Paternal Transmission of a Plastid Anomaly in some Reciprocal Crosses of Sugi, Cryptomeria japonica D. Don

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Introduction

Sugi, Cryptomeria *japonica* D. DON is one of the most important tree species in Japan and distributed naturally all over this country except Hokkaido and some of other small islands. It is planted in a large area totaling about 40 per cent of the area of annual artificial plantation. Wood of Sugi is mostly used for house building and furniture. Sugi belongs to genus Cryptomeria and is the only one species in it. It is monoecious and usually propagated by seeds, and in certain districts, by cuttings. There are a number of local varieties propagated by seeds and cuttings including horticultural varieties with the traits of such as dwarf, variegation, morphological variations in needles and shoots.

At the Institute of Radiation Breeding, Ministry of Agriculture and Forestry, plants of Sugi varieties propagated vegetatively have been irradiated with gamma rays chronically or acutely to induce somatic mutations. Kuma-Sugi, one of the local varieties propagated by cuttings, produced various kinds of somatic mutations in higher frequency and most of them were chlorophyllous changes. We found some somatic mutations which were large enough to be propagated by cuttings, showing the trait resembling to that of Wogon-Sugi.

Wogon-Sugi, Cryptomeria *japonica* D. DON form Wogon hort. ex IWATA and KUSAKA is a horticultural variety having a specific trait of color change in its new growth. The new shoots are white to yellowish white in spring and change to normal green in late summer. They repeat this reaction every year as shown in Fig. 1.

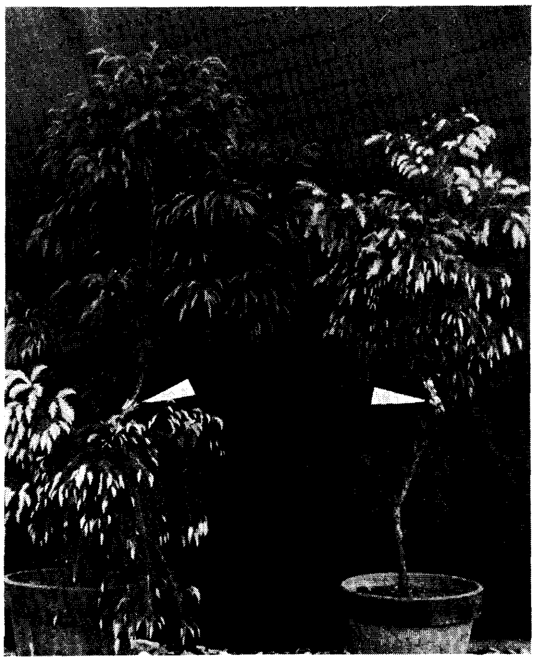

Fig. 1. — Grafted plant of *Wogon*-Sugi (right) and Kuma-Sugi (left) are shown. They are grafted exchanging the stock and the scion reciprocally in 1966 and they are keeping their own traits even at 4th year after the grafting. White arrows indicate the grafted points.

At the beginning, this experiment was planned to verify the genetically identity of these two phenotypically similar variants, namely, somatic mutants of Kuma-Sugi and Wogon-Sugi. After several breeding experiments, these two variants were found to have different genetical backgrounds. The variants from Kuma-Sugi seem to be induced by a recessive mutation with gamma ray irradiation, because the mother strain is heterozygous for the gene as reported by OHBA et al. (1967b), and for Wogon-Sugi the trait was mostly transmitted to the progeny when Wogon-Sugi was used as pollen parent. The second and the third breeding experiments were conducted to verify this highly paternal inheritance extensively in cooperation with the research workers of the Government Forest Experiment Station.

This report covers findings of these three breeding experiments in 1965, 1967 and 1968, and a grafting experiment in 1966 and chloroplast observation by electron microscope.

Materials and Methods

Plant materials used for the breeding experiments are summarized in Table 1. They are classified into Wogon-Sugi (Y-1, Y-2), or normal Sugi according to their phenotypes and are given their popular names or abbreviated symbols such as Y-1, Y-2, C-1 and etc. In crosses in 1965, the plants of Kuma-Sugi, about one meter high were planted in pots with 30 cm diameter, and Y-1, Y-2, Y-3 and GR were grown in a control field at the institute.

In the summer of 1964, these saplings were sprayed with 200 ppm gibberellin solution with 1 per cent urea to induce flower bud differentiation. Gibberellin is highly effective to induce flower bud differentiation in both sexes even in seedlings 2 to 3 years old. Pollen was collected from cut branches being watered in bottles, which had plenty of male strobili. In February 1965, the branches with female strobili were isolated with double-paper bags for controlled pollination. Self pollination was made with pollen from male cones enclosed in the bags and with artificial pollination with pollen gun. Pollination of Wogon pollen (Y-1 and Y-2) to the female strobili of Y-1, Y-2, Y-3 and GR began on March 5th, and was repeated six times every four days. Pollen of GR was pollinated four times. To the female strobili of Kuma, pollen of Wogon (Y-1 and Y-2) and G-5 were pollinated three to seven times.

