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Selective Regeneration of Plants from Diploid and Tetraploid Cells in Adventitious Shoot Cultures of Melon (*Cucumis melo* L.)

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The chromosome numbers of callus cells and regenerated plants from adventitious shoot cultures of two cultivars of melon (*Cucumis melo* L.) were determined. After five days of culture, 96. 5-98. 0% of the callus cells were diploid ($2x=24$) and 2. 0-3. 5% were tetraploid ($4x=48$). After seven days of culture, the frequency of diploid cells decreased to 76. 5-80. 0% and that of tetraploid cells increased to 11. 0-15. 5%. Small numbers of haploid ($x=12$), triploid ($3x=36$), hexaploid ($6x=72$) and octoploid ($8x=96$) cells were also observed. Within 14 days of culture, the frequency of diploid cells decreased further to 30. 0-44. 0% and that of tetraploid cells increased to 27. 5-35. 5% while more hyperploid cells ($>9x$) appeared. At all phases of callus culture, 2. 5-24. 5% of the observed cells were aneuploid. In the case of plants regenerated from adventitious buds, 69. 0-56. 0% were diploid and 26. 0-37. 0% were tetraploid while 5. 0-7. 0% were mixoploid, consisting of both diploid and tetraploid cells. No hyperploid or aneuploid plants were observed. The results indicate that the plants from adventitious shoot cultures of melon were regenerated selectively from diploid and tetraploid cells.

Introduction

Formation of adventitious shoots organogenesis from cotyledon and hypocotyl explants[1-3] and from leaf explants[3,4], somatic embryogenesis from cotyledon explants[5,6] and formation of shoot primordia from meristem tips[7] have all been reported in melon (*Cucumis melo* L.). Ezura *et al.*[8,9] reported a high frequency of tetraploidy (*ca.* 30%) in plants regenerated via adventitious shoot organogenesis and somatic embryogenesis in this species. Before these culture systems can be applied to the genetic manipulation of melon, for example, for cell selection, genetic transformation and micropropagation, it is important that we understand the mechanisms of the very frequent appearance of tetraploidy in the regenerated plans.

In this study, the chromosome numbers of callus cells and of plants regenerated from adventitious shoots of melon were analyzed.

Materials and Methods

1. *Adventitious shoot cultures*

Adventitious shoot cultures were generated by the method of Dirks and Buggenum[3]. Mature seeds of two cultivars of melon (*Cucumis melo L.*), cultivars Prince and Andes (Sakata Seed Co. Ltd., Japan), were used as the source of explants. Seeds, after decoating and sterilization, were soaked in sterilized distilled water for 6 hours. The cotyledons were cut into three sections. These explants were cultured at 25°C on Murashige and Skoog (MS) medium[10] with 1 mg/*l*

benzylaminopurine (BAP), 3% (w/v) sucrose and 0.4% (w/v) Gelrite (pH 5.8) in the light (16 h light per day at 5000 lux supplied by fluorescent lamps). Sterilization was achieved by decoating seeds for 15 sec. in 70% (v/v) ethanol, followed by a 15 min. treatment in a 1% (v/v) solution of sodium hypochlorite and three rinses in distilled water. After four weeks of culture, adventitious buds that had differentiated on the explant were excised and subcultured on MS medium with 3% (w/v) sucrose and 0.4% (w/v) Gelrite (pH 5.8) for elongation of shoot. After culturing, elongated shoots were subcultured on MS medium of the same composition for rooting and further cultured.

2. *Counting of chromosome in callus cells*

After 5, 7 and 14 days of culture on the medium for induction of adventitious buds mentioned above, cotyledon explants of each cultivar were stored for 1 day in distilled water at 5°C. They were then fixed in a mixture of ethanol and acetic acid (3 : 1, v/v). After washing with distilled water, a piece of callus excised from the cut surface of an explant was incubated in an enzyme solution that contained 4% (w/v) Cellulase RS (Yakult Co., Ltd., Japan), 1% (w/v) Pectolyase Y23 (Seishin Co., Ltd., Japan), 7.5 mM KCl and 7.5 mM EDTA (pH 4.0) for 50 min. in the dark at 37°C. After washing with distilled water, the cells were spread in a drop of the above mentioned fixative. Chromosomes were stained with 20% (v/v) of Giemsa's solution for microscopy (Merck, USA) in 0.067 M sodium phosphate buffer (pH 6.8) for 30 min. at room temperature. The chromosome numbers of 200 cells per experiment were counted on intact metaphase plates.

