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In Vitro Studies on *Pinus koraiensis*. II. Chromosomal Variation in the Callus[1]

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잣나무의 器內培養에 關한 硏究. II. Callus 細胞의 染色體變異[1]

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ABSTRACT

Excised mature embryos of *P. koraiensis* were plated on a series of G.D. basic media supplemented with growth regulators as follow; 0.1 mg/l NAA(M-1); 0.1 mg/l NAA and 2,4-D (M-2); 0.1 mg/l 2,4-D and BAP (M-3); 0.1 mg/l 2,4-D and kinetin (M-4). Cytological study of the callus showed that thercentage of occurrance of diploid cells was observed 52% in M-3 and 36% in M-4, while that was revealed 29% in M-1 and 17% in M-1. The frequency of diploid cells was increased on the M-3 and M-4 media. The stable chromosome state is a crucial factor for organogenesis. Therefore, it can be inferred that the callus tissues cultured on media supplemented with both auxin and cytokinin have the greatest possibility of organogenesis.

Key words: Chromosomal variation; organogenesis; auxin; cytokinin; Pinus koraiensis.

要 約

GD基本 培地에 서로 다른 生長調節物質을 添加한 4가지 培地에 잣나무 胚에서 由來한 Callus 를 1年 6個月間 培養시켜 染色體數 變異를 調査하였다. 그 結果 NAA 0.1 ㎎/ℓ와 NAA 0.1 ㎎/ℓ에 2,4-D 0.1 ㎎/ℓ를 添加한 培地 即 Auxin 單獨 및 複合 處理한 培地에서는 2n 比率이 各各 29%와 17%로 낮게 나타났다. 그러나 Auxin과 Cytokinin을 複合 處理한 培地 即 2,4-D 0.1 ㎎/ℓ와 BAP 0.1 ㎎/ℓ 에서는 52%, 2,4-D 0.1 ㎎/ℓ와 Kinetin 0.1 ㎎/ℓ에서는 36%로써 比較的 높은 頻度로 2n 細胞가 나타났다. 2n 細胞의 頻度는 器官分化와 相關이 높은 것으로 생각 할 수 있었다.

INTRODUCTION

The Genus *Pinus* consists of over one hundred species (Mirov, 1967) and most of them are economically important. Many workers have attempted to obtain organogenesis and clonal multiplication of pines through tissue culture techniques. Researchers have reported organogenesis from callus of *P. strobus* (Minocha, 1980), *P. sylvestris* (Bornman and Jansson, 1980) and *P. wallichiama* (Konar and Singh, 1980).

Chromosome instability of plant callus and cultured cells may often constitute a serious barrier, when the cultured cells are used in various approaches such as somatic cell genetics and mutation

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researches in which the preservation of a stable chromosome state is prerequisite.

Plant cell cultures *in vitro*, however, frequently exhibit an extensive chromosome variation. There is now a considerable amount of data available indicating that the chromosome stability of plant cell cultures is profoundly affected by cultural conditions and particularly by the composition of the culture media (Liu and Chen, 1976). But the cytology of the *Pinus* callus has been studied in only a few reports. Variation of chromosomes in the callus has been reported for *P. strobus* (Gautheret, 1956), *P. densiflora* (Tominaga and Oga, 1970) and *P. roxburghii* (Mehra and Anand, 1983).

This study deals with the chromosomal variations in callus of *Pinus koraiensis* cultured on media supplemented with various growth regulators.

MATERIALS AND METHODS

Callus induction;

Excised mature embryos of *P. koraiensis* were planted to a series of G.D. basic media (Gresshoff and Doy's medium, 1972) supplemented with growth regulators as follows; 0.1 mg/l NAA (M-1), 0.1 mg/l NAA and 2,4-D (M-2), 0.1 mg/l 2,4-D and BAP (M-3), and 0.1 mg/l 2,4-D and kinetin (M-4).

All cultures were subjected to a continuous temperature of 25 ± 3°C, and fluorescent illumination about at 3,400 lux with 9 hours photoperiod. Induced calluses were subcultured on the same medium every 6 months for one and a half year (Park and Kim, 1983).