In crosses in 1967, two *Wogon*-Sugi (Y-1 and Y-2) and young plants of 27 normal Sugi clones including 19 local clones propagated by cuttingc and nine individuals as listed in Table 1 were used. The nine trees are those preserved making clonal strains at the Institute of Radiation Breeding for the sake of their marker genes and other special traits. The young plants of the 19 clones which were used for the crosses were raised from the scions taken from a scion bank at the institute in 1964 which was established with plants introduced from the Forest Experiment Stations and Forestry Associations in respective districts. All of the 3-year old plants in which plants of Sibahara,

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Table 1. — Plant materials used for breeding experiments.

Crossing year	*Wogon*-Sugi: Variety or individual		*Wogon*-Sugi: Number of plants	Normal Sugi: Variety or individual		Normal Sugi: Number of plants
1965	Y-1	(♂, ♀)	1	GRa)	(♂, ♀)	1
	Y-2	(♂, ♀)	1	Kuma	(♀)	15
	Y-3	(♂, ♀)	1	G-5	(♂)	1
1967				Boka	(♂, ♀)	ca. 5
				Iwao	(♂, ♀)	ca. 5
				Kirisima-measa	(♂, ♀)	ca. 5
				Kuma	(♂, ♀)	ca. 5
				Kumotossi	(♂, ♀)	ca. 5
				Motoe	(♂, ♀)	ca. 5
				Obi-aka	(♂, ♀)	ca. 5
				Okinoyama	(♂, ♀)	ca. 5
				Ryuunohige	(♂, ♀)	ca. 5
				Sanbu	(♂, ♀)	ca. 5
	Y-1	(♂, ♀)	1	Tano-aka	(♂, ♀)	ca. 5
	Y-2	(♂, ♀)	1	Urasebaru	(♂, ♀)	ca. 5
				C-1	(♂, ♀)	2
				G-5	(♂, ♀)	5
				GR	(♂, ♀)	1
				Mor	(♂, ♀)	1
				Sibahara	(♂, ♀)	ca. 5
				Ao	(♀)	ca. 5
				Aya	(♂, ♀)	ca. 5
				Garin	(♂, ♀)	ca. 5
				Hiki	(♂, ♀)	ca. 5
				Masuyama	(♂, ♀)	ca. 5
				Tosa-aka	(♂, ♀)	ca. 5
				Yabukuguri	(♂, ♀)	ca. 5
				Bandai	(♂, ♀)	1
				Kuma (Individual)	(♂, ♀)	ca. 5
				Matusita No. 1	(♂, ♀)	ca. 5
				Midori No. 5a)	(♂)	2
1968	(GR.Y)	(♂, ♀)b)	14	(Y.GR).b)	(♂, ♀)	11
	Y-1	(♂, ♀)	1	Midori No. 1.a)	(♂, ♀)	1
	Y-2	(♂, ♀)	1	K 1	(♀)[1)]	1
				K 2	(♂, ♀)[1)]	1

Remarks: (♂, ♀); As male and female including selfing.
(♂), (♀); As male or female only.
(GR.Y), (Y.GR); F_1 hybrids of reciprocal crosses between *Wogon*-Sugi (Y) and GR in 1965.
a) Homozygous for a recessive marker gene, and the plants with this genotype keep fresh green in winter, while other plants change to reddish brown.
b) Heterozygous for the gene mentioned above.

Matusita No. 1 and G-5 were included, were planted in pots with 30 cm diameter, and two plants were planted in one pot as a rule. The 4- to 7-year-old plants of Y-1, Y-2, Bandai, Gr, Midori No. 5 and C-1 were grown in the field. In July 1966, they were sprayed three times with 100 ppm gibberellin solution to induce flower bud differentiation. Female strobili were bagged on February 25. Pollen was collected from cut-branches with the same methods as that previously stated or by extracting pollen from the pollination bags in which male strobili were also enclosed for self pollination. Five to seven times of pollination with the pollen gun were made every three to four days in March. For *Wogon*-Sugi (female), pollen of 18 normal Sugi were pollinated and, reciprocally, 27 normal Sugi were used as female.

In crosses in 1968, reciprocal F_1 hybrids between *Wogon*-Sugi and GR were used together with plants of Y-1, Y-2, Midori No. 1, K-1 and K-4. As mentioned in Tables 1 and 3, the plants, GR, Midori No. 1, K-1 and K-4, are homozygous for a recessive marker gene, and are fresh green in winter while other plants change color to reddish brown. The F_1 hybrids were grown in pots with 30 cm diameter. And gibberellin treatment was made in July to induce flowers. In the reciprocal F_1 hybrids, self pollination was made by enclosing male and female strobili in the same paper bags. These bags were shaken once a day or once for every two day to have good pollination. Artificial pollination for Midori No. 1, K 1 and K 4 were made three to six times during March 2nd to March 23rd and, sometimes, the bags were shaken to aid pollination.

Mature and immature cones were collected in the autumn and investigations on the rate of mature cone, seed production per cone (mg), 1000-seed-weight were made. In the seeds of Sugi, it is very difficult to discriminate full seeds from empty or resinous seeds. In this experiment, the seeds were selected by eye-discrimination, namely, small seeds or flat seeds are discarded taking seeds with large and round shape only.

