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On the occurrence of aneuploidy in the offspring of the artificially induced auto-tetraploid plants in Japanese radish (*Raphanus sativus* L.) and Chinese cabbage (*Brassica pekinensis* Rupr.)

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INTRODUCTION

Since the establishment of the colchicine technique a large number of the auto-polyploid plants or strains have been raised artificially in various cultivated species, and studies on the character and behaviour of those polyploid plants or strains have hitherto been to some extent accomplished. Excepting only few cases, with most examples we have not been, however, quite successful in obtaining results of certain practical value. Such a situation is considered to have been effected through the following circumstances which prevail on the breeding procedures accompanying chromosome doubling: that there usually exist urgent needs for preparing a large number of test strains covering many relating garden varieties, and also for rather many years of painstaking work on practical breeding (See Fukushima, Tokumasu and Oguro, 1949); that the auto-polyploid plants raised already in various crops have shown certain difficulties in the maintenance of their exact polyploid nature throughout successive generations. Moreover, with some of those tetraploid plants somewhat easy reduction of chromosomes towards the original diploid state during the course of sexual reproduction has also been reported (e.g., Kondo and Karitani (1947) in rice; Noguchi (1950) in *Helianthus annuus* L.). The present authors have also noticed not infrequently the spontaneous occurrence of a similar phenomenon in some strains of auto-tetraploid raised in several varieties

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of Cruciferous vegetables. The exact nature of those situations is yet quite obscure. To get a clue towards solving the problems concerning it may be a primary necessity in order to obtain clear insight into the chromosomal behaviours as well as the breeding procedures of those polyploid individuals or strains.

The present report covers the results of cytogenetic studies carried out with the polyploid individuals, including some hyper- and hypoploid ones, obtained in the offspring of the auto-tetraploid individuals which have been colchicine-induced by the senior author some years ago in the strains of Japanese radish and Chinese cabbage.[1]

MATERIAL AND METHOD

The auto-tetraploid strains of Japanese radish 'Osaka-Shijûnichi', 2n=36, and that of Chinese cabbage 'Tsujita', 2n=40, both of which were raised artificially by the senior author, were used as the materials.[2] These polyploid strains were maintained throughout several generations after their origin under the controlled pollinations or the selfing through bud-pollination technique. As compared with the normal diploid seeds, the seeds from autotetraploid individuals were clearly larger in size and took more or less uniform appearance, excepting a certain amount of abnormal seeds which occurred along with the normal large ones, but took much smaller sizes than the diploid seeds. The frequency occurrence of those abnormally small seeds varied among the individuals belonging to the same strain, and also showed a certain variation year after year. As well be made clear elsewhere, it was ascertained that those abnormally small seeds from the tetraploid individuals are autotriploid in nature.

The tetraploid individuals used in the present investigations were chosen from the strains which showed rather high occurrence of the exceptional seeds; i.e., Plant No. 3 (1950–51) in Japanese radish, *Raphanus sativus* L., and Plant No. 6 (1949–50) in Chinese cabbage, *Brassica peckinensis* Rupr., were used as the mother plants, the offspring of which were treated exclusively in the present report. Seeds produced

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[1] The present work was undertaken as a part of the general inquiries on the problems of seed growing in vegetable crops. The authors gratefully acknowledge the subsidies given from the Scientific Research Fund of the Ministry of Education.

[2] Since the season of 1937–38 a large number of the autotetraploid strains in Japanese radish and several *Brassica* species have been colchicine-induced by Fukushima, and some of those autopolyploid strains have hitherto been maintained and used as the materials for various studies. The two strains treated in the present investigation also have their origin among the first auto-tetraploid forms grown in the season of 1939–40.

under the open-pollination were collected from each individual, and their sizes were compared. Radish seeds were sown at 17th Sept. 1951 and the cabbage seeds at 13th Nov. 1951. The diploid seeds were sown as the controls at the same date as the polyploid ones. Examinations upon certain morphological characters were carried out with the individuals grown up from those seeds. With a part of those individuals the meiotic behaviours of chromosomes in their pollen mother-cells were observed, and their somatic chromosome numbers were ascertained at the same time. Cytological studies were carried through exclusively on the aceticorcein smear preparations of the pollen mother-cells.

RESULTS OF OBSERVATION

I. Size of seed and other morphological characters

The longer diameters of seeds obtained from the radish Plant No. 3 varied markedly as shown in Fig. 1, which was compiled on the data

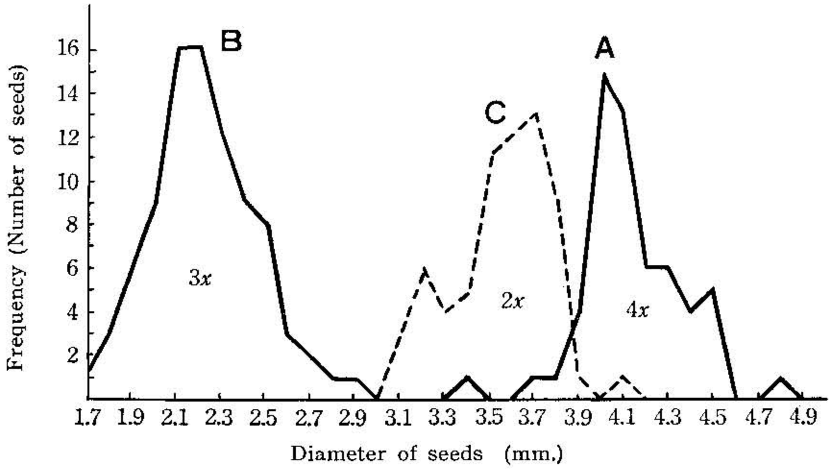

Fig. 1. Comparisons of the sizes of seeds with Japanese radish plants in a polyploidal series. N.B. A, 4*x* seeds; B, 3*x* seeds; C, 2*x* seeds.

of measurements with 231 seeds in total. They were dispersed into two distinct polygons; i.e. A, consisted of 144 seeds, and B, consisted of 87, whose mean values were 4.21 and 2.22 mm. respectively. Fig. 2 represents similar situation of seeds produced on the Plant No. 6 in Chinese cabbage. Two hundred and thirty-two seeds were distributed into two distinct polygons, each consisted of 141 (A) and 91 (B) respectively, their mean diameters being 2.20 and 1.36 mm. In both the species, the seeds showing larger mean diameters are considered to correspond to the tetraploid ones, and the rest with smaller diameters to the triploid.

