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Stability-related Parameters and Their Evaluation in a 17-Year Old Norway Spruce Clonal Test Series

By K. ISIK[1] and J. KLEINSCHMIT[2]

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Abstract

Rooted cuttings of 40 different clones (genotypes) of *Picea abies* L. Karst were planted on 7 contrasting test sites (environments) in northern Germany. Various concepts on stability and genotype x environment interactions (GxE) have been discussed using height data at age 17 years. Several parametric and rank-based measures concerning GxE and stability were estimated; and the relationships among them along with the total height were determined. In addition, 5 different rank-based measures *that combine both stability and performance* of genotypes simultaneously have also been estimated. Although ANOVA tests showed significant GxE, rank-based nonparametric tests using $S^{(m)}$ statistics showed no significant differences among the clones in their interactive behavior. Overall evaluation of various stability-related parameters indicate that significant GxE interaction detected by F tests is quite small in proportion (1.92%), clones contributed 7% and sites 50% within the total variance. The observed clonal contributions to interaction sum of squares are attributable mostly to changes in environmental conditions associated with site factors. In further selection and breeding with the clonal material at hand, GxE interaction appears to be of minor importance in Norway spruce, as it is also supported by the non-significance of rank-based tests. Any one of the 5 indices that combine stability and performance appears to be a better indicator than any other single stability-related parameter in detecting genotypes with both high stability and high performance.

Key words: Genotyp x environment interaction, ecovalence, stability, environmental sensitivity, rank-based tests.

Introduction

Picea abies L. Karst. (Norway spruce) is one of the important forest tree species in central and northern Europe. The species has been subject to intensive genetic selection and breeding since the 1960s. One of the large-scale selection and clonal propagation programs on Norway spruce has been developed at the Lower Saxony Forest Research Institute (LSFRI), Dept of Forest Genetic Resources (KLEINSCHMIT *et al.,* 1973, KLEINSCHMIT and SCHMIDT, 1977, KLEINSCHMIT, 1993). As part of this program, cuttings were taken from different provenances of outstanding performance, and rooted cuttings were serially propagated on a three-year cycle. Selection of the best clones has been carried out at each propagation cycle based on nursery and field performance of the clones. The genotypes used in this study are the outcome of such truncation selection.

Growth trends in 17-year-old Norway spruce clones at seven test sites in northern Germany were evaluated in an earlier study (ISIK *et al.,* 1995). The results of this study indicated significant differences among the test sites, among the clones, and significant clone x site (GxE) interaction. However, stability-related parameters were not covered in the same study.

The objectives of this study, using the data in ISIK *et al.* (1995), are to identify the most- and the least- interacting clones, to estimate various (parametric and rank-based) stability-related clonal parameters, and to make comparisons among them. Utilizing information obtained from these objectives and from the overall clonal performance, one can make inferences as to which genotypes to be selected for further breeding and plantation purposes.

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[1] Akdeniz Univ., Faculty of Arts and Sciences, Dept of Biology, 07058 Antalya- Turkiye.

[2] Lower Saxony Forest Res. Inst., Dept of Forest Genetic Resources, D-34355 Staufenberg- Escherode, Germany.

Stability-related Parameters: Brief Comments

In plant breeding, uniform performance of selected genotypes is expected across a given management unit of land. Further, when grown under different environmental conditions, there should be no or very little genotype x environment interaction ($G{\times}E$) for the set of genotypes selected. In other words, selected genotypes should display an acceptable stability level in their performance. In order to meet these requirements and to be able to identify stable genotypes, various parameters of uniformity, stability and/or interaction have been developed by several researchers.

Genotype Environment ($G{\times}E$) interaction is defined as the relative impact of differential environmental changes on phenotypes. That is, $G{\times}E$ is meaningful only for a set of genotypes, and not for a single genotype. Stability, on the other hand, is used in the context of homeostasis. It is the ability of a genotype (individual, clone, population) to keep its phenotype invariant despite the changes in environmental conditions. Therefore, stability is a characteristic of a single genotype.

Some of these stability-related parameters and associated concepts are briefly reviewed below.

Parametric measures

Measure of uniformity across the management units of lands (or test sites)

Environmental variance ($S^2_{X_i}$) is the phenotypic variance of the i_{th} genotype across the test environments. When clone i displays a uniform performance over all the test sites, then, by definition, environmental variance equals zero. From the viewpoint of a static concept of stability, genotypes with zero or small environmental variance are the stable ones. In reality, however, a certain positive response of a genotype is desirable as the quality of environment improves. Therefore, $S^2_{X_i}$, although a good measure of "uniformity" across the test sites, is far from being an acceptable stability index for most quantitative characters. Such a concept of stability is desirable for quality characters only, such as resistance to disease or stress etc (Becker and Léon, 1988).

Coefficient of variation (CV_i) is often considered as a measure of relative variability levels of different entities having different means. CV_i has the advantage over $S^2_{X_i}$ to measure uniformity of genotypes across environments, since it is independent both from the unit of measurement and from the magnitude of means of different genotypes. As applied in this study, it measures the relative uniformity of clones across the test sites. Theoretically, a clone with $CV_i = 0.0$ would have the same height at all test sites. Yet, the CV_i value does not reflect any level of interaction, since CV_i of each clone is independent of the individual test site means and the means of associated clones.

