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A method of estimating varietal stability for data of long-term trials

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Summary. A method is proposed to analyze the stability of cultivars in long-term varietal trials. The method involves the following steps: (i) regress a standard variety on environmental means; (ii) regress varieties under test on the standard variety; (iii) transform, through a procedure of reparameterization, the regression computed for each variety under test on the standard variety into the regression of the variety on environmental means. Although this method is proposed to analyze data sets from complex designs, it may also possess some advantages over conventional procedures for simpler designs.

Key words: Genotype-environment interaction – Stability – Regression – Reparameterization – Long-term varietal trials

Introduction

The effect of genotype-environment (GE) interaction on plant selection has long been a major concern to plant breeders and many methods have been developed to evaluate the performance of varieties grown in variable environments. Traditional analysis of variance procedures have been commonly applied to estimate the magnitude and the relative importance of the variance components from GE interactions in varietal trials over multiple years and locations (Comstock and Moll 1963). Plaisted and Peterson (1959) used an analysis of variance method to estimate the relative magnitude of the GE components for individual varieties, thus providing a measurement of the stability of each variety.

The technique most often used in measuring and comparing varietal stabilities is regression analysis. The genotypic mean yields are regressed on the environmental means (the mean yield of all genotypes tested in the environments), and the slope of a variety thus obtained measures the sensitivity of the variety to changes of environment. Among the several versions of this general approach which have been proposed, some include deviations from the fitted regression (Yates and Cochran 1938; Finlay and Wilkinson 1963; Eberhart and Russell 1966; Perkins and Jinks 1968) as a secondary estimate of the stability. This approach has, however, been criticized because dependency between genotypic means and the environmental means invalidates the analysis of the variance of regression (Freeman and Perkins 1971; Hardwick and Wood 1972). Nevertheless, the regression approach is widely used by breeders as a method to estimate varietal stability.

Varietal trials are often long term and old varieties are continuously replaced as new varieties are developed by breeding. Each variety will be ultimately discarded or released for commercial production after it has been tested for a number of years. Testing sites are not necessarily the same from one year to another. Moreover, the number of varieties involved in ongoing trials often vary from one location to another, even within the same year. Because the varieties included in trials represent the best genetic materials available at any given time, environmental means based on average yields of varieties tend to increase over time even though there may be no improvement in the test environments. The regression methods previously proposed cannot be applied to compare varieties that are grown at different periods of time because they do not account for this continuing improvement. Further, data obtained over time do not result in a completely balanced two-way interaction table. Methods of handling missing values are also difficult to apply in such situations, because a large number of the data points in the two-way table are missing (e.g. Freeman 1975).

The objective of this paper is to report a method of analyzing the data obtained from long-term variety trials that overcome some of the difficulties of the earlier procedures. A numerical example is provided to illustrate the method.

The method

First, let us describe the general situation of a long-term varietal trial. Suppose that two standard (tester or check) varieties and varieties that are being tested are being grown during a period of time. There is a period of time during which only the older standard variety is used (period I), followed by a period when both standard varieties are used (period II), and a third period when the newer standard variety is used alone (period III). Thus, at least one of the standard varieties is grown at any given location in a given year but the varieties under test and the locations may change from year to year.

The proposed method of analyzing varietal stabilities in such long-term trials involves the following steps of regression analysis.

1 Regress the standard varieties on environmental means

The mean yields of the standard varieties are regressed on the environmental mean yields in period II during which both standard varieties were grown. Suppose that there are n_2 such environments in this period with t_{2j} varieties (including standard varieties) in the jth environment. Let y_{2ij} be the mean yield of the ith variety ($i=1, 2, \ldots, t_{2j}$) in the jth environment ($j=1, 2, \ldots, n_2$), and Y_{2kj} be the mean yield of the kth standard variety ($k=1, 2$) in the jth environment. Thus k is a subset of i. Let e_{2j} be the jth environment effect which is estimated by

$$\hat{e}_{2j} = \sum_i y_{2ij}/t_{2j} - \Sigma\Sigma y_{2ij}/t_{2j}$$

and

$$y_{2kj} = \mu_2 + c_{2k} + e_{2j} + g_{2kj} + v_{2kj}, \tag{1}$$

where μ_2 is the grand mean of all varieties over all environments in period II, c_{2k} is the effect of the kth standard variety, and g_{2kj} is the GE interaction effect between the kth standard variety and the jth environment. The v_{2kj} is the random error.

