Triploids and Tetraploids of Sugi (*Cryptomeria japonica* D. DON) selected in the Forest Nursery

By Shigeru CHIBA

With one plate, three tables and two text-figures

Introduction

Since the discovery of the colchicine methods inducing the doubling of chromosomes in plants, many artificial tetraploids have been made in the forest trees. However natural polyploids are very rare in the conifers.

The polyploids recognized in the same genera that have been hitherto reported in the conifers are ***Pseudolarix*** (SAX and SAX 1933), ***Juniperus*** (SAX and SAX 1933, JENSEN and LEVAN 1941) and ***Sequoia*** (HIRAYOSHI and NAKAMURA 1943), and those recognized in the same species are triploid produced in hybrids between ***Larix decidua*** × ***L. occidentalis*** (SYRACH and WESTERGAARD 1938) and tetraploid in twin seedlings of ***Abies firma*** (KANEZAWA 1949).

In the summer of 1950 the writer and ZINNAI, I. discovered tetraploids of ***Cryptomeria japonica*** (2, 3-year-old seedlings) in the nurseries (ZINNAI and CHIBA 1951). In the same year the writer selected 18 tetraploids in the germination beds. The result was reported recently (CHIBA 1951). In this case some individuals were assumed to be triploids, and after the investigation they have been decided as triploids. This paper presents the results of comparative observations of diploids, triploids and tetraploids.

The writer wishes to express his appreciation to Dr. Masataka OHMASA, Chief of Silviculture Divison, and to Mr. Mitsuo IWAKAWA, Chief of Laboratory of Forest Breeding of Government Forest Experiment Station, under whose directions this study was made, for helpful advice; to Mr. Ryookichi TODA and Dr. Kazuo ITÔ for their kind help in preparing this paper; to Mr. Kôzô SHIBUKAWA for the photographic work.

Thanks are due to Mr. Gorô OKAZIMA, Chief of Reforestaion Section of Tokyo Regional Forestry Office, and others who helped in gathering materials.

Materials and Methods

The selection of polyploids was carried out in the summer of 1950 to the spring of 1951 at Kinuta Nursery of the Tokyo Regional Office at Seijo in Tokyo, Japan. The area of germination beds used for selection were 250 sq. m.

Seedlings, the needles of which were thick, tended to be twisted and showed gigantic form have been picked up, and the stomata of the needles were observed under the microscope. The chromosome number were determined from mitotic metaphase in the section of root tips, which were fixed with the modification of Nawashin's solution, inbeded in paraffin, sectioned at 10 to 12 μ and stained with Newton's gentian-violet-iodine. Drawings have been prepared by use of a Zeiss microscope equipped with a 90× oil immersion objective, N. A. 1.25, K. 15× and K. 20× ocullars. The stomata preparations were made by stripping off the epidermal tissue and stained in phloroglucine, conc. hydrochloric acid, drawings have been made by use of Abbé camera lucida and a level drawing board. The length of guard cell of the stomata was measured and the number of stomata per sq. 0.1 mm was counted.

In compariosn with the characters of polyploid and diploid, 18 tetraploids, 3 triploids and 18 diploids were used.

Results

1. Occurrence of polyploid in the germination bed

Thirty-nine seedlings which were assumed to be polyploid were selected. In these seedlings 18 tetraploids, 3 triploids and 2 diploids were found by chromosome observations and 10 were assumed to be tetraploids and 6 to be diploids by the stomatal appearances. The occurrence of polyploids in the germination beds were exceedingly low (ca. 0.0005%). They appeared not in group but solitarily.

2. Comparison in tetraploid, triploid and diploid seedlings

(1) Chromosome number　The chromosome number of *Cryptomeria japonica* were reported as follows: n=9~10 (LOWSON 1904), n=12 (SATÔ 1930, DARK 1932), n=11 (MATSUMOTO 1933, SAX and SAX 1933).

