Culture in vitro of Excised Ovaries in *Brassica campestris* L.

II. Development of Excised Ovaries in Various Carbon Sources

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Studies on development in vitro of excised ovaries in the sibmate of diploid *Brassica campestris* L. ssp. *chinensis* (L.) Makino cv. Seppaku-taina and in the sibmate of autotetraploid *Brassica campestris* L. ssp. *pekinensis* (Lour.) Olsson cv. Nozaki-hakusuai were carried out. Development in vitro of excised ovaries at four days after pollination was studied in various carbon sources and various sucrose concentrations. Results obtained were as follows. (1) Seed set in the capsules was observed in culture media with sucrose, glucose, raffinose and fructose in each sibmate of diploid Seppaku-taina and autotetraploid Nozaki-hakusuai. (2) Neither growth of the capsules nor seed set in the capsules were observed in the medium with mannose in both sibmates. (3) In diploid Seppaku-taina, seed set in the capsules was better in the medium with sucrose or glucose than with fructose or raffinose. (4) In autotetraploid Nozaki-hakusuai, seed set in the capsules was better in the medium with sucrose, glucose or raffinose than with fructose. (5) In the experiment of various sucrose concentrations on development in vitro of excised ovaries, the higher sucrose concentration in the medium, the better seed set in the capsules was observed in diploid Seppaku-taina. Seed set in the capsules was best in the medium with 210 g/l of sucrose. Percentage of germinated seeds was, however, somewhat less than that from the seed obtained in the medium with lower sucrose concentration. (6) In autotetraploid Nozaki-hakusuai, seed set in the capsules was good from 30 g/l to 70 g/l of sucrose in the medium. (7) Seed set in the capsules in development in vitro of excised ovaries was different between diploid Seppaku-taina and autotetraploid Nozaki-hakusuai.

**Introduction**

No viable seeds were obtained ordinarily under natural condition in reciprocal crosses between diploid and autotetraploid *Brassica campestris* L. (Nishiyama and Inomata 1966). Hybrid plants, however, obtained by embryo culture of $2x \times 4x$ and $4x \times 2x$, when the mebyros were taken out from the ovule before degeneration and cultured in the medium with embryo factor (Inomata 1967). Hybrid plants were also obtained by culture in vitro of the ovaries ($2x \times 4x$, $4x \times 2x$) at early developmental stage (Inomata 1968). But until now culture condition of excised ovarised ovaries in *Brassica campestris* has not been investigated in details. The previous paper dealt with development in vitro of excised ovaries under various conditions of culture media, temperature and light (Inomata 1976). The present paper deals with development in vitro of excised ovaries in the sibmate of diploid and autotetraploid *Brassica campestris* in various carbon sources.

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Materials and Methods

The materials used in the experiment were diploid *Brassica campestris* L. ssp. *chinensis* (L.) Makino cv. Seppaku-taina and autotetraploid *Brassica campestris* L. ssp. *pekinensis* (Lour.) Olsson cv. Nozaki-hakusai. The ovaries were obtained from each sibmate. When emasculated flower buds bloomed two days after emasculation, pollination was done with fresh pollen grains. Ovaries four days after pollination were removed from the plant and cultured in various conditions.

In the experiment on various carbon sources, the medium used was Nitsch's minerals (1951) added with White's vitamins (1963) and 8 g/l of agar. Fifty grams per liter of one of the following carbohydrates was added in the culture medium: sucrose, glucose, raffinose, fructose and mannose.

In the experiment on various sucrose concentrations, the medium used was Nitsch's minerals added with modified White's vitamins (Inomata 1976) and 8 g/l of agar. Sucrose concentrations used in the experiment were 10 g/l, 30 g/l, 50 g/l, 70 g/l and 90 g/l.

In the further experiment on various sucrose concentrations, the medium used was Nitsch's minerals added with White's vitamins and 8 g/l of agar. Sucrose concentrations used in the experiment were 0 g/l, 10 g/l, 30 g/l, 50 g/l, 70 g/l, 90 g/l, 120 g/l, 150 g/l, 180 g/l and 210 g/l.

Sterilization and explantation of the ovaries were the same as the previous paper (Inomata 1976). The medium was adjusted to pH 5.6 ~ 5.8 before autoclaving. All the ovaries in the experiment were cultured in a culture room at 22 ± 2°C and 300 ~ 500 lux of illumination by fluorescent lamp for 12 hours a day. Forty days after pollination (36 days after explantation), the cultured ovaries were taken out from the test tube. The length of the capsules was measured and seed set in the capsules was counted. Rate of germinated seeds was also examined.

