Tetraploids of *Larix Kaempferi* in the Nurseries

By

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With two plates, three text-figures and two tables

Introduction

In the forest trees, polyploidy has been studied recently from the standpoint of breeding, and many artificial tetraploids were induced by the colchicine treatment. In the other hand, however, it is useful to investigate the natural polyploids as well as artificial polyploids. In the conifers, spontaneous polyploids reported up to t e present time are as follows; the triploid produced in hybrids between *Larix decidua* × *L. occidentalis* (SYRACH and WESTERGAARD 1938), tetraploid in twin seedlings of *Abies firma* (KANEZAWA 1949), the triploid of *Picea Abies* (KIELLANDER 1950), tetraploid of *Larix decidua* (CHRISTIANSEN 1950), tetraploids and triploids of *Cryptomeria japonica* (ZINNAI and CHIBA 1951, CHIBA 1951) and tetraploids of *Pinus densiflora* (ZINNAI 1952), etc.

In the autumn of 1951, the writers discovered eight tetraploids of *Larix Kaempferi* SARG. (*L. leptolepis* MURR.) which were induced in different origin in the nurseries. *Larix Kaempferi* is one of the most important forest trees in Japan.

It is the purpose of this paper to report the cytological and morphological investigations of the spontaneous tetraploids in *Larix Kaempferi*.

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Matelials and methods

The localities materials were selected, areas of nurseries examined, the number of selected tetraploids and diploids for control are shown in Table 1.

Table 1. The localities materials were selected, areas of nurseries examined and the number of materials.

Name of Nurseries	No. of tetraploid selected	No. of diploid for control	Area examined and numbers of seedlings	Date of selection
Shinmachi Nursery of Mamurokawa D. F. O.	No. 41	No. 21	100m², 4900	15/VIII '51
Kemuriyama Nursery of Morioka D. F. O.	No. 42	No. 22	900m², 50000	20/VIII '51
Shiono Nursery of Iwamurata D. F. O.	No. 43～48	No. 23～28	11970m², 508200	3/X '51

(note) D. F. O.: District Forest Office.
Seed source: Yielded in Nagano Regional Forest Office.

In the preliminary selection, 2-year-old seedlings which have thick, twisted and sometimes connatus needles were marked under the careful observations, and immediately their root-tips were fixed with Nawashin's solution, and then, in the secondary selection, the stomata of needles of these plants were observed under the microscope.

In April and May of 1952 the chromosome numbers were investigated on the plates of somatic cell division in the root-tips and shoot-apexes which were fixed with Nawashin's solution, imbedded in paraffin, sectioned at 14 μ and stained with Ehrlich's acid haematoxylin. The stomata preparations were made by stripping off the epidermal tissues and stained with phloroglucine and conc. hydrochloric acid. Drawings were made by the use of Abbe's apparatus. The length of guard cell of the stomata and the number of stomata per sq. 0.1 mm were measured.

Observations

1. Occurrences of tetraploids in the nurseries.

In preliminary selection by the morphological appearances, 20 plants were picked up, and in the secondary selection by the observations of stomata, eight plants were obtained (No. 41～48) as shown in Table 1. These eight plants were determined as tetraploid by the cytological investigations.

The occurrence of spontaneous tetraploids in the nurseries were exceedingly low, an instance in Shiono Nursery was about 0.0012 percent, and they were appeared not in group but solitarily.

2. Comparison in tetraploid and diploid seedlings.

(1) Chromosome. The chromosomes were investigated in each 20 plants which had been selected in the preliminary selection. The investigations were made in the root-tips collected in October of '51 and April of '52 and also on the shoot-apexes collected in May of '52.

The choromosome number of *Larix Kaempferi* were confirmed to be n=12 by Ishikawa (1902), Sax (1932 a) and Sax & Sax (1933). By the writers' observations on the mitotic metaphases in the section of root-tips and shoot-apexes, the diploid number in ordinary plants were counted 2n=24, and this result agreed with the earlier investigators' reports. In tetraploids, 2n=48 were counted, but it is very difficult to confirm the numbers, as the chromosomes were long and meandering.