By the writer's observations on the mitotic metaphases in section of root tips

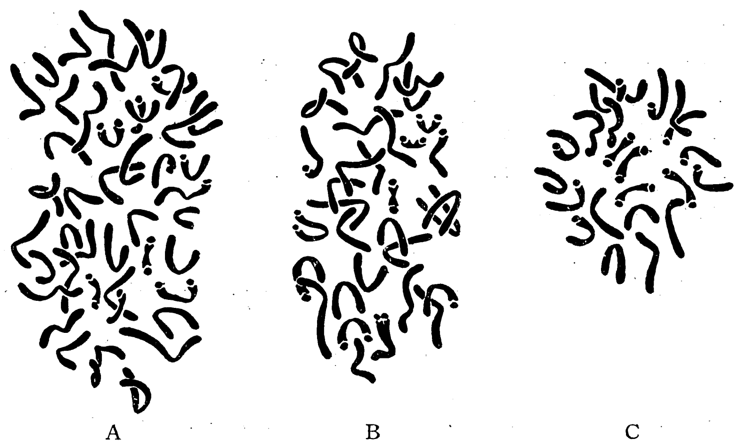


Fig. 1. Somatic metaphase plate of *Cryptomeria japonica*.

A) tetraploid 2n=44　B) triploid 2n=33　C) diploid 2n=22　× ca. 1500 they were counted 2n=22 in ordinary seedlings (diploid), and 2n=44 in tetraploid, and some individuals among gigantic form 2n=33, they are probably tetraploid and triploid. Metaphases plates are shown in Fig. 1.

(2) Morphological appearance Tetraploids showed gigantic form, the needles of them increased in both thickness and width, tended to be twisted and the stomata band showed the same irregular arrangement as the tetraploids induced by colchicine treatment. The triploids had shown somewhat gigantic form in several months after germination but this character gradually disappeared in a year. The needles of them were a little thicker than those of diploids. The appearances of tetraploids were easily distinguished from diploids, but triploids not easily (Plate. I, A, B, C).

(3) Cell size a) Stomata. In measuring the length of guard-cell, 200 cells from each of the seedlings of 18 tetraploids, 3 triploids, and 18 diploids were used. The results obtained are given in Table 1. In the case of tetraploid and diploid, the frequencies of average length of 18 seedlings and that of two seedlings showing Min. and Max. values are given in the table. The tetraploids showed on an average the largest values and triploids medium values. The ratio of the length of guard cell were about 130 : 118 : 100 in the tetraploids, triploids and diploids. Stomata are shown in Fig. 2.

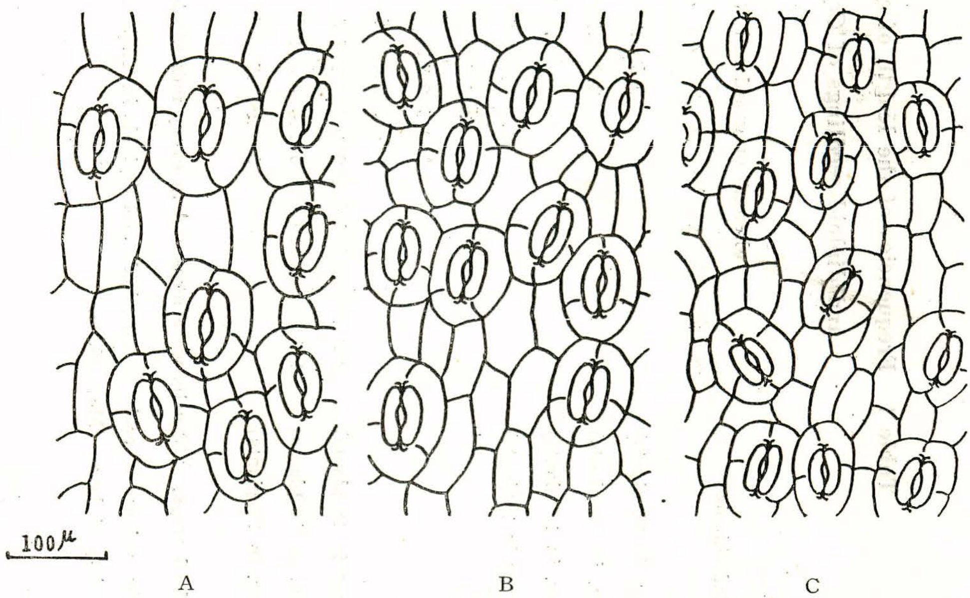


Fig. 2. Stomata from needles of *Cryptomeria japonica*.
A) tetraploid B) triploid C) diploid

