Analysis of Clones of Nango-hi, a Vegetatively Propagated Cultivar of *Chamaecyparis obtusa* ENDL. Based on Isozyme Genotypes

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Nango-hi is a vegetatively propagated cultivar of *Chamaecyparis obtusa* (Sieb. et Zucc.) ENDL. which had been directly planted from unrooted cuttings or rooted cuttings 200~300 years ago in the forest of the Aso area in Kumamoto. Three groups of individuals of the cultivar with different ages were analysed for their isozyme genotypes. One group consisted of 43 trees more than 100 years of age grown on the grounds of 9 shrines and one temple. Another group consisted of 331 trees about 50~100 years of age in 6 mature stands and the last group consisted of 216 rooted cuttings supplied by three seedling growers. The cultivar has been propagated vegetatively for long years, and is thought to be a mixture of clones. Therefore, based on the individual genotypes at 11 isozyme loci, individuals with the same genotype are deemed to be a presumed clone (hereafter; clone) and are grouped into a single clone. The number of clones in the group of old trees corresponded to 10 types with one major clone accounting for 72.1% of the total. In the mature stands, 22 clones were identified, with two major clones accounting for 67.9% and 11.9%. In the cutting gardens, 14 clones were identified with 3 major clones accounting for 47.7%, 18.1% and 16.3%, respectively. The genotype with the highest percentage was the same in the three groups, and two major clones displayed the same genotype in the mature stands and cutting gardens.

Nango-hi groups consisted of 27 different genotypes in all, and only 5 extra genotypes were found in the rooted cuttings.

KEY WORDS: *Chamaecyparis obtusa*, vegetative propagation, isozyme, clone analysis, cultivar

Introduction

Nango-hi is the only vegetatively propagated cultivar of *Chamaecyparis obtusa* (Sieb. et Zucc.) ENDL. (common Japanese name is hinoki) which was directly planted from unrooted cuttings or rooted cuttings 200~300 years ago in the forests of the Aso area in Kumamoto, Kyushu, Japan. This information is based on the report of SATO and MIYAJIMA (1956) who designated the cultivar as Nango-hi (hi, standing for hinoki and Nango, for the Nango valley). They carried out extensive studies on its specific traits, including straight bole, larger angle of branching, favourite wood color and quality, lack of hypertrophy and resinosis. Especially MIYAJIMA (1962) made a complete survey of Nango-hi whose timber is economically valuable.

MIYAJIMA (1962, 1989) stated that the stands of Nango-hi were composed of a mixture of clones with a high purity (a small number of clones with similar characteristics), because the characteristics of Nango-hi exhibited a consistency among different stands. TAJIMA and MIYA-

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ZAKI (1973) who analysed the clones of this cultivar in four stands based on the peroxidase isozyme pattern, disclosed 9 patterns in all, consisting of 2 to 4 types in each stand.

In studies on the natural distribution of *Chamaecyparis obtusa* (hinoki), no natural stands of this species were observed in Kyushu. However huge hinoki trees more than 100~200 years old or more were grown in sacred groves on the grounds of shrines and temples located in the Aso area (both in Aso valley (northern district) and Nango valley (southern district)) around Mt. Aso which is a famous active volcano.

Moreover there were old legends according to which the cuttings from these holy trees were collected for direct planting (unrooted cuttings) from olden times. The distribution of the mature stands of Nango-hi was strictly limited to the Nango valley district.

At present, rooted cuttings of this cultivar are predominantly used for artificial plantations. Rooted cuttings for commercial purposes are raised by private seedling growers from their own cutting stocks on a small scale of less than one hundred thousand plants annually (Personal communication with Mr. SHINGAI, Director of the Kumamoto Prefecture Forestry Research Station).

Concerning the inheritance of isozyme variants, UCHIDA *et al.* (1991) had already reported the presence of 14 loci with a total of 30 alleles for 10 enzyme systems.