From the results of investigations in each 20 plants which were selected preliminaryly, 12 plants were determined as diploids (2n=24) and eight plants were as tetraploids (2n=48) (Fig. 1, Plate Ⅱ).

However, two tetraploids (No. 44, 46) were different from other six plants. According to the detailed observations of the writers in october of '51, April and May of '52 on the mitotic metaphases of root-tips and shoot-apexes, following remarkable facts were cleared. In the case of No. 46, the roots were diploidy

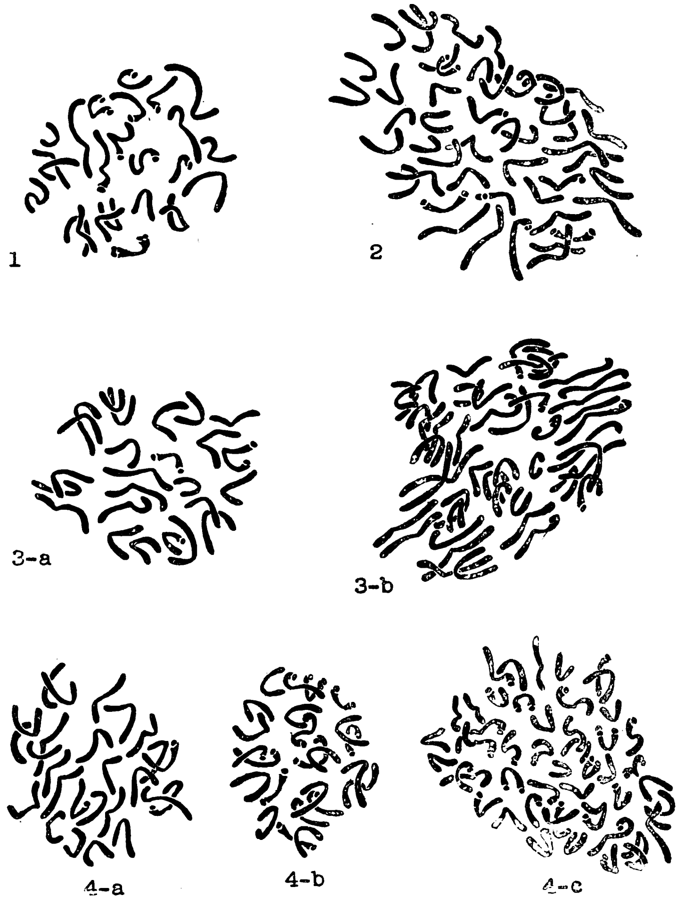
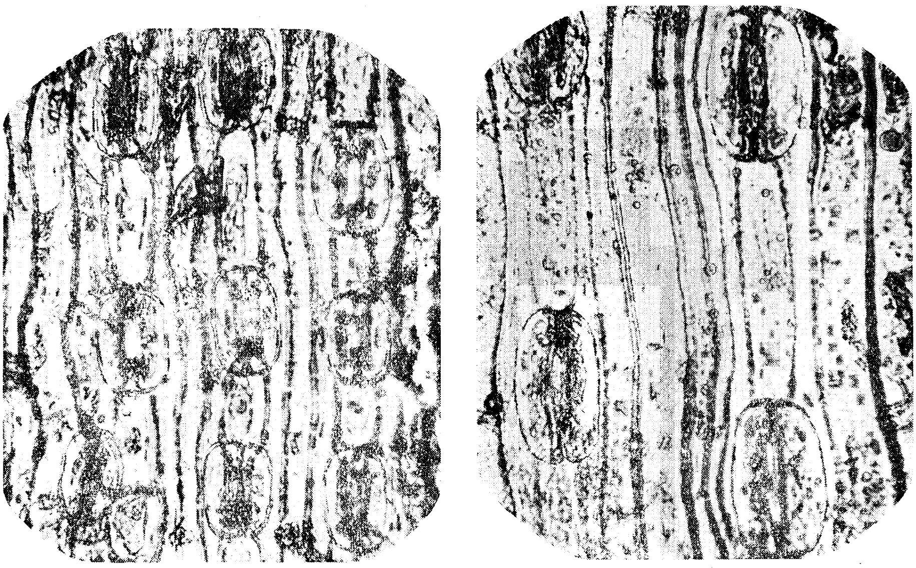


