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SPONTANEOUS CHANGES IN THE GENOME OF THE RED CLOVER (*TRIFOLIUM PRATENSE* L.) I. TRISOMITY[1]

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Summary. Aneuploides with $2n=16$ chromosomes, separated in the process of sib-mating, were intercrossed. The obtained hybrid plants had from 14 to 16 chromosomes in their somatic cells. This paper contains a description of 15-chromosome plants and secondary sister-euploids.

Trisomics were characterized by irregulations in meiosis and sporogenesis, whereas in sister disomics, reduction and division of chromosomes in the PMC[s] proceeded normally, like in highly fertile initial euploids.

Trisomic plants clearly differed from disomics. Such morphological characters like the height of shoots, the number of shoots and flower heads, the flower size and others, were of no use in the identification of 15-chromosome plants of red clover.

Studies of spontaneous aneuploids will contribute to a better knowledge of the genetics and cytogenetics of red clover.

No polyploid and aneuploid plants were found among cultivated forms and natural populations of red clover, except induced autotetraploid. The described European forms of *T. pratense* have $2n=14$ chromosomes in their somatic cells (Bleier 1925; Karpeczenko 1925; Wexelsen 1928; Evans 1962; Bijok, Góral 1971; Kazimierski et al. 1972 and others).

In the process of sib-mating we managed to separate plants and lines, the chromosome number of which appeared to be larger than $2n=14$ and amounted to $2n=16$ (Strzyżewska 1974, 1976). They gave rise to trisomics ($2n=15$). The characteristic of trisomics and secondary disomics ($2n=14$), which appeared after crossing $2n=16$ plants, is given in the present paper.

MATERIAL AND METHODS

Aneuploid plants with $2n=16$ obtained in I_3 local red clover forms during inbreeding and sib-mating were intercrossed. The chromosome number was estimated in the cells of the tip shoots and roots and fixed in Carnoy's solution. Meiosis was analysed on squashed preparations of anthers stained with acetocarmine supplemented with $FeCl_3$.

Measurements of the shoots, leaves, flowers and others were made at the same developmental stage.

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RE SULTS

Intercrossed aneuploids ($2n=16$) set seeds, which gave rise to plants with 14-16 chromosomes in the somatic cells. Two generations of hybrid plants were studied. It was found that the chromosome number in F_1 was $2n=15$ in 12 plants (Fig. 1) and $2n=16$ in 7 plants, i.e. the same as in the parental plants. The remaining 85 plants had the chromosome number $2n=14$, i.e. euploid (Fig. 2). This phenomenon was repeated in the second generation of aneuploid plants with $2n=16$. Out of 54 F_2 plants two had 15 chromosomes, six — 16 chromosomes and 46 had $2n=14$.

A. MEIOSIS IN 15-CHROMOSOME PLANTS

The meiosis of aneuploid plants with $2n=15$ was found to have deviation in the process of chromosome pairing and division. It was also found that during pachytene PMCs had single chromosomes, which did not pair along the entire length, and bivalents with shorter or longer unpaired fragments. There were also cells, with four nuclei at the pachytene stage.

During diakinesis and metaphase I the chromosomes occurred in the form of bivalents, trivalents and univalents. About 73.0% of the cells had the chromosome configuration $7_{II}1_{I}$ (Fig. 3), 13,8% — $6_{II}1_{III}$ (Fig. 4), and the smallest number of PMCs had the chromosome configuration $5_{II}5_{I}$ (Fig. 5) and $6_{II}3_{I}$. A single cell during metaphase I was found to have $3_{II}11_{I}$ and 4 fragments (Table 1). The total number of chromosomes and fragments in that PMC was 19. This number, larger than the aneuploid one, is difficult to explain. It seems to be explainable under the supposition that during mitosis chromosomes in the pramaternal cell of the meiocyte divided unequally, as a result of which one cell had 19 chromosomes and another — only 10. The fragments are likely to be chromatide arison from the division of univalents before metaphase I.

Bivalents during metaphase I were mostly straight and only rarely had the form of a ring.