This study aimed at defining the clone constitution of this cultivar in three groups of tree materials of different ages, namely old trees more than 100 years of age, mature trees in stands and rooted cuttings based on isozyme analysis.

Materials and Methods

In the group of old trees, the leaves of 43 trees standing on the grounds of 9 shrines and one temple which were located in the Aso and Nango valley districts were sampled as shown in Table 1.

Table 1. Location of nine shrines and one temple where old trees of hinoki, *Chamaecyparis obtusa* were grown and number of hinoki trees examined in relation to their isozyme genotypes

No.	Name of shrine and temple	Location	Number of sampled trees
①	Sugahara Shrine (S.)	Aso County, Chouyou Town	2
②	Aso-Nagano S.	Ibid	1
③	Nishinomiya S.	Ibid	17
④	Uzoe S.	Ibid	1
⑤	Kukino S.	Aso County, Kukino Village	4
⑥	Takamori-Aso S.	Aso County, Takamori Town	5
⑦	Yunoura-Shou-Hachiman S.	Aso County, Aso Town	5
⑧	Akamizu S.	Ibid	3
⑨	Baba-Hachiman S.	Aso County, Ichinomiya Town	3
⑩	Entsu Temple	Ibid	2
Total			43

A total of 5 mature stands of Nango-hi and one seedling-stand was selected at Kukino village (3 stands), Takamori town (2 stands) and Namino village (1 stand) as shown in Table 2. From each stand, 22~64 individuals were randomly selected for leaf sampling. For the comparison of isozyme genotypes, leaves from individuals of one stand derived from seedlings were sampled. The old trees and mature trees in which leaves were sampled were tagged with identifying symbols and figures and their position was mapped in each area and stand for future survey of the individual trees after isozyme analysis. Location of the shrines and the temple as well as of the mature stands mentioned above is illustrated in Fig. 1.

Two hundred and sixteen rooted cuttings obtained from tree seedling growers (72 rooted cutting each), were used for isozyme analysis. The growers own cutting gardens for cutting production as shown in Table 3.

The leaf samples were stored in glass vials at -80°C until they were used for electrophoresis. Polyacrylamide vertical slab gel electrophoresis described by DAVIS (1964) and ORSTEIN (1964) was applied with the small modifications described by TSUMURA *et al.* (1990).

Ninety mg of leaves of individual plants were ground to a fine powder using a mortar and pestle with liquid nitrogen. Then the powder was stirred after the addition of 70 mg of polyvinylpyrrolidone. One ml of extraction buffer [93 mM Tris-HCl buffer pH 7.5, 0.6% Tween 80, 2.3 mM DTT, 2.8 mM EDTA, 0.6 mM NAD, 0.5 mM NADP, 0.05% 2-mercaptoethanol, 0.8% BSA] was added immediately after stirring. The homogenate of individual samples was centrifuged at 15,000 rpm at 0°C for 40 minutes. For the detection of each enzyme, $10\ \mu\text{l}$ of the supernatant was used for electrophoresis. Running gels consisting

Table 2. Location and description of mature stands of Nango-hi and one seedling stand from which trees were selected for sampling, and number of sampled trees examined for their isozyme genotypes

No.	Location	Number of sampled trees	Stand age (year)	Stand area (ha)	Direction of slope of stand (degree)	Elevation of stand above sea level (m)
Nango-hi						
1.	Aso County, Kukino V. Hisaishi	64	70	0.5	N 30° E	520
2.	Ibid	59	60	0.3	N 30° E	520
3.	Aso County, Takamori T. Nakayama	62	70~100	0.6	N 20° E	620
4.	Aso County, Takamori T. Nakajima	22	50	0.4	N 30° W	640
5.	Aso County, Namino V.	61	70	0.9	S 80° E ~N 20° E	850
Sub-total		268				
Seedling hinoki stand						
6.	Aso County, Kukino V. Hisaishi	63	60	0.45	N 30° E	440
Total		331

Fig. 1. Location of 9 shrines and one temple, and 6 mature stands where leaf sampling were made for isozyme analysis.

