Evaluation methods and utilization of germplasm of annual crop species.

J.B. Smithson,
Centro Internacional de Agricultura Tropical,
Apartado Aereo 6713,
Cali, Colombia.

[1987]

ABSTRACT

The proper evaluation of the quantitative characteristics of large numbers of materials such as germplasm collections presents serious problems due to the effects of environment and g x e interactions on character expression. There is no complete solution to these problems but methods of measuring and reducing environmental variability are available.

General environmental effects may be reduced by adjustment of test entry performance according to the performance of regular checks or nearest neighbour analysis. Common checks enable adjustments between seasons.

G x e interaction is more difficult and can only be estimated by evaluating the same materials in more than one environment. This also provides a much better description of performance. Multivariate analysis can be used to select locations and accessions representing the total range of environments and genotypes. Emphasis should be given to the collection of environmental data to obtain a better understanding of the relationship between genotypes and their environments.

Lastly, long lists of accessions and their characteristics are not the best way to present the information recorded. A frequency distribution for each character together with short lists of materials with important characters or combinations of characters are more helpful to the breeder. Information on the environments in which the evaluations are conducted is important in identifying materials for particular circumstances. Sets of materials of different sizes, representing the total variability in the collection, will be helpful where knowledge of plant/environment relationships is incomplete.

Evaluation methods and utilization of germplasm of annual crop species.

J.B. Smithson,
Centro Internacional de Agricultura Tropical,
Apartado Aereo 6713,
Cali, Colombia.

INTRODUCTION

The proper evaluation of the very large germplasm collections now assembled for many crop species presents difficult problems, which arise principally from the affects of the environment on the expression of plant characteristics. For qualitative characteristics there is little difficulty as their expression is usually affected little by environment. Examples are seed coat and flower colour and colour pattern. A single evaluation is all that is required to characterise a set of materials in respect of such characteristics.

It is, however, the quantitative characteristics, and these are of most interest to the breeder, that are especially intransigent, as their expressions are always modified by environment to some degree, so that the separation of the contributions of genotype and environment to the phenotype require special techniques.

ENVIRONMENT AND GENOTYPE X ENVIRONMENT INTERACTION

The modification of plant characteristics by environment takes two forms. First, there is a general reduction or increase in expression of a character across all genotypes. Environmental features such as soil fertility, moisture availability, temperature and pathogens, pests and weeds may all affect plant characters in this way. The result is what is often termed "field variability" and this will always occur in a single evaluation at a single location in a single season. It also occurs across locations and seasons.

Secondly, there is the situation where all genotypes are not affected equally by differences in environment, normally described as "genotype x environment interaction". Such interactions are usually associated with a set of materials being grown in more than one location or season and indeed is only detectable so. It also occurs in a set of materials at a single location and season but is not separable from general environmental effects.

The two features of the relationship between plants and their environments pose serious problems in the evaluation of large numbers of materials, whether germplasm or breeding lines, and in the interpretation of the collected data. Two examples from

chickpeas illustrate the importance of environmental effects in evaluation and the misinterpretations that can arise as a result.

The first concerns seed protein percentage (unpublished). Seed protein percentages were routinely determined on seeds from successive evaluations of different germplasm accessions over a period of five years. The seeds of 100 accessions representing the largest and smallest seed protein percentages in each of the five years were grown in a replicated trial at ICRISAT Centre in 1982-83 and 1983-84 and the seed protein percentages of their produce determined. Examination of the data revealed very poor correlations between the seed protein percentages from the germplasm evaluation and those from the replicated trials in either of the two years. Large and small values from the germplasm evaluation tended towards the mean and much of the differences that had been demonstrated earlier disappeared. There were, however, good correlations between the protein percentages obtained from the trials in the two years, indicating that the discrepancies arose from environmental differences within or between seasons or both but illustrating that repeatable results can be obtained with appropriate methods.

The second case concerns days to flowering (ICRISAT, 1980). Based on germplasm evaluation data, five groups of lines flowering respectively in 45, 45-56, 57-68, 69-80 and more than 80 days after sowing were included in five replicated trials again at ICRISAT Centre to examine their adaptability to sowing one month earlier than normal. Mean flowering times for the trials were roughly according to expectation, the ranges of flowering times were, however, 33-80, 34-66, 41-80, 40-106 and 44-124, overlapping completely and with very poor correlations with the earlier records, and this is exactly what would be expected from the effects of different daylengths and temperatures on the flowering times of sets of materials differing in their photoperiod and temperature responses.

