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Karyotype Analysis in Meiosis: Giemsa Banding in the Genus *Secale* L.

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Summary. Terminal bands of meiotic chromosomes stained by the Giemsa technique are permanent genetic structures of the nucleus during PMC differentiation in 8 samples of wild, primitive, and cultivated species of rye. The characteristic meiotic banding pattern is probably identical with the heterochromatic regions of mitotic chromosomes of root meristem cells (RMC) which have so far been studied. Karyotype analysis can be significantly improved by quantitative studies of the number and size of the bands combined with certain well-known chromosome characters in diplotene and diakinesis. The chromosomes involved in multivalents of some natural and synthetic species hybrids are identified for the first time. The results are discussed both in relation to the problems of chromosome evolution and their significance for marker techniques in cytogenetics.

Introduction

Mitotic karyotype analysis of root meristem cells (RMC) by C-banding (preferential staining of heterochromatin segments) has been demonstrated with great success in some species of *Anemone, Hepatica* (Marks et al. 1974) and also in *Secale cereale* L. (Sarma et al. 1973; Schweizer 1974). Recently Marks (1974) has shown that Giemsa bands similar to those seen in the mitotic chromosomes are discernable at all the principal stages of meiosis in *Anemone blanda* L. If these experiments could be confirmed in those plants whose chromosomes can be studied more easily in PMC-meiosis than in RMC-mitosis, both quantitative studies of the structural chromosome differences and conclusions on their genetical and evolutionary significance might be improved. For this purpose we chose the genus *Secale* L., a popular subject for cytogenetics in the last three decades (Lima de Faria 1952; Jain 1960; Kranz 1973a). This paper describes for the first time a complete karyotype analysis of meiotic chromosomes in the principal species of a plant genus.

Material and Methods

The material studied consisted of the following eight samples:

Species	Experiment-No. (Seed No.)	Origin (Collection)	Plants studied
S. montanum Guss.	68-1 (68/43)	Bot.Gard.Univ. Frankfurt a.M.GFR	6
S. africanum Stapf	E 72	Dept.Agric.Techn. Serv.Pretoria,S.A.	3
S. silvestre Host.	E 72	Nürnberg (1960)	7
S. vavilovii Grossh.	73-9 (73-52,58,59,64)	Kuckuck et al. (1957)	6
S. kuprijanovii Grossh.	E 72	Genet.Inst.Univ. Lund Sweden	3
S. cereale L."Iran"	67-6,68-1,70-1 (67/54-57,68/11,70/6)	Kuckuck et al. (1957)	14
S. cereale L."Korea"	72-2 (70/8,9)	Lee (1968)	14
S. cereale L.'Petkuser Normal'	67-6 (67/66,73)	F.v.Lochow-Petkus, Bergen, GFR	5

Spikes were collected just before they had grown out of the flag leaf. Preparations were made by fixation in 3:1 v/v methanol: acetic acid for 24 hours, transfer to 90% ethanol for 8 hours and storage in 80% ethanol at 4°C. The stages of meiosis in each spikelet were checked in one of the 3 anthers of a flower by squash preparations in a drop of 45% acetic acid. Preparations for a detailed karyotype and chromosome analysis were made following the Giemsa banding procedures of Schweizer (1974) and Marks (1974). Adhesive for the PMC's was necessary but after staining the preparations were immediately mounted in distilled water, bordering the coverslips with lacquer (Glemadur). For quantitative studies, suitable PMC's were monitored under the light-microscope and photographed. The photos were used for the estimation of each character as follows:
Chromosome length (3 = long, 2 = medium, 1 = short); band number (0, 1 and 2), size of the first and second terminal band (0 = none, 1 = small, 2 = medium, 3 = large); mean and standard deviation were based on more than 20 chromosome estimations per species sample. Comparisons between the chromosome types were statistically tested by the G-test of independence (Sokal et al 1969).

