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A method of analyzing cultivar × location × year experiments: a new stability parameter *

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Summary. Assessment of cultivar performance in a cultivar × location × year experiment is often difficult because of the presence of a location × year interaction. Our objective is to demonstrate a method on separation of environment effects (location × year) into predictable and unpredictabel components. The analysis consists of two parts: (1) a regression analysis based on location effects (averaged over years), assuming that the location means represent predictable environmental variation; and (2) the estimation of stability (denoted type 4) based on the years within location mean squares, assuming that years within location represent unpredictable environmental variation. From the regression analysis in (1), a breeder can determine the optimum range of locations in which a cultivar is well suited, and from (2) he can choose the most stable cultivars. The advantage of type 4 stability is that it is independent of the other cultivars included in the test and of the regression coefficient estimated for predictable variation. Three sets of published data are used to illustrate the analysis. Type 4 stability is compared with type 3 stability (deviation mean square from regression on environmental index) for genetic consistency. The analyses suggest that type 4 stability is consistent and is therefore a potential genetic parameter, but type 3 stability is not.

Key words: Genotype × environment interaction – Regression analysis – Stability analysis

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Introduction

In a cultivar × location × year experiment, one of the problems associated with cultivar evaluation is that the effect of location can vary considerably from year to year. This is usually evidenced by a significant location × year interaction in the ANOVA. The presence of such an interaction presents a serious problem to anyone wishing to recommend a cultivar to a region, because such a recommendation is based on the premise that regional characteristics are persistent and that breeding locally adapted varieties is possible. One approach to solving this problem (which we call a two-way analysis) is to restructure the data as a cultivar × environment experiment, making a single factor out of the location × year combination, and to use Finlay and Wilkinson's (1963) regression analysis to provide general information on a cultivar's performance. However, because the environment factor in this analysis is a combination of locations and years, it is not helpful when recommendations of cultivars to specific locations are required.

Conceptually, the environmental effect on a genotype depends on two main elements: soil and weather. The soil element is usually persistent from year to year and can be regarded as fixed. The weather element is more complex, because it has a persistent part represented by the general climatic zone, and an unpredictable part represented by time variation (e.g. year to year). Once the environmental effect has been conceptually subdivided into predictable and unpredictable components, a similar subdivision can be made for the genotype × environment (GE) interaction. The separation of the environment effect into these two components was first advocated by Allard and Bradshaw (1964). They suggested that, while developing cultivars with specific adaptation to predictable specific environments (to cope with a cultivar × location interaction), plant breeders should also aim to produce cultivars that are adapted to withstand unpredictable transient environmental variation (such as year to year variation). A similar idea was also expressed by Breese (1969) who, in a slightly different context, argued that "since the linear regressions represent very definite and measurable responses to the environment, it is no longer profitable to consider this component of genotype-environment interactions as a measure of stability in the way described by Finlay and Wilkinson", and suggested that "the term 'stability' should now rather be reserved to describe measurements of unpredictable irregularities in the response to environment as provided by the deviations from regression". Although we disagree with Breese's specific parameter (see Lin et al. 1986), his suggestion to use the regression coefficient to allocate cultivars to regions, and of defining a stability parameter in a more restricted manner, is very sensible. Apparently, two different criteria are needed for cultivar selection: (1) for predictable variation, we can identify a cultivar's optimum range of responses from a regression on the environmental index; and (2) for unpredictable variation, we can find the cultivars with a small within-location variance. For a cultivar × location × year experiment we can assume that the cultivar × location mean averaged over years is the biological equivalent of cultivar × predictable variation, and years within location is equivalent to cultivar × unpredictable variation. Methodologically, we can use the regression analysis of the GE interaction (averaged over years) for the former (but not as a stability parameter) and simply use the mean squares (MS) within location as a stability statistic (which we call type 4) to measure a cultivar's ability to withstand unpredictable variation.

