Analysis of Genotype × Environment Interaction by Stability Estimates

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Genotype × environmental interaction (GEI) is an important consideration in plant breeding programs because it reduces the progress from selection in any one environment (Hill, 1975). Significant GEI results from the changes in the magnitude of differences between genotypes in different environments or changes in the relative ranking of the genotypes. Consistent performances across different sites and/or years are referred to as stability. Partitioning GEI into stability statistics assignable to each genotype evaluated across a range of environments is useful in selecting stable genotypes. Different stability estimates are proposed to measure the stability of genotypes tested under a wide range of environments (Fernandez et al., 1989; Hill, 1975; Pritts and Luby, 1990). The most popular methods (Eberhart and Russell, 1966; Finlay and Wilkinson, 1963) have used analysis of variance combined with joint regression analysis to determine whether GEI is a linear function of the additive environment. Genotypes having unit slope (Finlay and Wilkinson, 1963), insignificant deviation from regression (Eberhart and Russell, 1966), and a large coefficient of determination (r²) (Pinthus, 1973) are considered averagely stable.

One of the essential features in developing this regression technique was the estimation of the environmental index (EI), an independent variable, which is obtained by subtracting the environmental mean from the grand mean. Although joint regression analysis (Eberhart and Russell, 1966) has been extensively used to study stability, the statistical validity of this method has been questioned because genotypic means are not statistically independent of the EI on which they are regressed (Hill, 1975; Shukla, 1972). However, Hardwick and Wood (1972) claimed that when many genotypes are included in the experiment and the environmental range is such that the between-environments mean square is significantly greater than error mean square, any bias that results should not prove serious in practice. Freeman (1973) also agreed that this regression procedure is perfectly valid provided the model is fixed and inferences are drawn only about the sample of genotypes and environments used in the analysis.

Others have approached this problem by proposing different methods to overcome the limitation of regressing one variable onto another that is not independent of it. Wricke (1962) and Shukla (1972) measured stability on the basis of the contribution of a genotype to the GEI sums of squares and two stability parameters, ecovalue stability index (W, Wricke, 1962) and stability variance (σ²), which were developed. Kang et al. (1987) have shown that the estimation of ecovalue is an intermediate step in calculating stability variance and therefore stability variance is a coded value of ecovalue. Shukla’s method also provides additional information on stability by using covariate to remove the linear effect of environment from GEI. The remainder of the GEI variance can be partitioned into a component, S (adjusted stability variance), assignable to each genotype, and the significance of this component can be tested by an approximate F test. However, the methodology for computations of the stability variance and the S components was tedious, especially when many genotypes were evaluated across environments.

Tai (1971) proposed partitioning the GEI effect of the ith genotype into stability statistics α and λ, based on the principles of structural analysis. The α measures the linear response of the environmental effect, and λ measures the deviation from the linear response in terms of the magnitude of the error variance. A genotype having α = 0 and λ = 1 was considered of average stability. Approximate procedures for testing the hypotheses α = 0 and λ = 1 were given, and a method of obtaining the prediction interval for α = 0 and a confidence interval for λ, values so that genotypes can be distributed in different stability regions were also suggested (Tai, 1971).

The importance of assessing the stability of performance in horticultural crops was reported recently (Pritts and Luby, 1990). Because of the complex computations involved in the calculations, Tai’s and Shukla’s stability estimates are not extensively used in horticultural research. Kang (1985) developed a computer program written in the MATRIX programming language of Statistical Analysis System (SAS) (SAS Institute, Inc, 1988) to estimate Shukla’s stability estimates. However, MATRIX programming is quite complicated for the average user in horticultural science.

The use of SAS software in statistical analysis is rapidly increasing with the availability of command-driven SAS for personal computers (PC-SAS). In a recent study, PC-SAS was identified as one of the more versatile and easy-to-use software programs available (Milliken and Remmenga, 1989). In addition, PC-SAS supports powerful data management and is quite flexible in formatting output. Therefore, developing PC-SAS programs to estimate these stability estimates will increase the use of stability analyses in horticultural research.

