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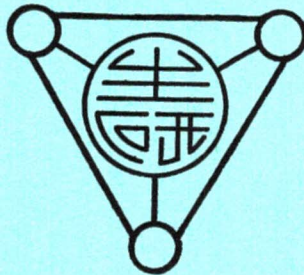
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**Studies on Cross Compatibility of Diploid, Triploid
and Tetraploid Barley, III**

Results of $3x \times 3x$ Crosses

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Studies on Cross Compatibility of Diploid, Triploid and Tetraploid Barley, III

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Introduction

Concerning the cross compatibility among diploids, triploids and tetraploids in barley, fertility and other problems arising in experiments with autotriploids should be considered at the same time, since they produce gametes with various chromosome numbers ranging from haploid ($n=7$) to diploid ($n=14$) and, hence, their progeny obtained by selfing is mainly controlled by the same mechanism which functions in crosses between diploids, triploids, and tetraploids.

Some results on fertility, seed germination and chromosome numbers in the progenies of selfed autotriploid barley have been reported in previous papers (TSUCHIYA 1952a, b, 1958a, 1960a). In this paper recently obtained results will be described including some previous findings related to the problem of cross compatibility between polyploids of barley.

Materials and methods

Materials used in this study are the same triploids as described in the 1st and 2nd papers of this series, namely, Early Golden Melon, Hakata No. 2, and *Hordeum spontaneum nigrum* (*H. spontaneum* C. KOCH var. *transcaspicum* VAV.) (TSUCHIYA 1958a, 1960b; cf. 1953a). All triploids were produced by crossing $4x \times 2x$. One of them was obtained from the cross Early Golden Melon $4x \times H. spontaneum nigrum$ $2x$ (TSUCHIYA 1952b, 1958a). The tetraploid plants are descendants of colchicine induced autotetraploids (ONO 1946, 1947; cf. TSUCHIYA 1953b, 1960b).

For self pollination of autotriploids the usual paper bags were applied 2 or 3 days before anthesis. Some florets flowered without paper bags but the plants were kept at a sufficient distance from other barley plants to avoid contamination. The anthers of almost all of the autotriploids dehisced well and scattered a sufficient amount of pollen, having more than 80% good pollen grains (TSUCHIYA 1952a, b, 1953a, unpublished).

Chromosome studies were made in root tips and meiosis was studied in microsporocytes using the aceto-carmin squash methods.

Seed fertility and germination of seeds

1. Hypotriploid plants with $2n=20$ chromosomes

From a selfed ear of a hypotriploid plant of Early Golden Melon 2 seeds from 25 florets were obtained which did not germinate. In hypotriploid *H. spont. nigrum* 23 seeds were obtained from 198 florets (self fertility=11.6%). Three of 20 seeds sown germinated, the effective crossing success being 1.67% (Tab. 1).

2. Eutriploid plants with $2n=21$ chromosomes

Generally speaking the average seed fertility was slightly better in eutriploids than in hypotriploids but the difference was not significant (Tab. 1). The germination of eutriploids was, however, significantly better than that of hypotriploids. Seed fertility varied in different varieties from the lowest 5.3% in Hakata No. 2 to the highest 22.6% in a 3x-hybrid, the average being 15.3%.

The germination percentage was high in the eutriploids; 46.5% on an average varying from 41.2% in Hakata No. 2 to 58.2% in the 3x-hybrid. The average value of crossing success is 7.11 varying from the lowest 2.2 in Hakata No. 2 to the highest 13.2 in the 3x-hybrid. The 3x-hybrid seems to be higher in seed fertility and germination percentage and, thus, in crossing success as well (Tab. 1). The same situation has been observed in the $3x \times 2x$ crosses (TSUCHIYA 1960).