Fig. 1. Mitotic metaphases of root- and shoot-apex of *Larix Kaempferi*. (ca. ×1200)
1) No. 28, Diploid (Cont.), root-tip 2n=24.
2) No. 43, Tetraploid, root-tip 2n=48.
3) No. 46, a) Root-tip, diploid: b) Shoot-apex, tetraploid.
4) No. 44, a) Root-tip, diploid: b) Shoot-apex, the 1st-year's parts, diploid.: c) Shoot-apex, the parts of the 2nd-year which have grown from the apical bud of stem, tetraploid.

and the shoots were tetraploidy. In the case of No. 44, the roots were diploidy, the shoots of the 1st-year (1950) were diploidy and all of the shoot which have grown in the 2nd-year (1951) only from the apical bud of stem were tetraploidy as presented in Figure 1 and Plate Ⅰ, C. These two plants are not entire tetraploid as others but partial tetraploids which were induced by the doubling of chromosome in somatic cells. These phenomena are in accord with the results of observations in stomata of needles.

(2) Stomata. On the length of guard cells and the number of stomata per unit area (1 unit = sq. 0.1 mm), 200 units were measured in each cases. The appearances of stomata in diploid and tetraploid under the microscope are shown in Figure 2. The frequencies of the length of guard cells and the number of stomata per unit area are presented in Figure 3a, b. Data obtained will be summarized in Table 2. From these results, the characteristics on the stomata in diploid and tetraploid are briefly described as follows; (1) Length of guard cells of tetraploids is larger about 23～40 percent than those of diploids. (2) Number of stomata per unit area of tetraploids are smaller about 25～44 percent than those of diploids. (3) Two characters in tetraploid and diploid plants above described, were recognized also in tetraploid and diploid parts of No. 44. (4) As shown in Figure 3, in the case of 2n-No. 23 and 4n-No. 41, it may be difficult to decide as tetraploid by the stomata only in many individuals. It is evident, however, from the data in Table 2, that the characteristics of stomata are very important indicator in the selection, although they are not an essential one in identification of diploid and tetraploid.



A　　　　B

Fig. 2. Stomata appearance in needle under the microscope.
A) diploid.　B) tetraploid.　(ca. ×500)

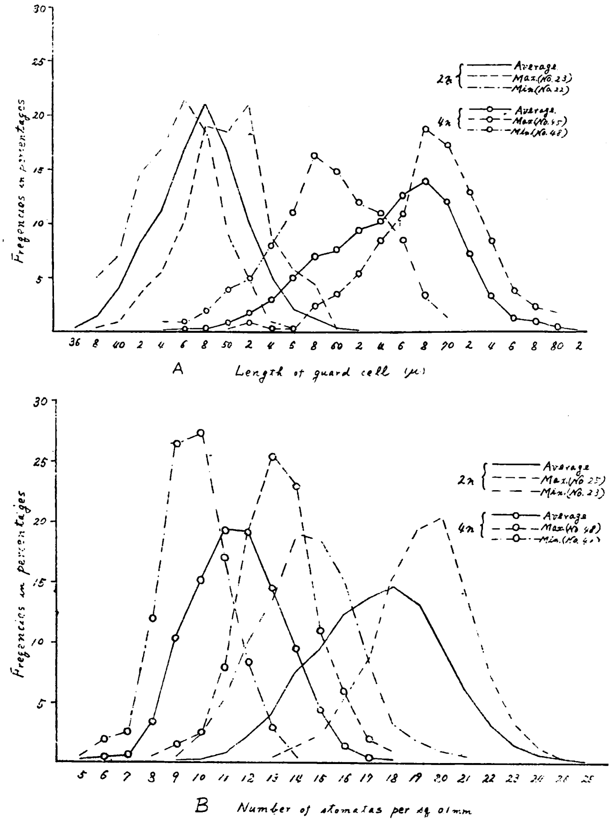


Fig. 3. A) Showing frequencies of the length of guard cell.
B) 〃 the number of stomata per sq. 0.1 mm.
(Average, Max. and Min. in each 8 plants of diploid and tetraploid.)

