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BARLEY HAPLOIDS AND HYBRIDS REGENERATED FROM CALLUS TISSUE OF *HORDEUM VULGARE* L. × *SECALE CEREALE* L. EMBRYOS[1]

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Summary. Barley monoploids and hybrids were regenerated from callus tissue of barley × rye undifferentiated embryos. The hybrids had a somatic complement of $2n=14$ (7 barley +7 rye chromosomes) and $2n=15$ (7 barley +8 rye chromosomes). Hybrid meiocytes exhibited chromosome instability in 3.7–22.7% of PMCs. The pairing of chromosomes was very low. Chiasma frequencies per cell varied from 0.03 to 0.13 in the haploids. In the hybrids with $2n=14$ the frequency was 0.02 and in the hybrids with $2n=15$ it ranged from 0.81 to 1.98. Chromosome pairing in the hybrids occurred nearly exclusively between rye chromosomes.

As a result of crosses between barley and rye the number of embryos is relatively high but the number of plantlets regenerated directly from embryos and that of survived plants is very low (Wojciechowska 1984, Pickering, Morgan 1985). In the experiments, undifferentiated embryos were induced to callus formation in an attempt to regenerate plants. Haploids and hybrids with $2n=14$ and $2n=15$ were found among the regenerates.

The present report describes the production, morphology, mitotic and meiotic chromosome behaviour of three cytotypes derived from barley × rye crossings combinations.

MATERIALS AND METHODS

Five varieties of *Hordeum vulgare* L. (Trumpf, Emir, Atlas, Valja, Jumbo) were crossed with *Secale cereale* L. (Strzekęcińskie, Dańkowskie Złote, Białe Tetra), which served as a pollen donor. The plant material used in the crosses is listed in detail in Table 1.

The technique of hybridization was described in the previous paper (Wojciechowska 1984). At 18 - 28 days after pollination the embryos were excised and plated on the B_5 medium (Gamborg et al. 1968). Embryos with some signs of differentiation were plated on the A medium (B_5 without 2,4-D) and undifferentiated

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ones — on the B medium (B_5+2 mg/l 2,4-D). After about six weeks the callusing embryos were transferred to the Murashige and Skoog (1962) medium (MS) with 2 mg/l 2,4-D and subcultured twice on the MS medium with 1 mg/l kinetin and 2 mg/l IAA or with 1 mg/l kinetin and 2 mg/l NAA.

Regenerated plantlets were transplanted into the soil and grown to maturity in a glasshouse.

The techniques used in the study on mitosis and meiosis were described earlier (Wojciechowska 1986).

In the paper the chromosomes of barley and rye are designated with the *V* and *R* letters, respectively.

RESULTS

PRODUCTION AND MORPHOLOGY OF HAPLOID AND HYBRID PLANTS

As shown in Table 1, haploids and hybrids were produced only from 3 out of 16 barley × rye combinations. All 34 plants were regenerated from callus tissues (Fig. 1) produced by undifferentiated embryos. Three plantlets produced directly from the embryos died at the early tillering stage after transferring them to the pots. Plants from Trumpf × Strzekęcińskie combination were derived from the callus tissue of four embryos (Nos. 34/1, 2, 45, 70). Plants from embryos Nos. 34/1, 2 had 7 and 15 chromosomes; plants from embryos No. 45 had $2n=14$, and those from embryos No. 70 — $2n=15$. One embryo (No. 90) from the combination Trumpf × × Dańkowskie Złote produced callus from which only haploid plants were regenerated. However, 10 plants with $2n=15$ regenerated from one callused embryo of the cross Emir × Strzekęcińskie.

Haploid plants morphologically resembled the barley haploids produced by bulbosum technique. Of the three obtained cytotypes the haploids tillered most profusely (Fig. 2). Hybrids with $2n=14$ (Fig. 3), were weak especially during the early tillering stage and their development was markedly slower than that of hybrids with $2n=15$ (Fig. 4). All the plants were shorter than either parent. Hybrid plants morphologically resembled rye by pubescent peduncles, stiff hair on the lemma keel and by the spike structure (Fig. 5). The spikelets had 2 - 3 florets. The haploids and the hybrids were completely sterile.

