Studies on Cross Compatibility of Diploid, Triploid and Tetraploid Barleys I. Seed Development in Reciprocal Crosses between Diploids and Induced Autotetraploids

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土屋 工*: オオムギにおける二倍体三倍体, および四倍体間の交雑親和性. 第1報.
二倍体と同質四倍体間の正逆交雑における種子稔性と次代の染色体数

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The present author has succeeded in producing in barley a considerable number of autotriploids during the past several years (Tsuchiya 1949, 1952a, b, 1953a). In the present paper, the experimental results regarding cross compatibility between diploid and induced autotetraploid barleys are described.

Materials and Methods

In the crossing experiments, diploids and induced autotetraploids of the following 5 cultivated varieties and 1 wild species were used. All autotetraploids used were produced by Dr. T. Ono by colchicine treatments (Ono 1946, 1947).

Hordeum sativum Jessen (cultivated varieties)

Early Golden Melon (two-rowed)

Hakata No. 2 (")

Hosokara No. 2 (six-rowed)

Hosomugi (")

Mochimugi (")

H. spontaneum C. Koch var. *transcaspicum* Vav. (two-rowed wild species)

Bonnett's method (Bonnett 1938) was used exclusively in the crossing experiments. To count the chromosome numbers in root tip cells, both paraffin method and aceto-carmin squashes were employed and the meiotic chromosomes were observed in temporary aceto-carmin preparations.

Results and Considerations

1) Crossing experiments

Adopting Bonnett's method (l. c.), the flowers were opened by slitting the lemma with a sharpened forceps. Through the slit made in the lemma the anthers were removed and pollinations were made. The results of the crosses are given in Table 1.

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Table 1. Cross fertility in reciprocal crosses of $4x \times 2x$ and germination of the seeds.

Materials and cross combin.	Year	Number of		Fertility (%)	Germination	
		florets	seeds ¹⁾		No. of seed	%
$4x \times 2x$ EGM. ²⁾	1947	94	3	3.19	1	33.33
	1952	49	6	12.24	0	—
	1953	370	274	74.05	1	0.36
HST. ³⁾	1952	793	130	16.39	12 ⁴⁾	9.23
	1953	956	468	48.95	12 ⁵⁾	2.56
Hakata No. 2	1952	77	1	1.29	0	—
	1953	206	63	30.58	2	3.17
Hosomugi	1952	47	0	0.00	—	—
Hosokara No. 2	1952	80	0	0.00	—	—
Mochimugi	1953	49	20	40.81	1 ⁶⁾	5.00
EGM. $4x \times$ HST. $2x$	1948	19	18	94.73	27 ⁷⁾	11.11
$2x \times 4x$ HST.	1952	21	19	90.48	0	—
Hakata No. 2	1952	40	39	97.50	0	—
Hosomugi	1952	16	0	0.00	—	—

1) Plump and shrivelled (parthenocarpic) seeds are involved.

2) Early Golden Melon.

3) *Hordeum spontaneum* C. Koch var. *transcaspicum* Vav.

4) 7 plants died in early seedling stage and the remaining 5 grew to maturity.

5) 1 plant died in the seedling stage and the remaining 11 grew to maturity.

6) Died early in the seedling stage.

7) 1 plant died early in the seedling stage and the remaining 1 matured.

No seeds were obtained from the crosses $4x \times 2x$ or $2x \times 4x$ in Hosomugi and $4x \times 2x$ in Hosokara No. 2, while many seeds were obtained from $4x \times 2x$ in Early Golden Melon. The cross of Early Golden Melon $4x \times H. spontaneum$ var. *transcaspicum* $2x$ (1948) gave a very high percentage of seed setting (94.7 %). From the cross $2x \times 4x$ (1952) in *H. spontaneum* var. *transcaspicum*, 19 seeds were obtained from 21 florets (90.5 %). In the reciprocal cross of 1952, $4x \times 2x$, the fertility was lower than in 1953. Hakata No. 2 showed a rather low percentage of seed setting in the cross $4x \times 2x$. The fertility, however, showed an increase from 1.3 % in 1952 to 30.6 % in 1953. The reciprocal cross, $2x \times 4x$, showed the highest fertility (97.5 %) in 1952. Mochimugi showed a fertility of 40.8 % in the cross $4x \times 2x$ in 1953.