Let

$$g_{2kj} = a_k e_{2j} + d_{2kj} \tag{2}$$

and

$$r_{2kj} = d_{2kj} + v_{2kj}, \tag{3}$$

where a_k is the regression coefficient of the kth standard variety on environmental means and d_{2kj} is the deviation from the regression. Thus, r_{2kj} represents the residual of the fitted model. Then model (1) becomes

$$y_{2kj} = \mu_2 + c_{2k} + (1 + a_k)\, e_{2j} + r_{2kj}, \tag{4}$$

or simply

$$y_{2kj} = \mu_{2k} + b_k e_{2j} + r_{2kj}, \tag{5}$$

where

$$\mu_{2k} = \mu_2 + C_{2k} \quad \text{and} \quad b_k = 1 + a_k.$$

The least square estimator of b_k is

$$\hat{b}_k = \sum_j (y_{2jk} - y_{2k.})\, \hat{e}_{2j}/\Sigma \hat{e}_{2j}^2,$$

with

$$y_{2k.} = \sum_j y_{2kj}/n_2.$$

The sum of squares due to regression of the kth standard variety on environmental means is

$$SSR_{2k} = \hat{b}_k \sum_j (y_{2kj} - y_{2k.})\, \hat{e}_{2j},$$

and the residual sum of squares is

$$SSr_{2k} = \sum_j (y_{2kj} - \hat{y}_{2kj})^2,$$

where

$$\hat{y}_{2kj} = y_{2k.} + \hat{b}_k \hat{e}_{2j}.$$

The error sum of squares (SSE_2) can be estimated by pooling the error sums of squares in the replicated trials over environments. The exact form of the estimator for SSE_2 and its degrees of freedom depend on the design of the varietal trials.

Assuming that d_{2kj} and v_{2kj} in (3) are independent of each other, the variance of r_{2kj} is,

$$V(r_{2kj}) = V(d_{2kj}) + V(v_{2kj}), \tag{6}$$

where V (*) represents the variance of *.

Denote the residual mean square by MSr_2 and the error mean square by MSE_2, and suppose that there are m_{2j} blocks in the jth environment. Then $V(r_{2kj})$ can be estimated by MSR_2 and $V(v_{2kj})$ by

$$S_e^2 = MSE_2/m_o,$$

where $m_o = n_2/\Sigma(1/m_{2j})$ is harmonic mean number of blocks over environments of period II. Thus, the ratio MSR_2/s_e^2 provides an F-test for the significance of the deviation from regression.

2 Regress the varieties under test on the standard varieties

The mean yield of each variety under test is regressed on the mean yield of the standard variety over the environments. The varieties grown in the first period will be regressed against the first standard variety and all other varieties will be regressed against the second standard variety in the analyses. If the ith variety has been grown in n environments and if there are t_j varieties in the jth environment, the yield of the ith variety y_{ij} can be expressed as

$$y_{ij} = \mu_i + b_i (y_{kj} - y_{k.}) + r_{ijk}, \tag{7}$$

where μ_i is the mean yield of the ith variety in all environments, b_i is the regression coefficient of the ith variety on the kth standard variety, and y_{kj} is the mean yield of the kth standard variety in the jth environment. $y_{k.}$ is the mean yield of the kth standard variety over the n environments where both the kth standard and the ith varieties were present. r_{ijk} is the residual which contains both deviation from the regression and the random experiment error.

As in the previous section, the least square estimator for b_i is

$$\hat{b}_i = \sum_j (y_{ij} - y_{i.})\,(y_{kj} - y_{k.})/\sum_j (y_{kj} - y_{k.})^2,$$

where

$$y_{i.} = \sum_j y_{ij}/n.$$

The sum of squares from the regression of the ith variety on the kth standard variety is

$$SSR = \hat{b}_i \sum_j (y_{ij} - y_{i.})\,(y_{kj} - y_{k.})$$

and the residual sum of squares is

$$SSr = \sum_j (y_{ij} - \hat{y}_{ij})^2,$$

where $\hat{y}_{ij} = y_{i.} + \hat{b}_i (y_{kj} - y_{k.})$. The error sum of squares can be found as described in the previous section by pooling error sums of squares over the n environments.

