86. **Maintenance and Mass-Propagation of an Aneuploid in Annual Haplopappus gracilis (2n=4) by Shoot Tip Cloning**

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Over years maintenance of a particular chromosomal type in an annual or biennial plant has been attained by means of the conventional sexual seed reproduction and the recently developed vegetative culture. However, such a method of seed reproduction requires much labor in screening the variants derived from meiosis and fertilization. And, in the case of the vegetative culture, it is difficult to propagate the plants for a long period of years as in perennial and it is in most cases impossible to redifferentiate the same type as the parent one from tissue cultured cells (D’Amato 1975; Krikorian 1982). Further, it is substantially difficult to maintain and mass-propagate such unusual chromosomal types and genotypes as, for instance, triploid, aneuploid, interspecific F1 hybrid, hybrid vigor, and heterogeneous genotypes and the like.

To solve this difficulty, Tanaka and Ikeda (1983) developed a method of the tissue culture of shoot tip meristems in an annual plant *Haplopappus gracilis*, Compositae, which is useful material in the study of cytogenetics by its low chromosome number 2n=4. The tissue cultured meristems, which were named as the “shoot primordia”, propagate vegetatively with very high rate and easily redifferentiate to new plants. In spite of a number of years of cultivation, all of the shoot primordia and redifferentiated plants showed the same karyotype as that of its mother plant.

Use being made of the method of tissue cultured meristem through the shoot primordia we have tested the plants with particular genetical property, i.e., triploidy in seedless water-melon, hybrid vigor in maize and rice, heterogeneous genotypes in morning glory and chromosomal variation in *Crepis capillaris*. From the re-

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sults of the tests, it was confirmed that in all of these annual or biennial plants the method of tissue cultured meristem through shoot primordia could be useful for the long years of vegetative maintenance and mass-propagation (Tanaka 1983). In the present paper the maintenance of an aneuploid for a long period of years and mass-propagating the aneuploid in a short period of time by the shoot primordia will be reported.

Material and method. An aneuploid with 2n = 5 chromosomes of *Haplopappus gracilis* (Nutt.) Gray (2n = 4) was used in this study. Its 2n = 5 chromosomes (Fig. 1A) were composed of two 1g chromosomes and two 2g chromosomes (nucleolar chromosomes) of *H. gracilis* and one 4r chromosome (nucleolar chromosome) of *H. ravenii*. According to Jackson (1962) and Tanaka (1967) the 4r chromosome is considered to be homologous to the whole short arm and to the proximal segment of the long arm of 2g chromosome. Therefore, this aneuploid is regarded as a segmental trisomic.

The method of Tanaka and Ikeda (1983) was used for the induction, subculture and redifferentiation of the shoot primordia: The dome of the shoot tips was cut off from the aneuploid mother plant and transplanted in the liquid medium of Murashige and Skoog (1962) supplemented with 2 mg/l 6-benzylaminopurine (MS16 in 25 squars method, as shown in Table I of Tanaka and Ikeda 1983) at about 22°C under rotation at 2 cycles/min while the circumstance was held in an illumination 2,000–9,000 lux continuously. The shoot primordia were subcultured at a period of about one month. When the shoot primordia were transplanted to solid media (diluted two times the MS7 shown in Table I of Tanaka and Ikeda 1983) to obtain young plants the condition was subjected to stationary culture at about 22°C and at the illumination of 4,000 lux for 16 hr and in the dark for 8 hr per day.

Chromosomes at mitotic metaphase were investigated in mother plant, shoot primordia, and in redifferentiated young plants. The chromosomes were treated by the aceto-orcein squash method of Tanaka (1959): They were fixed in 45% acetic acid at 10°C for 10 min, macerated in the mixture of 1N HCl and 45% acetic acid (2:1) at 60°C for 15 sec, stained with 1% aceto-orcein, then squashed.

Results. The induced shoot primordia were light green in color and in a form of globular conglomerate of about 3–10 mm in diameter (Fig. 1A), and have been actively propagating now for 17 months. The propagating rate of the shoot primordia was extremely high to such an extent that from one shoot primordium about four shoot primordia can be produced weekly. The propagation rate therefore
is about $4^n$, whereby $n$ is the number of weeks. This rate was found to be the same as that of the shoot primordia of normal *Haplopappus gracilis* reported by Tanaka and Ikeda (1983).

The chromosomes of the shoot primordia were observed as follows: All of the cells examined had the karyotype formulated as $2n=5=1g+1g+2g+2g+4r$ (Fig. 2B), which was the same as that of the mother plant, except for only three endotetraploid cells with $2n=10$ (Table I). This result indicates that the aneuploidy of $4r$ chromosome has been maintained constantly in cultured shoot primordia for a long period of time.

A large number of young plantlets were formed from the conglomerate of the shoot primordia (Fig. 1B). The observation of the chromosomes in the plantlets was made for the purpose of the inspection of the stability in inherency of the redifferentiated plants, and it was confirmed that all the plantlets examined had the same karyotype $2n=5=1g+1g+2g+2g+4r$ as the mother plant (Table II, Fig. 2C). The newly formed plantlets were found to have the same morphology in the leaves and stems as the mother plant. It is now

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**Fig. 1.** Vegetatively mass-propagating aneuploid *Haplopappus gracilis* with chromosomes $2n=5=1g+1g+2g+2g+4r$. A: A number of conglomerates of the shoot primordia in a vial with liquid medium propagated for three weeks after the subculture of one conglomerate. Bar indicates 10 mm. B: A cluster of fascicled young plantlets redifferentiated from one conglomerate of the shoot primordia. Bar indicates 10 mm.

**Table I.** Results of chromosome count in the shoot primordia of aneuploid *Haplopappus gracilis* with $2n=5=1g+1g+2g+2g+4r$

<table>
<thead>
<tr>
<th>Months in culture</th>
<th>Number of cells with following chromosome number</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$2n=5$ (%)</td>
<td>$2n=10$ (%)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>117 (100)</td>
<td>1 (1.0)</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>101 (99.0)</td>
<td>1 (1.0)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>102 (100)</td>
<td>1 (1.0)</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>98 (99.0)</td>
<td>1 (1.0)</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>105 (100)</td>
<td>1 (0.7)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>132 (99.3)</td>
<td>1 (0.7)</td>
<td></td>
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</tbody>
</table>
therefore possible to preserve the aneuploid vegetatively for many years, by using the shoot primordia, and to mass-propagate in a short period of time.

The results obtained in the present investigation provide a method for cloning over a long period of years and mass-propagating the annual or biennial plant resources by use being of tissue cultured meristem through the shoot primordia, while maintaining such unusual chromosomal types and genotypes as aneuploid, triploid, interspecific F₁ hybrid and the like.

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References


——: Cytologia, 32, 542–552 (1967).