2) Germination tests

The results of germination tests are given in the last 2 columns of Table 1. Though the seed setting was rather good in the cross $2x \times 4x$, combinations of *H. spontaneum* var. *transcaspicum* and Hakata No. 2, the seeds did not germinate.

Out of 283 seeds of the cross $4x \times 2x$ in Early Golden Melon, two triploid plants were obtained, namely 1 plant out of 3 seeds in 1947 and 1 out of 274 seeds in 1953, but all the 6 seeds obtained in 1952 failed to germinate.

Considerable number of seeds obtained in the cross $4x \times 2x$ of *H. spontaneum* var. *transcaspicum* germinated. In 1952, 12 out of 130 seeds germinated; 7 plants died in an early seedling stage and the remaining 5 grew to maturity (Tsuchiya 1953a). 12 seedlings emerged from 468 seeds in 1953 but 1 died by an accident and the remaining 11 plants grew up to maturity.

In Hakata No. 2, 1 seed of the cross $4x \times 2x$ (1952) did not germinate. Two seeds out of 63 obtained from the same cross in 1953 germinated and the plants matured.

Mochimugi gave one seedling from 20 seeds in the cross $4x \times 2x$ in 1953, which was killed by an accident in an early stage.

The results mentioned above show that seeds obtained in the crossing with diploid mother plants, $2x \times 4x$, do not germinate, though seed setting is very good, with the exception of Hosomugi. The situation is reversed in the reciprocal cross, $4x \times 2x$; that is, seeds were able to germinate in many cases, although seed fertility and percentage of germination were not always high. Similar relationship has been reported in *Raphanus* (Nishiyama 1949, 1952), *Brassica* (Nishiyama and Inamori 1952, 1953), *Capsicum* (Nishiyama and Karasawa 1954), *Hordeum* and *Zea* (Håkansson 1953), and other plants.

The fact that seed setting in selfing of diploids and induced autotetraploids of barley is very good shows that both pollen and embryo-sacs of diploids and tetraploids are functional (cf. Ono 1946, 1947, 1948, 1949a, b, 1952, Tsuchiya 1953b). However, only a few seeds obtained from the cross $4x \times 2x$ germinated and none of the seeds from the reciprocal cross, $2x \times 4x$. These results seem to show that the cross incompatibility between diploid and autotetraploid barleys may be ascribed mainly to zygotic sterility and not to gametic abortion.

Håkansson (1953) ascribed the failure of seed setting in reciprocal crosses between diploid and tetraploid plants of barley (and maize) to the "degeneration of endosperm and embryo" resulted from the unbalanced quantitative relations of chromosome numbers (genomes) within the seeds. According to him, "the changed relations between the maternal tissue and the endosperm were considered to be the initial cause of the irregular development and degeneration of the endosperm; the death of the embryo was secondary to the disturbed endosperm development". The significant differences seen in either seed setting or in germination percentage among materials and in different years, however, remain unsolved problem.

Smith (1951) and Håkansson (l. c.) reported that the production of autotriploids in barley by crossing induced autotetraploids with diploids was very difficult. The present experiments carried out during the past 4 years tend to confirm their results, namely only 19 autotriploids were obtained from the 2721 ($4x \times 2x$) and 77 ($2x \times 4x$) florets, i. e., 0.67 %.

3) Cytological observations

Among 31 plants obtained from the seeds of the cross $4x \times 2x$, 10 died in early seedling stage and the chromosome numbers of remaining 21 plants were counted as given in Table 2.

