Studies on the alloplasmatic effect in tribe Brassiceae : I. On the carinata-cytoplasmic Brassica pekinensis induced by the successive backcrosses

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Studies on the alloplasmatic effect in tribe *Brassiceae*
I. On the *carinata*-cytoplasmic *Brassica pekinensis* induced by the successive backcrosses*

Shoichi IWASA

Studies on the cytoplasmic inheritance, a branch of genetics comparable in its importance with that of the chromosomal inheritance, were started rather very early in the history of genetics by Correns (1908) and other workers, but they have since been far less fruitful than those of chromosomal inheritance for want of the adequate materials and methods. Nevertheless, the accumulated results of many years' studies of the subject on various kinds of plants (see Caspari, 1948; Michaelis, 1954), combined with the amazing progress in those of the same subject in microorganisms (see Sonneborn, 1950; Ephrussi, 1953), have thrown light on the specific behaviors of cytoplasm. Thus it is now manifest that the cytoplasm, besides playing an important role of participation to the activities of nuclear genes, has its own genetical bearing. The results thus gained are no doubt applicable to the practical plant breeding. As the examples of utilizing alloplasmatic individuals, the new field of plant breeding as proposed by Mizushima (1961), the male-sterility in *Nicotiana tabacum* having *N. debneyi*'s cytoplasm (Clayton, 1950), the repression of self-incompatibility in *Brassica oleracea* having *B. nigra*'s cytoplasm (Mizushima and Katsuo, 1953) and the artificial production of haploids in *Triticum vulgare* and Taylor's *Triticale* having *Aegilops caudata*'s cytoplasm (Kihara and Tsunewaki, 1962) could be referred and their success will be obtained in the near future.

The author has succeeded in producing the nucleus-substituted strain of *Brassica pekinensis* and carried out investigations upon the alloplasmatic effect with those forms. In this report the formation processes of these *pekinensis* plants having *B. carinata*'s

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cytoplasm and the heritable behaviors of their peculiar characteristics are dealt with.

MATERIALS AND METHODS

Brassica carinata Braun (a strain) and *Brassica pekinensis* Rupr. (the commercial variety, "Kashin-hakusai") were used for the substitution and the restoration of nucleus. These strains were obtained from the stock cultures raised at the Horticultural Laboratory, Kyushu University.

The following processes were followed for the nucleus-substitution: the amphidiploid F_1 plant (its genome constitution is *aabbcc*, 2n=54) was produced by the colchicine technique applied to the seedling of original F_1 hybrid raised from *B. carinata* (♀) × *B. pekinensis* (♂); the amphidiploid thus obtained had been backcrossed repeatedly with the pollen-grains of *B. pekinensis* (Table 1).

The nucleus-restored strain was obtained, in turn, from the progeny of the above-mentioned original F_1 plant through the successive backcrosses with the pollen-grains of *B. carinata* (Table 1).

The crossing was effected by the bud-pollination technique. The cytological observations were made using the smear method with aceto-carmine. The pollen-fertility was examined with the mature pollen-grains smeared in a mixture of aceto-carmine and glycerin, and the pollen-grains stained well and appeared normal in their shapes were taken as fertile ones, while the seed-fertility was denoted by the number of viable seeds produced per silique set under the open-pollination.

RESULTS

I. *The tri-genomous triploid hybrids and their amphidiploids*

The original F_1 plants obtained were more or less intermediate in their morphological characters and grew up quite vigorously. At the meiotic metaphase-I, PMCs showed the chromosome pairings, $(2—9)_{II}+(23—9)_I$. As the result, the later meiotic processes became severely irregular, the degenerating anthers remained closed, the pollen-fertility reduced to 0.4%, the percentage of siliques set was far from satisfactory, and the number of seeds collected per plant through the open-pollination amounted to around 30 at the maximum. The amphidiploid plants, on the contrary, were much more fertile than the original F_1 plants, their pollen-fertility being 38.8%, and their seed-fertility (denoted by the percentage occurrence of viable seeds with the ovules developed) amounting to 36.5% (under open-pollination) and to 16.0% (under self-pollination) respectively. At their metaphase-I of PMCs, which contained

no univalents or trivalents, showed the chromosome configurations, $(0—3)_{IV}+(27-21)_{II}$. In Fig. 1, 27 bivalent chromosomes are clearly found at metaphase-I. Subsequent meiotic behaviors were quite irregular, so that the metaphase-II plate consisted of the exact set of 27 chromosomes occurred with the 48 % of plates observed.