As usual, there is no complete solution. Nonetheless, methods of measuring and controlling environmental variation are available and some of these will be described and discussed in this paper.

The question of the need for evaluation of genetic resources and of the characters to be evaluated are not considered here. It is assumed that accessions are maintained discretely in order to retain character combinations and that some kind of evaluation is required for the purposes of utilization. In the text, materials being evaluated will be termed test entries or materials. Performance refers to the value assigned to any quantitative character, be it size, concentration or number of any plant component or rate of any plant process. It is also assumed that performance is being measured in field nurseries and with the necessary precision and accuracy. Disease and pest reactions are not considered since they require specialised techniques.

FIELD VARIABILITY

First, we will consider means of handling a set of test entries in a single location and season. Where numbers are relatively small (say 500 or less), orthodox statistical designs such as randomized blocks or lattices may be employed, utilizing techniques such as randomisation, replication and sub-grouping within replicates to measure and/or control environmental effects.

But germplasm collections and early generation breeding materials are usually too numerous to allow replication and, were replication even feasible, and with the most uniform field environment conceivable, the replicates would be too large to cope with field variability by orthodox analysis of variance. What then can be done to measure and/or control environmental effects in such situations?

1. Regular check entries.

The simplest means of obtaining a measure of environmental variability in an unreplicated set of entries is the inclusion of check entries. This is common practice in germplasm evaluation, comprising the inclusion of 2 or 3 different checks in the nursery at the rate of one check for every 10 test entries. Unfortunately, the mere inclusion of checks is not sufficient. They must also be used to assess and reduce field variability by some form of adjustment of the performances of the test entries according to the performance of the checks, and this is rarely practised.

The simplest form of adjustment is to express the performance of each test entry as a percentage or proportion of some measure of the performance of the checks in the same sector of the field. Where several different checks are included, the best measure is likely to be the mean of the performances of the nearest full set or sets of checks.

Alternatively, the performance of each test line may be adjusted by subtracting the deviation of the mean of the performances of the nearest full set of checks from the mean of the performance of all the checks. For example, if the mean number of seeds per pod of the nearest full set of checks is 9.5 and the mean for all checks is 9.0 seeds per pod, the number of seeds per pod of each test entry in the same sector of the field is adjusted downwards by 9.5-9.0 = 0.5.

Conceptually, the latter adjustment is more desirable than the first in that the actual and the adjusted date are of the same units. Both have the disadvantage of providing no estimate of error for a comparison of the differences among the test lines.

2. Augmented designs.

An extension of the regular check system is the augmented design

(Federer, 1956). The test materials are again unreplicated but are randomised and grouped in blocks of convenient size (say 20 to 50) for the number of materials, size of field and number of checks. An appropriate number of different check entries (2 to 5 according to block size) is then randomised within each block. Check performance can then be analysed in the form of a complete randomized block and the estimate of error so derived used to compare the performances of the unreplicated test lines.

The performances of the test lines may also be adjusted according to the deviation of the mean of the checks in the block in which they occur from the mean of all the checks, so that Bj-y.. is the adjustment for the performance of each of the test lines in the jth block, where Bj is the mean of the checks in the jth block and y.. is the overall check mean. Note that the variance of the difference of two test entries in the same block will be twice the error mean square, while that of two test entries in different blocks will contain an additional quantity for block differences.

The method assumes that the random components associated with the checks and the test lines are similar. This may not be so, but is more likely if the checks are chosen to represent the range of variability in the collection. However, it does provide a measure of environmental variation and a means of adjusting the performances of the test lines to remove some of the variability, and thus is an improvement on other methods.

One further point. It is common practice to include test lines in order of origin or group them in some other way, so that similar materials are compared more accurately. But in evaluating a set of materials, we are also interested in the relative performances of dissimilar materials, so in the absence of very compelling reasons for grouping, less biased comparisons are obtained by randomising the test lines.

3. Nearest neighbour analysis.

A third and less often used method of handling field variability is adjustment according to the performances of neighbouring plots, first proposed by Papadakis (1937) and described with a worked example in Pearce (1983). Adjustment by neighbouring plots may be applied to any replicated field layout and is especially useful for large sets of materials. It adjusts the performance of each plot according to the mean performance of its neighbours. In most cases, the four plots adjoining the ends and sides of each plot are used for the adjustment. Where plots are long and narrow, it is more appropriate to use only the plots along each side. In the case of end plots there are only three neighbours and corner plots have only two, but dummy plots can be sown around the outside of the nursery to provide four neighbours for end and corner plots also. It has been found that the first cycle of adjustment is often erratic, but it is possible to iterate (i.e. repeat the calculation with the adjusted values until the adjustments remain similar), as is usually done when estimating

values for more than a single missing plot.