SOMATIC CHROMOSOMES

The somatic chromosome number was counted in 28 plants. Six barley haploids had 7 chromosomes with 2 nucleolar organiser constrictions (Fig. 6). Two hybrids had $2n=14$ (7 barley+7 rye chromosomes) and 20 hybrid plants had $2n=15$ (7 barley+8 rye chromosomes). In the hybrids the parental chromosomes could be identified as all the *Secale* chromosomes were larger than the *Hordeum* ones (Figs

Table 1. Embryos cultured and plant regeneration from hybrids between *H. vulgare* and *S. cereale*

Parental forms		Number							
		florets pollinated	embryos cultured		embryos callused		plantlets regenerated		plants
Barley cv.	Rye cv.		A	B	A	B	directly from embryos	from callus	
Trumpf 2*x* (s)	Strzekęcińskie 2*x* (s)	140	8	18	2	11	3	16	21 (2*n*=7,14,15)
Trumpf 2*x* (s)	Dańkowskie Złote 2*x* (w)	291	20	10		1		9	3 (2*n*=7)
Trumpf 2*x* (s)	Białe Tetra 4*x* (w)	20		2		1			
Emir 2*x* (s)	Strzekęcińskie 2*x* (s)	40	1	2		2		2	10 (2*n*=15)
Atlas 2*x* (w)	Strzekęcińskie 2*x* (s)	120		1					
Atlas 2*x* (w)	Dańkowskie Złote 2*x* (w)	224		3					
Atlas 2*x* (w)	Białe Tetra 4*x* (w)	30		1					
Valja 2*x* (w)	Strzekęcińskie 2*x* (s)	36							
Valja 2*x* (w)	Dańkowskie Złote 2*x* (w)	88		3					
Valja 2*x* (w)	Białe Tetra 4*x* (w)	69	1						
Jumbo 2*x* (w)	Strzekęcińskie 2*x* (s)	19							
Jumbo 2*x* (w)	Dańkowskie Złote 2*x* (w)	18							
Jumbo 2*x* (w)	Białe Tetra 4*x* (w)	92							
Atlas 4*x* (w)	Strzekęcińskie 2*x* (s)	46	3						
Atlas 4*x* (w)	Dańkowskie Złote 2*x* (w)	52							
Atlas 4*x* (w)	Białe Tetra 4*x* (w)	81	3	3	2	2			

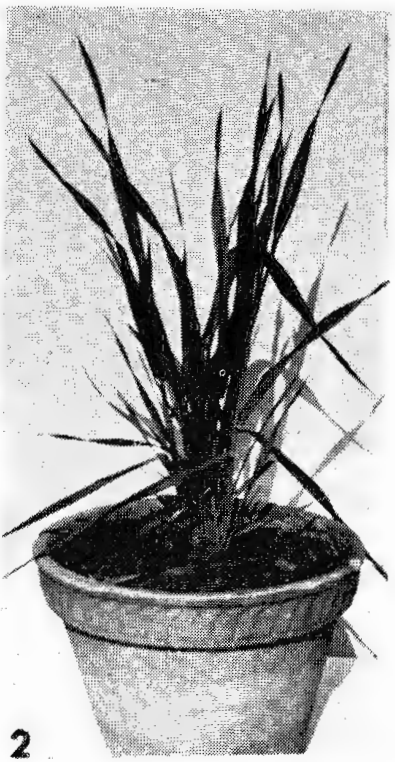
s – spring
w – winter

A – B5 medium without 2,4-D
B – B5 with 2 mg/l 2,4-D

Table 2. Chromosome numbers and mean configurations at MI cells of barley haploids and barley × rye hybrids

