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SPONTANEOUS CHANGES IN GENOME OF RED CLOVER (*TRIFOLIUM PRATENSE* L.). II. TETRASOMITY. MORPHOLOGICAL CHARACTERS AND FERTILITY[1]

CZESŁAWA STRZYŻEWSKA[2]

Institute of Plant Genetics, Polish Academy of Sciences, Poznań

Summary. Some morphological characters were studied in hybrids of red clover obtained from tetrasomics with $2n=16$. The progeny of 16-chromosome plants (F_1-F_4) was found to have a high per cent of secondary disomics. The fifth generation was characterized by a certain stability of the genome — the per cent of 14-chromosome plants in tetrasomics lines definitely decreased, whereas the fertility of tetrasomics increased. The progeny of tetrasomics was not phenotypically similar to the parents. Hybrids did not differ visibly from sister disomics.

The method of obtaining plants and lines with $2n=16$ from population of euploid red clover with $2n=14$ was described previously (Strzyżewska 1974, 1976). In the present paper some morphological characters of initial tetrasomics, hybrids and sister disomics were compared.

MATERIAL AND METHODS

Aneuploid plants with $2n=16$ obtained in I_3 were intercrossed. The succeeding hybrid generations were obtained by pollinating sister plants.

The chromosome number was estimated according to the method given in part I of this publication. Pollen viability was determined on staining pollen grains with Belling's fluid. The number and size of tracheal cells were estimated in the epidermis of the lower leaf side. Measurements of morphological characters of di- and tetrasomics were made at the same developmental stage.

RESULTS

Among 16-chromosome plants crossed in F_1 and F_2 we separated: secondary disomics — $2n=14$, trisomics — $2n=14+1$, tetrasomics — $2n=14+2$.

In the next generations of plants originating from crosses of 16-chromosome

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[1] Received for publication: June, 1983.

[2] Dr. Present address: ul. Strzeszyńska 30/36, 60-479 Poznań, Poland.

individuals no trisomics were found (Table 1), whereas secondary disomics constituted significant portion: in F_1 — 81.7%, in F_2 — 85.2%, in F_3 — 85.8%. Similarly the fourth generation of 16-chromosome plants did not become stable and out of 37 plants only 7 had $2n=16$ chromosomes, whereas the remaining plants were 14-chromosome. As follows from Table 1, plants with $2n=14$ chromosomes constituted 80% of F_1-F_4 hybrid plants. Like in the F_3 and F_4 generations, tetrasomic lines of F_5 were also observed to return to euploidy. However, the per cent of 14-chromosome plants clearly decreased. 16-chromosome plants made up 79.0% and they were by over 60.0% more numerous than in F_4.

Table 1. Percentage of secondary disomics, trisomics and tetrasomics in the progenies of red clover tetrasomics

Generation	Total number of plants	Type of plants		
		secondary disomics	trisomics	tetrasomics
F_1	104	81.7	11.5	6.73
F_2	54	85.2	3.7	11.1
F_3	40	85.8	—	14.2
F_4	44	81.1	—	18.9
F_5	111	21.0	—	79.0

The noticeable increase in the percentage of tetrasomics in F_5 may be considered to be the beginning of genome stabilization.

MORPHOLOGICAL CHARACTERS

Spontaneous tetrasomics of red clover could be distinguished from euploids comparatively easily, since they were significantly lower and less vigorous. They also differed from disomics by the size and shape of the leaf blade and by the habitus. They had few shoots, short internodes and light, small, shrivelled alongside nervuration, stiff leaflets.

Hybrid plants were not phenotypically similar to 16-chromosome initial forms (parents) and did not differ from secondary sister disomics by their habitus.

16-chromosome initial plants had, as mentioned above, rhomboid shrivelled

Table 2. The length, breadth and length to breadth ratio of the leaf blade of parental and hybrid plants with $2n=14$ (secondary disomics) and $2n=16$ (tetrasomics)

Generation	Chromosomes number $2n$	Leaf blade		
		length	breadth	length to breadth radio
Parental	14	27.1	17.2	1.60
	16	19.7	15.8	1.24
F_4	14	55.8	21.2	1.68
	16	51.4	19.8	1.50
F_5	14	39.0	25.0	1.56
	16	24.5	14.8	1.65

leaflets, which were light and stiff. The length to breadth ratio of leaves was somewhat larger than the unit. Hybrids had more or less elipsoid leaflets with the length to breadth ratio equal to 1.50 (in F_4) and 1.65 (in F_5). In comparison with 14-chromosome plants, leaflets of 16-chromosome individuals were somewhat smaller, but definitely larger than the leaf blades of initial tetrasomics (Table 2). No leaf differentiation was found between tetra- and disomics as it took place in the parental forms — reduction of leaf blade (one- or two-leaflet leaves) and characteristic of 16-chromosome plants of red clover "shriveling" of leaves.