Table 2. Length of guard cell, number of stmata per sq. 0.1 mm and chromosome number in diploid and tetraploid.

No. of plants	Length of guard cell (μ)				Number of stomata per sq. 0.1 mm				Chromosome number	
	Mean	Frequency	σ	Proportion	Mean	Frequency	σ	Proportion	Shoot-apex	Root-tips
Diploid No. 21	47.60±0.13 a)	36～58	1.884	99.6	16.97±0.17	11～23	2.396	97.6	2n=24	2n=24
No. 22	45.48±0.13	38～56	1.889	95.2	16.30±0.17	11～23	2.380	93.7	〃	〃
No. 23	50.06±0.14	38～58	2.035	104.7	14.45±0.15	9～21	2.189	83.1	〃	〃
No. 24	49.81±0.13	38～62	1.879	104.2	17.46±0.16	12～24	2.207	100.4	〃	〃
No. 25	47.18±0.14	36～62	2.198	98.7	19.28±0.15	13～25	2.100	110.9	〃	〃
No. 26	46.27±0.14	38～58	1.965	96.8	17.86±0.14	13～23	2.030	102.7	〃	〃
No. 27	48.51±0.14	40～58	1.939	101.5	18.31±0.16	12～23	2.254	105.3	〃	〃
No. 28	46.52±0.14	36～56	1.975	97.3	18.99±0.19	12～26	2.620	109.2	〃	〃
Average	47.79±0.05	36～62	2.140	**100.0**	17.39±0.07	9～26	2.731	**100.0**		
Tetraploid										
No. 41	67.12±0.20	54～82	2.844	140.4	9.76±0.13	5～14	1.513	56.1	2n=48	2n=48
No. 42	63.30±0.20	50～78	2.777	132.5	11.12±0.11	8～16	1.490	63.9	〃	〃
No. 43	63.25±0.22	46～78	3.076	132.3	12.65±0.13	8～17	1.783	72.7	〃	〃
No. 44	67.71±0.18	52～80	2.516	141.7	11.81±0.12	8～16	1.625	67.9	〃	} 24
(No. 44)	47.23±0.11	40～54	1.539	98.8	16.93±0.14	12～22	3.790	97.4	24	
No. 45	68.39±0.19	50～80	2.661	143.1	10.48±0.17	6～15	1.523	60.3	48	48
No. 46	64.68±0.20	52～80	2.800	135.3	12.10±0.13	7～17	1.868	69.6	〃	24
No. 47	65.42±0.21	50～80	3.025	136.9	11.34±0.12	7～16	1.652	65.2	〃	48
No. 48	59.03±0.18	44～70	2.608	123.5	13.25±0.12	8～18	1.700	76.2	〃	〃
Average	64.86±0.08	44～82	3.161	**135.7**	11.57±0.05	5～18	1.990	**66.5**		

(Note) a) Used standard error.
200 units were investigated both length of guard cell and number of stomata per unit area in every plants.

(3) Morphological appearances. The appearance of tetraploids dose not show remarkable gigantic form as in the case of *Cryptomeria japonica* (Plate I). According to the writers' observations, the characteristics induced by the doubling of chromosome number in *Larix Kaempferi* are briefly noted as follows; (1) The needles incresased in thickness, and can be easily distinguished by the tough feeling. (2) In many cases they have connatus needles as shown in Plate I D, II, C. (3) Needles tended to be twisted and needles on shoot are not so dence as those of diploid. (4) The color in needles varied from light to dark green in both diploids and tetraploids, then, the tetraploids can be hardly distinguished from diploids by this character.

According to the writers' experiences, the thickness of needles and the connatus needles are important indicators in the preliminary selection of tetraploid.

