

The results of the rooting experiment are shown in Table 3.

Transferring the rooting shoots into vermiculite substratum, fertilized with 0.1% Hyponex solution, developed healthy roots and good growths of plantlets (Fig. 3). Afterwards, plantlets were transplanted to plastic pots and acclimated successfully under conditions described previously (Fig. 4). In another experiment, successful acclimatizations of plantlets were obtained by transplanting the rooted shoots directly to pots inside the growth cabinet, omitting the previous phase for root developments and growths of shoots. Flasks containing vermiculite substratum kept under culture-room conditions. This method may reduce the period necessary for outplanting.

The potential benefits in the use of clonal planting stock in reforestation programs have long been recognized (8). However, most often, the feasibility of clonal stocks production by micropropagation is associated with mass production at competitive prices (3). Whenever vegetative propagation is possible economically, it generally is preferred to sexual propagation because genetic characteristics are maintained better (2).

Even though vegetative propagation of jacaranda is feasible, to date, a suitable method for mass production has not been found.

In these experiments, the possibility of rapid clonal production by micropropagation of jacaranda is proven by the culturing of the shoot-tips of seedlings. However, the feasibility of micropropagation by culture of mature stages remain to be determined.

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## 短 報

**An Application of a Best Linear Prediction to Clonal Tests  
of Sugi (*Cryptomeria japonica*)  
in the Northern Kanto Region\***

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**I. Introduction**

To develop an appropriate and robust system for the analysis of large progeny testing data is one of the essential subjects in promoting operational tree improvement programs efficiently, because it will provide plus tree rankings based on field tests for the purpose of roguing seed orchards and choosing parents for the second generation breeding population. However, the conventional methods of standardized score or least squares are much limited in their applicabilities and flexibilities in practical situations, because an unbalance of available data in traits or ages, differences in testing frequencies among plus trees, and heterogeneities of variances or precisions among tests are frequently met in operational tree-improvement programs (2, 7). A Best Linear Prediction (BLP) developed by animal breeders (4) seems to be more flexible and robust in treating such situations (6). WHITE and HODGE modified this procedure to make it applicable to progeny tests in forestry (7), and it was put into practice in some of the forest tree-improvement programs in the United States recently (5, 6).

In this paper, the application of BLP to operational clonal tests of sugi (*Cryptomeria japonica*) in Japan is presented. Although the basic method used here is the same as that outlined by WHITE and HODGE (7), we determine Type B correlations (1) by using the relative size of clonal variances and those of site  $\times$  clone interactions instead of estimating the correlations directly in order to process a large quantity of data more efficiently.

**II. Materials and Methods**

Data used in this study are from 45 clonal tests established from 1970 to 1985 in the northern Kanto Region (Table 1). As the data used to calculate BLP are the latest measurements in each test, they differ in ages or traits because of the differences in their years of establishment and intensities of measurements. The traits analyzed by BLP are height, stem straightness, and survival rate at fifteen years of age. The data on diameter at breast height and crookedness of stem bottom were added to increase the precisions of the predictions. Data on stem straightness and crookedness of stem bottom were expressed by scores of from 1 (extremely crooked) to 5 (very straight) and were available only in 10 and 15 years' measurements. The clonal tests were made with randomized complete block designs of two to four, mostly three, replications.

The method of calculating BLP with progeny test data was described in detail by WHITE and HODGE. The principal assumptions of a BLP is that the second moments used as elements of  $V$ , the matrices of variances and covariances among observations, and  $C$ , a matrix of covariances between the observations and the genetic values to be predicted, are known (7). Once the elements of these matrices are specified, the BLP of every parent in the tests can be calculated with the maximum use of available data, and their precisions of predictions will be reflected in the rankings of the BLPs. In clonal tests of sugi however, the elements of the matrices should be estimated directly from the data, because no estimates are available for this species. The procedure for calculating a BLP in this study is composed of the following four steps;

- 1) Analysis of data on each trait in each test,
- 2) Overall analysis across multiple tests to estimate variance and covariance components of each trait,

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Table 1. Data of clonal tests used in this study