Results

The present study demonstrates the potentialities of the Giemsa-staining technique for recognizing structural differences of chromosome pairs in PMC-meiosis, compared with the results of earlier karyotype observations on RMC-mitosis. The C-bands observed from pachytene up to anaphase II always appear in the

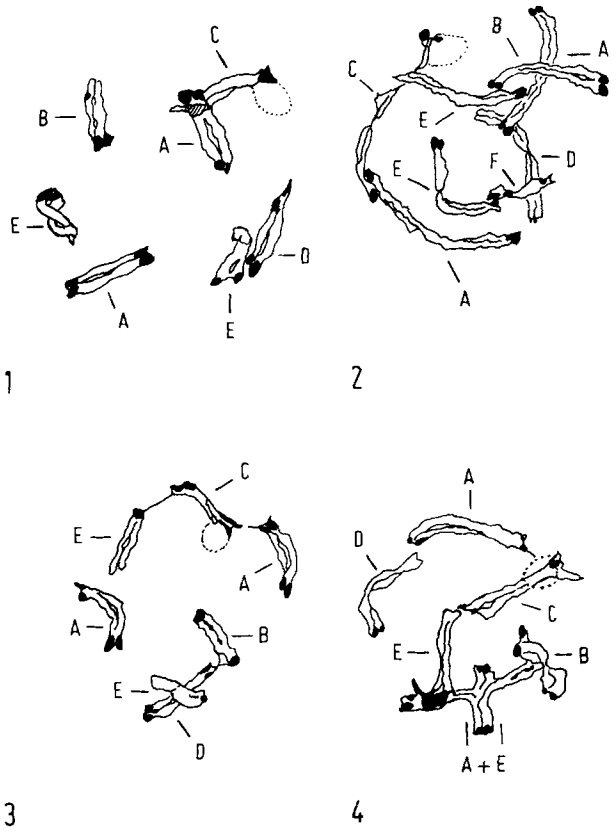

Fig. 1. Six groups of chromosome types (A to F) in diplotene-diakinesis of PMC's of 4 species samples in rye showing the C-band structure studied.
[1] *S. cereale* 'PN', [2] *S. cereale* "Korea" (access. sf = F),
[3] *S. cereale* "Iran", [4] *S. kuprijanovii* (A + E = quadrivalent)

10 µm

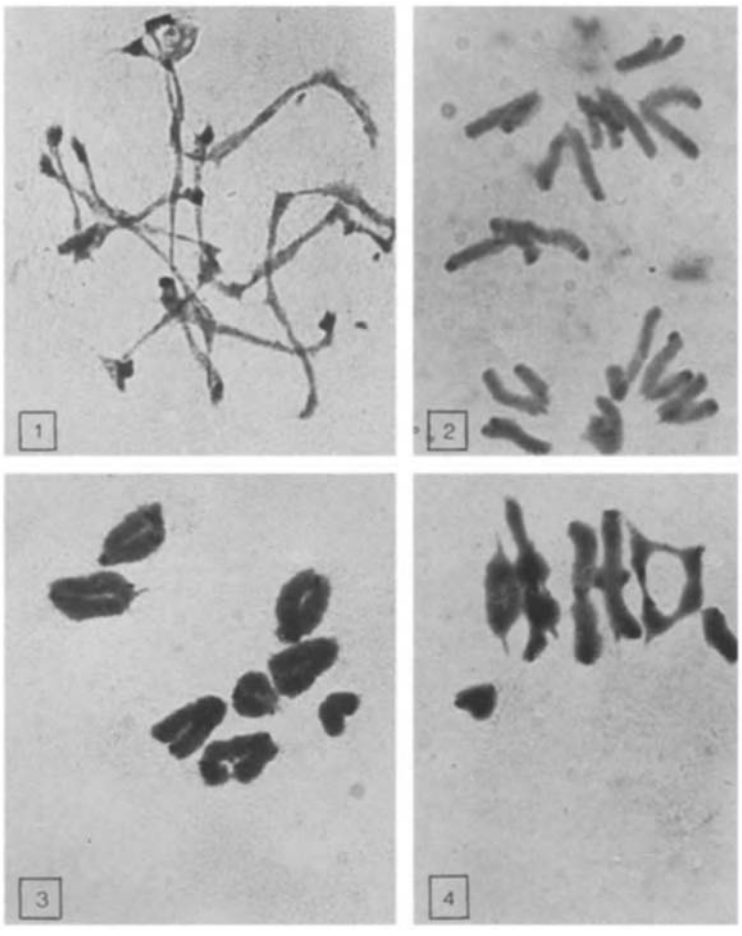

Fig. 2. Permanence of terminal/subterminal C-bands in different meiotic stages of rye PMC's.
[1] late pachytene (*S. cereale* 'PN')
[2] second anaphase
[3] first metaphase } (*S. vavilovii*)
[4] first anaphase

10 µm

heterochromatic, terminal/subterminal regions. Banding patterns of equal size have not been revealed so far in the euchromatic, interstitial regions of our material. This corresponds to the findings of other authors in RMC's; our results also agree with the classification of 5 types of C-banding in RMC-mitosis of *S. cereale* 'Dakold' by Sarma et al. (1973).