3 Reparameterize the regression of a variety under test on the standard variety to a regression on environments

To make the stability parameters of the varieties grown in different time periods comparable, the regression of a variety under test on the standard variety (in period I or III) is reparameterized using the regression of the standard variety on environmental means in period II. The reparameterization process results in a set of regression coefficients for the varieties under test as if they were obtained from the environments of period II. Replacing y_{kj} by the predicted y_{kj}, $\hat{\mu}_k + \hat{b}_k \hat{e}_{2j}$, equation (7) can now be rewritten as

$$\hat{y}_{ij} = y_i + \hat{b}_i(\hat{\mu}_k + \hat{b}_k \hat{e}_{2j} - y_{k.}) = y_i + \hat{b}_i \hat{b}_k \hat{e}_{2j}, \qquad (8)$$

where $\hat{b}_i \hat{b}_k$ is the reparameterized regression coefficient of the ith variety on environments of period II. The interpretations of the stability predicted from these coefficients will be the same as those proposed by previous workers. Note that the subscript j proceeded by a subscript 2 has different range of values than one without the subscript 2.

4 Comparison of the reparameterized regression coefficients

The method proposed by Geng et al. (1985) for calculating variance of compound variables is employed to estimate variances of these reparameterized regression coefficients. For a function of n random variables, f(x), the variance is given by:

$$V(f(x)) = \delta' \Sigma \delta,$$

where $x' = (x_1, x_2, \ldots, x_n)$

$\delta' = (\partial f(x)/\partial x)'$,

and is the variance-covariance matrix of x.

Assuming that b_i and b_k are independent of each other, the variance of the reparameterized regression coefficient ($\hat{b}_i \hat{b}_k$) is estimated by

$$V(\hat{b}_i \hat{b}_k) = \hat{b}_i^2 V(\hat{b}_k) + \hat{b}_k^2 V(\hat{b}_i). \qquad (9)$$

An example

The data used in this example were part of a series of cotton varietal trials conducted in California during the 18-year period, 1966–1983. The breeding aspect of the study will be reported separately (Geng et al., in preparation). For simple presentation, all varietal names were coded alphabetically. Two varieties of cotton were used as standard varieties: S-1 during the nine years from 1966–1974 and S-2 during 13 years from 1971–1983. In our analysis, these years were divided into three periods: Period I, the five years from 1966–1970; Period II, the four years from 1971–1974; Period III, the nine years from 1975–1983.

The mean yield of S-1 and S-2 for experimental sites with six or more varieties were plotted against the environmental means, S-1 in Fig. 1 and S-2 in Fig. 2. The regression coefficient (± SE) computed on environmental means for S-1 was 1.077 ± 0.046 in the period 1966–1970, and was 1.066 ± 0.039 in the period 1971–1974. These two regression coefficients were not significantly different, indicating that no marked increase of varietal mean yields occurred from period I to period II. The regression coefficient (± SE) for S-2 was 1.08 ± 0.032 in period II, 0.923 ± 0.050 in period III. A Students *t*-test shows that these two regression coefficients differ significantly at the 1% level. The significant reduction of the regression coefficients in period III suggested that significant improvements in the mean yields of varieties occurred in later years compared to period II.

The error variance obtained for this series of experiments was $s_e^2 = 762.31$. The mean square of residuals on environmental means for regressions is 1,744.9 for S-1 and is 1,538.6 for S-2; both were significantly larger than the random variance by the F-test. The statistic R^2, however, which measures the proportion of regression SS in total SS, was 95.7% for regression on environmental means of S-1 and 97.2% for S-2, indicating

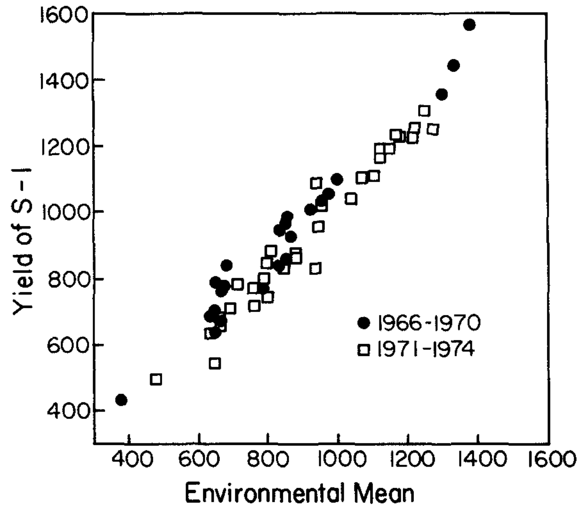

Fig. 1. The yields of S-1 plotted against environmental means for the periods 1966–1970 and 1971–1974

