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The chromosome constitution of plants derived from pollen of hexaploid triticale × common wheat F_1 hybrids

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Summary. The anthers of three F_1 hybrids of hexaploid triticale and common wheat ('Rosner'×'Kedong 58', 'Beagle'×'Kedong 58' and 'Beagle'×'Jinghua No. 1') were cultured on four media in vitro. More than 900 green plants were obtained. The chromosome numbers ranged from 17 to 27 for haploid derivatives and from 38 to 52 for diploid regenerates. The chromosome constitutions of the pollen plants reflect those of the gametes found in the donor plants (genome formula: AABBDR). The value of such pollen plants for genetical analysis of rye wheat addition and substitution lines, as well as for breeding purposes, is discussed.

Key words: Pollen plants – Cytological characteristics – Anther culture – Giemsa banding – Meiosis

Introduction

Anther culture is a means of producing simultaneously a large number of haploids, homozygous diploids as well as variant types (Larkin and Scowcroft 1981). In wheat, one of the important cereal crops, 90% of the pollen plants derived via anther culture are haploid or homozygous diploid, whereas the residual 10% are heteroploids and aneuploids (Hu et al. 1979). Various lines of pollen plants, including 5x, 7x, 8x, 9x, 11x, 12x, nullisomic, monosomic, trisomic, tetrosomic and telosomic plants, have been obtained in anther culture of wheat (Hu et al. 1978, 1979, 1980; Xi et al. 1981, 1982).

In cereal breeding triticale proved to be a successful example of utilizing distant hybridization, and nowadays crosses between 6x triticale and common wheat are widely used to improve the primary 6x triticale (Müntzing 1979). The F_1 hybrid between 6x triticale and common wheat has the genome formula AABBDR, and the gametes formed in the hybrid will contain 14–28 chromosomes since the D and R chromosomes remain univalent at meiosis and are distributed at random into the gametes. The aim of the present study is to see if the multitude of chromosome constitutions of the gametes can be represented in plants by regenerating pollen via anther culture into plants.

Materials and methods

The three types of F_1 hybrids of 6x triticale (Triticosecale Wittmack) and common wheat (*Triticum aestivum*) were obtained from crosses of 'Rosner'×'Kedong 58', 'Beagle'×'Kedong 58' and 'Beagle'×'Jinghua No. 1'.

Conditions for anther culture

Media used for callus induction comprised B_5 (Gamborg et al. 1968), Potato II (Chuang et al. 1978) and B_5 supplemented with either 5% or 10% potato extract. Medium 190-2 (Chuang and Jia 1980) was used for plant regeneration. Anther culture was conducted according to the standard procedure.

Investigation of chromosomes in somatic cells

Healthy root tips were taken from pollen plantlets grown in test-tubes, treated in ice-water for 24 h, fixed in 3:1 absolute alcohol:glacial acetic acid, macerated in an enzyme solution of cellulase and pectinase, then washed and refixed. A root tip was then immersed in a drop of modified carbol fuchsin (Kao 1975) on a slide, torn apart to give a cell suspension and squashed under a coverslip. More than 50 metaphases were examined for each plant.

Investigation of chromosomes in pollen mother cells (PMC)

Suitable young spikes were excised and fixed in 3:1 absolute alcohol:glacial acetic acid, stained in propionic acid-chloral hydrate-iron alum-hematoxyline (Li et al. 1978). Over 50 PMCs were examined for each plant.