With respect to the terminal/subterminal band number the chromosomes in PMC's can be classified into 3 groups: A(I), A(III) and C(VII) with 2 terminal bands; B(IV) and D(II) with 1.5 to 2 and 1 to 1.5 resp.; E(V) and E(VI) with 1 band. Table 1 and Fig. 1 show that in *S. cereale* 'PN' these 5 types can also be distinguished. In detail, comparisons between the 7 autosomes and 1 accessory chromosome (sf = F VIII) of the rye chromosome complement give results as follows: Using the G-test for statistical independence of each of the 4 chromosome characters studied our results (table 2) show that the 6 autosomes A(I), A(III), B(IV), D(II), E(V), and E(VI) can be identified in at least 2 chromosome characters at the 5 p.c. probability level (α); i.e. 1) the chromosome A(I) is frequently longer than A(III), B(IV) and E(V); 2) its band number is higher than in B(IV), D(II) and E(V); 3) its second band is larger than in those chromosomes; but, 4) its first band is larger than in D(II) only. The chromosome B(IV) is frequently longer than D(II) and E(V); its band number is higher and its second band is larger than in E(V). D(II) and E(V) are longer and their first bands are larger than those of E(V) and E(VI). The nuclear chromosome C(VII) and the accessory F(VIII) can be identified without any difficulty due to their typical structure (nucleolus, shortness).

Another aspect of our analysis is the permanency of the C-bands during all the stages of PMC-meiosis. Figure 2 demonstrates an example of the characteristic terminal bands in diplotene of *S. cereale* 'PN' and in metaphase II of *S. vavilovii*. Evidently terminal C-bands are also structures in interphase nuclei. Additionally we have studied and compared the band characters of *S. vavilovii*, *S. kuprijanovii* and *S. cereale* "Iran" and diplotene-diakinesis and anaphase I-II by use of the G-test. No significant independence between each of the 7 chromosomes in the meiotic stages was observed.

The aim of this study was to test the identity or the differences of the chromosomal types of *S. cereale* in the principal species of the genus. The results obtained are summarized in Table 1. A(I) and A(III) show complete identity in all species samples studied. B(IV) is identical only in 6 of the 8 samples; independence for band number and size is also significant between *S. cereale* 'PN' and "Korea" and *S. vavilovii*. In C(VII) no identity between *S. cereale* 'PN' and *S. silvestre* or *S. montanum* can be shown in all the characters. For D(II), identity exists between *S. cereale* 'PN' and "Iran" but in the other 6 samples this chromosome is significantly different from 'PN' mostly in band number and size of the second band. E(V) is identical in the species except in *S. cereale* 'PN' and *S. vavilovii* or *S. montanum* (in chromosome length, band size first) and between 'PN' and *S. africanum* or *S. montanum* (in band number and size). All in all we may conclude that there are structural differences between *S. cereale* 'PN' and the other species samples, mainly in the chromosomes B(IV), D(II), E(V), and C(VII). This result is most important for improving localization of the known structural differences in the chromosomes of different rye species (Kranz 1973).

Discussion

Meiotic karyotype analysis in PMC's is favourable for the following reasons:

1) Adequate numbers of cells (chromosome complements) at synchronized division stages can be studied and full use of quantitative, biometric tests can be made. Classification of 5 chromosome groups (A to E) in the *S. cereale* complement has been confirmed in another cultivar ('PN') and the primitive rye ("Iran"). The number and size of terminal/subterminal C-bands of chromosome pairs in diplotene-diakinesis are significant characters for the identification of the intra-group types of chromosomes. Using 3 C-band characters as well as the conventional chromosome length and the nucleolus, intra- and interspecific variability of chromosome structure has been estimated quantitatively in PMC's of a genus for the first time. Their chromosome variability had not been identified sufficiently in earlier studies of RMC's (Jain 1960, Bhattacharyya et al. 1960) or PMC's (Kranz 1971).

2) Known problems of chromosome identification in natural and synthetic hybrids can now be solved.