The polygons denoted with C in both figures were compiled from the data of diploid seeds respectively.

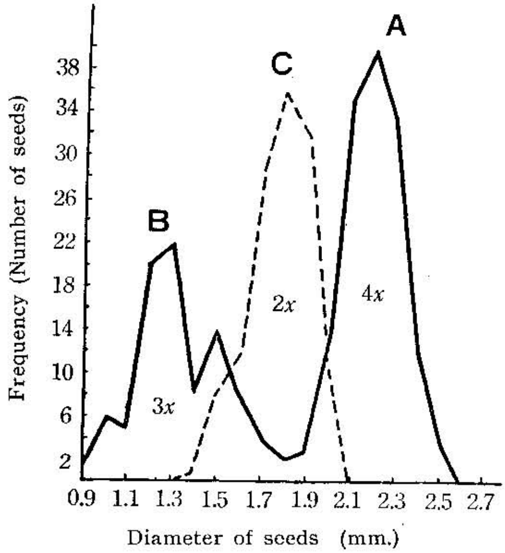

Fig. 2. Comparisons of the size of seeds with Chinese cabbage plants in a polyploidal series. N.B. A, 4*x* seeds; B, 3*x* seeds; C, 2*x* seeds.

Germinability of the small seeds, which are well defined as being 3*x* in nature, was rather normal and well comparable with the diploid ones. The 4*x* seeds showed, in turn, somewhat weaker germination capacity than the diploid ones.

Fully expanded cotyledons developed from those three kinds of radish seeds showed the following values in their breadth measurements: 27.0 ± 1.4 mm. in 4*x*, 11.7 ± 1.8 mm. in 3*x*, and 19.0 ± 1.3 mm. in 2*x* seedlings. The similar data of measurements on Chinese cabbage seedlings were as follows: 27.0 ± 2.2 mm. in 4*x*, 13.1 ± 1.8 mm. in 3*x*, and 21.5 ± 3.2 mm. in 2*x* seedlings respectively.

The first foliar leaves on the 4*x* seedlings showed most rapid expansion as compared with those of 2*x* or 3*x*. The 3*x* seedlings expanded their first leaves rather slowly, but they realized the most vigorous growing afterwards. Thus the number of leaves expanded before a certain date had shown clear correspondence to the mode of growing in each seedling, representing its developmental velocity. For examples, until 22nd Jan. 1952 4*x* seedlings had developed 28.3 leaves in average, 3*x* ones 30.4 leaves, and the normal 2*x* ones as many as 40.9 leaves. But the blooming had begun earliest in 2*x* individuals, being followed by 4*x* ones, and that in 3*x* ones had fallen behind the former two, sharply concurring to the growth pattern and vigour of the plants with different nuclear contents.

To compare the size of cells, the major diameters of stomatal guard-cells on the undersurface of fully expanded leaves were measured. The number of chloroplasts in each guard-cell was also counted as another indicator of nuclear structures (See Table 1). Cells of an individual grown up from the exceptionally small seeds in both the species took an intermediate size between those of the $4x$ and $2x$ ones, directly revealing that the individual is of $3x$ in nature. The number of chloroplasts contained in each cell showed a quite similar mode of variation as those of the cell size. The theoretical ratio of the diameters of those three kinds of the cells of different nuclear contents is calculated to be $\sqrt[3]{2a} : \sqrt[3]{3a} : \sqrt[3]{4a} = 1 : 1.14 : 1.26$. And the following actual ratios were obtained respectively; $1 : 1.19 : 1.37$ in radish, and $1 : 1.11 : 1.46$ in Chinese cabbage.

Table 1. Comparison of major diameters of stomatal guard-cells and number of chloroplasts in each cell.

Polyploidy		Average diameter of guard-cells (micrometer unit)	Number of cells measured	Average number of chloroplasts in each cell	Number of cells observed
Japanese radish	$4x$	19.2±2.3	222	11.4±2.1	186
	$3x$	16.6±2.5	232	8.4±1.4	232
	$2x$	14.0±1.8	238	5.4±1.1	268
Chinese cabbage	$4x$	21.8±1.7	165	10.3±1.7	204
	$3x$	16.6±1.4	195	8.7±1.6	223
	$2x$	14.9±1.7	183	6.9±1.3	268

II. Cytological observations

Meiotic divisions of the PMCs were examined with several individuals of Japanese radish and Chinese cabbage, which had grown up from the seeds collected from the auto-tetraploid plants selected. As stated elsewhere, all the plants grown from the seeds of exceptionally small sizes were ascertained to be of $3x$, chromosomal behaviours of PMCs in these $3x$ individuals are reproduced in the following:[1]

(a) Triploid radish, $2n=27$.

At heterotypic metaphse several tri- and univalent chromosomes

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[1] All the micrographs or camera lucida drawings of the chromosomes obtained were not presented here, and the reproduction of those figures was postponed to some future occasions, in which the problems on the variation or alteration of chromosome association during the years of repeated reproduction in the auto-polyploid forms will be thoroughly treated.

appeared always along with the bivalent ones. Tri- and bivalent chromosomes took regular orientation on the equatorial plane, but univalents were scattered outside of the plate or dispersed on the surface of the spindle sphere. Most III-chromosomes took either one of the following three kinds of shapes at I-Metaphase; , , . That of  shape occurred very rarely. No IV-chromosomes was encountered at all. Frequencies of various configurations of the chromosome associations are reproduced in Table 2. The somatic chromosome number of these individuals was exactly determined as 27, i.e., three times the basic set of 9. As shown in Table 2 each nucleus contained 7.35 III-chromosomes in average. From the mode of chromosome associations it is quite certain that those individuals observed would be of the auto-triploid in nature.

