

PLANT GENETIC RESOURCES - Newsletter
RESSOURCES GÉNÉTIQUES VÉGÉTALES - Bulletin
RECURSOS GENETICOS VEGETALES - Noticiario

41



INTERNATIONAL BOARD FOR PLANT GENETIC RESOURCES
CONSEIL INTERNATIONAL DES RESSOURCES PHYTOGÉNÉTIQUES
CONSEJO INTERNACIONAL DE RECURSOS FITOGENETICOS



FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
ORGANISATION DES NATIONS UNIES POUR L'ALIMENTATION ET L'AGRICULTURE
ORGANIZACION DE LAS NACIONES UNIDAS PARA LA AGRICULTURA Y LA ALIMENTACION

The Plant Genetic Resources Newsletter is published under the joint auspices of the Plant Production and Protection Division of FAO, and the International Board for Plant Genetic Resources. Contributions in English, French and Spanish are considered, and if accepted, will be published in the original language, with a summary in the other two.

Editor: J.T. Williams, AGP

Secretarial Assistant: Miss Dorothy Quaye, AGP
FAO, 00100, Rome, Italy

The designations employed, and the presentation of material in this newsletter, and in maps which appear herein, do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations (FAO) or the International Board for Plant Genetic Resources (IBPGR) concerning the legal or constitutional status of any country, territory or sea area, or concerning the delimitation of any frontiers. Similarly, the views expressed are those of the authors and do not necessarily reflect the views of FAO or the IBPGR.

FOREWORD

This issue of the Newsletter differs somewhat from recent ones. The Newsletter aims at providing up-to-date information through summaries of current activities and notes on crops, genetic resources centres, publications and meetings but there is, however, the need for review articles and papers on work which will interest the whole genetic resources community. Hence, this issue contains two papers of this type: one on evaluation and another on a new, more economic and accurate approach to monitoring the viability of accessions during storage in seed banks. Comments on these papers will be welcome.

A second item of interest relates to correspondents for this Newsletter. FAO has asked member governments to designate scientists who can act for their countries to keep us informed of on-going activities. Those correspondents nominated to date are listed in this issue.

AVANT-PROPOS

Le présent numéro du Bulletin diffère quelque peu des précédents. Le Bulletin vise à fournir des informations récentes au moyen de résumés des activités en cours et de notes sur les cultures, les centres de ressources génétiques, les publications et les réunions, mais il est néanmoins nécessaire d'y publier des articles et des documents passant en revue les travaux qui intéressent tous ceux qui s'occupent des ressources végétales. En conséquence, le présent numéro contient deux documents de ce type: un sur l'évaluation et un autre sur une approche nouvelle, plus économique et plus rigoureuse pour surveiller la viabilité des accessions au cours de l'entreposage dans les banques de semences. Toute observation sur ces documents sera la bienvenue.

Le deuxième point concerne les correspondants du Bulletin. La FAO avait demandé aux gouvernements membres de désigner des chercheurs qui puissent, au nom de leur pays, nous tenir informés des activités en cours. Les noms des correspondants désignés jusqu'à présent figurent dans le présent numéro.

PREFACIO

Este número del Noticiario difiere algo de los últimos. Con el Noticiario se pretende dar información actualizada a través de resúmenes de actividades en curso y notas sobre cultivos, centros de recursos genéticos, publicaciones y reuniones, pero existe, sin embargo, la necesidad de artículos y documentos de análisis sobre trabajos que interesen a toda la comunidad de recursos genéticos. Por ello, este número contiene dos artículos de este tipo: uno sobre evaluación y otro sobre un sistema nuevo, más económico y preciso, para vigilar la viabilidad de las entradas durante el almacenamiento en los bancos de semillas. Los comentarios sobre estos documentos serán bien acogidos.

Un segundo tema de interés se refiere a los corresponsables de este Noticiario. La FAO ha pedido a los gobiernos de los Estados Miembros que designen científicos que puedan actuar en nombre de sus países para mantenernos informados sobre las actividades en curso. En este número se incluye la lista de los corresponsales nombrados hasta la fecha.

Faint, illegible text in the upper left quadrant.

Faint, illegible text in the middle left quadrant.

Faint, illegible text in the middle section.

Faint, illegible text in the lower middle section.

Faint, illegible text at the bottom of the page.

A NEW, MORE ECONOMIC AND ACCURATE APPROACH TO MONITORING
THE VIABILITY OF ACCESSIONS DURING STORAGE IN SEED BANKS

R.H. Ellis^{1/}, E.H. Roberts^{1/} and J. Whitehead^{2/}

Provisional IBPGR recommendations for long-term storage

In most crop species, seed storage is the safest, cheapest and easiest method of conserving crop genetic resources. The IBPGR recommends storing orthodox seed for long-term conservation at -18°C , or less, with $5\pm$ percent moisture content (fresh-weight basis), this is the 'preferred' standard (IBPGR, 1976). This recommendation was based upon the observation that the seed survival period of the majority of crop species increases exponentially with a reduction in both seed moisture content and storage temperature. Such seed storage behaviour has been described as orthodox. There is a possibility of desiccation injury to seed of some orthodox species at moisture contents below 5 percent. In contrast, the recommended storage temperature is not based upon any physiological constraint, but is a pragmatic compromise between the implications of orthodox seed storage physiology and the availability, reliability and cost of refrigeration equipment. The IBPGR (1976) recommends that storage temperature could be relaxed from the preferred -18°C to -10°C only in special cases. ^{3/}

The preferred storage conditions are far superior to those in general use previously, but nevertheless some deterioration will occur under these conditions - albeit at a slow rate. This deterioration ('ageing') will eventually result in a reduction in the percentage viability of each accession. By itself, the random death of a few percent of a seed lot might be thought unimportant, but increases in the percentage of cells of surviving seeds which show visible chromosome aberrations and the incidence of mutant phenotypes in succeeding generations are correlated with loss in viability (Abdalla and Roberts, 1968, 1969). There is no threshold to this mutation in storage, but in order to minimize the loss of genetic integrity which precedes loss in viability of the individual seed the IBPGR recommends that accessions should be regenerated, (i.e. the old seed sown, and fresh seed recollected) as soon as a significant reduction of percentage viability is evident in storage. In addition, the IBPGR recommends that accessions which are less than 80 percent viable at receipt should be regenerated before storage or, in species which typically show low initial percentage viability, if the initial viability is less than 85 percent of the expected initial value. To detect whether a significant loss in viability has occurred in storage, accessions must be regularly monitored, that is a sample removed from storage for a germination test.

^{1/} Department of Agriculture and Horticulture, University of Reading, Earley Gate, Reading, RG6 2AT, UK

^{2/} Department of Applied Statistics, University of Reading, Whiteknights, Reading, RG6 2AN, UK

^{3/} See Note 1 on p. 16

Depletion of accessions

Three independent factors may contribute to the depletion of accessions in storage: loss in viability, removal of samples for monitoring viability (monitoring tests), and distribution of seed to replenish active collections - or possibly, directly to scientists (requests for accessions). The aim of all seed banks must be to minimize the first two depletory agents in order to maximize accession availability for future breeding and research programmes and to minimize the size of accession which needs to be collected and stored. One established seed bank has reported that it is not uncommon to distribute only 20 000 seeds annually, whilst in the same period nearly three million seeds are used for testing viability (Stanwood and Bass, 1978). By storing accessions in the IBPGR-preferred conditions, loss in viability will be minimized, but loss of seed through monitoring viability in store will remain the major cause of accession depletion. Consequently, efficient management of seed banks is necessary to minimize this depletion of accessions, for inadequate management may result in either an increase in the labour required to maintain accessions - for example, by monitoring too frequently, which would increase the workload and necessitate premature regeneration - or too frequent monitoring, which would result in unacceptable loss in genetic integrity or even complete loss of viability of the accession.

Estimating the probable regeneration interval

An estimate should be made, for each accession, of the probable regeneration interval (period between receipt and the point at which viability will have dropped to a level to make regeneration desirable). This estimate would then provide a rational basis on which to decide a calendar of monitoring tests to check on the expected loss of viability. We describe the period between one monitoring test and the next as the monitoring interval. The frequency of monitoring will in turn influence decisions on size of accession and estimates of future requirements for staffing, germination laboratories, and facilities (including land) for regeneration.

