On the Allocation to the Homoeologous Group 3 of the Gametocidal *Aegilops triuncialis* Chromosome in Common Wheat

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Introduction

Substitution of alien cytoplasms for the cytoplasm of wheat has been intensively investigated by several workers (Maan 1973, Panayotov and Gotsov 1975, Tsunewaki *et al.* 1976). The cytoplasm substitution was achieved by backcrossing F_1 hybrids between alien species as females and wheat as males to the recurrent pollen parents of wheat. In the process of backcrossing, especially in the earlier backcross generations, many wheat derivatives possessing one or more alien chromosomes were obtained. Recently, some such alien chromosomes were found to provoke atypical breeding behavior in wheat: When alien chromosomes were added to wheat in monosomic condition, they caused great reduction of seed fertility, and viable offspring produced by the backcross were always of the parental type. Endo and Tsunewaki (1975) isolated two such chromosomes separately from *Aegilops triuncialis* and synthetic *triuncialis* into common wheat. These chromosomes were distinguished from those of common wheat by their subterminal centromeres. Maan (1975) also reported two other *Aegilops* chromosomes showing similar breeding behavior in common wheat, one derived from *Ae. longissima* and the other from *Ae. sharonensis.* These workers independently studied the mechanism of the preferential transmission of those *Aegilops* chromosomes accompanied by the low seed fertility, and reached the same conclusion: When the critical *Aegilops* chromosome is present in common wheat in monosomic condition, only the gametes with the alien chromosome are functional, while gametes lacking it can not function, resulting in the preferential transmission of this chromosome.

The *triuncialis* chromosome was found to have been successfully substituted for a wheat chromosome as well as to have been added to the chromosomal complement of common wheat in disomic condition. Using these disomic *triuncialis* chromosome substitution and addition lines of common wheat, the present investigation was conducted to determine which common wheat chromosome was replaced by the *Ae triuncialis* chromosome, and to confirm the homoeologous group of this chromosome. The results are reported here.

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Materials and Methods

A chromosome of *Aegilops triuncialis* (2n=28, $CCC^{u}C^{u}$), which causes selective gametophyte sterility in common wheat (2n=42, AABBDD) was isolated into common wheat together with the *triuncialis* cytoplasm in either monosomic addition or substitution condition after several backcrosses of F_1 hybrid, *Ae. triuncialis* (♀) x common wheat (♂), to common wheat. As the *triuncialis* chromosome in common wheat was preferentially transmitted to the offspring of every backcross generation by virtue of the sterility of the egg cells lacking this chromosome, no special effort was needed to retain the chromosome in common wheat (rf. Endo and Tsunewaki 1975). The disomic *triuncialis* chromosome substitution plants which had 20 pairs of common wheat chromosomes and a pair of the *triuncialis* chromosomes were produced simply by selfing the monosomic *triuncialis* chromosome substitution plants: The disomic alien chromosome substitution plants used in this work were derived from two monosomic substitution lines whose pedigrees were *Ae. triuncialis*/cw^3/*T. aestivum* cv. Selkirk and *Ae. triuncialis*/cw^3/*T. spelta* var. *duhamelianum*[3], respectively. A disomic alien chromosome addition line in which a pair of the *triuncialis* chromosomes were added to the full complement of common wheat chromosomes was obtained by self-pollination of a monosomic alien chromosome addition plant which had the pedigree of *Ae. triuncialis*/cw^1/*T. aestivum* cv. Chinese Spring[6] (cw denotes common wheat other than those used in the later backcrosses). In spite of possessing the cytoplasm of *Ae. triuncialis*, the disomic alien chromosome addition and substitution plants had normal or almost normal pollen fertilities, and could be used as pollen parents.

In order to identify which common wheat chromosome had been replaced by the *triuncialis* chromosome in the disomic substitution lines, all of the 21 monosomic lines of Chinese Spring were crossed as the female parents with either of the two disomic substitution lines. F_1 plants having 41 chromosomes were selected by the cytological observation of root tip mitoses, and their meiotic configuration was studied in the PMCs at MI of two to four plants for each of 21 F_1 lines.

In this experiment the *triuncialis* chromosome was easily recognized by its extremly subterminal centromere at the metaphase of root tip mitosis. The observation of meiosis and mitosis was carried out by the usual aceto-carmine smear and squash methods, respectively.