Contribution of individual genotypes to the total interaction

Traditional *ANOVA* provides information on the significance level of $G{\times}E$, but not on the contributions of individual genotypes to total interaction. "Ecovalence" (W_i), proposed by Wricke (1962), estimates the contribution of individual genotypes to the total interaction sum of squares. Genotypes with low W_i are considered relatively stable among a given set of genotypes. Becker and Léon (1988) suggested that W_i is a more direct measure of $G{\times}E$, and proposed to name it "stability variance".

Measure of response or environmental sensitivity of individual genotypes

Environmental sensitivity or response of a genotype is simply the regression of its own phenotypic value (X_{ij} values) on the environmental value ($\bar{X}_{.j}$ values) (X_{ij} and $\bar{X}_{.j}$ values are given in *Table 1*). The regression coefficient (b_i) has been used as a measure of stability by several researchers (Finlay and Wilkinson, 1963; Owino, 1977; St. Clair and Kleinschmit, 1986; Li and McKeand, 1989; McKeand *et al.,* 1990). The value $b_i = 1.0$ indicates average stability, $b_i > 1.0$ low stability, and $b_i < 1.0$ high stability. However, what level of b_i value should be considered as "the most desirable" is a subjective choice, since it often depends on the objectives of breeder, crop species in consideration and homogeneity of land under management. For example, for an annual crop species to be grown with intensive care in a homogenous site, a farmer may prefer "responsive" (high b_i value) genotypes. On the other hand, a forest manager, who has to deal with forest trees that ought to endure fluctuations of various environmental factors both in space and time, may prefer "average b_i" genotypes. In low yielding sites, a breeder may prefer low b_i genotypes ($b_i < 1.0$) [Yet, no beeder would be interested in such environments; and as stated by Becker and Léon (1988), "there will be no commercial interest in developing special varieties for such environments"]. Therefore, in the real sense of stability, b_i value is not a good measure of stability *per se.* As indicated by Breese (1969), Powell *et al.* (1986) and also by Becker and Léon (1988) b_i characterizes the specific response of a given genotype to changing environmental factors. Thus, b_i should be considered as a response parameter and/or as a measure of environmental sensitivity or phenotypic plasticity of a given genotype.

Measure of proportion of GE that is attributable to regression

Coefficient of determination (r_i^2), as proposed by Pinthus (1973), is also a stability-related statistics obtained for i_{th} genotype by regression approach as b_i above. Value r_i^2 indicates how strongly the variability of a dependent variable (phenotypic value) can be explained by the independent variable (environmental value). It measures the proportion of $G{\times}E$ *accounted for* by regression (or by environmental sensitivity), and shows how well the linear model fits the data for a given genotype. Genotypes with higher r_i^2 value are more predictable across different environments. In addition, $CND_i = 1 - r_i^2$ is known as the coefficient of nondetermination (Sokal and Rohlf, 1995). It measures the proportion of $G{\times}E$ *not accounted* for by regression.

St. Clair and Kleinschmit (1986), and McKeand *et al.* (1990) took r_i^2 as a complementary parameter to be considered together with b_i to detect stable genotypes. Provided that other stability-related parameters are equal, genotypes with higher r_i^2 or smaller CND_i are more stable, thus more desirable for breeder.

Deviation mean squares (S^2_{di})

Deviation mean squares (S^2_{di}), first proposed by Eberhardt and Russel (1966), is widely used as stability parameter in both crop species and in forest tree breeding studies (see, for example, St. Clair and Kleinschmit, 1986; Skrøppa, 1984; Becker and Léon, 1988). Both b_i and W_i are the components of S^2_{di}. It is also strongly related to CND_i (Becker and Léon, 1988).

Rank-based Measures

Huehn (1996) argues that $G{\times}E$ may appear even without changes in relative performances of genotypes. He points out that the breeder is not interested in $G{\times}E$ interaction *per se*, but rather in clarifying whether the best genotype in any one particular environment is also the best in other environments. Therefore, the relative ranks of genotypes are more important than their absolute values. Similarly, Skrøppa (1984) states that plant breeders have essentially two groups of genotypes within their study material: one group is to be selected for further breeding and the other group to be culled. He is right in noting that changes in ranking between members within each group have no significance (for practical purpose). More important is the extreme rank changes between genotypes belonging to separate groups. Both because of these arguments, and because of their advantages over parametric methods, we also applied the following rank-based methods on our data.

Huehn's $S_i^{(1)}$ and $S_i^{(2)}$ parameters

If a genotype has identical rank across test sites, it is considered to have maximum stability, and shows $S_i^{(1)} = 0$ and/or $S_i^{(2)} = 0$. These parameters are independent from the yield level, since they are based on transformed X_{ijk} values (Huehn, 1990, 1996, and Nassar and Huehn, 1987).

Nassar and Huehn's $Z_i^{(m)}$ and $S^{(m)}$ statistics

To validate the assumption of no significant genotype x environment interaction, an approximate statistical test of significance has been developed by Nassar and Huehn (1987). The same test was further discussed by Huehn (1990), Huehn and Nasser (1991), Krenzer *et al.* (1992) and Piepho (1992).

