

## A method of choosing locations based on genotype × environment interaction for regional trials of rice

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### Abstract

An ideal test location with particular environmental conditions should generate a relatively large genotype × environment interaction (GE) value in regional trials. A method of selecting test locations based on GE was proposed. Using the statistic  $D_j$ , the ranking of ability to discriminate the best genotypes for locations could be obtained. The locations among the group with the smallest  $D_j$  value were deleted from the whole set step by step, and the number of locations was reduced to a rational level at which most (over 85%) of the GE sum of squares of trials were maintained. A comparative evaluation based on data collected from Zhejiang Province (China) rice regional trials showed that the new method was more feasible than two other methods without loss of accuracy and reliability.

**Key words:** *Oryza sativa* — genotype × environment interaction — locations — regional trials

The choice of experimental locations is an important part of crop regional trials. An ideal location for the assessment of yield potential of a set of genotypes should reflect the relative potential of those genotypes over a wide range of possible environments. However, locations suitable for crop yield trials and for identifying promising genotypes are often determined by arbitrary factors, such as convenience, administrative region, etc., and sometimes by chance.

Efforts have been made to choose 'representative' locations for field trials. Hamblin et al. (1980) proposed four criteria which they believed to be important in choosing areas for trials. One was that the differences between the genotypes at a test site should allow easy discrimination of the best genotypes and that the regression coefficient could be employed to estimate the 'discrimination' ability of the test site. Brown et al. (1983) used the regression coefficient of sites for the classification and evaluation of testing sites. Geng et al. (1990) designed an index based on an analysis of variance for estimating the suitability of test locations to evaluate genotypes. Lin and Butler (1988) and Lin and Morrison (1992) developed a clustering method using similarity of genotype × environment interaction (GE) as the distance measure. Based on this method, locations were grouped first and then locations with similar environmental responses were discarded. Two methods based on the different distance measures, i.e. similarity of GE and diallel correlation, were compared by Brandle and Arthur (1992). The conclusion was that neither technique could be deemed completely adequate. Cluster analysis procedures were also suggested for the classification of locations in regional trials where trial entries change over

years (Lawrence and Delacy 1993) and the shifted multiplicative model (SHMM) clustering method was applied to cluster test sites (Abdalla et al. 1997).

Evaluation trials of cultivars are normally conducted for consecutive years and at multiple locations. To obtain a successful cultivar assessment, a considerable number of test locations representing a reasonable range of the regional characteristics is required. However, owing to the limitation of resources and funds it often becomes necessary to reduce the number of test locations, but it is difficult to determine which and how many locations are needed to obtain adequate samples representing regional environments. If a few locations are chosen, the cultivars in the trials would not be fully exposed to the wide range of conditions prevalent in that region. Conversely, if too many locations are used, there would be a risk of redundancy in work and wastage of resources.

The purpose of this study was to design and describe a method for reducing the number of test locations to a rational level at which most of the GE sum of squares (SS) of trials were maintained.

### Materials and Methods

**Materials:** The data used were yield data for early season *indica* rice and later season *japonica* rice resulting from official variety performance tests in Zhejiang Province in China. In the experiment, 20 *indica* rice genotypes were tested at 15 locations and 11 *japonica* rice genotypes at 12 locations in 1997. A randomized complete-block design with three replicates was employed, and the plot size recommended to cooperators was 13.3 m<sup>2</sup>.

**Statistical procedures:** An ideal test location typifying the condition of a particular environment, such as climatic condition, plant disease complexes and soil types, should generate a relatively large GE in regional trials. A statistic,  $D_j$ , is designed to estimate the GE which a location generates in a multiple environment trial based on the additive main effects and multiplicative interaction (AMMI) model:

$$D_j = \sqrt{\sum_{r=1}^N IPCA_r^2} \quad (j = 1, 2, \dots, n) \quad (1)$$

where  $D_j$  is the distance of the  $j$ th location in the space of principal components analysis (PCA) axes from the origin and  $IPCA_j$  is an estimator of the  $j$ th location for interaction, i.e. the environment PCA score and is expressed as the principle component score for PCA axis  $r$  of the  $j$ th location ( $y_{jr}$ ) times the square root of the eigenvalue of the PCA axis  $r$  ( $\lambda_r$ ), i.e.

$$IPCA_j = \lambda_r^{0.05} \gamma_{jr}$$

$N$  is the number of principal components which are significant at probability levels of 0.01 or 0.05.