Test code	No. of clone	Traits						Test code	No. of clone	Traits						Test code	No. of clone	Traits					
		H	D	SB	ST	SV	H			D	SB	ST	SV	H	D			SB	ST	SV			
212	14	10	10	—	—	10	241	13	15	15	15	15	15	426*	11	15	15	15	15	15	15		
213	14	10	10	—	—	10	242	13	10	10	—	—	10	427	52	10	10	10	10	10	10		
214	9	10	10	—	—	10	243	12	10	10	—	—	10	428	11	10	10	10	10	10	10		
215	18	15	15	—	—	15	244	14	10	10	10	10	10	432	51	10	10	10	10	10	10		
216	14	15	15	—	—	10	393*	84	15	15	15	15	15	1426	15	10	10	—	—	—	5		
217	12	15	15	—	—	15	403*	28	15	15	15	15	15	1428	15	10	10	—	—	—	10		
218	15	10	10	—	—	10	404*	32	15	15	15	15	15	1430	9	5	—	—	—	—	5		
219	11	10	10	—	—	10	405	13	15	15	15	15	15	1431	8	5	—	—	—	—	5		
220	12	10	10	—	—	10	406*	31	15	15	15	15	15	1432	7	5	—	—	—	—	5		
221	12	10	10	—	—	10	407	12	10	10	—	—	10	1433	8	5	—	—	—	—	5		
222	11	10	10	—	—	—	411	47	10	10	—	—	10	1440	21	10	10	—	—	—	10		
227	17	15	15	—	—	15	419*	11	15	15	15	15	15	1491	12	15	15	15	15	15	15		
230	21	5	—	—	—	10	422*	11	15	15	15	15	15	1535	10	10	10	10	10	10	10		
233	21	10	—	—	—	10	423	53	15	15	10	10	15	1787	38	5	—	—	—	—	5		
240	12	15	15	15	15	15	425	11	15	15	—	10	15	1792	27	5	—	—	—	—	5		

Notes: Symbols for the traits are H, Height; D, Diameter at breast height; SB, Crookedness of stem bottom; ST, Straightness of stem and SV, Survival rate. Numbers in the table show the age of measurements.

\*Denotes the tests used for overall analysis.

3) Construction of  $V$  and  $C$  matrices for each clone to calculate the BLP,

4) Calculate the BLP for each clone.

As the purpose of this paper is to present the method of application of BLP to clonal tests, the term “genetic” used in the referenced papers (6~8) is replaced with “clonal” in this paper.

Conventional two-way analyses of variances using plot means ( $y_{jk}$ ) were made on every trait in each test in order to estimate phenotypic variances of clonal means ( $Vp$ ) and clonal variances ( $Vc''$ ). A linear model for a measured trait in each test is as follows,

$$y_{jk} = \mu + \beta_j + \gamma_k + \varepsilon_{jk} \quad (1)$$

where,  $\mu$ , A population mean in respective test;  $\beta_j$ , Fixed effect of  $j$ th replication;  $\gamma_k$ , Random effect of  $k$ th clone with  $E(\gamma_k) = 0$ ,  $\text{Var}(\gamma_k) = \sigma_c^2$ ;  $\varepsilon_{jk}$ , Random plot error of  $y_{jk}$  with  $E(\varepsilon_{jk}) = 0$ ,  $\text{Var}(\varepsilon_{jk}) = \sigma_p^2$ .

Least square estimates of  $\gamma_k$  are used as elements of data vector ( $y$ ), because some of the tests have missing plots. Then the estimates of the clonal variances were adjusted according to the size of their standard errors by using the following formula,

$$Vc' = [Vc'' + b \cdot Vp \cdot w / (1 + w)] / [1 + w / (1 + w)] \quad (2)$$

where  $b$  is the regression coefficient of clonal variance to the phenotypic variance on each trait, and  $w$  is a ratio of standard error to the estimate of clonal variance on a trait in each test.

Overall analyses of variances and covariances were made using plot means of seven tests of which complete data on twelve traits were available (Table 2). A linear model for the analysis is (3):

$$y_{ijk} = \mu + \alpha_i + \alpha\beta_{ij} + \gamma_k + \alpha\gamma_{ik} + \varepsilon_{ijk} \quad (3)$$

where,  $\mu$ , A population mean across the tests;  $\alpha_i$ , Fixed effect of  $i$ th test;  $\alpha\beta_{ij}$ , Fixed effect of  $j$ th replication in  $i$ th test;  $\gamma_k$ , Random effect of  $k$ th clone with  $E(\gamma_k) = 0$ ,  $\text{Var}(\gamma_k) = \sigma_c^2$ ;  $\alpha\gamma_{ik}$ , Random effect of interaction between site  $i$  and clone  $k$  with  $E(\alpha\gamma_{ik}) = 0$ ,  $\text{Var}(\alpha\gamma_{ik}) = \sigma_{sc}^2$ ;  $\varepsilon_{ijk}$ , Random plot error of  $y_{ijk}$  with  $E(\varepsilon_{ijk}) = 0$ ,  $\text{Var}(\varepsilon_{ijk}) = \sigma_p^2$ .