Discussion

Morpholoigcal characteristics in the plants induced by the doubling of chromosome number have been reported in various respects. In generally speaking, those of polyploid are as follows; (1) The increment of cell size (stomata, woodfiver, pollen, mesophyll, etc.), nucleus, cytoplasmic volume and some organs (flower, seed, fruit, etc.). (2) An increase of the thickness in leaves, and dark green in color of leaves.

From the results obtained by the writers', it may be said that the morphological characteristics of spontaneous tetraploids in *Larix Kaempferi* are agreed in general with those of ordinary polypoloid. The facts reported by ILLIES (1952) that the needles of tetraploid in *Larix decidua* induced by the colchicine is shorter and dark green in color than those of diploid, were not recognized by writer's. These discrepancies would be caused by the different origin of those tetraploids.

In the case of tetraploids in *Cryptomeria*, the difference of morphorogical appearances could not see between tetraploid induced by colchicine and spontaneous ones. From these aspects, it may be expected that there are difference in resistivity for the injury of colchicine treatment by the difference of plants used.

Stomata size as an index of polyploidy have been discussed by many authors, viz. MUNTZING (1936), SAX and SAX (1937), SAX (1938), JOHNSON (1940), TAKENAKA (1943), KANEZAWA and GO (1948) and KANEZAWA (1951), etc. According to these investigations, it is summarized that there is a marked relation between chromosome number and a size of stomata, while as pointed out by BERGSTRÖM (1940) and JOHNSON (1940), the proportional increasing of stomata size is not recognized in triploids and tetraploids poplar. Then, the stomata can not be used as an essential index in identification of polyploid and this relation was experienced in writers' investigations.

Abnormal nuclear and cell divisions in nature will be occurred by many agencies, species hybridization, temperature changes, regeneration of tissue, diseases, insects, osmotic changes and mechanical injuries, etc. They occur more easily in meiosis than mitosis by these agencies.

The origin of tetraploids reported here can not be fully explained but they would be induced following processes: (1) The doubling of chromosome occurred presumably in the early proembryonic division after fertilization by some stimulations. On this case, CLAUSEN and GOODSPEED (1925) reported in hybrid of *Nicotiana*, ICHIJIMA (1926) in *Fragaria*. The instances of induced tetraploids artificially by heat treatment at the stage of proembryonic division were reported by RANDORPH (1936) in maize, MUNTZING, TOMETOP and HUNDT-PETERSON (1936) in barley and DORSEY (1936) in *Triticum* and *Secale*. The occurrences of polyploids from the twin seedlings were reported by many authors. In coniferous tree, the tetraploid of *Abies* was reported by KANEZAWA (1949). In this paper, the entire tetraploids of *Larix Kaempferi* No. 41～48 excepted No. 44 and No. 46 are probably induced by the above described processes. (2) The occurrence of secondary doublings of chromosome from diploid tissue has been reported at the early stage of germination or in the growing points of shoot by some stimulations, but these phenomena, the occurrence of the doubling of chromosomes in somatic cell, is rare in nature. BELLING (1925) rerorted in *Stizolobium* occurred by temporariry chill and NEWTON and PELLOW (1929) in *Primula*. In this paper, No. 44 and No. 46 were probably induced by these processes. In the case of No. 46, it is also presumable that only the part of bud was doubled at the developing stage of proembryo. In the case of No. 44, the 1st-year's parts are diploidy and all the 2nd-year shoots grown from the apical bud of stem were doubled when the bud begins initial cell division in the following spring.

To know the direct stimulation which has caused the doubling of chromosome is difficult, but, considering from the climatic conditions of nurseries, it would be temporariry chill caused by spring frost at the buds sprouting.

Spontaneous mixoploid in forest trees have been reported by MEURMAN (1933) in *Acer* and by KANEZAWA and ODAIRA (1942) in *Rhus* and *Quercus*. In these cases, the tetraploid shoot may occur by the sprouting of tetraploid bud. .

From the results of investigations of chromosome both in the root-tips and shoot-apexes, reasonable aspects of mixoploid have not been recognized.

