Theor Appl Genet (1992) 83:968–972

THEORETICAL AND APPLIED GENETICS

Selection of test locations for regional trials of barley *

C. S. Lin [1,] and M. J. Morrison [2]**

[1] Research Program Service, Research Branch, Agriculture Canada, Ottawa, Ontario, K1A OC6 Canada
[2] Plant Research Centre, Central Experimental Farm, Ottawa, Ontario, K1A OC6 Canada

Received May 22, 1991; Accepted November 1, 1991
Communicated by A. R. Hallauer

Summary. Three sets of regional six-row barley (*Hordeum vulgare* L.) trial data, representing cultivar × location × year, were grouped for locations based on the similarity of genotype × environment (GE) interaction. Locations were selected from each group (cluster) so that the structure of the GE interaction generated by the subsets of the locations would be approximately similar to that of the whole set (all locations). The purpose of this paper is to determine the number of locations where the GE interaction structure generated by these selected locations would be fairly consistent over years. Two statistics were used to measure the success of the selected locations: (1) the ratio of GE mean square (MS) associated with the selected location set relative to that associated with the best set (which gives the highest GE interaction MS) and (2) the rank correlation between the cultivar means averaged over the selected locations and those based on the entire data set. The results show that, for eastern Canada, 10–13 locations based on the cluster method can achieve a fairly consistent GE interaction structure over years.

Key words: Genotype × environment interaction – Location – Classification

Introduction

One of the major objectives of a regional trial is to investigate the differential responses of the test cultivars. To have a successful cultivar assessment, test locations should be selected to represent a reasonable range of the regional characteristics. This may require considerable numbers of test locations. However, resources are limited, and one has to compromise between a desire to cover all possible regional characteristics and a need for economizing on the number of test locations. To achieve this objective, Lin and Butler (1988) proposed a numerical solution to obtain a subset of the locations, which would generate a similar genotype × environment (GE) interaction structure to that of the whole set. The method consisted of two steps. Step 1 identified the set of locations that gave the largest GE interaction mean square (MS) for each number of locations. Step 2 grouped the locations by similarity of their GE interaction using cluster analysis. The locations were then chosen empirically from each cluster. Although, in step 2, the cluster analysis has its own rule for determining the cut-off point for the clusters, plotting of the change in GE interaction MS by step 1 can provide an additional visual guide.

While a numerical solution is relatively simple, the critical question is whether the selected locations will generate a similar GE interaction structure year after year. There are two opposing views. One group argues that a consistent GE interaction pattern is unlikely to occur because the location × year interaction is known to be large in most regional trial data. The other side argues that there must be some degree of consistency; otherwise, the selection of cultivars for subregions is not meaningful. The truth may reside somewhere in between these two opinions. The difficulty lies in the fact that a location effect consists of two components; a fixed (e.g. soil) and a random (e.g. weather) effect, and they are confounded.

To deal with this dilemma, Lin and Butler (1988) suggested an ad hoc approach by using the set of cultivar × location means, averaged over years, assuming the mean over years at each location was representative of the fixed component. The assumption is that if the loca-

* Contribution no. R-078 from Research Program Service, Agriculture Canada, and contribution no. 1352 from Plant Research Centre, Central Experimental Farm
** To whom correspondence should be addressed

tions are grouped based on their fixed components, the consistency of GE interaction structure over years may be substantially improved. To test this hypothesis independent sets of data from different years were used to investigate the consistency of performance.

The purpose of our study was to determine how many locations (location-level) were required in order to obtain a fairly consistent GE interaction structure over years when the test locations are selected by the ad hoc method (Lin and Butler 1988). The historical data of the Eastern Cooperative Six-row Barley (*Hordeum vulgare* L.) Trial were used as the data base.

Data and method

The data were taken from the 1980–1987 annual reports of the Canadian Eastern Cooperative Six-row Barley Trial. These trials are one of two national trials in Canada used for variety registration and represent the Maritime, Quebec, and Ontario regions.

