A Cytological Study on the Intergeneric F_1 Hybrid between *Triticum sphaerococcum* and *Secale cereale*

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Introduction

In the hybridization between wheat of dinkel group ($n=21$) and *Secale cereale*, cytological and genetical studies on F_1 hybrid raised between *T. vulgare* and *S. cereale* have been carried out by many investigators, and the report concerning them came up to large numbers. Further, some cytological and genetical researches of F_1 plants raised between *T. Spelta* or *T. compactum* with *S. cereale* were reported by some authors (Aase 1930, Florel 1931, Kagawa 1933, Kagawa and Chizaki 1934). But, cytogenetical study on F_1 plant raised between *T. sphaerococcum* and *S. cereale* seems to be not yet carried out, and one of a series of cytogenetical studies on intergeneric hybrids between *Triticum* and *Sereale*, the present author tried to make out of a hybrid between *T. sphaerococcum* and *S. cereale* in 1951, and obtained 5 individuals of F_1 plant. Some cytogenetical studies on the F_1 plants were made and the results obtained will be dealt with in this paper.

Material and method

The chromosome numbers of *T. sphaerococcum* as mother plant, and *S. cereale* as pollen parent, were $n=21$, 7 and $2n=42$, 14 respectively. In 1951, 160 flowers on 10 spikes of *T. sphaerococcum* were pollinated with the pollen of *S. cereale*, resulting in 46 grains, the number of grains corresponding to 28.75% of the pollinated flowers. These 46 grains were sown in October of the same year in separate pots, and from them 5 F_1 plants were obtained. The proportion of individuals raised to the number of grains of the seed sown and that of pollinated flowers was 10.87 and 3.13 percent respectively.

The percentage of F_1 plants for the pollinated flowers in this case was higher than that of *T. Spelta* $\times$ *S. cereale* (0.48%) but was lower than that of *T. vulgare* $\times$ *S. cereale* (25.18%) which were reported by the present author in a previous paper (1952). The hybrid raised by the combination of *T. sphaerococcum* and *S. cereale* will be represented by the symbol TsphRF $_1$ -.

The anther was fixed in Carnoy's fluid and preserved in 70% alcohol for the study of maturation division in PMCs, and later PMCs were observed mainly in Belling's aceto-carmin smear method. The root tip

cells of F_1 plant, fixed with Nawashin's solution, were used for the observation of somatic chromosomes and the permanent preparations were made by paraffin method.

Photo. 1. Spikes of F_1 plant ($TsphRF_1$ -) and its parents, from left to right, *Triticum sphaerococcum*, F_1 and *Secale cereale*. $\times$ ca. $3/5 \times 2/3$.

All figures were drawn by the aid of Abbe's large camera using Zeiss apochromatic objective 1.5 mm, Leitz 1/12a fluorite objective and Zeiss $\times 15$ compensation ocular. Original magnification is $\times 3000$ for the somatic chromosomes and $\times 2300$ for the reduced chromosomes.

Results of observation

A. External characters of F_1 plants:—In the 5 F_1 individuals, obtained in 1951 by the hybridization of above combination, some differences in length of stem and spike were observed, but no marked differences in other characters among them. Comparison of the external characters of these 5 F_1 plants and that of the parental plants are as shown in the following Table 1 and Photo. 1.

As is evident from Table 1, the F_1 hybrid was intermediate between both parental plants in number of spikelet per spike, spike density and number of flowers per spikelet, but in the length of stem and spike it rather somewhat closely resembled to the mother plant. On the contrary, in length of

Table 1. Comparison of external characters of $TsphRF_1$ - and its parental plants

Plants	Chromosomes	Length of stems cm	Length of spikes cm	Length of awns cm	Number of spikelet per spike	Spike density (spikelet number: spike length)	Number of measurement (Tillering)	Number of flowers per spikelet
<i>T. sphaerococcum</i>		76.96	5.27	2.02	15.20	2.88	25	4
<i>S. cereale</i>		160.35	13.54	3.20	42.45	3.14	20	2
$TsphRF_1$ - No. 1		75.10	8.33	2.98	24.40	2.92	53	3
" 2		84.80	9.54	3.06	26.30	2.76	57	4
" 3		100.60	10.69	3.38	28.40	2.66	114	4
" 4		103.50	10.42	3.45	28.40	2.73	68	4
" 5		93.60	9.11	3.06	25.60	2.81	49	3

awns it was more approaching to the pollen parent. The colour of spike was intermediate between the parents. In general, the external characters of F_1 plants resembled to the parents, although more closely to *T. sphaerococcum* as the mother plant than the intermediate of both parents (Photo. 1). The hair on the neck of spike of F_1 plant was rich on 3 individuals (No. 2, 3 and 4), while in the remaining 2 (No. 1 and 5) it was not so rich.