An equation for estimating the probable regeneration interval for accessions from the result of initial germination tests has been described by Ellis and Roberts (1980). Already the equation has been applied to data on the longevity of barley seed. Investigations (supported by the IBPGR) are now under way on a number of species, and we believe that this equation (with the insertion of different values for the constants according to species) may prove applicable to seeds of most, if not all, orthodox species. It will be some time, however, before the appropriate values for the constants have been determined for all important crop species. In the meanwhile, the estimate of regeneration intervals for most species will have to be based on informed guesses. The unavoidable lack of precision in estimating regeneration interval will mean monitoring more frequently than might be necessary, but it does not alter the advantages attached to the procedures for monitoring loss of viability in store advocated here.

Regeneration of Accessions

We have noted that accessions should be regenerated to avoid excessive accumulation of mutation, but that there is no threshold to mutation in storage. It is therefore necessary to consider the criteria for initiating the regeneration process. At present the IBPGR recommends regeneration after a significant loss in viability has occurred in storage: but whether or not the difference between two germination test results is significant at any particular level of probability will depend upon the magnitude of the real loss in viability of the population, and on the number of seeds used on each accession.

Results of individual germination tests are frequently accorded more reliability than they deserve. Errors may be introduced in determining the percentage viability of a seed lot through imperfect sampling or differences in germination test conditions. However, for our purposes the variability of germination tests may be predicted because, in cereal seeds at least, at germination levels above 80 percent, variability is almost entirely due to the random selection of seeds (Thomson, 1963).

We have calculated the levels to which viability must fall for the loss to be significant ($P = 0.05$) on the basis of random selection of seeds for a combination of differing initial viability percentages and various sizes of germination test. This loss in viability varies between 13 and 2 percent, depending on initial viability (between 80 and 100 percent) and size of germination test (between 100 and 400 seeds), Table 1. Therefore, under the present recommendations these two factors must influence the length of the storage period before regeneration is called for. Further, the original viability of accessions has a dual influence on the estimate of regeneration interval. First, it affects the storage potential of the seed lot: the greater percentage viability at receipt, the greater the storage potential (Ellis and Roberts, 1980). Secondly, the initial percentage viability affects the loss of viability which will be detected by a germination test; the greater the initial viability the smaller will be the loss of viability needed to achieve significance. Table 1 provides estimates of the probable regeneration interval for barley accessions stored at -18°C with 6 percent moisture content, which clearly demonstrate the influence of both size of germination test and initial percentage viability. In Table 1 we have also included an estimate (based on Abdalla and Roberts, 1968) of the probable percentage of cells of viable barley embryos which would show visible chromosome aberrations as an indication of the loss in genetic integrity associated with loss in percentage viability to the levels indicated. The visible chromosome damage itself is probably unimportant since most, if not all of it is removed by diplontic selection during cell division and therefore does not persist; but it is correlated with point mutations which are carried through to subsequent generations (Abdalla and Roberts, 1969). These estimates of aberrant cells are very tentative indeed but nevertheless are sufficient to indicate that, according to current recommendations, regeneration is apparently called for at very different levels of genetic integrity. For example, if 400 seeds are used for each germination test, the incidence of aberrant cells may differ ten-fold between accessions, depending upon the initial viability of the accession. Moreover, the greatest incidence of

Table 1 The combined effect of initial percentage viability and germination test size on: (a) the maximum percentage germination at which a significant loss in viability ($P = 0.05$) may be recorded assuming the random selection of seeds; (b) estimated regeneration interval (years) for this loss of viability for barley seed stored at the IBPGR preferred conditions of -18°C with 6% moisture content (fresh-weight basis); (c) estimated percentage of cells of surviving barley seeds at this percentage viability which would show visible chromosome aberrations at regeneration

Initial Viability %		Seeds per test			
		100	200	400	1 000
100	(a)	94	97	98	99
	(b)	6 700 ^{1/}	5 880 ^{1/}	5 440 ^{1/}	4 710 ^{1/}
	(c)	0.5	0.3	0.2	0.1
95	(a)	85	89	91	92
	(b)	1 590	1 100	790	620
	(c)	1.5	1.2	0.9	0.7
90	(a)	78	82	85	87
	(b)	1 320	940	620	380
	(c)	2.2	1.7	1.5	1.4
85	(a)	72	76	79	81
	(b)	1 190	850	590	410
	(c)	2.6	2.3	2.0	1.8
80	(a)	66	71	73	76
	(b)	1 110	730	590	330
	(c)	3.3	2.7	2.5	2.3

(b) is calculated from an improved viability equation (Ellis and Roberts, 1980)

^{1/} assumes an initial population viability greater than 99.99%

Table 2 Regeneration interval estimates (years) for barley accessions of different initial germination percentages stored at -18°C, 6% moisture content (fresh-weight basis), if an 85% germination regeneration standard were adopted

<u>Initial Germination</u> (%)	<u>Regeneration Interval</u> (years) <u>1/</u>
100 <u>2/</u>	8 050
99	3 340
98	2 610
97	2 170
96	1 830
95	1 560
94	1 340
93	1 130
92	930
91	770
90 <u>3/</u>	620
89 <u>3/</u>	490
88 <u>3/</u>	340
87 <u>3/</u>	230
86 <u>3/</u>	100

1/ Calculated from an improved viability equation (Ellis and Roberts, 1980)

2/ Assumes an initial population viability greater than 99.99%

3/ Germination tests at first monitoring interval may initiate regeneration procedure (see Table 3 and Note 3)

aberrant cells indicated in Table 1 gives cause for concern since it is more than half the maximum observed on surviving seeds at much lower viability levels (Abdalla and Roberts, 1968). Inevitably therefore, the provisional recommendations must imply both differences in genetic integrity between accessions of each species at regeneration, and a poor minimum standard of genetic integrity at regeneration.

In view of the preceding discussion, we suggest that the provisional recommendation to initiate the regeneration procedure as soon as viability declines significantly is not entirely satisfactory. We suggest that it would be preferable to decide to regenerate all accessions of a particular species at a single level of viability - the regeneration standard.

A purely physiological approach to defining an appropriate single regeneration standard for all accessions would suggest that regeneration should be initiated after the smallest significant loss in viability from maximum, that is at 98 percent viability assuming 400 seed germination tests. Although this would minimize the accumulation of genetic damage, this high standard is impractical as the majority of accessions would require regeneration before storage. This very high level is also substantially greater than that apparently accepted by plant breeders at present - the minimum germination standard. This standard varies between crop species, but for the majority of species is between 80 percent and 90 percent germination. If the minimum germination standard were adopted as the regeneration standard for most species then the substantial differences in genetic integrity at regeneration between accessions would be reduced, and the genetic integrity of the poorest accessions would be safeguarded against further deterioration. We suggest that such common regeneration standards should be introduced, subject to the approval of the appropriate IBPGR Crop Committees or Working Groups.

In Table 2 we have provided estimates of the time taken for the viability of barley accessions to fall from the various levels of percentage germination which they might show at receipt to a prescribed common regeneration standard of 85 percent. The accuracy of these estimates, from the barley seed viability nomograph (Ellis and Roberts, 1980), will depend upon three distinct factors: the accuracy of the initial germination test of an accession at receipt, error in estimating the true rate of deterioration in storage (either in the predictive model or in determining and maintaining the assumed store environment), and the error probability to be attached to the decision to regenerate. This last factor was not considered in the provisional IBPGR recommendations. In this article a procedure is introduced in which the probability of deciding to regenerate accessions at each level of germination would be predetermined.

Germination tests to monitor loss of viability in storage

No prediction can be entirely reliable, especially one requiring considerable extrapolation. Thus it is necessary to monitor germination during storage to determine when regeneration is necessary, rather than relying on the estimate of the regeneration interval alone. This will be the case whether the estimate is provided by an equation or is the result of an informed guess. But how often should an accession be monitored? A seed

bank manager who monitored accessions frequently might be considered cautious - despite wasting seed; whereas an extended monitoring interval - which would reduce workload (and therefore costs) and conserve the seed in the accession - might be considered a risky policy. The dilemma facing the seed bank manager results from the destructive nature of the germination test.