Material	No. cells	No. chromosomes	% of total cells	Chromosome associations							X-ta per cell		
				I		II				III			
				V	R	$rod^{V/V}$	ring	$rod^{R/R}$	ring	R/R/R	V/V	R/R	total
Haploids													
99	170	7	100.00	6.78 (3–7)	–	0.13 (0–2)	–	–	–	–	0.13	–	0.13
34/1	120	7	100.00	6.90 (5–7)	–	0.05 (0–1)	–	–	–	–	0.05	–	0.05
34/2	159	7	100.00	6.93 (5–7)	–	0.03 (0–1)	–	–	–	–	0.03	–	0.03
34/7	64	7	100.00	6.93 (5–7)	–	0.03 (0–1)	–	–	–	–	0.03	–	0.03
Hybrids													
45/3 ($2n=14$)	173	14	77.23	6.99 (5–7)	6.99 (5–7)	0.01 (0–1)	–	0.01 (0–1)	–	–	0.01	0.01	0.02
	3	12	1.33	6.66 (6–7)	4.66 (4–5)	–	–	–	0.33 (0–1)	–	–	0.66	0.66
	24	13	10.71	6.75 (6–7)	6.25 (6–7)	–	–	–	–	–	–	–	–
	24	15	10.71	7.54 (7–8)	6.46 (5–8)	–	–	0.25 (0–1)	0.25 (0–1)	–	–	0.75	0.75
34/11 ($2n=15$)	41	15	89.13	7.00	5.92 (4–6)	–	–	0.10 (0–1)	0.90 (0–1)	0.02	–	1.98	1.98
	4	14	8.69	6.50 (6–8)	5.50 (5–6)	–	–	0.25 (0–1)	0.75 (0–1)	–	–	1.75	1.75
	1	16	2.17	8.00	4.00	–	–	1.0	1.00	–	–	3.00	3.00
50/2 ($2n=15$)	46	15	89.79	7.02 (7–8)	5.97 (4–6)	–	–	0.14 (0–1)	0.52 (0–1)	–	–	1.45	1.45
	6	14	11.32	6.83 (6–7)	4.83 (4–5)	–	–	0.66 (0–2)	0.50 (0–1)	–	–	1.66	1.66
	1	16	1.88	8.00	6.00	–	–	1.00	–	–	–	1.00	1.00
50/4 ($2n=15$)	68	15	85.00	7.00	5.91 (4–8)	–	–	0.48 (0–2)	0.55 (0–2)	–	–	1.58	1.58
	8	14	10.00	6.87 (6–8)	5.12 (4–6)	–	–	0.12 (0–1)	0.87 (0–1)	–	–	1.86	1.86
	1	16	1.25	8.00	6.00	–	–	–	1.00	–	–	2.00	2.00
	1	18	1.25	8.00	6.00	–	–	–	2.00	–	–	4.00	4.00
70/1 ($2n=15$)	51	15	96.22	7.00	6.62 (4–8)	–	–	0.37 (0–2)	0.22 (0–1)	–	–	0.81	0.81
	1	14	1.88	7.00	5.00	–	–	1.00		–	–	1.00	1.00
	1	16	1.88	7.00	5.00	–	–	–	2.00	–	–	4.00	4.00

V = chromosomes of *H. vulgare*; *R* = chromosomes of *S. cereale*



Figs 1 - 4. 1-Regenerated plantlets of *H. vulgare* × *S. cereale* from the embryo callus. 2 - 4. Plants of barley haploid and barley × rye hybrids. 2-Barley haploid. 3-Hybrid with $2n=14$. 4-Hybrid with $2n=15$

