In vitro culture of ovary in orchids (I)
Effects of sugar, peptone and coconut milk upon the growth of ovary of *Dendrobium nobile*

By ITSUHIKO ITO

Summary As a method of ovary culture, the "Partial sterile culture method" was proposed. The ovaries of *Dendrobium nobile* pollinated at the start of the culture process developed well on Nitsch's medium with inorganic salts and sugar; and any kind of additional organic substances was dispensable, although a slight supplement of peptone and coconut milk promoted development of ovary. And 6% sucrose seems to be optimum for ovary growth. Peptone was one of the most easily available nutriments for seed fertility.

Introduction

Since the early decades of this century, scientific research dealing with artificial germination of orchid seed has been carried out by Bernard[2,3] and Burgeff[3-8] and advanced by Knudson[23-26], Withner[49-54] and many other workers. Most of this research was carried out in relation to symbiotic germination of normal seeds and growth of seedlings, using immature seed in a few cases. A few years ago, the present author[15-17] presented a culture method of orchid ovaries of excised flowers for securing germinable seeds.

Of the reports which give valuable information in the field of ovary culture, the present author will cite Nitsch's articles[33-38] on the culture in vitro of fruit of several plants such as tomato, bean, tobacco and gherkin etc. Anantaswamy[1] examined the influence of colchcine upon the development of endosperm and embryo in excised ovaries of *Phlox drummondii* cultivated in vitro. He found considerable irregularities in the behavior of nuclei in early embryogenesis on the medium with and without colchicine and of endosperm grown in colchicine medium. After the experiment with *Papaver somniferum*[30], Maheshwari and Lal[31] cultured ovaries of *Iberis amamra* in vitro and studied the effect of several chemicals on the development of the pericarp and ovule. Culturing the ovary from the flower one day after pollination on sugar-agar medium containing minerals and vitamins, they obtained an almost exact duplication of natural fruit and reported that in about 2 weeks the growth of the ovary was completed, the seeds became brownish, and the embryo and the endosperm were

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The author wishes to express his hearty gratitude to Prof. Shun-ichiro Imamura and Prof. Minoru Hamada, Kyoto University, for their kind advices and valuable criticisms. Many thanks are also due to Prof. Yotaro Tsukamoto, Kyoto University, Prof. Makoto Shisa, Nagoya University, Prof. Carl L. Withner, Brooklin College and Prof. Haruyuki Kamemoto, University of Hawaii, Prof. S. Takashima and Prof. R. Nakahira, Kyoto Prefectural University for their encouragement and kind advice. And also the author wishes to express his gratitude to Mr. C. Takeda, Mr, H. Yamamoto, Dr. K. Miyake, Mr. T. Kawamura who are members of the Japan Orchid Society for their generous aid and encouragement.

similar to those obtained in nature. They[32] subsequently investigated the influence of several growth substances on the artificially reared ovaries, and obtained viable seeds from the ovaries excised one day after pollination. Moreover, there are many other papers dealing with ovule or ovary culture in vitro carried out in Maheshwari school at Delhi University. Using the Nitsch technique Rébei and Rédei[40] obtained viable plants from the very young embryo of wheat ovaries excised 4 days after pollination. Chopra[10] also produced parthenocarpical hollyhock fruit in vitro from ovaries excised 3 days after pollination. Sachar[42] and Sachar and Baldev[43] attempted culture in vitro of the ovaries of *Tropaeolum majus* collected 6 days after anthesis and of *Linaria maroccana* obtained 2 days after pollination, using Nitsch's basic medium with White's (1954) modified vitamin solution and they gained viable seeds of *L. maroccana* on media containing several substances or yeast extract. Better growth was gained on yeast extract and test tube fruit matured about 6 days earlier than controls. The seeds from fruit ripened in yeast extract (0.25%) germinated successfully.
Nitsch[37] observed only in the gherkin (*Cucumis anguria*) the formation of a few seeds per fruit which was excised from the plant 3 days after pollination; and 11 seeds per fruit excised 4 days after pollination, and 6% of the latter germinated. Jansen and Bonner[19] also achieved success in culturing ovaries of another species of tomato (*L. pinpinellifolium*), but they have never produced seeds. Kano[20] cultured fruit of the tomato in vitro parthenocarpic, using 2, 4 dichlorophenoxy acetic acid (5 ppm) and investigated the effects of these additions to medium, light, temperature and stages of floral development on the ovary growth. Israel,[13] with orchids as material, cultured the ovaries of *Dendrobium* hybrid in a test tube containing culture medium modified from Vacin and Went (1949) with various concentration (1-50ppm) of alpha-naphthalene acetic acid, and he reported that in every case a small sample of placenta produced many seedlings, and that an effective NAA concentration would be between 1 and 50 ppm.

