Chromosome Constitution of the Plants Induced from Callus Culture of the Daylily Hemercallis Flava L.

Yvonne C. Goeden

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CHROMOSOME CONSTITUTION OF THE PLANTS INDUCED
FROM CALLUS CULTURE OF THE DAYLILY HEMEROCALLIS FLAVA L.

BY
YVONNE C. GOEDEN

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science, Major in
Biology, South Dakota
State University
1976
CHROMOSOME CONSTITUTION OF THE PLANTS INDUCED
FROM CALLUS CULTURE OF THE DAYLILY *HEMEROCALLIS FLAVA L.*

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Advisor                        Date

Head, Botany-Biology Department       Date
ACKNOWLEDGMENT

I would like to express my gratitude to Dr. C. H. Chen, Professor of Biology, for his guidance during the course of this study.

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I also wish to thank my parents, Mr. and Mrs. Emil Goeden, and my fiance, Bob Kallemeyn for their encouragement throughout this investigation.

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INTRODUCTION

Variations in chromosome number and/or structure were reported to occur in long-term cultures (Heinz, Mee and Nickell, 1969; Heinz and Mee, 1971; Horak, Lustinec, Mesicek, Kaminek and Polackova, 1975; Kao, Miller, Gamborg, and Harvey, 1970; Mitra and Steward, 1961; Sacristan, 1971; Singh and Harvey, 1975a and b; Torrey, 1959). No change of chromosome number was found in the callus tissue or in the plants induced from the callus of *Hemerocallis flava* L. subcultured for six months (Chen and Holden, 1972).

In this study, karyotype of the plants derived from a callus culture of *H. flava*, which had been subcultured for 30 months was analyzed to determine any change in chromosome structure.

Meiotic studies of pollen-mother-cells of the plants were also made to determine the nature of aberration in the chromosomes.

Polyplloid plants derived from a colchicine-treated long-term culture of the daylily callus were also investigated cytologically for obtaining indirectly the evidence of chromosome stability in the callus culture.
LITERATURE REVIEW

Variations in chromosome number and structure in cultured cells were first reported by Torrey (1959). In the callus initiated from pea roots, the inability to form adventitious roots after a prolonged period of subculture was found to be associated with the elimination of the diploid cells from the culture. It was later discovered by Torrey (1967) that prolonged cultivation of pea callus resulted in a high frequency of the occurrence of polyploid cells, with lower chromosome numbers disappearing. The cells having chromosomes deviating from the diploid number lost their organ-forming capacity. The polyploid cells also showed structural changes of the chromosomes.

Mitra and Steward (1961) reported changes of chromosome number and structure in cell cultures of Haplopappus gracilis. Various ploidies were observed in a suspension culture subcultivated for six months. The majority of the polyploids were tetraploid. Structural changes observed included chromatid breaks, fragmentations and dicentric chromosomes. Singh and Harvey (1975a and b) investigated the effects of the physical condition of the medium on chromosome behavior of the cultures of H. gracilis. In both the callus and suspension cultures, number and/or structure change in chromosomes were observed. In agar medium, the frequency of polyploid cells increased with time, while in the suspension cultures, a very large increase in the frequency of diploids was observed but longer transfer intervals in the suspension cultures resulted in an increase in the
frequency of tetraploid cells. The authors suggested that endore-
duplication was the major factor involved in the increase of poly-
ploidy frequency. Among euploids investigated from callus cultures,
diploid cells appeared to be the most stable and have the highest
chance to survive. However, in callus cultures, polyploids had better
competitive advantages than diploid cells. Anaphase figures from
callus were observed with fragments, lagging chromosomes and chromatin
bridges in low frequencies. In cells suspension-cultured for 94 days,
chromosomal fragments, dicentric, ring and microchromosomes were
observed occasionally at metaphase. However, anaphase analysis in the
cultures 100 days old showed chromosomal fragments, bridges and lagging
chromosomes in higher frequency in suspension culture than in callus
cultures.

Kao, Miller, Gamborg and Harvey (1970) reported no variation in
number or structure in long term suspension cultures of *H. gracilis*.
They explained that the loss or gain of a chromosome in a species with
a low chromosome number as *H. gracilis* (2n=4) would sufficiently upset
the genetic balance; therefore, a high degree of stability would be
expected in cell cultures of this species.