3. *Counting of chromosomes in regenerated plants*

Root tips of plants regenerated from adventitious buds were stored for 1 day in distilled water at 5°C. They were then fixed in a mixture of ethanol and acetic acid (3 : 1, v/v). After washing with distilled water, they were incubated in the enzyme solution mentioned above for 1 hr in the dark at 37°C. After washing with distilled water, the cells were spread on a glass slide in a drop of the above fixative. The chromosomes were stained with 20% Giemsa's solution for 30 min. at room temperature. The chromosome numbers of 10 cells per plant were counted on intact metaphase plates. For each cultivar, the chromosome number of 100 regenerated plants were counted.

Results and Discussion

The chromosome numbers of callus cells and root tip cells of the regenerated plants derived from adventitious shoot cultures of the melon (*Cucumis melo* L.) cultivars Prince and Andes were counted. In cells from callus cultures, a variety of polyploid and aneuploid cells was observed (**Fig. 1** and **2**). The frequency of aneuploid cells was added to the frequency of cells with the closest corresponding ploidy in estimating the frequency of cells of each ploidy to allow us to follow the changes in ploidy.

After five days of culture, in cv. Prince, diploid ($2x=24$), tetraploid ($4x=48$) and aneuploid cells ($2n=22$-25) were observed. The frequency of aneuploid cells was 2.5%. Among dividing cells, 98.0% were diploid and 2.0% were tetraploid (**Fig. 3-A**). After seven days of culture, the frequency of diploid cells decreased to 76.5% and that of tetraploid cells increased to 11.0%. Small numbers of haploid ($x=12$), triploid ($3x=36$) and hexaploid ($6x=72$) cells (**Fig. 3-B**) were also observed. The frequency of aneuploid cells was 24.5%. After 14 days of culture, the frequency of the diploid cells decreased to 44.0% and that of the tetraploid cells increased to 35.5% while heptaploid ($7x=84$), octoploid ($8x=96$) and more hyperploid cells ($nx>100$) appeared (**Fig. 3-C**). The frequency of the aneuploid cells was 14.0%.

After five days of culture in the case of the cv. Andes, diploid, tetrapliod and aneuploid cells (2