Several methods for the sterile culture of whole plants have been described.[5, 11, 22, 27, 28, 41, 45] Blanchard and Diller[4] devised a method whereby only the roots are maintained in a sterile medium containing sucrose and other organic substances, and other parts remained outside of the culture vial, eanabling the testing of the effects of various substances susceptible to microbial contamination.

In the present study, plant organs such as ovary or fruit, which remained out of vials, were nourished by nutrient solution supplied aseptically in the vials. The technique has already been applied as a method for vegetative propagation of *Phalaenopsis* by Urata and Iwanaga.[47] Research dealing with ovary or fruit culture of the orchid has been carried out with this technique since 1956.

Material and methods

The plant material used was *Dendrobium nobile* Lindl. In this species the germination rate is high, flowers are numerous, fruit sets easily and the seed fertility is very high. As this species has often been used in studies with regard to cytology[12, 18, 35] and germination of immature seed[14] or culture of seedling in vitro,[15] it was

thought advantageous to use it in this study also. When the experiment was started, between January and March, plants were generally forced to bloom in a small 20–25°C room in greenhouse.

As nutrient medium Nitsch,s solution[36] with modified sugar concentration was adopted. The solution was made up as follows:

Nitsch's basic solution	
$Ca(NO_3)_2$ $4H_2O$	500mg
KNO_3	125mg
$MgSO_4$ $7H_2O$	125mg
KH_2PO_4	125mg
Distilled water	1000ml

Trace element solutions	
A : H_2SO_4 sp. gr. 1.83	0.5ml
$MnSO_4$ $4H_2O$	3000mg
$ZnSO_4$ $7H_2O$	500mg
H_3BO_3	500mg
$CuSO_4$ $5H_2O$	25mg
Na_2MoO_4 $2H_2O$	25mg
Distilled water	1000ml
B : $FeC_6O_5H_7$ $5H_2O$	10g
Distilled water	1000ml

To 1 liter of the basic solution, both 1 ml of trace element solutions and 60g of sucrose were added and the medium was adjusted to pH 6.0 before autoclaving.

In this species of *Dendrbium* the time between pollination and arrival of pollen tube to the inside of ovarian cavity is relatively long. Following histological observation of the growth of fruit, it was found that the embryos are formed within 110 days after pollination; it is presumed about 80 days after pollination. Considerable time being necessary for the pollen tube to attain to the ovarian cavity, it may be harmful for the pollen, pollen tube, and stigma to come into contact with any disinfectant for the sterilization. Perhaps, this was the reason why the culture tried by Nitsch with gherkin ovaries excised on the day of anthesis failed to yield germinable seed. Accordingly, the present author proposed a new method named "partial sterile culture method". In this method, the stigma, which is one of the most delicate parts of flower, does not come into contact with the disinfectant, so that the process of pollination and fertilization is not adversely affected.

For the measurement and adjustment of pH values of pH values of nutrient solutions, a glass electrode pH meter was used. The glass vessels used for the culture were small vials of 2.4 cm in diameter and 5.0 cm in length, and those of 2.5cm in diameter and 6.0 cm in length. These vials hold 15 and 20 ml of liquid media respectively. After charging with solution, the mouth of vial was closed with a rubber sheet (0.5 mm thick), washed with soap and distilled water a fine wire. Two holes were cut in the rubber sheet by means of a heated metal needle; one hole was made 1 mm in diameter while the other was 0.5 mm in diameter. Before autoclaving, the smaller hole was closed by means

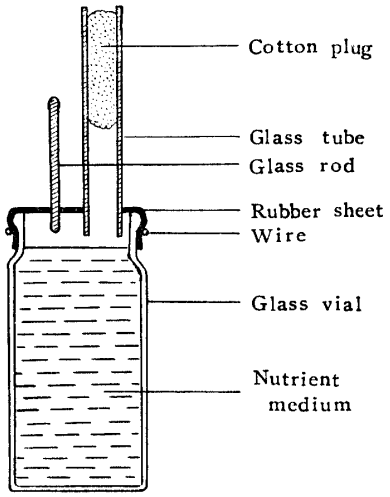

Fig. 1. Apparatus for ovary culture in vitro.

of a glass rod and in the larger hole a glass tube (5 cm long, 4–5 mm in diameter) plugged with cotton was inserted. The latter serves for aeration of the vial and is also necessary when autoclaving at 15 pound/in^2 over pressure for 10 minutes. The solution was never renewed.