Reinert and Kuster (1966) found no variation in chromosome struc-
ture or number in cells of *Crepis capillaris* after one year of sub-
culture. However, Sacristan (1971) reported that cell cultures of
*C. capillaris* subcultivated for one year became partially polyploidized.
Chromosome rearrangements were also observed. The frequency of aber-
ration was found to be much higher in polyploid cells than diploids.
The frequencies of these abnormalities increased rapidly with time, suggesting a positive selective value in respect to polyploid cells in vitro.

In *Nicotiana*, Shimada and Tabata (1967) discovered that in cultured stem pith cells, chromosome number varied widely, ranging from 40 to 215 at first mitosis after explanting. A similar variation was observed in second mitosis. These aneuploids contributed to 70 percent of the cell population. However, after a prolonged period of subculture (18 months), the cell population became much more uniform in chromosome number. The wide range of variation in chromosome number found in the early stage of culture suggested that the pith tissue of tobacco, at the time of explanting, had been made of cells containing different chromosome numbers. Shimada (1971) also observed chromosome numbers in tobacco cultures originated from roots and subcultured for three to seven months varying from 45 to 96 with 64 percent of the cells being diploid. After two years of subculture the spectrum of chromosome variation widened. The change of chromosome number in the course of subculture, in that instance, was similar to that shown in the callus cells originated from the pith tissue. Plantlets regenerated from tumorous and non-tumorous cultures in tobacco have been reported by Sacristan and Melchers (1969). The variation in chromosome number in the plantlets differentiated from the non-tumorous cell which had a mean range of 72 chromosomes whereas those regenerated from the tumorous cultures had a mean range of 63 chromosomes. More plantlets were obtained from the tumorous cultures than the non-tumorous.
Chromosome number changes were also reported in callus cells of *Saccharum* (Heinz, Mee and Nickell, 1969; Heinz and Mee, 1970; Heinz and Mee, 1971). The plantlets evolving from these callus cultures had different chromosome numbers than the "parental" cells. Univalents were found at meiotic metaphase I (MI) in the plants differentiated from callus cultures but not in the "parental" plants. This implied that abnormalities in chromosome number or structure were present in these plantlets. In cell suspension cultures no cells were observed to have chromosome number doubled (Heinz and Mee, 1970).

Similar results have been reported by Horak, Lustinec, Mesicek, Kaminek and Polackova (1975) in cultures of stem pith in Kale. Of the plantlets observed, 76 percent were tetraploid. The tetraploid plants appeared to arise from "parental" cells that were already tetraploid.

Nishi, Yamada and Takahashi (1968) reported that rice plantlets restored from callus contained uniform diploid numbers while the chromosome number in the callus varied widely.

Callus cells of common wheat and emmer wheat after subculturing for four years were examined by Shimada (1971). In common wheat 40 percent of the cells maintained the original chromosome number, while the rest were aneuploids. After prolonged culture, the wheat callus tissue did not greatly change its chromosomal constitution.

Kao, Miller, Gamborg and Harvey (1970) also found that both *Triticum monococcum* and *Triticum aestivum* had numerical and structural variation. In *T. monococcum*, the majority of cells in one culture were found to be
aneuploid and the other cultures were tetraploids with fragments. In
*T. aestivum* most cells were aneuploids; no tetraploids were found.
Acentric fragments, dicentric chromosomes and one chromosome that was
extremely long were observed. The dicentric chromosomes found in
cultured cells were not observed in the intact root-tips of plants
grown from the same seed source.

It had been reported by Asami, Shimada, Inomata and Okamoto (1975)
that after six months of subculture, four aneuploid groups of
*T. aestivum* exhibited a greater change in number than the disomic line.
This suggested that the disomic line had greater stability in the
karyotype than the aneuploid lines.

In cell suspension cultures of *Daucus carota*, the chromosome
number remained stable if at the diploid level (Wetherell and Halperin,
1973) but the loss of totipotency of the carrot callus was attributed
to the callus being aneuploid. Smith and Street (1974) cultured carrot
tissue and discovered that the callus that had no embryogenetic poten­
tial was of higher ploidy.