Table 2. Chromosome associations at I-Metaphase in $3x$ Japanese radish.

Configuration	Frequency
9_{III}	21
$8_{III}+1_{II}+1_{I}$	41
$7_{III}+2_{II}+2_{I}$	35
$6_{III}+3_{II}+3_{I}$	21
$5_{III}+4_{II}+4_{I}$	4
$4_{III}+5_{II}+5_{I}$	3
Total number of PMCs	125

Table 3. Frequency occurrences of lagging chromosomes and chromosomal bridges at I-Anaphase in $3x$ Japanese radish.

I-Anaphasic figures	Frequency	
with no laggard	89	59.0 %
with 1 laggard	39	38.4 %
〃 2 laggards	14	
〃 3 〃	4	
〃 4 〃	1	
with 1 bridge	3	2.6 %
〃 2 bridges	1	
Total number of PMCs	151	100 %

At I-Anaphase there appeared some laggards lying behind in the equatorial region, which are duly ascribed as a part of univalent at I-Metaphase. The tri- and bivalent chromosomes disjoined and proceeded to either one of the poles, and some univalents situated in the neighbourhood of the poles went intact to the respective poles with the disjoined chromosomes. And the rest of the univalents situated rather remotely from the equatorial region, remained as laggards between the poles, also showing, in turn, some movement towards the equator and their splitting. In some rare cases, a chromosomal bridge, which discloses the occurrence of some inversions, was encountered at I-Anaphase. Frequency occurrences of those lagging univalents and chromosomal bridges are presented in Table 3. Tri-polar spindles were also met with in some rare occasions.

As the results of uneven distribution of chromosomes at I-Anaphase, the number of chromosomes constituting the homotypic plates showed marked variation between 9 and 18, 13 or 14 being the most frequent. In or out of the equatorial plate consisting of the diad chromosomes there appeared a few monad ones, each of which was considered to be the split half of the univalent. Table 4 is compiled by the data of frequency distribution of chromosomes on each homotypic plate, which was composed exclusively of the diad chromosomes. As may be duly expected, there occurred also some lagging chromosomes at II-Anaphase, and these situations will be, again, ascribed as the cause of abnormal polyads appearing at sproad stage.

Table 4. Frequency distributions of diad chromosomes at II-Metaphasic plates in $3x$ Japanese radish.

Number of diad chromosomes in each II-plate	9	10	11	12	13	14	15	16	17	18	Total number of II-plates
Frequency	1	1	8	23	40	32	22	5	1	1	134

(b) Triploid Chinese cabbage, $2n=30$.

As the basic chromosome set in Chinese cabbage is 10, the autotriploid individuals are consist of 30 chromosomes. A quite similar association procedure of chromosomes as in $3x$ radish was met with at I-Metaphase of microsporogenesis (See Table 5). The average number of III-chromosomes appearing in each nucleus was 8.42. Behaviours of chromosomes in both the first and the second divisions were quite similar to those in $3x$ radish plants. Table 6 shows the lagging chromosomes appearing at I-Anaphase. Table 7 represents the frequency occurrence of monad chromosomes which are situated in the vicinity of sister plates at II-Metaphase. No I-Anaphasic bridge was encountered at all.

Table 5. Chromosome associations at I-Metaphase in 3*x* Chinese cabbage.

Configuration	Frequency
10_{III}	18
$9_{III}+1_{II}+1_{I}$	29
$8_{III}+2_{II}+2_{I}$	23
$7_{III}+3_{II}+3_{I}$	14
$6_{III}+4_{II}+4_{I}$	5
$5_{III}+5_{II}+5_{I}$	1
Total number of PMCs	90

Table 6. Frequency occurrences of lagging chromosomes at I-Anaphase in 3*x* Chinese cabbage.

I-Anaphasic figures	Frequency	
with no laggard	28	35.4 %
with 1 laggard	26	64.6 %
〃 2 laggards	15	
〃 3 〃	8	
〃 4 〃	2	
Total number of PMCs	79	100 %

Table 7. Frequency occurrences of II-Metaphasic plates with or without monad chromosomes in 3*x* Chinese cabbage. Countings were obtained with each set of sister plates.

Number of monad chromosomes appeared in		Frequency	
one plate	another plate		
0	0	68	52.7 %
1	1	25	47.3 %
0	2	18	
2	2	9	
1	3	8	
3	3	1	
Total number of PMCs		129	100 %

Homotypic plates, which consisted exclusively of the diad chromosomes and did not contain any monad ones, showed marked variation as presented in Table 8. Plates consisted of 10 or 20 chromosomes,

Table 8. Distributions of chromosomes at II-Metaphasic plates in $3x$ Chinese cabbage.

Number of chromosomes in each II-plate	11	12	13	14	15	16	17	18	19	Total number of plates
Frequency	2	3	17	17	27	21	12	2	1	102

i.e., the extreme numbers were not obtained, and the plates of 15 occurred most frequently. Several lagging monad chromosomes were also observed at II-Anaphase (See Table 9). In each $3x$ mother-cell there appeared 1.58 univalents in average at I-Metaphase (From Table 5), and 1.11 laggards also at I-Anaphase (From Table 6). Monad chromosomes derived through splitting of diads had amounted to 0.67 per cell in average (From Table 7). From the data calculated above it may be safely deduced that the 70 per cent of the univalents appeared at I-Metaphase remained as laggards on the I-Anaphasic spindles, and that the 56 per cent of the latter, again, have accomplished their splitting, while the remaining ones have proceeded intact towards either one of the poles. Furthermore, Table 7 shows that the monad chromosomes appearing in each II-Metaphasic nucleus were 1.24; and Table 9 reveals that the laggards appearing at II-Anaphase amounted to 1.03 per cell. So that the 80 per cent of the monad chromosomes occurring at I-Anaphase might have appeared again as the laggards at II-Anaphase. There is need to mention here, in addition, that some of the lagging chromosomes would have been excluded from the division spheres, and some of them contributed to the formation of supernumerary microspores.