Table 1. Origin of the nucleus-substituted and -restored strains.

1949	1950	1951
B. carinata (♀)	$\xrightarrow{o}$ F_1 (*abc*, 0.4 %) $\xrightarrow{o}$ F_2 (−, 65.0 %) $\xrightarrow{1)}$	
× *B. pekinensis* (♂)	⇓ chromosome doubling	
	F_1 (*aabbcc*, 38.8 %) $\xrightarrow{\times p}$ B_1F_2 ($10_{II}+17_I$, 70.2 %) $\xrightarrow{\times p}$ $\xrightarrow{2)}$	
		$\xrightarrow{3)}$

1952	1953	1954
$\xrightarrow{1)}$ F_3 (−, 68.6 %)	$\xrightarrow{\times c}$ B_1F_4 (−, 83.1 %)	$\xrightarrow{\times c}$ B_2F_5 (17_{II}, 99.1 %) $\xrightarrow{\times c}$ $\xrightarrow{1)}$
$\xrightarrow{2)}$ B_2F_3 ($10_{II}+5_I$, 58.0 %)	$\xrightarrow{o}$ B_2F_4 ($14_{II}+4_I$, 84.5 %)	$\xrightarrow{s}$ B_2F_5 (18_{II}, 93.5 %) $\xrightarrow{\times p}$ $\xrightarrow{2)}$
$\xrightarrow{3)}$ B_2F_3 ($10_{II}+1_I$, 90.7 %)	$\xrightarrow{\times p}$ B_3F_4 (10_{II}, 98.8 %)	$\xrightarrow{\times p}$ B_4F_5 (10_{II}, 99.4 %) $\xrightarrow{\times p}$ $\xrightarrow{3)}$

1955	1956	1957
$\xrightarrow{1)}$ B_3F_6 (17_{II}, 96.4 %)	→ Restored strain	
$\xrightarrow{2)}$ B_3F_6 ($1_{IV}+9_{II}+2_I$, 52.4 %)	$\xrightarrow{\times p}$ B_4F_7 (10_{II}, 98.0 %)	$\xrightarrow{\times p}$ B_5F_8 (10_{II}, 99.1 %) $\xrightarrow{1)}$
$\xrightarrow{3)}$ B_5F_6 (10_{II}, 97.0 %)	$\xrightarrow{\times p}$ B_6F_7 (10_{II}, 97.3 %)	$\xrightarrow{s}$ B_6F_8 (10_{II}, 99.7 %) $\xrightarrow{s}$ $\xrightarrow{2)}$

1958	1959	1960
$\xrightarrow{1)}$ Substituted strain		
$\xrightarrow{2)}$ B_6F_9 (10_{II}, 97.7 %)	$\xrightarrow{\times p}$ B_7F_{10} (10_{II}, 99.5 %)	$\xrightarrow{\times p}$ B_8F_{11} (10_{II}, 98.5 %) $\xrightarrow{\times p}$ $\xrightarrow{1)}$

1961
$\xrightarrow{1)}$ B_9F_{12} (10_{II}, 97.9 %) → Substituted strain

N.B. Genome constitutions or chromosome configurations at MI and pollen-fertilities are represented in parentheses.
Abbr. o……open-pollination, s……self-pollination,
p……*pekinensis*, *c*……*carinata*.

II. *The substitution process of nucleus*

The procedures effected towards the nucleus-substitution are summarized in Table 1. An amphidiploid F_1 hybrid raised was backcrossed by the pollen-grains of the original male parent, and 11 plants obtained