The AMMI model is

$$Y_{ij} = \mu + g_i + l_j + \sum_r^n \lambda_r \alpha_{ir} \gamma_{jr} + e_{ij} \quad (2)$$

where  $Y_{ij}$  is the yield of the  $i$ th genotype in the  $j$ th environment,  $\mu$  is the grand mean;  $g_i$  and  $l_j$  are the genotype and location deviations from the grand mean, respectively,  $\lambda_r$  is the eigenvalue of the PCA axis  $r$ ,  $\alpha_{ir}$  and  $\gamma_{jr}$  are the genotype and location principal component scores for axis  $r$ ,  $n$  is the number of principal components retained in the model and  $e_{ij}$  is the error term.

The statistic  $D_j$  gives the genotype  $\times$  environment interaction estimate of a particular location (the  $j$ th location) with a group of genotype samples. The greater the  $D_j$  value of a location, the greater the distance of the location from the origin of PCA axes. It also implies that there is a greater GE in a location with such a high  $D_j$  value, with this location being considered an ideal one to discriminate the best genotypes. If the  $D_j$  value is too small for a location, this location would fail to generate enough GE for the trials and be regarded as unsuitable to measure the intrinsic GE of cultivars and the accuracy of regional trials. This provides a measure of the degree of empirical success that the locations have provided.

**Ranking and optimizing:** For genotypic yields in multilocation trials, the AMMI analysis was conducted first. Based on the results of AMMI analysis, the value of  $D_j$  for every test location can be obtained using equation (1). Locations were ranked on the basis of their  $D_j$  values, and locations with smaller  $D_j$  values were removed until the percentage of the GE sum of squares (SS) of residual locations was just over 85%. The GE SS maintained included most ( $\geq 85\%$ ) of the GE SS in regional trials and would therefore ensure a representative GE, while the location level of regional trials was minimized.

As well as the  $D_j$  method, two other methods, i.e. coefficient of variation (CV) and  $b$ -value for the regression of set yield on overall mean yield, can be employed both to estimate the discrimination ability of test sites and to rank the test sites on the basis of their CV or  $b$ -values. The comparison of all the three methods was undertaken using data from the regional trials of rice in Zhejiang Province. The rankings of the locations by using the three methods separately were compared based on the GE as explained above.

The CV is defined as usual.

The regression model is

$$Y_{ij} = a_j + b_j y_{i.} + e_{ijk} \quad (3)$$

where  $Y_{ij}$  is the mean yield of the  $i$ th genotype at the  $j$ th location;  $a_j$  is the intercept of the  $j$ th location,  $b_j$  is the coefficient of regression of the

$j$ th location,  $y_{i.}$  is the mean yield of the  $i$ th genotype at all locations, i.e. the 'cultivar index' of the  $i$ th genotype, and  $e_{ijk}$  is the error term.

## Results

### Ranking and optimizing

The AMMI analysis of data from rice regional trials in Zhejiang Province showed that the GE was highly significant ( $P < 0.01$ ). F-tests significant at the  $P < 0.05$  level suggested including the first three interaction PCA axes, which covered 68.60% of the mass of the GE SS in only 33.83% of the interaction df in the model (Table 1). The  $D_j$  values for all the locations, measured by equation (1), could then be obtained based on the three PCA axes (Table 2). Table 2 indicated that the  $D_j$  values of Taizhou, Jiaxin, Ruian and Ninbo were less than 0.4 and their distances from the origin of PCA axes were among the shortest. This meant that those four test sites generated a small amount of GE in 1997 and had poor ability to discriminate the best genotypes. Accordingly, Zhoushan, Fuyang and Lishui had intermediate value while Wenzhou, Longquan, Shaoxin, Pujiang and Quzhou were the best in the year. This suggested that those locations with a greater  $D_j$  value were ideal locations for rice regional trials in Zhejiang Province based on the data from the trial of 1997.

In a regional trial, locations chosen for yield trials should be representative of regional characteristics, and this can be realized to a certain extent by maximizing the GE with the most reduced number of locations. The higher the GE, the

Table 1: Additive main effects and multiplicative interaction (AMMI) model analysis of variance of rice trials including the significant interaction principal component analysis (PCA) axes

Source of variation	df	Sum of squares	Mean squares
Treatment combinations	299	1679.11	5.61**
Genotype	19	214.35	11.28**
Environment	14	1180.15	84.29**
Genotype $\times$ environment interaction	266	284.59	1.06**
Interaction PCA1	32	102.62	3.20**
Interaction PCA2	30	52.17	1.77**
Interaction PCA3	28	40.45	1.44**
Residual	176	88.34	0.50
Error	600	74.33	0.12

\*\* Significant at  $P = 0.01$ .

