

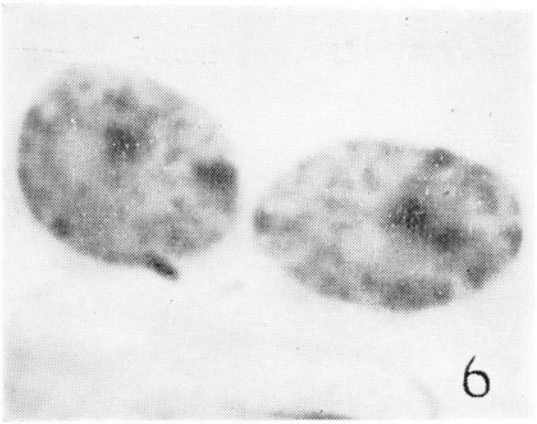
Cytological observations of Y chromosome heterochromatin were carried out on interphase nuclei from the anther stalk, tapetum cells and PMC's. Male plants with five different karyotypes were chosen for the observation:

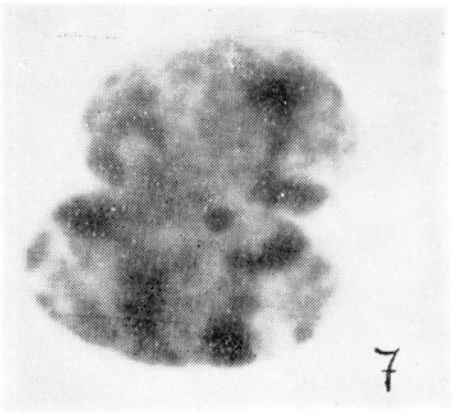
Plate I

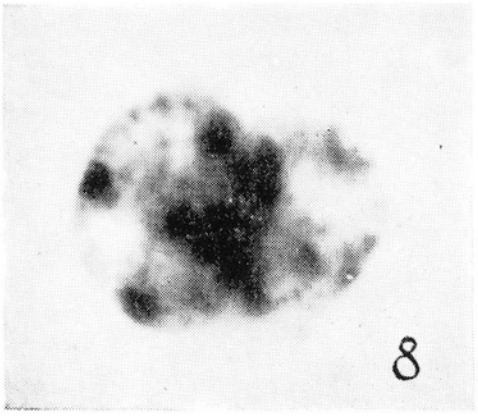


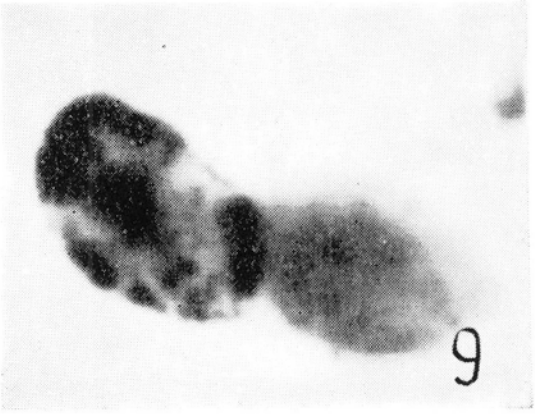
Figs 1—5. Interphase nuclei of anther stalk in plants with different karyotypes: *1*. 15=XYY++12A, *2*. 14=XY+12A, *3*. 16=XYYY+12A. *4*. 17=XYYYY+12A, *5*. 18=XYYYYY+12A. ×2,500.