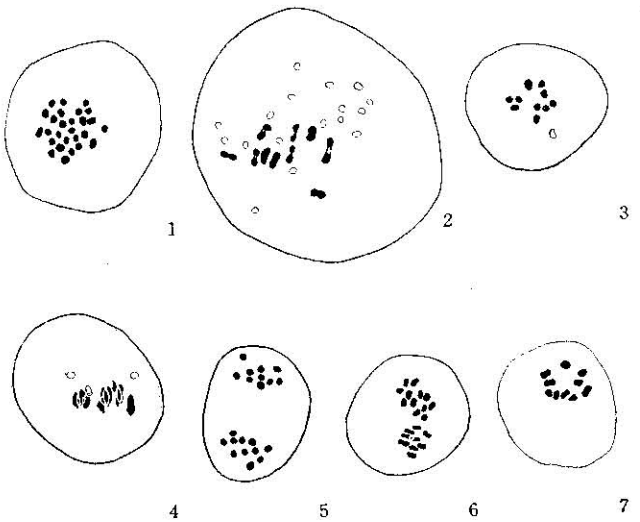
in B_1F_2* offsprings were quite similar morphologically to the F_1 hybrid, excepting the forms of their leaves. PMCs in one of these 11 plants revealed the chromosome pairings of $10_{II}+17_I$ (Fig. 2) at the metaphase-I, indicating unquestionably that this plant, B_1F_2 7–1, may be *aabc* (2n=37) in its genome constitution.

This plant was 70.2% in its pollen-fertility, clearly surpassing the original amphidiploid F_1 plant, and about 1.4 seeds per silique were obtained from the backcrossing with the *pekinensis* plant, used as the male parent. Twenty-five B_2F_3 seeds thus obtained showed wide variation in their sizes between 1.72 and 0.85 mm (in diameter). As shown in Table 2, with all the plants whose chromosome numbers were determined the relationship between the number of chromosomes of the plant and the size of seed from which the plant had grown up was made clear. The number of somatic chromosomes versus the diameter of seeds were 2n=21 vs. 1.72 mm with the plant 6–1; 2n=22 vs. 1.63 mm with the plant 6–2; 2n=25 vs. 1.53 mm with the plant 6–4; 2n=25 vs. 1.52 mm with the plant 6–7; and 2n=24 vs. 1.50 mm with the plant 6–10, respectively. These results show, in consequence, that the size of seed is inversely proportional to the number of somatic chromosomes with each B_2F_3 plant.

The aspect of chromosome pairings at metaphase-I of PMCs in the above-mentioned 5 plants is given in Table 2. In B_2F_3 6–1 there usually occurred the configuration $10_{II}+1_I$ (Fig. 3) and often $9_{II}+3_I$ (Fig. 4) as well, so that either 10–1–10 (one lagging) or 10–11 (Fig. 5), and rarely 12–9 (Fig. 6) distribution, was to be seen at metaphese-II of PMCs. At metaphase-I of PMCs in B_2F_3 plants the chromosomes derived from *carinata* genome appeared as univalents in plants 6–2 and –4, and as trivalents, in addition to univalents, in plants 6–7 and –10. The trivalent chromosome is duly considered to be made by conjugation of a bivalent from *pekinensis* with a univalent from *carinata*, clearly indicating that the *a*, *b* and *c* genomes in genus *Brassica* were in partially homologous. All the plants in B_2F_3 strain were very low in their fertility with the exception of plant 6–1 which showed nearly as high in the pollen- and seed-fertilities as in the normal *pekinensis* plants. However, the plants having 2n=21 chromosomes in the progeny, B_3F_4, of B_2F_3 6–1 (♀)×*pekinensis* (♂) were conspicuously retarded in the growth and showed low fertility, excepting two plants which showed quite similar chromosomal aberrations and high fertility as the B_2F_3 6–1. Moreover, these 2n=21 plants showed invariably the same configuration $10_{II}+1_I$ at metaphase-I. It is conceivable from these facts that the chromosomal aberrations occurring in B_2F_3 6–1 would be lost

* B_1F_2 denotes the 1st backcross generation obtained from the F_1 original form, so that B_2F_3 represents the next backcross generation of B_1F_2, and so on.

in these B_3F_4 plants having 2n=21 chromosomes, except the two plants above-mentioned. (N.B. It seems quite probable to consider that the chromosomal aberrations accompanied with B_2F_3 6–1 might have caused the inhibition of the *carinata*-cytoplasmic effect. Some detailed accounts of such *carinata*-cytoplasmic ($10_{II}+1_I$) plants will be given in the future report.)