In preparing the material about 1 week after anthesis, the flowers were removed from the plants several minutes or several hours before the start of the culture. The flower stalk was cut out 0.5 mm below the peduncle. Some withered bracts at the base of the peduncle were removed before sterilization, while in some experiments petals and sepals were removed after starting the culture. The removal of the petal and sepal had little influence upon the ovary growth. When the exclusion was done at the begining of the culture, slight injury due to dryness was observed in the part of stigma.

The ovaries and peduncles were cleansed with 75% ethyl alcohol and soaked for 10 to 15 minutes in calcium hypochlorite solution (Wilson's method). Only peduncles were inserted into the nutrient solution through the smaller hole on the rubber sheet. After this procedure, the ovaries were washed with un-sterilized water. If washing was omitted, the ovaries were apt to be seriously injured. The pollination was always done within ca. 20 hours after the culture was started. In general, self-pollination resulted. The cultures were placed in an incubator at 25°C which was furnished with double pane surroundings and set in a greenhouse. In warm weather the temperature inside the incubator frequently rose to 34°C in the daytime. One experiment (kind of sugar) was performed in an air-conditioned room of 20° ± ca. 2°C under artificial light at Kyoto University. Experiments were performed under natural day length.

The growth of ovaries and fruit was taken as being indicated by maximum width and length measured by means of calipers (Fig. 2). Weight of the cultured fruit without peduncle was measured, except in the case of the primal experiment. In most cases one lot consisted of 10 flowers at the start of experiment. Fruit with contaminated peduncles or the fruits which withered or died physiologically, were excluded. Therefore, there were often only a few fruit left to be measured at the end of experiment. The period of culture in the earlier part of the study was 4 weeks, and in the later experiments, 5 months was the general period used. In case of fruit cultured for 5 months, the fertility of seed was examined. The seed thus obtained was named "cultured seed".

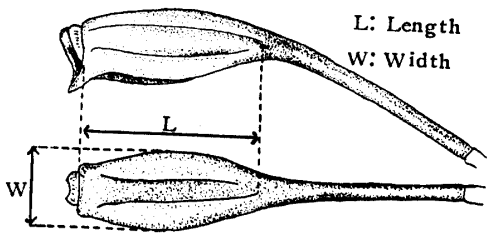

Fig. 2. Standard for the measurement of *Dendrobium* fruit.

Orchid ovaries frequently develop into seedless fruit. In *Dendrobium nobile* such phenomenon is seldom observed, but in the case of vitro culture, it occurs rather frequently. As the testa of *D. nobile* consists of one transparent layer, one can distinguish fertile seeds which contain well developed embryos from empty ones. Seed fertility was determined by the naked eye, or sometimes by means of a microscope