During anaphase I the following chromosome configurations were observed: 7-8 (44.7%), 7-1-7 (23.1%) and 6-1-8 (5.5%). After the first division the cytoplasm of some cells had lagging chromosomes, the number of which was from 1 to 5. At telophase I and interkinesis, chromosomes and chromosome bridges were found. During metaphase II the PMCs had as a rule two division plates with the chromosome number 7 and 8. Less numerous (23.1%) were cells with two 7-chromosome division plates and with a single chromosome beyond the plates.

During anaphase II, groups with 7 and 8 chromosomes were encountered. Cells with a single lagging chromosome were sporadically observed. After the second division there occurred tetrads and polyads of microspores. Tetrads were the most — 63.5%, pentads and hexads were less numerous. Sometimes diads and triads of microspores were also found. The nucleous of a microspore had usually a single nucleolus (rarely 2 nucleoli), but sometimes even 3-5 nucleoli.

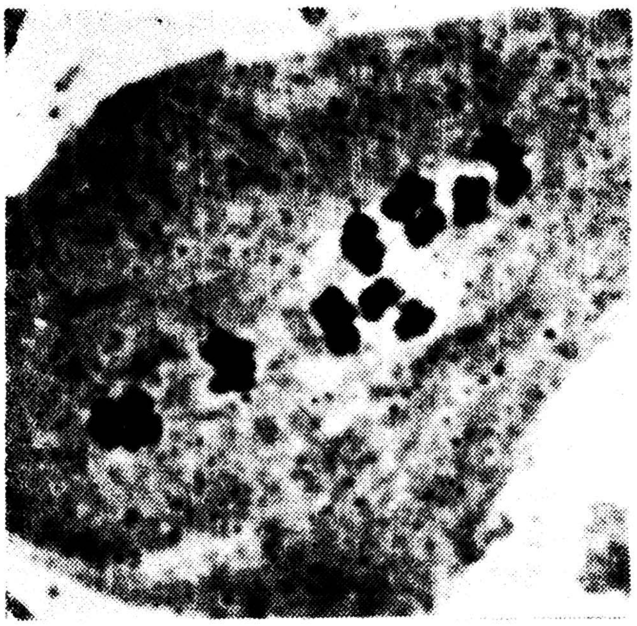
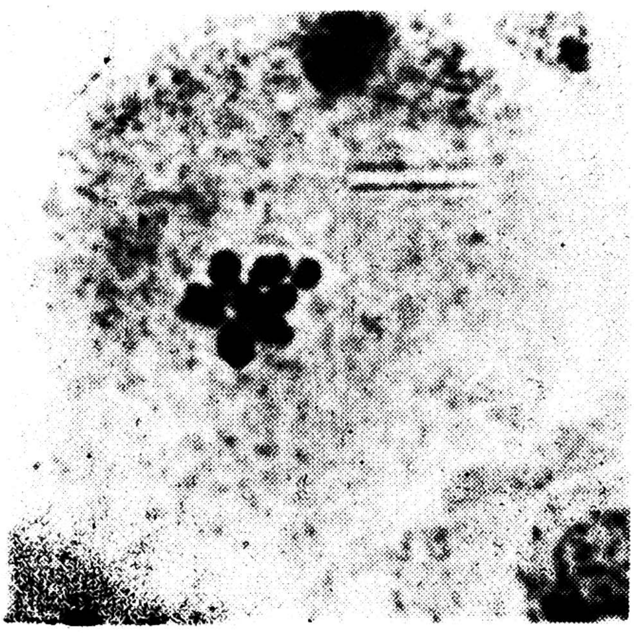


Fig. 1. Metaphase in the somatic cell of a trisomic plant with $2n=15$

Fig. 2. Metaphase in the somatic cell of a disomic plant with $2n=14$ (10×95)