Table 3. Description of three cutting stands (gardens) owned by private seedling growers

No.	Location	Stand age (year)	Stand area (ha)	Cutting collection
A	Aso County, Kukino Village	10	0.35	Annually
B	Ibid	7	0.25	Annually
C	Ibid	5~12	0.35	Annually

of 7.5% and 3.75% spacer gel were used. Electrophoresis was carried out at 4°C and 12.3 mA/cm² for 150 minutes.

After the electrophoresis, each gel was stained according to the procedure of TSUMURA *et al.* (1990). Namely, 9 enzyme systems such as 6-phosphogluconate dehydrogenase (6PGD), glucose-6-phosphate dehydrogenase (G6PD), glutamate dehydrogenase (GDH), peroxidase (POD), glutamate oxaloacetate transaminase (GOT), glucokinase (GK), phosphoglucomutase (PGM), es-

Table 4. Enzyme systems and loci analysed (abbreviation and E. C. number)

No.	Enzyme system	Abbreviation	Locus	E. C. number
1	6-Phosphogluconate dehydrogenase	6PGD	<i>6Pg-1, -2</i>	1.1.1.44
2	Glucose-6-phosphate dehydrogenase	G6PD	<i>G6p</i>	1.1.1.49
3	Glutamate dehydrogenase	GDH	<i>Gdh</i>	1.4.1.2
4	Peroxidase	POD	<i>Pod</i>	1.11.1.7
5	Glutamate oxaloacetate transaminase	GOT	<i>Got-1, -2</i>	2.6.1.1
6	Glucokinase	GK	<i>Gk</i>	2.7.1.2
7	Phosphoglucomutase	PGM	<i>Pgm</i>	2.7.5.1
8	Esterase	EST	<i>Est</i>	3.1.1
9	Leucine aminopeptidase	LAP	<i>Lap</i>	3.4.11.1
Total		9 enzymes	11 loci	

terase (EST), and leucine aminopeptidase (LAP) were stained to detect the isozyme band segregation at 11 loci (Table 4).

Results

Isozyme genotypes of 63 individuals taken from a seedling stand are shown in Table 5. Three loci, *Gdh*, *Got-2* and *Lap* showed a completely fixed genotype, while two loci, *Gk* and *6Pg-1* showed only a slight variation in the genotypes. At the other six loci, *G6p*, *Est*, *Pod*, *Got-1*, *6Pg-2* and *Pgm* the genotypes showed considerable variations. In this study, previously reported *Got* was named as *Got-1*, because new locus *Got-2* which migrated fast was found. Based on the genotypes at these 8 loci, 63 individuals were classified into 56 genotypes. Only a few individuals showed the same genotype within this stand, and 8 individuals showed 5 genotypes, Nos. 8, 11, 14, 19 and 23, similar to these of Nango-hi as indicated in Table 6 (column marked as duplicated genotypes).

The isozyme genotypes of the Nango-hi individuals in the three groups with different ages are shown in Table 6. There were 32 genotypes in all and 27 genotypes were detected in the old and mature groups. At five loci, *Gdh*, *Got-2*, *Lap*, *Gk* and *6Pg-1* out of 11 loci 27 genotypes were completely fixed as described in the remarks of Table 6. In the old group, 10 genotypes were detected with the major genotype of No. 14, being observed in 31 (72.1%) out of 43 individuals. In the mature group, the genotype of No. 14 was also dominant in all the five stands, and all the 22 individuals in the stand of No. 4 exhibited the same genotype as that of No. 14. Other 4 stands consisted 8~10 clones. The stands of Nos. 1, 2 and 5 exhibited the same major genotype up to 70 to 80%, respectively. However in the stand of No. 3 there was another major genotype, No. 18, as shown in Table 6. In total, 268 individuals were found to correspond to 22 genotypes, and 182 individuals (67.9%) to the genotype of No. 14. The genotype of No. 18 accounted for the second largest proportion 11.9% in the mature group.