Results and Discussion

Development *in vivo* of ovaries under natural condition

The mean length of the ovaries four days after pollination was 20.2 mm in diploid Seppaku-taina and 15.6 mm in autotetraploid Nozaki-hakusai. Table 1 shows the results on development *in vivo* of ovaries under natural condition. The mean length of the capsules in diploid Seppaku-taina was shorter than in autotetraploid Nozaki-hakusai. Seed set in the capsules was less in diploid Seppaku-taina than in autotetraploid Nozaki-hakusai. Percentage of germinated seeds was high in both sibmates.

Development of excised ovaries in various carbon sources

For the study on development *in vitro* of excised ovaries, ten ovaries each were cultured in medium with various carbon sources. Table 2 shows the results of the experiment. Indiplid Seppaku-taina, mean length of the capsules was about the same among four

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different media with sucrose, glucose, raffinose and fructose each. No growth of the capsules was observed in the medium with mannose. Seed set in the capsules was observed in all media used except with mannose. It was about the same between two different media with sucrose and glucose, and less in the other two media with raffinose and fructose. Percentage of germinated seeds was over 90% in the seeds obtained from each culture medium.

In autotetraploid Nozaki-hakusai, mean length of the capsules was about the same among four different media with sucrose, glucose, raffinose and fructose each. No growth in the capsules was observed in the medium with mannose. Seed set in the capsules was observed in all media used except with mannose. It was about the same in the medium with sucrose, glucose and raffinose, and less in the medium with fructose. Percentage of germinated seeds was over 80% except in the medium with fructose.

According to GAUTHRET (1959), in normal callus cells of Daucus carota, growth rate was best in the medium with sucrose. It was the same among three different media with glucose, maltose and raffinose, although it was more than in the medium with mannose. On the other hand, in the crown gall callus of Helianthus annus, the
growth rate was best in the medium with glucose or fructose. It was more in the medium with sucrose compared with that in the medium with maltose or raffinose. No growth of callus cells was observed in the medium with mannose. In normal callus cells of *Sequoia sempervirens*, the growth rate was about the same among the four media with sucrose, glucose, raffinose and mannose each, and it was more in the medium with fructose than in the medium with each of the above four different carbohydrates. In the present experiment *in vitro* of excised ovaries in *Brassica campestris*, sucrose, glucose, raffinose and fructose each could be utilized for development of excised ovaries from sibmate of diploid Seppaku-taina and autotetraploid Nozaki-hakusai. Mannose, however, could not be utilized. Seed set in the capsules was more in the medium with sucrose, glucose or raffinose than in the medium with fructose in both sibmates of diploid Seppaku-taina and autotetraploid Nozaki-hakusai.

**Development of excised ovaries in various sucrose concentrations**

For the study on development *in vitro* of excised ovaries in various sucrose concentrations, ten ovaries were cultured in each sucrose concentration. Table 3 shows the results of the experiment.

In diploid Seppaku-taina, mean length of the capsules was about the same among media with various sucrose concentrations. Seed set in the capsules was observed in all culture media and the seeds germinated. Seed set in the capsules was best in the medium with 30 g/l of sucrose and lowest in the medium with 10 g/l of sucrose. Percentage of germinated seeds was over 90% in each culture medium.

In autotetraploid Nozaki-hakusai, mean length of the capsules was a little shorter than in diploid Seppaku-taina. Seed set in the capsules was observed in all culture media and

<table>
<thead>
<tr>
<th>Material</th>
<th>Sucrose concentration (g/l)</th>
<th>No. of capsules examined (A)</th>
<th>Mean length of mature capsule (mm±SD)*</th>
<th>No. of seeds obtained (B)</th>
<th>Seed set (B/A)</th>
<th>Percentage of germinated seeds (%)</th>
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<tr>
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<td></td>
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<td>9</td>
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<td>4.8</td>
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<tr>
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<td>Autotetraploid</td>
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<td>0.2</td>
<td>50.0</td>
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<td></td>
<td>30</td>
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<td>32.5±2.69</td>
<td>8</td>
<td>0.9</td>
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<td>34.1±4.01</td>
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<td>1.5</td>
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<tr>
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<td></td>
<td>90</td>
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<td>33.0±3.85</td>
<td>2</td>
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<td>50.0</td>
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<td>32.4±3.63</td>
<td>37</td>
<td>0.8</td>
<td>67.6</td>
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</table>

* SD : Standard deviation.

