IN VITRO CULTURE OF OVARIES OF *BRASSICA* HYBRIDS BETWEEN $2x$ AND $4x$. I. CULTURE MEDIUM*.

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Synopsis : In order to overcome cross-incompatibility between *Brassica chinensis* ($2x$) and autotetraploid *B. pekinensis* ($4x$), excised ovaries of cross-pollinated flowers were cultured on various media containing IAA, kinetin, gibberellic acid, tomato juice, yeast extract, casein hydrolysate and coconut milk in different concentrations. Triploid hybrids were successfully obtained, and the results suggested that the reciprocal hybrids, $2x \times 4x$ and $4x \times 2x$, have different nutritional requirements.

Introduction

Since ovary culture became useful in *Lycopersicon* and *Cucumis* (NITSCH 1951), *in vitro* culture of excised ovules has been attempted with *Papaver* (MAHESHWARI 1958), *Citrus* (RANGA SWAMY 1959), *Hordeum* (CROLX *et al.* 1962), *Anethum* (JOHRI and SEHGAL 1963), *Gynodrropsis* and *Impatiens* (CHOPLA and SABHARWAL 1963), and some viable seeds have been produced.

Application of ordinary embryo culture method was almost unsuccessful in *Brassica* hybrids, because degeneration of ovules occurred soon after pollination.

For this reason, *in vitro* culture of excised ovaries was attempted with diploid *B. chinensis* and autotetraploid *B. pekinensis*, which are normally cross-incompatible when crossed each other. It was aimed here to investigate interaction between embryo and endosperm under artificial condition and to get triploid hybrid seeds.

Materials and Methods

Autotetraploid *B. pekinensis* Rupr. cultivar Chifu-hakusai and diploid *B. chinensis* L. cultivar Nikanme-taina were used. The autotetraploid *B. pekinensis* was produced by colchicine treatment by NISHIYAMA in 1950 (unpublished). Diploid *B. pekinensis* and *B. chinensis* possess the same genomes, AA ($2n=20$) (MORINAGA

1934). Their strains used in this experiment were the same as those used in a previous study (NISHIYAMA and INOMATA 1966).

The following crosses were made :

B. chinensis ($2n=20$) $\times$ *B. chinensis* ($2n=20$)

B. chinensis ($2n=20$) $\times$ *B. pekinensis* ($2n=40$)

B. pekinensis ($2n=40$) $\times$ *B. pekinensis* ($2n=40$)

B. pekinensis ($2n=40$) $\times$ *B. chinensis* ($2n=20$)

Flower buds were emasculated two days before their pollination with fresh pollen grains which were carried out in greenhouse. Suitable age of ovary for culture was determined by histological investigation of embryos. Until four days after pollination, developmental rate of hybrid endosperm was similar to that of the controls ($2x \times 2x$ and $4x \times 4x$) (NISHIYAMA and

Fig. 1. Explanted ovaries in test tubes.

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INOMATA 1966). At this age the ovaries were cut off from the plant, put in a 5% solution of calcium hypochlorite for 8 to 10 minutes and rinsed three times with sterilized water. Then, the ovaries were explanted in test tubes (one ovary per test tube) as shown in Fig. 1.