When Tai’s (α, λ) and Shukla’s (σ², S) stability statistics were compared using Spearman’s rank correlation, they were closely related for potato (Tai, 1979) and sweetpotato genotypes (Bacusmo et al., 1988). However, the statistical significance of these stability statistics was not in agreement. The reasons for this disagreement were not reported in the literature.

PC-SAS programs for calculating Tai’s and Shukla’s stability statistics and the similarities between Tai’s and Shukla’s stability analyses are presented here.

STABILITY STATISTICS

Tai’s stability statistics

The GEI component (ge) (Tai, 1971) for genotypes evaluated in a randomized complete block design over a series of environments equals

\[
(Y_{ij} - \bar{Y}_i - \bar{Y}_j + \bar{Y}_.)
\]

Where: \(Y_{ij}\) is the mean response over replicates of the ith genotype in the jth location. \(\bar{Y}_i\) = mean of ith genotype, \(\bar{Y}_j\) = mean of jth location. \(\bar{Y}_.\) = grand mean.

For the following calculations, let: \(m = \) number of genotypes; \(n = \) number of locations or environments; \(r = \) number of replicates or replicates × years, if applicable; \(\text{MSE} = \) mean square error; \(\text{MSL} = \) mean square location, \(\text{MSB} = \) mean square blocks.

Calculation of α and λ

Environmental index (EI), site mean adjusted for the grand mean:

\[
(\text{EI}_i) = \bar{Y}_j - \bar{Y}_.,
\]

Restricted (β = 0) least squares of the GEI component for ith genotype on EI:

\[
ge_i = T + \beta_0 + \beta_i(\text{EI}_j) + e_i
\]

where \(\beta_0 = \) intercept = 0; \(\beta = \) regression coefficient; α = regression coefficient adjusted for blocks. MSD = mean square de-
viation from the regression (Eq. [3]).

$$\hat{b}_i = \frac{\sum [ge_i \cdot EL_i]}{\sum E_i L_i}$$  \[4\]

$$\hat{d}_i = \frac{\beta_i MSL}{(MSL - MS_B)}$$  \[5\]

$$\text{M}S_D_i = \frac{S_i^2 \cdot (\alpha_i^2 + \alpha_i \cdot (E_i L_i))}{n - 1}$$  \[6\]

Because restricted least squares were used, the error df in the regression model was \(n - 1\).

$$\hat{\lambda}_i = \frac{\text{M}S_D_i}{(m - 1)\left(\frac{\text{M}S_E}{m r}\right)}$$  \[7\]

95% Prediction limits for \(a_i\), corresponding to \(a = 0\)

$$= \lambda_i \left(1 - \frac{\lambda_i(n - 1)\text{M}S_E \text{M}S_B}{(MSL - MS_B)(n - 2)\text{M}S_E - (\lambda_i)^2 + (n - 2)\text{M}S_B}\right)^{\frac{1}{2}}$$  \[8\]

Testing \(\lambda_i = 0\) at 0.05 level

\(\lambda_i\) value greater than \(F^*\) value is significant at \(P = 0.05\), where \(F^* = F_{0.05.2}^*\) value with \(n - 2\), \(n(m - 1)(m - 1)\) numerator and denominator df, respectively.

Shukla's stability statistics

Because the means of the genotypes over replication are used in the following computations instead of the sum of the values over all replications, the stability statistics are adjusted by multiplying by the number of replicates as suggested by Kang (1986).

Ecovalence (W) = The contribution of a genotype to the GEI sums of squares.

$$W_i = \left[\sum g_i e_i\right]^2 r$$  \[9\]

Ecovalence means squares = WMS

$$\hat{W}_i \text{MS} = \frac{W_i}{(m - 1)(n - 1)/m}$$  \[10\]


$$d_i^2 = \frac{1}{(n - 1)(m - 1)(m - 2)}$$

$$= \frac{1}{(m - 1)W_i - \sum W_i}$$  \[11\]

Stability variance (\(S_i^2\)) adjusted for EI from Shukla's Eq. [16]:

$$\hat{S}_i = \frac{m}{(m - 2)(n - 2)}\left(\frac{S_i}{S_i - \sum S_i}{r(m - 1)}\right)$$  \[12\]

Where: \(S_i = r[S_i(\sum e_i - \beta_1 L_i)]\)

The significance of WMS, \(d_i^2\), and \(S_i^2\) can be tested by an approximate F test,

$$F_{0.05} = \frac{W_i \text{MS}}{\sigma_i^2} \text{ or } \frac{S_i^2}{\sigma_i^2}$$  \[13\]

with \(n - 1\) and \(m(r - 1)\) df in which \(\sigma_i^2\) is pooled mean square error.