Table 1. Self-fertility and germination percentage in autotriploids ($2n=20$ and 21)

Materials	Number of		Fertility (%)	Number of seeds			Crossing success*
	florets crossed	seeds obtained		sown	germinated (%)		
3x-1 ($2n=20$)							
Early Golden Melon	25	2	8.0	2	0	—	—
<i>H. spont. nigrum</i>	198	23	11.6	20	3	(15.0)	1.7
Total (Average)	223	25	11.2	22	3	(13.6)	1.4
3x ($2n=21$)							
Early Golden Melon	170	36	21.2	36	18	(50.0)	10.6
Hakata No. 2	320	17	5.3	17	7	(41.2)	2.2
<i>H. spont. nigrum</i>	1,473	226	15.3	218	93	(42.7)	6.5
Total (Average)	1,963	279	14.2	271	118	(43.5)	6.0
3x-hybrid**	296	67	22.6	67	39	(58.2)	13.2
Grand total	2,259	345	15.3	338	157	(46.5)	7.1

* The percentage of seedlings per crossed floret.

** Early Golden Melon $4x \times H. spontaneum$ *nigrum* $2x$ (TSUCHIYA 1952b, 1958a).

Variation in fertility and germination between plants with the same chromosome number within a variety

A variation in fertility and germination capacity was found between the varieties used and also between groups with different chromosome numbers as mentioned above (Tab. 1). Further, variation in fertility and germination percentage was observed between plants having the same chromosome number within the same variety. Table 2 represents two year data for 12 eutriploids of *H. spont. nigrum*. Selfed seed fertility ranged from the lowest 5.3% in Triplo-4 in 1954 to the highest 22.9% in Triplo-3, the average being 20.2% in 1953 (3 plants), 12.0% in 1954 (9 plants) and 15.3% for all 12 triploids examined.

The fertility at open pollination of 9 eutriploid plants (1954) showed a similar variation as the bagged ears, varying from 9.4 to 25.4% with the average of 13.9% for all 9 triploids (Tab. 2).

Table 2. Seed fertility and germination percentage in 12 autotriploid plants of *Hordeum spontaneum nigrum* ($2n=21$)

Year	Materials	Seed fertility		Germination percentage*	Success in selfing
		self	free		
1953	Tri— 1	6.32	—	83.33	5.25
	Tri— 2	22.79	—	30.66	7.00
	Tri— 3	22.93	—	45.45	10.42
	Average	20.23	—	42.98	8.69
1954	Tri— 4	5.33	13.6	58.50	3.10
	Tri— 5	17.86	15.0	46.66	8.36
	Tri— 6	19.38	10.6	26.31	5.10
	Tri— 7	6.94	12.1	30.00	2.07
	Tri— 8	13.04	9.4	33.33	4.33
	Tri— 9	10.89	25.4	63.63	6.93
	Tri—10	17.65	18.2	53.33	9.43
	Tri—11	8.33	12.9	0	0
	Tri—12	21.62	12.5	37.50	8.10
	Average	11.95	13.9	42.30	5.08
Total (Average)		15.34	13.9	42.66	6.53

* Only selfed seeds were tested.

Germination was tested only in selfed seeds of all 12 triploids. In addition to the wide variation in self fertility, the germination percentage was found for 11 plants to show considerable variation from 30.7 to 83.3%. Only seeds of Triplo-11 did not germinate at all. The reason is not known.