The genotypes of the rooted cuttings derived from three different seedling growers corresponded to 6~9 clones. The genotype with the highest proportion was also that of No.

Table 5. Isozyme genotypes of individuals in one seedling stand of hinoki, *Chamaecyparis obtusa* ENDL.

No. of individulas		Isozyme loci										Duplicated genotypes ¹⁾	
		<i>Gdh</i>	<i>Got-2</i>	<i>Lap</i>	<i>Gk</i>	<i>6Pg-1</i>	<i>G6p</i>	<i>Est</i>	<i>Pod</i>	<i>Got-1</i>	<i>6Pg-2</i>		<i>Pgm</i>
1	A013	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>		<i>a/—</i> ²⁾	<i>a/a</i>	<i>b/b</i>	<i>a/b</i>	<i>a/a</i>	
2	A049	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/—</i>	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	<i>a/b</i>	
3	AO23	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>o/o</i>	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	<i>a/a</i>	
4	AO32	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>o/o</i>	<i>a/a</i>	<i>b/b</i>	<i>a/b</i>	<i>a/b</i>	
5	AO35	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>o/o</i>	<i>a/a</i>	<i>b/b</i>	<i>a/a</i>	<i>a/a</i>	
6	AO43	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>o/o</i>	<i>a/a</i>	<i>b/b</i>	<i>a/b</i>	<i>a/a</i>	
7	AO04	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/—</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	
8	AO46	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/—</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>a/b</i>	
9	AO51	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/—</i>	<i>a/a</i>	<i>b/b</i>	<i>b/b</i>	<i>a/a</i>	
10	AO14	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/—</i>	<i>a/a</i>	<i>b/b</i>	<i>a/a</i>	<i>a/a</i>	
11	AO12	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/—</i>	<i>a/b</i>	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	
12	AO44	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>o/o</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>a/a</i>	
13	AO25	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>o/o</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>a/a</i>	
14	AO60	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>o/o</i>	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	<i>a/a</i>	
15	AO36	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>o/o</i>	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	<i>a/b</i>	
16	AO39	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>o/o</i>	<i>a/a</i>	<i>b/b</i>	<i>a/a</i>	<i>a/a</i>	
17	AO56	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>o/o</i>	<i>a/a</i>	<i>b/b</i>	<i>a/b</i>	<i>b/b</i>	
18	AO61	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>o/o</i>	<i>a/a</i>	<i>b/b</i>	<i>a/b</i>	<i>a/b</i>	
19	AO28	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>o/o</i>	<i>a/b</i>	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	
20	AO05	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>o/o</i>	<i>a/b</i>	<i>a/b</i>	<i>a/b</i>	<i>a/a</i>	
21	AO01	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>o/o</i>	<i>a/b</i>	<i>a/b</i>	<i>a/a</i>	<i>a/a</i>	
22	AO42	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>o/o</i>	<i>a/b</i>	<i>b/b</i>	<i>a/a</i>	<i>a/b</i>	
23	AO11	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>o/o</i>	<i>a/b</i>	<i>b/b</i>	<i>a/b</i>	<i>a/a</i>	
24	AO64	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>		<i>b/b</i>	<i>b/b</i>	<i>a/a</i>	<i>a/a</i>	
25	AO15	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>a/—</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	
26	AO31	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>a/—</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	
27	AO03	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>a/—</i>	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	<i>a/a</i>	
28	AO02	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>a/—</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>a/b</i>	
29	AO19	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>a/—</i>	<i>a/a</i>	<i>b/b</i>	<i>a/a</i>	<i>a/b</i>	
30	AO59	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>a/—</i>	<i>a/a</i>	<i>b/b</i>	<i>a/b</i>	<i>a/b</i>	
31	AO65	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>a/—</i>	<i>a/b</i>	<i>a/b</i>	<i>b/b</i>	<i>a/b</i>	
32	AO21	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>a/a</i>	
33	AO09	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>a/a</i>	
34	AO29	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>a/b</i>	No. 11
35	AO08	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>a/b</i>	No. 11
36	AO28	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	No. 8
37	AO16	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	<i>a/b</i>	No. 14
38	AO48	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	<i>a/b</i>	No. 14

¹⁾ Individuals with the same genotype as that of Nango-hi were indicated by figures (cf. Table 6.)²⁾ “*a/—*” showed “*a/a*” or “*a/o*”, because the phenotypes could not be differentiated.