Cytogenetical observation;

The samples of callus tissues were pretreated in 0.03% 8-hydroxyquinoline for 24 hours at 5°C and then fixed in alcohol-acetic acid solution (3:1) for 4 hours. The fixed callus tissues were washed several times with 95% ethanol and then stained with aceto-carmine. The slides were prepared by routine squashing.

RESULTS AND DISCUSSION

Haploid cell are shown the 12 chromosomes (Fig. 1) and diploid cell with 24 chromosomes (Fig. 2). Triploid and tetraploid cells are shown in Fig. 3 and Fig. 4, while aneuploid cells are shown in Fig. 5 (2x-2 = 22) and in Fig. 6 (4x + 4 = 52).

As shown in table 1, the highest percentage of euploid cells occurred on M-3 supplemented with both auxin and cytokinin. The frequency of euploid is 61.9% in M-3, 58.6% in M-2, 48.5% in M-4 and 45.8% in M-1, respectively.

Table 1. Number of euploid and aneuploid of callus cells cultured in four different media

Medium	Ploid		No. of
	Euploid (%)	Aneuploid (%)	cells (%)
M−1	11 (45.8)	13 (54.2)	24 (100)
M−2	17 (58.6)	12 (41.4)	29 (100)
M−3	13 (61.9)	8 (38.1)	21 (100)
M−4	16 (48.5)	17 (51.5)	33 (100)

The variations in chromosome number of callus cultured on 4 different media are shown in Table 2. A considerable range of variation in number of chromosome from haploid to tetraploid was observed in the callus tissues on the M-1. The average number of chromosomes per cell in M-1 was 30 and that was more than other media.

The widest variation of the number of chromosomes was investigated in the callus cell derived from M-2 medium. The average number of chromosomes per cell in M-2 was fewer than that of

Table 2. Variations in chromosome numbers of calluses cultured in four different media.

Medium	No. of cells observed	Chromosome number																										$x \pm \sigma$	C.V. (%)
		10	11	12	13	14	20	21	22	23	24	25	26	32	33	34	35	36	37	45	46	47	48	49	50	51	52		
M−1	24			1	−	1		2	2	2	7			1	1	1	2	−	1	1	−	−	3					30.04 ± 1.726	5.7
M−2	29	1	−	5	1				3	4	5	1	1					2					5	−	−	−	1	26.76 ± 2.428	9.0
M−3	21		1	1					3	1	11				2	1	1											24.52 ± 1.367	5.5
M−4	33			1	1		2	2	6	5	12						1				1	2						24.88 ± 1.424	5.7

M-1 but more than M-3 and M-4. The coefficient of variation (C.V.) in M-2 was the highest among 4 media as 9.0%. In the case of M-3, the number of chromosomes per cell ranged from 11 to 36 and the average number was 24.5 of the smallest among the 4 media. C.V. of M-3, therefore, was also the lowest than others as 5.5%. As far as M-4, the number of chromosomes varied from 12 to 48. The average number and C.V. were 24.8 and 5.7%, respectively, similar to that of M-3.

Considering the C.V. and the average number of chromosomes per cell, M-2 gave the largest variation but M-3 and M-4 were shown stable.

The percentage of occurrance of diploid cells was observed 52% in M-3 and 36% in M-4, while that was revealed 29% in M-1 and 17% in M-2. The medium supplemented with auxin alone or the combination of auxins gave a lower percentage of diploid cells than that with the combination of auxin and cytokinin.

It is clear now that the growth regulators in the culture medium have a determinative role of chromosomal behavior in callus tissue, although the precise mechanisms are not proved at present.

Induction of callus using a variety of media supplemented with various plant growth regulators has been achieved in a number of only a few pines. Mehra and Anand (1983) reported that cells of *Pinus roxburghii* in 4 to 12-week-old-calli on the medium with 4 mg l^{-1} NAA or 2,4-D and 1 mg l^{-1} kinetin were predominantly diploid, though a few polyploid and aneuploid cells were also noticed.