On the rare occurrence of natural polyploids in gimnosperm, MUNTZING (1933) and SAX and SAX (1933) pointed out the following reasons; (1) The conifer dose not performed the double fertilization, and hybridization occurs between diploids and polyploids, therfore, polyploids would be disappeared gradually if they were not limited to the some districts. (2) As in the meiosis of the polyploids, occur frequently the interstitial chiasmata at the forming of tetrad, they would be produced sterile pollen caused by irregular distribution of chromosomes.

Many reports on the occurrence of spontaneous polyploid in conifers have been recently published are as follows; the triploids in *Larix* hybrid, *Picea Abies*, *Cryptomeria japonica* and the tetraploid in *Abies firma*, *Larix decidua*, *Cryptomeria japonica* and *Pinus densiflora*. From these instances, it may be expected to be the possibility of occurrence of polyploids also in other species.

The silvicultural or physiological characteristics of tetraploids presented in this paper have not yet known and they will be discussed in further papers. In the present state, the tetraploids of *L. Kaempferi* do not show the retardation of growth and remarkable differences in morphological characters compared with those of diploids, therefore in the nurseries they will not be thrown away as in the cases of the polyploids of *Cryptomeria* (CHIBA 1951). Then, it is presumable that the aged polyploid of *L. Kaempferi* grow in the forest as reported in *L. decidua* (CHRISTIANSEN 1950). It is also need to pay attention on the spontaneous polyploids as well as the induced polyploids artificialy from the view point of forest tree breeding.

Summary

In the present paper the writers deal with the results of cytological investigation and morphorogical chracteristics on the spontaneous tetraploids of *Larix Kaempferi* discovered in the nurseries in the autumn of 1951.

In the preliminary selection the seedlings which were likeley to be polyploid by macroscopic appearances were maked and stomata of needles of them observed. Cytological investigations were done on the mitotic metaphases in root-tips and shoot-apexes. From the 20 plants which were picked up in the preliminary selection, eight tetraploid plants (2n=48, No. 41～48) were obtained, and others were diploids (2n=24).

Among them, two tetraploids (No. 44, 46) were different from the others. In the case of No. 44, roots were diploidy and the 1st-year's parts were also diploidy, but all the shoots of the 2nd-year which have grown only from the apical bud of stem were tetraploidy. In the case of No. 46, roots were diploidy but shoots and stem were tetraploidy.

In comparison with the characteristics between diploids and tetraploids are briefly noted as follows; (1) The length of guard cell of tetraploids were larger about 23～40 percent than those of diploids. (2) Number of stomata per unit area of tetraploids were smaller about 25～44 percent than those of diploids. (3) Two characters in tetraploid and diploid plants above described, were recognized also in tetraploid and diploid parts of No. 44. (4) From the data in Table 2, it it evident that the characters of stomata is very important indicator in the identification of diploid and tetraploid but not essential one. (5) In the present state, the tetraploids do not show the retardation of growth and remarkable differences in appearances compared with those of diploids.

The origin of tetraploid reported in this paper can not be fully explained,

but they may be induced following processes. (1) The entire tetraploids of No. 41～48, excepted No. 44. and No. 46. are probably caused by the following way; the chromosome number would be doubled at the early proembryonic division after fertilization, or the tetraploid occured from the twin seedlings. (2) No. 44 and 46 induced by the secondary doubling of chromosomes from diploidy somatic cell. No. 46, would be doubled at the early stage of germination, or only the part of bud may be doubled at the developing stage of proembryo. In No. 44, the apical bud of 1-year's stem would be doubled at the beginning of sprouting in the following spring. They are not recognized for reasonable aspects of mixoploid, and seem to be autopolyploids.

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Explanation of Plates

Plate I.

A. Diploid in *Larix Kaempferi* SARG. 2n=24, Control, No. 21. (2-year-old seedling, Photographed 6, Oct. 1951).

B. Spontaneous tetraploid. 2n=48 in root-tips and shoot-apexes, No. 43.

C. Spontaneous tetraploid, No. 44, Roots and the 1st-year's parts were diploid (shown-2n) and the 2nd-year's shoots which grown only from the apical bud of the stem were tetraploid (shown-4n).