From the results of this analysis, the clonal variances of twelve traits ( $Vc$ ) and clonal correlations ( $r_c$ ) between two traits of all possible pairs were calculated directly. Then the degrees of freedom and the sums of squares or sums of cross products for clones and those for site  $\times$  clones interactions were pooled to estimate phenotypic correlations of clonal means among the traits ( $r_p$ ). In this study,  $r_{pij}$  between traits  $i$  and  $j$  is obtained by;

$$r_{pij} = \text{SCP}(c + sc)_{ij} / [\text{SS}(c + sc)_i \cdot \text{SS}(c + sc)_j]^{1/2} \quad (4)$$

Table 2. Analysis of variances for seven clonal tests using plot means

Source	d.f.	Expected mean squares
Site	6	—
Replication/Site	13	—
Clone	96	$\sigma_p^2 + 2.89 \sigma_{sc}^2 + 5.95 \sigma_c^2$
Site $\times$ Clone	105	$\sigma_p^2 + 2.79 \sigma_{sc}^2$
Experimental error	370	$\sigma_p^2$

Notes:  $\sigma_p^2$ ,  $\sigma_{sc}^2$  are  $\sigma_c^2$  are variances of plot errors, site  $\times$  clone interactions, and clones, respectively. Analyses of covariances were made using the same coefficients for each component of covariances.

$$V = \begin{bmatrix} Vp_{i/h}, & Cp_{ij/h}, & Cc_{i/hl}, & Cc_{ij/hl} \\ Cp_{ij/h}, & Vp_{j/h}, & Cc_{ij/hl}, & Cc_{ij/hl} \\ Cc_{i/hl}, & Cc_{ij/hl}, & Vp_{i/l}, & Cp_{ij/l} \\ Cc_{ij/hl}, & Cc_{ij/hl}, & Cp_{ij/l}, & Vp_{j/l} \end{bmatrix}$$

Fig. 1. A schematic notation of variances and covariances in  $V$  and  $C$  for traits  $i$  and  $j$  observed in tests  $h$  and  $l$

Notes:  $Vp$  and  $Cp$  are phenotypic variances and covariances of family means, respectively;  $Cc$  are clonal covariances.

where SS and SCP are pooled sums of squares and sums of cross products, respectively.

As the plus trees tried in the clonal tests might be unrelated to each other,  $V$  and  $C$  matrices can be set up for each clone separately (7). Although the sizes of the matrices were different among clones due to the differences in their tested frequencies, all types of elements used in the  $V$  matrix are represented as in Fig. 1. In obtaining the elements of  $V$ , we made two assumptions. First, phenotypic correlations within the tests ( $r_p$ ) are constant for all tests. Second, the relative ratio of site  $\times$  clone interaction variances to clonal variances ( $r_{sc}$ ) is constant in any pair of the tests. Thus  $r_{sc}$  was determined by,

$$r_{sc} = Vc/Vc' = \sigma_c^2/(\sigma_c^2 + \sigma_{sc}^2) \quad (5)$$

where  $Vc$  and  $Vc'$  are the estimates obtained from overall analyses.

With the two assumptions, all elements in  $V$  can be specified by using estimates of  $r_p$ ,  $r_c$ , and  $r_{sc}$  and variances of  $Vp$  and  $Vc'$ , of which the former are obtained by overall analyses, and the latter from single site analyses. Diagonal elements of  $V$  matrices ( $Vp$ ) are the phenotypic variances of clone means in each test, and off-diagonal elements are obtained by;

$$Cp_{ij} = r_{pij} \cdot [Vp_i \cdot Vp_j]^{1/2} \quad (6)$$

$$Cc_{ij} = r_{cij} \cdot [(r_{sci} \cdot Vc_i) \cdot (r_{scj} \cdot Vc_j)]^{1/2} \quad (7)$$

$Cp_{ij}$  is the off-diagonal element of the sub-matrix of the same test in  $V$  and generally is called the Type A covariance (1).  $Cc_{ij}$  is the remaining off-diagonal element in  $V$  and they are the product of clonal correlations and geometric means of the two corresponding clonal variances discounted by the magnitude of the site  $\times$  clone interaction.  $Cc_{ij}$  is regarded as a Type B covariance (1).