As far the reports of organogenesis in *Pinus* species, Sommer *et al.* (1975) induced the shoots and roots from callus tissue derived from embryos of *Pinus palustris* Mill on the medium supplemented with auxin and after subculturing the medium with cytokinin. The organogenesis with hormone control in *Pinus taeda* was by Mehra *et al.* (1978) and Mott and Amerson(1981), in *P. strobus* (Minocha, 1980), in *P. radiata* (Reilly and Brown, 1976; Reilly and Washer, 1977), in *P. thunbergii* (Sato, 1978), in *P. nigra* (Kolevska *et al.*, 1983), in *P. sylvestris* (Bornman and Jansson, 1980), and in *P. wallichiana* (Konar and Singh, 1980).

As some conifers, Douglas-fir was redifferentiated on the medium with 5 μM BAP and 5 μM NAA from callus tissue (Cheah and Cheng, 1978). In *Picea glauca*, redifferentiation from callus tissues occurred on the medium containing 10^{-5} M BAP and 10^{-7} NAA (Campbell and Durzan, 1975).

These results indicate that organogenesis is related to both the auxin and cytokinin. The mechanism of organogenesis from callus tissues in conifer species is not clear at present but we could suppose that the increment of the number of diploid chromosome cells are related to organogenesis.

Therefore, to induce the shoots and roots from callus tissue in *P. koraiensis*, it is necessary to

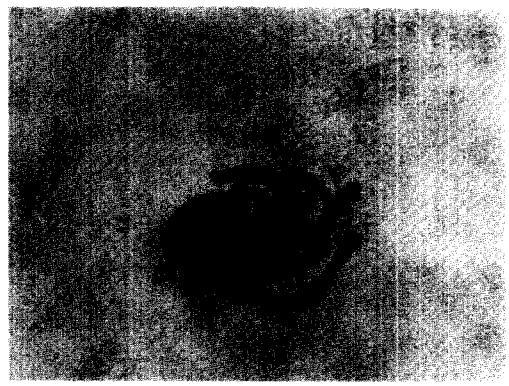

Fig. 1. Chromosomes of haploid cell (n = 12) in the callus derived from M-1.

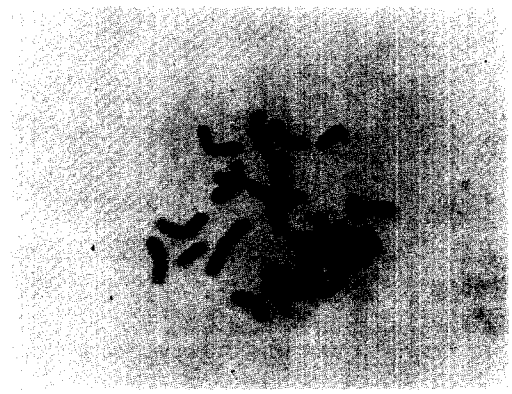

Fig. 2. Chromosomes of diploid cell (2n = 24) in the callus derived from M-2.

Fig. 3. Chromosomes of triploid cell (3n = 36) in the callus derived from M-3.

Fig. 4. Chromosomes of tetraploid cell (4n = 48) in the callus derived from M-4.

Fig. 5. Chromosomes of aneuploid cell (2n - 2 = 22) in the callus derived from M-1.

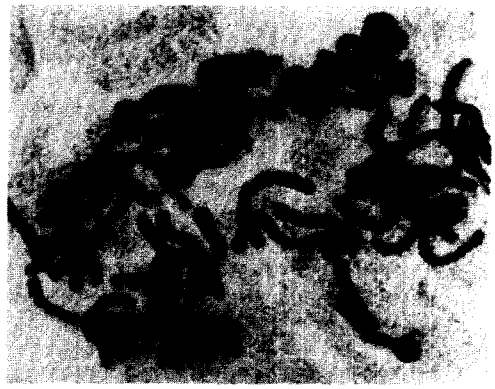

Fig. 6. Chromosomes of aneuploid cell (4n + 4 = 52) in the callus derived from M-2.

increase the cell population which have stable chromosome numbers, and we could suggested that the regeneration can be facilitated on proper medium by adjustment of concentration of auxin and cytokinin.

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