Table 1. The classification of 6 groups (A to F) of chromosomes (I to 4 tructural chromosome characters (schromosome length, band

Species	Character	Chromosome no.		
		I	II	III
	Chromos.length	3.00	2.67± .58	3.00
	number	2.00	1.00	2.00
S.montan.	band-	1.20± .45	1.33± .58	1.20± .45
	size	1.00		1.00
	type	A	D?	A
	Chromos.length	3.00	3.00	1.80± .45
	number	2.00	1.78± .44	2.00
S.africon.	band-	3.00	1.89± .33	3.00
	size	2.20± .45	1.78± .44	2.80± .45
	type	A	D	A
	Chromos.length	3.00	2.67± .49	2.00
	number	2.00	.75± .45	2.00
S.kuprij.	band-	2.46± .52	1.75±1.29	3.00
	size	1.54± .78		2.25± .62
	type	A	D?	A
	Chromos.length	3.00	3.00	3.00
	number	2.00	1.00	2.00
S.silvestre	band-	2.00	1.78± .67	1.00
	size	1.50± .53		1.00
	type	A	D?	A
	Chromos.length	3.00	3.00	2.00
	number	2.00	.90± .32	2.00
S.vavil.	band-	2.33± .72	1.80±1.14	2.50± .76
	size	1.67± .62		1.64± .74
	type	A	D?	A
	Chromos.length	3.00	3.00	2.00
S.cereale	number	2.00	1.00	2.00
"Korea"	band-	2.50± .53	2.50± .58	3.00
	size	2.13± .83		2.00± .71
	type	A	D?	A
	Chromos.length	3.00	3.00	2.00
S.cereale	number	2.00	2.00	2.00
"Iran"	band-	3.00	1.90± .30	3.00
	size	2.32± .68	1.71± .46	2.53± .64
	type	A	D	A
	Chromos.length	3.00	3.00	2.00
S.cereale	number	2.00	1.50± .55	2.00
'PN'	band-	3.00	1.67± .52	3.00
	size	1.90± .57	1.50± .55	1.88± .99
	type	A	D	A

The chromosomes included in multivalents can be identified. The structural differences yielding one quadrivalent in some plants of the Swedish Kuprijanovii material (Hrishi et al. 1960) show from band characters that one chromosome of each group A and E is involved in the pairing configuration (Fig.1).

This result agrees partially with the conclusion of Nürnberg (1967), that the origin of the structural heterozygosity in this species sample is probably spontaneous, i.e. only as far as the chromosome of group A is concerned; and, in the E-chromosome, interspecific hybridization with *S. montanum* may be

VIII) in the genus *Secale L.* Mean and standard deviation (x ± s) of number and size) in 8 species samples of rye

IV	V	VI	VII	VIII
2.00	2.00	1.67± .58	2.00	
2.00	2.00	1.00	1.33± .58	
1.67± .52	1.67± .52	1.67± .58	1.33± .58	
1.33± .52	1.33± .52		.33± .58	
B	?	E	?	
2.00	1.75± .50	2.00	2.57± .53	
2.00	2.00	1.29± .49	1.57± .53	
2.00	1.00	2.14± .69	2.57± .53	
2.00	1.00	.57± .98	1.43±1.13	
B	?	E	C	
2.00	1.81± .40	1.81± .40	2,85± .38	
2.00	1.00	1.00	2.00	
1.73± .49	1.94± .25	1.94± .25	2.62± .65	
1.43± .53			2.15± .69	
B	E	E	C?	
2.00	1.82± .40	1.50± .53	2.00	
2.00	1.09± .30	1.25± .46	1.00	
1.90± .57	2.00± .77	2.13± .64	1.88± .64	
1.40± .52		.25± .46		
B	E	E	?	
2.00	1.00	2.00	2.80± .42	
.45± .52	.71± .49	1.11± .33	1.90± .32	
.73±1.01	1.29±1.11	2.89± .33	2.40± .70	
		.22± .67	1.40± .70	
?	E?	E	C	
2.17± .75	1.83± .41	1.80± .45	2.67± .52	1.00
2.00	.50± .55	1.00	1.83± .41	1.00± .63
2.83± .41	1.50±1.64	1.60± .55	3.00	1.50±1.05
2.17± .41			2.00±1.26	.33± .83
B?	E	E	C	F
1.97± .17	2.14± .35	1.91± .30	2.11± .32	1.00
2.00	.86± .35	1.27± .47	1.92± .28	.78± .44
1.81± .40	2.50± .74	2.36± .67	2.58± .60	1.33±1.00
1.53± .51			1.75± .81	
B	E	E	C	F
2.00	2.00	1.11± .33	3.00	
1.50± .53	1.00	.89± .60	2.00	
1.75± .46	3.00	1.89±1.27	2.86± .38	
1.50± .53		.33±1.00	2.86± .39	
B	E	E	C	

another reason for the structural differences in this quadrivalent, as we can conclude from our results in Table 1 and 3. Comparison of the intra-complement variability in the band characters (Table 1) studied shows that chromosomes of different band characteristics are probably included in the known multivalents of species hybrids in rye. Figure 3 shows that in the case of quadrivalents (IV) at least 2 chromosomes are different and in hexavalents (VI) probably 3. Exceptions like the hybrids *S. montanum* × *silvestre*, *S. cereale* × *africanum* and *S. cereale* × *silvestre* may be interpreted as follows: 1) there are