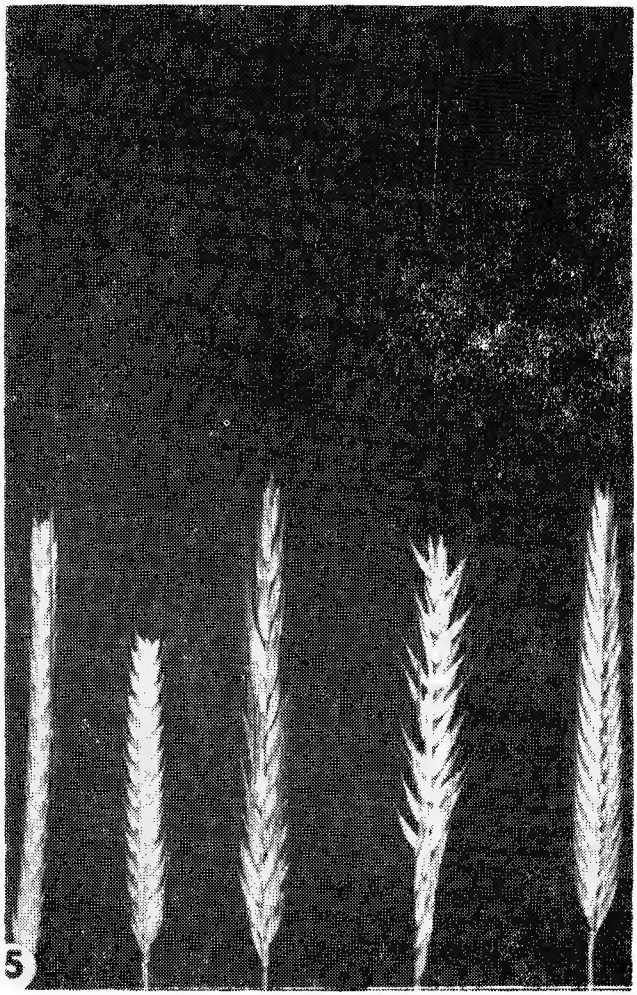
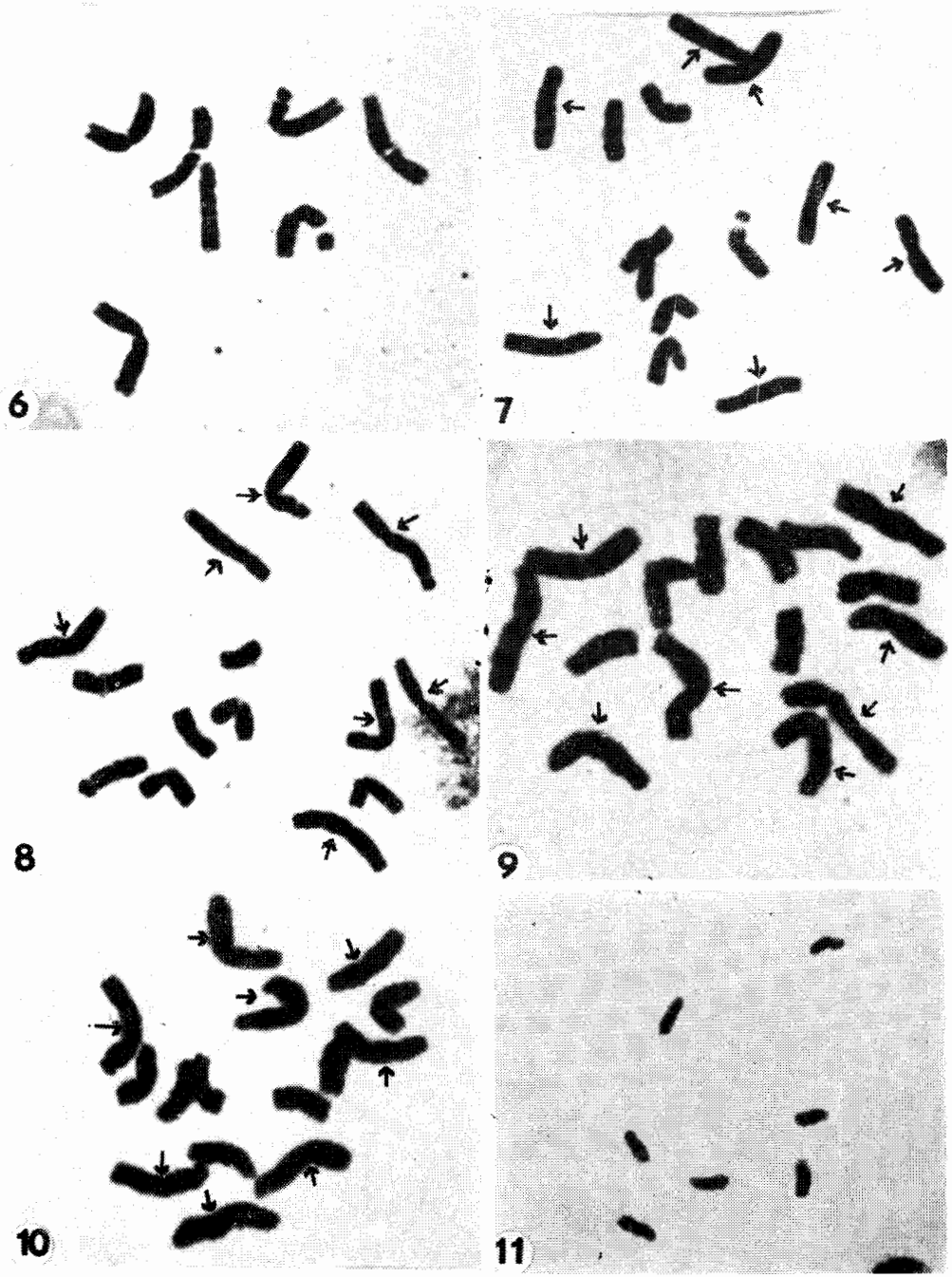