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Table 4. Further studies on effects of the medium with various sucrose concentrations on development in vitro of excised ovaries in the sibmate of diploid *Brassica campestris*

<table>
<thead>
<tr>
<th>Sucrose concentration (g/l)</th>
<th>No. of capsules examined (A)</th>
<th>Mean length of mature capsule (mm±SD)*</th>
<th>No. of seeds obtained (B)</th>
<th>Seed set (B/A)</th>
<th>Percentage of germinated seeds (%)</th>
</tr>
</thead>
<tbody>
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<td>26.5±3.50</td>
<td>0</td>
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<tr>
<td>10</td>
<td>11</td>
<td>28.4±4.43</td>
<td>0</td>
<td>0</td>
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<tr>
<td>30</td>
<td>11</td>
<td>29.9±5.76</td>
<td>4</td>
<td>0.4</td>
<td>100</td>
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<td>50</td>
<td>11</td>
<td>29.0±2.64</td>
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<td>1.4</td>
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<tr>
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<td>11</td>
<td>33.5±4.54</td>
<td>29</td>
<td>2.6</td>
<td>93.1</td>
</tr>
<tr>
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<td>11</td>
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<td>32</td>
<td>2.9</td>
<td>90.6</td>
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<td>120</td>
<td>12</td>
<td>35.7±3.28</td>
<td>63</td>
<td>5.3</td>
<td>96.8</td>
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<tr>
<td>150</td>
<td>11</td>
<td>34.0±3.09</td>
<td>57</td>
<td>5.2</td>
<td>100</td>
</tr>
<tr>
<td>180</td>
<td>11</td>
<td>32.9±1.97</td>
<td>61</td>
<td>5.5</td>
<td>96.7</td>
</tr>
<tr>
<td>210</td>
<td>11</td>
<td>34.4±3.47</td>
<td>86</td>
<td>7.8</td>
<td>75.6</td>
</tr>
<tr>
<td>Total or mean</td>
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<td>31.7±4.51</td>
<td>347</td>
<td>3.2</td>
<td>90.8</td>
</tr>
</tbody>
</table>

* SD: Standard deviation.

the seeds germinated. Seed set in the capsules was best in the medium with 50g/l of sucrose and lowest in the medium with 10g/l and 90g/l of sucrose. The mean of seed set in the capsules was about one sixth of those in diploid Seppaku-taina. Percentage of germinated seeds in autotetraploid Nozaki-hakusai was somewhat lower than in diploid Seppaku-taina.

In diploid Seppaku-taina, seed set in the capsules did not decrease remarkably in the medium with higher sucrose concentrations. Further study on development in vitro of excised ovaries in various sucrose concentrations was undertaken. Table 4 shows the results of the experiment. Eleven or twelve ovaries were cultured in each culture medium.

The mean length of the capsules was 31.7mm. Growth was somewhat observed in the medium without sucrose. Seed set in the capsules was remarkably low in the medium with 0g/l, 10g/l and 30g/l of sucrose. It became, however, more in the medium with higher sucrose concentrations. It was best in the medium with 210g/l of sucrose. Mean percentage of germinated seeds was over 90%. Percentage of germinated seeds was poorest in the medium with 210g/l of sucrose. Seed set in the capsules was more in the medium with 210g/l of sucrose than under natural condition.

The mean length of the capsules was shorter than in the previous experiment. Seed set in the capsules was poorer in the medium with 10g/l, 30g/l and 50g/l of sucrose than in previous experiment. Seed set in the capsules in the medium with 90g/l of sucrose was about half of the previous experiment.

In an ordinary way of tissue-, embryo-, ovule- and ovary- culture, sucrose concentration in the medium was from 20g/l (White 1963) to 50g/l (Niisch 1951). In growth rate of endosperm callus tissue in maize, the optimum concentration of sucrose in the medium was 2% on a fresh weight basis. However, on dry weight basis, 8% sucrose in the
medium was best (Straus and Larue 1954). Development in vitro of excised young ovules of Petunia hybrida Vilm, the most favorable osmotic value in the medium was equivalent to 8% sucrose solution (Wakizuka and Nakajima 1974). Nakajima pointed out that the placenta played an important role in development in vitro of excised ovule (Nakajima and Tatsumoto 1969, Nakajima 1970). In the present experiment on development in vitro of excised ovaries in diploid Seppaku-taina, seed set in the capsules was promoted when sucrose concentration became unexpectedly high in the medium. It seemed that the placenta played an important role in the development in vitro of excised ovaries.