Table 1. Results of anther culture

Material		No. of anthers plated	No. of calli produced	No. of green plants regenerated	No. of green plants examined
'Beagle' × 'Jinghua No. 1'	W	5,400	937	109	81
	S	1,328	123	20	14
'Beagle' × 'Kedong 58'	W	8,737	2,263	486	
	S	3,345	847	236	124
'Rosner' × 'Kedong 58'	W	2,881	278	62	27
Total		21,691	4,508	913	588

W: Sown in autumn; S: Sown in spring

Table 2. Number of chromosomes in pollen plants derived from different hybrids between triticale and wheat

F_1		Chromosome nos.																						
		17	18	20	21	22	23	24	25	26	27	38	40	42	44	46	48	50	52	I	II	III	IV	V
'Beagle' × 'Kedong 58'	W		1	4	5	21	50	51	18	12	2	1		18	9	42	18	5	3	3*[a]	34	30	5	10
	S	1*		1	8	5	13	15	8	1	4	1	1	9	5	8	4	1	2	2*[b]	13	14	6	2
'Beagle' × 'Jinghua 1'	W			1	1	5	8	9	6	4	2	1		2	2	8	4	1		2*[c]	10	10	2	3
	S					4		3	3			1				1					2			
'Rosner' × 'Kedong 58'	W				1	1	3	3	4	1	1					4					4	5		
Total nos. of plants		1	1	6	15	36	74	81	39	18	9	4	1	29	16	63	26	7	5	7	63	59	13	15

* This plant subsequently had 34 chromosomes
I: *[a] plants with 43, 45 and > 90 chromosomes; *[b] plants with 49 and 64 chromosomes; *[c] plants with 64 and > 90 chromosomes
II: Haplontic mixoploids
III: Diplontic mixoploids
IV: Haplontic chromosome structural variants
V: Diplontic chromosome structural variants
W and S: Sown in winter and spring, respectively

Table 3. Number of chromosomes in pollen plants derived from anthers of triticale × wheat F_1 hybrids cultured on different media

Medium	Chromosomes nos.																						
	17	18	20	21	22	23	24	25	26	27	38	40	42	44	46	48	50	52	I	II	III	IV	V
Potato II			2	2	9	19	20	8	4	1			9	3	18	4	2	1		13	14	4	4
B_5		1	3	6	23	42	44	23	11	5	2		12	9	38	19	4	3	6	40	36	5	9
B_5 + 5% PE	1		1	4	3	12	13	6	3	3	1		7	3	7	3	1	1	1	9	8	2	2
B_5 + 10% PE					1	1	4	2			1	1	1	1						1	1	2	

I, II, III, IV, V: see Table 2
PE: potato extract

Giemsa C-banding technique

Coverslips were removed from the slides which were then air-dried at room temperature for 2–3 days, treated for 15–18 min in 5% $Ba(OH)_2$ at 50–55 °C, incubated in 2×SSC (0.15 M NaCl, 15 mM Na-citrate) for 60 min at 60 °C, stained in Giemsa solution for 3–5 min, and mounted with Dammar balsam. A total of 20 cells were analysed for each plant.

Results

From the 21,691 anthers cultured, 913 green plantlets were obtained, 588 of which were cytologically examined (Table 1). The highest yield (11.5 green plantlets from 100 anthers ('Beagle'×'Kedong 58') of F_1 plants was achieved on Potato-II medium (Wang and Hu 1984).

Distribution of the chromosome numbers in the pollen plants

The 588 plants examined were derived from 38 donor plants of three F_1 hybrids on four different media, as detailed in "Materials and methods".

The distributions of the chromosome numbers in the pollen plants derived from the three types of hybrids were found to be very similar (Table 2). If the chromosome constitutions of the pollen plants regenerated on the four different media are compared no significant difference is observed (Table 3). The distribution of the chromosome number of pollen plants from the different donor plants was also compared, but again no significant differences were apparent. These results suggest that the chromosomal constitutions of the pollen plants are determined primarily by the chromosome content of the gametes and that, in this case, the genotypes of the plants in the cross and the culture conditions do not influence the chromosomal variation observed.

Ploidy status of the pollen plants

The examined pollen plants can be divided into haploids, mixoploids and plants with structurally altered chromosomes (Table 4). The structural alterations observed comprise isosomic and telosomic chromosomes. Pollen plants having more than 34 chromosomes are considered to have spontaneously doubled their chromosomes and thus are classified as homozygous diploids. There were also a few pollen plants having 43, 45, 49, 64 and more than 90 chromosomes (Table 2). Table 5 shows that the number of chromosomes of pollen plants varied from 17 to 27 and gives the frequencies of the chromosome classes observed. This range of variation applies also to mixoploids and chromosome structural variants.