Rank-based measures that combine stability and performance (CSP_i)

Genotypes, which are identified as "stable" and thus desirable based on their stability-related parameters, may not be, in many cases, the ones with desirable performance or yield. The problem, then, is to detect the genotypes with both desirable stability and high performance. Yet, in all of the stability-related parameters above, this problem still remains to be evaluated. *One proposal* to clarify this problem is to plot the stability-related values (on Y axis) of a genotype against the site means (on X axis) of the same genotype. On such a graph, the most desirable genotypes (genotypes with high performance and low stability value) would locate in the lower right corner of the plot (Finlay and Wilkinson, 1963; Skrøppa, 1984; St. Clair and Kleinschmit, 1986). *Another proposal* is to employ new parameters that consider stability and performance simultaneously. To meet this purpose we used five different measures in our study parameters (CSP_{i1}, CSP_{i2}, CSP_{i3}, CSP_{i4}, CSP_{i5}). The first two of these combined stability and performance (CSP_i) parameters were proposed by Huehn (1979, 1996), and the remaining three by Nassar, Léon and Huehn (1994).

Materials and Methods

Genetic Material and the Test Sites

Cuttings of the 40 clones used in this study were tertiary cuttings (third cycle of vegetative propagation started in 1968), which were rooted in spring 1974 and grown for three years in the nursery at the LSFRI (Kleinschmit et al. 1973; Kleinschmit and Schmidt, 1977). During spring 1977, cuttings and control seedlings were planted at 7 contrasting sites in northern Germany. Initially, 20 ramets per clone were planted at each test site. These sites represent environments where Norway spruce clones from this program can be planted in the future. Height of each tree was measured at age 17 years to the nearest 5 cm.

Bio-statistical Analyses on Stability-related Measures

Parametric Analyses

Overall analysis of variance was performed using untransformed data. The stability-related parameters have been estimated using the data in *Table 1*.

a. Analysis of variance (*ANOVA*)

ANOVA model was applied for overall comparisons (among test sites, among genotypes (clones), clone x site interactions) (Sokal and Rohlf, 1995). Model for this *ANOVA* was:

$X_{ijk} = \mu + C_i + S_j + CS_{ij} + e_{ijk}$, where

X_{ijk} = Observed value of the k_{th} individual (ramet) of the i_{th} clone at the j_{th} site;

μ = Overall expected mean (of $N = s.c.t$ individuals);

C_i = Effect of the i_{th} clone ($i = 1, 2 \ldots c, c = 40$);

S_j = Effect of the j_{th} site ($j = 1, 2 \ldots s, s = 7$);

CS_{ij} = Interaction between the i_{th} clone and j_{th} site;

e_{ijk} = Error term (within clone)

[k = 1, 2 ...*t*, *t* = number of trees (ramets) per clone, initial number of *t* = 20]

The *ANOVA* model for clonal comparisons to estimate clone x site interaction sum of squares was based on clonal means at the test sites (as presented in a two-way table with clone x site in *Table 1*). Model for this *ANOVA* was:

$X_{ij} = \mu + C_i + S_j + e_{ij}$ where

X_{ij} = Mean value of the i_{th} clone at the j_{th} site

μ = Overall mean (of $N = s \times c$ observed means)

b. Environmental variance ($S^2_{X_i}$) and coefficient of variation (CV_i)

Table 1. – Height means (cm) and ranks of 40 *Picea abies* clones at seven test sites.

Clone			Test site means (X_{ij}) for clones*							Clone ranks within test sites**						
Code	Rank	$\overline{X}_{i.}$ [a]	P	H	L	S	B	M	K	P	H	L	S	B	M	K
123	1	747	726	717	558	858	749	945	676	11	1	3	15	1	1	1
37	2	721	750	621	574	915	680	840	666	6	7	2	6	6	14	3
95	3	719	736	625	554	889	685	875	667	7	5	5	9	3	9	2
41	4	715	732	655	557	941	665	924	533	8	2	4	3	7	3	24
188	5	712	756	640	589	851	644	912	593	4	4	1	17	8	4	7
107	6	709	820	599	444	960	683	910	549	1	12	34	1	5	5	17
103	7	709	709	622	536	956	684	876	581	16	6	10	2	4	8	9
26	8	699	758	572	544	907	591	941	579	3	20	7	7	17	2	10
143	9	685	699	575	487	866	690	820	659	17	19	20	12	2	17	4
87	10	683	725	653	468	917	572	901	542	12	3	29	5	20	6	19
152	11	676	721	609	498	920	587	870	529	13	9	18	4	18	10	25
145	12	675	784	608	546	831	534	832	591	2	10	6	19	26	15	8
197	13	672	753	551	499	868	619	879	536	5	25	17	11	11	7	21
101	14	661	713	553	505	901	620	844	493	15	24	15	8	10	12	32
46	15	657	675	549	479	873	565	844	611	25	26	24	10	22	13	6
45	16	653	664	583	516	854	615	793	548	29	16	12	16	12	22	18
42	17	651	692	612	516	843	493	831	572	19	8	13	18	35	16	11
18	18	646	661	580	505	818	574	850	535	30	17	16	21	19	11	22
50	19	642	682	565	455	865	612	795	519	23	22	31	13	13	21	27
125	20	638	680	591	533	738	599	758	571	24	14	11	37	15	32	12
90	21	637	673	600	506	812	517	783	566	26	11	14	22	30	25	13
113	22	635	729	460	542	809	546	807	552	9	40	9	23	23	18	16
98	23	634	691	572	446	797	611	761	561	20	21	33	24	14	30	14
196	24	629	653	584	483	786	632	775	490	32	15	22	30	9	28	34
88	25	627	720	595	474	791	541	776	489	14	13	27	28	25	27	35
115	26	626	728	547	480	793	501	798	534	10	29	23	26	31	19	23
4	27	626	648	549	487	825	533	724	615	33	27	21	20	27	37	5
66	28	624	635	517	544	772	570	790	538	36	33	8	34	21	23	20
142	29	619	685	577	478	777	597	727	494	21	18	25	32	16	34	31
116	30	618	659	548	455	865	485	795	519	31	28	32	14	36	20	28
9	31	614	695	537	475	788	497	746	558	18	31	26	29	32	33	15
15	32	609	669	498	494	796	544	770	491	28	35	19	25	24	29	33
94	33	592	683	546	432	776	451	760	496	22	30	35	33	38	31	30
118	34	590	635	479	470	786	497	790	471	35	37	28	31	34	24	36
104	35	588	643	562	415	729	529	727	510	34	23	37	38	29	35	29
181	36	576	671	505	366	772	497	776	448	27	34	39	35	33	26	38
112	37	570	619	526	463	743	391	719	526	37	32	30	36	40	38	26
173	38	551	606	462	366	792	483	725	423	38	39	40	27	37	36	39
11	39	550	595	468	391	700	530	716	454	40	38	38	40	28	39	37
189	40	522	598	482	431	703	436	662	343	39	36	36	39	39	40	40
	$\overline{X}_{.j}$ [b]		**692**	**567**	**489**	**830**	**571**	**809**	**541**	$\overline{X}_{..}$ [c] **= 643**						