Germination tests were made in the field and in the greenhouse. Seeds were sown in wooden or plastic boxes filled with soil. As a rule, 200 seeds per seedlot were sown in a row 30 to 50 cm long. Germination was scored every two to three days for about one and a half months after sowing, and the final scoring of the variant seedlings with traits such as white primary leaves, chimera or normal green primary leaves was made two to three months after sowing. Segregation of a recessive gene responsible for winter discoloration was investigated after cold treatment.

Boxes of seedlings were brought into an unheated glass-house from the heated greenhouse in winter. The seedlings exposed to a minimum temperature of —5^0 C, showed a color change within two to three weeks; seedlings having the dominant gene had cotyledons and primary leaves of reddish brown to purple.

Results

In the crosses in 1965, percentage of mature cone was comparatively low owing to the damage by heat (sunburn). Full seed weight per cone varied between 49 and 202 mg among cross combinations of Y-1, Y-2, Y-3 and GR, and 1000-seed-weight also varied between 2.915 and 4.954 g. Germination was rather good except for self-pollinated seeds.

In the second breeding experiment, the rate of mature cones after outcross was 76 to 79 per cent, and, after self pollination of Y-1 and Y-2, their rates of mature cones were reduced slightly. Full seed per cone varied between 36 and 199 mg, and the average values were 93.5 and 81.9 mg for Y-1 and Y-2 respectively. In the same female parents, 1000-seed-weight ranged between 1.710 and 4.080 g by different pollen parents and their mean values were 2.611 and 2.553 g. Germination percentage was very high having mean values of 54.27 and 58.09 per cent respectively. Differences in germination percentage due to pollen parent and interaction between pollen parents and mother trees (Y-1 and Y-2) were statistically significant with the confidence level of 99 per cent.

In the reverse cross combination, 19 local clones and eight individuals as female parents, and *Wogon*-Sugi (mostly, Y-1) as male parent, the same sort of variation was noted for the full seed weight per cone and 1000-seed-weight. Their germination percentages varied widely and the average value was 18.91 per cent.

In the crosses in 1968, after self pollination of (GR.Y) and (Y.GR) plants, 140 and 288 mature cones were produced. Full seed per cone weighed 94.8 and 141.3 mg, respectively. In the pollination of (GR.Y) and (Y.GR) pollen to Midori No. 1, K-1 and K-4, full seed weight per cone varied 63.8 to 261.8 mg in each female tree. By the self pollination of (GR.Y) and (Y.GR) plants, average 1000-seed-weights were 2.209 and 2.686 g respectively. After use of pollen from *Wogon*-Sugi (Y-1 and Y-2), (GR.Y) and (Y.GR) on Midori No. 1, K-1 and K-4 as female, each female had varied 1000-seed-weight by different pollen parents as follows: in Midori No. 1, 1.992 to 3.416, K 1, 3.000 to 3.607 and K 4, 4.155 to 5.985. g.

Germination percentage of these seeds varied widely by cross combination, and in the selfed seeds and the seeds produced after pollination of (GR.Y) pollen, germination percentage was lower than 10 per cent as shown in *Table 4*.

After germination, almost all seedlings produced normal green cotyledons, but the color of primary leaves differed in reciprocal crosses. In seedlings derived from the crosses of *Wogon*-Sugi(female) × normal Sugi(male), the primary leaves were always normal green with the exception of a few seedlings. On the other hand, seedlings derived from crosses of normal Sugi(female) × *Wogon*-Sugi(male) produced white to yellowish white primary leaves (sometimes, light green), and in low frequency, primary leaves of chimera and normal green as shown in *Fig. 2*. Chlorophyll deficiency in the seedlings showing abnormal color recover to apparently normal green in late summer just like *Wogon*-Sugi. Some of these seedlings showed the chlorophyll deficiency in late fall too. Seedlings having primary leaves with white to light green color are called *Wogon* type, and seedlings with normal green primary leaves are called normal type hereafter.

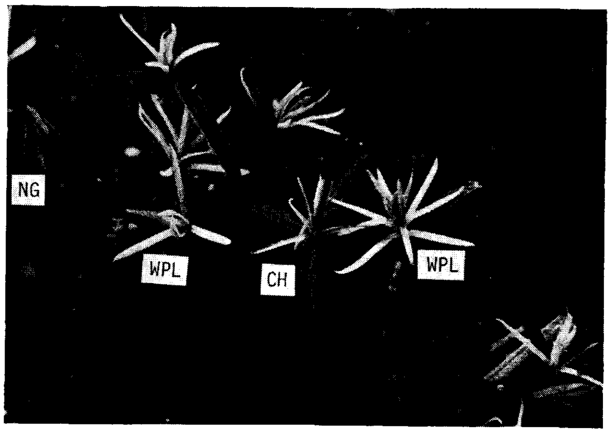

Fig. 2. — Phenotypes of the seedlings derived from a cross of C—1 × *Wogon*-Sugi (male). Most of the seedlings have white primary leaves (WPL), and some seedlings were with normal green primary leaves (NG) or with white — green chimeric primary leaves (CH).