On the counting the number of stomata of each seedling 100 unit areas were used; one unit=sq. 0.1 mm. The results obtained are given in Table 2. The ratio of the number of stomata were about 50 : 75 : 100 in tetraploids, triploids and diploids.

Table 1 Frequencies of the length of guard cell in tetraploid, triploid and diploid seedlings of *Cryptomeria japonica*.

Number of individuals	Length of guard cell (μ) (frequencies in percentages)																			N	M±m	Ratio
	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60	62			
Tetraploid (Average in 18 plants)							0.5	3.3	7.8	15.9	22.3	18.7	14.9	8.2	5.1	1.9	1.0	0.4		100	47.37±0.21 a)	134.6
No. 11 (Min. in 18 plants)							3.0	7.5	13.5	27.5	26.0	12.5	6.5	4.0						100	45.02±0.25	127.9
No. 17 (Max. in 18 plants)								0.5	1.0	4.5	11.0	21.5	22.5	16.0	14.5	5.5	3.5			100	50.25±0.25	142.8
Triploid (Average in 3 plants)						2.2	9.6	21.5	25.2	25.3	11.7	4.1	0.2	0.2						100	42.31±0.29	120.2
No. 1						2.0	10.5	20.0	27.5	25.0	10.0	5.0								100	42.36±0.20	120.3
No. 2						0.5	8.5	18.0	20.5	30.0	15.0	6.5	0.5	0.5						100	42.95±0.22	122.0
No. 3						4.0	10.0	26.5	27.5	21.0	10.0	1.0								100	41.71±0.19	118.5
Diploid (Average in 18 plants)	0.2	1.7	10.2	15.0	18.9	23.0	18.7	9.6	1.9	0.6	0.2									100	35.20±0,17	100.0
No. 17 (Min. in 18 plants)	1.5	10.5	28.0	25.0	18.0	12.0	4.0	1.0												100	32.09±0.25	91.2
No. 8 (Max. in 18 plants)				4.0	14.0	24.0	27.5	20.5	8.0	1.5	0.5									100	37.58±0.20	106.8

a) Used standard error. 200 cells were measured in every individuals.

(Note) The values of each 18 tetraploids and diploids in Table 1–3 were reported at the 59th Annual meeting of the Forestry Society of Japan (May, 1951).

Table 2 Frequencies of the number of stomata per sq. 0.1 mm in tetraploid, triploid and diploid seedlings of *Cryptomeria japonica*.

Number of individuals	Number of stomata per sq. 0.1 mm. (frequencies in percentages)																	N	M ± m	Ratio
	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
Tetraploid (Average in 18 plants)	1.5	8.4	26.1	29.6	20.5	8.9	3.3	1.3	0.4									100	7.08±0.14	50.4
No. 14 (Min. in 18 plants)	6	14	40	25	10	3	1	1										100	6.37±0.13	45.3
No. 4 (Max. in 18 plants)		6	10	22	23	24	8	6	1									100	7.84±0.19	55.8
Triploid (Average in 3 plants)			0.3	1.7	6.7	12.3	23.3	22.7	17.3	9.3	4.0	2.3	1.0					100	10.87±0.18	77.3
No. 1				3	6	11	16	21	20	11	6	4	2					100	11.18±0.20	79.5
No. 2			1	1	8	15	30	23	11	7	3	1						100	10.45±0.16	74.3
No. 3				1	6	11	21	24	21	10	3	2	1					100	10.98±0.17	78.1
Diploid (Average in 18 plants)					0.2	0.4	1.9	4.8	11.1	19.2	22.4	20.0	12.4	5.0	2.0	0.5	0.1	100	14.06±0.18	100.0
No. 2 (Min. in 18 plants)					3	5	8	15	16	20	15	8	7	1	2			100	12.61±0.22	89.7
No. 7 (Max. in 18 pants)									4	10	13	32	25	11	4	1		100	15.18±0.15	108.0