* Abbreviations for the test sites: P = Paderborn, H = Holzminden, L = Lautenthal, S = Syke, B = Binnen, M = Medingen, K = Kattenbühl.

** Clones are ranked in descending order of original height, the tallest clone having the rank of 1.

a, b, c: Mean height of clone across all test sites; mean of all clones in a test site; overall mean, respectively (cm).

To determine each of these parameters for a given genotype (clone) across the test sites, clonal values (X_{ij}) at each test site were taken as the basis by the equations below:

$CV_i = (SD \text{ x } 100) / \bar{X}_{i.}$, where

SD = Standard deviation = $\sqrt{S^2_{X_i}}$ and,

$\bar{X}_{i.}$ = Mean of clone i for all sites ($i = 1, 2 \ldots c, c = 40$);

$S^2_{X_i} = \sum_j (X_{ij} - \bar{X}_{i.})^2 / (s - 1)$ = Environmental variance as annotated by BECKER and LÉON (1988).

c. Ecovalence (W_i) values

It was calculated for each clone according to WRICKE 1962.

d. Regression coefficients (b_i)

To measure this parameter for a given clone, we followed the method of FINLAY and WILKINSON (1963).

e. Coefficient of determination (r_i^2) and nondetermination (CND_i)

These parameters for each clone were calculated from regression analyses, using the below equation and related data in *Table 1*.

$$r_i^2 = b_i \sum_j (X_{.j} - \bar{X}_{..})(X_{ij} - \bar{X}_{i.}) / \sum_j (X_{ij} - \bar{X}_{i.})^2$$

f. Deviation mean squares (S^2_{di})

It was calculated according to equation given by BECKER and LÉON (1988).

g. Correlation coefficients

Pearson Correlation Coefficients (r) were found to determine degree of associations between various clonal parameters

Rank-based Analyses

HUEHN's $S_i^{(1)}$ and $S_i^{(2)}$ parameters were annotated and calculated for each clone according to HUEHN (1990, 1996) and NASSAR and HUEHN (1987). NASSAR and HUEHN's $Z_i^{(m)}$ and $S^{(m)}$ statistics were found according to HUEHN (1996), using the original notation.

We calculated five different rank-based measures (CSP_{i1}, CSP_{i2}, CSP_{i3}, CSP_{i4}, CSP_{i5}) that combine stability and performance for a give clone. The first two of these parameters were calculated according to HUEHN (1979, 1996), and the remaining three according to NASSAR, LÉON and HUEHN (1994).

To perform rank-based tests (except calculating CSP_{i4} and CSP_{i5}), original X_{ijk} values were first transformed into $X_{ijk}{}^*$ values as proposed by HUEHN (1996). All the above parametric and rank-based biostatistical procedures were performed using SAS (SAS 1987) and our own programs.

Results and Discussion

Parametric Stability-related Measures

The results of overall analyses of variance are presented in *Table 2*. We found significant $G \times E$ among 17 years old *Picea abies* clones growing on seven test sites ($P < 0.001$). Yet, when the variance components were partitioned, the contribution of $G \times E$ on total variance was relatively small [i.e., $\sigma^2_{cs} / (\sigma^2_s + \sigma^2_c + \sigma^2_{cs} + \sigma^2_e) = 1.92\%$, and $\sigma^2_{cs} / (\sigma^2_s + \sigma^2_c + \sigma^2_{cs}) = 3.26\%$].