*Glycine max* and *Melilotus alba* were also subcultured and studied
by Kao, Miller, Gamborg and Harvey (1970). *G. max* had cells with
chromosomes less than the normal. Chromosome structure was not studied.
*M. Alba* was found to have no change in chromosome morphology but the
majority of the cells were found to be diploid or tetraploid: only a
few cells were found to contain aneuploids.

Plantlets obtained from callus tissue of *Lilium longiflorum* and
*L. philadelphicum* showed no change in chromosome number of root-tip
Chen and Holden (1972) observed a normal diploid number (2n=22) in cultured cells of *H. flava* and plantlets derived from the callus. The callus appeared to be cytologically stable even though it had been subcultured for as long as six months on the same medium.

It appeared that plantlets exhibiting variations might have been induced from callus cells which could already have had those variations at the time of explanting. The callus tissue which was chromosomally heterogeneous might lose its totipotency.

A defined karyotype of a species has been found to be useful in detecting chromosome aberrations. Karyotype analysis has been used as a tool in identification of similarities and differences among species. For example, a comparison of karyotypes in several species of bulbous *Iris* were made by Mitra and Randolph (1959). Based on camera lucida drawing of the mitotic chromosomes, idiograms were constructed. The chromosomes were numbered according to length starting with the longest to the shortest.

Bhattachargya and Jenkins (1960) measured with a Zeiss ocular screw micrometer the mitotic chromosomes of ten metaphase cells of *Secale cereale* L. cv. "Dakold." The mean of several measurements of each arm and total length of every chromosome was taken for more accurate results. An idiogram was then constructed using the arm index ratio.

Huziwara (1957) applied the information obtained from karyotype analysis to his studies of taxonomic relationships in the genus *Aster*.
In all species examined the chromosome numbers were nine or multiples of nine. No aneuploidy or structural changes were found.

Mensinkai (1939) made an extensive study of mitotic and meiotic chromosomes of *Allium* species. It was found that in some plants of *Allium cepa*, univalents at MI and bridges plus fragments at anaphase I (AI) were frequently present, indicating a heterozygous inversion was involved. In other plants of this species, only univalents were observed in meiotic cells. Homologous chromosomes that did not form chiasma failed to pair. Failure of chiasma formation in *Primula kevensis* (Upcott, 1939) and in *Paeonia japonica* (Haga and Ogata, 1959) was also accounted for by the presence of univalents in meiosis. In *Allium darwasicon* heteromorphic pairs were observed but pairing at meiosis was normal. In all species of *Allium* examined there was more than 20 percent inversion heterozygosity. This had a significant effect on the fertility of the plant.

Levan (1931, 1932) also did cytological studies in *Allium*. Karyotypes were drawn for several species. Measurements of the long arm, short arm and total length of the chromosomes were calculated. Fragments in somatic and meiotic cells were found. In one cell, the chromosome fragment attached perpendicularly on a chromosome near the centromere.

In a meiotic study of *Viola canina*, Clausen (1931) discovered an oscillating chromosome number. Twenty bivalents plus three fragments were found to behave irregularly during meiosis.
In *Tulipa* the presence of chromatin bridges and fragments at meiotic AI and/or AII indicated the involvement of heterozygous inversion (Upcott, 1937). In all triploid *Tulipa*, one or more inversions were found.

Ourecky (1970) found that in the genus *Sambucus* pairing of meiotic chromosomes was irregular. Univalents occurred in one species while chromosome bridges in AII appeared in another species. Karyotype analysis of individual plants revealed no apparent morphological differences within each of the species. This abnormality was caused by chromosome instability or structural hybridity. Univalents resulted from the cancellation of the end-to-end junctions of the bivalents that were paired only by terminal chiasmata (Haga and Ogata, 1956).

Reciprocal translocations and paracentric inversions in *Paeonia californica* were reported by Stebbins and Ellerton (1939) and Walters (1942). But inversion bridges and fragments were found more often than translocations.

In *Bromus* unequal length in homologous pairs arose from normal median pairs of chromosomes by means of two independent pericentric inversions, resulting in a heteromorphic pair. Ninety percent of the pollen was functional but bridge-fragments appeared at AI and AII (Walters, 1952).

Carson and Stalker (1947) found that in *Drosophila robusta* two pericentric inversions changed one chromosome from median to submedian.