The objective of this paper is to use three sets of published data to demonstrate the method of analyses. The genetic consistency of the proposed stability parameter (type 4) is compared with a conventional stability parameter (type 3) based on the residual from regression (Eberhart and Russell 1966), and the experimental conditions required for type 4 analysis are discussed.

Materials and methods

The three sets of data used for this study are:

Set 1

Seven cultivars of six row barley (Bruce, Conquest, Laurier, Leger, T-1, T-2, T-3)[a] were grown at 15 locations within Ontario, Quebec and Atlantic regions of Canada during 1982–1984. The yield data (kg/ha) of the 7 × 15 × 3 experiment are based on the Cooperative Trial of Eastern Canada [Ottawa Research Station (O.R.S) Report Nos. 144, 150, 158, respectively].

Set 2

Eleven cultivars of barley (B-1 to B-11)[a], each with two seeding rates (0.072 and 0.108 m^3/acre) were sown at three different times in each of seven locations in Ontario. Note that for convenience in comparing the genetic consistency of various parameters, cultivar × seeding rate is considered as a single factor and regarded as 22 genotypes in the analysis. The first seeding-time at each location was chosen locally and independently of the other locations; the succeeding two times were at 2 week intervals. The yield data (kg/ha) of this 22 × 7 × 3 experiment are based on the report from the regional production test, Province of Ontario (O.R.S. Report No. 156, 1984).

Set 3

Six cultivars of oats (O-1 to O-6)[a], each with two seeding rates (0.072 and 0.108 m^3/acre) (regarded here as 12 genotypes), were sown at three different times in each of seven locations in Ontario. Sowing times were structured as in set 2. The yield data (kg/ha)of this 12 × 7 × 3 experiment are based on the report from the regional production test, Province of Ontario (O.R.S. Report No. 155, 1984).

Based on the cultivar × location means averaged over years (set 1), or over seeding-time (sets 2 and 3), the regression coefficient was calculated for each cultivar on the environmental index, defined as the difference between the location mean averaged over cultivars and years (or seeding-rate) and the overall mean. In this paper, seeding-time (sets 2 and 3) is instrumental in creating a condition of unpredictable variation, and will be treated analogously with the year factor of set 1 (discussed below). The pooled years within location (Y/L) MS is calculated for each cultivar. This MS (Y/L) consists of two components: year (Y) and Y × L interaction effects, but they are not separated because both reflect the sensitivity of individual cultivars to unpredictable variation. The regression slope, b, is used as an indicator to identify recommended locations in terms of the index, and the MS (Y/L) is used to measure stability (type 4).

For comparison, the data were also analyzed by the two-way analysis, i.e. L × Y (or L × T) is considered as a single factor (environment). The observed value of each cultivar was regressed on the index, which is defined as the difference between the constructed environment mean averaged over cultivars and the overall mean. The resulting regression coefficients (b′) and the residual MS from the regression (type 3 stability) are then used for comparison.

Results

Summary statistics of the type 4 analysis and the two-way analysis are shown in Tables 1, 2, and 3 for sets 1, 2, and 3, respectively. In each of the three data sets, coefficients of determination (r^2) were all greater than 88%, indicating adequate linear fit.

The combined ANOVA (Table 4) shows that the b's are not homogeneous among cultivars for set 1 and set 2, but are homogeneous for set 3. Also, the residual MS are all substantially larger than the error MS, indicating heterogeneity of residuals among cultivars in each set. The heterogeneity of residuals is not important in type 4 analysis, because we do not use the residuals as a basis for a stability parameter.