Figs. 1–7. Meiotic divisions in PMCs of F_1 plant (*B. carinata* × *B. pekinensis*), and its progeny obtained by the successive backcrosses. ca. × 1000.
Fig. 1. MI cell of the original amphidiploid F_1 plant, showing 27_{II}.
Fig. 2. MI cell of B_1F_2 7–1, showing $10_{II}+17_I$.
Figs. 3 and 4. MI cells of B_2F_3 6-1, showing $10_{II}+1_I$ and $9_{II}+3_I$, respectively.
Figs. 5 and 6. MII cells of B_2F_3 6–1, showing 11-10 and 12-9 distributions, respectively.
Fig. 7. MI cell of B_3F_4 1-5, showing 10_{II}.

Nucleus-substituted plants could be obtained from the progeny of B_2F_3 6–1 and –7. The B_3F_4 seeds produced by B_2F_3 6–1 did not varied in their sizes, but when they were sown, only smaller ones out of those, not any of larger ones, grew up into ($10_{II}+1_I$) plants, showing that the situation was quite in accord with that in the preceding generation. In the plants (2n=20) of B_3F_4 strain, however, the chromosome association at metaphase-I of PMCs was exclusively 10_{II} (Fig. 7), and the subsequent meiotic divisions proceeded quite normally. These facts probably shows that in those plants, resembling morphologically to the normal *pekinensis* plant, the substitution process of nucleus had already been completed. Those plants showed highly fertile (the pollen-fertility was 99.8%; the seed-fertility was 10.3 seeds per silique)

under the greenhouse condition; but with several peculiar characters of their own, these nucleus substituted *pekinensis* plants could easily be

Table 2. Chromosome pairings and fertilities in progenies derived through backcrossings.

Exp. no. of individuals	No. of chromosomes	Chromosome configurations at MI (Frequencies)	Fertility denoted as: per cent of viable pollen-grains	Fertility denoted as: no. of seeds per silique
Part I with nucleus-substitution;				
F_1 2-1, 2	54	$27_{II}(8), 26_{II}+2_I(3), 1_{III}+25_{II}+1_I(2), 1_{IV}+25_{II}(5), 2_{IV}+23_{II}(1), 3_{IV}+21_{II}(2), 3_{IV}+20_{II}+2_I(1)$	38.8%	6.1
B_1F_2 7-1	37	$10_{II}+17_I(7)$	70.2	—
B_2F_3 6-1	21	$10_{II}+1_I(45), 9_{II}+3_I(12)$	90.7	10.7
-2	22	$10_{II}+2_I(20)$	68.2	1.4
-4	25	$10_{II}+5_I(22)$	62.9	3.1
-7	25	$10_{II}+5_I(9), 1_{III}+9_{II}+4_I(4), 2_{III}+8_{II}+3_I(4)$	58.0	2.8
-10	24	$10_{II}+4_I(58), 11_{II}+2_I(4), 1_{III}+9_{II}+3_I(25), 2_{III}+8_{II}+2_I(2)$	66.3	2.5
B_3F_4 1-1,2,3, 4,5,6, 7,8,9 10	20	$10_{II}(40)$	98.8	10.3
14,18, 21	21	$10_{II}+1_I(40)$	86.5	8.1
B_2F_4 22-16	32	$15_{II}+2_I(1), 14_{II}+4_I(30), 1_{III}+13_{II}+3_I(3)$	84.5	—
B_2F_5 3-2	36	$18_{II}(4), 16_{II}+4_I(2), 1_{IV}+16_{II}(1), 1_{IV}+15_{II}+2_I(1)$	93.5	8.2
B_3F_6 29-3	24	$10_{II}+4_I(1), 1_{IV}+9_{II}+2_I(6), 2_{III}+8_{II}+2_I(2)$	52.4	—
B_4F_7 15-8	20	$10_{II}(12)$	98.0	10.5
Part II with nucleus-restoration;				
F_2 1-4	—	—	65.0	7.4
F_3 1-12	—	—	68.6	8.8
B_1F_4 7-1	—	—	83.1	8.5
B_2F_5 10-8	34	$17_{II}(20)$	99.1	12.5
B_3F_6 3-1,2, 3,4,5,6, 7,8,9, 10	34	$17_{II}(40)$	96.4	13.0

distinguished from the normal ones. This strain was propagated through generations under successive selfings or backcrossings until the B_9F_{12}