Table 2. G-test of independence in the chromosome structure studied between the inhomologous autosomes of *S. cereale* 'PN' (below) and between the 7 homologous chromosomes of 8 species samples of rye (above). n.s.: not significant, +++: $\alpha \leqslant 0.001$, ++: $\alpha \leqslant 0.01$, +: $\alpha \leqslant 0.05$

Chromos. type	Species	Chromos. length	Band number	Band size first	Band size second
D (II)	*S.c.* PN/ *S.c.* Korea	n.s.	+++	n.s.	+++
	" / *S.c.* Iran	n.s.	+++	n.s.	n.s.
	" / *S.vav.*	n.s.	+	n.s.	+++
	" / *S.silv.*	n.s.	+	n.s.	+++
	" / *S.kuprij.*	+	+	n.s.	+++
	" / *S.mont.*	n.s.	n.s.	n.s.	+++
C (VII)	*S.c.* PN/ *S.silv.*	+++	+++	+	+++
	" / *S.mont.*	+++	+++	+	+++
E (V)	*S.c.* PN/ *S.vav.*	+++	n.s.	+++	-
	" / *S.afric.*	n.s.	+++	+++	+++
	" / *S.mont.*	n.s.	+++	+++	+++
B (IV)	*S.c.* PN/ *S.c.* Korea	n.s.	+	+++	+++
	" / *S.vav.*	n.s.	+++	+++	n.s.
A (I)/ A(III)	*S. cereale* 'PN'	+++	n.s.	n.s.	+
E (V)/ E(VI)		+++	n.s.	++	n.s.
A (I)/ B(IV)		+++	+++	n.s.	+++
A (I)/ D(II)		n.s.	+	+++	+++
A (I)/ E(V)		+++	+++	n.s.	+++
B (IV)/ D(II)		+++	n.s.	n.s.	+++
B (IV)/ E(V)		n.s.	++	n.s.	+++
D (II)/ E(V)		+++	+	+	n.s.

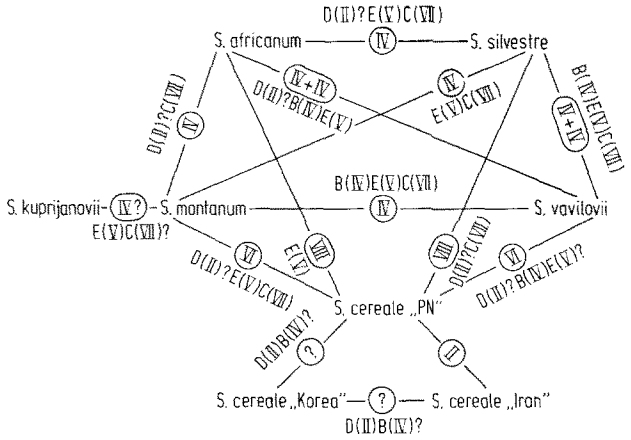

Fig. 3. Chromosomes probably involved in multivalent formation (○) of known species hybrids in *Secale* L.

structural differences not including the terminal bands, i.e. interstitial translocations; and 2) individual differences of the samples for both the translocations (Nürnberg 1967) and the C-bands (Marks et al. 1974).

3) With respect to the cytogenetic function of the C-bands, it has now been shown in a second plant genus that the bands are identical with the heterochromatic regions of mitotic chromosomes. Although we cannot yet equate the variation in the distribution of the chromosomal bands with certain function (Lewin 1974), the permanency of these regions in Giemsa-stained interphase nuclei gives an additional argument for their significant function during the process of cell division. Nevertheless these terminal heterochromatic regions may be structural and functional markers of linkage groups. Functional consequences following elimination and translocation of C-bands in nullisomes and trisomes will be an important aspect of further studies. Consequently the use of the C-band as markers of certain valuable gene blocks in transfer experiments will probably improve the chromosome technique of plantbreeders (Kranz 1973b).

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