The basal medium, which was employed, contained NITSCH's solution (NITSCH 1951), glycine 15mg, niathine 25mg, pyridoxine 0.5mg, sucrose 50 g, and agar 9 g, in every liter of distilled water. The basal medium was enriched, when necessary, with growth substances and some other nutrients; those are IAA, kinetin, gibberellic acid, tomato juice, yeast extract, casein hydrolysate and coconut milk. Their concentrations in various media are shown in Tables 1 and 2. pH of the media was adjusted to 6.0 with 1 N KOH and 1 N HCl before autoclaving, that was carried out for 10 minutes at a pressure of 1.2 kg/cm². Test tubes used were of 18×180 mm, to each of which 10 ml of medium was put. IAA employed was manufactured by the Merk & Co., Inc. in Germany, kinetin by the Sigma & Co., Inc. in U.S.A., gibberellic acid by the Association of Gibberellin in Japan, yeast extract by Takeda-Yakuhin Co., Ltd., and casein hydrolysate by Nissui-seiyaku Co., Ltd. Tomato juice was prepared from ripe tomatoes, which were bought at market, by filtrating raw juice with cotton tissue, and autoclaving the filtrate at a pressure of 1.2 kg/cm² for 10 minutes. It was again filtrated with filter paper before its addition to the medium. Coconut milk was taken from ripe coconuts, which were also bought at market, and after autoclaving at a pressure of 1.2 kg/cm² for 10 minutes, it was stored in refrigerator. It was filtrated when used.

The explanted ovaries were grown at 22±2°C under continuous illumination of 80 to 100 luxes with fluorescent lamps. The explanted ovaries of 36 days after explantation (40 days after pollination) were examined for their capsule length, seed setting, callus formation, etc.

Results

Crossing Experiment in Natural Condition

Capsule length, seed set percentage and germination rate were examined in mature capsules born on naturally grown plants. The results

Table 1. The concentrations of IAA, kinetin and gibberellic acid in different media (unit: mg/l)

Medium	IAA	Kinetin	G. A. ¹⁾
K-1	0	0	—
K-2	1	0	—
K-3	5	0	—
K-4	0	0.1	—
K-5	1	0.1	—
K-6	5	0.1	—
K-7	0	0.5	—
K-8	1	0.5	—
K-9	5	0.5	—
G-1	0	—	0
G-2	1	—	0
G-3	5	—	0
G-4	0	—	1
G-5	1	—	1
G-6	5	—	1
G-7	0	—	5
G-8	1	—	5
G-9	5	—	5

1) : Gibberellic acid

Table 2. Composition of supplemented media for excised ovaries

	None	IAA	K	GA	T
BM	NO	NI	NK	NG	NT
BM-TJ	TJO	TJI	TJK	TJG	TJT
BM-YE	YEO	YEI	YEK	YEG	YET
BM-Ch	ChO	ChI	ChK	ChG	ChT
BM-CM	CMO	CMI	CMK	CMG	CMT
BM-CM-Ch	CMChO	CMChI	CMChK	CMChG	CMChT

IAA : 1 mg/l, K : Kinetin 0.1 mg/l, GA : gibberellic acid 1 mg/l, T : IAA+K+GA, TJ : tomato juice (15%), YE : yeast extract (2 g/l), Ch : casein hydrolysate (300 mg/l), CM : coconut milk hydrolysate (300 mg/l)

Table 3. Results of the crossing experiments for control; no culture was made

Cross	No. of capsules examined	Capsule length (mm)	No. of seeds obtained	Seed set (%)	Seed germination (%)
2 x 2 x	37	29.5	126	18.8	92.0
2 x 4 x	12	32.0	0	0.0	0.0
4 x 4 x	70	52.1	359	20.3	95.1
4 x 2 x	13	37.8	0	0.0	0.0