Table 5. (Continuation)

No. of individuals		Isozyme loci											Duplicated genotypes ¹⁾
		<i>Gdh</i>	<i>Got-2</i>	<i>Lap</i>	<i>Gk</i>	<i>6Pg-1</i>	<i>G6p</i>	<i>Est</i>	<i>Pod</i>	<i>Got-1</i>	<i>6Pg-2</i>	<i>Pgm</i>	
39	AO37	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	<i>a/b</i>	No. 14
40	AO06	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/a</i>	<i>b/b</i>	<i>a/b</i>	<i>a/a</i>	
41	AO47	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/a</i>	<i>b/b</i>	<i>b/b</i>	<i>a/a</i>	
42	AO55	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/a</i>	<i>b/b</i>	<i>a/b</i>	<i>a/c</i>	
43	AO17	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/a</i>	<i>b/b</i>	<i>a/b</i>	<i>a/b</i>	No. 19
44	AO18	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/a</i>	<i>b/b</i>	<i>a/a</i>	<i>a/b</i>	
45	AO38	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/a</i>	<i>b/b</i>	<i>a/a</i>	<i>a/b</i>	
46	AO40	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/b</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	
47	AO52	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/b</i>	<i>a/b</i>	<i>a/b</i>	<i>b/b</i>	No. 23
48	AO34	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/b</i>	<i>b/b</i>	<i>a/b</i>	<i>a/a</i>	
49	AO50	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>b/b</i>	<i>b/b</i>	<i>b/b</i>	<i>a/b</i>	
50	AO30	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>a/a</i>	<i>a/—</i>	<i>a/b</i>	<i>b/b</i>	<i>a/b</i>	<i>a/a</i>	
51	AO07	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>a/a</i>	<i>o/o</i>	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	<i>a/a</i>	
52	AO54	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>a/b</i>	<i>o/o</i>	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	<i>a/b</i>	
53	AO63	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>a/b</i>	<i>o/o</i>	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	<i>a/b</i>	
54	AO57	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>a/b</i>	<i>o/o</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>a/a</i>	
55	AO20	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>a/b</i>	<i>o/o</i>	<i>a/a</i>	<i>b/b</i>	<i>a/b</i>	<i>a/b</i>	
56	AO62	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>a/b</i>	<i>o/o</i>	<i>a/b</i>	<i>a/b</i>	<i>a/b</i>	<i>a/b</i>	
57	AO45	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>a/b</i>	<i>o/o</i>	<i>b/b</i>	<i>b/b</i>	<i>a/a</i>	<i>b/b</i>	
58	AO53	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	<i>a/b</i>	
59	AO48	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/a</i>	<i>b/b</i>	<i>o/o</i>	<i>a/b</i>	<i>b/b</i>	<i>a/b</i>	<i>a/a</i>	
60	AO22	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>b/b</i>	<i>a/—</i> ²⁾	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	<i>a/b</i>	
61	AO24	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>b/b</i>	<i>a/—</i>	<i>a/a</i>	<i>b/b</i>	<i>a/b</i>	<i>a/a</i>	
62	AO23	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	<i>b/b</i>	<i>o/o</i>	<i>a/a</i>	<i>b/b</i>	<i>a/a</i>	<i>b/c</i>	
63	AO10	<i>a/a</i>	<i>a/a</i>	<i>a/a</i>	<i>a/b</i>	<i>a/b</i>	<i>b/b</i>	<i>o/o</i>	<i>a/a</i>	<i>b/b</i>	<i>a/a</i>	<i>b/c</i>	

¹⁾ Individuals with the same genotype as that of Nango-hi were indicated by figures (cf. Table 6.)