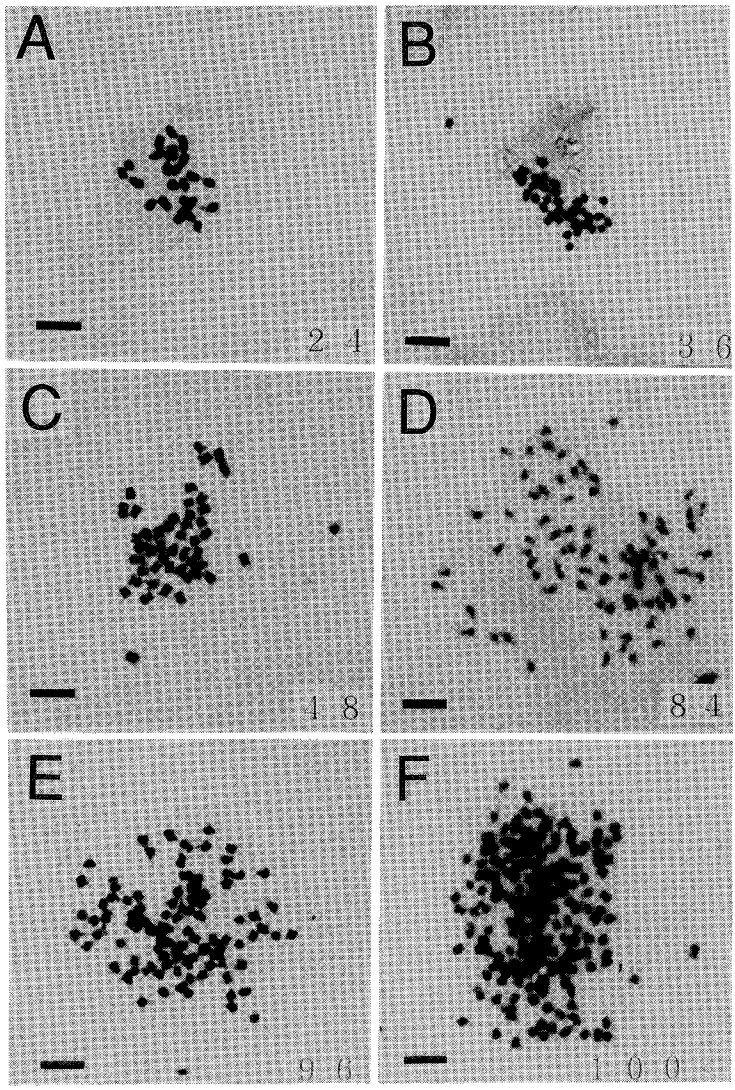

Fig. 1 Chromosomes of polyploid cells from callus induced in the medium for the formation of adventitious buds in a smooth-skined melon cultivar, Prince.
The number in each plate indicates the chromosome number. A, Diploid; B, triploid; C, tetraploid; D, heptaploid, E, octoploid; F, hyperploid. Bars : 5 μm.

n=21-23) were observed (**Fig. 4-A**). The frequency of the aneuploid cells was 3.0%. Among dividing cells, 96.5% were diploid and 3.5% were tetraploid. After seven days of culture, the frequency of diploid cells decreased to 80.0% and that of tetraploid cells increased to 15.5%. A small number of haploid, triploid, octoploid and aneuploid cells also appeared (**Fig. 4-B**). The frequency of aneuploid cells was 16.5%. After 14 days of culture, the frequency of diploid cells decreased to 30.0% and that of tetraploid cells increased to 27.5% while pentaploid ($5x$=60), hexaploid, heptaploid, and more hyperploid cells appeared (**Fig. 4-C**). The frequency of aneuploid cells was 9.5%.

Our data provide evidence that a change in the ploidy of the callus cells occured during the early stage, after one to two weeks of culture, of adventitious shoot organogenesis of melon. A variation in the ploidy of plant cells in tissue culture and cell culture has been reported in many plant spieces, for example, *Daucus carota*[11], *Kallstroemia pubescens*[12], and *Citrus sinensis*[13]. Ronichi *et al.*[14,15]

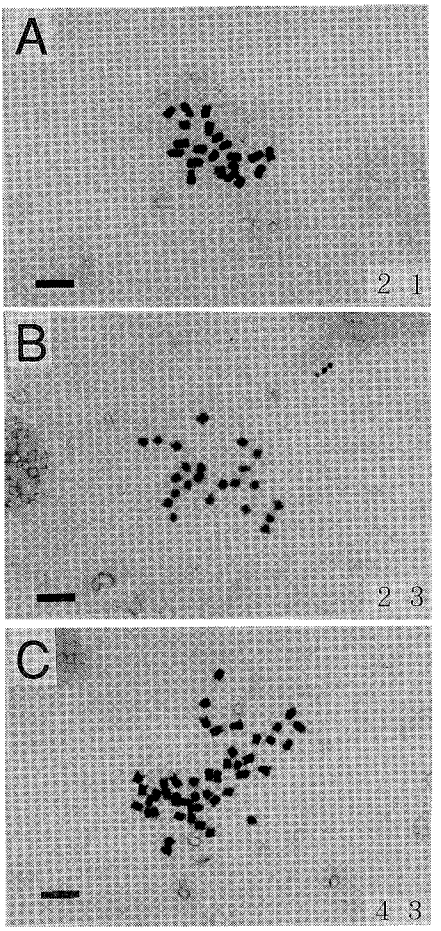

Fig. 2 Chromosomes of aneuploid cells from callus induced in the medium for the formation of adventitious buds in a smooth-skined melon cultivar, Prince.
The number in each plate indicates the chromosome number. Bars : 5 μm.

reported that the chromosomal variability was generated by a prophase reduction in chromosome number and somatic meiosis after polyploidization in cultured carrot cells. In *Kallstroemia pubescens*, 100% of the observed callus cells were diploid, having the original ploidy level, after 6 weeks of culture[12]. In *Citrus sinensis*, 89. 9-94. 3% of the observed callus cells were diploid, having the original ploidy level, after 12 weeks of culture[13]. However, in the adventitious shoot of melon, the ploidy of 56. 0-70. 0% of the observed callus cells differed from the original ploidy, namely, diploidy, even after a further two weeks of culture. Therefore, in the adventitious shoots of melon, it appears that the change in the ploidy of cultured cells progresses more rapidly than in the other species mentioned.