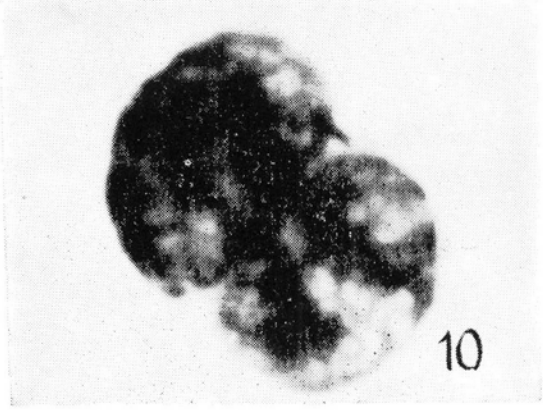
All preparations Feulgen staineds





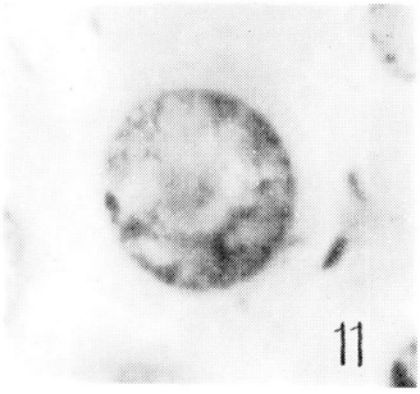


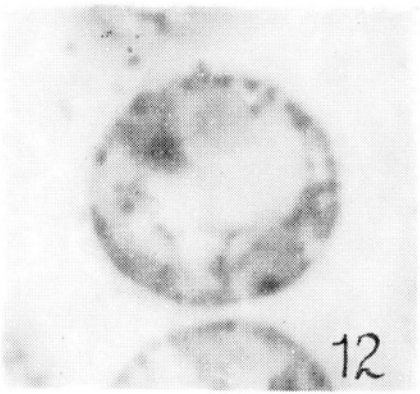


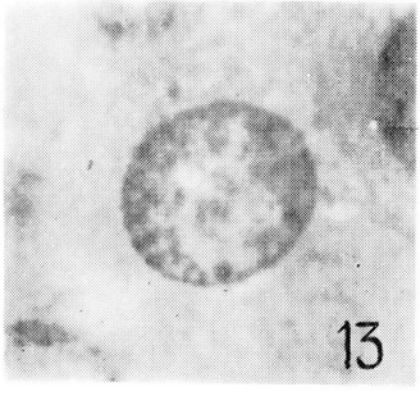


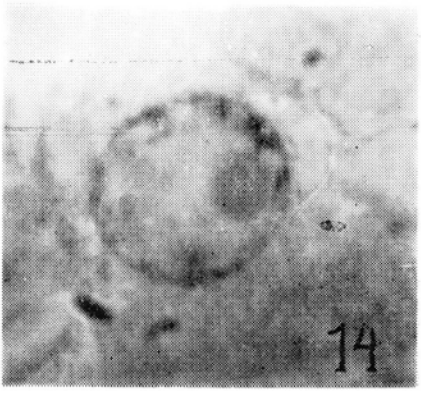
Figs 6—10. Interphase nuclei of tapetal cells in plants with different karyotypes: *6*. 14=XYY+ +12A, *7*. 15=XYY+12A, *8*. 15=XYYY+12A, *9*. 17=XYYYY+12A, *10*. 18=XYYYYY+ +12A. ×2500.

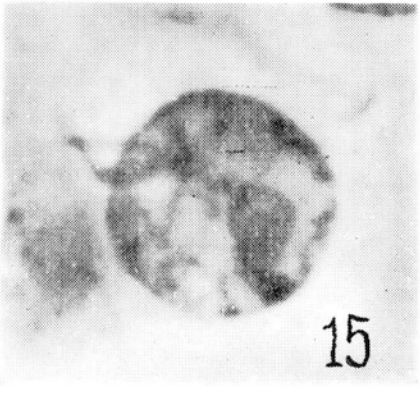
All preparations Feulgen stained











Figs 11—15. PMC's nuclei during ,,diffuse stage" in plants with different karyotypes *11*. 14=XY+12A. *12*. 15=XYY+12A, *13*. 16=XYYY+12A, *14*. 17=XYYYY+12A *15*. 18=XYYYYY+12A. X2500.