²⁾ "*a/—*" showed "*a/a*" or "*a/o*", because the phenotypes could not be differentiated.

14 (47.7%), followed by those of No. 18 (18.1%) and No. 8 (16.3%). Moreover, in the cutting gardens of these growers the spectra of 3 genotypes of Nos. 14, 18 and 8, respectively could be differentiated.

Nango-hi groups consisted of 27 different genotypes in all, and only 5 extra genotypes were found in the rooted cuttings.

Discussion

MIYAZAKI and SAKAI (1969) carried out an analysis of the cultivar of Kumotooshi (*Cryptomeria japonica*) a single clone. MIYAZAKI (1971) reported on the clone composition of vegetatively propagated cultivars of sugi, *Cryptomeria japonica* and old sugi trees which were

Table 6. Frequency of isozyme genotypes of Nango-hi individuals in three groups of different ages

Geno- type No.	Genotypes at loci based on isozyme analysis				Number of Individuals																							
	G6p	Est	Pod	Got-1	6Pg-2	Pgm	Shrines and temples										Sub-total (%)	Mature stands					Sub-total (%)	Cutting gardens			Sub-total (%)	Total (%)
							①	②	③	④	⑤	⑥	⑦	⑧	⑨	⑩		1)	2)	3)	4)	5)		A	B	C		
1.	a/b	o/o	a/b	b/b	a/b	a/b											—	1					1	1	1	1	2	
2.	b/b	a/—	a/a	a/a	a/b	a/b											—		1					1	1	2	2	
3.	b/b	a/—	a/a	a/a	a/b	b/b	1										1		4						6	11 (2.1)	2.1)	
4.	b/b	a/—	a/a	a/b	a/b	a/b											—	2	1					3	3	6	6	
5.	b/b	a/—	a/a	a/b	a/b	b/b				1							1						—	—	—	1	1	
6.	b/b	o/o	a/a	a/a	a/a	a/b											—	1	5	1		7 (2.6)	—	—	—	7	7	
7.	b/b	o/o	a/a	a/a	a/a	b/b											—		1			1	—	—	—	1	1	
8.	b/b	o/o	a/a	a/a	a/b	a/b											—	2	2	4				1	23	11	35 (16.3)	39 (7.4)
<hr/>																												
9.	b/b	o/o	a/a	a/a	a/b	b/b				2							2						—	—	—	—	2	2
10.	b/b	o/o	a/a	a/a	b/b	a/b											—	1				1	—	—	—	—	1	1
11.	b/b	o/o	a/a	a/b	a/a	a/b											—	1				1	—	—	—	—	1	1
12.	b/b	o/o	a/a	a/b	a/a	b/b											—	1				1	—	—	—	—	1	1
13.	b/b	o/o	a/a	a/b	a/b	a/a				2							2					1	1	1	1	1	4	4
14.	b/b	o/o	a/a	a/b	a/b	a/b	2	1	13	1	4	2	2	3	3	2	31 (72.1)	45	47	17	22	51	182 (67.9)	28	42	33	103 (47.7)	316 (60.0)
<hr/>																												
15.	b/b	o/o	a/a	a/b	a/b	b/b				2							2	2				1	3	—	—	—	5	5
16.	b/b	o/o	a/a	a/b	b/b	a/a				1							1					—	—	—	—	—	1	1
17.	b/b	o/o	a/a	a/b	b/b	a/b											—	5	6		2	13 (4.9)	—	—	—	—	13 (2.5)	2.5)
18.	b/b	o/o	a/a	a/b	b/b	b/b											—		1	31		32 (11.9)	13	4	22	39 (18.1)	71 (13.5)	13.5)
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19.	b/b	o/o	a/a	b/b	a/b	a/b	1										1	2	1			3	3	1	4	8	8	8
20.	b/b	o/o	a/a	b/b	b/b	a/b											—		1			1	—	—	—	—	1	1
21.	b/b	o/o	a/b	a/b	a/a	b/b											—	4				4	—	—	—	—	4	4