100 nuits (10 sq. mm) were counted in every individuals.

b) Cell size at metaphases　In comparing the size of cells at metaphases, 15 cells on the sections of 100～200 μ from the root tip of each seedling were measured, and the results obtained are given in Table 3. Both length (L) and width (W) of the tetraploids and triploids were larger than those of diploids. The ratio of the value of L×W were about 193 : 149 : 100 in the tetraploids, triploids and diploids.

Table 3　Cell size at metaphases of root tips in tetraploid, triploid and diploid seedlings of *Cryptomeria japonica*.

Number of individuals	Means of Length (L)	Means of Width (W)	L × W	Ratio
	(μ)	(μ)		
Tetraploid (Average in 18 plants)	27.35	16.34	419.59	192.5
No. 3 (Min. in 18 plants)	25.85	15.58	368.36	169.0
No. 14 (Max. in 18 plants)	31.90	18.52	599.57	275.0
Triploid (Average in 3 plants)	22.12	14.67	323.34	148.3
No. 1	22.18	15.03	333.25	154.2
No. 2	22.55	14.12	318.13	145.9
No. 3	21.63	14.85	318.63	146.1
Diploid (Average in 18 plants)	18.35	12.57	218.01	100.0
No. 10 (Min. in 18 plants)	15.95	11.43	190.58	87.4
No. 5 (Max. in 18 plants)	21.45	12.57	256.12	117.5

15 cells at metaphases were measured in every individuals.

Discussion and Conclusion

Morphological appearances of tetraploids occurred in nature were the same as those induced by colchicine treatment. The tendency of the needles to be twisted and the irregular arrangement of stomata band will disappear in several years as in the case of colchicine treatment (ZINNAI and CHIBA 1951). The seedlings of triploid showed gigantic form at the early stage as tetraploids but became gradualy normal in a year.

Cell size (stomata and at metapases of root tip) became larger by the result of the doubling of chromosome number. On the polyploids of poplar, BERGSTROEM (1940) and JOHNSON (1940) reported that the stomata length of polyploids was greater than that of diploids, but the opposite was true of diploids and tetraploids, and they concluded that the stomata length was not always proportional to the chromsome number. In this study, as shown in Table 1, Max. value in triploids did not over the Min. value of tetraploids and the same results were shown between diploids and triploids. However, the means of them approached each other and it was impossible to distinguish triploid from others by the stomata. In addition, the forest trees show large individual variation (JOHNSON 1940) and anticipated that the size of cell will vary with the change of appearance according to the acclimation as years go by (KANEZAWA 1940), moreover, especially treated by colchicine, it is confined partially the doubling of chromosome number (SATINA, BLACKESLEE and AVERY 1940, KANEZAWA 1942). In these reasons it can not be decided only by the stomata whether the tree is totally tetraploid, triploid or not. However each materials used in this experiment were seedlings of the same condition, it is clear that the size of stomata is important index to the preliminary selection of polyploids, but not their absolute index.

Origin of polyploidy reported here is unknown. Irregularities in nuclear and cell division can be induced by many different agencies. Abnormal division may be occurred by temperature changes, regeneration of tissue, diseases, insects, osmotic changes, mechanical injuries, and species hybridization etc. in natural conditions. Having only one species in the genera ***Cryptomeria***, species hybridization cannot be occurred, and these polyploids seem to be autopolyploid. Of the external factors, temperature is expected to be most effective in nature.