At present, the IBPGR recommends that germination tests be conducted according to International Seed Testing Association Rules (ISTA, 1976a, 1976b). This recommendation is well-founded since ISTA has a wealth of experience in seed testing problems to which seed bank personnel may refer. It is important that ISTA test procedures for substrata, temperature, light, test-period and dormancy-breaking treatments be followed where available, in particular to avoid confusing dormancy with loss in viability. Current ISTA germination test procedures have been devised to determine the percentage germination of seed lots of effectively infinite size in as short a time as possible. However, in a seed bank, seed numbers are severely restricted, but results of germination tests are not required as urgently. We believe that these differences are important and suggest that procedures for monitoring germination in seed banks are required which differ in one major respect from those of the ISTA.

The standard ISTA germination test relies upon a fixed number of seeds, simultaneously selected from the accession, set to germinate, and observed until germination can be determined. In contrast we suggest a sequential procedure, the form of which will be described in the next section.

Sequential tests to monitor germination

In the original, and simplest form of sequential analysis, a single article (for our purpose, one seed) would be sampled from a batch (accession) and tested: from this result (either positive or negative) a decision is made to accept the batch, to reject the batch, or to continue the sequential procedure and test one more article. The essential feature of sequential analysis is that the number of observations required by the procedure is not pre-determined; the decision to terminate a test depends, at each stage, on the results of all previous observations (Wald, 1947). Thus, a sequential test will require, on average, substantially fewer seeds than equally reliable germination test procedures based on pre-determined sample sizes.

If single seed germination tests were used in the above manner, the sequential test-period would be unacceptably long because of the time taken for each germination test. To minimize this problem, but maintain the advantages of the sequential test, it will be necessary to test a small group of seeds at one time. Briefly the sequential germination test procedure we envisage for monitoring accessions in a seed bank would be as follows. A group of seeds (we suggest 40) would be set to germinate, having been sampled from an accession in store. When the germination of this group has been observed three decisions are possible - depending upon how many seeds have germinated: satisfactory germination (abandon test and maintain accession in store), unsatisfactory germination (abandon test and regenerate accession as soon as possible), or test inconclusive (continue test). If the test needs to be continued, a further group of (40) seeds is sampled from the accession

in store and set to germinate; the accumulated germination result is then used for the subsequent decision-making process, and so on. ^{1/}

There is, however, a disadvantage in grouping observations: increasing the number of observations (seeds) in a group will increase the number of seeds which will need to be tested before a decision can be made, because the number of seeds tested must always be a multiple of the group size. An account of the theory involved in applying sequential analysis in this context is given by Whitehead (1980).

There are a number of quantities in the sequential test procedure which need to be fixed in advance: the regeneration standard (minimum percentage germination at which regeneration is desirable), the probability of regeneration if the accession is at or below the regeneration standard, the probability of unnecessary regeneration in error at a higher level of germination than the regeneration standard, and the number of seeds in a group. These quantities will all affect the average number of groups which will be tested at each level of germination before a decision can be reached. Values of all of them may be chosen at will, although they will interact in their effect on the average number of groups tested.

To demonstrate the advantage of a sequential monitoring test procedure over a conventional fixed sample test it has been necessary to choose certain values for these quantities. Although we have chosen these values deliberately because we believe they are particularly suitable for seed banks, our choices provide a conservative sequential test in that error probabilities are lower than present procedures. The theoretical probability of calling for regeneration when the viability of the accession as a whole is 85 percent (the assumed regeneration standard) will be 0.95 and the theoretical probability of calling for regeneration unnecessarily at 90 percent germination will be 0.05.

To achieve these requirements using a fixed sample test would require the use of 467 seeds, which is equivalent to 11.7 groups of 40 (Whitehead, 1980). Thus, the requirements are more exacting than the standard achieved by an ISTA test using only 400 seeds. The properties of a sequential test using groups of 40 seeds and achieving these requirements are presented in Table 3. This shows the probability of regeneration and the average number of groups, for various accession viabilities. The table shows that the number of groups varies according to the viability of the accession as a whole. In practice, the total number of seeds tested at each monitoring interval will be a multiple of the group size, which is 40 in this example. The probabilities of regeneration given in Table 3 refer to an individual monitoring test. The fact that a typical accession will experience a series of monitoring tests will increase the probability of regeneration before accession viability has fallen below 85 percent.

A comparison of the theoretical model with 1 000 simulated sequential monitoring tests at each level of germination (Table 3) shows that the

^{1/} See Note 2 on p. 16

Table 3 The theoretical probability of initiating the regeneration procedure (regeneration standard, 85%) and the average number of seed groups tested before terminating the sequential test procedure at different levels of germination. This procedure continues until $G_m - 34m \geq 5.17 + 1.058m$ in which case the accession should be maintained in store, or $G_m - 34m \leq -5.17 + 1.058m$, in which case the accession should be regenerated, where m is the number of groups of seeds tested (40 seeds per group) and G_m is the accumulated number of germinated seeds (see Figure 1). These theoretical calculations are compared with the results of 1 000 simulated sequential tests.

Percentage Germination of Accession as a whole	Probability of initiating regeneration procedure		Average number of groups required by the sequential test	
	Simulated	Theory	Simulated	Theory
85	0.947	0.95	6.00	5.67
87.645	0.498	0.5	9.21	9.66
90	0.054	0.05	6.17	6.36
92.5	0.002	0.001	3.55	3.51
95	0.000	0.000	2.49	2.36
97.5	0.000	0.000	2.05	1.74

agreement between theory and simulation is good, both with respect to the probability of regeneration and average sample size. The decision boundaries of this particular sequential test procedure can best be shown graphically (Figure 1). If test results cross the upper boundary then the test is terminated and the accession retained in store. If test results cross the lower boundary, the test is terminated and the accession regenerated. The sequential test procedure could also be outlined as a table for use by seed bank personnel for although the theory behind a sequential test is complicated, the practical application of the procedure can be very simple. Table 4 shows a sequential procedure in a form which could be reproduced for a seed bank manual.

It is clear from the average number of groups shown in Table 3 that the sequential germination test procedure offers a saving of seed at all levels of germination over the equivalent fixed sample size test of 467 seeds or 11.7 groups. In particular, the savings are greatest at levels of germination approaching 100 percent. This would be of great benefit

