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In Vitro Studies on *Pinus koraiensis*(I)[1]

—Establishment and Growth of Callus—

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잣나무의 器內培養에 關한 硏究(I)[1]

-Callus의 誘発과 生長-

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ABSTRACT

Embryo tissue from *Pinus koraiensis* was established in culture on G.D. and W.S. basal medium supplemented with the auxin (NAA, 2,4-D, IBA) and kinetin. The various combinations of growth regulators were studied in order to determine the specific requirements of the callus tissue *in vitro*. The inorganic nutrient combination of G.D. medium was found to be better than that of W.S. medium. G.D. basal medium supplemented with 0.1 ppm NAA and 0.1 ppm kinetin was the most successful nutrient combination for the growth of callus induced from the embryo of *P. koraiensis*.

Key words: Pinus koraiensis; in vitro; callus.

要 約

잣나무 胚에서 誘發한 Callus의 安定된 生長을 지속하는 最適培地를 밝혀내기 위하여 여러 가지 生長調節物質을 添加한 G.D.培地와 W.S.基本培地에 Callus를 移植하여 30日마다 生長量을 測定하였다. 生長物質에 對한 反應을 보기 위해 G.D. 基本培地에 2,4-D, NAA, IBA와 Kinetin을 각각 0.2ppm씩 첨가한 배지에 Callus를 移植 120日동안 每月 生長量을 調査하였다. 그 結果 2.0ppm의 高濃度에서는 거의 生長이 中止되었으며, 2,4-D와 NAA 0.2ppm의 低濃度에서는 높은 生長을 보였으나 Kinetin과 IBA의 低濃度에서는 약간의 生長을 보였다. 生長物質間 相互作用을 보기 위해 G.D. 培地에 2,4-D 0.1, NAA 0.1, IBA 0.1ppm에 對해 各各 Kinetin 0, 0.1, 1.0ppm씩 組合한 9가지 處理와 比較培地(生長物質無添加)를 만들어 Callus를 移植하여 生長量을 120日間 30日마다 調査하였다. 그 結果 Kinetin 1.0ppm을 添加한 培地에서는 거의 生長이 中止되었다. 그러나 NAA 0.1ppm에서 44倍, 2,4-D 0.1ppm에서는 35倍의 높은 生長을 보인 반면 NAA 0.1+Kinetin 0.1ppm에서 57倍, 2,4-D 0.1+Kinetin 0.1ppm에서는 43倍의 生長을 보여 低濃度에서 Auxin과 Kinetin의 相互作用이 크게 나타났다. W.S.基本培地에 對한 生長物質間 Callus 生長量調査結果 G.D. 培地보다 낮은 生長量을 나타냈다. 以上의 結果 잣나무 胚에서 誘發된 Callus는 培養室 溫度 25°C±3°C, 日長 9時間, 光度 3,400Lux下에서 G.D. 基本培地에 NAA 0.1과 Kinetin 0.1ppm을 添加한 培地에서 가장 높은 生長을 나타냈다.

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INTRODUCTION

Tissue culture in forest tree species is a very important technique in tree breeding. There are so many ways as using of the shortening of breeding period (Ishikawa, 1975), of the mass propagations (Kim *et. al.*, 1982), and of the physiological and genetical materials (Durzan and chalupa, 1976a, b).

The callus of gymnosperm plant cells is very useful materials for physiological and genetical studies (Chalupa and Durzan, 1976) and for making protoplast culture (Harn, 1978). Earlier, Ball (1950) cultured the callus from *Sequoia sempervirens*. Loewenberg and Skoog (1952) reported the establishment of tissue cultures of *Pinus banksiana* on a medium containing malt extract and NAA. Barnes and Naylor (1958) used Heller's medium supplemented with IAA, tyrosine and vitamines to initiate callus from *Pinus clausa, P. serotina, P. elliottii* and *P. taeda* in the dark. Hypocotyl segments of *P. gerardiana* were grown on complex liquid medium (Konar, 1963), while those of *P. palustris, P. taeda,* and *P. elliottii* were raised on a modified Murashige and Skoog's medium. Harvey and Grasham (1969) established callus culture of *P. albicaulis, P. contorta, P. flexilis, P. monticolas, P. nigra* and *P. ponderosa* on a chemically defined medium. Konar (1974) reported the establishment and growth of the callus of *Pinus gerardiana.*

The present report deals with an attempt to establish the callus tissues from *Pinus koraiensis* on G.D. (Gresshof and Doy's) and W.S. (Wolter and Skoog's medium) with the varying concentrations of growth regulators.