All preparations Feulgen stained

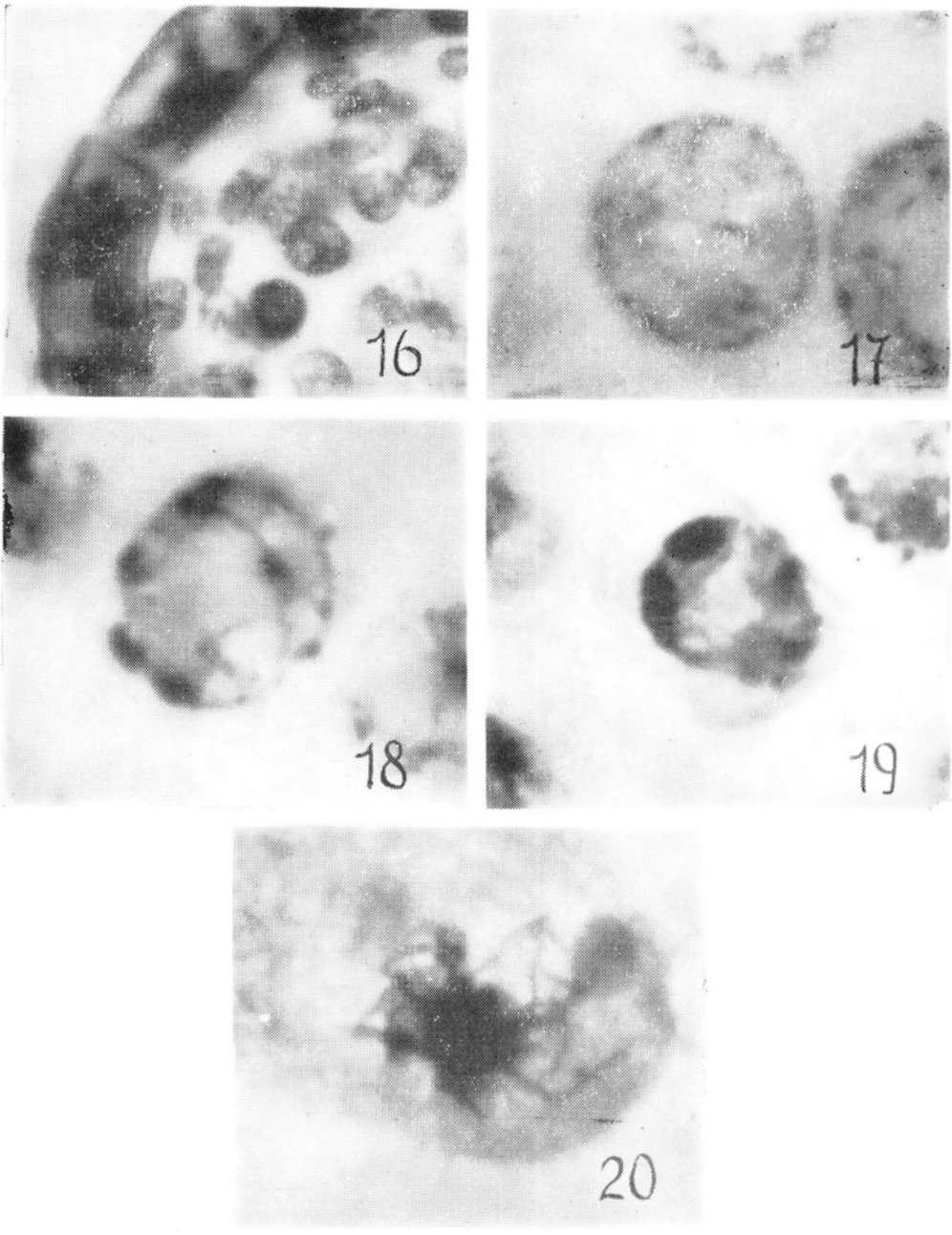


Fig. 16. Sporogenous tissue during premeiotic mitosis of normal male plants with karyotype 15 = XYY+12A. × 800

Fig. 17. Premeiotic "diffuse stage" in PMC of normal male plant with karyotype 15 = XYY+12A- × 2500.