The 8 years of trial data were divided into two groups: one group consisting of 3 years was used for selecting locations; and the other group consisting of single-year data, which were not involved in the cluster analysis, was used for measuring the consistency of performance by the selected locations. This process was repeated 3 times with different cluster sets, each using a different set of years. Three cluster sets and their corresponding test sets were as follows:

Cluster set	Test set
Set 1: 1980–1982 (14 × 6 × 3)	1984 (17 × 10)
	1985 (17 × 7)
	1986 (18 × 6)
	1976 (19 × 11)
Set 2: 1982–1984 (15 × 7 × 3)	1985 (17 × 7)
	1986 (18 × 6)
	1987 (19 × 11)
Set 3: 1984–1986 (17 × 5 × 3)	1987 (19 × 11)

where the figures within parenthesis represent location × cultivar × year. The locations selected from cluster set 1 were tested by four test sets; set 2 by three; set 3 by one. Several points need to be noted about the data: (1) cluster sets 1 and 2, and cluster sets 2 and 3 are interrelated, but the data of each cluster set and its corresponding test sets are independent; (2) cultivars in the test and cluster sets are not necessarily the same, while the locations of each test set always included at least 13 selected locations from the corresponding cluster set; (3) although there were only five to seven cultivars in the cluster sets, these were mostly checks that were representative of a range of response types observed in the trials [see Lin and Butler (1988) for further discussion].

Cluster sets were first analyzed using approach 1 of Lin and Butler (1988) to obtain the largest GE interaction MS for each number of locations. These largest MS were then plotted against the numbers of locations (designated as location-level in this paper) to be used to determine the optimum number of locations required (step 1). Secondly, the locations were grouped by the cluster analysis of Lin (1982), (see also Lin and Butler 1990), assuming cultivar × year as one factor (step 2). Note that for the

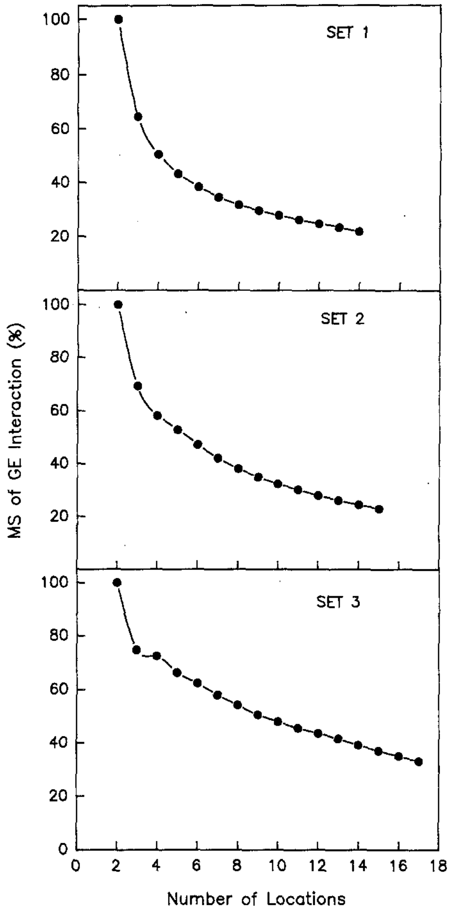

Fig. 1. The largest GE interaction MS for each numbers of locations, expressed as a percentage of the MS for the two locations

present cluster sets, this analysis is equivalent to the analysis based on the data set of location × cultivar means over years. The analyses of step 1 (Fig. 1) show that the (MS) curves tend to tail off after 7 location-levels, while the analyses of step 2, which is based on the ratio between the smallest dissimilarity index and the estimated error, also indicated that 7 or 8 groups are necessary. However, for the purpose of comparison, locations were selected for 4, 7, 10, and 13 location-levels based on the dendrograms of the cluster analysis (Fig. 2). Locations were chosen from each cluster based upon their biological relevance and their consistency over years. The selected locations for the three cluster sets were:

Set 1: KEMP, NORM, ALSA, FRED; LAPO, LIST, THUN; CHAR, ELOR, OTTA; RIDG, NOWL, STAN

Set 2: NORM, LIST, OTTA, FRED; LAPO, STRO, RIDG; CHAR, NEWL, HURO; ALSA, MAPP, KEMP

Set 3: CHAR, KEMP, LAPO, THUN; STAN, RIDG, ALSA; FRED, LIST, NEWL; NORM, PINT, STRO

The 13 selected locations are separated by semicolons into four segments: the first segment representing the 4 location-level, the first two segments representing the 7 location-level, etc.