MATERIALS AND METHODS

The entire female gametophytes of *P. koraiensis* were sterilized with 70% ethyl alcohol for one minute, washed with sterilized distilled water and a 0.02% mecuric chloride solution for one minute, and rinsed several times with sterile distilled water.

Excised embryos were placed on G.D. medium (Table 1) with 0.1 ppm 2,4-D and 0.1 ppm kinetin. After two months. a piece of callus with an average 30 mg of fresh weight was transferred to a new G.D. medium containing 0.2 and 2.0 ppm of NAA, 2,4-D, IBA, and kinetin, respectively, to compare growth rate in each growth regulator. To determine the effects of the interaction between

Table 1. The constituents of two basal media

Constituents	Basal medium (mg/liter)		Constituents	Basal medium (mg/liter)	
	G.D. *1	W.S. *2		G.D. *1	W.S. *2
$(NH_4)_2SO_4$	200	–	Na_2 Fe-EDTA	–	5.5
NH_4NO_3	–	50	$MnSO_4 \cdot H_2O$	10	9
$CaCl_2 \cdot 2H_2O$	150	–	$ZnSO_4 \cdot 7H_2O$	3	3.2
$Ca(NO_3)_2 \cdot 4H_2O$	–	425	H_3BO_3	3	3.2
$MgSO_4 \cdot 7H_2O$	250	764	$CuSO_4 \cdot 5H_2O$	0.25	–
KNO_3	1,000	170	$Na_2MoO_4 \cdot 2H_2O$	0.25	–
KCl	300	140	$CoCl_2 \cdot 6H_2O$	0.25	–
KI	0.75	1.6	Inositol	10.0	10.0
$NaH_2PO_4 \cdot H_2O$	90	35	Thiamin HCl	1.0	–
Na_2SO_4	–	425	Nicotinic Acid	0.1	–
Na_2HPO_4	30	–	Pyridoxine HCl	0.1	0.1
$FeSO_4 \cdot 7H_2O$	27.8	–	Sucrose	20,000	20,000
Na_2EDTA	37.3	–	Agar	7,000	10,000

*1. Gresshof and Doy's medium; *2. Wolter and Skoog's medium

various growth regulators, a piece of callus with an average fresh weight of 20 mg was transferred to a new G.D. medium with varying concentrations of 2,4-D, IBA, NAA and kinetin (Table 2). Observing the growth of callus on the various concentrations, the fresh weight of the callus was measured by every month during four months.

Investigating the effect of the W.S. medium, a piece of callus induced from W.S. with 0.1 ppm 2,4-D and 0.1 ppm kinetin was transferred to a new W.S. medium with the combinations of NAA at 0.1 or 2,4-D at 0.1 and kinetin at 0, 0.1, 0.5, 1.0 and 1.5 ppm, respectively (Table 4). The fresh weights of the callus were measured by every month during two months.

The callus pieces were removed from the test tubes and placed on sheets of filter paper to remove surface moisture and agar. Their fresh weights were measured, and then the callus tissues were inoculated on the same used medium.

The test tubes (ϕ 20 cm, L 15 cm) were subjected continually to a temperature of 25 ± 3°C and a fluorescent illumination of about 3,400 Lux with 9 hours of photoperiod throughout culture period.

RESULTS AND DISCUSSION

Table 2 shows the growth rate of the callus in G.D. medium with 0.2 and 2.0 ppm of NAA, 2,4-D, IBA and kinetin, respectively. The growth of the callus on all of 2.0 ppm of growth regulators was seriously depressed, while the medium of 0.2 ppm revealed the various growth rate of the callus.

Table 2. Growth of the callus induced from *P. koraiensis* embryos in G.D. medium with varying concentrations of growth regulators. The number is the mean fresh weights (mg) of the callus in 10 test tubes. The number in parenthesis is growth rate index (investigating fresh weight/inoculating fresh weight).

Growth regulators	Concentration (mg/liter)	Investigating days				
		0	30	60	90	120
NAA	0.2	22.9(1.0)	39.5(1.3)	136.5(4.6)	549.0(18.4)	825.8(27.6)
	2.0	23.9(1.0)	25.3(1.1)	25.0(1.1)	22.3(1.0)	23.3(1.0)
2,4-D	0.2	44.3(1.0)	120.2(2.7)	297.5(6.7)	717.1(16.2)	1012.6(22.8)
	2.0	56.8(1.0)	48.5(0.9)	49.9(0.9)	46.3(0.8)	44.5(0.8)
IBA	0.2	8.7(1.0)	15.4(1.8)	35.1(4.0)	86.6(10.0)	34.5(4.0)
	2.0	22.5(1.0)	22.5(1.0)	22.5(1.0)	22.0(1.0)	21.6(1.0)
Kinetin	0.2	15.5(1.0)	19.2(1.2)	24.5(1.6)	117.5(7.6)	124.4(8.0)
	2.0	14.7(1.0)	14.8(1.0)	14.5(1.0)	13.1(0.9)	12.5(0.9)