Table 4. Interacting effects of IAA and kinetin on explanted ovaries

Cross	Medium ¹⁾	Capsule length (mm)	Callus formation	No. of seeds obtained	Seed set (%)	Seed germination (%)
$2x \times 2x$	K-1	23.0	—	0	0.0	—
$2x \times 2x$	K-2	21.5	—	1	2.5	0.0
$2x \times 2x$	K-3	23.5	+	1	2.5	0.0
$2x \times 2x$	K-4	14.0	—	0	0.0	—
$2x \times 2x$	K-5	21.0	+	1	2.5	0.0
$2x \times 2x$	K-6	23.0	+	2	5.0	50.0
$2x \times 2x$	K-7	22.0	±	0	0.0	—
$2x \times 2x$	K-8	18.5	+++	0	0.0	—
$2x \times 2x$	K-9	20.0	++	1	2.5	0.0
$2x \times 4x$	K-1	16.5	—	0	0.0	—
$2x \times 4x$	K-2	23.0	± ²⁾	0	0.0	—
$2x \times 4x$	K-3	17.5	+	0	0.0	—
$2x \times 4x$	K-4	21.0	+	0	0.0	—
$2x \times 4x$	K-5	21.0	+	0	0.0	—
$2x \times 4x$	K-6	19.0	+++	0	0.0	—
$2x \times 4x$	K-7	19.0	±	0	0.0	—
$2x \times 4x$	K-8	19.5	+	0	0.0	—
$2x \times 4x$	K-9	18.5	+++	0	0.0	—
$4x \times 4x$	K-1	22.0	±	2	5.0	0.0
$4x \times 4x$	K-2	23.0	+	5	12.5	25.0
$4x \times 4x$	K-3	23.0	± ²⁾	2	5.0	0.0
$4x \times 4x$	K-4	18.0	—	0	0.0	—
$4x \times 4x$	K-5	18.0	++	1	2.5	0.0
$4x \times 4x$	K-6	20.0	+++	1	2.5	0.0
$4x \times 4x$	K-7	24.5	±	3	7.5	66.7
$4x \times 4x$	K-8	22.0	+	0	0.0	—
$4x \times 4x$	K-9	21.0	+++	6	15.0	16.7
$4x \times 2x$	K-1	34.0	±	0	0.0	—
$4x \times 2x$	K-2	28.5	± ²⁾	1	2.5	0.0
$4x \times 2x$	K-3	31.0	++ ²⁾	0	0.0	—
$4x \times 2x$	K-4	22.0	±	1	2.5	0.0
$4x \times 2x$	K-5	24.0	++	1	2.5	0.0
$4x \times 2x$	K-6	21.5	+++	0	0.0	—
$4x \times 2x$	K-7	32.0	±	0	0.0	—
$4x \times 2x$	K-8	31.5	±	0	0.0	—
$4x \times 2x$	K-9	30.0	+++ ²⁾	0	0.0	—

1) : Refer Table 1

2) : Roots were formed from the callus

are shown in Table 3. The seed fertility of the self-pollination, $2x \times 2x$ and $4x \times 4x$, was considerably low. It was probably due to harmful effects of inbreeding. Percentages of seed germination in $2x \times 2x$ and $4x \times 4x$ were 92 and 95%, respectively. The seed set percentage was estimated from the number of seeds set and that of placentas, which was 20 in $2x \times 2x$ and $2x \times 4x$, and 24 in $4x \times 4x$ and $4x \times 2x$. In reciprocal crosses between $2x$ and $4x$ many ovules showed a sign of slight development, but seeds in them collapsed in young embryonic stages and no hybrid seeds could be produced.

Artificial Culture of Cross-pollinated Ovary

(1) Interaction between IAA and kinetin
Interactions between various supplemented