Elements of  $C$ , which are the covariances between clonal values and the data, are calculated as (8);

$$Cc_{ti} = r_{cti} \cdot [Vc_t \cdot (r_{sci} \cdot Vc_i)]^{1/2} \quad (8)$$

where  $Cc_{ti}$ ,  $r_{cti}$  and  $Vc_t$  are covariance between the clonal values of target trait  $t$  and trait  $i$ , clonal correlation between target traits  $t$  and  $i$ , and clonal variance of target trait  $t$ , respectively. Because the target traits are the three measured traits in this study,  $r_{cti}$  and  $Vc_t$  are the parameters estimated in overall analyses.

The BLP for each clone ( $c$ ) is calculated by solving the following formula (7),

$$c = C' \cdot V^{-1} \cdot y \quad (9)$$

If the number of available data is  $n$ , the size of  $C'$ ,  $V^{-1}$  and  $y$  are  $3 \times n$ ,  $n \times n$ , and  $n \times 1$ , respectively. Then the vector of the predicted value for target trait ( $c$ ) will become  $3 \times 1$  in size. The correlations between the predicted and true clonal values are calculated as (8);

$$\text{Corr}(c, c') = [(C' \cdot V^{-1} \cdot C)/Vc_t]^{1/2} \quad (10)$$

### III. Results

A conventional two-way analysis of variance in each test showed that the ratio of clonal variance ( $Vc''$ ) to the phenotypic variance of clonal means ( $Vp$ ) for height, diameter, crookedness of stem bottom, stem straightness, and survival rate were 0.59, 0.60, 0.38, 0.53 and 0.66, respectively. Figure 2 shows the distribution of  $Vc''$  and  $Vc'$  of height. White dots are  $Vc''$ , and black dots are  $Vc'$  which were adjusted by

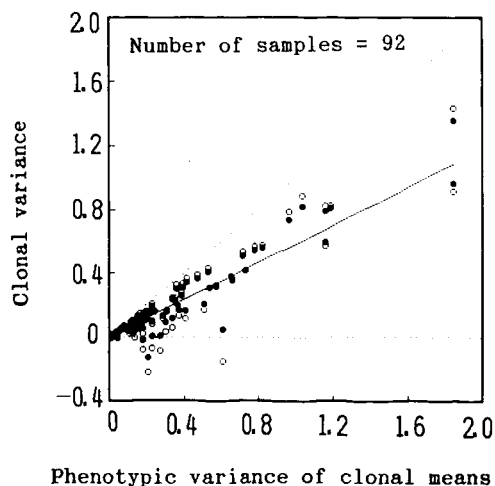


Fig. 2. Adjustment of clonal variances on height estimated in individual clonal tests

Notes: White dots are the original estimates, and black dots are the adjusted ones by Formula (2). The relative size of clonal variance to the phenotypic variance is shown by the regression line through the origin.

Formula (2). The amount of shrinkage toward the regression line is greater for the dots scattered below the regression line. This is probably because their standard errors are much larger than those above the regression line. Thus the tests having excessive estimates of clonal variances might be adjusted appropriately when they are used for BLP.

Variance component estimates on height, diameter, and survival rate seems to be accurate enough to be used for BLPs as can be seen from their smaller rates of standard errors, whereas the estimates for stem form traits are not so reliable (Table 3). The magnitude of site  $\times$  clone interaction variance is smallest in height, followed by diameter and survival, whereas the magnitude of the stem form traits are nearly the same sizes as their clonal variance components except for the 10 years' crookedness of stem bottom. This may be due to possible inconsistencies of criteria among

Table 3. Variance components of 12 traits estimated by analyses of variances across seven clonal tests