Fig. 18. Beginning of "compact stage" in PMC of normal male plant with karyotype 15 = = XYY + 12A. Note formation of chromocentres. × 2500

Fig. 19. More advanced "compact stage" in PMC of normal male plant with karyotype 15 = = XYY + 12A. Note two big chromocentres formed probably by Y chromosomes. × 2500.

Fig. 20. Meiotic prophase — typical "compact stage" in normal male plant with karyotype 15 = = XYY + 12A. Note big chromocenter formed in the middle and chriomatid threads going out. × 2500. All Prepratıons Feulgen stained except Fig. 19 which is stained with fast greeneosin according Bloch (1966)

XY+12A=14
XYY+12A=15
XYYY+12A=16
XYYYY+12A=17
XYYYYY+12A=18

In normal diploid male plants of *R. thyrsiflorus* (XYY+12A), Y chromosomes are usually visible in the interphase nuclei of the anther stalk as two distinct chromocentres (Fig. 1). In plant lacking one Y chromosome, only one chromocenter was observed in the corresponding tissue (Fig. 2). In plants with additional Y chromosomes the number of large chromocentres was usually equal to that of Y chromosomes present in the karyotype (Figs 3–5). Sometimes, however, the number of chromocentres was lower than the number of Y chromosomes, owing to the tendency of heterochromatin to fuse in the interphase nuclei. A similar correlation between the number of Y chromosomes and the number of chromocentres was found in tapetum cells (Figs 6–10). A different appearance of Y chromosome heterochromatin was observed in the last premeiotic interphase of PMC's. During this stage, all chromatin has a fuzzy appearance and notwithstanding how many Y chromosomes are present no chromocentres are visible in the nuclei (Figs 11–15). This finding may be interpreted as evidence that in PMC's before meiosis, Y chromosomes undergo despiralisation as other euchromatic chromosomes. It can be also assumed that in this stage Y chromosomes could be active in RNA synthesis. Therefore the rate of ^{3}H-uridine incorporation by microsporocytes was determined.

2. Rate of ^{3}H-uridine incorporation at various stages of PMC's development

The intensity of ^{3}H-uridine incorporation was analysed in plant with four Y chromosomes. Three easily distinguishable stages of PMC's development were chosen for grain counting:

1. Premeiotic cells — sporogenous tissue is already well differentiated but mitotic divisions are still going on. Tapetal cells are not yet differentiated (Fig. 16).

2. Premeiosis — all PMC's are in last premeiotic interphase, chromatin is fuzzy in appearance, large nucleoli occupying central position in the nucleus. Tapetal cells can be already distinguished from PMC's as more elongated and occupying a peripheral position in the anther (Fig. 17).

3. Meiotic prophase — "compact stage". It should be mentioned that in *R. thyrsiflorus* early pictures of meiotic prophase are somehow different than those typically observed in other plants. In this species meiotic prophase begins with formation of distinct chromocentres (Fig. 18, 19). As meiosis preceeds the chromocentres tend to fuse forming a common compact chromatin mass with single chromatin threads sticking out. A typical picture of the "compact stage" is given in Fig. 20. The "compact stage" is followed by a diplotene-like stage with chromosomes clearly visible as long threads.

The results of grain counting are summarised in Table 1. It is seen that in cells in

the stage of premeiotic mitoses ^{3}H-uridine incorporation hardly takes place (5.1 ± 0.53 grains per 25 μ^2). There were no distinct differences in labelling between the nucleus, nucleoli and cytoplasm. Incorporation of ^{3}H-uridine is much more intensive in the last premeiotic interphase (14.2 ± 0.64 grains per 25 μ^2, Fig. 21, 22, 23). It was noticed that in some specific period of tissue development, particularly intensive labelling occurred over nucleoli (Fig. 24, 25). At the beginning of meiotic prophase the rate of ^{3}H-uridine incorporation rapidly falls down (1.2 grain per 25 μ^2). The cytoplasm and nucleoli are almost completely unlabelled (Fig. 26).