Table 2. Chromosome number of plants obtained from the cross $4x \times 2x$ in barley

Materials	Year	Number of plants with respective chromosome numbers of			
		20	21	29	30
Early Golden Melon	1947 ¹⁾	1			
"	1953		1		
<i>H. spont.</i> var. <i>transcaspicum</i>	1952 ²⁾	1	3	1	
"	1953		10		1
Hakata No. 2	1953		2		
EGM. ³⁾ $4x \times$ HST. ⁴⁾ $2x$	1948 ⁵⁾		1		

1) Tsuchiya 1949, 1952a.

2) ——— 1953a.

3) Early Golden Melon.

4) *Hordeum spontaneum* C. Koch var. *transcaspicum* Vav.

5) Tsuchiya 1952b.

One plant of Early Golden Melon obtained from the cross $4x \times 2x$ in 1947 was a hypo-triploid with $2n=20$ chromosomes (Tsuchiya 1949, 1952a) and another plant from the same cross in 1953 had $2n=21$ chromosomes.

Among 5 matured plants of $4x \times 2x$ (1952) in *H. spontaneum* var. *transcaspicum* there were 3 eu-triploid with $2n=21$, 1 hypo-triploid with $2n=20$, and 1 hyper-tetraploid with $2n=29$ chromosomes. Somatic chromosomes of all 11 surviving plants from the cross $4x \times 2x$ in the same species (1953) were counted; 1 was hyper-tetraploid with $2n=30$ and the remaining 10 were eu-triploids with $2n=21$ chromosomes.

Two individuals from the cross $4x \times 2x$ of Hakata No. 2 made in 1953 were eu-triploid with $2n=21$ chromosomes. The hybrid plant raised from the cross Early Golden Melon $4x \times$ *H. spontaneum* var. *transcaspicum* $2x$ in 1948 was also eu-triploid with $2n=21$ chromosomes (Tsuchiya 1952b).

As described above, almost of all the plants obtained from the cross $4x \times 2x$ were hypo- or eu-triploids with $2n=20$ or 21 chromosomes. Unexpectedly, however, 29- and 30-chromosome plants were obtained from the cross $4x \times 2x$ in *H. spontaneum* var. *transcaspicum*. These two plants might have resulted from the union of hyperdiploid eggs in tetraploids and unreduced pollen which occur rarely in diploid barley as observed by Smith (1942) and Tsuchiya (1955).

Self-pollination resulting from unsuccessful castration should be taken into consideration as an alternative.

Similar cases of unexpected occurrence of tetraploid plants in the crosses between autotetraploids and diploids have been reported in *Raphanus* (Nishiyama

1949, 1952) and *Brassica* (Nishiyama 1953).

Eight seedlings (27.6 %) out of 31 obtained from the cross $4x \times 2x$ died in a very early stage. From the morphological features these seedlings were assumed to be triploid or nearly triploid. The difficulty in obtaining autotriploids from the cross $4x \times 2x$ in barley may partly be ascribed to a very low viability in early seedling stages.

Summary

1. Diploids and induced autotetraploids of 2 two-rowed and 3 six-rowed cultivated varieties of *Hordeum sativum* and a two-rowed wild species, *H. spontaneum* C. Koch var. *transcaspicum* Vav. were used in the crossing experiments.

2. Seventeen eu-triploids with $2n=21$ and 2 hypo-triploids with $2n=20$ chromosomes were obtained from a total of 2721 florets of the crosses $4x \times 2x$.

3. The cross $2x \times 4x$ gave a high percentage of seed setting with an exception of Hosomugi in 1952, the seeds, however, did not germinate.

4. Unexpectedly, two hyper-tetraploids, $2n=29$ and 30 , were obtained from the cross $4x \times 2x$ in *H. spontaneum* C. Koch var. *transcaspicum* Vav.

5. The percentage of seed setting and of germination differed in different cross combinations and different years.

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