For each test set, two statistics were determined. A percentage of the GE interaction MS generated by the selected locations (from the cluster set) over that of the best locations obtained in step 1. "Best locations" refers to the set of locations that give the

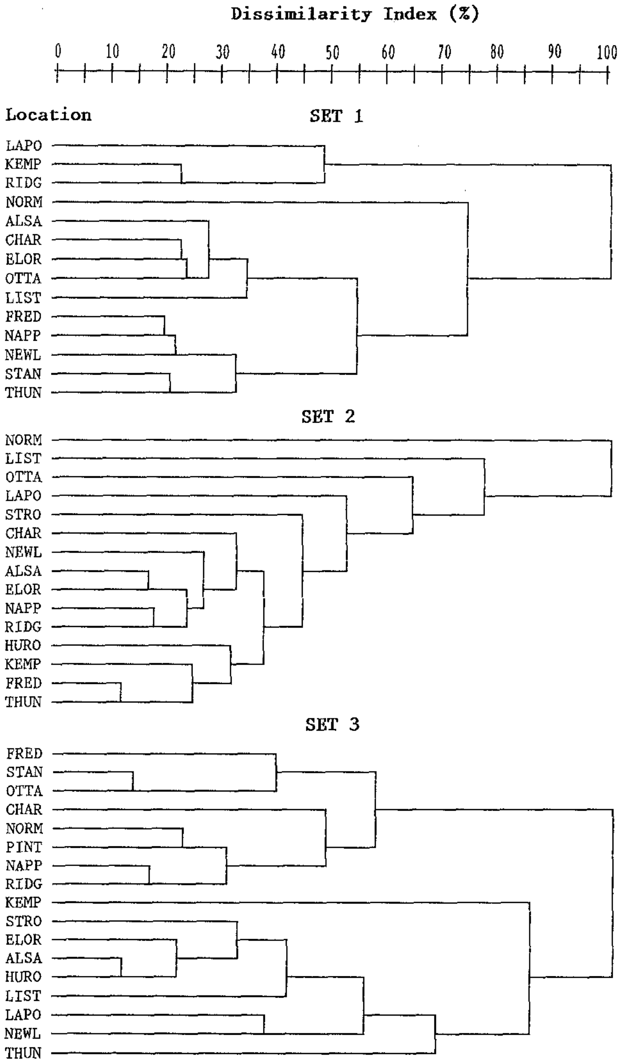

Fig. 2. The dendrograms of cluster analysis of location based on the method of Lin (1982)

largest GE interaction among all possible location combinations. This statistic, which shall be referred to as "% optimality", provided a numerical measure of degree to which the selected locations represented the GE interaction structure of the location-level. Secondly, two kinds of rank correlations were calculated: (1) the correlation of the cultivar means averaged over the selected locations with these from all locations (r_s) and (2) the correlation of cultivar means averaged over the best locations with those from all locations (r_b). If the GE interaction structure represented by the selected locations (or by the best locations) were basically the same as that generated by the whole set the correlation would be high, i.e. no significant change in cultivar ranking. This provided a measure of the degree of empirical success that the selected locations had accomplished.

One practical problem with these two statistics is a lack of standards, which provide the basis on which proper location-level can be decided. There are some empirical rules that can be used. (1) Use a % optimality of 70% as an approximate cut-off point. In the previous study (Lin and Butler 1988) it was observed that the ratio between the GE MS of the "Maximum set" (best set) and that of the "Minimum set" in the structured combination is 1.3 (77%). This suggests that 70% is not unrealistic.

Table 1. ANOVA of grain yield (kg ha^{-1}) for the three cluster sets

Source	Set 1		Set 2		Set 3	
	df	MS[a]	*df*	MS	*df*	MS
Year (Y)	2	2,425.4	2	33,064.5	2	7,365.5
Location (L)	13	6,354.7	14	19,324.7	16	8,923.3
Cultivar (V)	5	2,046.6	6	2,694.0	4	3,360.7
L · V	65	237.1	84	203.6	64	369.9
Y · L	26	4,802.0	28	4,561.6	32	2,744.7
Y · V	10	198.0	12	136.6	8	217.7
Y · V · L	130	141.6	168	111.7	128	139.3

[a] All MS should be multiplied by 1,000

(2) When both r_s and r_b of the test set are high, it is an indication that both sets generate similar GE interaction. The corresponding % optimality at this level also can be assumed to be a proper cut-off point. (3) Use the % optimality and the rank correlation (r_s) of the selected location-level from the cluster analysis. Because these two estimates are from the same data set from which the locations were selected, they can be considered to be the upper limit for corresponding estimates from the test sets.