Type of variances	Traits											
	H5	SV5	H10	D10	SB10	ST10	SV10	H15	D15	SB15	ST15	SV15
$\sigma_c^2$	0.058	0.399	0.212	0.595	0.008	0.010	0.529	0.624	1.497	0.041	0.042	0.510
$SD(\sigma_c^2)$	0.011	0.090	0.043	0.134	0.004	0.006	0.114	0.130	0.313	0.016	0.018	0.120
$\sigma_{sc}^2$	0.017	0.207	0.060	0.294	0.000	0.009	0.237	0.165	0.658	0.034	0.060	0.324
$r_{sc}$	0.776	0.659	0.778	0.670	1.00	0.527	0.690	0.790	0.695	0.534	0.412	0.611

Notes:  $SD(\sigma_c^2)$  is the standard errors of the estimate of clonal variance.  $r_{sc}$  is calculated as  $\sigma_c^2/(\sigma_c^2 + \sigma_{sc}^2)$ . Figures attached on the right hand side of the symbols denote the ages of the measurements of each trait.

observers in standardizations of the assessments of those traits.

Almost all of the phenotypic or clonal correlations among the twelve traits were positive except for that of crookednesses of stem bottoms and stem straightnesses at ten years' measurement (Table 4). Clonal correlations among the growth traits of different ages and those of survival rates exceeded 0.9. The phenotypic correlations between growth traits and stem straightnesses were less than 0.5, whereas clonal correlations were moderately large at 0.6.

The data of 45 clonal tests enabled us to predict clonal values of 190 plus tree clones on the three traits by BLP (Table 5). Although the amount of data per clone was 18.6 on the average, it varied from 2 to 108. Thus the precision of the predictions varies considerably with the amount of available data per clone (Fig. 3).

#### IV. Discussion

The precisions of the predictions as revealed by correlations between predicted values and true clonal values are the greatest for height, followed by survival, and smallest for stem straightness (Table 5). This order of precision is the same as in  $r_{sc}$ , the relative rate of  $V_c$  to  $V_c'$ . For stem straightness, direct measurements were available only for 113 plus trees. The precision of a prediction might mainly depend on the size of  $r_{sc}$ , but it is modified by the size of data for a trait used for the prediction.

Table 4. Correlations among 12 traits used for BLPs

Trait	1	2	3	4	5	6	7	8	9	10	11	12
1 H5	—	0.175	0.872	0.780	0.065	0.232	0.262	0.782	0.711	0.309	0.291	0.264
2 SV5	0.273	—	0.129	0.156	0.116	0.149	0.941	0.146	0.179	0.165	0.188	0.895
3 H10	0.945	0.279	—	0.904	0.138	0.362	0.235	0.923	0.882	0.385	0.377	0.248
4 D10	0.864	0.331	0.933	—	0.149	0.342	0.257	0.870	0.943	0.380	0.352	0.277
5 SB10	0.006	0.344	0.089	0.186	—	0.323	0.174	0.160	0.188	0.288	0.274	0.194
6 ST10	0.322	0.362	0.425	0.516	-0.01	—	0.248	0.408	0.415	0.617	0.649	0.277
7 SV10	0.393	0.960	0.386	0.479	0.374	0.328	—	0.256	0.304	0.308	0.275	0.969
8 H15	0.928	0.231	1.01	0.950	0.203	0.398	0.339	—	0.895	0.403	0.398	0.270
9 D15	0.807	0.372	0.908	0.994	0.236	0.544	0.506	0.937	—	0.427	0.426	0.328
10 SB15	0.414	0.273	0.544	0.684	0.253	0.940	0.439	0.611	0.714	—	0.627	0.336
11 ST15	0.455	0.419	0.618	0.758	0.027	0.841	0.441	0.658	0.698	0.627	—	0.296
12 SV15	0.435	0.956	0.409	0.526	0.261	0.330	0.996	0.339	0.543	0.466	0.490	—

Notes: Values above the diagonal are phenotypic correlations of clonal means ( $r_p$ ), and those below the diagonal are clonal correlations ( $r_c$ ). The clonal correlation coefficient between H10 and H15 was set to be 1.00 in calculating BLPs.