22.	b/b	o/o	a/b	a/b	a/b	a/b	a/b	—	1	1	—	1																						
23.	b/b	o/o	a/b	a/b	a/b	a/b	b/b	—	2	2	—	2																						
24.	b/b	o/o	a/b	a/b	a/b	a/b	a/b	—	1	1	—	1																						
25.	b/b	o/o	a/b	a/b	a/b	a/b	b/b	—	1	1	—	1																						
26.	b/b	o/o	a/b	a/b	b/b	a/b	b/b	1	—	—	—	1																						
27.	b/b	o/o	b/b	a/b	a/b	a/b	a/b	1	—	—	—	1																						

28.	b/b	a/—	a/a	b/b	a/b	a/b	a/b	—	2	2	2	2																						
29.	b/b	o/o	a/a	a/b	a/a	a/a	a/a	—	—	4	4	4																						
30.	b/b	o/o	a/b	a/b	a/b	a/a	a/a	—	—	1	1	1																						
31.	b/b	o/o	a/b	a/b	b/b	a/b	a/b	—	15	15 (6.9)	15 (2.8)	15																						
32.	b/b	o/o	a/a	b/b	b/b	a/a	a/b	—	—	1	1	1																						
Total number of individuals													2	1	17	1	4	5	5	3	3	2	43 (100)	64	59	62	22	61	268 (100)	72	72	72	216 (100)	527 (100)
Number of clones													1	1	5	1	1	3	3	1	1	1	10	10	8	8	1	8	22	9	6	6	14	32

Five loci out of 11 loci, namely *Gdh*, *Got-2*, *Gk*, *Lap* and *6Pg-1* were omitted from this table due to the complete fixation of the genotype as *a/a*.

considered to be the source of cuttings for the cultivars based on the analysis of the banding pattern of the peroxidase isozyme. He reported that 5 old Yabukuguri trees belonged to a single clone and old Measa trees consisted of a mixture of several clone groups.

TAJIMA and MIYAZAKI (1973) reported the results of an analysis of the peroxidase banding patterns of Nango-hi. They took samples from 15 to 35 individuals from 4 stands with different ages, 56, 37, 11, and 5 years, respectively. A total of 9 banding patterns was detected, and 2 to 4 banding patterns could be differentiated in the respective stands. They estimated that these stands may be consist of a single to a few clones. They also grouped the banding patterns with similar phenotypes and suggested the existence of genetic differences between the stands in the Aso valley and Nango valley.

TAIRA (1979) carried out the same type of studies on sugi cultivars in Toyama prefecture. However, the banding pattern of the isozyme may be vary depending on the season when sampling was performed, sampled tissues, age of sample and other factors. Therefore the use of the genotype after genetic study of isozyme variants was considered to be more reliable.

OKUIZUMI and OHBA (1990) carried out an analysis of the clones of vegetatively propagated sugi cultivars based on the genotypes at four isozyme loci. They found that 11 cultivars of the Obi-sugi group, Yabukuguri and Measa were composed of at least two to five clones.

For the hinoki isozyme, TAJIMA *et al.* (1977) reported on a peroxidase locus with simple Mendelian inheritance. SHIRAISHI *et al.* (1986) reported the presence of the

dimeric inheritance of glutamate oxaloacetate transaminase of needle and pollen tissues in hinoki. UCHIDA *et al.* (1991) carried out extensive genetic studies on the isozymes of hinoki, and they identified 14 loci after examining 12 enzyme systems in the species.

SEIDO (1992) identified 9 more loci with 18 alleles which could be used for genetic analyses of hinoki.