We estimated the contribution of each clone to the total interaction sum of squares by WRICKE's (1962) ecovalence values (W_i). The first 10 clones with the highest stability (lowest W_i, i.e., the lowest contribution to $G \times E$) were the clones with height ranks of 18, 32, 16, 39, 34, 5, 19, 38, 3 and 21 in that order (*Table 3*). Among these, only four (3, 5, 16, 18) had height growth above overall mean height, which is 643 cm. Clones with h_t ranks of 1, 4 and 6 had corresponding W_i ranks of 39, 31 and 40, respectively. The best performing clone (clone 123) had W_i rank of 39, and thus made the 2nd highest contribution to $G \times E$ interaction. There were no significant correlations between clonal height and W_i values. The positive tendency of relationship between clonal height ($\bar{X}_{i.}$) and W_i value ($r = 0.27$, $P < 0.09$) indicates that faster growing clones tend to be the less stable ones. ST. CLAIR and KLEINSCHMIT (1986) using the same genetic material from the test sites, when the trees were 10 years old, found similar trends ($r = 0.22$ ns).

Table 2. – ANOVA results for height of *Picea abies* clones at age 17 years.

Source of variation	**df**	**Mean square**	**F value and significance level**	**Variance components (σ^2)**		
A: Based on individual observations				due to	absolute	%
Sites = s	6	12125112	439.83 ***	σ^2_s	17960	50.1
Clones = c	39	315012	11.43 ***	σ^2_c	2510	7.0
Clone x Site interaction	234	27568	1.88 ***	σ^2_{cs}	690	1.9
Within	4440	14697		σ^2_e	14697	41.0
TOTAL	**4719**				**35857**	**100.0**
B: Based on clonal means				due to	absolute	%
Sites = s	6	731765	456.52 ***	σ^2_s	18254	81.7
Clones = c	39	19064	11.89 ***	σ^2_c	2494	11.1
Clone x Site interaction	234	1603		σ^2_{cs}	1603	7.2
TOTAL	**279**				**22351**	**100.0**

***: Significant at the 0.1% level.

Clonal relative variability level CV_i value had significant negative relationship with clonal height, which indicates that taller clones exhibited greater uniformity (smaller CV_i) among the test sites, which is a desirable outcome for the interests of a breeder. Among the top 10 height performers, 5 genotypes (those with height ranks 1, 2, 3, 5 and 9) would also be among the best 10 CV_i clones in that order. CV_i detected best performing genotypes better than did W_i.

CND_i, (i.e., $1 - r_i^2$), estimates the proportion of $G \times E$ not accounted by regression slope. In our study, 34 out of 40 clones had very low (less than 10%) CND_i values, which indicates relatively high stability for the clones involved.

S^2_{di}, estimated by employing regression approach, is another parameter that describes the contribution of each genotype to total $G \times E$. It is highly correlated with W_i ($r = 0.75$***) and CND_i ($r = 0.93$***).

If the regression coefficient (b_i) values are considered as stability measures, four best clones in height growth (height ranks 4, 6, 8 and 10) would have been determined as "sensitive" (because each had $b_i \geq 1.17$), and thus they have been eliminated if selection had been based on b_i value alone. Furthermore, b_i did not show any statistically significant relationships with W_i and S^2_{di}, as it was also the case in an earlier study on the same species (ST. CLAIR and KLEINSCHMIT, 1986). Studies with maize and barley also reported no significant relationships of b_i with W_i and (BECKER, 1981). BREESE (1969), POWELL *et al.* (1986) and BECKER and LÉON (1988) also indicated that b_i characterizes the specific response of a given genotype to environmental factors, not the "stability" *per se.* All these and our results show that b_i should not be treated as stability parameter by itself. We regard bi as a measure of "response" or "environmental sensitivity" of a genotype rather than as a direct measure of stability.

Table 3. – Various parametric stability-related indices* for 40 clones at seven test sites.