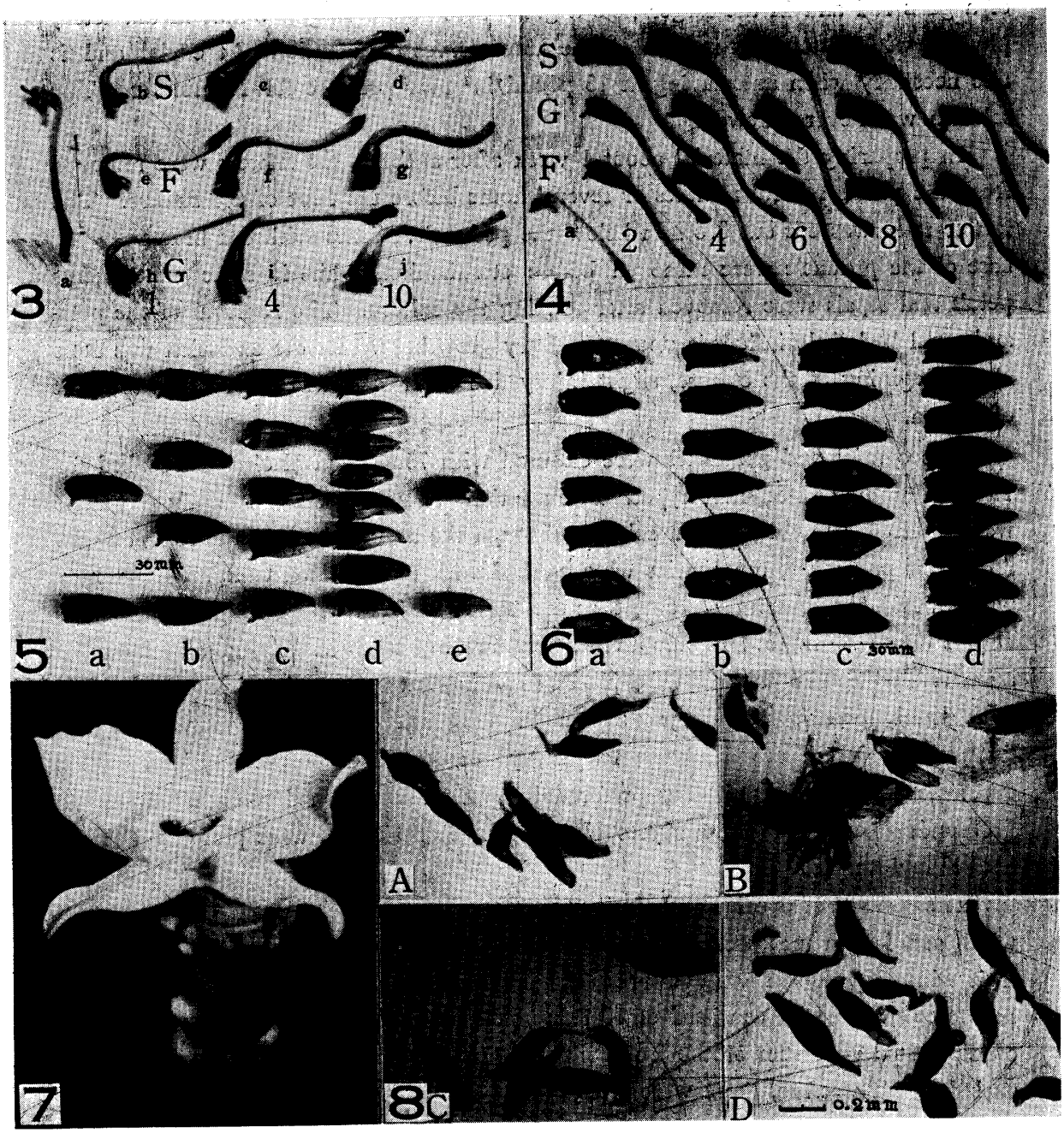

Fig. 3. Fruit cultivated for 3 weeks on Nitschs's media containing different concentration of sugars. From left to right; a: control (at anthesis); b, e, h: 1%; c, f, i: 4%, and d, g, j: 10%. Top row: sucrose; center row: fructose; bottom row: glucose.

Fig. 4. Fruit cultivated for 6 weeks, Top: sucrose, center: glucose and bottom: fructose. From left to right: 2, 4, 6, 8 and 10%. a: control (at anthesis).

Fig. 5. Fruit cultivated on Nitsch's media with 6% of sucrose and various concentrations of peptone. a: 500 ppm; b: 100 ppm; c: 50 ppm; d: 10 ppm; e: control.

Fig. 6. Fruit cultivated on Nitsch's media with 6% of sucrose and different concentrations of coconut milk. a: control; b: 5%; c: 10%; d: 20%.

Fig. 7. Apparatus of culture with a flower of *Dendrobium nobile* in vial containing liquid medium.

Fig. 8. A-B. Seed formation in cultured fruit. A: peptone 50 ppm; B: control of peptone experiment; C-D. Seed formation in pod grown on plant. C: Seeds from 5 months old immature pod. D: Seeds from 13 moths old pod.

of low magnification. The seed formation of a fruit was quantitatively demonstrated with 5 degrees according to the number of fertile seeds per fruit.

10^0–10^1	+	10^3–10^4	||||
10^1–10^2	++	10^4–10^5	|||||
10^2–10^3	+++		

For determining seed size, 40 or 50 seeds from the ovaries of highest fertility were measured, both in length and width of embryo or only in width, under a microscope of low magnification.