From the observations in many metaphases in these polyploidy seedlings chimeras were not found, so the doubling of chromosome number seems to be occurred in zygote after fertilization or at the early stage of embryo development by some stimulation. Gametes, which gave somatic numbers of chromosome formed by non-reduction, may unite and produce polyploidy zygotes. In this case triploid might be produced by such gametes, however the probability of occurrence of the tetraploidy is little. Triploid might be produced by hybridization between diploid and tetraploid ***Cryptomeria*** trees.

The silvicultural or physiological characteristics of polyploids are not yet known and they will be discussed in furthe paper.

In the selection of polyploids in the nurseries we must care about following facts: almost all of the polyploids were picked up and thrown away by nursery men at the weeding, thinning and transplanting by their unfamiliar appearances, and then the negative selection of the polyploid were carried out artificially. This report is an example of selection of the polyploids in the nursery. Many polyploids which have been selected in the nurseries from different localities and origins may contribute, with induced polyploids, to the breeding of Sugi.

Summary

In the summer of 1950 to the spring of 1951 the writer carried out the selection and gained polyploid seedlings of ***Cryptomeria japonica*** in the germination beds of the forest nursery. In these selected seedlings, 18 seedlings were tetraploids (2n=44) and 3 were triploids (2n=33).

In the preliminary selection seedlings of gigantic form were picked up and the stomata size and the numbers per unit area were observed, and then chromosome number were determined by the mitotic metaphases of root tips.

In comparison with the tetraploids, triploids and diploids the following

characteristics are noted. (1) The tetraploids showed the gigantic form, triploids did not show gigantic form but had a little thicker needles than those of diploids. (2) In the length of guard cell, the tetraploids were about 30 percent larger than diploids, the triploids were medium. As for the number of stomata per unit area, the tetraploids were about 50 percent smaller than diploids, and triploids were medium. The length and width of the cells at metapases of root tips increased by the doubling of chromosome number.

Though the origin of polyploid is unknown, tetraploid seems to be doubled at the early stage of zygote after fertilization or embryo development. Triploid may be probably produced by the fertilization of an unreduced 2nd gamete or by the hybridization of diploid and tetraploid trees of *Cryptomeria japonica*. These polyploids seem to be autopolyploid, for the genus *Cryptomeria* contains only one species.

LABORATORY OF FOREST BREEDING,
GOVERNMENT FOREST EXPERIMENT STATION,
MEGURO, TOKYO, JAPAN.

Literature cited.

BERGSTROEM, I. (1940) On the progeny of diploid×triploid *Populus tremula* with special reference to the occurrence of tetraploidy. Hereditas 26, 191～201.

CHIBA, S. (1951) Selection of natural tetraploids of Sugi (*Cryptomeria japonica* D. DON) in the forest nursery. (Japanese with English résumé, reported at the 59th Annual meeting of the Jap. Forestry Soc. 1951). Jour. Jap. Forestry Soc. (in press)

DARK, S. O. S. (1932) Chromosomes of *Taxus*, *Sequoia*, *Cryptomeria* and *Thuja*. Ann. Bot. 46, 965.

HIRAYOSHI, K. and NAKAMURA, Y. (1943) Chromosome number of *Sequoia sempervirens*. (Japanese with English résumé) Bot. Zool, 1, 73～75.

JOHNSON, H. (1940) Cytological studies of diploid and triploid *Poplus tremula* and of crosses between them. Hereditas. 26, 321～352.

KANEZAWA, R. and GO, M. (1948) On the morphological changes in some polyploid forest trees which were induced by colchicine treaments. (Japanese with English résumé) Jap. Jour. Genet. 23, 77～81.

—— (1949) Polyploids appeared in twin seedlings of forest trees. (Japanese with English résumé.) Jour. Jap. Forestry Soc. 31, 22～24.

LOWSON, A. A. (1904) The gametophyes, fertilization, and embryo of *Cryptomeria japonica*. Ann. Bot. 18, 417～444.

MATSUMOTO, K. (1933) Ueber die Chromosomenzahl von *Cryptomeria japonica* und *Taiwania cryptomerioides* HAYATA. (Japanese with German résumé) Bot. Zool. 1, 1751～1756.