The heteromorphic chromosome pairs found by Boyes and van Brink (1967) in *Syrphidae* frequently exhibited reduced somatic pairing affinity
in mitotic metaphase. They separated from each other as two univalents at meiosis.

Lagging chromosomes at AI in Crepis capillaris were observed by Richardson (1936). The lagging chromosomes would migrate to each pole before the nuclear membrane was formed. Occasionally the lagging chromosomes would be excluded from the daughter nuclei.

In Trillium kantschaticum abnormalities in meiotic chromosomes such as tangling of chromosome ends and premature separation of chromatids resulted in pollen abortion. They attributed the abnormality to the effect of high temperature (Haga and Kayano, 1955; Matsuura and Haga, 1940).

Marenah and Holden (1967) used a karyotype to differentiate nine chromosome deficient lines of Avena sativa. Karyotype and idiogram of root-tip chromosomes were constructed. Chromosomes were classified on the presence or absence of satellites and on the position of the centromere. Chromosomes were placed in four groups: satellited, median, submedian and subterminal groups established by Rajhathy (1963) for A. sativa.

Chromosomes of Hemerocallis were first studied by Belling (1925). Meiotic chromosomes of H. fulva were found to associate in trivalents, bivalents and univalents at metaphase I. This species was identified as a triploid and was highly sterile.

Takenaka (1929) analyzed karyotypes of some species of Hemerocallis using camera lucida drawings. No clear structures of the chromosome pairs were distinguishable. Later, mitotic and meiotic
Chromosomes of some *Hemerocallis* species were depicted by Dark (1932). At meiosis no univalents were observed in any species studied except in the triploid *H. fulva*. Chromosomes of all other species studied paired to form eleven bivalents. He then concluded that, in the genus *Hemerocallis*, the somatic number of 22 was a rule for all species. Stout (1932) obtained the same conclusion.

Arisumi (1971) observed that it was necessary to differentiate cytologically the causes of sterility in *Hemerocallis* by counting of the chromosomes. It was also reported that spontaneous chromosome doubling in daylilies was very low.
MATERIALS AND METHODS

Thirty-two plants, which were originated from callus cells of the daylily *Hemerocallis flava* L. subcultured for 30 months, were used for this study. The methods of culturing and maintenance of the callus were described by Chen and Holden (1972). For somatic chromosome analysis root-tips were taken from the plants reared in clay pots in the greenhouse. Material used for meiotic studies were taken from plants growing in the field condition.

The tips of three young roots from each plant were prefixed in 0.2 percent aqueous solution of colchicine at 5°C for two to three hours before being fixed in acetic alcohol (one part glacial acetic acid: three parts absolute ethanol) for 24 hours. The roots were then hydrolyzed in 1N HCl at 60°C for ten minutes and stained with Feulgen reagent for 15 to 30 minutes. In preparing the microscope slides, a modified squash technique described by Darlington and LaCour (1960) was applied. Briefly, a root-tip, 1 mm in length was restained in a drop of 1 percent carmine in 45 percent acetic acid and macerated in the center of the slide. The root-tip cells were then squashed under a coverslip. The slide was made permanent by using the quick freezing method which was essentially the same as described by Conger and Fairchild (1953).

Chromosome measurements were made under an oil immersion objective with the aid of an ocular screw micrometer. These measurements of each arm of a chromosome were made and averaged. A photomicrograph of
every cell measured was taken with an automatic camera attached to a Leitz compound microscope.

A karyotype as well as an idiogram was established from the chromosome measurements of six randomly sampled plants. Chromosome constitution of the root-tip cells taken from the rest of the plants were then compared to the "standardized" karyotype for determining if there was any variation in chromosome number or structure.