Location code number	Location	$CV_j$		$b_j$		$D_j$	
		Value	Rank	Value	Rank	Value	Rank
1	Changxin	6.22	15	0.57	13	0.79	8
2	Jiaxin	7.86	8	1.13	3	0.31	14
3	Jinhua	8.19	6	0.89	8	0.98	6
4	Quzhou	9.55	3	0.30	15	1.12	5
5	Lishui	6.42	13	0.77	11	0.56	9
6	Longquan	20.41	1	2.07	2	1.56	2
7	Ninbo	6.82	11	0.90	7	0.38	12
8	Pujiang	9.45	4	1.06	5	1.29	4
9	Shaoxin	9.08	5	0.54	14	1.37	3
10	Fuyang	6.29	14	0.76	12	0.41	10
11	Taizhou	7.65	9	0.85	9	0.19	15
12	Ruian	7.16	10	1.09	4	0.32	13
13	Wenzhou	16.34	2	2.32	1	1.75	1
14	Zhoushan	6.79	12	0.80	10	0.40	11
15	Zhuji	6.17	16	0.94	6	0.84	7

Table 2: Estimated  $CV_j$ ,  $b_j$  and  $D_j$  values and their ranks at all 15 locations in the *indica* rice trials (see text)

greater the reliability for resolution among cultivars for their different responses to environments (locations). Therefore, the main consideration for selecting locations for a regional trial does not focus on the number of locations but on the quality of the locations that can generate a representative GE of the region (Lin and Morrison 1992). A good method to solve this problem should provide a representative GE when a proper location-level is to be determined. As a result, some locations which generate the smallest GE may be removed from the regional trials.

In the present example, the  $D_j$  method was efficient enough to solve the problem. The locations with the smallest  $D_j$  values were removed until the ratio of the GE SS of the residual locations (i.e. the GE SS of the residual locations compared with that of all the locations) was just greater than 85%. This ensured that a representative GE (i.e. 85% of total GE) would be maintained when some locations were removed and the number of locations (location-level) minimized. The results (Fig. 1) show that the curve reduced slowly at first and then tended to tail off when the locations with the smallest  $D_j$  values were deleted step by step. After Ninbo (the fourth location among those with the smallest  $D_j$  value) was deleted, the ratio was 89.88%, when the fifth location (Zhoushan) was discarded, the ratio reached nearly 84%. This means that if a  $D_j$  value was used as a sole criterion for measurement and 85% set as a lower limit, then 11 residual locations might be required in rice trials in Zhejiang Province. The other four locations with the smallest GE would be removed to conserve resources.

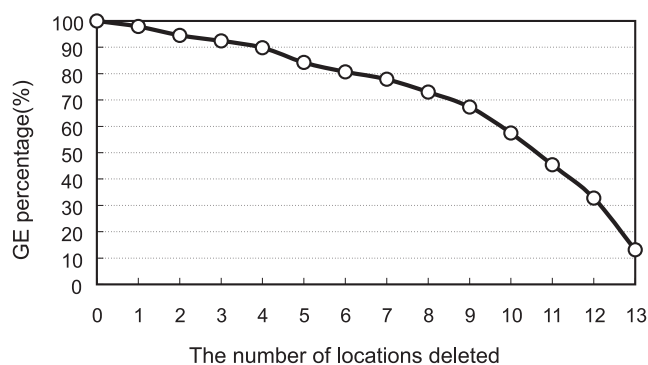


Fig. 1: The curve of the genotype  $\times$  environment interaction (GE) of the residual locations over that of all locations when the locations with the smallest  $D_j$  values were deleted

### A comparative evaluation

Based on the same example, a comparative evaluation of three methods of selecting locations was undertaken. The  $D_j$ ,  $b_j$  and  $CV_j$  values of all locations were obtained by using equations (1) and (3), respectively (Table 2). The results of the ranking based on the three methods are given in Table 2. The results indicated that the three rankings, except for a few locations, were similar in general, especially for the locations with high discrimination ability. This suggested that relatively similar conclusions could be reached by all three methods, although they were based on different theories, except that some locations had different rankings, such as numbers 2 (Jiaxin), 9 (Shaoxin) and 15 (Zhuji).

Based on three locations (numbers 2, 9 and 15), the three methods could be compared in terms of the amount of the GE SS at these three locations. After deleting one of the three locations from the whole set (total 15), the GE SS of the residual locations (total 14) were calculated. The results showed that the GE SS of residual locations were 275.21 for Jiaxin (2), 252.10 for Shaoxin (9) and 270.35 for Zhuji (15). When the GE SS of the three locations were calculated, ranking was achieved as  $9 > 15 > 2$ . When comparing this ranking with the rankings of the three methods, only  $D_j$  was similar. The rankings using  $CV_j$  and  $b_j$  were  $9 > 2 > 15$  and  $2 > 15 > 9$ , respectively, which implies that the  $D_j$  method was a more rational than the other two.