SATÔ, K. (1930) Ueber die Zahl der Chromosomen von *Cryptomeria japouica* D. DON. (Japanese with German résumé) Jour. Jap. Forestry Soc. 12, 396～399.

SATINA, S. BLACKESLEE, ee, A. F. and AMOS, G. A. (1949) Demonstration of the germ layers in the shoot apex of *Datura* by means of induced polyploidy in percrinal chimeras. Amer. Jour. Bot. 27, 895～905.

SAX, K. and H. J. SAX. (1933) Chromosome number and morphology in the coniffers. Jour. Arn. Arb. 14, 356～357.

SYRACH, L. C. and M. WESTERGAARD. (1938) Contributions to the cytogenetics of forest trees. Genetics. 36, 523～530.

ZINNAI, I. and CHIBA, S. (1951) Tetraploids of Sugi (*Cryptomeria japonica* D. DON) discovered at forest nurseries. (Japanes with English résumé) Jap. Jour. Breeding. 1, (in press)

苗畑にて選抜されたスギの3倍体及び4倍体

（摘　要）

農林技官　千　葉　　茂

昭和25年（1950）夏より26年春にわたり，東京営林局砧苗畑（東京都世田谷区成城町）の播種床にてスギの倍数体の選抜を行つた。

選抜は初め外部形態が Gigas type を示す個体を選び，それらの気孔を観察し，更に根端細胞の細胞学的観察により倍加を認定した。

選抜した個体は39本であり，其の中4倍体は18個体，3倍体は3個体であつた。他に気孔の観察のみで4倍体と推察されるもの 10 個体を得た。針葉樹に於て同一種内の3倍体は，人為倍数体をも含めて現在の所，Larix の種間交配により得られたという報告があるのみである (Syrach and Westergaard, 1938)。苗畑にて倍数体の得られた率は極めて小さく約 20～30 万本に1本の割合であつた。

染色体数は4倍体 2n＝44，3倍体 2n＝33，2倍体（対照植物）2n＝22 を観察した。

外部形態は，4倍体は Gigas type を示し濃緑色で茎葉共に肥厚する。針葉が初めの中は捩れ，気孔帯の排列が乱れる傾向がある。3倍体は初期は Gigas type を示すが漸時薄らぎ2倍体と4倍体の中間的形態を示し，針葉は2倍体に比して肥厚している。