generation was reached. The offspring of B_2F_3 6-7 was raised by the open-pollination. The plant, B_2F_4 22-16 (2n=32), showed the $14_{II}+4_I$ at metaphase-I in most PMCs and $1_{III}+13_{II}+3_I$ or $15_{II}+2_I$ in some cases. From B_2F_4 22-16, B_2F_5 3-2 (2n=36) was obtained by selfing, and it showed 18_{II} at metaphase-I in the half of the PMCs examined and $16_{II}+4_I$, $1_{IV}+16_{II}$, or $1_{IV}+15_{II}+2_I$ in the rest. B_3F_6 29-3 (2n=24), obtained from B_2F_5 3-2 through the backcrossing with the pollen-grains of *pekinensis*, showed most frequently $1_{IV}+9_{II}+2_I$ or $2_{III}+8_{II}+2_I$, and $10_{II}+4_I$ in some rare cases. The presence of those multivalent chromosomes higher than trivalent was likely to indicate that there had occurred certain chromosomal aberrations during the propagation through generations (B_2F_3—B_2F_5). B_3F_6 29-3, through backcrossing again with *pekinensis*, could produce 11 individuals in the next B_4F_7 generation, 4 ones out of those 11 showing close morphological resemblance to *pekinensis*. None the less these 4 plants, like nucleus-substituted descendants from B_2F_3 6-1, had conspicuous characters of their own, and in one of them, B_4F_7 15-8, the chromosome association at metaphase-I was exclusively 10_{II}, and the subsequent divisions were quite normal, realizing its high fertility under the greenhouse environment.

III. *The restoration process of nucleus*

It may be possible that the nucleus-substitution would bring about certain cytoplasmic changes towards the F_1 plant or its progeny, and that such changes, if thus induced, could be detected upon the more or less modified appearances realized through the comparison of those nucleus-restored plants with the normal intact *carinata* plants. Those nucleus-restoration processes, as shown in Table 1, were examined in detail for this purpose. (The most desirable method to be used for this purpose, is considered to repeat the backcrossings between the nucleus-substituted *pekinensis* plants and the normal intact *carinata* plants, taken as the pollen provider, but this attempt, though extensively tried in repetition over a period of years, have ended in vain.) The strain adopted for the nucleus-restoration was that of the progeny produced through twice open-pollinations from the original F_1 plant (Table 1). F_2 1-4 and F_3 1-12, which were selected for the pedigree culture through the open-pollinated generations, were found to resemble more closely in their morphological characters to *B. carinata* than to the original F_1 plant, and they showed relatively higher fertility, though none of them could be examined cytologically. F_3 1-12 through twice backcrossings with *carinata* (the pollen provider) produced B_2F_5 10-8 (2n=34), which was highly fertile, and was also morphologically indistinguishable from *carinata* plant, forming exclusively 17_{II} at metaphase-I of its PMCs. The offspring (B_3F_6 derived from B_2F_5 10 8 by backcrossing with *carinata*) was found to be quite identical with

carinata plant karyologically and morphologically as well. From these facts it may be safely deduced that the processes of nucleus-restoration have been practically completed in those forms. Such nucleus-restored plants, however, did not revealed any sign of peculiar characteristics effected cytoplasmically.

IV. *Chlorophyll deficiency in the nucleus-substituted plants and its genetic behavior*

Carinata-cytoplasmic *pekinensis* plants (will be denoted in short as *ca-pekinensis* plants in the following description) revealed certain specific characteristics. Chlorophyll deficiency, the most conspicuous one of those characters, served as the most reliable criterion of discriminating the nature of *ca-pekinensis* plants. In the seedlings of *ca-pekinensis* plants, the cotyledons look rather pale yellow in color at first and soon became green as normal ones, and the first few foliage leaves developed the pale yellow or yellowish pale green coloring at