Figure 1 shows the growth rate of the callus on the G.D. medium with the four growth regulators at 0.2 ppm. The growth rate at the concentration of 0.2 ppm NAA reached almost 28 folds and that of 0.2 ppm 2,4-D almost 27 folds, while that of 0.2 ppm kinetin was shown 8 folds and 0.2 ppm IBA only 4 folds after 120 days. The growth rate at 0.2 ppm NAA was lower than that at the same concentration of 2,4-D after 60 days, while this tendency was opposite after 90 days.

Table 3 shows the growth rate on G.D. media combined with the auxin (NAA, 2,4-D, IBA) at

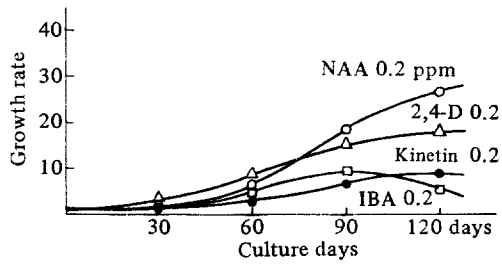

Fig. 1. Growth rate curve of the callus showing the effect on the G.D. mediums supplimented with 0.2 NAA, 0.2 2,4-D and 0.2 ppm kinetin after 120 days culture.

Table 3. Growth of the callus induced from *P. koraiensis* embryos in G.D. medium with the varying concentrations of auxin and cytokinin. The number is the mean fresh weights (mg) of the callus in 10 test tubes. The number in parenthesis is growth rate index (investigating fresh weight/inoculating fresh weight)

Growth regulators			Investigating days				
Auxin (mg/liter)		Kinetin (mg/liter)	0	30	60	90	120
NAA	0.1	–	31.4(1.0)	248.9(7.9)	833.0(26.5)	1327.5(42.3)	1376.5(43.8)
NAA	0.1	0.1	18.2(1.0)	61.6(3.4)	382.9(21.0)	857.2(47.1)	1031.9(56.7)
NAA	0.1	1.0	21.5(1.0)	22.3(1.0)	26.7(1.2)	41.9(2.0)	21.1(1.0)
2,4-D	0.1	–	36.3(1.0)	62.3(1.7)	277.7(7.7)	990.9(27.3)	1273.9(35.1)
2,4-D	0.1	0.1	16.1(1.0)	67.6(4.2)	313.1(19.5)	415.3(25.8)	689.4(42.8)
2,4-D	0.1	1.0	17.9(1.0)	21.0(1.2)	23.0(1.3)	45.0(2.5)	33.4(1.9)
IBA	0.1	–	17.8(1.0)	35.2(2.0)	67.8(3.8)	103.7(5.8)	63.2(3.6)
IBA	0.1	0.1	26.2(1.0)	37.1(1.5)	58.4(2.2)	100.3(3.8)	64.5(2.5)
IBA	0.1	1.0	26.5(1.0)	25.0(0.9)	35.5(1.3)	36.5(1.4)	35.1(1.3)
Control	–	–	31.3(1.0)	46.0(1.5)	56.3(1.8)	60.0(1.9)	48.7(1.6)

0.1 ppm with kinetin at 0, 0.1 and 1.0 ppm. The media with 1.0 ppm kinetin and with no growth regulators did not induce much growth of callus. Figure 2 illustrated the tendency of growth rate on the media combined with six growth regulators. There was so much variation in the growth rate. The medium supplemented with 0.1ppm NAA and 0.1 ppm kinetin was found out to induce the fastest growth rate as 57 folds, while the medium of 0.1 2,4-D and 0.1 ppm kinetin showed the fairly good growth rate as 43 folds after 120 days. The growth rate was 44 folds on the medium with 0.1 ppm NAA alone and 35 folds on that with 0.1 ppm 2,4-D alone. The medium with 0.1 ppm IBA alone showed 4 folds and the medium with 0.1 ppm IBA and 0.1 ppm kinetin revealed the low growth rate of the callus as 3 folds after 120 days.