**RESULTS AND DISCUSSION**

Published data from Tai (1971; table 1) and Shukla (1972; table 1) were used to verify the PC-SAS programs. SAS statements used to compute Shukla's and Tai's stability estimates are given in Table 1 and Table 2, respectively. Explanations for major steps are given as comments within the SAS program.

To verify the working and accuracy of the program, the stability estimates obtained from this program were compared with the values reported by Tai (1971) and Shukla (1972). The results of the stability analysis (Tables 3 and 4) confirm these SAS programs and compute Tai's (1971) and Shukla's (1972) stability estimates correctly. The stability estimates Alpha and Lambda (Table 3) agree with \(a_i\) and \(\lambda_i\) from Tai's (1971) table 4.

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Similarly the Stabvar, F-stbvar, Sigsq, and F-sigsq (Table 4) agree with \(d_i^2\), \(\sigma_i^2\), \(S_i\), and \(F\), respectively, from Shukla's (1972) table 4.

These PC-SAS programs provide a way of computing stability estimates using 'PROC GLM', 'PROC REG', 'PROC MEANS' and data manipulations that are familiar to the average user. Both these programs required \(\approx 4\) to 5 min to estimate the components with a Zenith, L-286-Lp AT computer, with a math coprocessor, 640 K RAM and 40 megabytes hard disk drive. With the development of these programs the use of the stability estimates should become more frequent in plant breeding programs in horticultural research.

Comparisons between Tai's and Shukla's stability analyses

Tai's data (1971; table 1) were used to compare Tai's and Shukla's stability analyses. The comparisons of the stability estimates by these two methods are presented in Table 5. Although both \(a_i\) and \(S_i\) statistics were closely associated with the rank correlations (Table 6), the significance level was not in agreement for the potato genotype 'Katahdin' (Table 5). When the two stability analyses were compared, the following similarities and dissimilarities were found:

i) Both methods used the GEI component,
Tai's method uses interaction means while Skukla's method uses interaction totals in the analysis.

Both methods used the EI as a covariate to remove the linear effect of the environment from the GE component in a regression model $g_e = b_e + b_i E_i$ (Eq. [3]) with a restriction on $b_e = 0$. Thus, Tai's $\lambda_i$ is closely related to Shukla's $\lambda_i$ except that $\alpha$ is adjusted for the block effect (Eq. [4]).

Deviation from the regression is used to estimate both $\lambda_i$ and $S_i^2$. However, $\lambda_i$ is estimated by dividing the mean square deviation (MSD) from regression by the experimental error component $[(m - 2)\text{MSE}/(m - 1)]$ (Eq. [7]), where $m$ = no. of genotypes and $n$ = no. of replicates. Thus, $\lambda_i$ always will be positive and unitless. In $S_i^2$ computation, an adjustment is made for those genotypes included in the analysis by subtracting $[2 \sum\text{total deviation}/(m - 1)]$ from the total deviation of $i$-th genotype and is multiplied by $[m/(m - 2)]$ (Eq. [12]), where $n$ = no. of sites. Thus, $S_i^2$ can be negative.

The significance of $\lambda_i$ is based on a 95% confidence interval, where a $\lambda_i$ value greater than $F_{0.05} - value$ for the ratio $\text{df} = n - 2$ is considered significant at $P = 0.05$. The significance of $S_i^2$ in terms of MSE is derived by an approximate F-test with $\text{df} = n - 1$ and $n(m - 1)$ as numerator and $n(m - 2)$ as denominator. Thus, $\lambda_i$ values can be compared for adequacy of the $F$-test.