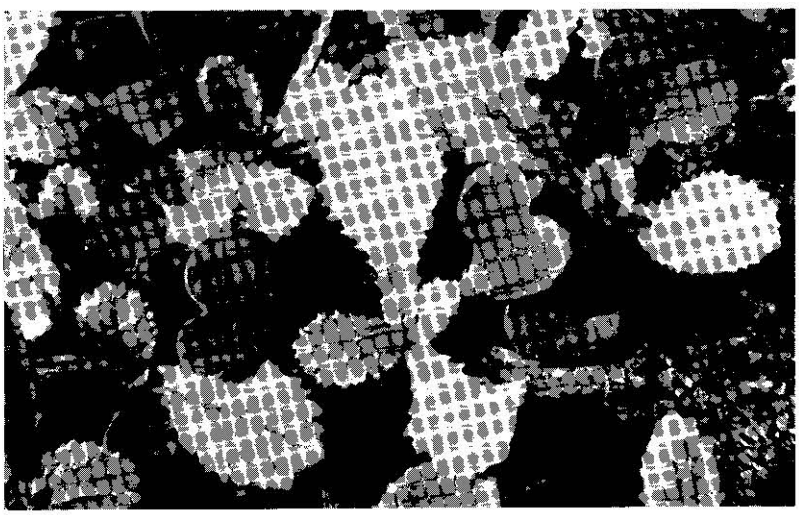


Fig. 8. General appearance of *carinata*-cytoplasmic *pekinensis* plants (B_8F_{11}) in the early stage of growth, showing clear deficiency in chlorophyll formation. (1960)

the early stage and they grew up to the variegated ones. Fig. 8 shows the chlorophyll-deficient young leaves on those plants, the cotyledons being fully green, while the foliage leaves pale yellow, and some of them turning normal green in their vein-regions. After this stage, the following leaves developed in succession have become as green as normal with the progress of growing and the plants appeared

more or less normal. But when those plants were removed to a cold place, the young leaves newly formed became chlorophyll-deficient, and, moreover, such environmental effectiveness was somewhat depended upon the duration of the low temperatures. Fig. 9 shows F_1 plants selected from two different lines, generated through reciprocal crosses

Table 3. Genetic behavior of chlorophyll deficiency in various strains.

Nature of cytoplasm	Generation	Descent of strains	Chlorophyll character under greenhouse Nor.	Chlorophyll character under greenhouse Def.	Chlorophyll character under field Nor.	Chlorophyll character under field Def.	Total no. of plants examined	Experiment years
pekinensis	F_1	normal *pekinensis* × B_3F_4	37	0	39	0	76	1953–54
		〃 × B_7F_{10}	—	—	20	0	20	1959–60
		〃 × B_8F_{11}	18	0	51	0	69	1960–61
	F_2	F_1 selfed	93	0	—	—	93	1954–55
		$F_1 \times B_4F_5$	20	0	—	—	20	〃
	F_3	F_2 selfed	20	0	—	—	20	1955–56
		$F_2 \times B_5F_6$	19	0	—	—	19	〃
	Totals		207	0	110	0	317	
carinata	F_1	B_3F_4 × normal *pekinensis*	0	28	0	68	96	1953–54
		B_4F_5 × 〃	0	35	—	—	35	1954–55
		B_5F_6 × 〃	0	37		—	37	1955–56
		B_6F_9 × 〃	0	40	—	—	40	1948–59
		B_7F_{10} × 〃	—	—	0	18	18	1959–60
		B_8F_{11} × 〃	0	20	0	75	95	1960–61
	F_2	B_4F_5 selfed	0	54	—	—	54	1954–55
		B_6F_7 〃	0	17	—	—	17	1956–57
	F_3	B_4F_6 〃	0	61	—	—	61	1955–56
		B_6F_8 〃	0	20	—	—	20	1957–58
	Totals		0	312	0	161	473	

between *ca-pekinensis* plant and normal *pekinensis* plant. The photograph was taken with plants which had been removed from the greenhouse and cultured thoughout in the open field from December through February. The hereditary behavior of this chlorophyll deficiency has been examined in continuation from 1953 through 1961.

Table 3 is compiled with whole the data obtained with the progeny raised through the crossings and as well as the selfings. All the