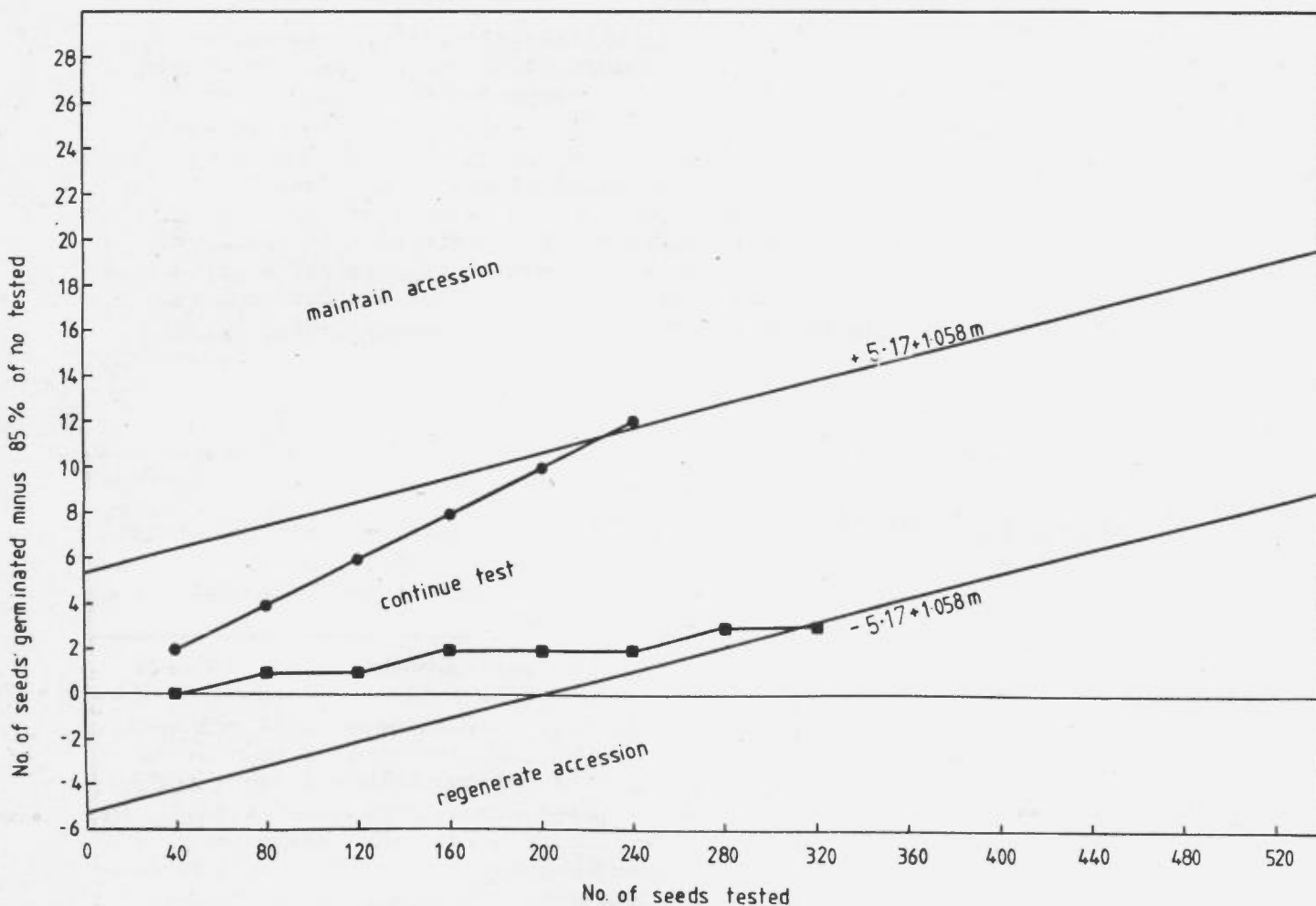


Figure 1

Diagram of sequential analysis monitoring procedure which continues until $G_m - 34m \geq 5.17 + 1.058m$ or $G_m - 34m \leq -5.17 + 1.058m$ where m is the number of groups of seeds tested (40 seeds per group), and G_m is the number of germinated seeds. The horizontal line corresponds to 85 percent germination (i.e. when $G_m/100 = 34/40 = 85\%$). Seeds are tested in groups of 40. When the response of the first group has been determined, the number of seeds germinated minus 40×0.85 can be plotted against 40 on a diagram similar to Figure 1. After m groups have been tested, the number of seeds germinated minus $40 \times 0.85m$ is plotted against $40m$. If the plotted point lies on or above the upper boundary then the accession will be retained in store and this monitoring test will be discontinued; after the next monitoring interval, a further test will be conducted. If the plotted point lies on or below the lower boundary then the accession will be regenerated. If the plotted point lies between the boundaries, then a further group of 40 seeds will be tested. Examples of hypothetical test results are shown in which sequential tests consistently record 86% (■) or 90% (●) germination, and terminate when the rejection or acceptance boundaries are crossed respectively.

Table 4. Sequential germination test plan for 85 percent regeneration standard, testing seeds in groups of 40, and probabilities of regeneration shown in Table 3

No. of seeds tested	Regenerate if no. of seeds germinated is equal to, or less than	Re-test accession if no. of seeds germinated between	Keep in store if no. of seeds germinated is equal to, or greater than
40	29	30 - 40	
80	64	65 - 75	76
120	100	101 - 110	111
160	135	136 - 145	146
200	170	171 - 180	181
240	205	206 - 215	216
280	240	241 - 250	251
320	275	276 - 285	286
360	310	311 - 320	321
400	345	346 - 355	356
440	380	381 - 390	391
480	415	416 - 425	426
520	450	451 - 460	461
560	485	486 - 495	496
600	520	521 - 531	532

to seed banks, for relatively few seeds would need to be sacrificed during early monitoring tests. The number of seeds tested would only begin to approach that required by the fixed sample test when the viability of the accession has fallen to between 85 and 90 percent - where the need for regeneration is probably imminent.

Regeneration of an accession may need to be initiated as a result of either loss in viability or depletion of seed numbers mainly through destructive monitoring tests. Thus, with the sequential monitoring test procedure advocated here, depletion of seed numbers would in most cases necessitate regeneration only if the percentage germination of an accession already borders upon the regeneration standard, where regeneration is advisable anyway because of genetic changes ^{1/} In this context it is

^{1/} See Note 3 on p. 17

necessary to make one further observation regarding the average sample sizes shown in Table 3. These are only average figures and the distribution of actual sample sizes will be skewed such that more sample sizes are less than average than are above. However, in a few of those instances where the number of seeds tested is above average, the number tested before a decision could be made would be very large. In these few cases it would be advisable to interrupt the sequential test procedure before its conclusion and regenerate the accession if there were a danger of excessively depleting the size of the accession. In terms of Figure 1 this would occur where the germination results did not cross either the upper or lower boundary, but remained between the decision boundaries in the re-test zone. However, from Table 2 and Figure 1 it is clear that this would only be a major problem where the viability of the accession as a whole is, in this example, close to 87.645 percent where the probability of deciding to regenerate is 0.5 and thus regeneration is advisable anyway to avoid further loss of genetic integrity.

The sequential test has various advantages over a fixed sample size test and, because of its flexibility, it could be adapted by seed banks in various ways as follows:

- (a) to reduce the probability of making an erroneous decision while using the same number of seeds as recommended for the fixed sample test, or
- (b) to maintain the same error probability as that for the fixed sample size test previously recommended, but reduce the number of seeds which need to be tested, or
- (c) to effect a compromise between the two preceding objectives and reduce both the error probability compared to the fixed sample size test previously recommended and to reduce the number of seeds tested to levels intermediate between 1 and 2 above.

In this article the example chosen to illustrate a sequential test procedure for use by seed banks shows both reduced error probabilities and reduced seed numbers when compared to the provisional IBPGR recommendations. If sequential test procedures are adopted for monitoring loss in viability in seed banks then a balance will need to be struck between saving valuable seed and the error probabilities attached to decisions to regenerate or to maintain accessions in store. If the numbers of seeds tested are minimized then error probabilities will be greater. Consequently, some accessions would be regenerated prematurely, entailing unnecessary and costly work, whilst other accessions would be regenerated too late, allowing unwanted genetic deterioration. On the other hand, if the error probabilities are set too low, more seeds would be used in the monitoring tests and many valuable seeds would be destroyed.

Conclusions

The proposals made in this article concern those factors which will directly influence both the cost and success of genetic resources conservation by long-term seed storage. The aim has been to suggest procedures which could be adopted as models for seed bank management which would reduce wastage of seeds in seed banks and improve the probability of appropriate decisions being taken with regard to regeneration. The concept of a single regeneration standard for all accessions of each species and the proposal to replace conventional monitoring germination tests of fixed sample size by a sequential test procedure will need further discussion, especially by those responsible for the management of seed banks and by IBPGR Crop Committees. The example we have chosen to illustrate the proposed test procedure would, we believe, be appropriate for most cereals and other crops where the initial percentage viability can be expected to be high. To summarize, views are needed on the following parameters appropriate for different crops (the values in parenthesis being those we believe would be appropriate for cereals):

Regeneration standard (85%)

Probability of not regenerating accessions
at the regeneration standard ($P = 0.05$)

Probability of regenerating accessions in
error at levels of germination above the
regeneration standard ($P = 0.5$ at 90%
germination)

Size of sequential test groups (40)

Notes

1. A second, less satisfactory standard for long-term storage exists: the "acceptable standard" where seeds are stored at 5°C or less, either in air-tight containers at 5-7 percent seed moisture content, or in a store atmosphere controlled at not more than 20 percent relative humidity (Report of the sixth session of the FAO Panel of Experts on Plant Exploration and Introduction. FAO, Rome, 1975). This "acceptable standard" recognized existing practice, but recommended that where practicable, such facilities should be up-graded to the preferred standard.