Tai's analysis also provides a method of determining the genotypic stability. Thus, Tai's method is more informative than Shukla's method.

SUMMARY

When Tai's and Shukla's stability analyses were compared, these stability estimates were closely associated in rank correlations. However, the significance levels of Tai's $\lambda_i$ and Shukla's $S_i^2$ were not in agreement because, for a given significance level ($P = 0.05$), the $F$ value chosen for $F_{0.05}$ test and the numerator $\text{df} = n - 2$ vs. $n - 1$ are contradictory between Tai's and Shukla's stability statistics, respectively. Thus, the genotype 'Katahdin' was identified as stable by Tai's analysis and not stable by Shukla's analysis because of the differences in performing these significance tests. Shukla's $F$ test is appropriate only when the number of genotypes included in the analysis is large. Tai's analysis provides a method of calculating stability values so that genotypes can be distributed graphically in different stability regions. Shukla's method uses only $S_i^2$ in determining the genotypic stability. Thus, Tai's method is more informative than Shukla's method.

Table 2. PC-SAS program statements for computing Tai's stability statistics.

```
data tai;
  infile "b-tal.dat";
  do line=1 to 8; do loc=1 to 6;
    input yield @;
    output; end; end;
  cards;
  proc glm; class loc line;
  model yield=loc line/nouni;
  output out=res r=gei; run;
  proc sort data=tai by loc; run;
  proc means mean noprint; by loc; var yield;
  output out=lj=mean=lj; run;
  proc means mean noprint; var lj;
  output out = gm mean = g; run;
  data envi; if _n_ = 1 then set gm; set lm;
  el=lj-g;
  proc sort data=res; by loc; run;
  proc data combine; merge res envi; by loc; drop _type__nreq__; run;
  proc sort data=combine; by line; run;
  proc reg; by line;
  model gei=el; restrict intercept=0; run;
  data lambda;
  input line mean bi dms @;
  *From reg output;
  *No. of regs (R), lines (L), locations (N), mean square error (MSE), mean square location (MSE), and mean square blocks (MSB);*
  r=8; m=8; n=6; mse=4.68; msl=428.17; msb=8.17;
  alphain=(bi*(msl/msb)); alphal=abs(alphain);
  lambda=bi/(msb*(m-1))/(msb);*
  *Calculation of 5% prediction interval for alpha=0;
  t95=inv(0.95);
  num=lambda*(m-1)*msb/msl;
  den=((msl-msb)*((n-2)*msl)*((t95**2)+(n-2)*msb));
  pialpha=abs(t95*sqrt(num/den));
  signi='ns'; if pialpha > pialpha then signi='*';
  n1=n-2; n2=n*(m-1)/(m-2); prob=1-prob(pialpha,n1,n2)*2;
  cards;
  1 11.20 - .0447 5.743 2 12.04 -.2026 1.544 3 17.79 - .0470 3.442
  4 9.65 - .158 5.067 5 15.35 .2094 20.526 6 17.99 -.242 12.653
  7 13.95 .3746 2.698 8 14.9 .1109 1.237
  proc print;
  var line mean alphain lambda prob;
```

Table 3. Results from PC-SAS output containing stability estimates that agree with those from table 4 of Shukla, 1972.

```
line  eco  f-eco  p-eco  stab-var  stab-var  stab-var  signi  f-signi  p-signi
  1  25.233  1.627  0.153  25.879  1.111  0.355  34.100  1.464  0.202
  2  22.114  1.424  0.216  19.600  0.841  0.521  40.485  1.739  0.126
  3  23.679  1.525  0.182  22.731  0.976  0.433  42.805  1.838  0.106
  4  12.08  0.959  0.000  22.53  9.687  0.000  79.638  3.420  0.005
  5  50.154  3.231  0.007  75.680  3.250  0.007  23.263  0.999  0.419
```