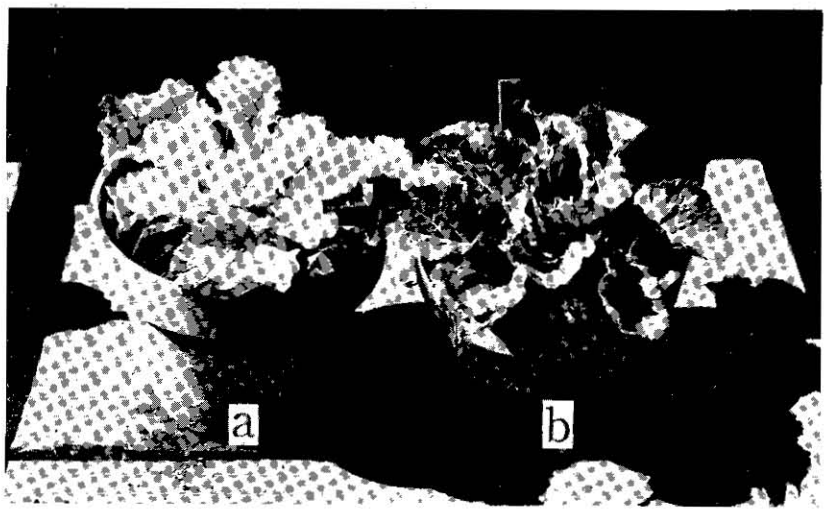


Fig. 9. Different appearances of the chlorophyll development in two F_1 plants raised reciprocally. a, *carinata*-cytoplasmic F_1 plant; b, *pekinensis*-cytoplasmic F_1 plant. (1955)

plants having *carinata*'s cytoplasm manifested exclusively the defect in chlorophyll characters, irrespective of the year or the environmental condition in which they were grown.

Discussion

The occurrence of *ca-pekinensis* forms was first noticed after the three times repetition of the backcrossings of the original F_1 hybrid. It appears rather important that such nucleus-substitution could be accomplished during a short period of time without accompaning any probable cytoplasmic changes or a certain alteration in the substituted genome if any. On the other hand, it has been disclosed that there prevailed a definite interrelationships between the somatic number of chromosomes and the size with each individual seed during the processes of nucleus-substitution. Those seeds enlarged in their sizes with parallel to the decreasing of their somatic chromosome numbers approching to 20 at the maximum. This phenomenon, combined with the following facts that in the tribe *Brassiceae* the true F_1 seeds raised by the interspecific or intergeneric crosses are generally so remarkably reduced in their sizes as to be readily distinguishable from any false F_1 seeds (Hosoda, 1946: Iwasa, 1951; Mizushima, 1952) and, moreover,

that in the artificially induced polyploid or anuploid forms there exist also a definite relationship between the somatic number of chromosomes and the size with those seeds (Fukushima and Tokumasu, 1959; Tokumasu, 1961), will suggest the advantage of selecting large-sized seeds for the use of the nucleus-substitution which would be accomplished in a short period of time, particularly when the polyploid forms were concerned.

The *ca-pekinensis* plants revealed certain conspicuous characters. These characters, though varied considerably from individual to individual and even under different environmental conditions, persisted intact from the B_3F_4 through B_9F_{12} generations. The detailed results of observations with those characters will be dealt with in a separate paper. Chlorophyll deficiency, the most conspicuous one of those characters, have been transmitted following the typical cytoplasmic way, as may be clearly seen from the facts compiled in Table 3. Further, the *ca-carinata* plants which were reorganized through the nucleus-restoration appeared quite normal, compared with *ca-pekinensis* plants. Therefore, those specific characters in *ca-pekinensis* plants will be considered to have arised through the disharmony or unbalanced condition derived between *carinata*'s cytoplasm and *pekinensis*' nucleus.

Summary

1. The *pekinensis* plants having *carinata*'s cytoplasm were obtained from the amphidiploid F_1 plant which had been produced by crossing *B. carinata* (♀) with *B. pekinensis* (♂) and which had been successively backcrossed with *B. pekinensis*, the pollen provider, for the nucleus-substitution. The *carinata* plants having *carinata*'s cytoplasm were reproduced from the progeny of the original F_1 plant by successive backcrossing with *B. carinata*, the pollen provider, for the nucleus-restoration. Comparative examinations disclosed that the nucleus-restored plant was perfectly normal as the normal *carinata* plant, and also that the nucleus-substituted plant developed certain peculiar characters, which could not be detected in the normal *pekinensis* plant.

2. Various specific characters were always observable throughout generations from B_3F_4 through F_9B_{12}. The chlorophyll deficiency, the most conspicuous and recognizable character, served as a norm for use in detecting the *carinata*-cytoplasmic *pekinensis* plant and transmitted as a typical case of cytoplasmic inheritance.

3. The conspicuous characters appeared in the *carinata*-cytoplasmic *pekinensis* plant are likely to arise from the disharmony

effected between *carinata*'s cytoplasm and *pekinensis*' nucleus.

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