Chromosome constitutions in the selfed progenies of autotriploid plants

The chromosome constitutions in the progenies of a hypotriploid plant of

Table 3. Chromosome constitutions in the selfed progenies of autotriploids

Chromosome number (2n)	Frequency of plants with respective chromosome numbers						
	<i>H. spont. nigrum</i>		Early Golden Melon		Hakata No. 2	Total plants studied	
	2n=20	2n=21	2n=20	2n=21	2n=21	number	%
14	1 (33.3)	20 (20.4)	6 (35.3)	1 (5.6)	2 (28.6)	30	21.0
14+1f	1 (33.3)	1 (1.0)	—	1 (5.6)	—	3	2.1
15	1 (33.3)	46 (46.9)	6 (35.3)	8 (44.4)	2 (28.6)	63	44.1
15+1f	—	3 (3.1)	—	—	—	3	2.1
16	—	19 (19.3)	2 (11.8)	1 (5.6)	1 (14.4)	23	16.1
17	—	5 (5.1)	—	—	—	5	3.5
18	—	1 (1.0)	—	—	—	1	0.7
19	—	—	—	—	—	0	0
20	—	—	—	—	—	0	0
21	—	1 (1.0)	—	—	—	1	0.7
25	—	1 (1.0)	—	—	—	1	0.7
Not identified	—	1 (1.0)	3 (17.7)	7 (38.9)	2 (28.6)	13	9.1
Total	3	98	17	18	7	143	

Early Golden Melon and hypo- and eutriploid plants of *H. spontaneum nigrum* have been reported in the previous papers (TSUCHIYA 1952a, 1958b, 1960a, b). In Table 3 are summarized all the results including the previous findings. In agreement with the previous descriptions (TSUCHIYA 1958b, 1960a, b) of the results with *H. spont. nigrum*, most frequent were simple trisomics having $2n=15$ chromosomes with 44.1% on the average for 3 varieties investigated including *H. spont. nigrum*. Next in frequency were the diploids followed by double trisomics with $2n=16$ chromosomes. Chromosome fragments were observed in 4.2% of the examined plants; 3 plants (2.1%) had $2n=14+1$ fragment and other 3 had $15+1$ fragment.

At meiosis in all simple trisomic plants ($2n=15$) the chromosome configurations of $1_{III}+6_{II}$, $7_{II}+1_I$ or derivative types have been observed which revealed the primary nature of the extra chromosomes (TSUCHIYA 1952a, 1960a). In 16-chromosome plants appeared chromosome associations of $2_{III}+5_{II}$, $1_{III}+6_{II}+1_I$, $7_{II}+2_I$ or derivative types, disclosing that the plants were not tetrasomic but double trisomics. From these findings it may be concluded that the 17- and 18-chromosome plants were triple and quadruple trisomics (TSUCHIYA 1960f).

Triple and quadruple trisomics were obtained in 3.5% (5 plants) and 0.7% (1 plant), respectively. One triploid and a plant having $2n=25$ chromosomes were also observed. The triploid showed the chromosome configurations of 7_{III} , $6_{III}+1_{II}+1_I$ and other derivative types thereby indicating that it was an autotriploid.

Among all 143 plants obtained from the $3x \times 3x$ crosses 13 plants which have not been investigated cytologically were more or less deviating morphologically

from normal diploids and showed weak viability thereby indicating their aneuploid nature.

Consideration

The crossing results of $3x \times 2x$ and $3x \times 4x$, especially in the former combination, show that the function of the gametes of autotriploid barley is high on the male and the female side (TSUCHIYA 1952b, 1958a, 1960b). It is remarkable that the pollen fertility of autotriploid barley is so high (TSUCHIYA 1952a, b, 1953a, unpublished). Selfed autotriploids had also well functioning gametes as Tables 1, 2 and 4 show (cf. TSUCHIYA 1952a, b, 1953a).

In Table 4 the seed fertility in various combinations of $4x$, $3x$ and $2x$ is represented. The seed fertility is higher in $4x \times 2x$ and $2x \times 4x$ than in $3x \times 2x$ and $2x \times 3x$. The fertility is lowest in $3x \times 3x$ combinations but the difference from $3x \times 2x$ is not considerable. However the germination is much higher in the combinations of $2x \times 3x$, $3x \times 2x$ and $3x \times 3x$ than in $4x \times 2x$ and $2x \times 4x$, the last one not germinating at all. Thus the frequency of germinated seeds per crossed floret is lowest in $2x \times 4x$ (0%) and $4x \times 2x$ (1.13%) and highest in $2x \times 3x$ (51.8%); $3x \times 2x$ (11.9%) and $3x \times 3x$ (6.5%) being intermediate.