For meiotic studies, young floral buds, 10 mm long, were collected from the plants grown in the field in the early afternoon during the latter part of June and early part of July and fixed in acetic alcohol for 24 hours. They were then transferred into 70 percent ethanol and stored in the refrigerator until used. The aceto-carmine squash technique for pollen-mother-cells described by Chen and Gregory (1973) was employed. Slides were temporarily sealed with wax.
RESULTS

Twenty-two somatic chromosomes were observed in all 545 cells of 32 plants. The result of chromosome counts agreed with those of Stout (1932), Dark (1932), Takenaka (1952) and Chen and Holden (1972) in the genus *Hemerocallis*. Figure 1 shows a root-tip cell of *H. flava* L. having 22 chromosomes. Measurements of these chromosomes from 17 cells are presented in Table I. The information given on this table includes the average lengths of the short and the long arms, and total length of each individual chromosome. Standard errors for these measurements were also calculated. A pairing t-test was made to ascertain any real difference in length between any of these homologous pairs. With the exception of the chromosome pair 3, the calculated t-value for each of the pairs showed no significant difference. The differences in short arm and total length of chromosome pair 3 were significant at 1 percent level whereas the long arm was at 5 percent significant level. It appeared that the heteromorphic pair might result from a change of lengths in both arms of the homologues.

A standardized karyotype was constructed based upon the chromosome length beginning with the longest (Fig. 2A). The chromosomes were placed into five groups, each of which was characterized as follows:

Group 1

Chromosome pairs number 1 and 2 belonged to this group. Both pairs were submetacentric with centromeric indices of 0.42 and 0.41,
Figure 1. Somatic chromosomes of a diploid *Hemerocallis flava* L. plant at mitotic metaphase (2n = 22) x3600.
<table>
<thead>
<tr>
<th>Chromosome #</th>
<th>Length of short arm</th>
<th>Length of long arm</th>
<th>Total Length</th>
<th>Centromeric index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.77 ± .06</td>
<td>3.78 ± .09</td>
<td>6.56 ± .15</td>
<td>0.42</td>
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<tr>
<td></td>
<td>2.77 ± .07</td>
<td>3.77 ± .08</td>
<td>6.55 ± .14</td>
<td>0.42</td>
</tr>
<tr>
<td>2</td>
<td>2.54 ± .05</td>
<td>3.63 ± .08</td>
<td>6.17 ± .10</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>2.50 ± .05</td>
<td>3.63 ± .08</td>
<td>6.12 ± .11</td>
<td>0.41</td>
</tr>
<tr>
<td>3</td>
<td>2.40 ± .08</td>
<td>2.56 ± .08</td>
<td>5.09 ± .15</td>
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<tr>
<td></td>
<td>2.10 ± .07</td>
<td>2.70 ± .09</td>
<td>4.78 ± .15</td>
<td>0.43</td>
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<tr>
<td>4</td>
<td>1.67 ± .05</td>
<td>3.07 ± .07</td>
<td>4.74 ± .11</td>
<td>0.35</td>
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<tr>
<td></td>
<td>1.65 ± .05</td>
<td>3.08 ± .08</td>
<td>4.73 ± .11</td>
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</tr>
<tr>
<td>5</td>
<td>1.50 ± .03</td>
<td>2.76 ± .07</td>
<td>4.26 ± .08</td>
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<tr>
<td></td>
<td>1.51 ± .03</td>
<td>2.77 ± .06</td>
<td>4.27 ± .07</td>
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</tr>
<tr>
<td>6</td>
<td>1.38 ± .03</td>
<td>2.77 ± .08</td>
<td>4.15 ± .10</td>
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</tr>
<tr>
<td></td>
<td>1.39 ± .04</td>
<td>2.78 ± .08</td>
<td>4.17 ± .11</td>
<td>0.33</td>
</tr>
<tr>
<td>7</td>
<td>1.64 ± .05</td>
<td>2.50 ± .06</td>
<td>4.13 ± .09</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>1.65 ± .04</td>
<td>2.51 ± .05</td>
<td>4.17 ± .09</td>
<td>0.40</td>
</tr>
<tr>
<td>8</td>
<td>0.97 ± .03</td>
<td>3.07 ± .05</td>
<td>4.04 ± .06</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>0.99 ± .04</td>
<td>3.02 ± .05</td>
<td>4.01 ± .06</td>
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<tr>
<td>9</td>
<td>0.38 ± .02</td>
<td>3.50 ± .07</td>
<td>3.88 ± .08</td>
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<td>0.36 ± .02</td>
<td>3.56 ± .07</td>
<td>3.92 ± .07</td>
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<tr>
<td>10</td>
<td>0.70 ± .02</td>
<td>2.81 ± .07</td>
<td>3.51 ± .09</td>
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<tr>
<td></td>
<td>0.74 ± .02</td>
<td>2.82 ± .07</td>
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TABLE I (contd.)