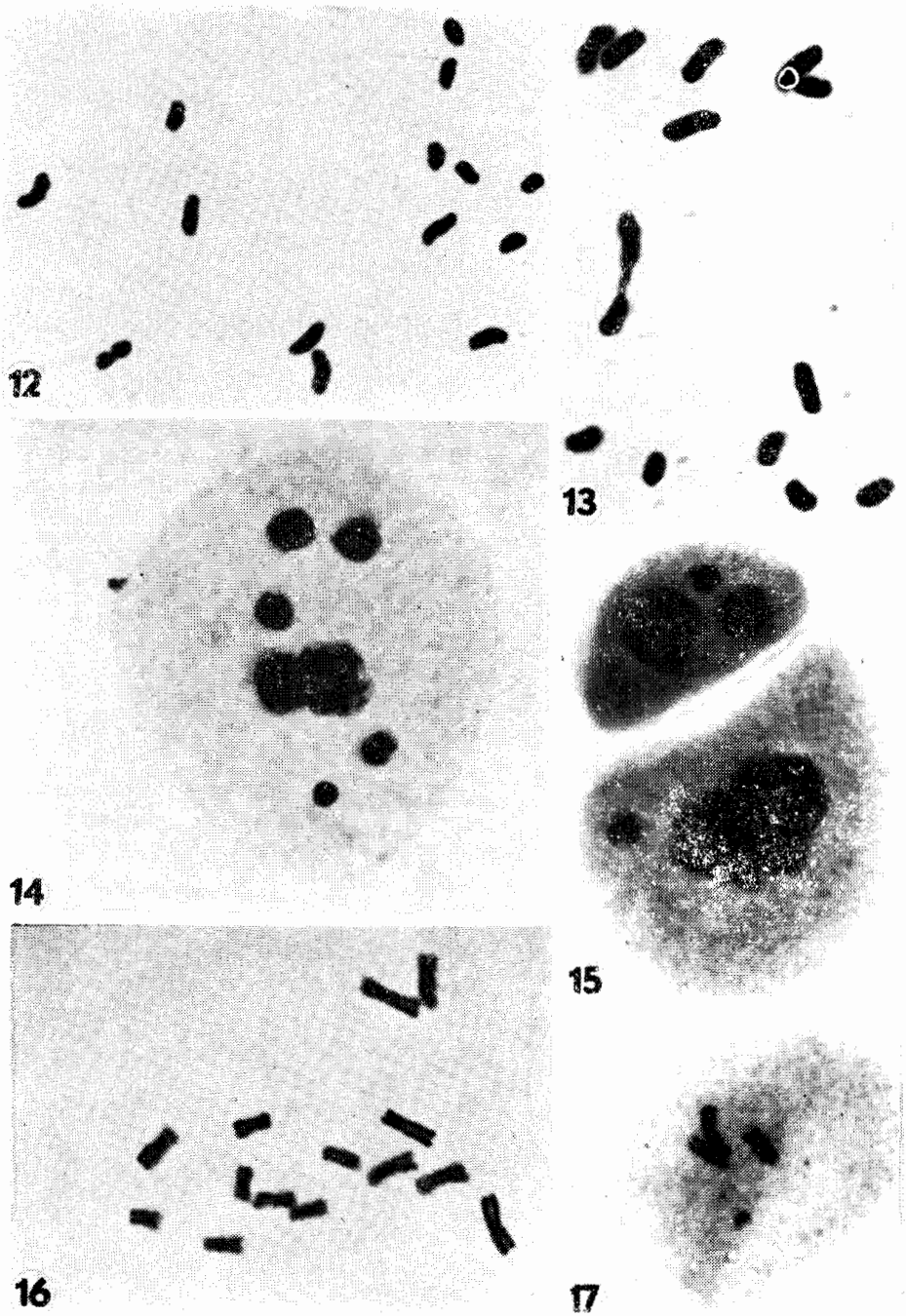