Results

1. Influence of sugar.

Thirteen kinds of sugar, 7 monosaccharides, 5 disaccharides and 1 trisaccharide, were employed. For some monosaccharides and for cellobiose, 90% of the total sugar was replaced with glucose. The results after 5 weeks of culture show that disaccharide is superior to monosaccharide for ovary growth (Table 1). The growth of ovaries

Table 1. Effect of sugars on the development of excised ovaries[1)]

Sugar and its concentration	Width of ovary (mm)	Length of ovary (mm)	Number of ovaries	Number of ovaries with healthy stalk
Frucose 4%	5.5±0.7	9.0±1.3	10	0
Galactose 4%	5.2±0.8	9.7±1.9	9	0
Glucose 4%	5.1±1.3	8.5±1.3	7	0
Mannose 4%	4.8±1.0	9.1±1.6	9	0
Glucose 3.6% plus arabinose 0.4%	5.2±0.6	9.3±1.2	8	0
Glucose 3.6% plus fructose 0.4%	6.0±0.8	10.6±1.5	10	0
Glucose 3.6% plus galactose 0.4%	6.0±0.8	10.6±1.5	10	0
Glucose 3.6% plus mannose 0.4%	4.8±1.5	8.4±1.7	9	0
Glucose 3.6% plus xylose 0.4%	5.4±0.8	9.3±1.6	10	0
Glucose 3.6% plus cellobiose 0.4%	5.4±0.8	9.3±1.6	10	0
Lactose 4%	6.7±0.9**	12.1±1.1*	10	7
Maltose 4%	6.4±1.0*	12.1±1.5*	10	7
Sucrose 4%	5.2±1.4	10.1±2.4	9	2
Raffinose 4%	4.2±1.4	8.9±1.6	2	2

1) Ovaries were measured after 5 weeks of culture.
* Significantly different from the value of sucrose, at the 5% level.
** Significantly different from the value of sucrose, at the 1% level.

in the solution containing raffinose seems to be inferior to that of others, though the color of ovaries turned dark green. Among the disaccharides, maltose and lactose are significantly superior to sucrose. In general, monosaccharides rapidly killed the peduncle tissue, while di- and trisaccharides did not, except for a small part near the excised surface of it. In the peduncle some changes always took place with the progress of the culture, generally after 2 or 3 months. The color changed to white, brown or black; and sometimes withering took place. These changes occurred

without any contamination. Therefore, it is supposed that the growing fruit can receive nutrient even through the changed or withered tissue of peduncle.

Two experiments were carried out to examine the effect of sugar concentration. At first, ovaries were cultured in the following media: 1) Nitsch's solution without sucrose, 2) 4% sucrose in distilled water, 3) distilled water and 4) Nitsch's solution with 4% sucrose. The ovaries cultured in the medium containing only inorganic salts showed the most inferior growth, and at 10 days after the start of the culture, the growth ceased; rot progressed from about the stigma and the ovaries soon withered. The ovaries cultured in distilled water lived 2 weeks longer than those cultured in inorganic solution. In distilled water with only sugar the ovary could live 3 or 4 weeks.

In the second experiment, the ovaries were cultured on Nitsch's medium with 1, 2, 4, 6, 8 and 10% of glucose, fructose and sucrose.

Table 2. Influence of sugar concentration on the growth of excised ovaries[1]

Kind of sugar		Concentration of sugar (%)					
		1	2	4	6	8	10
Fructose	Width(mm)	—	7.1	6.8	7.4	7.1	7.2
	Length(mm)	—	13.4	13.4	14.2	12.5	11.2
	Weight(mg)	— (0)[2]	543 (2)	610 (3)	780 (1)	665 (1)	650 (1)
Glucose	Width	—	6.3	7.2	7.1	8.4	6.1
	Length	—	13.7	15.1	15.7	16.5	10.4
	Weight	— (0)	570 (1)	853 (8)	878 (3)	950 (3)	425 (2)
Sucrose	Width	—	6.8	8.0	8.1	8.5	10.5
	Length	—	15.6	17.6	15.8	16.2	17.8
	Weight	— (0)	697 (4)	1046 (5)	1029 (8)	1032 (3)	1545 (2)

1) Ovaries were measured or weighed after 6 weeks of culture.
2) The number in parentheses shows the number of measured ovaries.

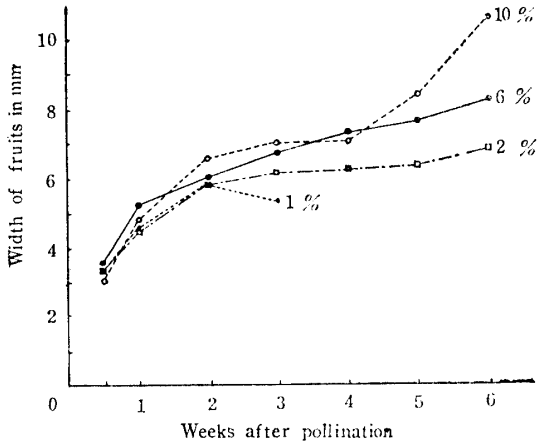

Fig. 9. Growth of ovaries grown in different concentrations of sucrose.