Table 3. The length and breadth of stomata and their number (in the field of vision in the microscope) of the parental and hybrid red clover with $2n=14$ (secondary disomics) and $2n=16$ (tetrasomics)

Plants	Chromosomes number $2n$	Stomata		
		length (μ)	breadth (μ)	number in the field of vision
Parental	14	16.66	12.10	19.9
	16	19.44	13.60	17.3
Hybrid F_1	14	17.91	12.05	18.1
	16	20.01	14.46	17.1

Hybrids and initial forms had similar number and shape of trachea. In 16-chromosome plants the tracheal apparatus were longer and broader and the unit leaf area had them more than did 14-chromosome sister plants (Table 3).

Other morphological characters of the F_1 and F_4 progenies were characterized by a large variation (Table 4). The shoot height of tetrasomics in F_1 ranged from 29 to 74 cm; on the average, the hybrid shoots were 25.9 cm longer than those of the initial forms. The range of the shoot length in F_4 individuals was also large — from

Table 4. Number of shoots and inflorescences per plant, shoot length and number of flowers per inflorescence of the parental and red clover hybrid plants with $2n=14$ (secondary disomics) and $2n=16$ (tetrasomics)

Generation	Chromosomes number $2n$	Number per plant						Shoot length			Number of flowers per inflorescence		
		shoot			inflorescences								
		min.	max.	$\bar{x}$	min.	max.	$\bar{x}$	min.	max.	$\bar{x}$	min.	max.	$\bar{x}$
Parental	14	10	39	29.5	23	69	36.2	36.9	57.3	27.9	21	175	91.6
	16	2	9	3.8	1	14	7.2	21.0	35.3	27.5	46	118	76.4
F_1	14	9	21	11.1	19	41	21.0	32	61	49.4	21	164	89.2
	16	6	14	4.8	23	37	29.5	29	74	53.1	28	114	71.5
F_4	14	6	9	7.5	19	39	20.3	41	73	50.5	48	175	84.1
	16	5	10	5.1	27	51	31.2	32	85	51.1	25	101	68.4
F_5	14	1	4	2.3	15	30	18.6	44	57	43.3	50	148	83.6
	16	3	10	3.9	22	48	33.7	30	88	49.8	36	108	60.0

32 to 85 cm. F_4 plants, on the average, had shorter shoots than F_1, however, they were deefinitely higher in comparison with those of the parents. Generally, shoots of the tetrasomic progeny were averagely 23.8 cm longer.

No significant differences in the shoot number of 16-chromosome plants were found at succeeding generations. Hybrids had on average 4.6 shoots, i.e. by 0.9 more than their 16-chromosome parents. On few shoots tetrasomics formed averagely 29.5 heads in F_1 and 31.2 heads in F_4, i.e. over 4-fold more than did the initial generation (7.2).

Regarding the floret number per head the F_1 and F_4 progenies did not differ from their parents. Tetrasomics of F_1 had on average by 4.9, and in F_4 — by 8.0 florets less in their head than did the parental forms. At the same time the variation in the number of florets per inflorescence was the same as that of their parents.

Tetrasomics of F_1 and F_4, as compared to sister disomics, had somewhat longer shoots, on average, by 2.3 cm, and the shoot number was smaller. Tetrasomics formed on average 6.2 and disomics 1.3 inflorescences per shoot. Inflorescences of 16-chromosome plants were smaller and their floret number was smaller by 16.8 than that of the sister disomics in F_1 and F_4.

The height of 16-chromosome plants of F_5 ranged from 30 to 88 cm (most frequently between 40 and 60 cm). The mean height of tetrasomic plants of F_5 was 49.8 cm. From Table 4 it follows that hybrid plants were higher than the initial forms, averagely by 22.3 cm, and formed more inflorescences on the shoots, their number being 7.2 in the parents and 33.7 in tetrasomic F_5 plants. Inflorescences of the parental forms had 46 - 118 florets, averagely 76.4. Heads of F_5 hybrids were smaller. Inflorescences of such plants had 60 florests each. Usually tetrasomic inflorescences were smaller than heads of disomics (Table 4). No differences were observed in the number of shoots between parental plants and their progeny. Hybrids did not differ from sister disomics in the shoot length and number, but produced more small heads on their shoots — by 55.1%.

Tetrasomic forms originate from 4 individuals, which belong to two separate lines. As a result of intercross between these plants within the same line one of them in F_1 was found to have 5 tetrasomic plants, whereas another had two plants. The remaining 16-chromosome lines were found to return to euploidy.