Table 9. Lagging chromosomes apparent at II-Anaphase in $3x$ Chinese cabbage. Countings were made with each set of homotypic spindles in a PMC.

Number of laggards appeared on		Frequency
one spindle	another spindle	
0	0	50
0	1	11
0	2	11
1	1	11
1	2	6
2	2	5
1	4	1
Total number of PMCs		95

All the plants grown up from the seeds of larger sizes, which were produced on the auto-tetraploid individuals, disclosed that they could be divided into two different categories, the former of which was of euploid, and the later was of certain heteroploid, i.e., hyper- and hypo-tetraploid. Results of cytological examinations with those plants are presented in the following:

(c) Tetraploid radishes.

(i) Eu-tetraploid plants, $2n=36$.

Two plants, Nos. 10 and 11, were determined to contain 36 chromosomes in somatic state, revealing that they are of eutetraploid. Reduction divisions of the PMCs in these plants proceeded quite regularly, forming exclusively IV- and II- chromosomes at I-Metaphase (See Fukushima, Tokumasu, and Oguro, 1949). Any other multivalent chromosomes were not encountered at all. But the univalent chromosomes were noticed in some rare cases. Table 10 represents the frequency appearances of the IV-chromosomes per nucleus in Plant Nos. 10 and 11 respectively. Heterotypic anaphase proceeded quite regularly in those plants. In Plant No. 11, however, only one laggard appeared not infrequently. Two or more laggards did not appear in any one cell at I-Anaphase. Homotypic nuclear division progressed also in a regular manner, excepting the occurrence of plates containing one or two chromosomes oriented outside of the plates. So the homotypic laggards were met with at some rare occasions. The number of chromosomes oriented on each II-plate is shown in Table 11. In Plant No. 10 chromosomal bridges were also encountered at I- and II-Anaphases.

Table 10. Frequency occurrences of IV chromosomes at I-Metaphase in $4x$ Japanese radish.

Number of IV-chromosomes in each nucleus		9_{IV}	8_{IV}	7_{IV}	6_{IV}	5_{IV}	4_{IV}	3_{IV}	2_{IV}	1_{IV}	0_{IV}	Total number of PMCs
Frequency	Plant No. 10				5	9	8	14	8	4	2	50
	Plant No. 11	1	1	3	10	9	16	11	8	3	1	63

Table 11. Frequency occurrences of chromosomes arranged on each II-Metaphasic plate in $4x$ Japanese radish.

Number of chromosomes in each II-plate		16	17	18	19	20	Total number of PMCs
Frequency	Plant No. 10		15	80	16		111
	Plant No. 11	2	17	109	17	1	146

(ii) Hypo-tetraploid plants, $2n=34$.

Two plants, Nos. 6 and 21, were ascertained to be of hypo-tetraploid, containing 34 chromosomes in somatic state. Association procedures of I-Metaphasic chromosomes were $(7\sim2)_{\mathrm{IV}}+(2\sim0)_{\mathrm{III}}+(12\sim0)_{\mathrm{II}}+(2\sim0)_{\mathrm{I}}$ in Plant No. 6, and $(6\sim0)_{\mathrm{IV}}+(2\sim0)_{\mathrm{III}}+(15\sim2)_{\mathrm{II}}+(2\sim0)_{\mathrm{I}}$ in Plant No. 21. From these facts the two missing chromosomes in both plants were duly inferred to be non-homologous to each other. At I-Anaphase one or two univalents appeared sometimes as laggards, and these univalents did not, in general, split to their halves and remained intact throughout the I-division, and some of them were excluded out of the division sphere. In Plant No. 6 the latter univalents were observed not infrequently, i.e., in the 11.6 per cent of cells examined. Tripolar spindles were also found in some rare cases. Table 12 show the frequency occurrences of the number of chromosomes arranged on the sister plates of II-Metaphase. Division processes after the II-Anaphase stage proceeded quite regularly, resulting in the formation of normal sporads.

Table 12. Chromosomes distributed to each II-Metaphasic plate in hypo-tetraploid, $4x$-2, Japanese radish.

Number of chromosomes in each II-plate		15	16	17	18	19	20	Total number of plates
Frequency	Plant No. 6	11	16	50	13	8	1	99
	Plant No. 21	7	19	32	18	2		78

(iii) Hyper-tetraploid plant, $2n=37$.

Plant No. 20 had contained 37 chromosomes in somatic state, consisting of the tetraploid number of chromosomes and an extra one in addition. Chromosome associations at I-Metaphase showed the configuration, $(7\sim0)_{\mathrm{IV}}+(1\sim0)_{\mathrm{III}}+(18\sim3)_{\mathrm{II}}+(1\sim0)_{\mathrm{I}}$. One pentavalent chromosome was, however, observed on some rare occasions. One or two lagging chromosomes appeared at II-Anaphase. Diad chromosomes distributed on the II-Metaphasic plates showed the variation depicted in Table 13. A few monad chromosomes usually occurred at II-Metaphase, though such plates were excluded from the compilation of the table.

Table 13. Chromosome distributed to each II-Metaphasic plate in hyper-tetraploid, $4x+1$, Japanese radish.

Number of chromosomes in each II-plate	16	17	18	19	20	21	Total number of plates
Frequency	2	4	21	27	4	1	59

(d) Tetraploid Chinese cabbage.

(i) Eu-tetraploid plants, $2n=40$.