Results

Cluster sets

ANOVA of the three cluster sets of data (Table 1) showed a large year × location interaction as expected. To measure the degree of the fixed component generated by location, the variance components of location (σ^2_L) and location × year (σ^2_{LY}) were estimated, and the ratio $\sigma^2_L/(\sigma^2_L + \sigma^2_{LY})$ was calculated. The results show that the ratio is 9.7% for set 1, 52.5% for set 2, and 43.8% for set 3. This suggests that the fixed component was about 50%, except for set 1. Note that the small ratio in set 1 was due to the different yield response in 1981 data.

To check the appropriateness of the cluster analyses, a combined ANOVA based on seven groups of locations was calculated for each set (Table 2). The results indicated that the percentages of total GE interaction sum of squares, attributable to the between group difference, are 87.3% for set 1, 82.1% for set 2, and 77.1% for set 3, suggesting that grouping has been successful for differentiating locations. If we assume seven groups as a proper level for the cluster sets, then practically the highest % optimality that can be expected from the test sets would be around 82% (Table 3) and the corresponding r_s would be 0.88 (Table 4). These values would be used as a guide for determining the required location-level.

Test sets

The % optimality (Table 5) showed that at the 4 location-level, the GE interaction generated by the selected loca-

Table 2. Combined ANOVA for each cluster sets based on seven groups of locations

Source	*df*	MS
Cluster set 1		
GE[a] interaction	221	717,969
Between group	102	1,358,349
Within group	119	169,071
Cluster set 2		
GE	280	584,297
Between group	120	1,119,249
Within group	160	183,082
Cluster set 3		
GE	224	577,373
Between group	84	1,186,839
Within group	140	211,694

[a] G represents cultivar × year, and E represents locations. Note that GE SS is equivalent to the pooled SS of V · L, Y · L and Y · V · L of Table 1

Table 3. Percentage GE interaction MS generated by the selected set as compared to that of the best sets (% optimality) for three cluster sets

Cluster set	Location × (V × Y)[a]	Location-level			
		4	7	10	13
1	14 × 18	55.5	87.4	82.4	93.9
2	15 × 21	75.4	85.1	92.5	93.7
3	17 × 15	83.9	72.2	94.6	93.0
Mean		71.7	81.6	89.8	93.5

[a] The combined factor of cultivar × year

Table 4. The rank correlations between the cultivar means of the selected locations and that of all locations (r_s); and the correlations between the cultivar means of the best set and that of all locations (r_b) for cluster sets

Cluster set	Location × (V × Y)[a]		Location-level			
			4	7	10	13
1	14 × 18	r_s	0.76[b]	0.82	0.88	0.95
		r_b	0.73	0.85	0.97	0.99
2	15 × 21	r_s	0.95	0.98	0.99	0.99
		r_b	0.96	0.98	0.99	1.00
3	17 × 15	r_s	0.78	0.85	0.95	0.98
		r_b	0.90	0.94	0.94	0.97
Mean		r_s	0.83	0.88	0.94	0.97
		r_b	0.86	0.92	0.97	0.99

[a] The combined factor of cultivar × year
[b] All correlation coefficients are significant at $P < 0.01$

Table 5. The percentage of GE interaction MS generated by the selected set as compared to that of the best set (% optimality) for the test sets

Test set	Location-level			
	4	7	10	13
	Cluster set 1			
1984 (17)[a]	35.7	55.0	59.7	72.9
1985 (17)	36.4	72.5	82.0	83.9
1986 (18)	72.4	90.8	92.8	89.8
1987 (19)	38.9	42.9	61.8	72.5
	Cluster set 2			
1985 (17)	52.6	56.0	81.8	86.3
1986 (18)	63.4	53.4	59.8	74.5
1987 (19)	54.3	66.7	74.7	88.0
	Cluster set 3			
1987 (19)	44.1	44.3	59.8	69.2
Mean[b]	48.9	56.1	68.7	77.3

[a] Number of locations
[b] Unweighted mean

Table 6. Rank correlations between the cultivar means of the selected and that of all locations (r_s); and the correlations between the cultivar means of the best set and that of all locations (r_b) for the test sets