In Table 1, the results of grain counting over tapetal cells are also presented. In the last premeiotic interphase the uptake of isotope by tapetum cells is not different from that observed for PMC's (14.8 ± 0.39). At the beginning of meiotic prophase, when the rate of isotope incorporation by PMC's has greatly decreased, labelling of tapetal cells (Fig. 26) is even more intensive than in the last premeiotic interphase (19.3 grains per 25 μ^2). Thus it may be concluded that in tapetal cells, RNA synthesis goes on intensively both in premeiotic interphase and during meiotic prophase, while in PMC's development the most intensive RNA synthesis takes place in the premeiotic interphase.

The formation of chromocentres observed in *R. thyrsiflorus* in PMC's at the very beginning of meiosis gives the possibility to compare the rate of RNA synthesis by hetero- and euchromatin. It was observed that, in the last premeiotic interphase, when all chromatin is in fuzzy state, the nuclei of PMC's are uniformly labelled (Figs 21, 22, 23). It means that in this stage the intensity of RNA synthesis is more or less the same for eu- and heterochromatin including that of Y chromosomes. In more advanced stages of PMC's development when distinct chromocentres are visible in the nuclei (beginning of meiotic prophase) differential labelling of eu- and heterochromatin was noted. At this stage nuclei were labelled either only in euchromatin or in hetero- and euchromatin or else labelled mainly in heterochromatin. Such differences in the labelling pattern are difficult to interpret. However, it is doubtless that in this stage label is present both in eu- and heterochromatin. If the chromocentres visible at the beginning of the meiotic prophase are actually formed mainly by Y chromosomes, then the labelling observed over chromocentres may be considered as a proof of active RNA synthesis by Y chromosomes.

This conclusion was further confirmed by the results of staining of PMC's with methyl-green-pyronin. It was found that nuclei of PMC's in the last premeiotic

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Plate V

Figs. 21, 22, 23. Pattern of labelling with ^{3}H-uridine of PMC during "diffuse stage". Note rather uniform labelling of nuclei.

Figs 21, 22 × 800, Fig. 23 × 1500.

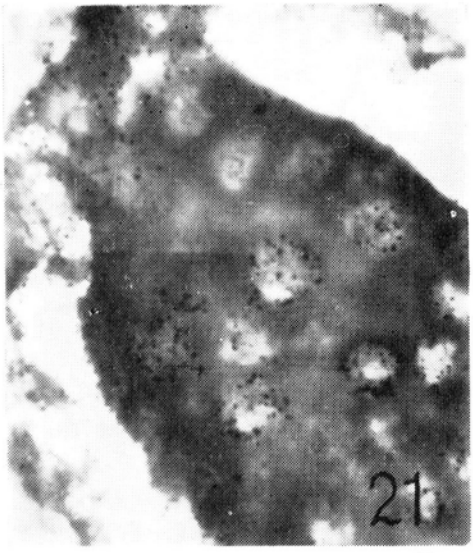
Figs 24, 25. Intensive labelling of nucleiol with ^{3}H-uridine.

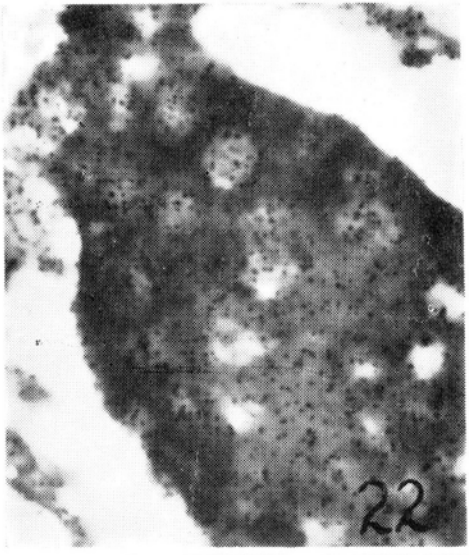
Fig. 24. × 800 Fig. 25. × 1500.