In the case of plants regenerated from the adventitious buds of the cv. Prince, 69. 0% were diploid, 26. 0% were tetraploid and 5. 0% were mixoploid, consisting of diploid and tetraploid cells (**Fig. 3-D**). In our previous report[9], 21. 6% of the plants regenerated from adventitious buds of the cv. Prince were tetraploid. The frequency of the tetraploid plants in the present experiment was very similar. No hyperploid or aneuploid plants were observed among the regenerated plants.

In the case of plants regenerated from the adventitious buds of the cv. Andes, 56. 0% were diploid, 37. 0% were tetraploid and 7. 0% were mixoploid, consisting of diploid and tetraploid cells (**Fig. 4-D**). In our previous study[9], 34. 4% of the corresponding regenerated plants were tetraploid. The

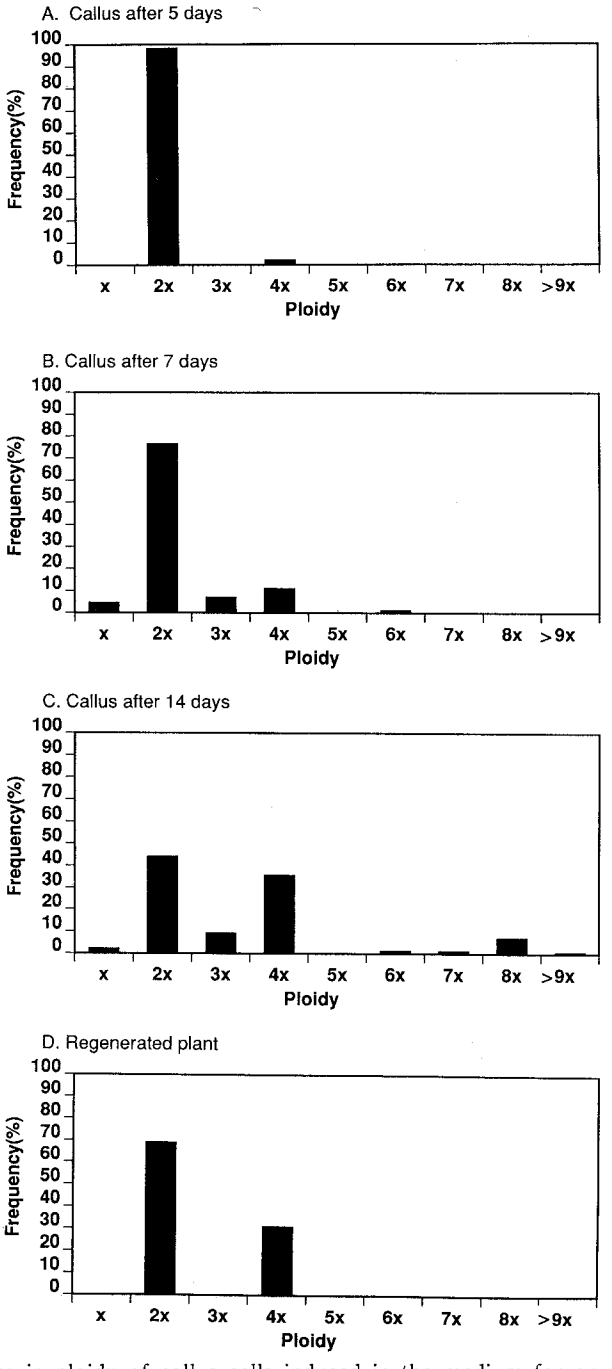

Fig. 3 Variations in ploidy of callus cells induced in the medium for an adventitious bud formation and root tip cells of plants regenerated via adventitious bud in a smooth-skined melon cultivar, Prince.