As shown in the first experiment, it was found that sucrose was superior among the 3 kinds of sugars used. As a whole, it was indicated that sugar solution of 4 or 6% concentration was optimum for ovary culture in *Dendrobium nobile*.

In 1% solution of any sugar, the growth of ovary became gradually slower after 2 weeks and no ovary remained alive after 6 weeks (Fig. 9). A few ovaries survived in 10% solution of sucrose and showed excellent growth. But this was an extraordinary

case and the repeated experiments using the same (10%) concentration usually proved a complete failure as to the growth of ovary. From these results 6% sucrose was adopted in the subsequent experiments.

As the concentration rose higher, the width of the fruit increased and the ratio of length per width gradually decreased (Table 2). The fruit color was dark green at low concentration; it became pale and light yellow in 10% sucrose. The presence of physiologically withered fruit tended to increase in number as concentration rose.

2. Influence of peptone.

Media containing various concentrations of peptone (0, 10, 50, 100 and 500 ppm) were prepared with Nitsch's solution with 6% sucrose. After adjusting the pH value at 6, they were autoclaved at 20 pounds/in^2 overpressure. As shown in Figure 4, the addition of peptone seems to promote the growth of fruit, especially at 50 and 100 ppm. The growth of the fruit stopped, in general, at approximately 100 days after the start of culture and then fruits began to wither.

Table 3. Effect of different concentrations of peptone on the growth of fruit in vitro

Concentration of peptone (ppm)	No. of fruit measured	Width of fruit (mm)	Length of fruit (mm)	Weight of fruit (mg)
Control	3	9.3±0.5	21.5±2.2	855±185
10	8	9.7±0.8	21.7±2.2	1033±300
50	5	9.7±0.4	23.6±1.9**	1138±170
100	4	10.3±0.6	23.2±0.7	1159±232
500	3	10.0±0.3	24.1±0.3	1193± 6

Start of culture: 29. Feb. 1960. Medium: Nitsch's solution with 6% of sucrose, pH 6.0. Fruit was measured after 150 days of culture.
** Significant at the 1% level.

The results at the end of 5 months of experiment are given in Table 3. Compared with the control, the growth in length, width and weight of the fruit cultured on the media containing peptone was in general excellent (Fig. 10). After 5 months of culture with the addition of peptone (50, 100 and 500 ppm) the seed fertility was also comparatively high (Table 3). The length and width of cultured embryos were more or less inferior to the control (Table 5).

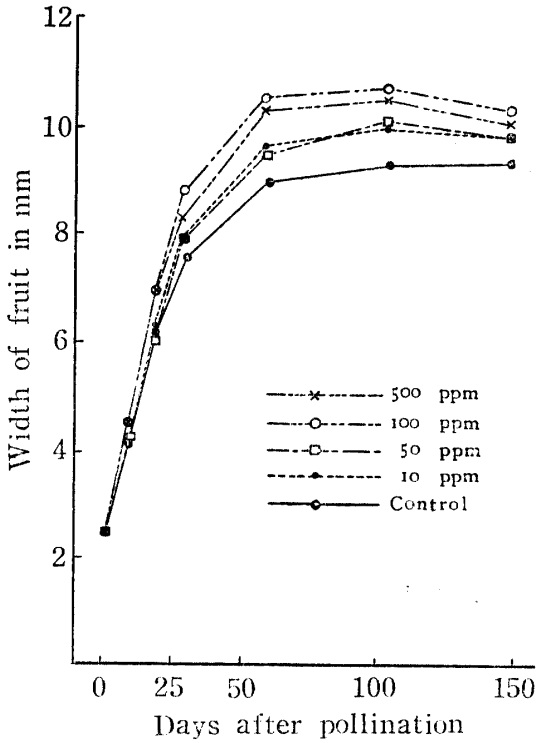

Fig. 10. Growth curves of fruit in vitro ; media with different concentrations of peptone.

3. Influence of coconut milk.

The coconut milk used in this experiment was brought from Hawaii. It was obtained from adequate imm-

Table 4. Fertility of fruit and abundancy of seeds in the fruit cultured in vitro in media of different concentrations of peptone

Concentration of peptone (ppm)	No. of sterile fruit	No. of fruit with various grades of fertility + ++ +++ ++++ +++++	Number of fertile fruit	Total number of fruit	Maximum number of seeds in the best fruit (unit : thousand)
Control	0	– – 2 1 –	3	3	ca. 5
10	1	– – 3 4 –	7	8	8
50	2	– – – 1 2	3	5	30
100	2	– – – – 2	2	4	20
500	0	– – – 1 2	3	3	30

Culture: 29. Feb. 1960 —27. July 1960. Observation: 30 days after the end of the culture (27. Aug. 1960).

