Application of a generalized Grubbs' model in the analysis of genotype–environment interaction

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The presence of genotype–environment interaction is a common feature of yield trial data. Estimating variance components attributable to the interactions of single cultivars is a useful means of assessing phenotypic stability. The stability variance is one such measure. In this paper it is argued that the stability variance is appropriate only if the error variances are homogeneous across genotypes and the number of replicates is the same in each environment. An alternative is suggested for the case that either of these two assumptions is violated.

Keywords: genotype–environment interaction, Grubbs' estimate, heteroscedasticity, MINQUE, phenotypic stability, yield trial.

Introduction

Genotype–environment interactions play an important role in the analysis of regional yield trials. It is common to interpret interaction in terms of stability. Large interactions of a genotype with different environments are often seen as an indication of low stability of that genotype. According to this concept maximally stable genotypes do not interact with environments. Many other concepts exist; they have been comprehensively reviewed elsewhere (Lin *et al.*, 1986; Westcott 1986; Becker & Léon 1988; Crossa, 1990).

It is desirable to find a measure that assesses instability so defined with regard to interaction as indicative of instability. Wricke (1962) proposed the so-called ecovalence, which is the contribution of a genotype to the total genotype–environmental interaction sum of squares. Similar measures have been proposed by Plaisted & Peterson (1959), Plaisted (1960) and Shukla (1972). Lin *et al.* (1986) have shown that all these measures are statistically inter-related and that they are equivalent for ranking purposes.

We prefer Shukla's estimator because it has certain statistical advantages such as unbiasedness. This estimator may be shown to be equivalent to Grubbs' (1948) estimator of measurement errors (Piepho, 1992). Its interpretation as a measure of phenotypic stability, however, rests on two important, rather restrictive assumptions. Firstly, error variances are assumed to be homogeneous, and secondly, the data

from which the stability variance is estimated must be balanced.

In a previous paper (Piepho, 1994a), we have proposed procedures to estimate stability, when data are unbalanced because of a lack of information on some genotype–environment combinations. In this paper we will deal with data displaying unbalancedness in the number of replications per environment. Attention will be restricted to randomized complete block designs (RCBs) because these prevail in the vast majority of yield trials (Gauch, 1992). We will also allow for heterogeneous error variances and explore the consequences this has with regard to the definition and estimation of stability.

A definition of stability

Let us assume that in each of N environments, the same set of K genotypes was tested in a RCB design with R_j ($j = 1, \dots, N$) replicates. The model for the observation y_{ijr} of the i th genotype from the r th block in the j th environment can be written as:

$$y_{ijr} = \mu + \tau_i + p_j + g_{ij} + b_{r(j)} + e_{ijr} \quad (1)$$

$(i = 1, \dots, K; j = 1, \dots, N; r = 1, \dots, R_j)$,

where μ , τ_i , p_j , g_{ij} , $b_{r(j)}$ and e_{ijr} are, respectively, the grand mean, the effect of the i th genotype, the j th environment, the interaction of genotype i and environment j , the effect of the r th block in the j th

environment and the error associated with y_{ijr} . It is assumed that environments, blocks and interactions are random whereas genotypes are fixed. We allow for both the interaction variance and the error variance to be heterogeneous among genotypes. Therefore, these variances will be denoted as σ_{gi}^2 and σ_{ei}^2 , respectively. Also, the number of replicates need not be the same in each environment. Now consider the means across replications, for which the model can be written as:

$$\begin{aligned}\bar{y}_{ij} &= \mu + \tau_i + p_j + g_{ij} + \bar{b}_{\cdot(j)} + \bar{e}_{ij} \\ &= \mu + \tau_i + p'_j + g_{ij} + \bar{e}_{ij} \\ &= \mu + \tau_i + p'_j + v_{ij},\end{aligned}\quad (2)$$