Among the individuals grown up from the seeds of larger sizes, as stated elsewhere, two plants, Nos. 1 and 2, were ascertained to be of eu-tetraploid. In most I-Metaphasic nuclei there appeared IV- and II-chromosomes, and the exceptional occurrence of a few I- and III-chromosomes was noticed in Plant No. 1. Table 14 shows the number of IV-chromosomes occurring in each I-Metaphasic nucleus, their average number amounting to 4.33 and 4.83 in Plant Nos. 1 and 2 respectively. Lagging chromosomes at I-Anaphase were observed to be rather usual. Chromosomal bridges were noticed rarely in Plant No. 2 (See Table 15). The future destiny of these laggards was quite similar to that of the laggards described above. Accompanying Tables 16 and 17 will suffice to give a clear insight into the division processes in these eu-tetraploid individuals. Some irregularities, such as the cell with

Table 14. Frequency occurrences of IV-chromosomes at I-Metaphase in $4x$ Chinese cabbage.

Number of IV-chromosomes in each nucleus		8_{IV}	7_{IV}	6_{IV}	5_{IV}	4_{IV}	3_{IV}	2_{IV}	1_{IV}	Total number of PMCs
Frequency	Plant No. 1	1	2	3	6	4	3	4	1	24
	Plant No. 2	3	6	11	11	11	5	5	1	53

Table 15. Occurrences of lagging chromosomes and chromosomal bridges at I-Anaphase in $4x$ Chinese cabbage.

PMCs	Frequency	
	Plant No. 1	Plant No. 2
with no laggard	83	82
〃 1 laggard	14	9
〃 2 laggards	2	
〃 1 bridge		2
Total number of PMCs	99	93

Table 16. Frequency of chromosomes composing each II-Metaphasic plate in $4x$ Chinese cabbage.

Number of chromosomes in each II-plate		18	19	20	21	22	Total number of PMCs
Frequency	Plant No. 1	1	13	58	10	1	83
	Plant No. 2		12	82	15		109

three homotypic spindles, or with the homotypic bridges, were also met with in a few exceptional cases.

(ii) Hyper-tetraploid plant, $2n=43$.

A Chinese cabbage, Plant No. 3, had contained 43 chromosomes in somatic state, the number being $4x+3$. Chromosome conjugations at I-Metaphase had shown the configuration; $(8\sim0)_{IV}+(3\sim0)_{III}+(18\sim3)_{II}+(2\sim0)_{I}$. Only in some exceptional cases one pentavalent was encountered. From the mode of chromosome associations it would be duly appreciated that the three extra chromosomes added may be non-homologous among each other. From one to three chromosomes lagging behind were observed at I-Anaphase. As shown in Table 18,

Table 17. Lagging chromosomes apparent at II-Anaphase in $4x$ Chinese cabbage. Countings were made with each set of homotypic spindles in a PMC.

Number of laggards in		Frequency	
one spindle	another spindle	Plant No. 1	Plant No. 2
0	0	28	84
0	1	2	11
1	1		1
0	2		1
Total number of PMCs		30	97

Table 18. Frequency occurrences of chromosomes composing each II-Metaphasic plate in hyper-tetraploid, $4x+3$, Chinese cabbage.

Number of chromosomes in each II-plate	19	20	21	22	23	24	Total number of PMCs
Frequency	8	17	50	31	7	2	115

II-Metaphasic plates consisted of from 19 to 24 chromosomes. One or more supernumerary II-spindles were observed not infrequently. A mothercell was encountered, containing a large II-plate of 43 chromosomes, which may have been derived through the restitution phenomenon. At II-Anaphase some lagging chromosomes occurred usually.

III. Fertility

Microspores at sporad stage showed some irregularities in all the polyploid individuals examined. The types and frequencies of those abnormal sporads are shown in Table 19. The hypo-tetraploid radish, Plant No. 6, $2n=34$, and the hyper-tetraploid Chinese cabbage, Plant No. 3, $2n=43$, showed the most prominent abnormality, the extents of

Table 19. Frequency occurrences of various types of sporads formed by polyploid and aneuploid individuals in Japanese radish and Chinese cabbage.

		Polyploidy		Types of sporads*									Total number of sporads	% of sporads other than ④
				①	②	③	④	⑤	⑥	⑦	⑧	⑨		
Frequency	Japanese radish	3x		1	–	–	850	6	2	–	–	–	859	1.0
		4x	Plant No. 10	1	–	–	361	2	–	–	–	–	364	0.8
			Plant No. 11	–	–	–	520	3	–	–	–	–	523	0.6
		4x−2	Plant No. 6	–	–	–	351	90	95	18	3	1	558	37.1
			Plant No. 21	1	2	–	348	2	–	–	–	–	353	1.4
		4x+1		–	–	–	750	–	–	–	–	–	750	0.0
	Chinese cabbage	3x		–	–	–	482	29	2	–	–	–	513	6.0
		4x	Plant No. 1	–	–	–	516	16	3	–	–	–	535	3.6
			Plant No. 2	–	–	–	365	5	1	–	–	–	371	1.6
		4x+3		–	1	–	328	42	3	2	–	–	376	12.8

N.B. * Type ④ denotes the normal tetrad; type ⑤ or ⑦ the pentad or heptad; and type ① the monad.

which far exceeded the expectations derived from the highly irregular behaviours of their meiotic chromosomes. Namely, in those plants pentads or still higher polyads were produced much more frequently than the estimations due to the results of karyological observations. The causes of such a situation is yet, however, not quite certain to the authors. The 3*x* individuals had shown, in turn, only a slight amount of abnormalities in the formation of their sporads, though each tetrad consisted of four spores, taking various sizes and markedly differing in each nuclear content. Thus again, the situation seems not to concur with the expectations deduced from the chromosomal behaviours.

Matured pollen grains were stained with the aceto-carmine, and the morphologically normal and stainable grains were identified as the viable ones. From Table 20, it is clear that the 3*x* plants of both the radish and Chinese cabbage have shown somewhat low degrees of pollen viability, but the percentage values were remarkably higher than those of the auto-triploid plants in various other plant species. Thus the triploidy accompanying such a comparatively high pollen viability seems to be confined to the special characteristics of *Brassica* and *Raphanus*. The radish hypo-tetraploid, Plant No. 6 (4*x*−2), and the hyper-tetraploid Chinese cabbage, Plant No. 3 (4*x*+3), both of which had produced remarkably higher percentages of abnormal polyads, showed clearly, in

Table 20. Morphological viability of pollen grains of polyploid and aneuploid individuals in Japanese radish and Chinese cabbage.