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<thead>
<tr>
<th>Chromosome #</th>
<th>Length of short arm (µm)</th>
<th>t</th>
<th>Length of long arm (µm)</th>
<th>t</th>
<th>Total Length (µm)</th>
<th>t</th>
<th>Centromeric index</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>1.36 ± .04</td>
<td></td>
<td>1.86 ± .08</td>
<td></td>
<td>3.22 ± .11</td>
<td></td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>1.35 ± .05</td>
<td></td>
<td>1.87 ± .07</td>
<td></td>
<td>3.22 ± .11</td>
<td></td>
<td>0.42</td>
</tr>
</tbody>
</table>

* and ** 5% (t = 2.120) and 1% (t = 2.921) significant levels, respectively.
respectively. An average length for pair number 1 is 6.6 µm while pair number 2 had an average length of 6.2 µm. The two pairs were distinguishable from each other by the short arm of pair 1 being noticeably longer than that of pair number 2. The long arms of these chromosome pairs appeared to be nearly the same length.

**Group 2**

The chromosomes of pair 3 were placed in this group; these two chromosomes were heteromorphic. One chromosome of the pair was sub-metacentric, 4.8 µm long, with a centromeric index of 0.44, while its apparent homologue was metacentric which was unique in the karyotype. The metacentric chromosome had an average length of 5.1 µm with a centromeric index of 0.47. The submetacentric chromosome had a characteristic rounding at the end of the short arm.

**Group 3**

Five pairs of chromosomes, designated chromosome pairs 4 through 8, belonged to this group. All members of this group showed little difference in chromosome length. The average chromosome lengths in this group varied ranging from 4.78 µm to 4.07 µm. Chromosome pair 7 had a relatively high centromeric index (0.40), indicating the centromere was nearly in median position while chromosome pair 8 had a nearly terminal centromere with a centromeric index of 0.24.

**Group 4**

Two pair of satellite chromosomes, designated pair 9 and 10 respectively, belong to this group. They were identified not only
by presence of a satellite but the position of the centromere. The 
centromeric indices for pair 9 was 0.10 and chromosome pair 10 was 
0.20. These calculated indices do not include the satellite. In both 
cases the satellite which was located on the short arm was extremely 
difficult to measure. All measurements of the satellites was done by 
the use of photomicrographs. The satellites were so small, at times 
they were folded under or bent down to where they could not be 
discernable. The satellite on one chromosome of each pair is shown 
in Figure 1. The short arm of pair 9 (0.37 \( \mu \text{m} \)) was almost half the 
size of the short arm of pair 10 (0.72 \( \mu \text{m} \)). A noticeable difference 
in the length of the long arm also distinguished between these two 
pair of chromosomes.

**Group 5**

Chromosome pair 11 belonged to this group. The average length 
of this chromosome was 3.2 \( \mu \text{m} \) with a centromeric index of 0.42. It 
was the shortest chromosome pair in the karyotype.

Based on the information present in Table I an idiogram for the 
plant population was constructed (Fig. 2B). The idiogram represented 
the chromosome length, the location of the centromere and the presence 
or absence of a satellite.

Marenah and Holden (1967) indicated that if colchicine was used 
to contract the chromosomes, it was essential to determine if the 
degree of contraction was proportional to chromosome length. In other 
words, in order for the idiogram to be valid, the tendency for the 
variation of chromosome length among the cells should be the same.
To test the validity, an analysis of correlation was made using the longest and the shortest chromosomes of each cell as the two variables. A significant positive correlation coefficient, $r=+0.57$, was calculated. A linear regression of the shortest on the longest chromosome is expressed in Figure 3, suggesting that there was a tendency for the chromosomes in a cell to contract proportionally to their length.

In 75 mitotic cells examined, no chromosomes were found to be abnormal with the exception of the heteromorphic pair, which was found consistently in all of the cells examined. This structural abnormality appeared to have existed at the time of explanting.

