The Extremely Frequent Occurrence of Haploids in Some Plants of a Common Wheat Strain with the *Aegilops triuncialis* Cytoplasm

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Introduction

Many attempts have been made to obtain haploid plants of various species, with some positive results. Except for anther culture, however, all methods so far have been successfully applied to only a very limited number of species: i. e., pollination of X-rayed pollen grains in *Triticum monococcum* (Katayama, 1934) ; selection of specific strains with effective screening in *Zea mays* (Chase, 1949); use of an alien cytoplasm in *T. aestivum* (Kihara and Tsunewaki, 1962) ; and embryo culture in *Hordeum vulgare* (Kasha and Kao, 1970) and *T. aestivum* (Barclay, 1975). At present, alien cytoplasms are quite useful for inducing haploids in a single strain of common wheat, *T. aestivum* strain Salmon, and their effect on haploid induction in this strain is rather prominent and stable. Eight species of the genus *Aegilops*, a related genus of *Triticum*, are known to possess cytoplasms that induce haploids when incorporated with Salmon's nucleus (Tsunewaki et al., unpubl.). *Ae. triuncialis* is one of them. This year we found four plants of Salmon with the *Ae. triuncialis* cytoplasm that produced so many haploid and twin seedlings that maintenance of the line became almost impossible.

We here report the extraordinarily high frequency of haploids and twins observed in the four plants of this line, and discuss briefly the cause.

Materials and Methods

The materials used were plants belonging to two generations of *Triticum aestivum* strain Salmon (2n=42, genome formula AABBDD) with the cytoplasm of *Aegilops triuncialis* (2n=28, CCC^uC^u). This line has the nucleus of Salmon and the cytoplasm of *Ae. triuncialis*, and is designated (*triuncialis*)-Salmon. Salmon is a strain of commonwheat derived from a cross between two octoploid Triticales. According to Tsunewaki (1964) and Zeller (1973), it possesses almost the same chromosome complement as that of normal *T. aestivum*, except for slight structural modifications of chromosomes 1B and 2B. *Ae. triuncialis* is a tetraploid *Aegilops* species belonging to the section Polyeides, which contains four other species having haploid-inducing cytoplasms (Tsunewaki et al., 1974). The (*triuncialis*)-Salmon used in this study was produced by backcrossing an F_1 hybrid, *Ae. triuncialis* (♀) x *T. aestivum* (♂) as the female parent to normal Salmon seven to eight times to become completely male sterile.

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Four individual plants, the bulk of eight plants of the B6 generation of (*triuncialis*)-Salmon; and three plants of the B7 generation were grown under different conditions, then studied for their efficiency in producing haploid and twins. Table 1 shows the chromosome numbers of these materials and their growing conditions. Plants I, II, and III with 2n=43 chromosomes were planted in the field in the fall, flowering early the next summer. Plant IV and group V were densely planted in seeding flats together with other plants of the same line, and were grown under a semitransparent plastic roof from October to April. Their chromosome numbers were not studied. Plants VI, VII, and VIII were produced by backcrossing a diploid twin (2n=42) of the B6 generation to Salmon. Consequently, they had 2n=42 chromosomes. These three plants were grown in a greenhouse, and flowered three months from seeding. All plants were pollinated with the normal pollen of common wheats.

Table 1. Materials used, their chromosme numbers and growing conditions

Plant or group	Backcross generation	No. of chromosomes (2n)	Growing condition	
			place	period**
I	B6	43	field	Oct., '74–June,'75
II	〃	〃	〃	〃
III	〃	〃	〃	〃
IV	〃	?	seeding flat under roof	Oct., '74–Apr.,'75
V*	〃	?	〃	〃
VI	B7	42	greenhouse	Mar.,'75-June, '75
VII	〃	〃	〃	〃
VIII	〃	〃	〃	〃

* Consisting of eight plants. ** From germination to anthesis.

Seeds obtained from each plant or group by artificial pollination were germinated on wet filter papers. Twin seedlings were identified when two shoots emerged from a single seed. Root tips were collected from the respective seedlings in order to examine cytologically their ploidy (diploid or haploid) and chromosome numbers. We confirmed the ploidy of almost all the seedlings.

Results and Discussion

Table 2 shows the frequencies of haploid and twin seedlings in the progeny of each plant or group. The average frequencies of haploids and twins in the B6 and B7 generations of (*triuncialis*)-Salmon were higher than those of the preceding B5 generation (32.1%) (Tsunewaki et al., unpubl.). Furthermore, we found extraordinarily high frequencies of haploids and twin seedlings in four plants; IV, VI, VII, and VIII. Plant IV produced 24 haploids and two four seedlings in 32 germinated seedlings. In the total of 62 offspring from three plants; VI, VII, and VIII, grown in the greenhouse, 49 haploids and five twin pairs were found. All twin pairs included at least one haploid twin, and almost all twins were the haplo-diplo type, with an exceptional haplo-haplo type. Therefore, 82.4 to 89.2% of the viable offspring of the four plants produced haploids. Such high frequencies of haploids have never been observed in other lines of Salmon with this and other alien cytoplasms.