Results

Identification of the wheat chromosome substituted with the *triuncialis* chromosome in the disomic alien substitution lines

As the disomic *triuncialis* chromosome substitution plants were nullisomic for a certain wheat chromosome and disomic for the *triuncialis* chromosome, their 2n=41 progenies from the crosses with respective 21 monosomic lines were expected to have one of the following two meiotic configurations: In the critical line, in which the substituted wheat chromosome of the former is homologous to the monosomic chromosome of the latter, the plants should show a meiotic configuration of $20''+1'$; in the non-critical lines, in which those two chromosomes are non-homologous, the 2n=41 plants should have a $19''+3'$ chromosome configuration.

The cytological study of the 21 hybrids between all 21 monosomic lines and the disomic substitution line of Chinese Spring revealed that only the 2n=41 F_1 hybrids from the cross involving the mono-3D had a meiotic configuration of $20''+1'$ in all four plants examined (Fig. 1). All other 2n=41 F_1 plants from the crosses involving monosomics other than mono-3D always had $19''+3'$ (Fig. 2). The F_1 plants with $20''+1'$, which were nullisomic for wheat chromosome 3D and monosomic for the *triuncialis* chromosome, had a normal growth vigor, but their seed fertility was very low (31.3% when crossed with normal pollen and 23.8% when selfed). Almost all the F_1 offspring from selfing the $20''+1'$ F_1 plant had 42 somatic chromosomes including two subterminal *triuncialis* chromosomes (29 out of 31 plants examined). These results clearly demonstrated that the *triuncialis* chromosome had been substituted for wheat chromosome 3D in the original alien chromosome substitution lines of common wheat, and has some genetic affinity with chromosome 3D.

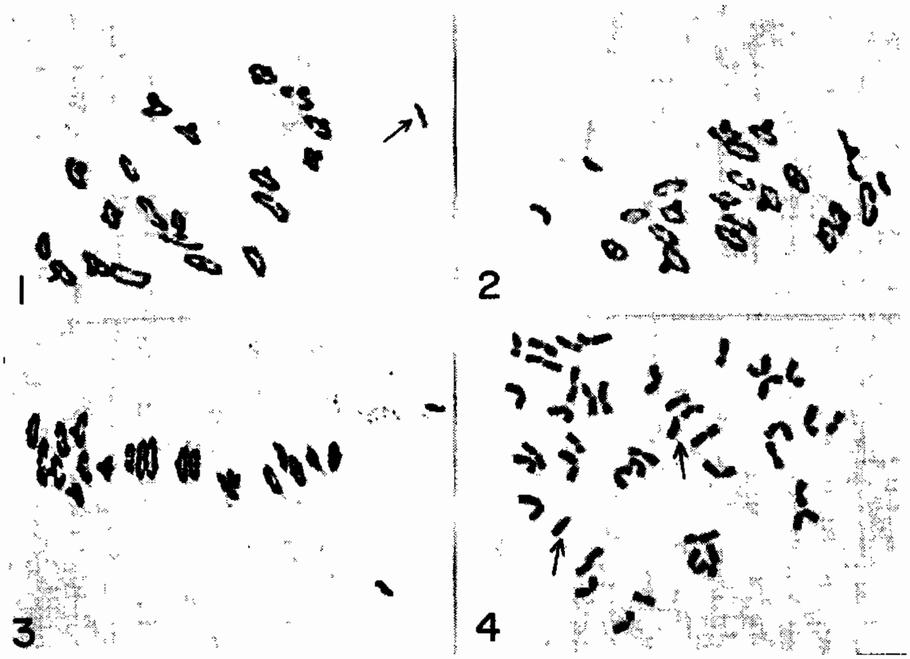
Table 1. Seed and pollen fertilities of F_1 hybrids monosomic for both a wheat and the *triuncialis* chromosome

Monosomic wheat chromosome	Crossed seed fert.*			Selfed seed fert.*			Pollen fert.**
	No. florets	No. seeds set	%	No. florets	No. seeds set	%	%
3A	176	54	30.7	624	167	26.8	46.0
3B	146	32	21.9	552	112	20.3	50.0
3D	124	31	25.0	336	104	31.0	48.0
1A	158	34	21.5	424	91	21.5	46.7
5A	188	25	13.3	672	61	9.8	45.3

*: 1st and 2nd florets were examined.
**: Pollen grains with two sickle-shaped germ nuclei and one vegetative nucleus were taken as normal.