The analysis proceeds as follows:

(a) Compute the deviations of each plot from the mean of all plots of that treatment.

(b) Compute the mean deviations (X) of the neighbours of each plot.

(c) Compute treatment totals and means for the X values.

(d) Compute an analysis of covariance of the actual values (Y) on X.

(e) Compute the regression (b) of Y on X.

(f) Adjust the Y value for each plot by subtracting b(X-x).

(g) Iterate the above steps until the adjustments are the same.

(h) Compute the analysis of variance of the adjusted values.

Since the test entries must be replicated the area required will be large but the method allows for adjustment for patchy field variability which is not possible in an orthodox analysis of variance in addition to providing an estimate of error.

Summary

It should be noted that these methods of analysis are not mutually exclusive. Common checks ought always to be included to enable adjustment across seasons and, provided there is replication and randomisation, both types of adjustment are theoretically possible. Augmented designs and nearest neighbour analysis both provide estimates of error for comparison of differences among entries; nearest neighbour analysis takes up more more land because at least two replicates are required, but this may be accommodated to some extent by reducing plot size; computer facilities are desirable for all because of the large volume of material to be examined; nearest neighbour analysis can be expected because of replication and the opportunity to adjust for patchy field variation and (see next section) to assess the magnitude of g x e interaction.

EVALUATION ACROSS SITES AND SEASONS

Because of g x e interaction, the relative performance of a set of materials in one environment is unlikely to reflect their relative performances in other locations and seasons. The value of a set of data obtained in one environment therefore is doubtful especially so in a country as varied as Ethiopia.

Furthermore, the very large numbers now present in germplasm collections virtually preclude the possibility of evaluating all materials at a single time. The whole available CIAT bean collection (over 17,000) was evaluated for resistance to angular leaf spot in hill plots this year (CIAT, 1987) but this is a special case. There is also the case of continuing collection and the need to evaluate newly assembled groups of materials. For these reasons, the evaluation of germplasm collections in successive seasons and/or at more than a single location is inevitable.

Care can be taken to ensure that environments are as uniform as possible. For example (IITA, 1974) in the evaluation of the IITA cowpea collection in two successive years, sowing was on the same date and exactly the same cultural practices were used, with sufficient fertilizer and irrigation to reasonably eliminate any soil nutrient or moisture stress. Nevertheless, it must be accepted that the removal of all the environmental variability likely to affect performance is impossible.

General environmental effects can be accommodated to some degree by the inclusion of common checks and using augmented designs to adjust performance across seasons in the same manner as within seasons.

Genotype x environment interactions cannot be handled in this way. Various methods of assessing their magnitude and of characterizing them have been developed. They include: combined analysis across locations and seasons; regression of individual entry performance on environment mean performance or some other environmental measure; and, more recently, multivariate analysis. A comprehensive account of these techniques is given by Hill (1975). Such methods have helped in the understanding of g x e interaction but are not appropriate to the evaluation of large numbers of materials.

There does, therefore, appear to be no escape from the need to evaluate the same test materials in more than a single environment. Seasons are unsatisfactory as they are unpredictable, so this means testing at a number of locations. Multivariate analysis can be used to chose locations that represent the range of environments in which the test entries are likely to be utilized. The greater the number of locations the more complete will be our characterization, but practically, three or four (say two extremes and two intermediates) may be all that is feasible or, for some characters, even necessary. For example, based on growth cabinet studies with chickpeas and lentils, Roberts et al., (1985) and Summerfield et al., (1985) conclude that evaluation in field nurseries at three properly chosen locations is sufficient to characterize accessions of these species for their flowering responses to photoperiod and temperature. Alternatively, the numbers of environments may be increased and the numbers of materials reduced by selecting representatives of the total variability by some form of multivariate analysis.

Finally, environmental data is every bit as important as plant character data in any evaluation if we are to understand variation in performance across environments. It is vital, therefore to characterize the physical and biological environments in which evaluations are conducted as thoroughly as we characterize our plants. Physical factors should include, at least: latitude; altitude; maximum and minimum temperatures and rainfall on 10 day mean bases during the growing season; and physical and chemical properties of soils. The biological environment will include diseases, insects and weeds.

SUGGESTED PROCEDURES

Based on the above considerations it is possible to suggest optimal procedures for germplasm evaluation.