Table 5. Interacting effects of IAA and gibberellic acid on explanted ovaries

Cross	Medium ¹⁾	Capsule length (mm)	Callus formation	No. of seeds obtained	Seed set (%)	Seed germination (%)
$2x \times 2x$	G-1	18.0	—	0	0.0	—
$2x \times 2x$	G-2	21.0	±	1	1.2	0.0
$2x \times 2x$	G-3	20.5	±	1	1.2	0.0
$2x \times 2x$	G-4	22.0	—	1	1.2	0.0
$2x \times 2x$	G-5	27.5	±	0	0.0	—
$2x \times 2x$	G-6	19.0	± ²⁾	0	0.0	—
$2x \times 2x$	G-7	23.5	—	0	0.0	—
$2x \times 2x$	G-8	21.5	—	0	0.0	—
$2x \times 2x$	G-9	18.5	±	0	0.0	—
$2x \times 4x$	G-1	22.5	—	0	0.0	—
$2x \times 4x$	G-2	22.0	—	0	0.0	—
$2x \times 4x$	G-3	28.5	± ²⁾	0	0.0	—
$2x \times 4x$	G-4	31.5	—	0	0.0	—
$2x \times 4x$	G-5	21.5	± ²⁾	0	0.0	—
$2x \times 4x$	G-6	20.5	± ²⁾	0	0.0	—
$2x \times 4x$	G-7	21.5	—	0	0.0	—
$2x \times 4x$	G-8	27.0	± ²⁾	0	0.0	—
$2x \times 4x$	G-9	20.5	±	0	0.0	—
$4x \times 4x$	G-1	27.5	—	3	3.6	33.3
$4x \times 4x$	G-2	23.5	± ²⁾	5	6.0	40.0
$4x \times 4x$	G-3	24.0	± ²⁾	5	6.0	40.0
$4x \times 4x$	G-4	25.0	±	3	3.6	33.3
$4x \times 4x$	G-5	27.0	± ²⁾	4	4.8	75.0
$4x \times 4x$	G-6	31.5	± ²⁾	1	1.2	0.0
$4x \times 4x$	G-7	30.0	—	4	4.8	0.0
$4x \times 4x$	G-8	38.0	±	2	2.4	50.0
$4x \times 4x$	G-9	31.5	± ²⁾	2	2.4	50.0
$4x \times 2x$	G-1	30.5	—	0	0.0	—
$4x \times 2x$	G-2	24.5	—	0	0.0	—
$4x \times 2x$	G-3	30.0	—	0	0.0	—
$4x \times 2x$	G-4	29.5	±	0	0.0	—
$4x \times 2x$	G-5	28.0	—	0	0.0	—
$4x \times 2x$	G-6	26.0	± ²⁾	0	0.0	—
$4x \times 2x$	G-7	29.5	—	0	0.0	—
$4x \times 2x$	G-8	29.0	—	0	0.0	—
$4x \times 2x$	G-9	29.5	—	0	0.0	—

1) : Refer Table 1

2) : Roots were formed from the callus

nutrients, including growth substances, were investigated using various explanted ovaries for 40 days after pollination. The explanted ovaries were little infected by moulds and bacteria. Percentage of seed set was estimated about the same as that found in natural condition.

Result on the interaction between IAA and kinetin is shown in Table 4. Developmental rate of capsules estimated by their length was best in $2x \times 2x$. In $2x \times 4x$ and $4x \times 2x$ the development of capsules was about half of that observed in natural condition. In certain cases, callus was formed from cut end of ovaries as shown in Fig. 2. Its formation was influenced by growth substances, especially, by concentration of IAA. High concentration (5 mg/l)

Fig. 2. Callus formation at the cut end, right to left ; $\pm, +, ++, +++$, showing different degrees of callus formation.

Table 6. Interacting effects of growth substances and tomato juice, yeast extract, casein hydrolysate and coconut milk on the growth of explanted ovaries : data from five different media in regard to growth substances were pooled