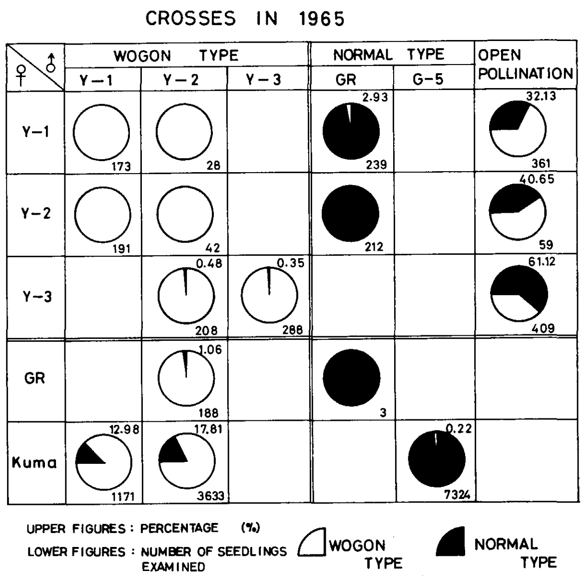

Fig. 3. — Scored result of the seedlings with white primary leaves (*Wogon*-type) and normal green primary leaves (normal type) from various cross combinations (cf. *Table 1*). It shows that the *Wogon* type and the normal type are mostly transmitted to the progeny by the pollen and not through the female gamete.

Results from the crosses in 1965 are presented in *Fig. 3*. In the crosses between *Wogon*-Sugi (Y-1, Y-2 and Y-3) and their selfing resulted nearly 100 per cent of *Wogon* type seedling. The seedling derived from Kuma-Sugi × G-5, both being normal Sugi, were normal type seedlings. Seedlings derived from Y-1 × GR and Y-2 × GR were normal type and those of GR × Y-2 showed *Wogon* type. Seedlings derived from Kuma-Sugi(female) × *Wogon*-Sugi (Y-1 and Y-2, as male) showed a little different coloration in primary leaves compared with those of the hybrids of GR × Y-2. Namely, the seedlings from this combination had primary leaves with color ranging white to yellowish white and further to light green, and normal green seedlings were observed in rather high frequency in comparison with the seedlings of GR × Y-2 as shown in *Fig. 3*. Those seedlings from Kuma-Sugi × *Wogon*-Sugi showing various coloration were separated to make further observation on development of their traits as classified in *Table 2*.

Table 2. — Results of the re-examination of seedlings showing various traits such as *Wogon* type, chimera and normal type derived from the crosses of Kuma-Sugi(female) × *Wogon*-Sugi(male) at different dates. In May, percentage of normal type seedlings was about 36 per cent in a germination test. One hundred and ninety three seedlings showing various traits were separated to follow their phenotypes. Meanwhile, by the scoring in November, most of the normal green seedlings in May showed white to yellowish green leaves and chimeric leaves indicating the effect of the pollen of *Wogon*-Sugi.

Type of varied primary leaves (May 15, 1966)		Number of seedlings examined	November 11, 1966				Percentage of variants
			Wogon type		Normal type		
			White — yellowish white	Yellowish green	Chimera	Normal green	
							(%)
Wogon type	White — Yellowish white	30	23	7	0	0	100.0
	Yellowish green	81	28	53	0	0	100.0
	Chimera	12	3	1	8	0	100.0
Normal type	Normal green	70	9	56	2	3	95.7
Total		193	63	117	10	3	98.4

Table 3. — Segregation of varied seedlings such as *Wogon*-type, chimera, and normal type after reciprocal crosses between *Wogon*-Sugi and normal Sugi in 1967 (cf. *Table 1*).

Female	Male	Number of seedling examined	Number of seedling			Percentage		
			Wogon type	Chimera	Normal	*Wogon* type	Chimera	Normal
Wogon (2)	Normal (18)	8,689	22	49	8,618	0.3	0.6	99.1
Normal (27)	*Wogon* (2)	3,781	3,386	284	111	89.6	7.5	2.9
						97.1 (Wogon type + Chimera)		

Figures in parentheses: Number of clones used.

The seedlings with *Wogon* type retained their traits, and apparently normal green seedlings which were classified in May 15, 1966 were found to be affected by the pollen of *Wogon*-Sugi, and most of them showed light green leaves in the late fall in 1966. All of the seedlings having *Wogon* type which were selected for long term observation and for breeding experiments have been showing the same coloration in their sprouts as *Wogon*-Sugi in every spring up to 1969.

The 1967 crossing results are presented in *Table 3*. *Wogon*-Sugi (Y-1 and Y-2) females crossed with 18 different male clones produced 8689 seedlings of which 99.2 percent were normal, 0.25 percent were *Wogon*-type and 0.57 percent were chimeric. Reciprocal crosses were made between 27 normal clones as females and *Wogon*-sugi (mostly Y-1) males. Of 3781 seedlings examined, 89.6 percent were *Wogon* type, 7.51 percent were chimeric and 2.9 percent were normal type. More than 10 percent chimeric seedlings were obtained in crosses involving Aya, Garin, Masuyama, Motoe, Obi-aka, Ryuunohige, Sanbu, C-1 and Sibahara clones as females (*Fig. 4*). Both in 1965 and 1967 Kuma-Sugi female × *Wogon*-Sugi male produced a high percentage of normal seedlings. A high percentage of normal seedlings was also obtained in 1967 when the Garin, Masuyama, Motoe, Obi-aka, C-1 and G-5 clones were used as female parents in crosses with male *Wogon*-Sugi. This may be due to earlier recovery of normal green color in the chlorophyll-deficient seedlings. There was no correlation between the results of reciprocal crosses.