It is obvious that the cross fertility (Tab. 4) is very high in the crosses $2x \times 4x$ and $2x \times 3x$ in which diploids were used as the female parents. However, the germination is rather higher in $3x \times 2x$ and the reciprocal cross, $2x \times 3x$. This result shows that cross fertility (seed setting) mostly depends on the gametic function of the female parent, while the germination of the obtained seeds from a cross depends on the zygotic chromosome assortment; seeds having diploid or nearly diploid chromosome complements (for example, simple trisomics) are able to germinate but those with extremely deviating from normal diploid

Table 4. Crossing success in various cross combinations among diploids, triploids and tetraploids

Cross combinations	Number of		Fertility (%)	Number of seeds		Success (%)
	florets	seeds		sown	germinated (%)	
$4x \times 2x$	2,721	980	36.01	980	31 (3.16)	1.13
Reciprocal	77	58	75.32	58	0	0
$3x \times 2x$	931	215	23.09	215	111 (51.63)	11.92
Reciprocal	56	32	57.14	32	29 (90.62)	51.79
$4x \times 3x$	206	62	30.09	62	10 (16.13)	4.85
Reciprocal	26	10	38.46	10	0	0
$3x \times 3x$	2,482	371	14.95	360	160 (44.44)	6.45

Note: Triploids include both eu- ($2n=21$) and hypotriploids ($2n=20$).

Tetraploids in $4x \times 3x$ include eu- ($2n=28$) and hypertetraploids ($2n=29$).

complement may not germinate well.

In fact fertilization occurred frequently in the reciprocal crosses between $4x$ and $2x$ as has been seen from the relatively high cross fertility (TSUCHIYA 1958a, unpublished). The seeds developed well until about 10 days after pollination. But the content of the hybrid seeds changed to a hyaline liquid which evaporated after harvesting. Consequently almost all of the obtained seeds became extremely shrivelled and did not germinate (TSUCHIYA unpublished).

In other words, the mechanism controlling cross fertility and germination is different in polyploid barley. The former is assumed to depend mainly upon the gametic function and the latter upon the chromosome constitutions of the seeds. Thus the success in obtaining triploids from the cross $4x \times 2x$ and aneuploids from crosses of $3x$ with $2x$, $3x$ and $4x$ is assumed to be determined both factors, the number of germinated seeds per crossed floret or "success" being the result of their combined effect.

The seed fertility of $3x \times 3x$ crosses was the lowest among all possible cross combinations among $2x$, $3x$, and $4x$ plants (Tab. 4). According to the above assumption, this may be explained mainly by the low function of female and to some extent male gametes of the triploids in comparison with normal function of the gametes of diploids. The germination was, however, good in $3x \times 3x$ progeny; it ranks third following $2x \times 3x$ and $3x \times 2x$.

About 67 percent of the plants raised from selfed triploids ($3x$ and $3x-1$) had the chromosome numbers $2n=14$ and $15^{1)}$. As has been reported (TSUCHIYA 1952a, 1958b, 1960a) the simple trisomics with $2n=15$ chromosomes have good viability and grow vigorously. Double trisomics²⁾ were raised frequently and had also good viability (TSUCHIYA 1952a, 1958b, 1960a). The good viability of simple and double trisomics as well as that of normal diploids in the $3x \times 3x$ progeny may be accounted for the unexpectedly high germination of $3x \times 3x$ seeds.

Among the various chromosomal types raised from $3x \times 3x$ crosses, diploids and simple trisomics amounted to about 70% in total in the offspring of hypotriploids and to 64% in that of eutriploids. In the former ($3x-1$), when selfed, diploids and simple trisomics occurred at the same frequency (35% each) in comparison with 50% ($2x$) and 39% ($2x+1$) respectively in $(3x-1) \times 2x$ cross (TSUCHIYA 1960b). In the latter cross ($3x$), when selfed, simple trisomics were most frequent followed by diploids. This is very similar to the $3x \times 2x$ cross (TSUCHIYA 1960b). In sum total the simple trisomics were most frequent as shown in Tables 3 and 5 (cf. TSUCHIYA 1958b, 1960a). In conclusion, autotriploids

1) Three of 63 plants had $2n=14+1$ fragment.