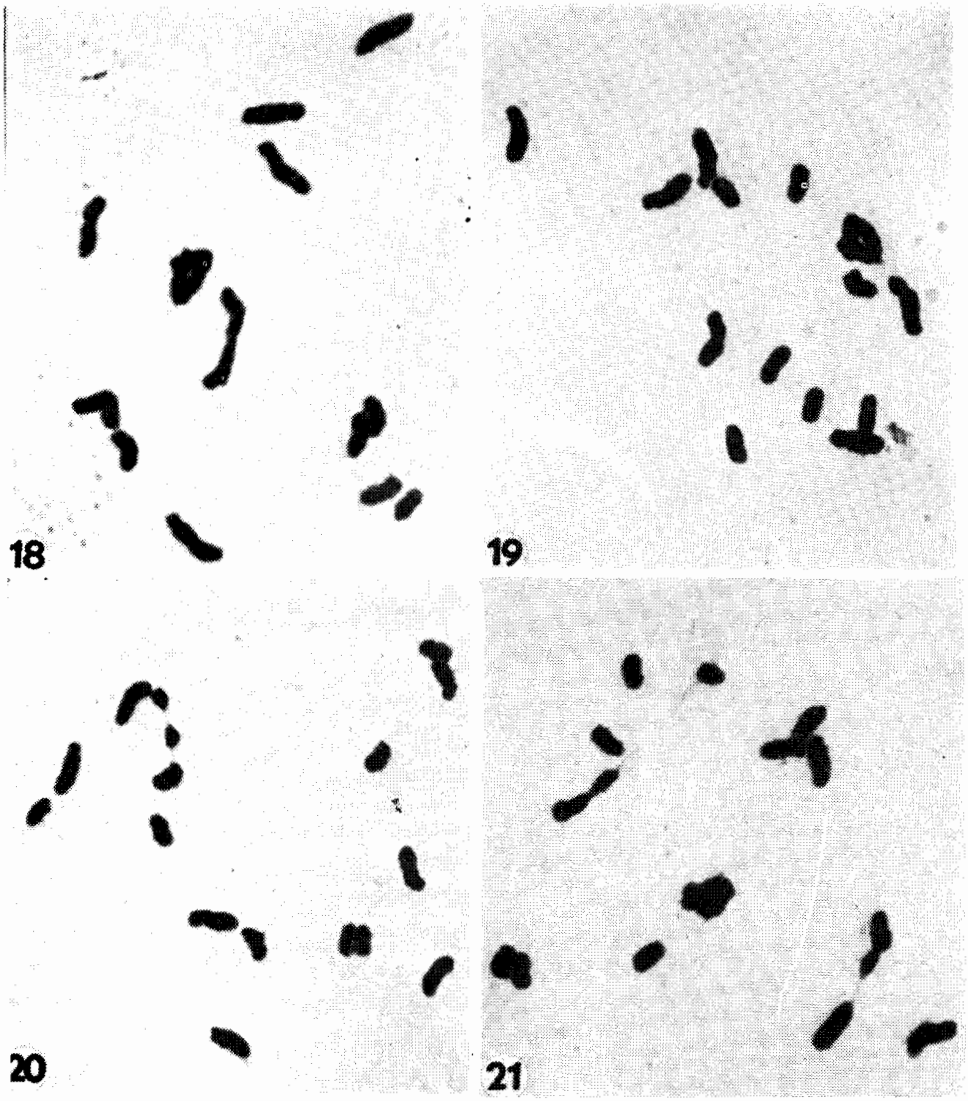
Fig. 5. Spikes. Left to right. Barley (♀), barley haploid, barley × rye hybrid with $2n=14$, barley × rye hybrid with $2n=15$, rye (♂)