where $p'_j = p_j + \bar{b}_{\cdot(j)}$, $\bar{b}_{\cdot(j)} = \sum_r b_{r(j)}/R_j$, $v_{ij} = g_{ij} + \bar{e}_{ij}$, and $\bar{e}_{ij} = \sum_r e_{ijr}/R_j$. Apart from the block mean, which has no influence on any of the estimates to be discussed in this paper, this is the model used by Shukla (1972) to estimate the stability variance. He assumes that $R_j = R$ for all j and $\sigma_{ei}^2 = \sigma_e^2$ for all i , and defines stability in terms of σ_{gi}^2 . Maximum stability occurs if $\sigma_{gi}^2 = 0$. One could seek an estimate of σ_{gi}^2 but Shukla (1972) suggests estimating $\sigma_{vi}^2 = \sigma_{gi}^2 + \sigma_e^2/R$ instead, which is computationally simpler and yields the same information, as we are basically interested in comparisons among genotypes, i.e. in the contrasts $\sigma_{gi}^2 - \sigma_{git'}^2$. It is easily seen that under the restrictive assumptions made by Shukla (1972) this is equivalent to estimating $\sigma_{vi}^2 - \sigma_{vit'}^2$. The variance σ_{vi}^2 is referred to as the stability variance (Shukla 1972).

Matters become slightly more complicated when error variances are heteroscedastic. In this case it is more useful to estimate both σ_{gi}^2 and σ_{ei}^2 and assess stability in terms of σ_{gi}^2 . In what follows we will give estimates for these two variances for the general case that the number of replicates is not the same in all environments.

Estimating σ_{gi}^2 and σ_{ei}^2

To assess stability as defined above, we need estimates of σ_{gi}^2 and σ_{ei}^2 when error variances are heterogeneous (a test for homoscedasticity is described in the next section). The model in eqn 1 is equivalent to the generalized Grubbs' model used by Deutler (1991), who discussed estimation of σ_{gi}^2 and σ_{ei}^2 . Estimation is different for $K=2$ and $K \geq 3$. We will restrict our attention to $K \geq 3$ and refer the reader to Deutler (1991) for the case $K=2$. In order to clarify the relationship to Shukla's estimate of the stability variance, we will express the estimates in a form different from that given in Deutler (1991). Estimates S_{ei}^2 and S_{gi}^2 of σ_{ei}^2 and σ_{gi}^2 are given by:

$$S_{ei}^2 = \frac{K(K-1)U_i - \sum_i U_i}{(\sum_j R_j - N)(K-1)(K-2)},$$

where

$$U_i = \sum_{j=1}^N \sum_{r=1}^{R_j} (y_{ijr} - \bar{y}_{ij} - \bar{y}_{\cdot jr} + \bar{y}_{\cdot j})^2 \quad \text{and}$$

$$S_{gi}^2 = S_{vi}^2 - \frac{N}{\sum_j R_j} S_{ei}^2,$$

where

$$S_{vi}^2 = \frac{NK(K-1)Z_i - N\sum_i Z_i}{\sum_j R_j(N-1)(K-1)(K-2)},$$

with

$$Z_i = \sum_j R_j (\bar{y}_{ij} - \bar{y}_{i..} - \bar{y}_{\cdot j} + \bar{y}_{\cdot \cdot})^2.$$

S_{gi}^2 and S_{ei}^2 are MINQUE (Minimum Norm Quadratic Unbiased Estimators) as is Shukla's estimator of the stability variance (Shukla 1972).

Assessing stability

S_{vi}^2 is an unbiased estimator of

$$\sigma_{gi}^2 + \frac{N}{\sum_j R_j} \sigma_{ei}^2.$$

Therefore, estimation of σ_{gi}^2 is only worthwhile if error variances are heterogeneous. Otherwise the contrasts $S_{vi}^2 - S_{vit'}^2$ and $S_{gi}^2 - S_{git'}^2$ are both unbiased estimators of the stability difference $\sigma_{gi}^2 - \sigma_{git'}^2$. In the latter case it is better to use S_{vi}^2 because S_{gi}^2 will show a higher sampling error because of the subtracted multiple of S_{ei}^2 . To decide whether S_{gi}^2 or S_{vi}^2 is appropriate, a preliminary test of heteroscedasticity of σ_{ei}^2 is in order. This may be carried out for each environment by any test for homoscedasticity in a RCB design. Three such tests are those by Anscombe (1981), Shukla (1982) and Brindley & Bradley (1985) (see also Piepho (1993)). Unfortunately, these tests are rather sensitive to departures from the normality assumption (Brindley & Bradley, 1985; Piepho 1992). Unless there is evidence that errors e_{ijr} are normally distributed, these tests cannot be recommended. We suggest the following distribution-free procedure, which is an extension of a test suggested by Piepho (1994b).