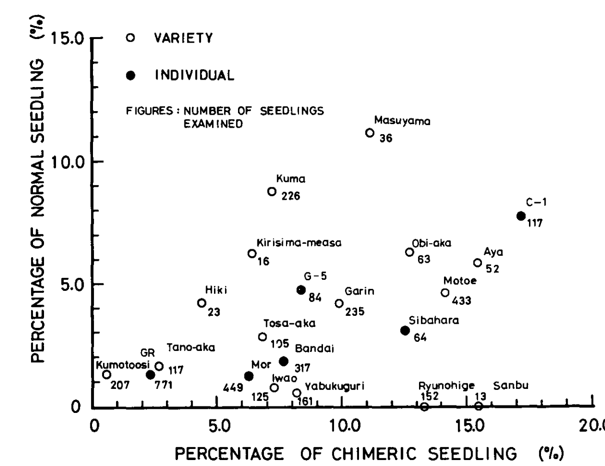

Fig. 4. — Relation between the percentages of seedlings with *Wogon* type and of chimera from crosses of normal Sugi (female) × *Wogon*-Sugi(male) in 1968 (cf. *Table 1*). No linear correlation can be observed between both percentages.

Table 4. — Segregation of varied seedlings such as *Wogon* type, chimera and normal type after crosses between *Wogon*-Sugi, reciprocal hybrids of *Wogon*-Sugi and normal Sugi in 1968

Male / Female	*Wogon*-Sugi								Normal Sugi			
	Y-1, Y-2				(GR. Y)				(Y. GR)			
	Wogon	Chimera	Normal	Total	*Wogon*	Chimera	Normal	Total	*Wogon*	Chimera	Normal	Total
(GR. Y)					5139	341	6.64					
					272	6	4	282				
					(96.5	2.1	1.4	100)				
(Y. GR)									7226	667	9.23	
									1	0	653	654
									(0.2	0	99.8	100)
Midori No. 1	2400	852	35.50		3251	319	9.80		2386	822	34.15	
K 1[1])	749	44	19	812	273	20	13	306	0	0	819	819
K 4[1])	(92.3	5.4	2.3	100)	(89.2	6.5	4.3	100)	(0	0	100	100)

Remarks: Figures in parentheses: Percentages of the seedlings classified. Figures with underline: Number of seed sown, germination and germination percentage.

In *table 4*, it is shown that *Wogon*-type seedlings were produced when *Wogon*-Sugi, such as Y-1 and (GR.Y) were used as pollen parents. Segregation of a recessive marker gene was investigated in selfed progenies of (GR.Y) and (Y.GR), and in back-crossed progenies with Midori No. 1, K-1 and K-4 as female. For winter discoloration, segregation ratios in selfed and back-crossed progenies agree with the theoretical ratios 3(red) : 1(green) and 1(red) : 1(green), respectively.

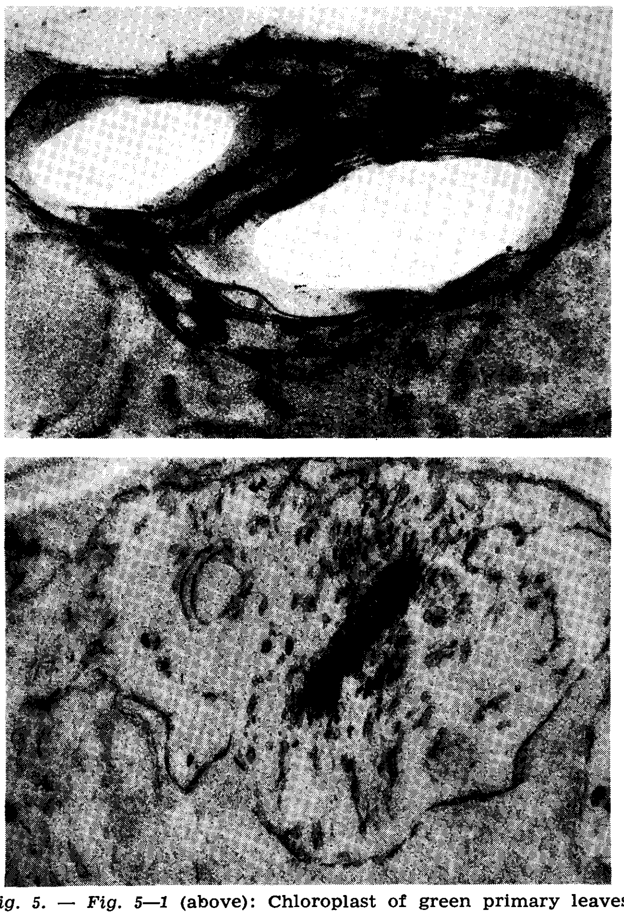

Fig. 5. — *Fig. 5—1* (above): Chloroplast of green primary leaves of a seedling after a cross of *Wogon*-Sugi (female) × GR (male). It has developed lamellar system with starch grains. (3332 ×). — *Fig. 5—2* (below): Chloroplast of white primary leaves of a seedling after a cross of GR (female) × *Wogon*-Sugi (male). Underdeveloped lamellar system is seen. A cluster in the center of the picture seems to be a clump of the grana. (3332 ×).