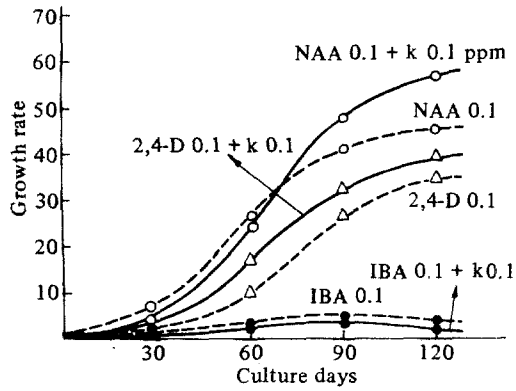

Fig. 2. Growth rate curve of the callus showing the effect on the G.D. mediums of 0.1 ppm auxin alone and of combining the auxin (0.1 NAA, 0.1 2,4-D, 0.1 IBA) with 0.1 kinetin respectively, after 120 days culture.

Table 4 shows the growth rate on W.S. medium with 0.1 NAA or 0.1 ppm 2,4-D with 0, 0.1, 0.5, 1.0 and 1.5 ppm kinetin, respectively. The mediums combined with 0.1 NAA or 0.1 2,4-D with 0.5, 1.0 and 1.5 ppm kinetin were not found out the growth of callus. The medium with 0.1 NAA and 0.1 ppm kinetin revealed the growth rate about 7 folds, while that with 0.1 2,4-D and 0.1 ppm kinetin about 9 folds. The growth rate in W.S. is much lower than that in G.D. medium which revealed the 21 folds on the medium of 0.1 NAA and 0.1 kinetin and the 20 folds on that of 0.1 2,4-D and 0.1 ppm kinetin after 60 days. The growth rate was about 20 folds on the W.S. medium of 0.1 ppm NAA alone and on that of 0.1 ppm 2,4-D alone. However, the growth rate in the W.S. medium with 0.1 2,4-D is higher than that in G.D. medium of 2,4-D which reveals 8 folds, the growth

Table 4. Growth rate of the callus from embryos of *P. koraiensis* cultured in 10 different concentrations of auxin with kinetin in W.S. medium. The number is the mean fresh weights (mg) of callus in 10 test tubes.

Growth regulators			Investigating days		
Auxin	(mg/liter)	Kinetin (mg/liter)	0	30	60
NAA	0.1	–	23.2(1.0)	142.8(6.2)	444.4(19.2)
NAA	0.1	0.1	25.4(1.0)	58.1(2.3)	167.6(6.6)
NAA	0.1	0.5	28.9(1.0)	22.9(0.8)	19.9(0.9)
NAA	0.1	1.0	29.7(1.0)	21.6(0.7)	20.6(0.7)
NAA	0.1	1.5	27.7(1.0)	21.1(0.8)	20.0(0.7)
2,4-D	0.1	–	25.7(1.0)	127.9(5.0)	503.4(19.6)
2,4-D	0.1	0.1	27.1(1.0)	62.4(2.3)	232.2(8.6)
2,4-D	0.1	0.5	29.7(1.0)	23.8(0.8)	27.3(0.9)
2,4-D	0.1	1.0	20.2(1.0)	14.8(0.7)	16.4(0.8)
2,4-D	0.1	1.5	18.2(1.0)	16.0(0.9)	19.4(0.9)

rate in the W.S. medium of 0.1 NAA alone is also lower than that in G.D. of 0.1 NAA which shows 27 folds after 60 days. Although the culture periods on W.S. at only 60 days is shorter than that on G.D. medium at 120 days culture, the growth rate on G.D. shows the higher than that on W.S. medium. G.D. medium was more satisfactory than W.S. for establishment and growth of callus of *Pinus koraiensis*.

Winton (1972a) lists some 40 species of gymnosperms which have been initiated to form calli. The success of subculturing has been limited. Saito (1979) reported that the callus induced from the hypocotyls of *Cryptomeria japonica* showed vigorous growth on the W.S. medium with 0.04 BAP 0.2 NAA, 1.0 GA_3 and 10.0 ppm *IBA*. The callus of Douglas-fir on a Brown and Lawrence medium with 1.0 ~ 5.0 ppm NAA and 0.01 ~ 1.0 ppm BAP grew best (Winton, 1972b). Harvey *et. al.* (1969) reported that the callus of five *Pinus* species (*P. albicaulis, P. contorta, P. flexillis, P. nigra, P. ponderosa*) showed the best growth on the basal medium with 1.0 ~ 0.5 ppm NAA or 0.1 ppm 2,4-D and 1.0 ~ 0.5 ppm kinetin.

In the present investigations the best growth of callus induced from the embryo of *Pinus koraiensis* was shown on the G.D. basal medium with 0.1 ppm NAA and 0.1 ppm kinetin.

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