Whole collections should be evaluated at 3 to 4 locations selected to represent a range of situations. Operationally, this will have to proceed in groups of around 2000 entries. These should be selected at random from those available for evaluation - they should be deliberately grouped only if absolutely necessary - for example, bush and climbing types. They should be sown in an augmented design with with a set of frequent, common checks, chosen to cover the total variability in the collection as far as possible. If possible, two replicates should be sown at each location to allow a nearest neighbour analysis. Plant and environment data should be collected on each evaluation.

Multivariate analysis of the data should be conducted to select sets of different sizes representing the variability in the collection, as was done with the IITA cowpea collection in the late seventies (Rawal et al., 1977). These sets should be of different size (say 100, 500, 1000 and 2000) to accommodate different capacities. the evaluation of these sets by breeders in other environments and the return of the data for continuous updating of information should be vigorously encouraged.

Finally, a note of caution. The techniques described are merely tools to aid evaluation. In most circumstances they can be expected to be useful and we have a duty to use them. But there is always the possibility of them distorting differences rather than reducing environmental variation. There is, therefore, no substitute for knowledge of the crop, for careful observation of the test materials in the field, for careful examination of the actual and adjusted data and for the application pf common sense in its interpretation.

DISSEMINATION OF INFORMATION

A further area requiring thought is method of presentation of data. Pulse germplasm catalogues include those for cowpea (IITA,

1974), beans (CIAT, 1983) and chickpea (Singh et al., 1983). All comprise long lists of accessions and characters recorded. For cowpea there are 46 characters for 4224 accessions; for beans it is up to 7 characters on nearly 17,000 accessions; and for chickpea, it is 29 characters for about 3,300 accessions.

Such forms of presentation are very difficult for an aspiring breeder to assimilate and are therefore not very useful. The information is important for the institution conducting the evaluation but can be on computer file for manipulation as it is unlikely that a hard copy of the complete information is ever going to be required.

For the breeder, what would be more useful would be a summary for each character perhaps in the form of a histogram showing its frequency distribution together with an estimate of the total variability such as the coefficient of variation. This should be accompanied by relatively restricted lists of accessions with extremes of important characters (for example disease resistance) or important combinations of characters. Data on the environments in which the evaluations are carried out and a summary of the check performance should also be included. The intending breeder can then see easily what kind of variability is available and can request whatever number of materials, having the range of characteristics and adaptation in which he is interested, that he is capable of handling.

These procedures presuppose that the intending breeder knows what he requires. But in many cases this may not be so. In other situations, materials adapted to a wide range of environments are needed. These are additional reasons why performance in different environments is important and environmental information is required for every evaluation.

In this situation, the different sized sets representing the variability are important. The breeder evaluates a set of the size he can handle in his own environment, identifies the most promising materials, requests additional accessions of similar origin and character from the distributing institution and returns the data for up-dating the information base.

The development of these kinds of relationships between genetic resources specialists and breeders is vital if we are to make the most effective use of our genetic resources.

REFERENCES

CIAT (1983). Catologo de Frijoles. Centro Internacional de Agricultura Tropical, Cali, Colombia.

CIAT (1987). Annual Report 1986. Centro Internacional de Agricultura Tropical, Cali, Colombia.

Federer, W.T. (1956). Augmented (or Hoonuiaku) designs. Hawaian Planters Record, 55, 191-207.

Hill, J. (1975). Genotype x environment interactions - a challenge for plant breeding. Journal of Agricultural Science, Camb., 85, 477-493.

ICRISAT (1980). Annual Report 1979. International Crops Research Institute for the Semi-Arid Tropics, Hyderabad, India.

IITA (1974). Cowpea Germplasm Catalogue No. 1. International Institute for Tropical Agriculture, Ibadan, Nigeria.

Papadakis, J. (1937). Methods statistique pour des experiences sur champ. Bull. Inst. Amel. Plantes, Salonique (Grece), 23.

Pearce, S.C. (1983). The Agricultural Field Experiment. John Wiley & Sons, Chichester.

Rawal, K.M., Kaltenhauser, J. and Snyder, M.J. (1977). Agronomy Abstracts. 69th Annual Meeting, American Society of Agronomy, 68.

Roberts, E.H., Hadley, P. and Summerfield, R.J. (1985). Annals of Botany, 55, 881-892.

Singh, K.B., Malhotra, R.S. and Witcombe, J. (1983). Kabuli Chickpea Germplasm Catalogue. ICARDA, Aleppo, Syria.

Summerfield, R.J., Roberts, E.H., Erskine, W. and Ellis, R.H. (1985). Annals of Botany, 56 659-671.