D. Spontaneous tetraploid (In the middle part of the stem, the connatus needles are shown. Such needles offen appeared in tetraploid seedlings.).

Plate II.

A. Cross section of needle in diploid plant of *Larix Kaempferi* SARG. Ph: Phloem, Xy: Xylem, St: Stomata band.

B. Section of needle in tetraploid.

C. Section of connatus needle in tetraploid.

D. Mitotic metaphase of root-tip in diploid plants, 2n=24, No. 28, control. (ca. ×800).

E. Mitotic metaphase of tetraploid, root-tip, 2n=48, No. 43.

F. No. 46: diploid in root-tip.

G. 〃 : tetraploid in shoot-apex.

H. No. 44: diploid in root-tip.

I. 〃 : 〃 in shoot-apex of the 1st-year's parts.

J. 〃 : tetraploid in shoot-apex, the parts of the 2nd-year which have grown from the apical bud of 1st-year's stem.

苗畑に現われたカラマツの四倍体
(摘　要)

農林技官　千　葉　茂
農枝技官　渡　邊　操

林木の同一種内に於ける自然倍数体は，既に数種の樹木について報告されている。著者等は1951年夏より秋にわたり，眞室川，盛岡，岩村田営林署管内の苗畑にてカラマツの倍数体の選抜を行い8個体の四倍体を得た。

外部形態による予備選抜の結果20個体が選抜され，更に気孔の観察，根端及び芽の細胞学的観察により，8個体は四倍体（染色体数 2n=48）であり（No. 41～48），他の12個体は二倍体（2n=24）であることが確認された。四倍体の出現率は極めて低く塩野苗畑の例では0.0012% であつた。

四倍体の形態的特徴は概ね一般倍数体の特徴を有しているが，外部形態はスギの場合の様に明かな Gigas type を示さない。針葉の色は二倍体と同様に濃緑から淡緑の種々な色調を呈している。但し針葉は肥厚し従つて手ざわりが剛く且着生も粗になる傾向がある。

細胞の大いさに就て見ると，気孔閉塞細胞は四倍体は二倍体に比較して 23～40% 増大し，単位面積当りの気孔数は 25～44% 減少している。其の他根端，芽及び葉肉等の細胞に於ても増大していることが観察された。細胞の増大殊に気孔の大いさと染色体数との関係に就ては種々なる論議があるが，これは倍加認定の絶対的指標とはなり得ないが，選抜に当つては最も有力な指標である。

細胞学的観察の結果，選抜された8個体（No. 41～48）の中 No. 44，46 を除いた6個体は，根端及び芽の何れも四倍体であつた。これら6個体の四倍体は，授精後胚発生の初期の分裂に於て染色体数が倍加したものと思われる。No. 46 に於ては，根は二倍体であり，地上部は何れの幹枝も四倍体であつた。No. 44 は，根は二倍体であり且地上部の第一年生の部分も二倍体であるが（Plate I C—2n 部），一年生幹の頂芽から生長した第二年目の幹枝は何れも四倍体であつた（Plate I C—4n 部）。気孔の観察結果も染色体数の関係と一致していた。この二個体は明かに体細胞染色体数の倍加により生じたものである。その時期に関しては，No. 46 は胚発生以後胚の完成迄に或いは発芽の際に芽の部分のみが倍加したものと推定される。No. 44 は発芽当年は二倍体で，幹の頂芽が第二年目の春季分裂開始の際に倍加したものと思われる。

倍加を誘発した直接の原因は明かでないが No. 44，46 に於ては，苗畑の気象条件より推察

すると，発芽開始当時に晩霜による低温の刺戟によるものと思われる。以上の倍数体は四倍性細胞の混在せるキメラとは認められなかつた。No. 44，46 は明かな同質四倍体であるが其の他も同様と思われる。

カラマツの四倍体の生長其の他の生理，生態的性質については今後の研究に待たねばならないが，現在のところ四倍体は二倍体に比し，著しい外形の相異もなく且生長減退の現象も認められない。