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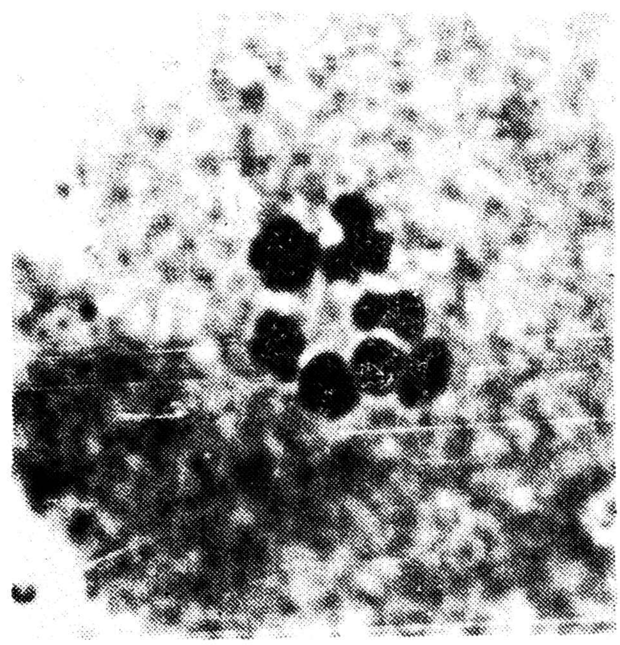

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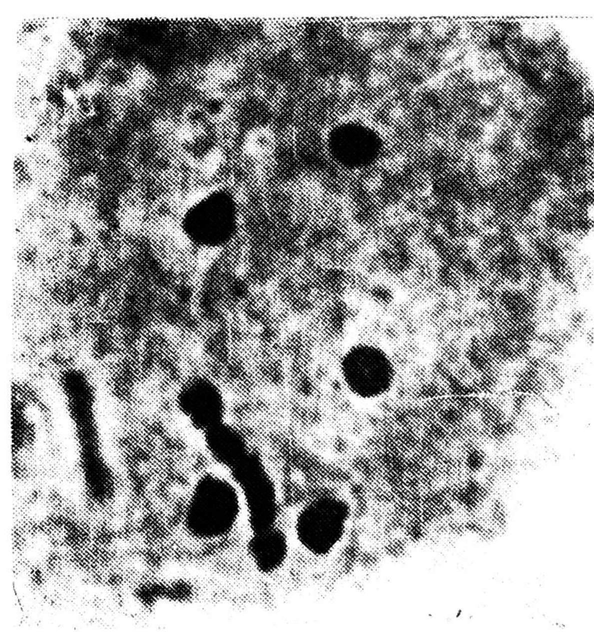

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Figs. 3, 4, 5. Meiosis in trisomic plants Metaphase I: Fig. 3 - $7_{II}1_{I}$; Fig. 4 - $6_{II}1_{III}$; Fig. 5 - $5_{II}5_{I}$

Table 1. The number of chromosome configurations in 15- and 14-chromosome plants of red clover during diakinesis and metaphase I

Chromosome number	Plants	Chromosome configurations							Analysed no. of PMCs
		7_{II}	$7_{II}1_{I}$	$6_{II}2_{I}$	$6_{II}1_{III}$	$6_{II}3_{I}$	$5_{II}5_{I}$	$3_{II}1_{I}$+4 fragments	
2n=14 (initial euploids)	1017/5	222		2					224
2n=14 (secondary euploids)	96/3	218		9					227
2n=15	23/2		24		4	1			29
	23/5		3		2				5
	78/2		21		4	2	5		32
	86/1		38		4	3			45
	79/5		20		7		11	1	39
	63/1		8		1				9
	50/2		18		3	1			22
Totally			132		25	7	16	1	181
%			72,9		13,8	3,8	8,8	0,5	

B. MEIOSIS IN SECONDARY EUPLOIDS (2n=14)

Meiosis in the PMCs of euploids (2n=14), found in the progeny of hybrid aneuploid plants proceeded without any deviations. Metaphase I had occasionally $6_{II}2_{I}$. Bivalents were chiefly straight, very rarely — ring-shaped.

Meiotic divisions in the PMCs of euploid initial plants proceeded in a similar way (Table 1).

C. MORPHOLOGICAL CHARACTERS OF TRISOMICS

A comparison was made between morphological characters of sister euploids and 15-chromosome plants.

Leaf blades of aneuploids were ellipsoid with the length-to-breadth ratio of 1.92. Plants with 15 chromosomes had leaflets somewhat longer and narrower than did 14-chromosome plants (1.69).