Table 5. Size of embryos of the fruit cultivated in media of different concentrations of peptone for 150 days after anthesis

Concentration of peptone (ppm)	Sign of fruit individual	Length of embryo* (μ)	Width of embryo* (μ)
Control	p-52	136.2±10.6	86.2±11.4
10	p-49	124.3±15.0	77.4± 8.0
50	p-32	137.5±18.0	84.8±10.1
100	p-24	119.6± 3.5	82.6± 9.0
500	p-17	125.7± 6.0	79.7± 7.9

Seeds were measured 30 days after the end of culture (27. Aug. 1960).
* Average of 40 embryos.

ature nuts and had been autoclaved at 10 pounds/in^2 overpressure for 10 minutes on the day when the nut was picked from the palm plant. This milk was added after autoclaving to Nitsch's media of pH 6 with 6% sucrose to make, 5, 10 and 20%. As shown in Table 6, the fruit cultured in any of the concentrations was bigger than that of the control; in the case of the 20% concentration, both width and weight of the fruit were significantly large. Fertility of seed cultured at any concentration was higher than that of the control. The size of the seeds was inferior to that the control (Table 7 & 8). Seed fertility was not so high as was expected from the appearance of the fruits (Table 7). Furthermore, the fruits cultured on some media with the milk showed excellent growth and their surfaces are apparently deep green and glossy

Table 6. Effects of different concentrations of coconut milk on the growth of fruit in vitro

Concentration of coconut milk	No. of fruit measured	Width of ovaries at anthesis (mm)	Width of fruit after 5 months (mm)	Length of fruit after 5 months (mm)	Weight of fruit (mg)
Control	7	2.80±0.14	9.9±0.8	22.7±1.6	1093±219
5%	7	2.82±1.38	9.7±1.3	25.0±2.3*	1349±383**
10%	8	2.75±0.17	10.2±0.7	24.9±2.9	1328±286**
20%	9	2.81±0.15	11.2±0.5**	26.9±2.2**	1578±227**

Start of culture: 24 April 1960. Medium: Nitsch's solution with 6% of sucrose. Temperature: 20–34°C.
* Significant at the 5% level.
** Significant at the 1% level.

Table 7. Fertility and abundancy of seeds in fruit cultured in vitro in media of different concentrations of coconut milk

Concentration of coconut milk	No. of sterile fruit	No. of fruit with various grades of fertility + ++ +++ ++++ +++++	Number of fertile fruit	Total number of fruit	Maximum number of seeds in the best fruit(unit: thousand)
Conrol	1	- - 2 3 -	5	6	ca. 5
5%	2	- - - 3 2	5	7	20
10%	0	- - 1 1 6	8	8	10
20%	0	- - 2 4 2	8	8	20

Observations were made 30 days after the end of culture (20 Oct. 1960).

Table 8. Size of embryos in fruit cultured in vitro in media of different concentrations of coconut milk during 150 days after anthesis

Concentration of coconut milk	Sign of fruit individual	Length of embryo[1] (μ)	Width of embryo[1] (μ)
Control	cm-2003, 2007	106.6±22.3	66.2±12.1
5%	cm-1903, 1907	95.8±18.8**	64.7± 9.5
10%	cm-1705, 1706	97.9±12.3	65.0± 9.3
20%	cm-1601, 1603	103.2±21.1	60.5±11.8**

Embryos were measured 30 days after the end of culture (20 Oct. 1960).
1) Average of 80 embryos in 2 fruit.
** Significant at 1% level.

(Fig. 10). Namely, it was found that addition of 5-20% of coconut milk was highly effective with regard tothe growth of the fruit from a morphological point of view.

Discussion

Using tomato and gherkin ovaries and applying the culture technique on ovaries excised from the plant, the study of fruit physiology has been extened by Nitsch.[33-38] He stated that the main characteristics of fruit growth is unalterable in culture in vitro and the effect of pollination, the shape of the growth curve, and the ripening process seem to follow similar patterns in vitro and in situ. In the essential point, it was also evident in the case of *Dendrobium nobile* reported herein. The growth of the cultured ovary is superior to that on the plant during the first 2 weeks or so. The ovary can develop even with distilled water for about 10 days after the start of culture. Presumably, in the early stage, only sugar is necessary to maintain the growth of ovary.