Figs 6 - 11. Mitotic and meiotic metaphase chromosomes. 6 - 10. Mitotic chromosomes. 6-Haploid of barley. 7, 8 — Barley × rye hybrid with $2n=14$ (7 chromosomes of barley + 7 chromosomes of rye). 9, 10 — Barley × rye hybrids with $2n=15$ (7 barley + 8 rye chromosomes). Arrowheads indicate rye chromosomes. 11 — Metaphase I of barley haploid



Figs 12 - 17. Meiosis in barley × rye hybrid with $2n=14$. 12—MI: 14 I. 13—MI: 1 barley — rye bivalent-like structure + 12I. 14—PMC with unequal size nuclei and micronuclei without undergoing cell division after MI. 15-Diad cells. 16, 17—MII with various chromosome numbers



Figs 18 - 21. Metaphase I in barley × rye hybrdis with $2n=15$. 18-2IIR+1II. 19-1IIR+13I. 20-1IIR+13I. 21-2IIR+1 barley-rye bivalent-like structure +9I

7 - 10). In the both hybrid cytotypes the barley chromosomes tended to be grouped nearer the centre of the mitosis contrary to the rye chromosomes (Figs 7 - 10). Two satellite chromosomes of barley were clearly visible in the both hybrid cytotypes, however, at metaphase up to three rye chromosomes with faint secondary constrictions were observed.

MEIOTIC BEHAVIOUR

Haploids. At metaphase I, the chromosomes occurred mostly as univalents (Table 2). One rod bivalent was formed in 3.13 - 11.76% of the cells; two rod bivalents were found in one cell of one haploid. Chiasma frequencies per cell varied from 0.03 to 0.13. Univalents were scattered in the cell (Fig. 11). Secondary associations were infrequent and mainly of the e-e type.

Hybrids. Meiocytes of both hybrid cytotypes exhibited hypo- and hyperploid chromosome instability in 1.33 - 10.71% PMCs of the hybrid with $2n=14$ and in 1.25 - 11.32% PMCs in hybrids with $2n=15$.

In hybrids with $2n=14$ most meiocytes contained 14 chromosomes (Table 2). The chromosomes formed mostly univalents scattered in the cells (Fig. 12). Meiotic configurations at MI were very infrequent. The number of bivalents per cell ranged from 0 to 1 for *Hordeum*, *Secale* as well as for *Hordeum-Secale* chromosomes. The mean chromosome pairing was 0.02 for meiocytes with 14 chromosomes (Table 2). Chromosome pairing in meiocytes with an unstable chromosome number was comparatively higher and ranged from 0.66 to 0.75 per cell (Table 2). A heteromorphic bivalent-like structure between parental chromosomes was observed on one case (Fig. 13). Meiotic stages after MI were highly irregular. Some PMCs remained undivided (Fig. 14), however, others produced asymmetrical dyads (Fig. 15). At MII the number of chromosomes was very variable (Figs. 16 and 17).

In hybrids with $2n=15$, 2.50 - 7.20% of the PMCs (with 15 chromosomes) contained two rye bivalents mainly of one ring and one rod type (Fig. 18). Most probably one of the bivalents was formed by homologous rye chromosomes and the second one by homeologous chromosomes. 21.60 - 90.20% of the PMCs contained only ring bivalent, however, 9.70 - 44.10% of the cells had only one rod bivalent (Fig. 19 and 20). Chiasma frequencies in the PMCs with 15 chromosomes varied from 0.81 to 1.98 (Table 2) in four hybrids. Very rarely bivalent-like configurations of barley-rye chromosomes were found (Fig. 21).

DISCUSSION

Three plant cytotypes with 7, 14, 15 chromosomes in the somatic cells were obtained from barley×rye crosses through embryo callus culture. Most probably the varying chromosome numbers of produced plants were due to the varying chromosome numbers of cultured embryos.

At the somatic metaphase of the hybrid plant the spatial separation of the parental genomes was similar to that reported for other *Hordeum*×*Secale* hybrids (Finch, Bennett 1981, Finch et al. 1981, Wojciechowska 1985) and for *Hordeum* hybrids (Linde-Laursen, Jensen 1982). The barley chromosomes tended to be nearer the cell centre than the rye chromosomes.

In haploid plants of *H. vulgare* low level of bivalent formation were recorded. It was noted that 1.24 - 5.00% of the PMCs had 0.03 - 0.13 rod bivalents per cell, which gave the mean chiasma frequencies equal to 0.06. Fedak (1979) noted the chiasma frequency of 0.04 per cell in the haploids of barley produced from barley × ×rye combination.