After a grafting experiment exchanging stock and scion reciprocally, as shown in *Fig. 1*, clones preserved their own phenotypes for four years, and non-transmissibility of their traits by this mode of grafting is obvious.

Electron-microscope observation of the chloroplasts in the primary leaves of *Wogon* type and normal type five months after germination are presented in *Figs. 5—1 and 5—2*. A normal chloroplast has a fully developed lamellar structure. On the other hand, chloroplasts in the primary leaves of *Wogon* type seedlings have patch-like structures with no clear differentiation. Number per cell and size of chloroplasts were lower than in normal seedlings. The appearance of *Wogon* type is caused primarily by underdeveloped chloroplasts.

Discussion

There are a few papers reporting inheritance with recessive genes in Sugi, *Cryptomeria japonica* D. Don, such as Chiba (1954), Fukuhara (1963) and Ohba *et al.* (1967 a), (1967 b). On the other hand, Jitumori published more than ten papers distributed personally since 1964, describing highly paternal inheritance of variegation in the seedlings derived from a spontaneously variegated Sugi which had been found in a plantation. In *Wogon*-Sugi, which is originally not a chimera, an apparently paternal transmission of the abnormal plastids was found.

Concerning the cytoplasmic inheritance in chlorophyll anomalies, a large number of reports have been published after Correns (1909) and Baur (1909), (1911). It is natural that there are chlorophyll deficiencies and variegations inherited to the progeny through the Mendelian genes. At present, it is commonly believed that the plastid has its own platstid DNA, and is an autonomous- and independent self-reproducing entity being capable of undergoing mutation. There are two types of the cytoplasmic inheritance in variegation, viz. status *albomaculatus* and status *paral-*

bomaculatus. Status *albomaculatus* means that chlorophyll deficiency is inherited to progeny through female only and pollen has no effect on the character of the progeny as shown in *Antirrhinum* and *Mirabilis jalapa*. In the status *paralbomaculatus*, as reported firstly by BAUR (1909) in *Pelargonium*, the chlorophyll deficiency and variegation are transmitted to the progeny through both sexes, usually lesser ratios from pollen, in reciprocal crosses. As reviewed by HAGEMANN (1963), there are two explanations for the development of variegation: (1) the variegation is due to the presence of genetically different types of plastids and their somatic segregation and sorting out during development and (2) the variegation is due to the determinants distributed in the cytoplasm, and not in the plastids. In addition to these two explanations, the variegation in some sepcies results from an interaction between gene complement and cytoplasm from both parents as reported by CASPARI (1948), MICHAELIS (1954) and OEHLKERS (1964). Moreover, not only the plastid variation, but also the shape of the leaf, hypanthis length, photoperiodic behavior and stem growth are affected by the cytoplasm (see, HAGEMANN 1963).

It is well known in *Epilobium* and *Oenothera* that the male transmission is usually infrequent (JINKS 1965). TILNEY-BASSETT (1967) made an extensive review on the plastid inheritance in higher plants. Concerning the plastid inheritance in a certain reciprocal cross combination of *Pelargonium zonale*, he found a fact that the proportion of green plastids is unexpectedly lower when they are transmitted through the female than when they are transmitted through the male gamete. But, in *Nepeta cataria* and *Pelargonium zonale*, a high level of the transmission of normal and abnormal plastids from both male and female is noted as high as 27 or 30 per cent. And in a cross of a female with normal plastids and a male with abnormal plastids in *Pelargonium zonale*, 70 per cent of the progeny possess the abnormal plastids of the pollen parent, and some of the progeny possess only plastids with paternal phenotype (JINKS 1965).

Effects of paternal cytoplasm on characters of progeny are also reported on maize by FLEMMING and CAMPBELL (1966) and on *Drosophila* by L'HERITIES and TESSIER (see, CASPARI 1948). Though the quantity of the cytoplasm transmitted to the embryo by the pollen is far less in comparison with that of female gamete, JINKS (1965) stated that male transmission occurred in some species ranging from exclusively maternal transmission to approximately equal maternal and paternal transmission of the determinants that control the phenotype of the plastids. He also stated that, in such species, post-zygotic or pre-zygotic reduction which was caused by unequal formation or lethality of gametes in both sexes, affected to increase paternal contribution.