2. Two assumptions concerning seed storage physiology are necessary to allow the adoption of a sequential test procedure. Firstly, the percentage germination of the accession is assumed to remain constant during the course of the sequential test. This is an acceptable assumption for seed banks because under the storage conditions recommended by the IBPGR, loss in viability is very slow. Secondly, it is assumed that frequent sampling of

accessions will not reduce the longevity of seed remaining in store. This is also an acceptable assumption since the period for which the accession is subject to a higher rate of deterioration in a less suitable environment (for example, 20°C with 5 percent moisture) than the IBPGR recommends will be extremely short and only marginal in effect, assuming the accession is stored in bulk, removed for a sample to be taken in a low humidity environment and then the bulk returned to store whilst the monitoring test is carried out. Even if the whole accession were withdrawn from the cold store for the whole of the test period, this period would nonetheless be very short compared to the total storage period.

3. An anomaly will arise if a fixed sample germination test of 400 seeds (ISTA, 1976a) is made on receipt of an accession, but a sequential test procedure is adopted for subsequently monitoring the viability of the accession during storage. At the first monitoring test (however short the first monitoring interval) many of those accessions initially showing percentage germination values slightly greater than the regeneration standard will require regeneration. (In the example outlined here, this would arise for accessions showing between 85 percent and 90 percent germination at receipt (see Table 3). This is because each monitoring test will determine whether or not the viability of an accession is significantly greater than the regeneration standard. The initial germination test ought to do the same, and with at least as much accuracy. There are strong reasons for favouring a germination test of fixed sample design on receipt of accessions - mainly to minimize the problem of high initial workloads when receiving large collections and to determine the initial viability of accessions in as short a period as possible. Thus, we advocate that an initial fixed sample germination test is required which is equivalent to the sequential procedure used to monitor accession viability during storage. In the example outlined here, this would require a 467 seed germination test: accessions showing germination values greater than 87.645 percent would be accepted for long-term storage; those showing values lower than 87.645 percent would be temporarily stored at the "preferred" conditions but regenerated as soon as the workload allowed.

References

- Abdalla, F.H. and Roberts, E.H. Effects of temperature, moisture and oxygen
1968 on the induction of chromosome damage in seeds of barley, broad beans
and peas during storage. *Ann. Bot.*, 32:119-136.
- Abdalla, F.H. and Roberts, E.H. The effects of temperature and moisture on the
1969 induction of genetic changes in seeds of barley, broad beans and peas
during storage. *Ann. Bot.*, 33:153-167.

- Ellis, R.H. and Roberts, E.H. Towards a rational basis for testing seed quality.
1979 In, P.D. Hebblethwaite (ed.) Seed Production, Butterworths, London,
p. 605-635.
- Ellis, R.H. and Roberts, E.H. Improved equations for the prediction of seed
1980 longevity. Ann. Bot. (in press)
- IBPGR Report of the IBPGR Working Group on Engineering, Design and Cost
1976 Aspects of Long-term Seed Storage Facilities. IBPGR Rome.
- ISTA International rules for seed testing: Rules 1976. Seed Sci. & Technol.,
1976a 4:3-49.
- ISTA International rules for seed testing: Annexes 1976. Seed Sci. & Technol.,
1976b 4:51-177.
- Stanwood, P.C. and Bass, L.N. Ultracold preservation of seed germ plasm. In,
1978 P.H. Li and A. Sakai (eds.) Plant Cold Hardiness and Freezing Stress
Mechanisms and Crop Implications, Academic Press, London, p. 361-371.
- Thomson, J.R. New tolerances in seed testing. J. Natn. Inst. agric. Bot.,
1963 9:372-377.
- Wald, A. Sequential Analysis, J. Wiley and Sons, Inc., New York.
1947
- Whitehead, J. The use of the sequential probability ratio test for monitoring
1980 the percentage germination of accessions in seed banks. (in preparation)

Summary

The provisional guidelines of the IBPGR concerning long-term seed storage facilities suggest that accessions be regenerated when their viability has fallen by a significant amount. In this paper we suggest that a policy of regenerating accessions when their viability has fallen to a prescribed level would be preferable. Furthermore, rather than following the example of the ISTA rules on germination tests and using a sample of fixed size, we advocate the adoption of sequential germination tests. Both fixed sample and sequential germination tests will detect whether the viability has fallen to the prescribed level with given probabilities of error, but the sequential test will use far less seed in doing so.

A method of estimating longevity from the results of an initial germination test which could be used to predetermine the monitoring interval of each accession has been described in another paper (Ellis and Roberts, 1980). The combination of sequential germination tests and predetermination of monitoring intervals, would provide an integrated and economic system for monitoring the viability of accessions and deciding when to regenerate. However, it will be some time before the necessary information to predetermine monitoring intervals is available for most species. Meanwhile, there would be considerable benefits in adopting a sequential germination test procedure as soon as possible.

RESUME

Les directives provisoires du IBPGR concernant les installations d'entreposage prolongé des semences suggèrent que les accessions soient régénérées dès que celles-ci perdent une part appréciable de leur viabilité. Notre opinion, comme on le verra dans le présent document, est qu'il serait préférable d'adopter une politique de régénération quand la viabilité des accessions tombe à un niveau prescrit. En outre, au lieu de suivre les règles de l'ISTA en matière d'essais de germination et d'utiliser un échantillon d'une taille fixe, nous préconisons l'adoption d'essais de germination séquentiels. L'échantillon fixe et les essais de germination séquentiels permettent l'un comme l'autre de déceler si la viabilité est tombée au niveau prescrit avec une probabilité d'erreur donnée, mais l'essai séquentiel consommera pour cela beaucoup moins de semences.

On trouvera dans un autre ouvrage (Ellis et Roberts, 1980) la description d'une méthode permettant d'estimer la longévité d'après les résultats d'un essai de germination initial; cette méthode pourrait être utilisée pour établir la périodicité des contrôles pour chaque accession. En combinant les essais de germination séquentiels et des intervalles de contrôle fixés au préalable, on disposerait d'un système intégré et économique pour surveiller la viabilité des accessions et pour décider du moment où il convient de les régénérer. Il faudra toutefois un certain temps avant que l'on ait les renseignements nécessaires pour établir la fréquence des contrôles pour la plupart des espèces. Entre temps, on aurait avantage à adopter dès que possible un protocole pour les essais de germination séquentiels.

RESUMEN

Las directrices provisionales del IBPGR sobre medios de almacenamiento de semillas a largo plazo sugieren que se regeneren los ejemplares cuando su nivel de viabilidad descienda de manera significativa. En este documento sugerimos que sería preferible una política de regeneración de ejemplares cuando su viabilidad haya descendido a un nivel predeterminado. Es más, en lugar de seguir el ejemplo de las normas de la Asociación Internacional para el Ensayo de Semillas (ISTA) sobre pruebas de germinación y emplear una muestra de tamaño fijo, aconsejamos la adopción de ensayos de germinación sucesivos. Tanto las muestras fijas como los ensayos de germinación sucesivos detectarían - con determinadas probabilidades de error - si la viabilidad ha descendido al nivel establecido, pero con el sistema de ensayos sucesivos se empleará muchísima menos semillas para lograrlo.

En otro documento (Ellis y Roberts, 1980), se ha descrito un método para estimar la longevidad a partir de los resultados de un ensayo de germinación inicial que podría emplearse para predeterminar el intervalo de control de cada ejemplar sometido a examen. La combinación de ensayos de germinación sucesivos y la predeterminación de intervalos de control, supondría un sistema integrado y económico para probar la viabilidad de los ejemplares y decidir cuándo proceder a la regeneración. Sin embargo, transcurrirá aún cierto tiempo antes de que se disponga de la información necesaria para fijar de antemano los intervalos de control para la mayor parte de las especies. Mientras tanto, se lograrían beneficios considerables adoptando lo antes posible un procedimiento de ensayos de germinación sucesivos.

THE PRINCIPLES, PROBLEMS AND RESPONSIBILITIES OF THE PRELIMINARY
EVALUATION OF GENETIC RESOURCES SAMPLES OF SEED-PROPAGATED CROPS

W. Erskine and J.T. Williams 1/

Introduction

The International Board for Plant Genetic Resources (IBPGR) has a remit to accelerate the collection and conservation of genetic variation of cultivated plants for use in both present and future plant breeding programmes. However, samples of genetic resources in store are not of immediate application in breeding programmes until they have been evaluated. For a full exploitation of the variability, the plant breeder needs information on the attributes of the samples to serve as a basis for his selection decisions.