Medium ¹⁾	No. ovaries examined	Capsule length (mm)	Callus formation	No. of seeds obtained	Seed set (%)	Seed germination (%)
NO	7.5	27.8	—~ $\pm$	8	1.2	75.0
NI	7.5	24.2	—~+	0	0.0	—
NK	7.0	25.8	$\pm$ ~+	6	1.0	33.3
NG	7.5	27.6	$\mp$ ~ $\pm$	4	0.6	25.0
NT	7.5	24.2	—~++	1	0.2	—
TJO	7.5	28.9	—~+	0	0.0	—
TJI	7.3	25.8	—~ $\mp$	0	0.0	—
TJK	7.5	29.3	$\pm$ ~++	0	0.0	—
TJG	7.3	30.5	$\pm$ ~+	0	0.0	—
TJT	7.3	24.9	$\mp$ ~+	0	0.0	—
YEO	7.0	26.5	$\mp$ ~ $\pm$	6	1.0	33.3
YEI	6.5	29.2	$\pm$	12	2.3	58.3
YEK	6.8	28.6	$\pm$ ~+	5	0.9	—
YEG	7.0	25.6	$\pm$ ~+	9	1.6	22.2
YET	7.3	26.5	+	16	2.5	12.5
ChO	7.5	29.0	$\pm$ ~+ ²⁾	26	3.9	50.0
ChI	7.3	21.7	$\pm$ ~+	15	2.4	—
ChK	7.5	29.0	+ ²⁾	13	2.0	61.5
ChG	7.5	28.2	$\mp$ ~+	23	3.5	82.5
ChT	7.3	23.9	+~++	9	1.4	—
CMO	7.5	29.2	+~++ ²⁾	14	2.1	78.6
CMI	6.8	27.0	+~++	8	1.3	—
CMK	7.5	30.4	+~+++	9	1.4	55.6
CMG	7.0	30.1	+~++ ²⁾	9	1.5	66.7
CMT	7.3	27.5	++	8	1.3	—
CMChO	8.0	30.4	+	10	1.4	60.0
CMChI	7.5	27.2	+~++ ²⁾	8	1.2	37.5
CMChK	7.5	30.2	+~++ ²⁾	18	2.7	55.6
CMChG	7.0	29.2	+~+++	15	2.4	40.0
CMChT	7.5	27.1	++~+++	15	2.3	60.0

1) : See "Methods"

2) : Roots were formed from the callus

of IAA caused frequent callus formation. Root never developed from the cut end but did from the callus. The root development was not influenced by the degree of callus formation. Number of seeds obtained was six in $2x \times 2x$, 17 in $4x \times 4x$ and three in $4x \times 2x$ crosses. No seed was produced in $2x \times 4x$. Seeds from $4x \times 2x$ were, however, not viable. The rate of seed set was about three times better in $4x \times 4x$ than in $2x \times 2x$. Though the callus formation was influenced by growth substances, the seed set did not respond to these substances. Germination of the seeds was good.

(2) Interaction between IAA and gibberellic acid

Result on the interaction of IAA and gibberellic acid is shown in Table 5. Growth of capsules was the same or better than that cultured in the medium containing IAA and kinetin. Their growth rate was not obviously influenced by different amount of the growth substances. Callus formation was only observed in media containing IAA. As the concentration of IAA was increased, the degree of callus formation became better. Gibberellic acid did not exert direct influence on the callus formation. Root development from the callus was observed in all media containing gibberellic acid, particularly in the medium that contained 1 mg/l of IAA and 5 mg/l of gibberellic acid (G-6). Three mature seeds were found in $2x \times 2x$. In $4x \times 4x$ mature seeds were obtained in every medium, seed set being from 2.1 to 8.3 per cent. Germination rate was from 50 to 100 per cent. On the other hand, no seeds were produced in reciprocal crosses between $2x$ and $4x$. The seed set was not influenced by the supplemented growth substances except the cross of $4x \times 4x$.

(3) Interacting effects of growth substances and other nutrients

Interacting effects of growth substances and other nutrients on the explanted ovaries were investigated, and the result was given in Table 6. Explanted

capsules grew longer when $4x$ was used as the maternal plant than when $2x$ was used as female. The development of capsules was not influenced by the growth substances. Though rate of callus formation was different in certain cases, no obvious effect of culture conditions was detected, and the development of callus formation was generally poor. Seed set was better in $4x \times 4x$ than in $2x \times 2x$, and their germination rate was within a range of 50 and 100 per cent. The best seed set and seed germination were obtained when the basal medium containing no growth substances was used. As to the reciprocal crosses between $2x$ and $4x$, a single hybrid seed of the cross, $4x \times 2x$, was obtained in the medium containing only gibberellic acid; it, however, did not germinate. In $2x \times 4x$, no hybrid seed was obtained.