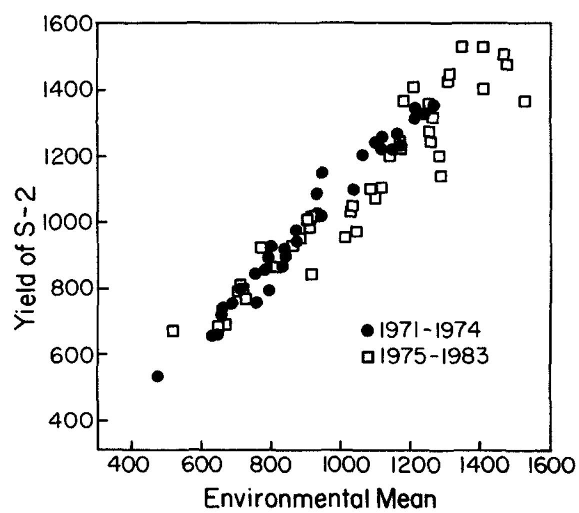

Fig. 2. The yields of S-2 plotted against environment means for the periods 1971–1974 and 1975–1983

Table 1. Stability parameters estimated for yields of five cotton varieties

Variety	Regression on environmental means of its own period		Regression reparameterized for period II						
	Coefficient	S.E.	Coefficient	S.E.	95% C.I.	R^2	M.S. residual	Error variance	F
TA	0.905	0.116	0.799	0.159	0.481–1.118	0.668	16,060	576	27.9
TB	1.047	0.043	1.001	0.063	0.875–1.127	0.945	3,124	530	5.9
TC	0.811	0.148	0.622	0.172	0.277–0.966	0.775	12,267	889	13.8
TD	0.918	0.064	1.076	0.128	0.821–1.332	0.845	9,425	745	12.6
TE	1.060	0.048	1.371	0.189	0.994–1.749	0.903	4,605	859	5.4

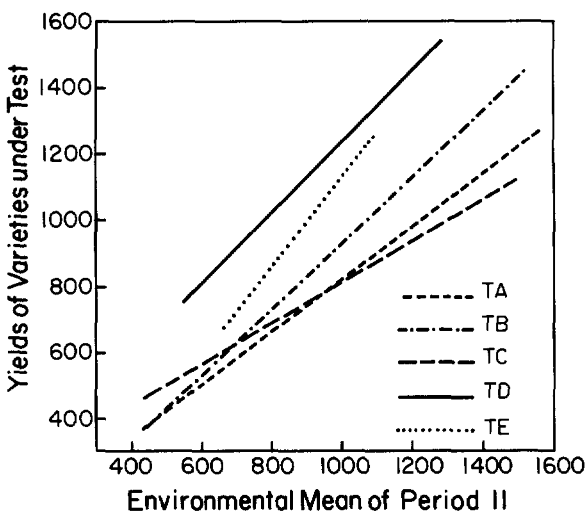

Fig. 3. The regression lines of five varieties under test on environmental means of period II derived through reparameterization. The range of the regression line of a variety was determined by the range of the yield for that variety

that the deviations from regressions for both varieties were trivial.

Five varieties under test (TA, TB, TC, TD, and TE) were selected to illustrate the analysis. TA was grown in the period 1966–1967, TB in 1966–1968, TC in 1969–1971, TD in 1982–1983, and TE in 1983 only. The coefficient for regression of each variety on environmental means in its own period of test is listed in Table 1. The coefficients for regression of these varieties on the standard varieties were computed by using formula (7). The first three varieties were regressed on S-1 and the other two on S-2. Formula (8) was used to reparameterize the regression of each variety to the environmental effects of period II. The variance of each derived regression coefficient was computed by using formula (9). The derived regression coefficients and their standard errors are also shown in Table 1 and graphically illustrated in Fig. 3.