Clone code	Height rank	$S^2_{X_i}$	CV_i	b_i	r^2_i	S^2_{di}	W_i	$\% S^2_{di}$	$\% W_i$
123	1	15592	16.7	0.83	0.81	3623	21321	6.76	5.68
37	2	14830	16.9	0.88	0.95	950	6437	1.77	1.72
95	3	15544	17.3	0.90	0.95	917	5711	1.71	1.52
41	4	26502	22.8	1.17	0.95	1619	11364	3.02	3.03
188	5	16674	18.1	0.93	0.96	844	4691	1.57	1.25
107	6	37331	27.2	1.39	0.95	2227	28048	4.15	7.48
103	7	23948	21.8	1.11	0.94	1618	9456	3.02	2.52
26	8	28576	24.2	1.23	0.97	930	10595	1.73	2.82
143	9	17168	19.1	0.89	0.85	3148	17030	5.87	4.54
87	10	30621	25.6	1.27	0.97	1143	13926	2.13	3.71
152	11	27492	24.5	1.22	0.99	357	7058	0.67	1.88
145	12	18147	20.0	0.95	0.91	2013	10354	3.76	2.76
197	13	25590	23.8	1.17	0.98	725	6748	1.35	1.80
101	14	26703	24.7	1.19	0.96	1158	9591	2.16	2.56
46	15	22591	22.9	1.08	0.95	1338	7454	2.50	1.99
45	16	16019	19.4	0.92	0.97	613	3754	1.14	1.00
42	17	20317	21.9	1.01	0.92	1972	9872	3.68	2.63
18	18	18855	21.3	1.00	0.97	617	3085	1.15	0.82
50	19	21858	23.0	1.08	0.97	835	4801	1.56	1.28
125	20	7545	13.6	0.64	0.98	152	15240	0.28	4.06
90	21	15162	19.3	0.89	0.95	935	6084	1.74	1.62
113	22	20419	22.5	0.98	0.87	3297	16519	6.15	4.40
98	23	15153	19.4	0.88	0.94	1041	6692	1.94	1.78
196	24	14865	19.4	0.86	0.91	1546	9837	2.88	2.62
88	25	18053	21.4	0.97	0.94	1196	6111	2.23	1.63
115	26	19909	22.5	1.02	0.95	1264	6344	2.36	1.69
4	27	13943	18.9	0.81	0.87	2174	14653	4.06	3.91
66	28	12967	18.3	0.81	0.92	1187	9930	2.21	2.65
142	29	13105	18.5	0.82	0.94	1019	8711	1.90	2.32
116	30	25459	25.8	1.16	0.97	953	7615	1.78	2.03
9	31	16028	20.6	0.91	0.94	1164	6766	2.17	1.80
15	32	17943	22.0	0.98	0.97	632	3227	1.18	0.86
94	33	21236	24.6	1.05	0.94	1479	7624	2.76	2.03
118	34	21622	24.9	1.07	0.97	759	4350	1.42	1.16
104	35	13730	19.9	0.85	0.95	757	6382	1.41	1.70
181	36	26542	28.3	1.19	0.98	639	7256	1.19	1.93
112	37	16974	22.9	0.88	0.83	3394	18567	6.33	4.95
173	38	25740	29.1	1.18	0.99	256	4885	0.48	1.30
11	39	15632	22.7	0.91	0.97	644	4140	1.20	1.10
189	40	17894	25.6	0.93	0.89	2465	12854	4.60	3.43
Sum:						**53598**	**375080**		

* See the text for explanations of indices.

Rank-based Stability-related Measures

We estimated $S_i^{(1)}$ and $S_i^{(2)}$ values based on transformed height values for each clone (NASSAR and HUEHN, 1987). Genotypes with low $S_i^{(1)}$ and/or $S_i^{(2)}$ values are considered to have the high stability. To test whether a given genotype is significantly different in its $S_i^{(1)}$ value, we estimated $S^{(1)}$ statistics, which is the sum $Z_i^{(1)}$ values determined for each genotype (NASSAR and HUEHN, 1987). Overall test based on $S^{(1)}$ statistics ($S^{(1)} = 34.73$) showed that there were no significant differences among the stability values of the 40 different clones in our study.

To test significance of $S_i^{(2)}$ values of the clones, $Z_i^{(2)}$ values and the corresponding $S_i^{(2)}$ statistics were used and interpreted in the same way as $S_i^{(1)}$ and $Z_i^{(2)}$ statistics (NASSAR and HUEHN, 1987). Again, overall $S^{(2)}$ statistics [$S^{(2)} = 46.65$] were found to be smaller than Chi-square value, $\chi^2 = 55.76$ (d.f. 40, $\alpha = 0.05$), which indicates overall non-significance as it was the case with $S^{(1)}$ statistics.

Application on our data demonstrated that HUEHN's $S_i^{(1)}$ and $S_i^{(2)}$ stability measures are preferable to parametric measures in many ways. For example, $S_i^{(1)}$ and $S_i^{(2)}$ showed strong relationships both among themselves and with W_i, S^2_{di} and CND_i. Higher magnitudes of these empirical relationships between $S_i^{(1)}$ and $S_i^{(2)}$ and other stability-related parameters is a strong indication that rank-based stability parameters are compromising measures of stability, detecting measures also measured by other stability-related parameters. Furthermore, these parameters are based on ranks, less sensitive to errors of measurement than the parametric estimates, and addition or deletion of one or a few observations does not cause great deviation from the estimate, even if they could be outliers (NASSAR and HUEHN, 1987; BECKER and LÉON, 1988; HUEHN, 1990).

Relationships among parametric and rank-based stability-related parameters

The following statistically significant correlations were found among the parametric stability-related parameters: (Very strong, positive) between CV_i and S^2X_i, between CV_i and b_i, between b_i and S^2X_i, between W_i and S^2_{di}, between W_i and CND_i, between CND_i and S^2_{di}. (Strong, negative) between CND_i and b_i. (Moderate, negative) between CND_i and CV_i, CND_i and S^2X_i. Similarly, rank-based stability-related parameters, $S_i^{(1)}$ and $S_i^{(2)}$ showed very strong positive relationships both among themselves and with W_i, S^2_{di}, and CND_i.