In order to determine the nature of aberration of chromosome pair 3, meiosis of pollen-mother-cells from eight plants was analyzed. The chromosome configurations obtained in the first division of meiosis are presented in Table II. Of 297 cells observed in metaphase I (MI), 285 pollen-mother-cells or 95.6 percent had 11 bivalents (Fig. 4A), while 12 or 4.0 percent of the cells showed 10 bivalents and two univalents (Fig. 4B). At anaphase I (AI) 954 cells or 97.8 percent showed a normal separation of the homologous chromosomes (Fig. 4C), while 21 cells or 2.1 percent of the cells examined showed two lagging chromosomes (Fig. 4D) which appeared to be derived from the two unpaired chromosomes seen at MI. No quadrivalents which might otherwise be an indication of the presence of reciprocal translocation were observed at MI, nor chromatin bridges and fragments for inversions at AI. The occurrence of the two univalents appeared to be related to the heteromorphic pair. Thus, the heteromorphic pair appeared to result from a chromosomal addition and/or deletion in these two chromosomes.
Figure 3. A regression line of the shortest chromosome ($y$) upon the longest chromosome ($x$).
Length of shortest chromosome (µm)

\[ \hat{Y} = 0.43x + 0.41 \]

Length of longest chromosome (µm)
**TABLE II**

CHROMOSOME CONFIGURATION OF *H. FLAVA* *L.*

METAPHASE I AND ANAPHASE I

<table>
<thead>
<tr>
<th>Metaphase I</th>
<th>Anaphase I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td><strong>11 II</strong></td>
<td><strong>Total</strong></td>
</tr>
<tr>
<td><strong>10 II</strong></td>
<td><strong>+ 2 I</strong></td>
</tr>
<tr>
<td><strong>No.</strong></td>
<td><strong>%</strong></td>
</tr>
<tr>
<td>285</td>
<td>95.6</td>
</tr>
</tbody>
</table>
Figure 4. Pollen-mother-cells of *H. flava*. (A) 11 bivalents, (B) 10 bivalents plus 2 univalents, (indicated by arrows), (C) normal separation of homologous chromosomes at AI, and (D) 2 lagging chromosomes at AI (indicated by arrows) x1700.
DISCUSSION

No variation in chromosome number or morphology in the plants obtained from a long-term culture of C. soldanella could be found. The tissue was karyologically stable.

The contradictory reports on chromosome variation observed in long-term cultures of some plant species might be due to differences in: (1) the material used; (2) the method of culturing; and (3) the age of the culture. All tetraploid plants examined showed normal chromosome arm type. However, the ploidy was not always the same in all parts of the plant. Table III shows a pattern of chromosome arm variation in the plants examined. Table III indicates that the chromosome arm type varied under the experimental conditions. Plants 1-4 showed a consistent pattern, while the remaining 7 (number 15-19) showed more variation.

Pack (1966, 1968) has reported that the ploidy of *Horseradish* is composed of three chromosomes, each of which was responsible for the development of certain morphological characters.
DISCUSSION

No variation in chromosome number or structure in the plants obtained from a long-term culture of *H. flava* L. would indicate that the tissue was karyologically stable.

The contradictory reports on chromosome variation observed in long-term cultures of some plant species could be due to the differences in: (1) the media used for the cultures; (2) the duration of subculture; (3) varieties or species; (4) tissues of the plant used in the culture.

Earlier, polyploid plants were induced after colchicine-treatment of the differentiating callus tissue (Chen and Goeden, 1974). Cytological studies of the tissues derived from different histogenic layers were made on 17 polyploids by chromosome-count in root-tips (Fig. 5), measuring sizes of stomata (Fig. 6A-C) and pollen grains (Fig. 6D and E) to further ascertain whether these polyploid plants evolved spontaneously or as a result of colchicine treatment. Morphologically, except for an octaploid plant, which had miniature stature, all tetraploid plants exhibited gigantic characters (Fig. 7). However, the polyploidy was not always cytologically homogeneous among the plants examined. Table III shows cytological variations among the polyploid plants. Plant numbers 1 through 9 were uniformly tetraploid while the remaining 7 (numbers 10-16) were cytochimeras.