Inspection of Table 1 shows that the derived regression coefficients are lower in value than those obtained from the direct regressions in period I (TA, TB, TC), whereas the reverse is true for varieties grown in period III (TD, TE). Inasmuch as these differences are in the directions expected when genetic improvement occurs, the proposed reparameterization procedure appears to be effective in correcting for the differences in environmental effects caused by genetic improvements.

The deviation from the reparameterized regression line is significant for every variety (Table 1). The values of R^2 show that the regressions of TB and TE account for more than 90% of the total variation, whereas the regressions of the other three varieties explain more than 67% of the total variation. Thus the significant F tests may not reflect important components of nonlinearity.

The approximate 95% confidence interval of the regression coefficient of variety TC is 0.277–0.966, indicating that the true regression coefficient is significantly smaller than unity. The 95% confidence interval of TE has 1.00 as its lower limit. Thus, the derived regression coefficient almost certainly is larger than unity. Further comparisons of values of R^2 and F tests of regression coefficients show: (i) that variety TC is less sensitive than the average variety to changing environment but with greater random variation; and (ii) that variety TE is more sensitive to changing environment but with smaller random variation. The derived regression coefficients for the other three varieties are not significantly different from unity. Had these coefficients not been corrected by reparameterization, none of them would be found significantly different from unity and TC could not be distinguished from TE by traditional regression procedures.

Discussion

We have proposed a method of analyzing varietal stabilities from long-term varietal trial data. The proposed method appears to have two distinct advantages. The first advantage is that it reduces the dependency between environmental indices and varietal means, which Freeman and Perkins (1971) correctly pointed out is a major drawback of conventional regression method. This dependency would be even more serious in data sets of long-term varietal trials in which unequal numbers of varieties are tested in different environments. In our analysis, environmental means are used only for analyzing the regressions of standard varieties, and only data collected from environments in which a relatively large number of varieties under test are used in the calculations. Comparisons and interpretations of varietal stability are based on the derived regression coefficient obtained through reparameterization of the regression coefficient of the varieties under test on the standard varieties. Thus, most of the environmental means are not used directly in the computational process and the analysis is no longer adversely affected by the dependence of the environmental means on varieties tested.

The second advantage is that the method allows for correction of genetic effects of varietal improvement confounded with the environmental means of different periods. A major feature of long-term trial data is that the varieties grown in later years have higher yields than those in earlier years. Thus, varieties grown in different time periods which have similar regression coefficients, as estimated by conventional methods may in fact differ considerably in response to environmental changes. If all the experimental environments are averaged, the conventional method would, in general, overestimate the regression coefficients for the varieties grown in earlier years and underestimate the regressions of the varieties in later years. Thus, direct comparisons of regression coefficients estimated by the conventional method for data sets of this kind could be misleading. But our method first regresses the varieties under test on the standard variety, and then transforms these regressions into regressions of the varieties on the relevant range of environments, so that comparisons are made as if all the varieties had been grown in the same set of environments. The environmental time ranges are chose so that they fall within intermediate stages of varietal trials; the use of environments at both the beginning and the end of the trials is to be avoided. As shown in the example, the reparameterization accomplished the purpose of adjusting the regression coefficients to correct for genetic improvement overtime. Therefore, this method provides a means of comparing varieties grown in different periods on a common basis.

We have tried several alternative methods, one of which is worth mentioning. This method involves a two-stage adjustment. Each variety is first regressed on the mean of the environment in which it was grown, and then each regression coefficient is adjusted by using the ratio of the regression coefficients of a standard variety on environments computed from data of different periods, so that comparisons can be made between varieties grown in different time periods. Stability parameters were also computed by using this method for the data set of our example. The rank correlation between regression coefficients by our primary and alternative methods is 0.78. The disagreement between these two methods presumably results from two causes. First, the greater dependence of the environmental means on the varieties in the alternative method may adversely affect results because, in some cases, as few as three varieties were grown in one environment. Second, the regressions may have been improperly adjusted for the varieties overlapping the two periods considered because the regression coefficients of the standard varieties are computed separatly for the two different time periods. This problem of overlap occurs because there is no time in variety trials when all the experimental varieties are renewed. However, the alternative method may be useful in analysis of data sets which are free of these two problems.

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