Under natural condition seed set in the capsules was less in diploid Seppaku-taina than in autotetraploid Nozaki-hakusai. In development in vitro of excised ovaries, however, seed set in the capsules was better in diploid Seppaku-taina than in autotetraploid Nozaki-hakusai. It seemed that the difference of seed set in the capsules between diploid Seppaku-taina and autotetraploid Nozaki-hakusai was due either to the difference of polyploidy or subspecies.

Acknowledgement

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Literature Cited

Brassica campestris における子房の人工培養
II. 種々の炭素源による培養子房の発育

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Brassica campestris の子房の人工培養において、種々の炭素源による培養子房の発育について検討を行なった。用いた実験材料はそれぞれ 2 倍体 Brassica campestris L. ssp. chinensis (L.) Makino 黒羽菜と同質倍体 Brassica campestris L. ssp. pekinensis (Lour.) Olsson 栽培品種野崎白菜とであった。2倍体野崎白菜の株間交配と同質 4 倍体野崎白菜の株間交配は、各々雌株後 2 日目に開花した柱頭上に当日開花した花粉をかけて行なった。交配後 4 日目の子房を植物体から切り取り、殺菌後試験管（18×180 mm）の繊維培地に植え込んだ。試験管に植え込んだ子房は植え込み後、36 日目に取り出し、其の長さを測定し、また英ににおける着粒率と得られた種子の発芽率を調査した。

種々の炭素源の培地における子房の人工培養による発育を調査した実験において、用いた培地組成は Nitsch (1951) の無機物に White (1963) のビタミン類と炭素（8 g/l）を加えたものであった。用いた種々の炭素源はサッカロース、グルコース、ラフィノース、フルクトースとマンノースであった。各炭素源は各々 50 g/l の割合で培地に添加した。

種々の炭糖濃度の培地における子房の人工培養による発育を調査した実験において、用いた培地組成は Nitsch の無機物に修正 White のビタミン類と炭素（8 g/l）を加えたものであった。培地に添加した炭糖濃度は各々 10 g/l, 30 g/l, 70 g/l と 90 g/l であった。

種々の炭糖濃度の培地における子房の人工培養による発育を調査した結果、2倍体野崎白菜においては英における着粒率にはほとんど差異がみられなかったので、更に種々の炭糖濃度の培地における子房の人工培養を行なった。用いた培地組成は Nitsch の無機物に White のビタミン類と炭素（8 g/l）を加えたものであった。培地に添加した炭糖濃度は各々 0 g/l, 10 g/l, 30 g/l, 50 g/l, 70 g/l, 90 g/l, 120 g/l, 150 g/l, 180 g/l と 210 g/l であった。

植え込んだ子房は 22±2°C の温度と 300~500 ルクスの蛍光灯による 12 時間照明の培養室で培養を行なった。次の実験結果を得た。

（1） 2 倍体野崎白菜と対照 4 倍体野崎白菜の各々の株間交配において、発実種子が得られたのはサッカロース、グルコース、ラフィノースとフルクトースを添加した培地においてであった。

（2） マンノースを添加した培地では、いずれの株間交配を行なったものでも、英の生長はなく、着粒種子も得られなかった。

（3） 2 倍体野崎白菜の発実種子は、サッカロースあるいはグルコースを培地に添加した方がフルクトースあるいはラフィノースの場合よりも良かった。

（4） 同質 4 倍体野崎白菜の発実種子は、サッカロース、グルコースあるいはラフィノースを培地に添加した方がフルクトースの場合よりも良かった。

（5） 2 倍体野崎白菜の発実種子は炭糖濃度が高くなるにつれて良好になった。最高の着粒率は 210 g/l の炭糖を培地に添加したときにあった。しかしながらその条件で得た種子の発芽率は、低濃度の炭糖を培地に添加して得た種子の発芽率より低かった。

（6） 同質 4 倍体野崎白菜の発実種子は 30 g/l から 70 g/l まで栽培の間の炭糖濃度の時が良かった。

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