Homoeology of the *triuncialis* chromosome with the wheat chromosomes of homoelogous group 3

The successful substitution of the *triuncialis* chromosome for wheat chromosome 3D implied a close genetic relationship between the *triuncialis* chromosome and the wheat chromosomes of group 3. Three monosomic lines of Chinese Spring that were monosomic for chromosome 3A, 3B, and 3D, respectively, and, as controls, two other monosomic lines for respective chromosomes, 1A and 5A were pollinated with the disomic *triuncialis* chromosome addition line with $22''$. Hybrids from the crosses with respective monosomics were cytologically examined in root tip cells, and the plants with 42 somatic chromosomes including one subterminal *triuncialis* chromosome were selected. They were further checked at meiosis to have a meiotic configuration of $20''+2'$ (Fig. 3). It was confirmed by the reduced seed and pollen fertilities that one of the two univalents of those F_1's was always the *triuncialis* chromosome (Table 1). F_2 offspring were obtained from selfing F_2 plants with $20''+2'$, and were subjected to cytological study of mitosis.



Figs. 1—4. : Fig. 1. Meiotic configuration of a 41-chromosome F_1 hybrid from the cross between mono-3D (♀) and the disomic *triuncialis* chromosome substitution line (♂) of common wheat, $20''+1'$, the univalent (arrowed) being the *triuncialis* chromosome. Fig. 2. Meiotic configuration of a 41-chromosome F_1 hybrid from the cross between mono-4A (♀) and the disomic *triuncialis* chromosome substitution line (♂) of common wheat, $19''+3'$, one of the univalents being the *triuncialis* chromosome. Fig. 3. Meiotic configuration of a 42-chromosome F_1 hybrid from the cross between mono-3D (♀) and the disomic *triuncialis* chromosome addition line (♂) of common wheat, $20''+2'$, one of the univalents being the *triuncialis* chromosome. Fig. 4. Somatic chromosome constitution of an F_2 plant with 42 chromosomes including two subterminals, obtained by selfing an F_1 hybrid doubly monosomic for wheat chromosome 3D and the *triuncialis* chromosome. The two subterminal chromosomes (arrowed) are the *triuncialis* chromosomes.

Before describing the results, we should consider the unique action of the *triuncialis* chromosome on a common wheat cultivar, Chinese Spring. The data of reciprocal crosses between euploid Chinese Spring and its monosomic *triuncialis* chromosome addition line ($21''+1'$) revealed that the *triuncialis* chromosome was transmitted to almost all the offspring through female gametes, while it was transmitted to about 40% of the progeny through male gametes. The crossed seed and pollen fertilities of the monosomic addition line were 16.4% and 46.7%, respectively. It seems likely that the *triuncialis* chromosome in Chinese Spring exerted its gametocidal effect on almost all the female gametes lacking this chromosome, but the effect was not extended to all the male gametes without the alien chromosome.

Table 2. The expected chromosome constitutions of male and female gametes produced by F_1 plants doubly monosomic for a wheat chromosome and the *triuncialis* chromosome and of F_2 progenies from selfing

Female gamete	Male gamete			
	20	21(1tr)	21	22(2tr)
21(1tr)	41(1tr)	42(2tr)	42(1tr)	43(2tr)
22(1tr)	42(1tr)	43(2tr)	43(1tr)	44(2tr)

Note) 1tr and 2tr in parentheses indicate the presence of one and two *triuncialis* chromosomes, respectively.

Therefore, the F_1 lines of Chinese Spring doubly monosomic for a particular wheat chromosome and the *triuncialis* chromosome are expected to produce two types of female gametes and four types of male gametes (Table 2). Table 2 also gives the possible somatic chromosome constitutions of F_2. Female gametes of the alien chromosome addition (n=22) and substitution types (n=21) are expected to function in a ratio of one to three in all the F_1 hybrids. It is also certain that chromosome-deficient male gametes (n=20) could rarely function in every $20''+2'$ F_1 line, and that male gametes of the alien chromosome addition type (n=22) can normally function in fertilization as shown in the monosomic alien chromosome addition line of Chinese Spring mentioned above. For male gametes of the alien chromosome substitution type with 20 wheat and one *triuncialis* chromosome, two possibilities are considered: (1) the *triuncialis* chromosome has such close genetic homology with the missing wheat chromosome that male gametes of the alien chromosome substitution type function normally in fertilization ; (2) the *triuncialis* chromosome can not compensate for the missing wheat chromosome, so that alien chromosome substitution male gametes are no better than the chromosome-deficient gametes (n=20). Thus if the *triuncialis* chromosome has a close genetic homology with the wheat chromosome which is monosomic in the F_1 with $20''+2'$, we can expect a considerable number of the disomic alien chromosome substitution plants (2n=42 chromosomes including two subterminal *triuncialis* chromosomes) from selfing the F_1's. Conversely, if the wheat chromosome monosomic in the F_1 hybrids has no genetic homology with the *triuncialis* chromosome, few disomic chromosome substitution plants would be expected.