Combined (stability + performance) measures

Among the several available methods that consider selection of both high stability and high performance simultaneously, we applied those proposed by HUEHN (1979, 1996) and NASSAR, LÉON and HUEHN (1994). Two of these parameters (CSP_{i1} and CSP_{i2}) have been applied by several researchers and were found to be very useful for selecting genotypes with both high stability and high performance (e.g., LÉON, 1986; KANG and PHAM, 1991). Application of both these and three other combined stability and performance parameters in our study also indicated that, all are very promising to detect genotypes with both acceptable (relatively high) stability and high performance. Suppose that top 25% of genotypes (10 clones) are selected based on height growth alone, clones with height ranks 1 to 10 would be selected (clones 123, 37, 95, 41,188, 107, 103, 26, 143, 87 in descending order). On the other hand, when selection is made on the basis of index CSP_{i1} alone, 6 of these top performers (i.e., 188, 95, 37, 41, 103 and 26) would also be included among the selected genotypes. Similarly, index CSP_{i2} would include 5 of the top performers (i.e., 188, 95, 37, 41, 26) and index CSP_{i3} would include 6 of them. Two recently proposed indices by NASSAR, LÉON and HUEHN (1994) (i.e., CSP_{i4} and CSP_{i5}) would include higher numbers of top growth performers: CSP_{i4} would have 8, and CSP_{i5} would have 9 of the top 10 growth performers [Clone 107 (height rank 6) would not be chosen by CSP_{i4}, and clone 87 (height rank 10) would not be chosen by both].

To view it from a different perspective, among the 25% to be selected, CSP_{i1} would select height ranks 5, 3, 2, 4, 18, 7, 8, 11, 17 and 16 in that order of preference. CSP_{i5} would select height ranks 1, 3, 2, 5, 4, 7, 8, 6, 12 and 9. Such sequencings can be made in similar ways for CSP_{i2}, CSP_{i3} and CSP_{i4} indices. It can be seen from these orderings that CSP_{i4} and CSP_{i5}, compared to the first three indices, gave more weight to performance than stability. In other words, CSP_{i4} and CSP_{i5} are relatively more "conservative" than CSP_{i1}, CSP_{i2} and CSP_{i3}, in that they are giving higher priority to high performing genotypes. An empirical observation in our data may clarify the point further: Clones 123 (height rank 1) and 143 (height rank 9) are among the first 10 top performers. Both appears less stable according to both $S_i^{(1)}$ and $S_i^{(2)}$ parameters (ranks 39 and 37, respectively in both parameters). These two clones would not be included among the top 10 clones selected by CSP_{i1}, CSP_{i2} and CSP_{i3}. However, they would be included among the top 10 clones detected by both CSP_{i4}, and CSP_{i5}.

Nassar *et al.* (1994) proposed a method to test significance of combined stability and performance (CSP_i) measures. In cases when there are 3 or more replications (i.e. 3 or more two-way c x s tables) one can use F statistics from *ANOVA* to test for differences and carry out a posteriori (multiple comparisons) tests among genotypes in regard to CSP_i measures.

There were very strong positive correlations among the combined stability and performance indices. All showed strong negative correlations with clonal height ($\bar{X}_{i.}$). Their significant negative relationships with clonal height imply that selecting for fast growing genotypes will also result in a base population with relatively stable individuals. Kang and Pham (1991) found similar relationships for CSP_{i1} and CSP_{i2} in several sets of maize trails. By looking at the magnitudes of the correlation coefficients, one may conclude that CSP_{i5} was a better predictor of height (performance) rank than the other indices. The order of predictive powers of CSP_i indices for performance was: CSP_{i5}, CSP_{i4}, CSP_{i3}, CSP_{i1} and CSP_{i2}.

CSP_i indices generally did not show any significant relationships with the parametric stability-related measures. Only CV_i (which is a "static concept" parameter as called by Becker and Léon, 1988) was moderately ($P < 0.05$) correlated with four of them, and nearly so with CSP_{i2} ($P < 0.06$). This means that any clone that show less variation (more uniformity) among the test sites had higher probability of being detected by any of the CSP_i indices, which is an advantage for the breeder.

The correlation coefficients between CSP_i indices and rank-based stability measures [$S_i^{(1)}$ and $S_i^{(2)}$] were also not significant, except in one case: CSP_{i5} showed moderate negative relationships ($r = -0.35$, $P < 0.05$) with $S_i^{(2)}$, and nearly so with $S_i^{(1)}$ ($r = -0.31$, $P < 0.07$). These negative relationships between CSP_{i5} and $S_i^{(1)}$, CSP_{i5} and $S_i^{(2)}$ parameters are additional indication that CSP_{i5} gives more weight to height than stability values. The results suggest that CSP_{i5} is better than the other four CSP_i indices. In accordance with the above considerations, Huehn (1996) in his review suggest that CSP_{i5} is preferable since it has a slightly higher power than the indices CSP_{i3} and CSP_{i4}. One additional convenience is that it does not require transformation of the original data.

The lack of significant correlations between CSP_i indices and the other stability-related parameters can be explained by the modified nature of the CSP_i indices. They combine both stability and performance levels, which is not considered simultaneously by the other measures.

In regard to combined stability and performance indices (CSP_i), there remains one question to be settled: Should stability and performance be weighted equally? Kang and Pham (1991) discussed and compared several indices developed by differential weighting procedures. They concluded that index that assumed equal weight for yield (performance) and stability would be preferable to those that give 2-, 3-, 4- and 5-times more weight to performance than stability. They also found Huehn's CSP_{i1} and CSP_{i2} indices (before the three others were proposed) are more useful than "weighted" indices in detecting genotypes with high performance and stability simultaneously. The general expectation concerning interaction of different genetic entities is that clones exhibit higher $G \times E$ than populations and progenies, since they have not the genetic buffering possessed by populations and/or progenies against environmental heterogeneity. This buffering prevents high $G \times E$ by favoring different genotypes under different environments, and thus stabilizing the entity (Kleinschmit, 1983; Huehn, 1992). In our study, however, clonal material (which is growing under very contrasting environments) showed relatively low level of interaction. Regarding stability, these results are somewhat different from the general expectation. One explanation for this may be the fact that the clones in this study are originating from third propagation cycle, which implies three previous selection steps under different annual and – as far as field tests are included – spatial environments. This procedure by itself indirectly includes a selection for uniformity with an apparent consequence that the resulting individual genotypes (clones) can be relatively stable over a quite considerable range of environments. In view of faster environmental changes such genotypes are preferable.