A similar result was obtained based on the data of later 1997 season *japonica* rice regional trials tested in 12 locations with 11 *japonica* cultivars. Several locations, such as numbers 2 (Jiaxin), 4 (Lishui) and 9 (Taizhou), had different rankings with the three methods (Table 3). The ranking of three locations (2, 4 and 9) based on the GE SS was  $2 > 4 > 9$ . The rankings of the three locations by the three methods ( $CV_j$ ,  $b_j$  and  $D_j$ ) were  $4 > 9 > 2$ ,  $9 > 2 > 4$  and  $2 > 4 > 9$ , respectively. The ranking using  $D_j$  was the only one that fitted to the ranking of GE SS.

Another way to evaluate the three methods was to compare the percentages of GE SS retained after removing the last four rank locations using all three methods. Table 4 shows the results after removing the four locations, respectively, based on all three methods. The results show that the percentage of GE SS using the  $D_j$  method was 89.88% when the fourth location was removed. But when the first and second locations were removed using the  $b_j$  and  $CV_j$  methods, respectively, the percentages were 89.75% and 88.87%, i.e. less than the percentage (89.88%) of the  $D_j$  method. This suggests that

Table 3: Estimated  $CV_j$ ,  $b_j$  and  $D_j$  values and their ranks of at 12 locations in the *japonica* rice trials (see text)

Location code number	Location	$CV_j$		$b_j$		$D_j$	
		Value	Rank	Value	Rank	Value	Rank
1	Changxin	8.36	4	0.88	5	0.64	8
2	Jiaxin	7.09	10	0.67	10	1.05	2
3	Quzhou	9.19	1	1.33	3	0.87	4
4	Lishui	8.31	5	0.65	11	0.66	7
5	Longquan	7.11	9	0.67	9	0.56	9
6	Ninbo	7.73	6	1.60	2	0.83	5
7	Pujiang	6.96	11	0.62	12	0.70	6
8	Fuyang	8.84	2	1.98	1	1.18	1
9	Taizhou	7.47	7	0.73	8	0.25	12
10	Wenzhou	6.57	12	0.82	7	0.46	11
11	Zhoushan	7.43	8	0.87	6	0.56	10
12	Zhuji	8.45	3	1.19	4	1.03	3

Number of locations deleted	Residual GE		Residual GE		Residual GE	
	SS by $D_j$	Percentage	SS by $CV_j$	Percentage	SS by $b_j$	Percentage
0	284.59	100	284.59	100	284.59	100
1	278.52	97.87	270.35	95.00	252.92	88.87
2	268.94	94.50	255.41	89.75	218.16	76.66
3	263.18	92.48	244.46	85.90	197.50	69.40
4	255.78	89.88	236.72	83.18	186.11	65.40

Table 4: The percentages of genotype  $\times$  environment interaction sum of squares (GE SS) which were retained after removing the last four rank locations using the  $CV_j$ ,  $b_j$  and  $D_j$  methods in the *indica* rice trials, respectively (see text)

the  $D_j$  method is a relatively good one to optimize regional trials in rice.

## Discussion

The performance of a number of genotypes evaluated in a broad range of environments (multilocation) is always affected by GE. The primary aim of multilocation trials in plant breeding programmes is to estimate genotypic yields. Thus, the selection of locations needs to take the GE into consideration and the selected locations should ensure that the GE characteristics of the cultivars are assessed properly.

As mentioned earlier, the  $D_j$  method based on the GE that a location creates is valuable. A significant feature of the method is that the  $D_j$  value includes all environment PCA scores which were significant at the 0.05 or 0.01 probability levels, whereas only the first one or two genotype PCA scores are used widely for cultivar evaluation (Cross et al. 1991). A large part of associated and biologically meaningful GE used to be relegated to the residual term of the AMMI analysis. However, the  $D_j$  method, involving more important GE information, avoided the fear of such over-simplification. In the present example, it was found that the first three significant PCA ( $P < 0.05$ ) accounted for the majority (69%) of the total GE SS. However, the over-simplification, which resulted in a small sacrifice (31%) of GE SS, as shown in the example, should also be recognized.

From a practical viewpoint, one important consideration is whether the selected locations will generate a similar GE structure year after year. The investigations of Lin and Butler (1988), Lin and Morrison (1992) and Fan et al. (2001) showed that some degree of consistency for GE pattern exists. It is possible that a location effect consists of two components, a fixed (e.g. soil) and a random (e.g. weather) effect, which are confounded. This suggests that the evaluation of locations based on the GE will depend upon a proper year-level in order

to cope with unexpected GE, thus arbitrary conclusions based on data from one or two years would be avoided.

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