Table 4. Results from the PC-SAS output containing stability estimates that agree with those from table 4 of Tai, 1971.

```
line  mean  alpha  pallet  signi  lambda  prob
  1  53.17  -0.045  0.71992  ns  5.6098  0.0069
  2  57.51  -0.206  0.37328  1.5082  0.4074
  3  58.15  -0.047  0.55754  3.3621  0.0037
  4  58.8  -0.15  0.6762  4.9495  0.0019
  5  59.33  -0.131  1.36102  20.049  0.0000
  6  60.32  -0.246  1.06895  12.359  0.0000
  7  7.99  -0.381  0.4953  2.6364  0.07416
  8  14.9  0.113  0.33412  1.2083  0.62121
```

Mean = Mean tuber yield (kg/plot); Alpha = $\alpha_i$; Pialpha = 95% prediction interval for $\alpha_i$ = 0; Signi = Significance level; Lambda = $\lambda_i$; Prob: Significance levels for testing $\lambda_i = 1$. 

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obtaining the prediction interval for $\alpha_i = 0$ and a confidence interval for $\lambda_i$ values so that genotypes can be distributed graphically in different stability regions. Shukla’s method uses only $S_i^2$ in determining the genotypic stability. Thus, Tai’s method is preferred, especially when the number of genotypes included in the analysis is small ($< 8$).

**APPENDIX**

**Glossary of terms**

Ecovalence ($W_i$): The contribution of a genotype to the genotype × environmental interaction (GEI) sums of squares.

Environmental index (EI): A measurement on the mean performance of an environment over genotypes adjusted for the overall mean (grand mean).

Shukla’s stability variance ($\sigma_i^2$): The contribution of a genotype to the GEI sums of squares after adjusting for the average genotypic contribution to the GEI sums of squares.

Shukla’s adjusted stability variance ($S_i^2$): Stability variance adjusted for the environmental index as covariate.

Stability: Consistency of genotypic performance evaluated in different years and/or locations.

Tai’s $\alpha_i$ is a component of GEI sums of squares measured as the linear response of a genotype to the environmental index.

Tai’s $\alpha_i$ is a component of GEI sums of squares measured as the deviation from linear response in terms of the magnitude of the error variance.

**Literature Cited**


### Table 5. Comparison of the stability estimates for Tai’s (1971) 1965-66 data.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Tai’s estimates</th>
<th>Shukla’s estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\alpha_i$</td>
<td>$\lambda_i$</td>
</tr>
<tr>
<td>F5317</td>
<td>-0.046</td>
<td>5.61***</td>
</tr>
<tr>
<td>F5751</td>
<td>-0.026</td>
<td>1.51NS</td>
</tr>
<tr>
<td>F5815</td>
<td>-0.048</td>
<td>3.36*</td>
</tr>
<tr>
<td>F5820</td>
<td>-0.161</td>
<td>4.95**</td>
</tr>
<tr>
<td>F5933</td>
<td>0.213</td>
<td>20.05***</td>
</tr>
<tr>
<td>F6032</td>
<td>-0.246</td>
<td>12.36***</td>
</tr>
<tr>
<td>Keshulina</td>
<td>0.262</td>
<td>2.63**</td>
</tr>
<tr>
<td>Sebago</td>
<td>0.113</td>
<td>1.20NS</td>
</tr>
</tbody>
</table>

*Linear response to EI adjusted for the blocks.

**Deviation from the linear response.

*Stability variance.

Stability variance adjusted for the covariate, EI.

**NS**: Not significant or significant at $P = 0.05$, 0.01, or 0.001, respectively.

### Table 6. Correlation coefficients between Tai’s and Shukla’s stability statistics.

<table>
<thead>
<tr>
<th>Linear correlation</th>
<th>Spearman’s rank correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_i^2$ and $\beta_i^2$</td>
<td>0.96***</td>
</tr>
<tr>
<td>$\lambda_i^2$ and $\sigma_i^2$</td>
<td>0.99***</td>
</tr>
<tr>
<td>$\lambda_i$ and $S_i^2$</td>
<td>1.00***</td>
</tr>
</tbody>
</table>

*Linear response to EI adjusted for the blocks (Tai, 1971).

**Linear response to EI (Shukla, 1972).

**Deviation from the linear response (Tai, 1971).

*Stability variance (Shukla, 1972).

**Stability variance adjusted for the covariate, EI (Shukla, 1972)

***Significant at $P = 0.001$.