2) Three of 26 plants had $2n=15+1$ fragment.

Table 5. Chromosome numbers of plants from various cross combinations between 2x, 3x and 4x

Chromosome numbers (2n)	Cross combinations				Total no. of plants
	4x×2x	4x×3x	3x×3x	3x×2x	
14	—	—	30 (21.0)	18 (24.3)	48
15	—	—	66 (46.2)	35 (47.3)	101*
16	—	—	26 (18.2)	12 (16.0)	38**
17	—	—	5 (3.5)	3 (4.1)	8
18	—	—	1 (0.7)	—	1
19	—	—	—	—	0
20	2 (9.5)	—	—	1 (1.4)	3
21	17 (81.0)	—	1 (0.7)	—	18
22	—	—	—	—	0
23	—	2 (28.6)	—	—	2
24	—	—	—	—	0
25	—	—	1 (0.7)	—	1
26	—	—	—	—	0
27	—	1 (14.3)	—	—	1
28	—	2 (28.6)	—	—	2
29	1 (4.8)	2 (28.6)	—	—	3
30	1 (4.8)	—	—	—	1
Not identified	—	—	13 (9.1)	5 (6.8)	18
Total	21	7	143	74	245

* Three of 101 is 14+1f.

** Four of 38 is 15+1f.

can be considered to be the best source of barley trisomics (cf. KERBER 1954; TSUCHIYA 1958b, 1960a, b).

In Table 5 the chromosome constitution of plants obtained from various cross combinations among 2x, 3x, and 4x so far carried out is represented. As Table 5 shows, almost all of the chromosome numbers from 2n=14 to 30 were observed with the exception of 2n=19, 22, 24 and 26 which have not been found in my experiments as yet. Recently, however, a plant having 2n=19 chromosomes was obtained in the progeny of a selfed autotriploid (TSUCHIYA unpublished) and also a 19-chromosome plant has been reported by MOCHIZUKI and REINBERGS (1958) in a C₄ population from colchicine induced tetraploids. Further, 26-chromosome plants have been reported in a twin plant obtained from a tetraploid hybrid (TSUCHIYA 1953c) and the progeny of induced tetraploids (DORSEY 1939; ROSENDAHL 1944). In addition, a 24-chromosome plant was reported by ROSENDAHL (1944). From the results mentioned above it may be concluded that in barley all plants with chromosome numbers from 2n=14 to 30 are viable though the viability is different by the chromosome constitution; plants with 2n=22 chromosomes have not been reported as yet. Haploids (2n=7) were also viable (TOMETORP 1939; SUZUKI 1959; TSUCHIYA unpublished). In conclusion it can be said that the production of autotriploids from crosses between 4x and 2x is very difficult owing to the great difficulty in germination of the hybrid seeds

(TSUCHIYA 1958a; cf. SMITH 1951). On the contrary, the production of aneuploids with various chromosome constitutions is rather easy from crosses of autotriploids with $2x$, $3x$ and $4x$, thanks to the relatively high function of $3x$ gametes in various cross combinations and the unexpectedly good germination of the obtained seeds as has been clearly shown in previous reports (TSUCHIYA 1952b, 1960b; cf. 1958b, 1960a) and in the present one (Tabs. 4 and 5).

Summary

In this paper the experimental results of $3x \times 3x$ crosses are described with some considerations on cross fertility, seed germination, and the chromosome complements of the derived plants from all possible combinations among $2x$, $3x$, and $4x$ barley. The main results are summarized as follows:

1. The average seed fertility of hypotriploids ($2n=20$) is similar to that of eutriploids ($2n=21$) (Tab. 1) though a variation was observed between strains or between plants within the strains.