Table 5. A summary of the results of BLPs

Traits	Numbers of data			Nos. of clones	Means	Standard deviations		Correlation coefficients
	5yr.	10yr.	15yr.			BLP	L.S.E.	
Heights (m)	119	409	383	190	6.54	0.562	0.936	0.769
Diameters (cm)	0	397	383	151	8.90	—	1.451	—
Crooks. b. Stems	0	208	258	113	4.51	—	0.308	—
Stems straight	0	219	258	113	4.41	0.094	0.332	0.436
Survival rates (%)	112	419	369	190	86.0	4.040	8.910	0.641

Notes: The unit of data is the deviation of each clonal mean from the respective test mean. Values in the columns of mean and correlation coefficients are the averages of 15 years' measurements of respective traits and those of correlation coefficients calculated for each plus tree clone.

One of the desirable properties of a BLP is that it tends to rank the clones with little precision around the mean, even if the absolute values of the clones in the data vector are large. It enables us to make more effective selections of plus-tree clones compared with the least-squares method (7). This trend is recognized clearly in the frequency distributions of the three traits in Fig. 4, in which clones are classified into three groups, that is no direct measurements, one to four measurements, and more than four measurements. Clones having more than four measurements are observed in all classes, whereas most of the clones with less than four measurements fell only into the three middle classes.

Two assumptions used in this study, that is a constant phenotypic correlation and a constant  $r_{sc}$  are prerequisites for treating data with small sample sizes as shown in Table 1. The assumption of constant phenotypic correlations ( $r_p$ ) might be safer than using the estimates obtained in each test, because the number of clones used in the present tests are too small to get reliable estimates in many instances. The difference in the sizes of site  $\times$  clone interactions between pairs of clonal tests was neglected in this analysis by assuming that the ratio of  $r_{sc}$  was constant because the two-way table of test  $\times$  clone was so sparse that we could not get reliable covariances between each pair of tests.

With these assumptions, labor to construct  $V$  and  $C$  matrices was greatly reduced. Because once the parameters ( $r_p$ ,  $r_c$ ,  $r_{sc}$ ) were estimated and data vector ( $y$ ) with their variances ( $Vp$ ,  $Vc'$ ) were prepared, elements of  $V$  and  $C$  could be obtained by conducting a similar matrix multiplication. In the case of slash pine (*Pinus elliottii*) (6), a lot of multiple regression equations were developed to estimate the parameters; however, proper regression equations always were not detected, especially when the number of traits was large. The procedure proposed here might be more straightforward and flexible to handle data on many traits or many different sets of progeny test data.

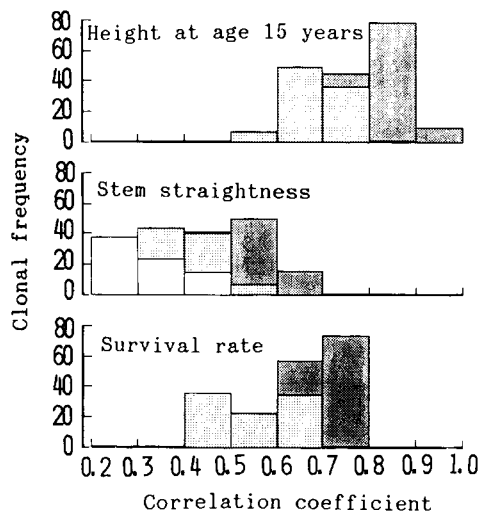


Fig. 3. Frequency distributions of correlation coefficients between predicted and true values

Notes: Correlation coefficients between predicted and true values were calculated using Formula (10) for each plus tree clone. Clones having more than four measurements are shown as dark gray areas. Those having one to four measurements and having no direct measurements are shown in light gray and white areas.

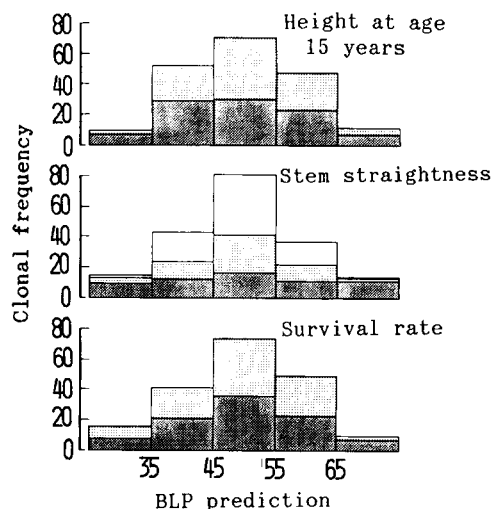


Fig. 4. Frequency distributions of predicted clonal values on each trait

Notes: BLPs for plus tree clones were converted to standardized scores having 50 for means and 10 for one standard deviation unit. Groupings of clones are the same as used in Fig. 3.