Table 2. Frequencies of diploid, haploid, and twin seedlings

Plant or group	Diploid		Haploid		Twin		Total
	No.	%	No.	%	No.	%	
I	25	55.6	18	40.0	2	4.4	45
II	19	82.6	3	13.0	1	4.3	23
III	48	48.5	41	41.4	10	10.1	99
IV	4	12.5	24	75.0	4	12.5	32
V	15	51.7	12	41.4	2	6.9	29
VI	2	11.8	13	76.5	2	11.8	17
VII	3	17.6	13	76.5	1	5.9	17
VIII	3	10.7	23	82.1	2	7.1	28

Because of the low seed-set and germination rate of (*triuncialis*)-Salmon, we believe that the following are the two probable causes for the unusually high frequency of haploids; a high frequency of haploid parthenogenesis in the egg cells, and the advantage of the haploids for survival. Table 3 gives the seed-setting rates of the individual plants and the group together with the germination rates and percent of success of the cross.

Table 3. Seed-set and germination rates in relation to the frequency of haploids

Plant or group	Seed-set (%)	Germination (%)	Success* of cross (%)	No. of haploids / floret
I	33.1	55.1	18.2	0.08
II	37.3	42.4	15.8	0.03
III	52.9	44.4	23.5	0.12
IV	32.4	66.7	21.6	0.19
V	30.5	74.4	22.7	0.11
VI	30.3	73.9	22.4	0.20
VII	25.8	38.6	10.0	0.08
VIII	38.6	82.4	31.8	0.28

* (Seed-setting rate) x (germination rate).

As to the cause of the different frequencies in haploids among the plants and the group of (*triuncialis*)-Salmon, we will consider two factors, environmental and genetic. The four plants that produced haploids at very high rates were grown under more or less severe conditions. But we can not ascribe the extremely high frequencies of haploids only to the severe conditions, because group V was raised under the same conditions as plant IV, but did not give so high a frequency of haploids as did plant IV. The high frequency of haploids may be attributed to the genetic peculiarity of the four plants: The chromosome numbers of plants VI, VII, and VIII were $2n=42$, and that of plant IV also seems to have been $2n=42$; as only $2n=42$ diploids and $2n=21$ haploids were recovered in its offspring. In contrasts, plants I, II, and III had

2n=43 chromosomes, and group V probably included 2n=43 plant(s) because some of its progeny had 2n=43 chromosomes. Consequently, the major genetic difference between the four plants, which produced haploids at extremely high frequencies, and the other three plants and the group, which produced haploids at ordinary frequency, is whether they carry an extra chromosome, because they were backcrossed to normal Salmon at least seven times. This extra chromosome is regarded as one of the *Ae. triuncialis* chromosomes because of its behavior in meiosis, and has been preserved in the line of (*triuncialis*)-Salmon, presumably, by preferential transmission through the female gamete to its offspring. Therefore, this high frequency of haploids may have been due to the loss of this chromosome. If that is the case, the cytoplasm of *Ae. triuncialis* essentially has a very strong power for haploid induction in Salmon, while the extra chromosome suppresses the occurrence of haploids in 2n=43 plants by some mechanism. Among the offspring of plants I, II, and III that had 2n=43 chromosomes, 2n=43 diploids were found far more frequently than 2n=42 ones (47 out of 59 diploids examined), while 2n=21 haploids were about three times as many as the 2n=22 ones (49 2n=21 to 17 2n=22 haploids). Thus, the difference in the frequency of the haploid occurrence between the 2n=42 and 2n=43 plants of (*triuncialis*)-Salmon may be as follows: Although the cytoplasm of *Ae. triuncialis* is able to induce a very high frequency of haploids in Salmon, the extra chromosome existing in the 2n=43 individuals reduces the frequency of haploids in their progeny by preferentially disturbing the egg cells without this chromosome, the 2n=21 ones, in their normal fertilization or successful parthenogenesis. In contrast, the extra chromosome improves the 2n=22 egg cells ability to be fertilized and to develop into diploid embryos. This type of preferential fertilization of egg cells with an extra chromosome is observed in other common wheat lines with the *Ae. triuncialis* cytoplasm (Endo and Tsunewaki, 1975). Although this assumption seems to be true in view of the present data, we are reluctant to draw any definitive conclusion until there has been further systematic investigations. We must also not neglect environmental factors, though they can not be solely responsible for the unusually high frequency of haploids in (*triuncialis*)-Salmon.

Summary

While replacing the cytoplasm of the *Triticum aestivum* strain Salmon (2n=42, AABBDD) with that of *Aegilops triuncialis* (2n=28, CCC^uC^u) by successive backcrosses, we found one plant in the B6 and three in the B7 generation that gave haploids and twin seedlings at the rate of 82 to 89% of their viable offspring. These frequencies were too high to be ascribed to deviation by chance. Possible roles of environmental and genetic factors were briefly discussed in relation to this high frequency of haploids. Unusual growing conditions were seemingly responsible to some extent. The possibility was also suggested that the loss of one of *Ae. triuncialis* chromosomes retained throughout the six backcrosses might have been involved in the extremely frequent occurrence of haploids, though further study is needed to obtain conclusive evidence.

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