At present, the evaluation of samples is undertaken largely in response to the current selection criteria of the breeders. This approach is extremely haphazard because the probability of a sample being examined for a particular attribute is solely dependent on the breeder's present needs. A more systematic approach to the evaluation of germplasm would help to provide information suited to future requirements. Nevertheless, the magnitude of such a task can be exemplified by one crop alone, e.g. wheat - of which there are *ca.* 300 000 accessions in collections around the world - and a range of attributes would need to be measured and recorded.

This paper assesses the problems associated with "preliminary evaluation" of genetic resources samples. Particular reference is made to three crops, rice, maize and winged beans. These crops were chosen because they represent different breeding systems and hence patterns of variation, and two of them are crops within the mandate of International Centres. Also, the history of evaluation in these three crops has been diverse.

Information requirements from evaluation

Clearly, information pertinent to present breeding aims is needed; and some genetic resources samples are being evaluated for current agronomic merit by breeders. However, it is not possible to predict the goals of the improvement programmes of the future. For example, at the turn of the century, short stature was not envisaged as a breeding aim for small grains.

In the absence of guidelines of the future information needs of breeders, two approaches are possible:

1/ Consultant and Executive Secretary respectively, IBPGR, Rome
W. Erskine now at ICARDA, P.O. Box 5466, Aleppo, Syria

- (i) samples should continue to be screened solely for the purpose of selecting for current breeding objectives; or
- (ii) samples should be systematically described (i.e. characterized) now, so that information will be available for the breeding programmes of the future.

In the short term, systematic description of samples is useful both in distinguishing between populations and identifying duplicate samples; it also provides information on the total breadth of variation within collections. In brief, the more documentation on a collection, the more rational will be its exploitation. For example, details on the site where a particular sample was collected.

Rice samples collected in areas with particular problem soils "jump the queue" for evaluation at IRRI and they are rapidly screened for tolerance to the trait concerned. At CIMMYT, the maturity characteristics of maize samples are recorded as part of the programme of systematic description. Shorter maturity is a current breeding aim at CIMMYT and consequently, more rapid exploitation of germplasm samples can be made on the basis of information from systematic characterization.

Traits for systematic characterization

The ideal description of the variability of a sample is genetic, and the eventual aim must be to describe the variation by an inventory of the differences between and within samples in the sequence of nucleotides in the DNA. This is not currently possible, and a compromise in description has to be made. At present, the study of protein variants (allozymes) by electrophoresis is the most convenient method available for detecting genetic differences close to the DNA (Brown, 1978). It may be argued that the variation in allozymes is of little immediate interest to the plant breeder and genetic conservationist because its correlation with agronomic characters is obscure. However, protein variant "markers readily monitor the comparative diversity of various kinds of genetic resources, the extent of co-adaptation and the degree of allozyme-environment correlation. Such data foreshadow the use of the variants in plant breeding programmes to an increasing extent" (Brown, 1978). It is expected that the technique of studying protein polymorphism by electrophoresis will be increasingly used in the description of genetic resources samples, and in the elimination of duplicate stocks through bulking.

A start to the description of the genetic variation of the field pea (*Pisum*) has been made and a type collection of the described genes is held in the Weibullsholm- *Pisum* Genetic Association Type collection (Blixt, 1977 and 1978).

In other crops, the systematic description of samples for discrete traits has been limited to cataloguing the phenotypic variation because of problems in relating genotype with phenotype. Discrete morphological traits which are environmentally stable are often used in systematic characterization because they are visible whenever the crop is grown. The characters may also be of immediate importance to plant breeders (e.g. kernel type in maize).

These qualitative traits will obviously continue to be important in the systematic characterization of genetic resources samples.

Quantitative morpho-agronomic traits are also currently used in characterization. These traits are controlled by many genes of small effect, and the effect of single genes is blurred by the environment. Consequently, the correspondence between the genotype and phenotype is obscured.

For some quantitative characters there is the additional problem that the ranking of samples changes from environment to environment. For these unstable traits the measurements made in one environment cannot provide an adequate description of the character concerned, and a description in each of several environments is required. Details of the growth environment must also be recorded. It is clear that a distinction between those traits that are environmentally stable and those that show genotype by environment interaction is necessary. This can only be done on the basis of a profound knowledge of the particular crop, and experienced plant breeders are the most able to comment on the environmental stability of individual traits.

Finally, there are those cryptic traits, such as disease resistance and tolerance to adverse soils for which variation is only visible in particular environments. *Cryptic traits are often extremely important economically, but special field or glasshouse conditions are required for their expression. Such traits cannot be recommended for use in systematic characterization. (But if, for example, a disease infection does develop on a growing sample, the worker should record the sample as susceptible).*

In summary, the systematic characterization of all genetic resources samples is a huge open-ended task but initial characterization should be for those traits which are morpho-agronomic and also have a reasonably high heritability.

Any communication or exchange of information requires a common language. It is vital that any trait to be used in the description of genetic resources samples is given an internationally-agreed definition. It is only with a standard language of description that data compatibility is ensured and a meaningful exchange of data can take place. For some years now, the terms "Descriptors" and "Descriptor States" have been used for these definitions and the IBPGR has accepted responsibility for developing descriptor systems for the major crops.

Progress in the systematic characterization of the case crops

The crops discussed below are:

- (i) rice (a self-pollinated crop of major global importance),
- (ii) maize (an outcrossing crop of major importance), and
- (iii) winged bean (a largely self-pollinating crop of minor importance).

Background

RICE

At IRRI, in excess of 47 000 samples of rice held in the genebank have been systematically characterized for 38 morpho-agronomic characters. This mammoth task has been under way at IRRI since the mid-1960's, when a research scientist, a college graduate and two technicians studied an average of 1 200 plots per season. Since 1973, the scale of operations has increased to more than 4 000 plots per season, covering up to 7 ha. Eight college graduates and four technicians are now involved in the characterization. 11 454 newly received samples await, or are under, description. The major limiting factor to the work is the availability of land at IRRI.

MAIZE

CIMMYT holds one of the largest collections of maize germplasm in the world. Between 8 000 and 9 000 samples from a total of 13 000 accessions have already been characterized at CIMMYT. The European collections, except those in Hungary and Bulgaria, have also been described. The backlog of material at CIMMYT awaiting characterization is due to the limited number of seeds of these accessions and the problem of the multiplication of maize seed, a cross-pollinated crop. This problem is discussed in the section on cross-pollination (p. 26).

WINGED BEAN

The winged bean collections in Papua New Guinea, the Philippines and Thailand, have been characterized, whereas those in Indonesia and Malaysia await description.

Summary

Major progress has already been made in the systematic characterization of all three crops. So much so, that they can be considered as *atypical* of crop germplasm samples. The proportion of characterized to undescribed samples is lower in most other major crops. The backlog of uncharacterized samples is, thus, a problem that is not highlighted by the specific crops in this paper.

Traits for characterization

RICE

When the systematic characterization of rice samples was started at IRRI more than a decade ago, at that time the 38 morpho-agronomic traits used in characterization were chosen for their value in agronomy, taxonomy, and, in some cases, academic studies. All the chosen traits have a reasonably high heritability and have been defined in Chang and Bardenas (1965). A list of the descriptors and descriptor states used in characterization has been produced by the IBPGR/IRRI Rice Advisory Committee (FAO/IBPGR Newsletter No. 38).

IRRI has also pioneered a standard system of description for those traits that are currently of interest to rice breeders as part of the International Rice Testing Program (IRRI, 1975). The growth stages of rice are also documented, so that the physiological age of a plant can be recorded at the same time that a trait is scored (Zadoks, Chang and Konzak, 1974). The definitions used at the start of the characterization at IRRI are similar to those defined later by the IBPGR/IRRI Rice Advisory Committee; so that the data collected at IRRI are all compatible.

MAIZE

A minimum list of descriptors and descriptor states has been produced by the IBPGR Maize Germplasm Advisory Committee (FAO/IBPGR Newsletter No. 37). All but two of the descriptors (number of kernel rows per ear and endosperm colour) have been used for some years for characterizing germplasm at CIMMYT. (These two particular traits are of limited interest to CIMMYT at present).