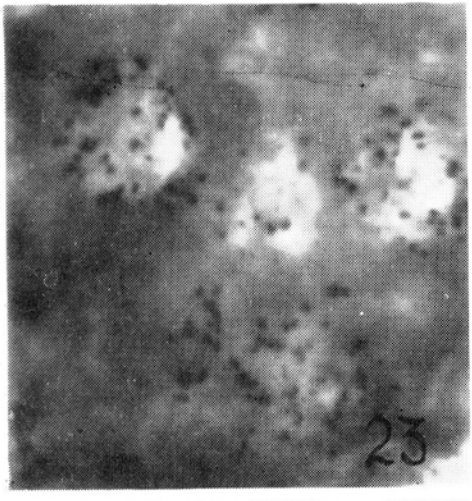
Fig. 26. Intensive labelling of tapetal cells with ^{3}H-uridine at the beginning of meiotic prophase. × 800.

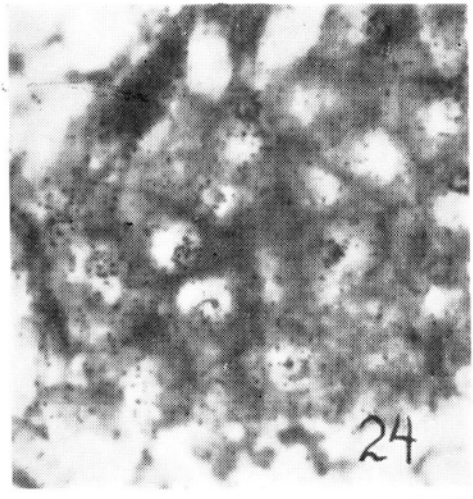
All preparations stained with methyl-green pyronin

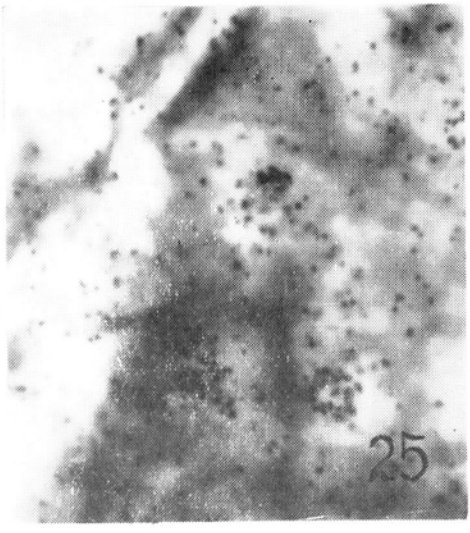
Plate V

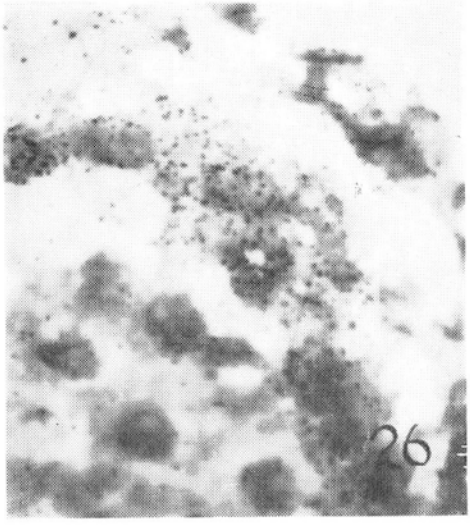












interphase (diffuse stage) stained like cytoplasm violet-red a colour characteristic for high RNA content. When chromocentres were formed at the very beginning of the meiotic prophase (Fig. 18, 19), heterochromatin stained a blue-green characteristic for DNA and the rest of chromatin was violet-red. This differential staining may be interpreted as reflection of the genetic activity of both eu- and heterochromatin in PMC's.