Chromosome constitution of the pollen plants

The chromosomes in 12 pollen plants derived from ('Rosner'×'Kedong 58') F_1 hybrids were identified with the Giemsa C-banding technique. Root tip cells of these 12 plants had 2–7 rye chromosomes and 18–21 wheat chromosomes, including 14 chromosomes of the A and B genomes as well as 4–7 chromosomes of the D genome (Table 6).

The chromosome constitutions also vary among plants having the same number of chromosomes. Thus, plants numbers 22, 37 and 38 for example, all had 23

Table 4. Classification of pollen plants from anther cultures of triticale × wheat F_1 hybrids

	Plants with n = 17–27	Mixo-ploid	Variants with chromosome aberrations	Total	%
Haploid	280	63	13	356	60.5
Diploid	158	59	15	232	39.5
Total	438	122	28	588	100
%	74.5	20.7	4.8	100	

Table 5. Comparison of the frequencies of pollen plants with 17 to 27 chromosomes with the frequencies expected if the 7 rye and the 7D wheat univalents are distributed at random in the meiosis of the triticale × wheat F_1 hybrid. Haploid and diploid pollen plants have been combined in the individual crosses

No. of chromosomes	17 (34)	18	19 (38)	20 (40)	21 (42)	22 (44)	23 (46)	24 (48)	25 (50)	26 (52)	27
No. of plants	1	1	4	7	44	52	137	107	46	23	9
Frequency observed	0.002	0.002	0.009	0.02	0.1	0.1	0.3	0.2	0.1	0.05	0.02
Frequency expected	0.020	0.06	0.1	0.2	0.2	0.2	0.1	0.06	0.02	0.006	0.0009

Table 6. The number of wheat and rye chromosomes in 12 pollen plants with chromosome numbers varying from 21 to 27. The chromosomes were identified by Giemsa banding

No. of plants	68	32	22	37	38	69	11	36	63	56	24	46
No. of chromosomes	21	22	23	23	23	24	24	25	25	25	26	27
No. of rye chromosomes	2	3	3	4	5	4	3	5	5	4	7	7
No. of wheat chromosomes	19	19	20	19	18	20	21	20	20	21	19	20

Table 7. Number of univalents and bivalents at metaphase I in pollen mother cells of 6 haploid and 1 diploid pollen plant

No. of chromosomes	21	23	24	25	26	27	46
Chromosome configuration	21I	23I	24I	25I	26I	27I	23II

chromosomes, but there were 3, 4 and 5 rye chromosomes combined with 20, 19 and 18 wheat chromosomes, respectively.

Chromosome configurations of metaphase I in PMC of the pollen plants

Metaphase I in PMCs was studies in pollen plants having 21, 23, 24, 25, 26, 27 and 46 chromosomes (Table 7). The metaphases of the 6 haploid plants contained univalents and no clearcut examples of bivalents. The chromosomes of the diploid plant had formed 23 bivalents as could be expected from a doubled haploid.

Discussion

F_1 hybrids between 6x triticale and common wheat have the genome constitution AABBDR. The gametes formed by meiosis are expected to contain 14–28 chromosomes. There will be 7 A chromosomes, 7 B chromosomes, 0–7 R chromosomes and 0–7 D chromosomes, depending on the segregation of the univalents. Through culture of anthers from F_1 hybrids, pollen plants with chromosome numbers ranging from 17 to 27 and the corresponding diploids after spontaneous chromosome doubling were obtained. Giemsa banding analysis of 12 pollen plants derived from the ('Rosner' × 'Kedong 58') F_1 hybrid showed that they comprised 11 different numerical constitutions of rye chromosomes with wheat chromosomes of the D genome. It can thus be concluded that many, if not all, combinations of rye and D wheat chromosome combinations can be obtained by regeneration of pollen grains from triticale × wheat hybrids.