Step 1. Compute U_{ij} , the error sum of squares associated with the i th genotype in the j th environ-

ment:

$$U_{ij} = \sum_{r=1}^{R_j} (y_{ijr} - \bar{y}_{ij.} - \bar{y}_{.jr} + \bar{y}_{.j.})^2.$$

Step 2. Arrange the resulting estimates in a two-way table of K rows and N columns.

Step 3. Perform the Friedman test (Gibbons, 1985) on this table of U_{ij} s, regarding genotypes (rows) as treatments and environments (columns) as replicates.

If the Friedman test is significant, it is reasonable to assume that the σ_{ei}^2 s are heterogeneous, in which case estimation of σ_{gi}^2 is worthwhile.

It is interesting to note that, if $R_j = R$ and $\sigma_{ei}^2 = \sigma_e^2$, S_{vi}^2 reduces to Shukla's estimator of the stability variance:

$$\hat{\sigma}_{vi}^2 = \frac{K(K-1)W_i - \sum_i W_i}{(N-1)(K-1)(K-2)}$$

with

$$W_i = \sum_j (\bar{y}_{ij.} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y}_{...})^2,$$

which then estimates $\sigma_{gi}^2 + \sigma_e^2/R$, where σ_e^2 is the common error variance. In summary, we suggest assessing stability by S_{gi}^2 when error variances are heterogeneous. When variances are homogeneous, one may use S_{vi}^2 if the number of replicates is not the same in all environments, while Shukla's estimate of the stability variance is appropriate if the number of replicates is constant.

Biological example

To exemplify the procedure outlined in this paper, we employed a *Faba* bean data set from German registration trials. It comprises the yields of 14 genotypes tested in nine environments. In each environment the trial was laid out as a RCB design with four replications. The fourth replication in the ninth environment was deleted to create unbalancedness for demonstration purposes.

The Friedman statistic of the procedure suggested in the foregoing section is 192.3, which is highly significant ($P < 0.001$), indicating that error variances are heterogeneous. Thus, we assess stability by S_{gi}^2 . The estimates are shown in Table 1.

For selection purposes, usually one is not primarily interested in the numerical magnitude of the variance estimates but rather in the rank order of these estimates. Therefore, Table 1 includes the stability ranking in brackets following the estimates. In this example, genotype 2 is the most stable genotype in

Table 1 Estimates of σ_{vi}^2 , σ_{gi}^2 and σ_{ei}^2 in 10^{-2} kg²/ha² for *Faba* bean data set, block 4 in environment 9 deleted

Genotype	S_{gi}^2	S_{vi}^2	S_{ei}^2
1	25688 (14)	25939 (14)	975
2	536 (1)	1051 (1)	2003
3	3814 (10)	3971 (9)	612
4	1295 (4)	1579 (4)	1107
5	2447 (6)	3006 (7)	2176
6	3663 (9)	4258 (10)	2313
7	12369 (12)	12655 (12)	1113
8	2898 (8)	3298 (8)	1556
9	724 (2)	1214 (2)	1907
10	918 (3)	1302 (3)	1494
11	1694 (5)	2057 (5)	1413
12	3848 (11)	4290 (11)	1719
13	20767 (13)	21432 (13)	2586
14	2489 (7)	2694 (6)	798

Rank order in parentheses.

terms of S_{gi}^2 . This genotype also has the smallest S_{vi}^2 . Generally there is a close agreement between the stability rank order given by S_{gi}^2 and the rank order of S_{vi}^2 . Only two rank changes are observed for genotypes 5 and 14 and for genotypes 3 and 6. Nevertheless, the numerical changes are appreciable. The three most stable genotypes are quite close in terms of S_{vi}^2 (values 1051, 1214 and 1302) whereas they are farther apart in terms of S_{gi}^2 (values 536, 724 and 918).

On the whole, in this example the rank changes between S_{vi}^2 and S_{gi}^2 are not very dramatic. This may be different in data sets where the error variances are larger than the interaction variances, a situation which is not uncommon in multilocation trials (see Utz & Laidig, 1989).

Concluding remarks

In this paper we have considered unbalancedness resulting from an unequal number of replications per environment, assuming that the set of genotypes is the same in each environment. If data on some genotype by environment combinations is lacking, one could use the method of moments or a MINQUE procedure in a similar fashion as described in Piepho (1994a) for $R = R_j$ and $\sigma_{ei}^2 = \sigma_e^2$.

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