	Chromosomal constitution		Number of pollen grains examined	Number of viable pollen grains*	% of viable pollen grains
Japanese radish	$2x$		1377	1265	91.9
	$3x$		1553	1266	81.5
	$4x$	Plant No. 10	655	645	98.5
		Plant No. 11	1064	1022	96.1
	$4x-2$	Plant No. 6	2426	2019	83.2
		Plant No. 21	776	749	96.5
	$4x+1$		1385	1335	96.4
Chinese cabbage	$2x$		598	592	99.0
	$3x$		1818	1597	86.9
	$4x$, Plant No. 2		1099	1076	97.9
	$4x+3$		769	711	92.5

N.B. * Viability of pollen grains was only inferred from their outward morphology and the staining reaction to aceto-carmine of their plasmatic inclusions.

concurrence with the above situation, slightly lower values of pollen viability than those values in the other tetraploid members. Among the other members of the present tetraploid strains, however, there could not be noticed any clear differences in pollen fertility.

Diameters of the socalled viable pollen grains were measured and compared (See Table 21). Diameters of the grains produced on the $3x$ individuals showed always much greater variability than those of $4x$ or $2x$ ones, though the average diameter took the intermediate value of the latter two. The above circumstances were considered to concur well with those of the meiotic procedures stated elsewhere. Any clear differences in the sizes of the viable pollen grains could not be obtained among the various tetraploidal forms treated above, so it may be ascertained that such a slight variation in the gametic content of chromosomes, extending only to the addition or subtraction of one or two chromosomes, could not be reflected so much on the size of pollen grains.

All the polyploid individuals, including some aneuploid ones, showed rather good setting of the pods after blooming, not showing any noticeable differences among the sister individuals. Owing to the adverse conditions of growing, the exact nature of which could not be made certain, the seed development in Chinese cabbage was very poor, so the

Table 21. Comparisons of diameters of pollen grains in polyploid and aneuploid individuals in Japanese radish and Chinese cabbage.

Chromosome constitution		Classes of diameters of pollen grains (micrometer unit) 15	16	17	18	19	20	21	22	23	24	25	26	Total number of pollen grains	Mean diameter
Japanese radish	$2x$	10	121	134	197	38	9	1						510	17.32
	$3x$	6	8	15	45	67	25	28	18	11	1		1	225	19.30
	$4x$				1	11	101	126	110	12	1			362	21.03
	$4x-2$				11	41	98	131	106	25	11	1		424	20.95
	$4x+1$				2	22	91	112	105	36	13	1		382	21.21
Chinese cabbage	$2x$	25	58	56	50	21	1							211	16.94
	$3x$			5	21	57	129	126	72	34	9		1	454	20.64
	$4x$				2	7	26	59	70	54	33	21	5	277	22.22
	$4x+3$				1	11	23	52	52	36	23	12	3	213	21.98

data of their seed fertility were discarded. The radish strains, in turn, had provided very reliable data of seed fertility as shown in Table 22. It is worthy of note that the $3x$ individuals of radish produced a fairly large number of good seeds, well competing with those of the $2x$ or $4x$ individuals. Furthermore, the authors could not obtain certain clear differences in seed fertility among the eu-tetraploid and hyper-tetraploid radishes treated in the present studies.

Table 22. Comparisons of seed-fertility in polyploid and aneuploid individuals in Japanese radish.

Chromosome constitution	Number of pods produced	Number of viable seeds	Number of non-viable seeds	Viable seeds/pod	Non-viable seeds/pod
$2x$	429	992	50	2.31	0.12
$3x$	1172	1808	237	1.54	0.20
$4x$	397	697	9	1.76	0.02
$4x-2$	314	550	50	1.75	0.16
$4x+1$	350	603	34	1.72	0.10

CONSIDERATIONS

In the offspring of the autotetraploid plants raised artificially, it is generally experienced that there usually appear the hyper- and hypo-tetraploid individuals, together with the eu-tetraploid ones. Aneuploidy encountered in the offspring of the present tetraploid strains of *Raphanus* and *Brassica* is a quite similar example. Since the chromosome numbers of the mother plants had not been ascertained beforehand, the exact origin of those aneuploid individuals is not quite certain, but the results of cytological observations with the eu-tetraploid individuals, which have been produced along with those aneuploid ones in the offspring, will suffice to make the situation clear. Uneven distribution of chromosomes at I-Anaphase has occurred not infrequently. As shown in Tables 11 and 12, even from 25 to 30 per cent of the II-Metaphasic plates observed consisted of the chromosomes, whose number was more or less the exact diploid number. The similar phenomenon was also reported by other workers in some species of *Brassica*. Howard (1939 a) reported that in the autotetraploid *B. oleracea* L. there appeared the II-Metaphasic plates consisting of from 16 to 20 chromosomes, the regular plate of 18 amounting to 57 per cent of the plates examined. Yakuwa (1944) also obtained the similar data from his autotetraploided *B. chinensis*. L. Chromosomes constituting the homotypic plates varied between 17 and 22, and the frequency of the regular plate of 20 chromosomes was 64.4 per cent. If the homotypic plates consisting of the unbalanced chromosomes, which have been formed through the uneven chromosome distribution, are capable of growing up into the mature viable gametes, the hyper- or hypo-tetraploid zygotes will naturally result.