B. The number of chromosomes and maturation division in PMCs of F_1 plants:—The number of somatic chromosomes in root tip cells of these 5 F_1 plants was counted to be 28 (Fig. 1) in every individuals and corresponds to the sum of the gametic chromosome numbers of the parents. Further,

Fig. 1-9. 1. Somatic chromosomes in root tip cell of F_1 (TsphRF $_1$ -). 28 chromosomes. $\times 1500$. 2-9. Heterotypic division in PMCs of F_1 hybrid *T. sphaerococcum* $\times$ *S. cereale* (TsphRF $_1$ -). $\times 920$. 2. Metaphase, 28 univalents scattered in spindle. 3. Side view of heterotypic metaphase. 1 $_{III}$ +26 $_I$. 4. do. 2 $_{II}$ +24 $_I$. 5. do. 2 $_{II}$ +24 $_I$. 6. do. 1 $_{III}$ +1 $_{II}$ +23 $_I$. 7. do. 3 $_{II}$ +22 $_I$. 8. do. 4 $_{II}$ +20 $_I$. 9. Unbalanced distribution in ana-telophase. 1 $_{II}$ +26 $_I$:0.

in meiosis of PMCs 28 chromosomes were observed as the somatic number (Figs. 2-8). At first metaphase in maturation division in PMCs of these F_1 plants, 0-4 bivalents and consequently 28-20 univalents were observed (Figs. 2-8). The frequency of bivalents in one pollen mother cell was shown in Table 2.

Table 2. Frequency of bivalents at first metaphase in maturation division of PMC's of F_1 plants

Bivalents and univalents		0II+28I	1II+26I	2II+24I	3II+22I	4II+20I	Mode (%)	Total
Individuals								
TsphRF ₁ -	No. 1	392	171	27			0II (66.44)	590
"	2	398	161	87	26	2	0II (59.05)	674
"	3	512	186	96	11	4	0II (63.29)	809
"	4	455	187	90	10		0II (61.32)	742
"	5	476	187	111	21		0II (59.87)	795
Total		2233	892	411	68	6	0II	3610
%		61.86	24.71	11.39	1.88	0.17	61.86	100.01

Trivalents were counted as bivalents

As will be seen from Table 2 as a whole, the number of bivalents in PMC's was 0~4 as mentioned above, but it shows differences according to the individuals, that is, 0~2 in TsphRF₁-1, 0~3 in TsphRF₁-4 and 5, and 0~4 in TsphRF₁-2 and 3. The case of zero in the number of bivalents appeared to be the mode in every F_1 plants as will be understood from Table 2.

The bivalents consist of two chromosomes of equal size and in most case, stick-shaped bivalents (bipartite) of which two chromosomes conjugated end to end loosely were observed, and as a rare case, ring-shaped one (geminus) of which two chromosomes closely conjugated was also observed (Fig. 5). Further, the formation of interstitial chiasma was observed on stick-shaped bivalent in rare cases (Fig. 5). Like case was already reported by the present author in a previous paper (1952). Trivalents were observed besides bivalents at the first metaphase, but no tetravalent was found.

The bivalents up to 4 found in one PMC of TsphRF₁-plants may have been resulted from syndesis between A and B, or among A, B and D genomes of *T. sphaerococcum* as the mother plant, which accord with the results of cytological researches on the F_1 hybrids raised between dinkel wheat and *Secale* by Kihara (1924), Meister and Meister (1924), Thompson (1926), Aase (1930), Kagawa (1933), Kagawa and Chizaki (1934), Kattermann (1934), Nakajima (1952) and many other investigators.