WINGED BEAN

Descriptors and descriptor states for the winged bean have been produced by an IBPGR *Ad Hoc* Working Group (IBPGR, 1979). The plant traits in the descriptor list for the winged bean are divided into "morphological" ones and ones for agronomic evaluation. This division suggests that only the morphological traits are sufficiently stable to be used in systematic characterization. However, some of the traits listed under agronomic evaluation data, like pod length (cm), seeds per pod, 100 seed weight (g), and shelling percentage, show little genotype by environment interaction (Erskine, 1979) and are very useful in characterization. It would be preferable in future descriptor lists to show the demarcation more clearly between, on the one hand, those environmentally stable traits that are useful in systematic characterization, and, on the other hand, those traits with a lower heritability of little use in this regard.

Summary

In the three crops, decisions have been made on the suitability of various data relating to collection and morpho-agronomic traits by meetings of plant breeders convened by the IBPGR. The traits to be used for characterization have then been defined as descriptors, and the descriptor states have also been agreed. In short, a standard language for the systematic characterization of samples of all three crops is available; and, more importantly, the language is finding widespread international acceptance and use.

Triple aims of planting new samples

When samples arrive at a genetic resources centre, two procedures are followed as routine: (i) growing out for multiplication, especially for seed crops, to provide sufficient fresh and clean material for storage, distribution and evaluation, and (ii) characterization of the samples. Maintenance

of the genetic composition of the samples^{1/} is vital because plant populations are polymorphic to different degrees, and radical changes in the genetic composition of the collected samples can occur in one growing cycle if certain procedures are not followed. The principles and methodology of growing out the new genetic resources samples are discussed by Shevchuk (1973) and important points are outlined in the paragraphs below.

Principles and methodology of planting newly-arrived samples

Adaptation of samples

The ecological environment of growth should be as near as possible to that of the collection location. This will minimize the effect of natural selection on the genetic composition of the sample and will also ensure an adequate harvest of seed. Since collected samples differ widely in their adaptation, it is clear that no single location will be suitable for all the samples of one crop.

CIMMYT uses two locations in Mexico for the multiplication of maize samples. Those samples that originate from an altitude below 1 500 m are planted at Ttaltizapán, Morelos (altitude 940 m); whereas those samples from above 1 500 m are grown at El Batán (altitude 2 249 m). Even so, the material of Andean origin is difficult to multiply in Mexico, and a cooperative arrangement with Universidad Nacional Agraria, La Molina, Peru, has been reached to grow these high-altitude samples. *There is a very real need to involve national genetic resources centres in the initial planting of samples and, later on, their help should be invoked in the regeneration of samples.* International collaboration is discussed on p. 29.

Avoidance of repeated cycles of regeneration

Seed multiplication should be completed in one cycle and repeated regenerations should be avoided. One cycle of multiplication minimizes the change in the genetic composition of samples due to natural selection and also reduces the probability of the inadvertent mixing of samples. For effective seed multiplication, a population of not less than fifty plants will be needed to minimize the fixation of genes through genetic drift and for the description of the variability within the samples.

The actual plot size will depend on a consideration of:

- (i) the quantity of seed available for planting
- (ii) the minimum population size for the maintenance and description of samples

^{1/} It is necessary to preserve the genetic composition of those collected populations which are directly the products of crop evolution. But for those collected populations which are only indirectly the products of evolution, e.g. market samples and biased field samples; the aim should be to maintain the genes but not necessarily the gene frequencies within populations.

- (iii) the local seeding rate
- (iv) the amount of seed required for storage and distribution
(the unpredictable nature of future seed requests is a problem)
- (v) the expected seed yield (only intermediate yields can be expected because most samples will be unadapted to the particular environment of growth)
- (vi) the availability of resources

At CIMMYT the repeated regeneration of samples is avoided, and sufficient maize seed is obtained from one cycle of multiplication using plots with eight 11m-long rows. The plots are thinned to 270 plants, if there is adequate seed.

At IRRI, the number of cycles of multiplication and regeneration of rice samples is also kept to a minimum. Two seasons of planting are necessary to produce sufficient seed and to distribute the systematic description work. Each viable accession is planted twice in three-row plots (more recently in two-row plots), once in the wet season and again in the dry season (Chang *et al.*, 1977). Forty single-plant hills in a transplanted culture or two 5-m long drilled rows is the minimum for each accession; 60 to 100 plants is about optimum (Chang, 1976).

In contrast to the situation for these two major crops, the genetic resources samples of winged bean are being repeatedly regenerated because of (i) inadequate seed storage facilities and a rapid loss of seed viability, and (ii) the expense of the trellis supports for the crop dictating that less than 20 plants per sample are grown. In turn, this gives an insufficient quantity of seed for both storage and distribution. During each cycle of regeneration some cross-pollination occurs between samples, changing radically the genetic composition of the samples. It is clear that the seed planted for regeneration should come from the initial planting, whenever possible. The use of new storage facilities for the winged bean collections in Indonesia, Thailand and the Philippines will reduce the need for such frequent cycles of regeneration and also spread the work of rejuvenation over seasons. In turn, this will allow an improved control of pollination and a larger population size per accession.

The control of pollination

The control of pollination is a major consideration in growing out genetic resources samples. The pollination between samples should be controlled to prevent gene migration; and the pollination within samples should be regulated to maintain the genetic composition of the samples. The latter entails either the enforcement of random mating within the sample, or first sampling at random and then the bulking of the self-pollinated progenies of a number of single plants.

For cross-pollinated crops, isolation plots, cages or bags may be needed to control the pollination. Square plots may be preferable to traditional row planting because the extent of cross-pollination may increase towards the plot borders.

At CIMMYT hand pollination is used in the multiplication of maize samples. The developing ear shoots, before the silks have emerged, are covered with a small glassine "shoot" bag. All the tassels in a plot are bagged and the pollen mixed. The appropriate "shoot" bags are then replaced by tassel bags. This is a system of bulk pollination. The control of pollination of maize, as in other cross-pollinated crops, is a very laborious and expensive task, and as such, the major logistic constraint to planting newly-arrived samples. For example, with all the backing of an International Centre, a maximum of only 300 accessions from the maize bank can be grown for seed multiplication at CIMMYT at one time.

Because of the expense of pollination control, there is a strong temptation to reduce the number of stored samples by compositing them on the basis of either morphological similarity (e.g. racial composites) or of adjacent locality of origin. The formation of composites reduces the number of samples and hence, the expense of their upkeep, but in so doing, the genetic composition of the original samples is lost, as is the flexibility in their use. In addition, composite formation makes a useful evaluation more difficult to achieve. Clearly while composite formation may be an important procedure in taxonomy and plant breeding, it is not a genetic resources procedure if the original samples are lost. The two recourses to this procedure in genetic resources work are (i) the merging of duplicate samples and (ii) the compositing of samples from one area if there is inadequate seed available of each particular sample to form a viable population.

Some form of pollination control is also needed for those crops which, although largely self-pollinating, show some degree of cross-pollination. The winged bean is a case in point with outcrossing estimated to vary from 0 to 8 percent (Erskine, 1979). To the present, the control of pollination during multiplication of winged bean samples has generally been far from adequate. Either inflorescences should be bagged or isolation (temporal or spatial) plots should be used to control outcrossing. For crops like rice in which the level of cross-pollination rarely exceeds one percent, in general there is little need to take measures for pollination control.

Two optional procedures, sometimes followed during the planting of samples of self-pollinated crops irrevocably alter the genetic composition of the samples. These are: (i) the roguing of off-types, and (ii) the practice of pure line selection for morphological variants within samples.

For rice, the policy at IRRI on the roguing of off-types is "when the original sample contains detectable variousness or separable sub-strains, it is important to first distinguish between obvious contaminants (off-types) and inherent variants within a population. Obvious off-types or contaminants, such as awned and bold-grained plants in an awnless and slender-grained population, should of course, be removed. (Chang, 1976)."

The recognition of off-types is a subjective decision introducing a measure of selection into the conservation process. It would appear more appropriate either to maintain the composition of the original samples without roguing or, if roguing is considered useful, to take a more conservative attitude and store the off-types as separate accessions.

Pure line selection for morphological variants within samples has been practised on winged bean germplasm. But with this procedure, the genetic composition of the original sample is lost. In spite of the fact that roguing off-types and pure line selection are well-known useful techniques in plant breeding, these two procedures cannot be condoned as genetic resources activities.