Table 5. Pollen grains viability of tetrasomic red clover hybrid plants in five generations

Generation	Number of plants	Number of plants with the per cent viable pollen grains			
		61 - 70	71 - 80	81 - 90	91 - 100
F_1	7	3	4	—	—
F_2	6	1	2	2	1
F_3	5	2	—	2	1
F_4	7	—	5	1	1
F_5	49	9	14	17	9
Totally	74	15	25	22	12

Five tetrasomics distinguished in F_1 gave rise to F_5 plants characterized by a certain stability of their genome. The progeny of two remaining 16-chromosome plants returned to euploidy. Already in F_3, plants of that subline had $2n=14$

chromosomes in their somatic cells. Pollen viability of tetrasomic initial plants of two lines, from which 16-chromosome F_1-F_5 plants were derived, ranged between 26.4 and 92.0%. Sister disomics had from 76.1 to 94.5% viable pollen grains.

Tetrasomic F_1 - F_5 plants were grouped depending on the percentage of stainable pollen grains (Table 5). Distinguished 16-chromosome plants had from 67.1 to 99.2% viable pollen in their anthers. Sister 14-chromosome individuals had from 74.3 to 99.7%.

FERTILITY

Tetrasomic fertility was examined by pollinating 10 florets in each inflorescences with pollen from other tetrasomic plants. Seed setting in F_1 - F_3 was satisfactory.

In F_1 generation 268 seeds were obtained from 770 pollinated flowers. The percentage of set seeds in relation to the general number of pollinated flowers of 16-chromosome plants was higher than that in the case of 14-chromosome plants. A similar dependence was observed in F_2 and F_3 of tetrasomics (Table 6). In F_1 tetrasomics

Table 6. Fertility of secondary disomics ($2n=14$) and tetrasomics ($2n=16$) red clover hybrid plants in five generations

Generation and combination of back crossing	Chromosomes number 2n	Pollinated flowers No.	Pods				Mature seeds	
			without seeds		with seeds			
			No.	%	No.	%	No.	%
F_1	14	760	510	67.1	250	66.0	165	21.7
	16	770	290	37.7	480	55.8	268	32.5
F_2	14	515	310	60.1	205	75.1	154	20.0
	16	707	177	23.6	430	72.0	310	30.5
F_3	14	648	437	67.4	211	72.0	152	23.4
	16	805	295	36.6	510	57.0	291	36.1
F_4	14	360	164	44.4	200	93.0	186	51.4
	16	810	500	61.7	310	43.2	134	16.5
F_5	14	421	116	27.5	305	89.1	272	64.6
	16	790	190	24.0	610	81.1	495	62.6
$F_1 \times P$	16	300	220	73.3	80	43.7	35	11.6
$P \times F_1$	16	100	40	40.0	60	35.0	21	21.0

gave 10.8% more mature seeds in comparison with disomics; in F_2 and F_3 this percentage was 9.5 and 12.7, respectively, on the average, tetrasomics gave 11.3% more seeds. Comparing the number of pollinated flowers which gave no seeds in F_1 - F_3, with the general number of pollinated florets in di- and tetrasomics it was found that the percentage of pods without seeds in the latter is smaller than that in disomics — by 29.4, 36.5 and 30.8%, respectively, on the average, by 32.3%. However, after subtraction of incompatible crosses (without seeds) from the general number of pollinated flowers and after calculation of the per cent of mature seeds

only in relation to compatible plants it appeared that the highest percentage of seeds was in disomic plants.

In the fourth generation, a marked decline in the fertility of hybrids from the crosses $2n=16\times 2n=16$ became evident. That decline constituted 45.5% in comparison with the fertility in F_1 - F_3 and in F_5. Tetrasomics of F_4 set 49.8% less seeds in comparison with sister disomics.

The fifth generation of 16-chromosome individuals had the fertility similar to that of sister 14-chromosome plants. Out of 790 pollinated flowers over half (62.6%) set normally developed seeds. Sister disomics set only 8.0% more seeds (Table 6). The fertility increase of tetrasomics in F_5 in comparison with F_4 was above 90.0%.

DISCUSSION

Tetrasomics distinguish by a stronger expression of traits determined by a supplementary number of chromosomes (Pala 1975). In *Matthiola incana* plants with broad leaves have $2n=14$ chromosomes. Plants with $2n=14+1$ have narrower leaflets and those with $2n=14+2$ — the narrowest (Malinowski 1967).

It is known that the phenotypic expression induced by a quaternary dosage of the *Q* gene (tetrasomic regarding the *5A* chromosome) is encountered among irradiated wheat mutants of the cultivar Chinese Spring. This gene has influence on the size, shape and turgor of cells, which in turn determines the development of plants, thickness of the stem, the size of the leaf blade, spike density and so on (Muramatsu 1963, Swaminathan 1966). A tetrasomic plant ($2n=44$) found in the progeny of a wheat trisomic ($2n=43$) did not differ from normal plants by the habitus (Sears 1939).