As an example of the use of these analyses, if the cultivars are to be chosen for high yielding environments

[a] Coded names refer to test cultivars

Table 1. Summary statistics for the two analyses of set 1 (kg/ha)

Genotype	Mean (kg/ha)	Type 4 analysis (7 × 15 × 3)			Two-way analysis (7 × 45)		
		b	MS[a] of Y/L	(type 4)	b′	MS[a] of residual	(type 3)
Bruce	4,068	0.85	912	(4)[b]	0.89	141	(5)
Conquest	3,864	0.98	880	(2)	0.97	107	(4)
Laurier	4,264	0.95	850	(1)	0.94	174	(7)
Leger	4,650	1.08	1,113	(7)	1.08	153	(6)
T-1	4,364	1.16	1,037	(5)	1.11	80	(1)
T-2	4,273	0.96	1,093	(6)	1.01	91	(2)
T-3	4,329	1.02	884	(3)	0.99	93	(3)

[a] Obtained for each genotype separately; each entry divided by 1,000
[b] Ranking

Table 2. Summary statistics for the two analyses of set 2 (kg/ha)

Cultivar	Mean (kg/ha)		Type 4 analysis (22 × 7 × 3)				Two-way analysis (22 × 21)			
			b		MS[a] T/L (type 4)		b′		MS[a] of residual (type 3)	
	Rate 1	Rate 2	Rate 1	Rate 2	Rate 1	Rate 2	Rate 1	Rate 2	Rate 1	Rate 2
B-1	3,564	3,835	1.15	1.05	553 (11)[b]	561 (11)	1.17	1.09	113 (9)	88 (4)
B-2	3,408	3,604	0.99	0.98	551 (10)	407 (5)	1.04	1.01	80 (5)	41 (1)
B-3	3,673	3,840	0.81	0.84	314 (4)	230 (2)	0.83	0.83	81 (4)	67 (2)
B-4	3,662	3,718	0.82	0.74	453 (8)	453 (7)	0.86	0.81	113 (8)	99 (5)
B-5	3,982	4,121	1.09	1.18	186 (1)	221 (1)	0.99	1.09	179 (11)	148 (10)
B-6	3,452	3,604	1.05	1.03	416 (7)	497 (10)	1.05	1.05	82 (6)	116 (8)
B-7	3,522	3,657	0.91	0.99	378 (5)	449 (6)	0.93	1.01	61 (2)	84 (3)
B-8	3,527	3,675	1.08	1.11	297 (3)	379 (4)	1.05	1.08	63 (3)	100 (6)
B-9	3,321	3,462	1.00	1.03	240 (2)	305 (3)	0.93	0.98	161 (10)	164 (11)
B-10	3,683	3,801	1.06	1.03	398 (6)	491 (9)	1.06	1.05	51 (1)	101 (7)
B-11	3,714	3,822	1.01	1.05	479 (9)	461 (8)	1.04	1.06	106 (7)	118 (9)
SED[c]	86.7		0.16				0.08			

[a] Obtained for each cultivar separately; each entry divided by 1,000
[b] Ranking within each rate
[c] Standard error of difference between two seeding rates

Table 3. Summary statistics for the two analyses of set 3 (kg/ha)

Cultivar	Mean (kg/ha)		Type 4 analysis (22 × 7 × 3)				Two-way analysis (22 × 21)			
			b		MS[a] T/L (type 4)		b′		MS[a] of residual (type 3)	
	Rate 1	Rate 2	Rate 1	Rate 2	Rate 1	Rate 2	Rate 1	Rate 2	Rate 1	Rate 2
O-1	3,708	3,795	1.07	1.11	607 (2)[b]	473 (2)	1.04	1.05	175 (5)	218 (6)
O-2	3,831	3,931	0.92	0.92	819 (4)	955 (6)	0.97	0.98	98 (3)	160 (5)
O-3	3,500	3,625	1.02	1.04	669 (3)	592 (3)	1.02	1.01	58 (1)	102 (2)
O-4	3,882	4,031	0.96	0.97	382 (1)	262 (1)	0.91	0.88	147 (4)	114 (4)
O-5	3,673	3,904	0.96	1.02	822 (5)	760 (4)	1.00	1.04	75 (2)	64 (1)
O-6	3,779	3,926	0.98	1.04	973 (6)	915 (5)	1.03	1.08	209 (6)	109 (3)
SED[c]	89.4		0.14				0.07			

[a] Obtained for each cultivar separately; each entry divided by 1,000
[b] Ranking within each rate
[c] Standard error of difference between two seeding rates