In reciprocal crosses between *Wogon*-Sugi and other normal Sugi, it is found that the trait of *Wogon*-Sugi is mostly transmitted to the progeny through the pollen and not through the female gamete. And this mode of the transmission is kept even in selfing or back-crossing of the F_1 hybrids derived from reciprocal crosses. Although, germination percentage varied largely in various cross combinations including reciprocal crosses, it is not proved any specific reduction or mortality of gametes or zygotes that may result this highly paternal plastid inheritance. Until now, we may say that no report has ever been published concerning genomic and cytoplasmic differentiation among Sugi varieties, and behavior in transmission of cytoplasm at fertilization. But, as seen in *Fig. 4*, frequencies of normal green- and chimeric seedlings after pollination of *Wogon*-Sugi pollen, varied largely among families and this might be explained by a postulation of varied interaction between the nucleus of *Wogon*-Sugi and the cytoplasm of normal Sugi varieties. On the other hand, pollination to *Wogon*-Sugi as female parent with the pollen of normal Sugi resulted in 99 per cent of normal type. About one per cent of the seedlings with *Wogon* type and chimera do not seem to be resulted from pollen contamination. It is because of the ratio of *Wogon* type : chimera which is 1 : 2 in the cross combinations of *Wogon*-Sugi(female) × normal Sugi(male) and, if these variants came from miss-cross of *Wogon*-Sugi pollen, it should be in the ratio of 12 : 1 as expected from the results of the crosses of normal Sugi (female) × *Wogon*-Sugi(male) just as presented in *Table 4*.

JITUMORI estimated that the male transmission in the progeny from a variegated Sugi is about 90 per cent. Meanwhile, the male transmission in *Wogon*-Sugi is about 97 per cent including chimeric seedlings.

WETTSTEIN (1961) described the developmental sequence of the plastids in normal plants and various mutants under various amount of light, and blocking of the developmental pathways occurring in various stages. Concerning the plastome and plasmon effects on the structure of chloroplast, he stated that, in status *albomaculatus* of *Nicotiana tabacum* and status *paralbomaculatus* in two *Oenothera* mutants, normal chloroplast are formed in the cells carrying the plastome defect. The plastids are destroyed secondarily and the whole lamellar system is broken down. In the primary leaves of *Wogon* type seedlings underdeveloped lamellar system were observed from the beginning, and later, as they recover to green, the developmental sequence of *Wogon* type plastids is somewhat different from those mentioned above. Their development seems to be stimulated at higher temperature.

Owing to insufficient information on genetical background of Sugi, it is, at present, difficult to make a decisive explanation on this predominantly paternal inheritance of the plastids. It is necessary to promote research works in genetics, cytology and cytochemistry of Sugi.

Aside from these basic studies, practical usages of this inheritance are possible to investigate on pollen dispersion in the natural conditions, selective fertilization with mixed pollen, pollen tightness of various crossing bags and pollination rooms, optimum stage of pollination and etc.

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Summary

In Sugi, *Cryptomeria japoninca* D. DON, an example of highly paternal transmission of the plastids is reported after three breeding experiments.

Wogon-Sugi, *Cryptomeria japonica* D. DON form *Wogon* hort. ex IWATA et KUSAKA, is a horticultural variety and all of their newly developing shoots are white to yellowish white in spring, and, in late summer, they usually recover

to apparently normal green. They repeat this reaction every year.

Cotyledons are always green in the progeny of reciprocal crosses between *Wogon*-Sugi and normal Sugi. Meanwhile, primary leaves of almost all, i. e. 99 per cent of the seedlings derived from crosses of *Wogon*-Sugi(female) × normal Sugi(male) are normal green. And more than 90 per cent of the seedlings raised from normal Sugi(female) × *Wogon*-Sugi(male) and selfing of *Wogon*-Sugi had primary leaves and sprouts with white to yellowish white (sometimes, light green) in color. In the same crossings, about 5 per cent (in average) of the seedlings showed chimeric primary leaves and eventually developed into chimeric plants, and there were a few per cent of normal seedlings. The ratios of the seedlings of chimera and normal type after crosses of normal Sugi(female) × *Wogon*-Sugi(male) varied among female parents.

After investigation of segregation on a recessive marker gene, theoretically expected ratios of 3 : 1 (selfing of heterozygous F_1 hybrids), and 1 : 1 (backcrossing to homozygous female with F_1 hybrids) are secured and no specific gametic or zygotic elimination is shown for the gene. By these breeding experiments, *Wogon* type seedlings with homozygous for the recessive gene were obtained.

After a grafting experiment, non transmissibility of the trait of *Wogon*-Sugi was proved. In addition, by electron microscopic observation, underdeveloped lamellar system in the *Wogon* type chloroplast is shown.