The results are given in Table 3. High frequencies of the seedlings with 42 chromosomes including two subterminal *triuncialis* chromosomes (Fig. 4) were found among all the F_2 progenies from the parents in which each of the three wheat chromosomes of homoeologous group 3 had been monosomic. It is evident that the male gametes with the *triuncialis* chromosome substituted for chromosome 3A, 3B, or 3D could function normally in competition with male gametes having 21 wheat chromosomes and those of the alien chomosome addition type (n=22). The F_1 parents monosomic for chromosome 1A or 5A, however, produced no and only two F2 plants with 40 wheat and two *triuncialis* chromosomes, respectively. This meant that n=21

Table 3. Frequencies of various chromosome constitutions in the F_2 generation of F_1 lines doubly monosomic for a wheat chromosome and the *triuncialis* chromosome

Monosomic wheat chromosome	Somatic chromosome constitution							
	41(1tr)	42(1tr)	42(2tr)	43(1tr)	43(2tr)	44(2tr)	Others	Total
3A	6	6	20	2	12	3	1	50
3B	1	8	14	3	10	4	2	42
3D	3	9	30	3	8	1	2	56
1A	1	13	0	4	15	13	4	50
5A	0	17	2	6	13	9	2	49

Note) 1tr and 2tr in parentheses indicate the presence of one and two *triuncialis* chromosomes, respectively.

pollen with the *triuncialis* chromosome instead of wheat chromosome 1A or 5A functioned no more frequently (less than 2% of pollen grains functioned) than the chromosome-deficient pollen. Therefore, there is no doubt that the *triuncialis* chromosome had a close genetic homology with all chromosomes of homoeologous group 3. The wheat plants nullisomic for 3A, 3B, or 3D and disomic for the *triuncialis* chromosome are expected to have normal growth vigor ; this has been confirmed for chromosome 3D.

Discussion

In view of the successful substitution of the *triuncialis* chromosome for wheat chromosome 3D in monosomic and disomic conditions, and the normal function of male gametes with the *triuncialis* chromosome instead of wheat chromosome 3A, 3B, or 3D, it became clear that the *triuncialis* chromosome has a close genetic homology with the wheat chromosomes of homoeologous group 3. On the other hand, male gametes deficient in chromosome 1A or 5A and monosomic for the *triuncialis* chromosome rarely functioned in spite of the advantage of the gametes with the *triuncialis* chromosome over those without it in gametogenesis. This fact implies substituting ability of the *triuncialis* chromosome for specific wheat chromosomes. Such a specific substituting ability of alien chromosomes has been reported by Riley (1965) for rye chromosome II, by Johnson (1966) for an *Agropyron* chromosome, and by Riley *et al.* (1966) for an *Ae. comosa* chromosome. Riley (1965), for example, was able to substitute rye chromosome II for all three wheat chromosomes of group 6 but no others. The ability of the *triuncialis* chromosome to substitute for wheat chromosomes other than those tested in the present study is under investigation.

In the knowledge of the authors, this is the first report dealing with the substitution of a chromosome of tetraploid *Aegilops* species for all three wheat chromosomes of a particular homoeologous group. It has been proved by genome analysis (Kihara 1940) that *Ae. triuncialis* (2n=28, CCC^uC^u) originated from a hybrid between two diploid *Aegilops* species, *i. e.*, *Ae. caudata* (2n=14, CC) and *Ae. umbellulata* (2n=14, C^uC^u). We can therefore trace the origin of the *triuncialis* chromosome, or the gene