The shoot length ranged from 81 to 87 cm with the mean equal to 84 cm. Sister euploids were on the average lower — 64.2 cm, but their shoot length was not equalled and ranged from 41 to 83 cm. Aneuploids and euploids averagely formed the same

Table 2. The height of plants and the number of shoots and inflorescences in diploids and aneuploids of red clover

Plants	Plant height (cm)			Number of shoots			Number of inflorescences		
	min.	max.	mean	min.	max.	mean	min.	max.	mean
2n=14	41	83	64.2	9	23	11.7	21	111	76.7
2n=15	81	87	84.0	10	13	11.5	81	91	86.0

Table 3. The number of flowers per inflorescence and the length of flower, calyx and ovary in euploid and aneuploid plants of red clover

Plants	Number of flowers per inflorescence	Length of (cm)			Ovary length (in μ)
		flower	calyx	ovary and pistil	
$2n=14$	58.2 (33-147)	14.0	2.9	10.0	1074.32
$2n=15$	59.4 (39-93)	13.4	2.8	10.8	1006.74

number of shoots — 11.5 and 11.7, respectively. However, in euploid plants the shoot number ranged from 9 to 23 and in aneuploids—from 10 to 13. Aneuploid plants, therefore, displayed a lower variation here like in the shoot length (Table 2). Less variable were also the number of heads per plant and the number of flowers per head (Table 2).

Trisomic plants did not differ from disomic plants also in the flower size (Table 3). However, 15-chromosome plants had a shorter ovary, on the average by 65.5 μ.

DISCUSSION

Meristematic cells of the root tips in diploid red clover have 14 chromosomes, which are small, from 1.9 to 2.9 μ (Kazimierski et al. 1972). One of the chromosome pairs with satelites is longer than the remaining ones (Wexelsen 1928; Kazimierski et al. 1972; Strzyżewska 1976 and others). The additional chromosome in trisomics was short (less than 2 μ), had no satelite, and little differentiation of the chromosome length, as well as their faint staining made difficult the determination of the additional chromosome affinity to a definite pair.

A larger than aneuploid number of chromosomes and fragments in one of the PMCs during metaphase I may be interpreted by unequal division of chromosomes in the pramaternal cell of the meiocyte, and the presence of fragments may be explained by division of univalents.

Trisomcs of red clover were characterized by significant disturbances in meiosis. The PMCs were found to have lagging chromosome and chromosome bridges. The chromosome occurring as a univalent probably was not included into progeny nuclei like in trisomics of other plants (Gajewski 1972). As follows from the literature, $n+1$ gametes have frequently a decreased viability and can be eliminated in the process of fertilization, when competing with gametes having n chromosomes (Gajewski 1972). A proper pairing of homologous chromosomes in the cells of secondary euploids of red clover at the pachytene stage, the occurrence in the PMC of 7 bivalents at the stage of diakinesis and metaphase I, as well as a normal course of the second division, indicate that gametes with an additional chromosome were eliminated.

A high per cent of euploids in the progeny of aneuploids of red clover — 81.7 in F_1 and 85.2 in F_2 — permits to suggest that microspores and gametes with an additional chromosome are less viable than normal ones. Hence, a frequently encountered return to euploidy in the progeny of aneuploids of red clover. Therefore, the karyotype $2n+1$ is not stable.

Trisomics ($2n+1$) occur sporadically in many diploid organisms (Dawson 1962; Brewbaker 1970), and in polyploid species of grasses of our flora they constitute a small per cent (Falkowski 1982). Trisomics of many plants do not differ from diploid plants by their morphological structure. In *Datura stramonium* 12_{I} various morphological types concerning the fruit shape can be observed. Fruits of each of these types ($2n+1$) differ from one another and differ from fruits of $2n$ plants (Malinowski 1967).

According to Frost the additional chromosome $2n=14+1$ in *Matthiola incana* has an influence on the narrowing of leaf blades (cited after Malinowski 1967). Generally taking, trisomics of grasses have narrower leaves, are less vigorous than disomic plants (Ahloowalia 1981). There are well-known opinions that morphological differences between euploids and aneuploids were imperceivable (Jauhar 1978). Under natural conditions wheat aneuploids occur very rarely (1%). Most monosomics and trisomics do not differ morphologically from normal plants (Pala 1975). Contrary to wheat trisomics, barley trisomics are not phenotypically similar either between each other or to typical diploid plants with regard to various chromosomes. Series of barley trisomics are easy to distinguish from plants with complete chromosome set (Tsuchiya 1960; Ramage 1960; Tsuchiya 1964, 1967; Das, Bhowmik 1971; Das, Kundu 1973 and others).