2. The germination of seeds is shown to be significantly higher in the eutriploids than in the hypotriploids. No striking difference has been observed in the average germination percentage between varieties with the exception of a $3x$ -hybrid which showed a relatively high germination (Tab. 1). However, germination varies in different plants of a variety within the group having the same chromosome numbers (Tab. 2).

3. In the selfed progeny of the hypotriploids ($3x-1$), chromosome constitutions of $2n=14$, $14+1f$, 15 and 16 were observed among which 14 - and 15 -chromosome plants were most frequent amounting to 35% each. In the selfed progenies of eutriploids ($3x$) were observed the chromosome numbers of $2n=14$, 15 , 16 , 17 , 18 , 21 and 25 ; The most frequent were 15 -chromosome plants followed by diploids and 16 -chromosome plants (Tab. 3).

4. From the study of meiotic chromosome behavior it was concluded that (1) the extra chromosomes of all trisomics were of primary nature, (2) the 16 -chromosome plants were not tetrasomic but double trisomics, (3) 17 - and 18 -chromosome plants were triple and quadruple trisomics, and a 21 -chromosome plant was autotriploid.

5. It is very easy to produce many aneuploid types in barley by crossing triploids with $2x$, $3x$, and $4x$, though it is very difficult to produce autotriploids from crosses between $4x$ and $2x$.

References

- DORSEY, E. 1939. Chromosome doubling in the cereals. *Jour. Hered.* **30**: 393-395.
- KERBER, E. R. 1954. Trisomics in barley. *Science* **120**: 808-809.
- MOCHIZUKI, A. and REINBERGS, E. 1958. A nineteen-chromosome barley plant. *Canad. Jour. Plant Sci.* **38**: 106-110.
- ONO, T. 1946. Fertile tetraploid barley obtained by the colchicine method. A preliminary note (Jap.). *La Kromosomo* **2**: 81-83.
- 1947. A short report on polyploids in *Hordeum* and *Eleusine* (Jap.). *Jap. Jour. Genet.* **22**: 55-56.
- ROSENDAHL, G. 1944. Cytologische Untersuchungen an tetraploiden Gersten. *Kühn-Arch.* **60**: 238-252.
- SMITH, L. 1951. Cytology and genetics of barley. *Bot. Rev.* **17**: 1-51.
- SUZUKI, H. 1959. Karyomorphological studies in barley (Jap. with Eng. Rés.). *Bull. Brew. Sci.* **5**: 43-57.
- TOMETORP, G. 1939. Cytological studies on haploid *Hordeum distichum*. *Hereditas* **25**: 241-254.
- TSUCHIYA, T. 1952a. Cytogenetics of hypo-triploid barley and its progeny (Jap. with Eng. Rés.). *Mem. Beppu (Women's) Univ.* **2**: 19-42.
- 1952b. Cytogenetic studies of a triploid hybrid plant in barley (Jap. with Eng. Rés.). *Seiken Zihô* **5**: 78-93.
- 1953a. Some notes on autotriploid barley (Jap. Abst.). *Jap. Jour. Genet.* **28**: 189.
- 1953b. Fertility of autotetraploids and their hybrids in barley, I. *Seiken Zihô* **6**: 46-52.
- 1953c. Cytogenetic investigations of twin plants found in a tetraploid barley hybrid. *Seiken Zihô* **6**: 53-56.
- 1958a. Studies on cross compatibility of diploid, triploid and tetraploid barleys, I. *Bot. Mag. Tokyo* **71**: 48-52.
- 1958b. Studies on the trisomics in barley, I. (Jap. with Eng. Rés.). *Seiken Zihô* **9**: 69-86.
- 1960a. Cytogenetic studies on trisomics in barley. *Jap. Jour. Bot.* **17**: 177-213.
- 1960b. Studies on cross compatibility of diploid, triploid and tetraploid barleys, II. *Jap. Jour. Genet.* **35**: 337-343.

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