In barley×rye hybrids ($2n=14$ and $2n=15$) the meiotic pairing was generally very low. Pairing was observed between the parental chromosomes, especially between rye ones, whereas, bivalent-like structures were noted between barley and rye chromosomes. The mean chiasma frequencies in the hybrids ranged from 0.02 to 1.98. The chiasma frequency in the hybrids with $2n=14$ was 0.01 for *Hordeum* and 0.01 for *Secale* chromosomes. It was lower than in the haploid of barley as well as in those of rye (Nordenskiöld 1939, Levan 1942). Fedak (1979) found higher chiasma frequencies of 0.22 per cell in barley×rye hybrids. Bivalents in the hybrids with $2n=15$ were formed only between rye chromosomes with a low frequency of 0.81 - 1.98 and most probably between homologous chromosomes. A higher chiasma frequency of rye bivalents (1.00 - 3.00) was found in hybrids between barley and alloplasmic rye (Wojciechowska 1985), whereas the barley bivalents were infrequent.

The present study confirms earlier findings (Fedak 1979, Thomas, Pickering 1979, Wojciechowska 1985) concerning a considerable differentiation between parental genomes. A complete lack of homology between parental chromosomes or the effect of a genetic system are probably responsible (Fedak 1979) for a very low (Fedak 1979) or no meiotic pairing between barley and rye chromosomes like in the earlier or present studies (Thomas, Pickering 1979, Wojciechowska 1985). The produced barley×rye hybrids (Kruse 1967, Fedak 1979; Thomas, Pickering 1979, Wojciechowska 1985) originated only from a few cultivars of barley and rye. Production of hybrids from a more differentiated initial material and a higher number of cultivars may lead to a discovery of more compatible parental forms.

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HAPLOIDY JĘCZMIENIA I MIESZAŃCE ZREGENEROWANE Z TKANKI KALUSOWEJ ZARODKA *HORDEUM VULGARE* L. × *SECALE CEREALE* L.

Streszczenie

Haploidy jęczmienia oraz rośliny mieszańcowe otrzymano poprzez regenerację z tkanki kalusowej powstałej z niedojrzałych zarodków *Hordeum vulgare* × *Secale cereale*.

Komórki somatyczne mieszańców zawieraly $2n=14$ (7 chromosomów jęczmienia +7 chromosomów żyta) lub $2n=15$ (7 chromosomów jęczmienia +8 chromosomów żyta). W mejocytach form mieszańcowych stwierdzono niestabilność liczby chromosomów w 3,7 - 22,7% KMP.

Otrzymane rośliny charakteryzowały się bardzo niskim poziomem koniugacji. Częstotliwość chiasm u roślin haploidalnych jęczmienia wynosiła 0,03 - 0,13 w KMP, natomiast u mieszańca z $2n=14$ wynosiła 0,02, a u roślin z $2n=15$ — 0,81 - 1,98. Koniugacja chromosomów zachodziła prawie wyłącznie między chromosomami żyta. Wszystkie badane rośliny były całkowicie niepłodne.

ГАПЛОИДЫ ЯЧМЕНЯ И ГИБРИДЫ, РЕГЕНЕРИРОВАННЫЕ ИЗ КАЛЛУСНОЙ ТКАНИ ЗАРОДЫШЕЙ *HORDEUM VULGARE* L. × *SECALE CEREALE* L.

Резюме

Моноплоиды ячменя и гибриды регенерировались из каллусной ткани недифференцированных зародышей ячмень × рожь. Гибриды имели соматический комплемент $2n=14$ (7 хромосом ячменя + 7 хромосом ржи) и $2n=15$ (7 хромосом ячменя + 8 хромосом ржи). Гибридные мейоциты показывали нестабильность хромосом в 3.7 - 22.7% МКП. Коньюгация хромосом была очень низкой. Частота хиазмат на клетку колебалась от 0.03 до 0.13 у гаплоидов. У гибридов с $2n=14$ их частота составляла 0.02, а у гибридов с $2n=15$ она колебалась от 0.81 до 1.98. Коньюгация хромосом у гибридов почти исключительно имела место между хромосомами ржи.