In this study, 11 loci for 9 enzyme systems were examined in relation to the genotypes of 527 individuals in total. As Nango-hi was propagated vegetatively and thought to be a mixture of clones, the results were analysed based on the isozyme genotypes of individuals, and the individuals which exhibited the same genotype at the analysed loci were deemed to be a presumed clone (hereafter; clone) and were grouped into a single clone. With this procedure, the genotypic data of the individuals from three groups of different ages and from mature stands or plant groups of rooted cuttings were analysed. Isozyme genotypes identified in 63 individuals of a seedling-stand showed a large variation with 56 genotypes.

Meanwhile in the Nango-hi groups, a total of 32 genotypes was detected, and although the number of samples varied considerably, the number of genotypes ranged from 1 to 10 in each sacred groves of the shrines, each mature stands and each plant groups of rooted cuttings owned by the growers, respectively.

Moreover, there might be 1 to 3 common major clones in the groups, corresponding to the genotypes of Nos. 14, 18 and 8. Although there were 3 groups based on the age, the major genotype of No. 14 was common, confirming the old legend on the establishment of Nango-hi. As Nango-hi was usually propagated by direct planting of unrooted cuttings, there may have been a very strong selection pressure on its rooting ability even in the establishment of holy groves. Successive procurement of the cuttings from these trees which grew successfully may have resulted in a mixture of a few clones called Nango-hi with a higher rooting ability even for the old trees. Such an example was also found in a vegetatively propagated sugi cultivar named Sanbu-sugi (OKUIZUMI, personal communication) which showed a very high rooting ability (up to 80%) even when the cuttings were collected from trees more than 200 years old.

Nango-hi might consist of one major clone, No. 14 and two other clones, Nos. 18 and 8, as well as of several minor clones depending on the age groups. The performance of each clone should be evaluated in the field. By using the technique of clone identification based on the isozyme genotype, Nango-hi plantations could be converted into progeny testing stands with a single plant plot scheme involving an uneven number of individuals for each clone. Money, time and labour could be saved to establish new testing stands by the planting of the rooted cuttings. After clone analysis, clonal performance in term of growth, bole traits, wood quality and resistance to pests and diseases could be evaluated by taking the samples from various sites and from trees of different ages. Then conventional stem analysis may enable to study the growth habit of Nango-hi at different sites and under different age conditions by collecting the sampled trees after isozyme analysis. Field surveys on tree damage caused by pests and diseases may also enable to analyse relationship between the respective clones and isozyme genotypes on their productive traits.

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ヒノキ (*Chamaecyparis obtusa* (Sieb. et Zucc.) ENDL.) の栄養系品種、南郷檜のアイソザイム
遺伝子型によるクローン分析

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南郷檜とは、九州、熊本の阿蘇周辺で200～300年前から直ぐしあるいはさし木苗で造林されてきたヒノキ (*Chamaecyparis obtusa*)である。異なった樹齢の3グループの個体についてアイソザイム遺伝子型を分析した。すなわち、社寺10ヶ所の樹齢100年以上の古い境内木43個体 (Table 1) と樹齢50-100年の6林分の331個体 (Table 2) および3人の苗木生産者の採穂園から入手したさし木苗216本である。南郷檜は200～300年にわたりさし木繁殖で造林され、混合クローンとみなされてきたことから、調査したアイソザイム11遺伝子座について、同じ遺伝子型と判定された個体は想定同一クローン (以下：クローン) とみなして分別した。その結果、社寺の老木は10クローンに分別でき、その内の1つのクローンが72.1%を占めていた。成熟林分では22のクローンに分別でき、主要な2つのクローンで67.9%と11.9%を占めていた。3人の苗木生産者の採穂園では14のクローンに分別でき、主要な3つのクローンでそれぞれ、47.7%、18.1%、16.3%を占めていた。3グループで最も高い割合を占めていた遺伝子型は同じものであった。成熟林分と採穂園での主要な2つのクローンは同じ遺伝子型であった。南郷檜全体では、27の異なった遺伝子型を持っていることが判り、さし木苗ではそれ以外に5遺伝子型が検出された。