The presence of additional chromosome in the genome of red clover manifests itself in the phenotype in a little visible way. The habitus, shoot length, the number of shoots and inflorescences, the flower size and other characters cannot be useful in the identification of trisomics of red clover. The loss or addition of one chromosome should not lead to the occurrence of easily observed changes in the phenotype.

The performed studies will permit to learn better the genetics and cytogenetics of red clover.

CONCLUSIONS

1. Plants obtained as a result of crossing aneuploids with $2n=16$ chromosomes had from 14 to 16 chromosomes in the somatic cells.

2. The analysed trisomics were characterized by significant disturbances in meiosis. The most frequently encountered chromosome configurations in the PMC during diakinesis and metaphase I were: $7_{\mathrm{II}}1_{\mathrm{I}}$, $6_{\mathrm{II}}1_{\mathrm{III}}$. During metaphase I one cell was found to have $3_{\mathrm{II}}11_{\mathrm{I}}$ and 4 fragments.

3. Meiosis in secondary sister euploids ($2n=14$) proceeded without disturbances.

4. A high per cent of euploids in the progeny of aneuploids permits to suggest that microspores and gametes with additional chromosomes are eliminated.

5. Trisomics did not differ phenotypically from sister disomics.

6. The performed studies will permit to learn the genetics and cytogenetics of spontaneous aneuploids separated in red clover for the first time.

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SPONTANICZNE ZMIANY W GENOMIE KONICZYNY CZERWONEJ (*TRIFOLIUM PRATENSE* L.) I. TRISOMICZNOŚĆ

Streszczenie

Wyodrębnione w procesie chowu siostrzanego aneuploidy z $2n=16$ chromosomów przekrzyżowano między sobą. Otrzymano rośliny mieszańcowe mające w komórkach somatycznych od 14 do 16 chromosomów. W pracy opisano rośliny 15-chromosomowe i siostrzane euploidy wtórne. Trisomiki charakteryzowały się zakłóceniami w mejozie i sporogenezie, natomiast u siostrzanych disomików redukcja i podziały chromosomów w KMP przebiegały prawidłowo, podobnie jak u wysoko płodnych euploidów wyjściowych.

Wysokością pędów, liczbą pędów i główek kwiatowych, oraz wielkością kwiatu rośliny trisomiczne nie różniły się w sposób widoczny od disomików. Cechy morfologiczne nie były pomocne przy identyfikowaniu roślin 15-chromosomowych koniczyny czerwonej.

Badania spontanicznych aneuploidów przyczynią się do lepszego poznania genetyki i cytogenetyki koniczyny czerwonej.

СПОНТАННЫЕ ИЗМЕНЕНИЯ В ГЕНОМЕ ЛУГОВОГО КЛЕВЕРА (*TRIFOLIUM PRATENSE* L.)
I. ТРИСОМАТИЧНОСТЬ

Резюме

Анэуплоиды с числом хромосом $2n=16$, выделенные в процессе скрещивания сибсов, скрещивались между собой. Полученны гибридные растения имели от 14 до 16 хромосом в своих соматических клетках. В настоящей работе описаны 15-хромосомные растения и вторичные сестринские эуплоиды.

Трисомики характеризовались нарушениями в мейозе и спорогенезе, в то время как у сестринских дисомиков редукция и деление хромосом в материнских клетках пыльцы протекали нормальне, подобно как у высоко плодоносных исходных эуплоидов.

Трисомические растения отчетливо отличались от дисомиков. Такие морфологические признаки, как высота побегов, число побегов и цветочных головок, величина цветов и другие, не были необходимы для идентификации 15-хромосомных растений лугового клевера.

Исследования спонтанных анэуплоидов помогут личше познать генетику и цитогенетику лугового клевера.