DISCUSSION

In the present paper convincing evidence is presented that in *R. thyrsiflorus* during the last premeiotic interphase heterochromatic Y chromosomes undergo full despiralisation, and that in this stage of PMC's development intensive RNA synthesis goes on. According to the observations here presented, premeiotic interphase is interpreted as a "diffuse stage". The presence of diffuse stage during meiosis is well established in fungi (Singleton, 1953; Carr and Olive, 1958; Żuk and Swietlińska, 1965; Rossen and Westergaard, 1966). Moens (1964) presented evidence that the diffuse stage during meiosis exists also in *Lycopersicon esculentum* and in some other higher plants as well. According to Moens the diffuse stage is observed after schizonema and before diplonema. The situation seems to be different in *Rumex*, as in this plant the diffuse stage of chromatin (including Y chromosomes) was confined only to the premeiotic interphase and was not observed in prophase nuclei of PMC's. Moreover, in early meiotic prophase, the compact state of chromatin was observed as a very persistent stage of meiosis in *R. thyrsiflorus*. It is difficult to speculate on the basis of the present data whether the characteristic feature of the meiotic prophase in *R. thyrsiflorus* is the result of occurrence of Y chromosome heterochromatin or not.

According to Clever (1968), the despiralised state of heterochromatin cannot be considered as an absolute proof of its genetical activity. However, there are many data suggesting that this is true for *Rumex*. Firstly, genetical data strongly suggest that Y chromosomes are necessary for normal pollen development (Żuk, 1970 a). Secondly, after staining with fast green-eosine after Bloch (1966) the Y chromosome chromatin stains green during the premeiotic interphase, which suggests its genetical activity (Żuk, 1969 b). Also after staining with methyl-green-pyronin the nuclei of PMC's during the diffuse stage are uniformly stained violet-red characteristic for a high content of RNA. On the other hand, in more advanced stages of PMC's development, when partial condensation of chromatin takes place, all chromatin and especially heterochromatic bodies stain blue-green characteristic for DNA. Finally the observations concerning the rate of ^{3}H-uridine incorporation by PMC's has shown that the despiralised state of Y chromosomes before meiosis coincided with intensive RNA synthesis. We assume that in *R. thyrsiflorus* premeiotic RNA synthesis, in which Y chromosomes take part, is responsible for normal pollen development. Intensive RNA synthesis was observed also by Sauter (1968) in premeiosis of *Paeonia tenuifolia*. Thus *R. thyrsiflorus* is not exceptional in that respect

and intensive premeiotic RNA synthesis observed in this species can not be considered only as a result of genetic activity of Y chromosomes.

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Dalsze obserwacje nad aktywnością heterochromatyny chromosomu Y u Rumex tflhyrsiorus

Streszczenie

Badano zachowanie się chromosomów Y u pięciu roślin męskich *R. thyrsiflorus* zawierających od 1 do 5 chromosomów Y. Obserwacje cytologiczne jąder premejotycznych wykazały, że w tym stadium wszystkie chromosomy Y znajdują się w stanie dyfuzyjnym i nie tworzą chromocentrów. Natomiast w jądrach nitki pręcikowej oraz w jądrach komórek tapetalnych obserwuje się duże chromocentry w ilości odpowiadającej liczbie chromosomów Y stwierdzonych w danej roślinie. Jest to dalszy dowód, że w stadium bezpośrednio poprzedzającym mejozę wszystkie chromosomy Y są genetycznie aktywne. Ponadto wykazano, że w ostatniej interfazie przedmejotycznej zachodzi silna inkorporacja ^{3}H-urydyny. Zatem dyfuzyjny stan chromosomów Y zbiega się z intensywną syntezą RNA.