As to the effects of tomato juice, supplement of it with growth substances gave a worse influence on seed setting than the growth substances alone. Callus formation was observed in the all media containing tomato juice, though difference between ovaries was great. Seed was not produced at all.

Yeast extract caused better development of capsules than the control. Callus formation at the cut end became also better. Rather abundant seeds were obtained, whose germination rate was, however, considerably low. Two hybrid seeds were obtained from the cross, $4x \times 2x$, in the medium containing only yeast extract (YEO); one of them germinated, giving rise a triploid hybrid. Though seed set was good in $2x \times 2x$, no hybrid seed was obtained from $2x \times 4x$.

As to casein hydrolysate, growth of capsules was not influenced by co-enriched growth substances. The callus formation was generally good. Its development apparently under influence of casein hydrolysate. Roots were developed from the callus in two media, ChO and ChK. The number of seeds obtained was seven in $2x \times 2x$, 77 in $4x \times 4x$ and two in $4x \times 2x$. Two hybrid seeds from $4x \times 2x$ were produced in the media, ChO and ChK. No seed was formed in the cross, $2x \times 4x$.

Coconut milk caused better growth of capsules, when $2x$ than $4x$ was used as the female. Generally the callus formation was

good. Root development was also stimulated, particularly, in the medium containing coconut milk and IAA. Number of seeds obtained was 29 in $2x \times 2x$, 17 in $4x \times 4x$ and two in $2x \times 4x$. The seed formation was better in $2x \times 2x$ than in $4x \times 4x$. No hybrid seed was got from $4x \times 2x$. Germination of seeds was better in $4x \times 4x$ than in $2x \times 2x$. Two seeds, which were obtained from $2x \times 4x$ in the media containing coconut milk only and coconut milk and gibberellic acid (ChO and CMG), gave rise triploid hybrids.

When coconut milk, casein hydrolysate and growth substances were supplemented together, development of capsules was better in $4x \times 4x$ than in $2x \times 2x$. The callus formation was found the same or a little better in this medium than in those containing coconut milk and growth substances. Number of seeds obtained was five in $2x \times 2x$, 60 in $4x \times 4x$ and one in $4x \times 2x$. Germination of seeds of $4x \times 4x$ was poor. One hybrid seed was obtained from the cross, $4x \times 2x$, in the medium, CM-ChG. The seed germinated, giving a triploid hybrid.

Hybrid Plants Produced by Ovary Culture

Number of viable hybrid seeds which were obtained by ovary culture was two in both $2x \times 4x$ (CMO and CMG) and $4x \times 2x$ (YEO and CMChG) (Fig. 3). Size of the seeds of $2x \times 4x$ was intermediate between those of $2x \times 2x$ and $4x \times 4x$, while that of $4x \times 2x$ was about the same or a little larger than that of $4x$.

Fig. 3. Hybrid seeds produced by ovary culture.

The hybrid plants raised from those seeds were grown in pots, and their morphological characteristics were investigated (Figs. 4-9), which were intermediate of those of the parents, $2x$ and $4x$. Cytological examination revealed that all of them were triploid. The hybrids of the reciprocal crosses, $2x \times 4x$ and $4x \times 2x$, were resembled each other.

Fig. 4. Diploid *Brassica chinensis*. (a) A whole plant, (b) its leaves showing both sides.

Fig. 5. Autotetraploid *Brassica pekinensis*. (a) A whole plant, (b) its leaves showing both sides.

Figs. 6 and 7. Two F_1 hybrids from the cross, *B. chinensis* (2x) $\times$ *B. pekinensis* (4x). (a) Whole plants, (b) their leaves showing both sides.