Tetrasomics of red clover separated in I_3 did not differ from disomics by the vigour, leaf length and breadth, by tracheal apparatus, leaf habitus and by the size of the perianth (Strzyżewska 1974). Phenotypically the progeny of tetrasomics was not similar to the parents, and, therefore, hybrid chromosomes occurring in the same number as in parents determined other morphological traits of the progeny. The reasons of that phenomena may be found in segregation of heterozygous progeny and in increase of genetic variation. It may be suggested that in the case of crossing tetrasomics $2n=16\times 2n=16$, the univalents observed in the PMCs disperse to the gametes randomly (Strzyżewska 1976), therefore, hybrid plants with the same chromosome number may differ phenotypically and certain additional differentiation may occur as a result of the chromosome number in the zygotes arisen from the gametes $n-1$, $n+1$, $n+2$.

After crossing tetrasomic plants the percentage of set seeds in relation to the number of pollinated flowers, except F_4, was higher than that of disomics. In 14-chromosome plants a high percentage of crosses without seeds (F_1 - F_3) indicates most probably the incompatibility of pollinated plants. The percentage of incompatible crosses of 16-chromosome plants was lower. This may suggest leveling of incompatibility by sumplementary chromosomes.

An increase of the seed number in the succeeding generations indicates a gradual, through slow stabilization of the tetrasomics genome.

Our dates do not permit to determine exactly the reasons of a significant decline of F_4 plant fertility. Plants used for crosses distinguished by a high pollen viability, and it may, therefore, be suggested that viability of pollen grains of tetrasomic red clover is determined by a certain system.

Tetrasomics grew in the glasshouse. It may be that 16-chromosome individuals are more sensitive to environmental conditions than disomics. Temperature or humidity could be of decisive importance in crossing and fertilization. The influence of temperature and humidity on the fertility of crossed tetraploids of red clover was revealed by Zosimowicz, Nawalichina et al. (1974). It cannot be excluded, however, that the cause of a worse seed setting in F_4 was inbred depression caused by sibling mating. As a result of breeding using sib-mating in clover the ability to seed setting decreases somewhat more slowly than in inbred mating, when the action of subvital recessive alleles becomes evident at once in the first generations (Williams 1939, 1947, 1948, Thomas 1955, Strzyżewska 1974 and others).

CONCLUSIONS

1. In F_1 - F_4 of tetrasomics high percentage of secondary disomics was found; F_5 was characterized by a certain genome stabilization.
2. 16-chromosome hybrids, contrary to the initial forms, did not differ perceivably from 14-chromosome plants.
3. Seed setting in tetrasomics in F_4 significantly decreased. We failed to find a direct reason of that fact.
4. A decrease in the percentage of secondary disomics in tetrasomic lines of F_5 was accompanied by the fertility increase.

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SPONTANICZNE ZMIANY W GENOMIE KONICZYNY CZERWONEJ (*T. PRATENSE* L.) II. TETRASOMICZNOŚĆ. CECHY MORFOLOGICZNE I PŁODNOŚĆ

Streszczenie

Badano niektóre cechy morfologiczne u mieszańców koniczyny czerwonej otrzymanych z tetrasomików $2n=16$.

W potomstwie roślin 16-chromosomowych (F_1-F_4) stwierdzono wysoki odsetek disomików wtórnych. Piąte pokolenie charakteryzowało się pewną stabilizacją genomu — odsetek roślin 14-chromosomowych w liniach tetrasomicznych zdecydowanie się zmniejszył, natomiast wzrosła płodność tetrasomików.

Potomstwo tetrasomików nie było fenotypowo podobne do form rodzicielskich. Mieszańce nie różniły się także w sposób widoczny od siostrzanych form disomicznych.

СПОНТАННЫЕ ИЗМЕНЕНИЯ В ГЕНОМЕ КРАСНОГО КЛЕВЕРА (*T. PRATENSE* L.) II. ТЕТРАСОМИЧНОСТЬ. МОРФОЛОГИЧЕСКИЕ ПРИЗНАКИ И ПЛОДОРОДНОСТЬ

Резюме

Исследовались некоторые морфологические признаки у гибридов красного клевера, полученных из тетрасомиков $2n=16$.

В потомстве 16-хромосомных растений (F_1-F_4) обнаружен высокий процент вторичных дисомиков. Пятое поколение характеризовалось определённой стабильностью генома — процент 14--хромосомных растений в тетрасомических линиях явно уменьшился, в то время как плодородность тетрасомиков возросла. Потомство тетрасомиков в фенотипическом отношении не было похоже на родителей. У гибридов не обнаружено также видимых различий от сестринских дисомиков.