Literature Cited

Baur, E.: Das Wesen und die Erblichkeitsverhältnisse der „Varietates albomarginatae hort." von *Pelargonium zonale*. Z. Vererbungslehre 1: 330—351 (1909). — Baur, E.: Untersuchungen über die Vererbung von Chromatophorenmerkmalen bei *Melandrium*, *Antirrhinum* and *Aquilegia*. Z. Vererbungslehre 4: 81—102 (1911). — Caspari, E.: Cytoplasmic inheritance. Advances in Genetics 2: 1—66 (1948). — Chiba, S.: Inheritance of coloring of needles in the fall of Sugi (*Cryptomeria japonica* D. Don) (in Jap.). J. Jap. Forestry 35: 286—289 (1954). — Correns, C.: Vererbungsversuche mit blaß(gelb) grünen und buntblättrigen Sippen bei *Mirabilis jalapa*, *Urtica pilulifera* und *Lunaria annua*. Z. Vererbungslehre 1: 291—329 (1909). — Flemming, A. A., and J. B. Campbell: Effects of male cytoplasm in a double cross maize hybrid. Abstracts of the Annual Meeting of the American Society of Agronomy, Oklahoma Sate Univ. 1966, Plant Breed. Abstr. 37: 66—67 (1967). — Fukuhara, N.: Inheritance of needle discoloration of Sugi (*Cryptomeria japonica* D. Don). World Consultation of Forest Genetics and Tree Improvement, Stockholm, Section (1), Tree Genetics and Cytology, 02695 (1963). — Hagemann, R.: Advances in the field of plastid inheritance in higher plants. Genetics Today, Proc. XI ICG, The Hague, Sept. 1963, 3: 613—625 (1963). — Jinks, J.: Extrachromosomal inheritance. Prentice-Hall Inc. (1965). — Jitumori, I.: No English title is available. Personal Publications (in Jap.) (1964—1968). — Kirk, J. T. O., and R. A. E. Tilney-Basset: The plastids. Freeman (197). — Michaelis, P.: Cytoplasmic inheritance in *Epilobium* and its theoretical significance. Advances in Genetics 6: 287—401 (1954). — Oehlkers, F.: Cytoplasmic inheritance in the genus *Streptocarpus* Lindley. Advances in Genetics 12: 329—370 (1964). — Ohba, K., and M. Murai: Studies on radiosensitivity of forest trees V. Difference in radiosensitivity of self- and cross-pollinated seeds, and of seeds with a marker gene in Sugi, *Cryptomeria japonica* D. Don (in Jap.). J. Jap. Forestry 49: 45—52 (1967 a). — Ohba, K., M. Murai, G. Sugimura, M. Saito, K. Okamoto, M. Watanabe, and T. Noguchi: Studies on variation of forest trees III. Cross-fertility between Kuma-Sugi and some other cutting varieties of Sugi (*Cryptomeria japonica*), growth of F_1 seedlings and two single recessive genes detected in Kuma-Sugi (in Jap.). J. Jap. Forestry 49: 361—367 (1967 b). — Wettstein, D. von: Nuclear and cytoplasmic factors in development of chloroplast structure and function. Can. J. Botany 39: 1537—1545 (1961).

Polycross Analysis of Pollen Radiosensitivity in Picea glauca[1])

By D. B. Houston and G. R. Stairs[2])

Introduction

The sensitivity of forest trees to ionizing radiation has been studied extensively during the past few years. Whole tree and seed tolerances to chronic and acute irradiation have now been adequately defined for several species, especially in the genus *Pinus*. To date, however, relatively few studies have been accomplished with forest trees species in the realm of gametic, or pollen, irradiation. Of those investigations reported, only a small proportion have been oriented toward definition of pollen radiosensitivity for subsequent employment in a breeding program.

Breeding programs must first be preceded by studies to determine the radiosensitivity of the pollen in the desired species, and to include the delimitation of exposures most suitable for the specific purpose. The purpose of the study herein reported was to characterize the radiosensitivity of white spruce (*Picea glauca* [Moench] Voss) pollen through the use of a polycross mating scheme as tested in two plantations in different environments. Radiosensitivity was judged by germination percentages of seed derived from the polycross, electron spin resonance spectroscopy of irradiated pollen, and germination of irradiated pollen *in vitro*. The study was designed to evaluate nuclear radiosensitivity, cytoplasmic radiosensitivity, and the possibility of parthenogenesis induction through the use of irradiated pollen.

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Review of Literature

Studies of chronic and acute ionizing radiation effects on forest trees have been conducted with seeds, pollen, and somatic tissues. Recent literature surveys of these studies have been published by Erikson *et al.* (1966) and by Lynn (1967). In addition, acute gamma irradiation survival data for 28 species of woody plants, including many forest tree species, have been compiled by Sparrow *et al.* (1968).

Pollen irradiation has received somewhat less attention than other aspects of forest tree research. This situation may be due to difficulties in handling arising from the limited span of viability and hygroscopic nature of the microspore. Brewbaker and Emory (1962) reviewed irradiation studies with mature angiosperm pollen, finding LD_{50} values for pollen germination *in vitro* ranging to 550KR (kiloroentgens), and a median lethal dose for germination of 250KR. In contrast, a median dose of only 250R (roentgens) was reported for pollen tube divisions. Vidaković (1963) fertilized *Pinus sylvestris* with *P. nigra* pollen irradiated at 800R to 1200R and obtained putative hybrid progeny from this otherwise incompatible cross. The investigator theorized that gamma radiation stimulated pollen tube growth and fertilization subsequently occurred, or, as a result of the radiation, certain chemical changes occurred in the pollen thereby stimulating development of the female gametophyte and fertilization. Naturally occur-