Conclusions

1) Variance component due to $G \times E$ (clone test site) interaction was statistically significant by parametric *ANOVA* tests. However, when rank-based tests by $S^{(m)}$ statistics were applied on the same data, $G \times E$ interaction was not statistically significant. In this respect, *ANOVA* tests, which require several firm assumptions to be fulfilled, appear to be more conservative than rank-based tests.

2) Our results showed that there were no significant relationships between clonal W_i and height values. W_i is a good measure to determine interactive behavior of individual clones, yet it is not a preferable stability parameter *per se*, to select good performers.

3) In general, genotypes that had low regression slopes ($b_i < 1.0$) have been considered "stable" and thus desirable in many of the earlier studies. Our results showed that many genotypes, in contrast to their relatively low b_i values, had relatively high W_i values (i.e., greater contribution to interaction), or *vice versa*. There were no significant relationships between b_i and $\bar{X}_{i.}$, b_i and W_i, b_i and S^2_{di} values. Therefore, according to our results, b_i, *per se*, is also not a good measure for stability of a given genotype.

4) Clonal CV_i value derived from environmental variance ($S^2{}_{X_i}$) is better indicator of relative uniformity across the test sites than $S^2{}_{X_i}$ itself. CV_i had a significant negative relationship with average clonal height, indicating that taller clones exhibited greater uniformity among the test sites, which is a desirable outcome for the breeder's interests. Among the top 10 height performers, 5 genotypes (those with height ranks 1, 2, 3, 5 and 9) would also be among the best 10 CV_i clones in that order.

5) CND_i indicates the proportion of interaction not accounted by regression slopes. In other words, relatively small CND_i values in our study show that higher proportion of $G \times E$ is attributable to environmental changes associated with site conditions. Very strong positive correlations of CND_i with S^2_{di}, and also with W_i indicate that genotypes identified by either S^2_{di} or W_i for their high interaction can also be detected by CND_i with a very high probability.

6) Rank-based stability measures estimated in our study (i.e., $S_i^{(1)}$ and $S_i^{(2)}$) are free from the rigid assumptions of parametric tests, and they are less sensitive to errors of measurement than the parametric estimates. Both had strong relationships with each other, and with Wi, S^2_{di} and CND_i; implying that $S_i^{(1)}$ and $S_i^{(2)}$ detect effects also measured by other stability-related parameters. According to our results, $S^{(m)}$ test, which employs $S_i^{(1)}$ and $S_i^{(2)}$ indices, appear to be more liberal than *ANOVA* tests to detect significance in $G \times E$. Overall, both $S_i^{(1)}$ and $S_i^{(2)}$ could be a highly compromising indices for stability, where $S_i^{(1)}$ was a better measure than $S_i^{(2)}$.

7) Genotypes, which are identified as "stable" and hence desirable based on their stability-related parameters, may not be, in many cases, the ones with desirable growth performance.

Therefore, we estimated five separate indices (CSP_{i1} to CSP_{i5}) that combine stability and performance simultaneously. All of these indices were very effective to detect genotypes with both desirable stability and performance levels. Among the five indices CSP_{i5} was the best index both in its predictive power and in its applicability.

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Inducing Male Flowering by Applying Gibberellic Acid has no Effect on the Cry j 1 Content in *Cryptomeria japonica* Pollen

By Y. Goto[1*], T. Kondo[1] and H. Yasueda[2]

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Abstract

Cryptomeria japonica pollinosis has recently become a serious problem in Japan. Two major allergens of *C. japonica* pollinosis, Cry j 1 and Cry j 2, have been isolated and characterized. Cry j 1 and Cry j 2 are basic proteins with molecular weights of 41-46 kDa and 37 kDa, respectively, and it was reported that more than 90% of *C. japonica* pollinosis patients had IgE specific to both of them. Several studies have found large variations in the content of Cry j 1, a major allergen of *C. japonica* pollinosis, suggesting that pollinosis could be reduced by replacing current *C. japonica* varieties with trees that produce less Cry j 1. In this study, Cry j 1 contents were compared in pollen produced with and without inducing male flowering by applying gibberellic acid (GA), which is a very useful technique for stimulating pollen production in targeted trees. No effect of

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[1] Forest Tree Breeding Center, 3809-1 Ishi, Juo, Taga, Ibaraki, Japan

[2] Clinical Research Center for Allergy and Rheumatology, National Sagamihara Hospital, Sakuradai 18-1, Sagamihara, Kanagawa, Japan

[*] Corresponding author. Forest Tree Breeding Center, Ishi 3809-1, Juo, Taga, Ibaraki 319-1301, Japan. Tel: +81-293-32-7000. Fax: +81-293-32-7306. E-mail: gomama@affrc.go.jp