It is generally appreciated that the sterility phenomenon accompanying the autopolyploidy would be mainly derived through the meiotic irregularities caused by the occurrence of multivalent associations of homologous chromosomes. As mentioned previously, the eutetraploid individuals of both the radish and Chinese cabbage did not show any great irregularities in their meiotic processes. At heterotypic metaphase, the most chromosomes appeared in IV or II, and III- or I-chromosomes occurred with much less frequencies. Some of those IV-chromosomes, however, were observed to disjoin non-disjunctionally or unevenly at I-Anaphase, and thus a certain percentage of the II-plates with unbalanced chromosomes were formed. In general, the gametes grown up from those nuclei containing unbalanced chromosomes are considered to realize more or less lethal effects upon the reproductive processes. The auto-tetraploid individuals of both the radish and Chinese cabbage produced the II-plates consisting of an exactly balanced number of chromosomes, i.e., 18 or 20 respectively, in 70—75 per cent of the II-plates examined, while the percentages of the viable pollen grains produced by those individuals have amounted to as high as 96—98. Thus in the present eutetraploid individuals it may be duly suggested that the microspores containing unbalanced nuclei can grow up into the matured grains which are supposed to be effective on the fertilization.

As shown in Table 22, the seed fertility of the eutetraploid radishes is somewhat inferior to that of normal diploid ones. And such differences in seed fertility can not be simply attributed to the higher lethality of the zygotes set on the tetraploid individuals, because the percentage occurrence of the nonviable seeds in the tetraploid pods is certainly less than that of the normal diploid ones. Thus the low fertility will be caused by the following alternative: that the eutetraploid individuals may produce the pods, containing a definitely smaller number of placentæ than that of diploid, or that the diploid gametes produced may have decisively lower reproductive power than the normal haploid ones.

In both the Japanese radish and Chinese cabbage, the gametes with unbalanced chromosomes could develop almost regularly and effect the fertilization, producing viable seeds. Such a circumstance is most outstanding on the present autotriploid forms, because almost all the gametes produced are containing the nuclei of more or less unbalanced nature. Table 20 indicates that the percentages of the morphologically viable pollen grains in those $3x$ radish and Chinese cabbage plants were 82 and 87 respectively. And moreover, the $3x$ radish produced almost as many good seeds as those of the $4x$ individuals (See Table 22). The greater variation ranges of the diameter readings prevailing on the matured pollen grains in the $3x$ individuals is considered to reflect, no doubt, the situation in the nuclear contents. It thus appears that the $3x$ individuals can reproduce under self-fertilization various kinds of individuals, whose chromosomal constitutions varied markedly between the $2x$ and $4x$ numbers.

Occurrences of the hyper- or hypotetraploid individuals in the offspring of the present autotetraploid strains would be, in turn, attributable to the conjugation of the viable gametes having the aneuploid number of chromosomes. Aneuploid individuals thus resulting, having one or two chromosomes in addition or in subtraction to the eutetraploid number, did not show any discernible morphological differences from the eutetraploid individuals in the same offspring. And certain irregularities in the meiotic processes, accompanying the aneuploidy, did not, however, lead to the diminution, even to a very slight extent, of the apparent pollen viability and also of the actual seed fertility. Such circumstances may have been derived from the fact that there were no clear differences on the effectiveness to fecundation between the two categories of gametes, one consisting of the euploid number of the chromosomes and the other of the heteroploid ones. And furthermore, size of matured pollen grains developed from the heteroploid microspores, so far as the heteroploidy of the present cases is concerned, could not realize any discernible distinctions from the ordinary euploid ones. Thus it seems very interesting to infer that those aneuploid individuals were almost indistinguishable from the sister euploid ones in the several characteristics depicted as follows: the general morphological appearances of the plant status; the percentage of pods set per flowers; the sizes and the viability of the matured pollen grains; and the actual seed fertility.[1]

As mentioned previously, it has been experienced usually that the artificially raised tetraploid strains return unawares to the original diploid ones under cultivation through generations, being deprived of the polyploid nature of nuclei. The causes of such regressional tendencies in autopolyploidy are duly considered by the present authors to be attributable, in the main, to the usual occurrence of aneuploid individuals in the offspring, because once the diminution of the chromosomes has commenced it will progress steadily and rather rapidly towards the final diploid state. As mentioned elsewhere, the present tetraploid strains of both the radish and Chinese cabbage have already passed through several generations and have been maintained as such with some stability. But, as was presented in the authors' results, the individuals with an exact tetraploid number of chromosomes had still produced a certain number of tetravalent chromosomes and developed many viable gametes consisting of various aneuploid numbers of chromosomes. In consequence, when an original polyploid plant is once induced artificially, its descendants will henceforth continue to separate out the aneuploid individuals in a certain progressive ratio through successive generations. And then, if the seed gatherings were intended to be made under self-pollinations from only a very few member in a definite strain, for an experimental or a practical culture, there may always accompany the opportunities for selecting the undesirable aneuploid individuals, and thus the heteroploidal tendencies in much stronger degrees may come to prevail over the future generations, providing finally the diploid condition. And moreover, it is not very easy to avoid such circumstance since it is quite difficult to identify the exact nature of those aneuploid individuals from the euploid ones throughout their whole vegetative stages and even at their blooming time. To maintain a certain tetraploid strain, we are accustomed, in general, to make the examinations upon the size and stainability of matured pollen grains with each individual. But as stated elsewhere, the size of the pollen grains of both the radish and Chinese cabbage does not reveal its gametic nature exactly, so that the actual chromosome counting

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[1] The characters and behaviours of the present aneuploid individuals may be taken as rather exceptional ones, and the noteworthy case of the offspring of the colchicine-induced eutetraploids of ***Antirrhinum majus*** (Straub, 1941) seems to be one of the typical examples. Even the aneuploids such as $4x+1$ or $4x-1$ forms could be easily identified through their several morphological characters and their definitely low pollen viabilities.

becomes a necessary task to make clear the genetic status of each individual. For the practical seed collecting with the tetraploid strains, the counting of chromosomes with each plant seems, however, to be near to improvable measures, and thus the more practicable task of maintaining the polyploid status is to collect seeds from as many individuals as possible. And further, it must be taken into consideration that the seeds of 3*x* constitution are quite small in size and are identifiable very easily. Howard (1939b, 1942) reported that the 3*x* seeds of *B. oleracea* and of *B. chinensis* are very small in size, taking about one third of the ordinary size, but filling fully the testa. Nishiyama (1949b) has also noticed that the 3*x* seeds of *Raphanus* were similarly small in size, and that he could easily sift out such small ones from all the other seeds produced on the 4*x* forms. As described elsewhere, with both of the present 3*x* radish and Chinese cabbage a quite similar situation in seed size was ascertained by the authors.[1]