Table 4. Combined ANOVA for each set (kg/ha)

Source	Set 1 (7 × 15 × 3) DF			MS[a]	Set 2 (22 × 7 × 3) DF			MS[a]	Set 3 (12 × 7 × 3) DF			MS[a]
Genotype (G)	6			2,709	21			745	11			479
Environment (E)	44			(10,183)	20			(24,816)	20			(21,288)
Location (L)		14		18,997		6		66,262		6		53,925
Year (Y)/L[b]		30		6,070		14		7,053		14		7,301
GE	264			(145)	420			(111)	220			(138)
G × L		84		(206)		126		(184)		66		(263)
heter. of *b*			6	361**			21	228**			11	91
residuals			78	194**			105	175**			55	297**
G × Y/L[a] (Error)		180		116		294		79		154		84
Total	314				461				251			

** Significant at the 0.01 probability level
[a] Each entry divided by 1,000
[b] For sets 2 and 3, seeding-time (T) replaces year (Y)

(i.e. large b) and with high stability (i.e. small years within location MS), then T-3 of set 1 (if only test cultivars are compared), B-5 of set 2, and O-4 of set 3 are the most promising candidates.

Discussion

The conceptual difference between type 4 analysis and the two-way analysis is that the former separates the environmental variation into predictable and unpredictable, while the latter does not. Separation of these two types of variation is important because predictable variation can be controlled to some extent by selecting cultivars with specific adaptability to regions, while unpredictable variation cannot be controlled: one must rely on the homeostatic property of the cultivar itself. For the type 4 analysis, two selection criteria are used: one from the regression analysis based on cultivar × location yields averaged over years, and the other from the stability analysis based on individual year yield within each location. The results of the regression analysis are similar to those of the two-way analysis: the simple correlations between the regression slopes obtained from the type 4 analysis (b) and those obtained from the two-way analysis (b′) are 0.95, 0.92 and 0.62 for sets 1, 2, and 3, respectively (Tables 1, 2 and 3). The key difference between the two methods is the measure of stability. In the two-way analysis, stability is defined either by slope (Finlay and Wilkinson 1963) or by both slope and residual MS from regression (Eberhart and Russell 1966). In the type 4 analysis, the regression slope is not used as a stability parameter but as a means to identify the optimum range of locations, and the residuals from the regression are not used in measuring stability. Rather, the stability parameter is defined from part of the data structurally independent of the regression analysis.

Comparison of type 4 with other measures of stability

In a critical literature review of stability, Lin et al. (1986) classified the conventional stability concept into three types. A genotype is considered to be stable: (1) if its among-environment variance is small (type 1); (2) if its response to environment is parallel to the mean response of all genotypes in the trial (type 2); and (3) if the residual MS from a regression model on the environmental index is small (type 3). Type 3 stability, to which the two-way analysis belongs, was criticised because the regression model in the context of GE interaction is a data-based descriptive model and not a prediction model as the argument for this stability assumes (note that the primary distinction between these two models made by the authors is that if the independent variable can be measured prior to the test, it is a prediction model; otherwise, it is descriptive). They also suggested that type 2 stability suffers from inferential limitations because this stability depends on the other cultivars in the test, and that type 1 stability lacks general practical value because a cultivar that yields evenly in all locations is usually poor. In contrast to these three types of stability, type 4 stability is a measure of unpredictable variation only, while the predictable or persistent part of the location effect is excluded. Furthermore, type 4 stability is statistically independent of the regression analysis and of the other genotypes in the test.