Figs. 8 and 9. Two F_1 hybrids from the reciprocal crosses, *B. pekinensis* (4x) $\times$ *B. chinensis* (2x). (a) Whole plants, (b) their leaves showing both sides.

Discussion

Although many histological and embryological investigations on cross-incompatibility following interspecific hybridizations have been made, its physiological or biochemical investigations are rather rare. No normal seed development was observed in the cross, *Aegilops squarrosa* $\times$ *Triticum dicoccum* (NISHIKAWA 1959), because of the degeneration of endosperm occurred soon after pollination. In this process, activity of the growth substance was measured. During 3 to 7 days after pollination, the activity was always higher in *Aegilops* $\times$ *Triticum* F_1 than in *Aegilops* itself. Investigation of excised tobacco pith revealed that real somatic cell divisions occurred when IAA and kinetin were added to the medium (Das *et al.* 1956). Growth and organ formations were regulated by the interactions of

IAA and kinetin, when plant tissues were artificially cultured (SKOOG and MILLER 1957). Effects of gibberellic acid on cell division were also reported (SACHS *et al.* 1959; SACHS and LANG 1957; SCHROEDER and SPECTOR 1957; GREULACH and HAESLOOP 1958), and further activity of α -amylase in barley endosperm became high when gibberellic acid was added to the reaction mixture (VARNER and CHAUD 1964). In artificial culture of excised anthers, pollen mother cells in leptotema to zygotema developed to pollen tetrads in the medium containing IAA and gibberellic acid (VASIL 1957). In ovule culture of *Papaver*, better development was observed in the medium containing IAA and kinetin (MAHESHWARI 1958), and by the *in vitro* culture of ovaries of *Anethum*, matured embryos were obtained four weeks after explantation in the medium containing IAA and gibberellic acid (JOHRI and SEHGAL 1963).

Based on those results, IAA, kinetin and gibberellic acid were chosen as the growth substance, and their interacting effects on the artificial culture of ovaries of certain cross-incompatible *Brassica* hybrid were investigated in the present experiment. Remarkable influence of growth substances was observed upon callus formation at the cut end of ovary. Seed formation became better in $4x \times 4x$ than in $2x \times 2x$, when IAA and gibberellic acid were added to the medium. A few hybrid seeds were obtained but a clear effect of the different concentrations of the growth substances could not be found in seed setting.

According to NITSH (1954), addition of tomato juice to the culture medium exerted stimulative effect on cultured tissues, which was comparable to that of coconut milk. Later, it was proved that the tomato juice contained a kinetin-like substance (NITSH 1960). It became also known that the tomato juice from both ripe and unripe, green fruits was effective to development of artificially cultured ovules (RANGA SWAMY 1959; NITSH 1954, 1963). Callus was induced from maize endosperm, when yeast extract was added to medium (STRAUS and LARUE 1954; TABATA and MOTOYOSHI 1965). *In vitro* culture of the pollen of *Ginkgo biloba* was also successful with yeast extract (TULECKE 1957). Casein hydrolysate was effective not only on development of artificially cultured embryos (ZIEBER *et al.* 1950) but also for culture of plant tissues (SHANTZ and STEWARD 1959). Coconut milk was proved to be an excellent growth factor for very young embryos (van OVERBEEK *et al.* 1941) and for callus from plant tissues (CAPLIN and STEWARD 1948; SHANTZ and STEWARD 1959). Using coconut milk, *in vitro* culture of pollen of *Ginkgo biloba* was made, and normal development of pollen grains was observed (TULECKE 1957). The ovary of *Anethum* was developed normally in the medium containing coconut milk (JOHRI and SEHGAL 1963). The medium containing coconut milk and casein hydrolysate promoted the growth of plant callus (SHANTZ and STEWARD 1959). Based on those results of previous workers, tomato juice, yeast extract, casein hydrolysate and coconut milk were added to the culture media in various combinations to the growth substances, and their effects on