細胞の大いさは，染色体数の倍加に従い増大することが認められた。4倍体は2倍体に比し

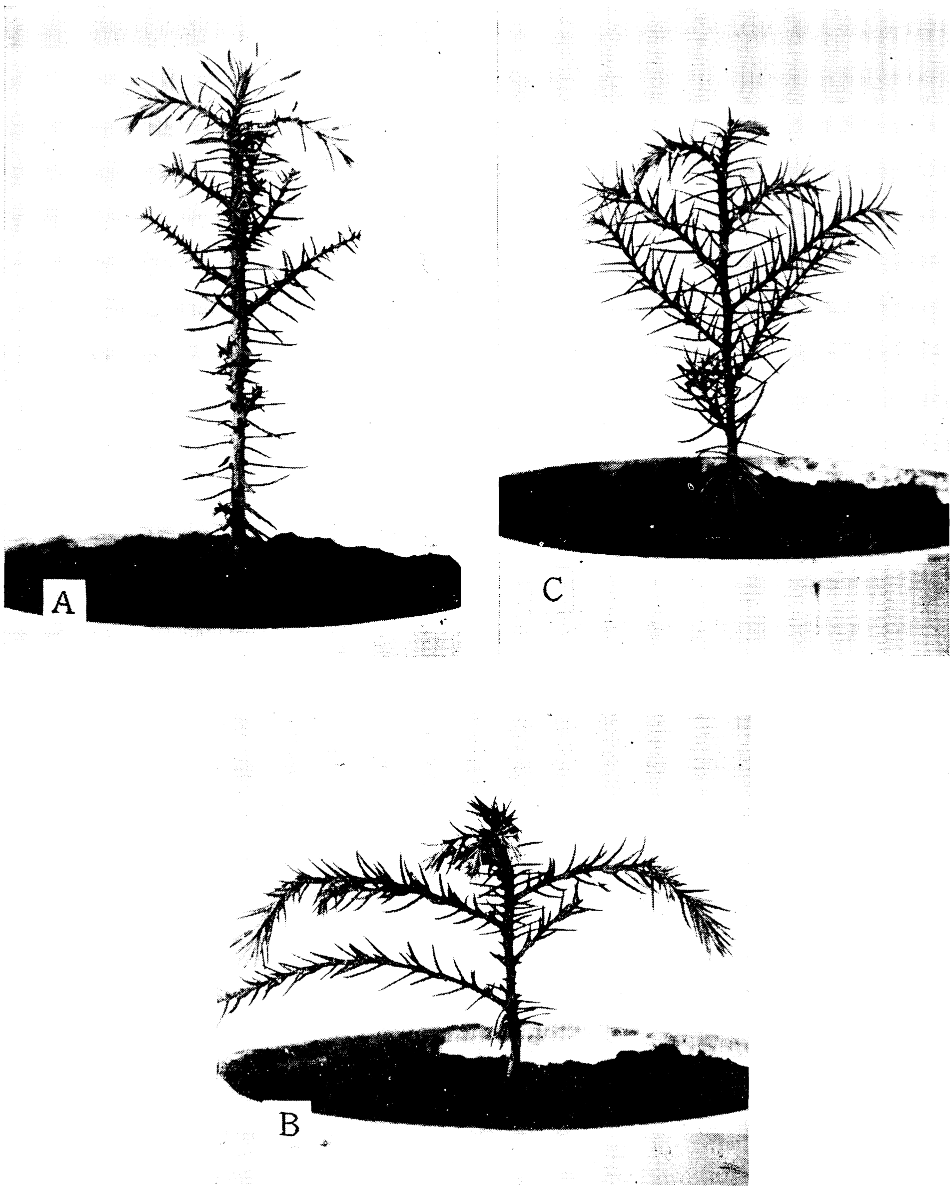
気孔閉塞細胞に於て約 30～40% 増大し，単位面積当りの気孔数は 40～50% の減少を示している。3倍体は何れもその中間に位する。根端細胞の分裂中期の細胞の大いさも同様のことが認められた。気孔の大いさによる染色体数倍加の認定に就ては論議があるが，筆者の選抜の結果では，4倍体は気孔による推定と染色体数観察の結果とは良く一致したが3倍体は気孔のみでは4倍体2倍体何れとも判別し得なかつた。倍加の認定には細胞学的観察を必要とする。

選抜された倍数体の成因は不明であるが，混数体が見られなかつた点より，4倍体は授精後胚発生の時期に，或いは針葉の捩れる点を考慮すれば胚完成後発育の段階に何等かの刺戟により倍加したものと推察される。3倍体は，非還元配偶子の授精により，或いは4倍体と2倍体との交雑により生じたものと思われる。

現状では倍数体の示す特殊な外部形態のため苗畑に於てその大部分は間引，床替の際に棄却され人為的に負の淘汰を受けていることは選抜に当り注意しなければならない。

今後諸地方にて選抜を行い性質の異る系統を蒐集し更に選抜を加えることにより，人為倍数体と共に自然倍数体も育種の有力な材料となり得るものと思われる。尚選抜されたる3倍体及び4倍体の生長其他の育林上の性質に就ては今後の研究に待たねばならない。

苗畑にて選抜されたスギの3倍体及び4倍体 （千葉）



Polyploid seedlings of *Cryptomeria japonica* selected in the nursery.

A) tatraploid 2n=44

B) triploid 2n=33

C) diploid 2n=22 (ordinary plants)

Note: 1-year-old seedlings, in the same scale (ca. 1/3). In the plate, the hight of triploid is lower than others, for the apical bud was injured by insects.