The distribution of chromosomes to the opposite poles at the anaphase in heterotypic division proceeded at random, but in most cases, the number of chromosomes distributed at the poles was usually not so different for both poles, viz. 14:14~27:1 were generally observed (Figs. 10 and 11). But the unbalanced distribution of chromosomes was rarely observed (Fig. 9).

In the stage after homotypic division, the nuclear division was proceeding more or less irregularly, consequently, lagging chromosomes and chromosome bridge were frequently observed in this stage.

In normal cases, univalents are scattered in the spindle at metaphase of heterotypic division, but in this case, it was rarely observed that all the univalents show the longitudinal splitting and consequently the formation of the restitution nucleus (Fig. 13). The tetrads consisted of 4 cells of almost equal size were observed in most cases, but also those consisting of 1~7 cells were rarely observed (Fig. 15 and Table 3).

Figs. 10-15. 10-12. Homatypic division in PMCs of F_1 hybrid *T. sphaerococcum* $\times$ *S. cereale* (TsphRF $_1$ -). $\times 920$. 10. Polar view of metaphase, 16 chromosomes. 11. do. 27 chromosomes. 12. Side view of ana-telophase, 2 lagging chromosomes. 13. Side view of heterotypic meta-anaphase, 28 univalents splitting longitudinally and growing to restitution nucleus. 14. Diad in tetrad stage with 2 nucleoli. 15. Hexad in tetrad stage, 3 of them having a nucleolus.

The micronuclei resulted from irregular division were observed frequently in the cells consisted of tetrads. Remarkable differences in the number of

Table 3. Frequency of cells consist of tetrad in tetrad stage

Number of cells		1	2	3	4	5	6	7	Mode	Total
Individuals										
TsphRF $_1$ -	No. 1		3	3	197	72	39		4	314
	2		87	17	212	56	19		4	391
"	3	4	217	22	131	8	2		2	384
"	4	3	177	44	87	47	32		2	390
	5		192	18	94	107	88	19	2	518
Total		7	676	104	721	290	180	19	4	1997
%		0.35	33.94	5.22	36.19	14.56	9.04	0.95	36.19	100.25

m micronuclei were observed according to individuals, viz. 0~12 micronuclei.

In the case of the pollen grains resulted from irregular meiosis, they become imperfect or abortive, the size and contents of chromosomes being unequal.

As the anther of these F_1 plants was not opened, the pollen grains showed almost sterile, viz. 4 individuals of 5 plants of F_1 were shown perfect sterile in pollen grains, but on rare occasions some anthers were opened and consequently a few grains of seed (5 grains) were obtained on 1500 spikelets of 75 spikes in natural selfing ($TsphRF_1-2$); and from these 5 grains one F_2 was germinated in 1952.

Summary

1. In the present investigation, cytological studies on the F_1 plants, raised from *Triticum sphaerococcum* and *Secale cereale*, was carried out.

2. 160 flowers on 10 spikes of *T. sphaerococcum* ($n=21$) were pollinated with the pollen of *S. cereale* ($n=7$), and 46 grains were obtained. From these grains 5 F_1 plants were raised. The percentage of the raised individuals to the number of all the pollinated flowers was 3.13 per cent.

3. The external characters of F_1 plants seem to have resemblance to the parents, although much more closely to the mother plant (*T. sphaerococcum*) than the intermediate of the parents (Photo. 1 and Table 1).

4. The number of somatic chromosomes of F_1 plants counted to be 28 (Fig. 1) and it corresponds to the sum of the reduced chromosome numbers of the parents.

5. At first maturation division in PMCs of the F_1 plants, 0~4 bivalents and 28~20 univalents were observed (Figs. 2~8). The frequency of bivalents in PMCs was shown in Table 2, and the case of zero in the number of bivalents appeared to be the mode. These bivalents seem to be derived from synthesis between A and B or among A, B and D genomes of *T. sphaerococcum* which is the mother plant. As there seems to be no homology between A, B, D genomes of *T. sphaerococcum* and R genome of *S. cereale*, synthesis will not occur between their chromosomes.

6. At ana-telophase in heterotypic division of PMCs, the chromosomes were distributed to the opposite poles from 14:14 to 27:1.

7. The anther of these F_1 plants was not opened and pollen grains showed almost sterile, but on rare occasions, some anthers were opened and consequently a few seed (5 grains) were obtained in natural selfing. From these 5 grains one F_2 plant was raised.

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