It is well inferred that the tetraploid strains grown in the vicinity of the diploid relatives will easily produce such 3*x* seeds by the natural crossings. The authors noticed that the frequency occurrence of those 3*x* seeds had varied more remarkably among the individuals belonging to the same 4*x* strain than among those belonging to the different varieties or strains. Nishiyama (1949b) has also reported that the natural crossability between 4*x* and 2*x* individuals of the same variety decreased according to the increase of planting distance between 4*x* and 2*x* forms, and it showed again marked variations among the different combinations of varieties undertaken in the adjacent planting. Thus it may be safely concluded that even among the tetraploid individuals of the same strain there may exist rather definite differences on the crossability with the pollen grains from the same diploid source. So far as the present studies have gone, the causes of such a situation are not yet made clear, although some environmental and genetic causes may well be inferred.

During the procedures of chromosome diminution towards the diploid state in the tetraploid offspring through the aneuploidy, a certain amount of seeds in extremely unbalanced state of nucleus, such as those in the triploid or its neighbourhood, will, no doubt, result. And the latter seeds are duly supposed to have very small sizes. To the

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[1] In connection with such a situation, the authors remember the fact that the various interspecific crosses in *Brassicas*, covering the diploid, tetraploid, and the amphidiploid species or strains raised artificially, there usually occurred true F_1 hybrid seeds of very small size; or, in other words, that the true F_1 seeds could only be gathered among the seeds of extremely small sizes produced on the crossings (See Howard (1942), Hosoda (1949), and Iwasa (1951)). It seems rather an easy task to exclude those small hybrid seeds occurring spontaneously from the seeds to be collected for maintaining a definite polyploid strain.

authors, the exact relationship between the smallness of size of seeds and their unbalanced states of chromosome construction is not yet certain,[1] but the selection of seeds by their sizes will be certainly taken as one of the practical measures for maintaining a certain tetraploid strain of vegetables, such as Japanese radishes or some *Brassicas*.

SUMMARY

1. To maintain the chromosome constitution of certain newly raised auto-tetraploid forms through generations is one of the urgent claims in plant-breeding by the chromosome doubling, because it has been generally experienced that the auto-tetraploid forms are apt to return always to the former diploid state even under the usual seed-growing measures. Some cytological evidences concerning such procedure of chromosome diminution are presented.

2. The auto-tetraploid individuals of both Japanese radish and Chinese cabbage produced a large number of $4x$ seeds of ordinary size together with a fairly large number of $3x$ seeds, which are quite full but very small in size, taking almost one third of the $2x$ size. Triploid seeds are considered to be due to the natural crossings between $4x$ and the pollens from diploid sources. Diploid seeds took, in turn, a somewhat intermediate size between the $4x$ and $3x$ ones.

3. Comparisons of certain morphological and physiological characters were made with many individuals grown up from those seeds set on the eutetraploid plants. Sizes of cotyledons of $4x$ plants were the largest, those of $2x$ medium, those of $3x$ being the smallest. Triploid individuals showed quite vigorous growing as a whole and their blooming was most delayed and fell behind $4x$ and $2x$ ones. Sizes of

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[1] Howard (1942) presented some considerations for the explanations of the occurrence of those small $3x$ seeds in *Brassicas*. He has suggested the application of Watkins' (1932) general rule in crosses of the type high-chromosome number × low-chromosome number that better seed production was obtained with the high-chromosome number one as female and it may be due to the genomic embryo : endosperm relation. And the small size of the $3x$ seeds was explained as being due to an abnormal embryo : endosperm relation, a $3x$ embryo developing in a $5x$ instead of in a 4.5-ploid endosperm (c.f., the normal-sized seeds from both $2x$ selfed and $4x$ selfed which have normal embryo : endosperm ratios of 1 : 1.5). For the explanation of the reduction in seed size in the interspecific crosses he has extended the above idea of embryo : endosperm ratio from the total genomes present to numbers of each type of genome present. Thus in a cross, e.g., *B. chinensis* female × *B. carinata*, 1 *chinensis* genome in the embryo is balanced against 2 instead of 1.5 *chinensis* genomes in the endosperm, and 1 *carinata* genome in the embryo against 1 instead of 1.5 *carinata* genomes in the endosperm. Such a disturbance of normal embryo : endosperm ratios is considered to take place in every interspecific cross.

the stomatal guard-cells and the number of chloroplasts in each guard-cell were measured and compared, revealing the following sequence of decreasing order with both items: $4x$, $3x$, and $2x$ plants.

4. Among the offspring of both the $4x$ radish and Chinese cabbage there usually appeared a few hyper-or hypo-tetraploid individuals along with the eutetraploid ones. Occurrence of those aneuploid individuals was suggested as due to the chromosomal behaviours of $4x$ meiosis and the resultant formation of the not less amount of viable gametes consisted of the aneuploid numbers of chromosomes.

5. The formation of such viable and effective aneuploid gametes in both $4x$ radish and Chinese cabbage concurs well with the fact that their $3x$ relatives undertook nearly regular meiotic behaviours and showed, in consequence, rather high pollen and seed fertility, being only slightly lower than those in the euploid forms, $4x$ or $2x$.

6. Through the above situation of producing the effective aneuploid gametes in some definite ratios to the typical $2x$ gametes, the $4x$ strains are always confronted with contamination of a fraction of heteroploid forms, so that the possibility of picking up undesirable heteroploid seed-plants for the reproduction is not so small that we may be almost free of the diminishing tendency of chromosomes in that strain, which is destined to restore easily the original $2x$ condition without special precautions.

7. In order to maintain a certain polyploid strain, the counting of chromosomes with a large number of individuals is the most desirable task, but that is rather difficult and troublesome in practice. Therefore, it may be duly suggested that we should gather the seeds for propagation from as many seed-plants as possible.

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