excised ovaries of cross-incompatible *Brassica* hybrids were investigated in the second part of the present experiment. Development of callus was better in the media containing those substances than in the basal medium. Some differences of the callus formation were shown by those media; the media containing coconut milk and casein hydrolysate promoted remarkably the callus formation. The medium containing tomato juice with or without the growth substances did not cause any seed formations. Other substances were effective in more or less extent on seed formation, and some differences were found between them; while the co-supplemented growth substances had almost no effect on cultured hybrid ovaries. It was of interest to notice that the rates of seed formation of $2x \times 2x$ and $4x \times 4x$ were greatly changed by the media; those containing yeast extractor coconut milk resulted in better seed formation of $2x \times 2x$ than $4x \times 4x$, while casein hydrolysate caused much better seed setting of $4x \times 4x$ than $2x \times 2x$. Seed germination rate was comparatively low, when yeast extract was added while it became high when coconut milk was supplied to the medium. Triploid hybrid seeds were obtained from the cross, $4x \times 2x$, in the medium enriched with yeast extract and casein hydrolysate, and those of $2x \times 4x$ with coconut milk. No media gave rise triploid hybrid seeds of the both crosses, $4x \times 2x$ and $2x \times 4x$. It might be due to a different physiological requirement of the reciprocally crossed hybrid seeds for their development.

Summary

Excised ovaries of cross-incompatible *Brassica* hybrids, *B. chinensis* ($2x$) $\times$ autotetraploid *B. pekinensis* ($4x$), were cultured with the media containing growth substances (IAA, kinetin and gibberellic acid) and other nutrients (tomato juice, yeast extract, casein hydrolysate and coconut milk) in different concentrations. The callus formation at the cut end of ovaries was influenced by concentration of the growth substances and other nutrients; remarkable callus development was observed in the media containing IAA, coconut milk and casein hydrolysate. The seed formation of excised ovary was not influenced by the

growth substances but by the other nutrients. Hybrid seeds were produced with the enriched media containing IAA and kinetin, yeast extract, casein hydrolysate and coconut milk, and triploid hybrid plants were obtained from them. However, in no cases, the reciprocal crosses, $4x \times 2x$ and $2x \times 4x$, were successful with the same medium. The hybrid plants thus obtained were morphologically similar, showing intermediate characteristics of their parents.

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3倍性 *Brassica* 雑種の子房の試験管培養

1. 培養基について

猪俣伸道

二貫目体菜 (*Brassica chinensis* L., $2x$) とチーフ白菜 (*B. pekinensis* Rupr., $4x$) は交雑不和合である (第3表)。その生理学的機構を解明する一手段として、交雑授粉した子房を人工培養し、子房の発達・雑種胚の形成などを観察した培養基には Nitsch の基本培地に生長促進物質 (IAA, カイネチン, ジベレリン酸) や他の栄養物質 (トマトジュース, イースト抽出物, カゼイン加水分解物, ココナットミルク) を種々の組合せで添加したものをを用いた。 $2x \times 4x$, $4x \times 2x$, $2x \times 2x$, $4x \times 4x$ (後2者は対照) の交配を行なった子房を授粉4日後に切りとり、上記の培養基に植え、 22°C , 100 ルックスで36

日間培養した。 $4x \times 2x$ 雑種はイースト抽出物 (1個体), カゼイン加水分解物 (1個体), カゼイン加水分解物+カイネチン (1個体), カゼイン加水分解物+ココナットミルク+ジベレリン酸 (1個体) を含む培養基から、 $2x \times 4x$ 雑種はココナットミルク (1個体), ココナットミルク+ジベレリン酸 (1個体) を含む培養基から得られた。イースト抽出物, カゼイン加水分解物の添加からは $4x \times 2x$ 雑種が, ココナットミルクの添加からは $2x \times 4x$ 雑種が得られたので、相反交雑の雑種胚は異なった栄養要求性をもつと考えられる。