A, B and C show the data for callus cells after 5, 7 and 14 days of culture. D shows the data for regenerated plants. Data for tetraploids in D were the summation of data for tetraploid plants and mixoploid plants with diploid and tetraploid cells.

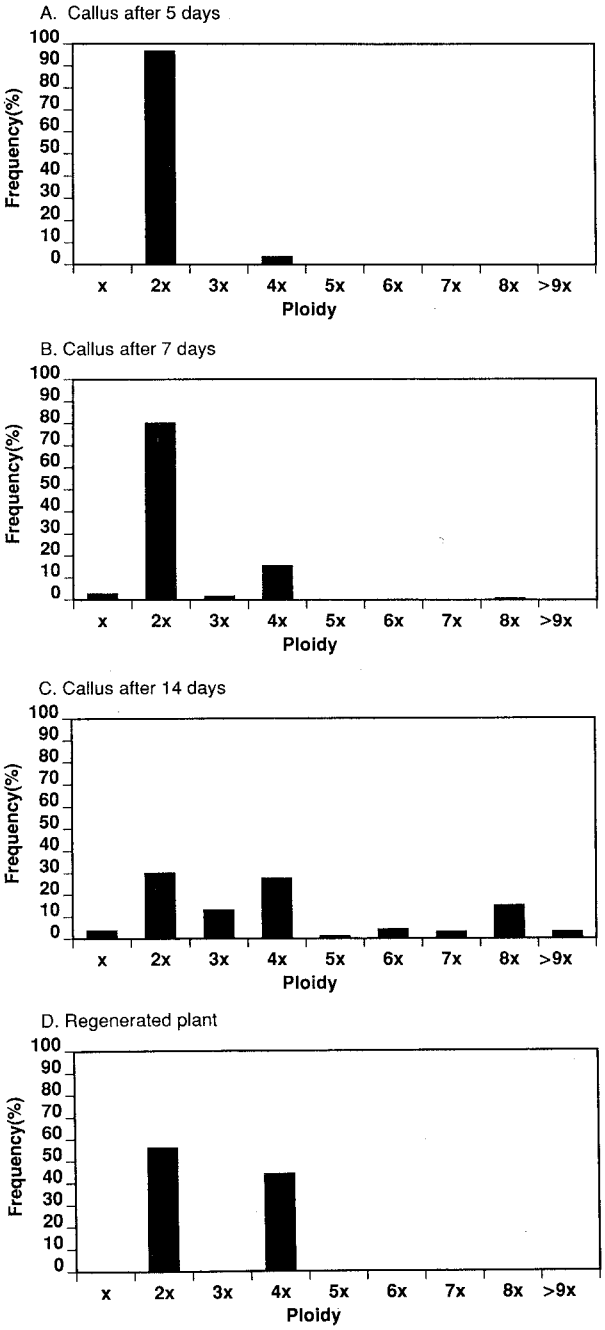

Fig. 4 Variations in ploidy of callus cells induced in the medium for formation of adventitious buds and of root tip cells of plants regenerated via adventitious bud in a net-skined melon cultivar, Andes.
A, B and C show the data for callus cells after 5, 7 and 14 days of culture. D shows the data for regenerated plants. Data for tetraploids in D were the summation of data for tetraploid plants and mixoploid plants with diploid and tetraploid cells.

frequency of tetraploid plants in the present experiment was again similar to previously reported value. No hyperploid or aneuploid plants were observed among the regenerated plants.

No aneuploid plants were observed among the plants regenerated from the adventitious shoots of melon. Aneuploids have been useful for genetic analysis of plants. Regeneration of plants from

tissue culture has been used as a method for obtaining aneuploid plants. In *Festuca arundinaua*[16] and *Triticum aestivum*[17], 36% and 29% of plants regenerated from callus were aneuploid. In *Lotus corniculatus*[18], no aneuploid plants were found among 72 plants regenerated from callus. Our results indicate that the formation of adventitious shoots in melon is not a useful method for producing aneuploid plants.