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## 短 報

## シラベおよびウラジロモミのアイソザイムの遺伝\*

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## I. はじめに

近年、わが国においても、林木の集団間や集団内の遺伝変異の調査や集団の繁殖構造などを調査するため、アイソザイムが標識遺伝子として用いられてきている(1, 4, 10, 11, 13)。その標識遺伝子を探索するため、すでに、イチョウ(14)、クロマツ(9)、スギ(15)、アカマツ(2)、チョウセンゴヨウ(12)、ヒノキ(16)、オオシラビソ(11)など、さまざまな樹種でアイソザイムの遺伝子分析が行われている。

ところで、マツ科モミ属に属するシラベ(*Abies veitchii* LINDL.)は、日本固有の種で、本州と四国の亜高山帯に分布し、同属に属するウラジロモミ(*Abies homolepis* SIEB. et ZUCC.)も、日本固有の種で、本州の関東、中部および四国の温帯上部から亜高山帯下部にかけて分布しており、シラベは下部でウラジロモミと接している(17)。この両種は、亜高山帯または温帯上部における主要な種でもあり、かつ、精英樹が選抜されている(3)など育種面からも重要な種である。この両種の精英樹のクローン管理、特性調査、さらに天然林集団の遺伝変異の解明などに用いるためのアイソザイムの標識遺伝子の探索を行った。

シラベについては、すでに清藤(5)が、天然木15個体の雌性配偶体を用いたアイソザイムの遺伝子分析により、7酵素種を支配する7遺伝子座の14対立遺伝子を明らかにしており、本研究では、清藤(5)が報告をしていない4酵素種を含め、計7酵素種について分析を行った。ウラジロモミについても、7酵素種について分析を行った。

## II. 材料と方法

シラベの精英樹は26本が、ウラジロモミの精英樹は37本がそれぞれ選抜されている(3)。そのうち、シラ

ベは18クローンが、ウラジロモミは34クローンが、林野庁林木育種センター長野事業場内の採種園に集植されている。

材料は、その採種園に集植されている精英樹クローンのうち、シラベは17クローンから、ウラジロモミは15クローンからそれぞれ採取された自然交配種子の雌性配偶体を用いた。種子採取された母樹の精英樹クローンは、両種とも天然林から選抜されたもので、シラベは、11クローンが長野県内から、他の6クローンは山梨県内から、ウラジロモミは、9クローンは静岡県内から、他の6クローンは長野県内から、それぞれ選抜されたものである。なお、裸子植物の雌性配偶体(胚乳)は減数分裂で生じる母親由来の半数体組織( $n$ )であり、母樹別の雌性配偶体を用いれば人工交配家系でなくてもアイソザイムの遺伝子支配を容易に調査することができる(6)。

種子は実験に供するまで5°Cの種子貯蔵庫内に保存した。アイソザイム分析の前処理として、4°Cの低温湿層処理を約1カ月間行つたのち、種子の雌性配偶体を随時実験に供した。

アイソザイム分析のうち、抽出、ポリアクリルアミドゲルによる電気泳動および染色は、白石(7, 8)の方法にほぼ従った。遠心分離は、11,000× $g$ 、0°Cで30分間行つた。

分析した酵素種は、シラベとウラジロモミともに、シキミ酸脱水素酵素(ShDH)、グリセリン酸脱水素酵素(G2DH)、リンゴ酸脱水素酵素(MDH)、6-ホスホグルコン酸脱水素酵素(6PGD)、グルタミン酸脱水素酵素(GDH)、アスパラギン酸アミノ転移酵素(GOT)およびフマラーゼ(FM)の7酵素種である。

バンドの観察された分離比とメンデルの法則から期待される分離比(1:1)との適合性検定を $\chi^2$ 検定により行つた。なお、同じバンド(群)が分離する家系が二つ以上ある場合は、不均一性の検定を行い、家系間

\* Masuo MIYATA and Masatoshi UBUKATA: Inheritance of isozyme variants in shirabe (*Abies veitchii* LINDL.) and urajiro-momi (*Abies homolepis* SIEB. et ZUCC.)

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