Genetic property of type 4 stability

An important question from the practical point of view is whether type 4 stability (or any other) is truly representative of a genetic characteristic. Data sets 2 and 3 provide a rare opportunity to investigate this question, because each set consists of cultivars with two seeding rates. If the parameter is genuinely genetic, the rank order of the genotypes at either seeding rate should remain approximately the same; otherwise the parameter is nongenetic and selection for such a parameter would be useless. This was investigated with respect to type 4 and type 3 stabilities. Spearman's ranking coefficient (e.g. Steel and Torrie 1960) was used to measure correlation of ranking order between the two seeding rates for data sets 2 and 3: the rank correlations between seeding rates for type 4 were 0.76 and 0.83, for sets 2 and 3, respectively (both significant: $P<0.05$), while for type 3 they were 0.50 and 0.54, respectively (neither significant: $P<0.05$). These results suggest that type 4 stability may be a genetic parameter while type 3 stability is not. As a further check, type 3 stabilities were calculated separately for each year for set 1; the resulting ranking orders (Table 5) were not consistent ($P>0.05$) by Friedman's test (e.g. Steel and Torrie 1960). The inconsistency of type 3 stability with respect to seeding rates and years indicates the circumstantial (nongenetic) nature of this parameter. The theoretical argument (Lin et al. 1986) that type 3 stability is merely an indicator of goodness-of-fit, but not an indicator of stability, is supported by these data.

Table 5. Residual mean squares from regression[a] for each cultivar and for each year in set 1 (kg/ha)

Cultivar	Year					
	1982		1983		1984	
Bruce	91[b]	(4)[c]	61	(2)	163	(5)
Consquest	84	(3)	137	(6)	99	(3)
Laurier	247	(7)	130	(5)	182	(6)
Leger	66	(2)	200	(7)	212	(7)
T-1	38	(1)	66	(3)	129	(4)
T-2	161	(6)	36	(1)	67	(2)
T-3	138	(5)	66	(4)	57	(1)

[a] The location index was based on each years data
[b] Each entry divided by 1,000
[c] Ranking

Genetic property of b

To investigate if b is genetically consistent, we subdivided the years within location sum of squares of set 1 into three components: year, b × year, and error (Table 6). The b × year interaction was not significant when tested against the error MS, suggesting that b was consistent among years. For sets 2 and 3, no such investigation was done since the time factor was nested within locations. For two data sets 2 and 3, consistency of b was investigated by comparing the difference between two seeding rates for each cultivar (Tables 2, 3). The results show that none of the differences was statistically significant in each set, suggesting that the estimate of b was genetically stable irrespective of seeding rates.

Conditions required for estimation of type 4 stability

Although the years within location MS is recommended as a stability parameter, not all within location MS can be so used. For example, for a data set that includes cultivar × location × replication, replication within location MS cannot be regarded as a proper measure of type 4 stability because ordinary field replications represent only local variation of the micro environment, which is inadequate to represent the macro view of unpredictable variation considered here. To obtain a meaningful estimate of type 4 stability, the experiment in a series of trials should have a time factor in addition to C × L, because only then can unpredictable variation (weather) be isolated from predictable variation (soil). A year factor is ideal, but to a lesser extent seeding-time also can be considered as a time factor, because different seeding-times represent differential weather sequences for the same developmental phase of a genotype, thus constituting a degree of unpredictable variation. For this reason both types of data set, with year (set 1) and with seeding-time (sets 2 and 3), can be analyzed for type 4 stability. Obviously, a type 4 stability estimate obtained using a year factor has a different biological interpretation from that obtained using a seeding-time factor. However, both factors have a common ground because a cultivar with

Table 6. Investigations of consistency of b-value over years' in set 1 (kg/ha)

Source	DF		MS[a]						
			Bruce	Conquest	Laurier	Leger	T_1	T_2	T_3
Year within location (Y/L)[b]	30		912	880	850	1,113	1,037	1,093	884
Year		2	3,853	3,544	3,893	5,659	5,301	6,254	5,085
b × year		2	1,854	1,085	1,294	458	1,213	1,157	573
Error		26	613	659	581	813	696	691	585

[a] Each entry divided by 1,000
[b] See Table 1

greater tolerance to seeding-time (better adjustment of its life cycle to local weather conditions) is likely to be more stable with respect to year differences. If evidence were found to support this conjecture, the use of both year and seeding-time could be useful for reducing the test period (years) of regional trials.

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