(s) causing selective gametophyte sterility, back to either of the diploid parental species, unless the gene(s) on the *triuncialis* chromosome arose by mutation after the amphidiploidization of the hybrid between *Ae. caudata* and *Ae. umbellulata.* Although the occurrence of chromosomes such as the *triuncialis* chromosome has not yet been found in either *Ae. caudata* or *Ae. umbellulata,* the following fact can be considered as indirect evidence that the *triuncialis* chromosome is directly derived from one of the parental *Aegilops* species: A chromosome extracted from a synthetic *triuncialis* into common wheat was proved to be very similar to the *triuncialis* chromosome in its unique function and morphology (Endo and Tsunewaki 1975); the synthetic *triuncialis* concerned was produced as an amphidiploid of the F_1 hybrid *Ae. caudata* (♀) x *Ae. umbellulata* (♂), only about 30 years ago (Kondo 1941). In the karyotypes of *Ae. caudata* and *Ae. umbellulata* studied by Senjaninova-Korczagina (1932) and Tanaka (1970), we can find subterminal chromosomes similar to the *triuncialis* one in both species. So it is evident that there is a chromosome of the same kind as the *triuncialis* chromosome in either *Ae. caudata* or *Ae. umbellulata.*

Maan (1975) also noticed during substitution of alien cytoplasms into common wheat that an alien chromosome once isolated into common wheat from *Ae. longissima* (2n=14, S^lS^l) and *Ae. sharonensis* (2n=14, S^lS^l) could not be eliminated from common wheat by backcrosses. He demonstrated that both of the *Aegilops* chromosomes had a gametocidal action of the same mechanism as was revealed with the *triuncialis* and synthetic *triuncialis* chromosomes. By the crosses of the disomic alien chromosome substitution line with the Chinese Spring telocentric stocks, the critical *Ae. longissima* chromosome was proved to have substituted for wheat chromosome 4A (Maan 1976).

Besides the gametocidal chromosomes of *Ae. triuncialis,* synthetic *triuncialis, Ae. longissima,* and *Ae. sharonensis,* some chromosome of *Ae. cylindrica* (2n=28, CCDD) and one other chromosome and a chromosome fragment of *Ae. triuncialis* are also thought to induce similar gametophytic sterility and transmit preferentially in common wheat. It seems that the gene(s) causing selective gametophyte sterility in common wheat is located on different chromosomes in different species of the genus *Aegilops.* Studies on the distribution of the gene(s) and on the homoeology of the carrier chromosomes will provide an additional approach to the full understanding of the phylogenetic relationships among the *Aegilops* species and their evolutionary processes.

Summary

It is known that a subterminal chromosome of *Aegilops triuncialis* (2n=28, CCC^uC^u) causes gametophytic sterility in common wheat having this chromosome in the monosomic condition, through making gametes without it functionless in fertilization, resulting in its preferential transmission to offspring. The *triuncialis* chromosome was noticed to be able to replace a pair of wheat chromosomes. By crossing all 21 monosomic lines of Chinese Spring with the disomic *triuncialis* chromosome substitution lines of common wheat, the wheat chromosome substituted with the *triuncialis*'s

was determined to be chromosome 3D.

Five monosomic lines of Chinese Spring for chromosomes 3A, 3B, 3D, 1A, and 5A were pollinated with the disomic *triuncialis* chromosome addition line of Chinese Spring. The F_1 hybrids with 20″+2′, where one of the two univalents was the *triuncialis* chromosome, were self-pollinated, and the chromosome constitutions of plants were examined in root tip mitosis. The *triuncialis* chromosome was recognized by its extremely subterminal centromere. A high frequency of the disomic alien chromosome substitution plants in the F_2, which were judged by 2n=42 somatic chromosomes including two subterminal ones, was obtained in all offspring of the F_1 hybrids in which chromosomes of homoeologous group 3 had been monosomic. On the other hand, two F_2 offspring from the hybrids doubly monosomic for the *triuncialis* chromosome and a wheat chromosome 1A or 5A yielded few seedlings with 2n=42 chromosomes including two *triuncialis* chromosomes. These results clearly indicate that the male gametes having the *triuncialis* chromosome substituted for any wheat chromosome of homoeologous group 3 could normally function in fertilization in competition with normal wheat male gametes, while those with the *triuncialis* chromosome substituted for chromosome 1A or 5A rarely functioned. Consequently, the *triuncialis* chromosome causing selective gametophyte sterility in common wheat is considered to be homoeologous with the wheat chromosomes of group 3. In addition, the possibility was discussed that the gametocidal gene(s) on the *triuncialis* chromosome had been derived directly from either of the parental species of *Ae. triuncialis*, namely *Ae. caudata* (2n=14, CC) and *Ae. umbellulata* (2n=14, C^uC^u).

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