Nitsch[37] added 5% sucrose to the medium for ovary culture. He[38] stated that carbohydrates are the important factor and tomato ovaries supplied only with auxin and mineral salts do not grow in vitro without some kind of organic carbon; growth curve rises steeply between 0 and 1% of carbohydrate, and at 5% it attains to a rather flat optimum. In the present experiment with *Dendrobium nobile*, the author recomends 6% of sugar such as sucrose, because the number of physiologically withered fruits is rather few at concentration from 3 to 6%. However, the ovaries of

vigorous flowers grow well even on the higher concentration than 6%, while for those of small flowers 4 or 5% of sucrose was preferable.

Every kind of sugar except raffinose influences the development of the *Dendrobium* ovary. The peduncle, particularly about the cut end, tends to be physiologically harmed. At least, for the growth of fruit (but not for the fertility), disaccharide, especially maltose, seems to be most preferable.

For the germination of orchid seed, much research was tried with 1.5-2% of sugar, while, for the tissue culture in some higher plants even higher concentrations of sugar have been adopted. The ovary seems to require rather high concentration of sugar. Such high concentration can not be taken for granted as necessary for nutriment. It seems very probable that sugar plays a special role in the fruit development under the present experimental conditions. The concentration of viscous liquid taken from the fruit tissue cultured both in vitro and on plant at 4 months after pollination corresponded roughly to 5 and 6% sucrose solution.

Several investigators[8,49] reported that the application of most amino acids had no effect in the promotion of seed germination and seedling growth. But, Curtis[9] stated that the seedlings of *Paphiopedilum*, *Phajus* and *Vanda* grew better and more regularly in the presence of peptone (50 ppm). Addition of peptone (10-500 ppm) showed good results in the growth of fruit as well as in the growth of seedlings, particularly, in the increase of seed fertility. The effect of peptone on the growth of fruit is coincident with that for seedling growth.[9,21]

Steward and Shantz[46] showed that leucoanthcyanin was one of the active principles present in coconut milk and Radkey and Dear[39] reported the occurrence of gibberellin-like substances in it. In the present experiment, 30-40 thousand seeds were possible to form in a fruit cultured on the media containing 10-50 ppm of peptone; while the fruits grown on the media containing 5-20% of coconut milk could form only 10-20 thousand seeds in maximum in respective concentrations. Disparity between best growth of fruit and comparatively low seed fertility in case of media with coconut milk, abnormality in the ovular development and disorder of fertilization are supposed to be due to an existence of abundant gibberellin-like substances in the milk.

In the early stage the ovary grows better in culture than on the plant; but, thereafter, the growth of young fruit becomes slow and at the end of the cultured period (5 months) even well-developed fruit are a quarter or one third in volume of those developed on the plant. It may be physiologically unimportant that the size of cultured fruit is small, just as Nitsch[37] stated.

Owing to the reduced seed number, it seems that the developmental process of seeds is scarcely affected by the artificial coditions, and seed similar to that normally matured can be produced. The decreased fertility seems to be attributable either to the fact that the fertilization was suspened, or the development of ovules after pollination was discontinued in the early stage of growth of embryo, though the pollen tube had reached the ovarian cavity.

It is interesting to note that in the fruit cultivated in vitro the seed-fertility was

usually low. In several plants the fruit cultured in vitro did not develop seeds, except in the case of the gherkin ovaries cultivated by Nitsch which bore a few fertile seeds. In the study dealing with self-pollination in orchids, Von Kirchner[47] described 3 operative factors causing sterility; inability of pollen to germinate on the stigma, inability of pollen tubes to grow into the ovary, and the production of seeds without embryos. In the present study the sterility of cultured fruit seems in most cases to be attributable to the disturbance of ovule development beofre fertilization or the failure of growth of embryo in the early stage after fertilization, because a great number of pollen tubes can be observed at the neighborhood of ovules 80-90 days after pollination. The fertilized seeds are found rather at the proximal end of the fruit than at the distal one, though it is nearer to the stigma.

摘 要 デンドロビウム・ノビルの開花時に切り取った花の子房を用いて，培養管外の器官を無菌的に培養する部分無菌培養法（器外器官培養法）により，稔性のある種子を含む果実を培養した．糖の種類および濃度は子房の発育に影響を与え，6％のしょ糖が果実の発育に適していた．果実の発達には糖以外の有機栄養は必要ではないが，少量の有機物質の添加は発育を助長した．50～500ppm のペプトンの添加は，果実の発育を促し，種子稔性を高め，ココナットの添加は，特に果実の発育に有効であった．

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