Test set		Location-level			
		4	7	10	13
		Cluster set 1			
1984 (10)[a]	r_s	0.83	0.92	0.78	0.99
	r_b	0.92	0.92	0.98	0.92
1985 (7)	r_s	0.89	0.96	0.89	1.00
	r_b	0.79	0.86	0.96	0.96
1986 (6)	r_s	0.94	0.43 ns[c]	0.94	0.83
	r_b	0.43 ns	0.66 ns	0.89	0.94
1987 (11)	r_s	0.78	0.81	0.81	0.90
	r_b	0.90	0.85	0.90	0.95
		Cluster set 2			
1985 (7)	r_s	0.71 ns	0.93	0.93	0.96
	r_b	0.79	0.86	0.96	0.96
1986 (6)	r_s	0.89	1.00	1.00	0.94
	r_b	0.43 ns	0.66 ns	0.89	0.94
1987 (11)	r_s	0.61	0.89	0.92	0.97
	r_b	0.90	0.85	0.90	0.95
		Cluster set 3			
1987 (11)	r_s	0.81	0.87	0.96	0.95
	r_b	0.90	0.85	0.90	0.95
Mean[b]	r_s	0.81	0.86	0.90	0.94
	r_b	0.76	0.87	0.92	0.95

[a] Number of cultivars
[b] Unweighted mean
[c] The "ns" refers to not significant ($P > 0.05$). All unspecified correlations are significant

tion was about 50% of that of the best set. This percentage increased as the location-level increased, and at the 13 location-level it reached nearly 77%. This means that if we use the % optimality as a sole criterion, and consider 82% as an upper limit, then 13 locations or more may be required. However, if we use the correlation of cultivar means between selected and all locations (r_s) as a criterion, then 10 locations may be satisfactory because a correlation of 0.90 (Table 6) was higher than the standard of 0.88. It is worth noting that r_s and r_b were very similar when location-level was 10 or greater, which implies that a % optimality around 70% may be adequate for these data sets.

Discussion and conclusion

In a regional trial, selection of locations (test sites) requires that they be representative of regional characteristics, which can be realized to a certain extent by maximizing the GE interactions MS with the smallest possible number of locations. The higher the GE interaction the greater the resolution among cultivars for their differential responses to environments (locations). Therefore, the important consideration for selecting locations for a regional trial is not so much the *number* of locations but *which* locations should be included so that a representative GE interaction of the region can be generated. Lin and Butler's (1988) method solves this problem numerically. However, a critical problem is that in a national trial, the same locations are likely to be used year after year. Thus, the selection of locations needs to take the location × year interaction of each region also into consideration. In general, the higher the ratio of the fixed component of location effect (i.e. the smaller the location × year interaction as compared to the main effect of location) the smaller the number of locations that is needed. However, if the ratio is small as in the present study (less than 50%), more locations are needed to cope with unexpected location × year interaction.

As mentioned earlier, one of the practical problems in the present investigation was to determine the proper location-level based on the calculated % optimality and the rank correlations. Although we can use the values from the cluster set as standards, they often represent the upper limits, which may be too high. Perhaps a more practical guideline would be to choose the location-level at which r_s and r_b are about equal and high, because it is an indication that the selected locations at that location-level are near the optimum number.

Table 7. Years within location MS for 17 locations in each cluster set

Location	Set 1	Set 2	Set 3
CHAR	111[a]	458	868
FRED	196	100	1,727
NAPP	622	187	79
LAPO	2,408	966	491
STAN	287	–	1,131
NORM	3,211	5,094	266
ALSA	728	539	155
ELOR	222	379	211
KEMP	697	186	2,452
NEWL	573	204	413
OTTA	714	2,229	1,070
RIDG	1,210	464	120
LIST	1,091	2,792	1,177
THUN	436	205	897
SYRO	–	1,244	435
HURO	–	258	158
PINT	–	–	554

[a] Divide by 1,000

Although 7 locations seem to be appropriate for generating the GE interaction structure for the cluster sets in this study, it appears that 10–13 locations would be necessary in order to cope with the unpredictable location × year interactions. One important consideration when the additional locations are to be selected for this purpose is an examination of type 4 stability for each location, i.e. years within location MS averaged over all cultivars as advocated by Lin and Binns (1988). For the present example (Table 7), we found that type 4 stability was very large for NORM, OTTA, and LIST, indicating that these locations had a large year variation. Although the underlying factors have to be investigated individually, if no apparent reason (drought, disease, etc.) can be found it is still desirable to include such locations in the test since they are more likely to create larger location × year interactions.

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