The plants regenerated from melon callus were diploid, tetraploid or mixoploid, consisting of diploid and tetraploid cells, while the callus was composed of haploid, diploid, triploid, tetraploid, pentaploid, hexaploid, heptaploid, octoploid and more hyperploid cells. This phenomenon was similar for both cultivars, Prince (smooth-skined melon) and Andes (net-skined melon). In *Kallstroemia pubescens*[2] and *Citrus sinensis*[13], regenerated plants were all diploid even though a variety of ploidies was observed in the callus. In the case of plants regenerated via adventitious shoots of melon, it appears that the plants were regenerated selectively from the diploid and tetraploid cells.

References

1) Moreno, V., M. Garcia-Sogo, I. Granell, B. Garcia-Sogo, L. A. Roig, 1985. Plant Cell Tissue Org. Cult., **5**: 139-146.
2) Bouabdallah, L., M. Branchard, 1986. Z. Pflanzenzucht., **96**: 82-85.
3) Dirks, R., M. Buggenum, 1989. Plant Cell Rep., **7**: 626-627.
4) Kathal, R., S. P. Bhatnagar, S. S. Bhojwani, 1988. Plant Cell Rep., **7**: 449-451.
5) Oridate, T., K. Oosawa, 1986. Jpn. J. Breed., **36**: 424-428.
6) Kageyama, K., K. Yabe, S. Miyajima, 1991. Jpn. J. Breed., **41**: 273-278.
7) Nagai, T., Y. Nomura, K. Oosawa, 1989. J. Jpn. Soc. Hort. Sci., **58** (Suppl. 1): 208-209.
8) Ezura, H., H. Amagai, K. Yoshioka, K. Oosawa, 1992. Jpn. J. Breed., **42**: 137-144.
9) Ezura, H., H. Amagai, K. Yoshioka, K. Oosawa, 1992. Plant Science, **85**: 209-213.
10) Murashige, T., F. Skoog, 1962. Physiol. Plant., **15**: 473-479.
11) Mok, M. C., W. H. Gabelman, F. Skoog, 1976. J. Am. Soc. Hort. Sci., **101**: 442-449.
12) Sengupta, J., G. C. Mitra, A. K. Sharma, 1987. Cytologia, **52**: 767-770.
13) Gimitter, F. G., X. B. Ling, C. Y. Cai, J. W. Grosser, 1991. Plant Sci., **74**: 135-141.
14) Ronchi, V. N., L. Giorgetti, M. Tonelli, G. Martni, 1992. Plant Cell, Tissue Org. Cult., **30**: 107-114.
15) Ronchi, V. N., L. Giorgetti, M. Tonelli, G. Martni, 1992. Plant Cell, Tissue Org. Cult., **30**: 115-119.
16) Eizenga, G. C., 1989. Genome, **32**: 373-379.
17) Karp, A., S. E. Maddock, 1984. Theor. Appl. Genet., **67**: 249-255.
18) Damiani, F., D. Mariotti, M. Pezzoti, S. Arcioni, 1985. Z. Pflanzenzucht., **94**: 332-339.

《和文要約》

メロンの不定芽培養系における二倍体および四倍体細胞からの選択的植物体再生

江面　浩・大澤勝次

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メロン（*Cucumis melo* L.）の不定芽培養系におけるカルス細胞と再生植物体の染色体数の変化を市販の二品種を用いて観察した．培養5日後ではカルス細胞の96.5-98.0%が二倍体（$2x=24$），2.0-3.5%が四

倍体 ($4x=48$) であった．培養7日後には二倍体細胞の頻度が 76.5-80.0% に減少し，四倍体細胞の頻度が 11.0-15.5% に増加した．また半数体 ($x=12$)，三倍体 ($3x=36$)，六倍体 ($6x=72$) および八倍体 ($8x=96$) 細胞もわずかに出現した．培養14日後には二倍体細胞の頻度が 30.0-44.0% にまで減少し，四倍体細胞の頻度が，27.5-35.5% にまで増加した。また新たに9倍体以上の高次倍数体細胞も出現した．カルス細胞では，観察した培養14日後までに 2.5-24.5% の頻度で異数体が観察された．再生植物体では 56.0-69.0% が二倍体，26.0-37.0% が四倍体であった．また 5.0-7.0% は，二倍体と四倍体細胞よりなる混数体であった．高次倍数体や異数体植物は観察されなかった．以上よりメロンの不定芽培養系では，二倍体と四倍体細胞から選択的に植物体が再生するものと考えられた．