Plate I

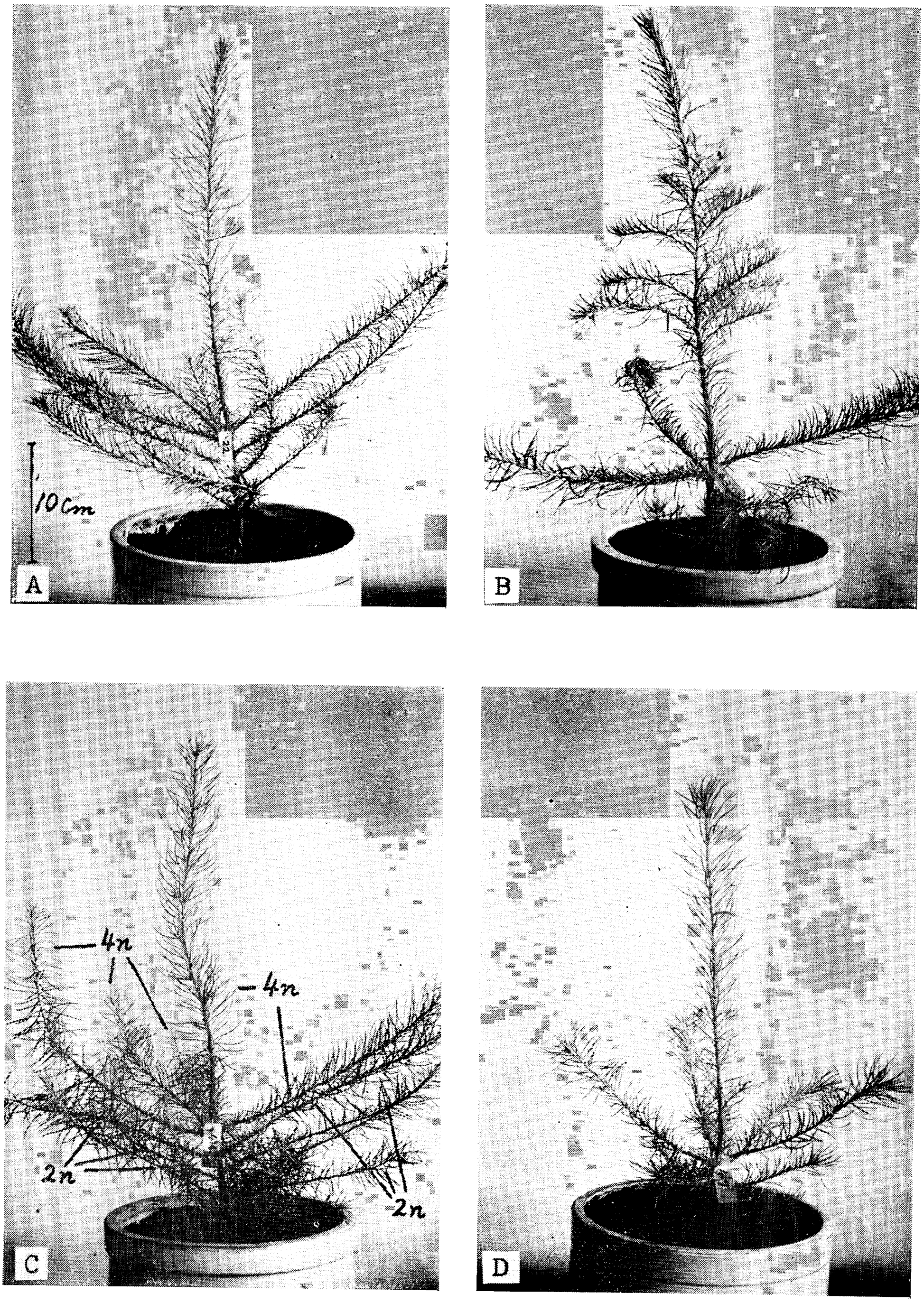

Plate Ⅱ