Peck (1957, 1968) and Arisumi (1972) indicated that the stem apex of *Hemerothallis* consisted of three histogenic layers, each of which was responsible in the development of certain plant tissues and organs.
Figure 5. Root-tip cells of (A) diploid, (B) tetraploid and (C) octaploid plants x1000.
Figure 6. Comparisons of stomata and pollen grains among various polyploid daylily plants. (A) diploid, (B) tetraploid, and (C) octaploid stomata (x320). Pollen grains of (D) diploid and (E) tetraploid (x140).
Figure 7. (A) Diploid, tetraploid, and octaploid daylily plants and (B) flowers of diploid and tetraploid plants.
TABLE III

CYTOLOGICAL VARIATIONS AMONG 16 POLYPLOID PLANTS EVOLVED AFTER COLCHICINE-TREATMENT OF A DIFFERENTIATING CALLUS OF *H. FLAVA*

<table>
<thead>
<tr>
<th>Plant No.</th>
<th>Stomates</th>
<th>Pollen</th>
<th>Root-tips</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4n</td>
<td>4n</td>
<td>4n</td>
</tr>
<tr>
<td>2</td>
<td>4n</td>
<td>4n</td>
<td>4n</td>
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<tr>
<td>3</td>
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<tr>
<td>8</td>
<td>4n</td>
<td>4n</td>
<td>4n</td>
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<tr>
<td>9</td>
<td>4n</td>
<td>4n</td>
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<tr>
<td>10</td>
<td>2n</td>
<td>2n</td>
<td>4n</td>
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<tr>
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</tr>
<tr>
<td>16</td>
<td>2n</td>
<td>4n</td>
<td>4n</td>
</tr>
</tbody>
</table>
According to Derman (1960), the outermost layer, designated L-I, gave rise to epidermis; the middle layer, or L-II, developed into spores; and the innermost layer, or L-III, was responsible for the formation of internal tissues of all organs including secondary roots. If polyploidization should have been spontaneous and occurred before cellular differentiation, the differentiated cells which eventually organized into a plant body would have been karyologically uniform. On the other hand, if the differentiating tissue should be treated with colchicine, cytochimera would be expected to occur. The occurrence of cytochimera in a relatively high frequency would then indicate that the polyploid plants appeared to have been induced by colchicine treatment, not the result of prolonged subculture of the tissue.

The heteromorphic pair, which occasionally separated as two univalents at meiosis appeared to be caused by a chromosomal deletion and/or addition. This pair must have been present in the "parental" tissue since they were observed consistently in all somatic cells. If the original tissue should not have been karyologically uniform, chromosome variation among the plants evolved from callus would have been found. Through asexual reproduction which appears to be the unique way of propagation for this stock, the propagates may retain the aberrant chromosome constitution.

Diploid plants of this stock did not set seed. The sterility was due to self-incompatibility in the style as reported by Jost (1907) and Brewbaker and Gorrez (1967). Analysis of pollen grains
showed that only 80 percent of the grains were stainable and normal in shape. Thus, the univalents observed in meiosis appeared to contribute the pollen sterility.

Since callus culture of daylily is karyologically stable, plants evolved from the callus would be expected to form a genetically homogeneous population. Thus, tissue culture appears to be an ideal method for rapid cloning of this ornamental plant. Furthermore, colchicine-treatment of daylily callus followed by induction of differentiation into plantlets would provide an effective method of producing polyploid plants.
SUMMARY

Karyotype analysis of root-tip cells in the plants, which were initiated from a long-term callus culture of *Hemerocallis flava* L., indicated no variation in chromosome or structure. A heteromorphic pair, designated chromosome pair 3, appeared consistently in the plants examined suggesting that the aberrant pair was present in the tissue at the time of culturing.

Meiotic studies revealed the presence of two univalent chromosomes in a small percentage of the pollen-mother-cells, which appeared to result from the unpaired members of chromosome pair 3. Since no pollen-mother-cells with multivalents at metaphase I or chromatin bridges at anaphase I were observed, the presence of heterozygous reciprocal translocation or inversion could be ruled out. Chromosomal deletion and/or addition might be involved in the aberration.

The occurrence of univalent chromosomes in some cells could account partially for sterility of this species of *Hemerocallis*.

A high frequency of polyploid plants arose after colchicine-treatment of the differentiating daylily callus. Cytological examination of the tissues derived from different histogenic layers revealed the presence of cytochimera sectors in the colchicine derivatives, suggesting that polyploidization took place after colchicine-treatment of the callus. No polyploid cells were found in the plants differentiated from the culture grown on a medium devoid of colchicine.
LITERATURE CITED