Production of plants with the genotype of the gametes

The present results reveal that many of the genotypes arising in gametes of F_1 hybrids can be propagated by anther culture into callus and pollen plants. A comparison between the number of different chromosome classes obtained as pollen plants and those expected, if the 7 rye univalents and the 7 wheat univalents of the D genome are distributed at random to the daughter nuclei at metaphase I, has been carried out in Table 5. While the most commonly expected chromosome numbers of the gametes are also the most common ones among the pollen plants, there are significant deviations. The frequencies of plants with less than 21 chromosomes were lower than that expected from the calculated gamete distribution. On the other hand, the proportion of pollen plants having more than 22 chromosomes was higher than one might expect from the calculated gamete frequency. This may be accounted for by a differential capacity for regeneration of the various gametes in anther culture. Gametes having more than 22 chromosomes may have a stronger ability for regeneration than those having a low chromosome number. The absence of pollen plants having 14–16 or 28 chromosomes in the present sample may be due to their low regenerating ability and/or to a less frequent formation of the corresponding gametes.

Production of lines with wheat-rye genome combinations

Gametes with a great variety of chromosome constitutions are formed in hybrids between 6x triticale and wheat (Müntzing 1979). Since the plants are predominantly self-pollinating, the ultimate result after a number of generations would theoretically be a complete range of plants having all possible chromosome combinations. However, certain combinations are difficult to achieve because of selective fertilization and differences in the viability of the genotypes as gametes or plants. This difficulty may be partly or completely overcome by the technique of anther culture. Non-functional gamete genotypes may be regenerated into plants in culture. Plants having 14–28 chromosomes (and their corresponding diploids) can be analysed and tested by chromosome identification procedures. It will then emerge if certain combinations of rye and wheat D chromosomes are incompatible in the presence of the A and B genomes. Thus, plants obtained by anther culture are equally useful for chromosome analytical works and as breeding resources.

For practical purposes, pollen plants derived from triticale-wheat hybrids are useful for wheat breeding. Most of the pollen plants have a mixture of D- and R-chromosomes, and it is possible to select substitution, addition and translocation lines from the doubled haploids since they are homozygous

Table 8. Possible system for studying individual chromosomes in a fixed genomic background

Crosses	Genome constitutions of F_1 hybrid	Chromosomal constitutions of the gametes	Chromosomes of the genome which can be studied
AABBDD × AABBDDRR	AABBDDR	ABDR (0–7)	R
BBDDRR × AABBDDRR	ABBDDRR	A (0–7) BDR	A
AADDRR × AABBDDRR	AABDDRR	AB (0–7) DR	B
AABBRR × AABBDDRR	AABBDRR	ABD (0–7) R	D

and stable. Plant No. 68 (see Table 6) for example, possesses 19 chromosomes of wheat and two chromosomes of rye. After chromosome doubling, an alien substitution line of wheat with two rye chromosomes is produced. Various substitution lines of wheat D-chromosomes with 1–6 rye chromosomes can be directly obtained from pollen plants.

In addition to triticale-wheat hybrids, other primary distant hybrids of wheat have been created. The technique of anther culture may also play an active role in analysing and improving them.

Prospects for investigation of individual alien chromosomes in a stable genomic background

The presence and behaviour of rye chromosomes in the wheat-rye substitution lines have been studied (Merker 1975; Gustafson and Zillinsky 1978). However, multitude of population was limited then due to gamete selection during the process of fertilization. The technique of anther culture will enable the production of wheat-rye substitution lines of a much wider spectrum. Individual rye chromosomes may be studied in further detail at the haplontic level using pollen plants derived from plants having the AABBDDR genotype. Gametes formed after meiosis would contain 0–7 rye chromosomes together with 21 chromosomes of the A, B and D genomes (Table 8). Thus, the behaviour of individual rye chromosomes may be studied in the pollen plants. Individual chromosomes of other genomes in triticale can be studied with pollen plants regenerated from other hybrids (Table 8).

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