Use of local cultivation practices

Local agronomic practices should be followed for the crop in relation to nutrient and water requirements, and also pest, weed and disease control. This will encourage crop growth, facilitate sample description, and maximize the production of seed. Weeds, especially volunteer plants from a previous crop, must be strictly controlled to avoid the contamination of samples. Similarly, pest and disease control is needed to ensure an adequate yield of clean seed for both storage and distribution.

Methodology prior to planting

Prior to planting the genetic resources samples should be divided with half kept in case of crop failure. Germination tests may also be run prior to planting, because some of the incoming seed samples are likely to be inviable or show low viability. If pests are a problem and there is not enough seed of an incoming sample to fill a plot, it may be necessary to plant the few available seeds in a screened house for a preliminary multiplication. This is sometimes done against leaf hopper attack on rice at IRRI.

Experimental design

One or more standard varieties should be planted as checks at regular intervals, such as every 20 rows. No replication of samples is warranted at this preliminary stage of evaluation. Any cultivars with the same vernacular name can be planted together to facilitate their comparison as possible duplicates. If lodging is a problem, then it may be necessary to use either blank rows or separator crops between the plots to avoid the contamination of samples.

Collection of data

Every planting of newly-arrived samples must be considered as a separate experiment in terms of the collection of data. For example, the environment of growth must be described by such factors as geographic location, soil type and its chemical properties, cultural practices and the climate during growth. The same facilities and care in field operations to avoid any seed mixing are needed for these plantings as in ordinary plant breeding experimentation. Thus, field plans and field books should be prepared prior to planting. All plots should be carefully labelled. Data should be collected in duplicate in field books coded ready for entry to the computer. The data must then be both checked and validated.

It is appropriate to discuss here the problem of the description of variability within samples. It is, of course, simpler to describe a homogenous sample than a heterogeneous one. A number of plants for each sample must be examined to ensure an adequate description of a heterogeneous population. For quantitative characters the mean and either the range or the standard deviation (assuming normal distribution) are amply descriptive of within-sample variability. At present the mean alone is used in the description of maize, rice and winged bean samples. Polymorphisms for qualitative characters must also be described. For rice the presence of a polymorphism for a qualitative character is described by a descriptor state ($S =$ segregating), whereas for maize and winged bean only the predominant character state in a sample is recorded. The computer programs for the documentation of germplasm collections are designed for a single biodatum per descriptor per accession. But the preceding comments on the description of heterogeneity within samples dictate that changes in the usage of these programs must be made to allow for the characterization both of polymorphism within samples and also of the mean, range and/or standard deviation of quantitative characters. In altering the usage of the existing programs it is axiomatic that the ability to query and retrieve sub-sets of information is retained. Thus the mean, range and standard deviation for a character can be stored each in different descriptors. The presence of the different descriptor states of a quantitative character may also have to be stored in more than one descriptor.

Harvest methodology

At harvest, some material may be collected as herbarium specimens. Scrupulous care must be taken to avoid the contamination of samples at harvest. All bags and containers should be carefully cleaned prior to use. Threshing machinery must also be thoroughly cleaned between samples.

International collaboration (who does the characterization?)

Base collection at an International Agricultural Research Centre

When a crop is being investigated at an International Agricultural Research Centre or a large genetic resources centre, then usually the base collection and the largest active collection are in the same place. All newly collected samples should be sent to the appropriate Centre where they should be planted with the triple aims of characterization, seed multiplication and maintaining the genetic composition.

The respective International Centres are already undertaking the characterization of samples of many of those food crops which have been given a high priority (1 & 2) by the Board (IBPGR, 1976) (e.g. sorghum and millets at ICRISAT; rice at IRRI; maize at CIMMYT; *Phaseolus* at CIAT; chickpea and groundnut at ICRISAT; cowpea at IITA; cassava at CIAT; potato at CIP; sweet potato at IITA and AVRDC, and soyabean also at AVRDC). The appropriate International Centre will maintain a data bank of information on the germplasm collection and should also handle the requests from active collections/plant breeders for both data and seed. The relationship between the active collections and the Centre is important in relation to (i) the initial planting and subsequent regeneration of those samples which are ill-adapted to the environment of the International Centre, and (ii) the eventual flow of germplasm into breeding programmes. Clearly, the Centres should pinpoint their needs in relation to the growing of unadapted material and then both initiate and coordinate the necessary regional/

national cooperative programmes. This material must be grown for (i) initial seed multiplication; (ii) regeneration of stored seed with a low viability, and (iii) regeneration of exhausted stocks.

A rice network has already been established (IRRI, 1978), and the following collaborative plan to preserve and rejuvenate conserved rice seedstocks agreed:

The following comprehensive plan of action suggests a division of responsibilities among the participating countries and international institutes.

- (a) A complete set of conserved stocks (the base collection) should be preserved in long-term seed storage at IRRI. National and international centres should provide to IRRI fresh and healthy seed of those stocks not already conserved there and of stocks that IRRI cannot effectively rejuvenate (see points (c), (d), (e) and (h)).
- (b) IRRI should preserve, rejuvenate, and distribute the indica and javancia cultivars and breeding lines of *O. sativa* and other *Oryza* species for those from Africa.
- (c) Japan should preserve, rejuvenate, and distribute as many of the japonica varieties of East Asia as possible.
- (d) The United States should preserve, rejuvenate, and distribute varieties from the US, temperate South America and the Mediterranean area; the US should also continue to store duplicate samples of conserved IRRI stocks.
- (e) IITA should preserve, rejuvenate, and distribute cultivars of *O. glaberrima* and wild species of Africa. IRAT plans to collaborate with IITA on seed multiplication. IRAT, ORSTOM and WARDA plan to collaborate with IITA on medium-term storage.
- (f) The above centres should exchange and carefully compare accession lists to minimize the maintenance of obviously duplicate accessions within single collections and to ensure that no distinct accession or ecostrain is overlooked in the inventorial process.
- (g) Major germplasm centres are urged to keep complete duplicate sets of accession records at separate locations to avoid loss through fire or other disasters. In the acquisition or exchange and use of accessions from major collections, original names and accession numbers should be included in the continuing records for cross reference purposes. Major germplasm centres are encouraged to standardize record systems. Where such standardization is impractical, compatibility of separate systems should be assured.

- (h) Each national and regional centre should preserve and rejuvenate its complete collection or at least a working collection, and assist the major germplasm centres to increase and rejuvenate accessions that are poorly adapted to the growing conditions at the centres.

There are problems in translating the above statement of intent into action. For example, Japan has strict quarantine regulations for rice and has taken little action in rejuvenation. The USA is trying to help in rejuvenation but is short on manpower for this work.

This network of active collections is also vital in relation to the eventual utilization of the germplasm in that it ensures some exchange of seed and information between plant breeders and thus facilitates the flow of germplasm into breeding programmes.

Two points merit a mention concerning the free exchange of both genetic resources samples and information. Firstly, some International Centres like CIMMYT respond to requests for germplasm, but put a relatively low priority on such exchange because they prefer to send out more advanced breeding material. Secondly, the reticence of breeders to divulge the results of evaluation for fear of plagiarism should be noted.

Base Collection and the Larger Active Collections in Different Locations

Wheat and the Asiatic *Vigna* spp. are the only non-industrial food crops which have been given a high priority for genetic conservation (Priorities 1 & 2, IBPGR, 1976) and are not being characterized at an IARC. The exceptional case of wheat was recognized by the IBPGR Wheat Advisory Committee, which recommended the initiation of a pilot wheat evaluation project. This project, run by the IBPGR, is in progress, but the results are not yet available. The Asiatic *Vigna* spp., in common with many other crops of lower priority, are of major regional importance, but of lesser global importance. It is clear that a regional approach will be needed for the characterization of these crops (but first, the base and active collections need establishment or recognition).

When the base collection and the larger active collections of a crop are in different locations within a region, the responsibilities of the various genetic resources centres in the characterization, preservation and regeneration of samples must be defined. Clearly, a very flexible approach is needed in the coordination of the institutions involved. It is important that the unnecessary multiplication of data banks is avoided in order to facilitate the retrieval of information. Scientists involved in this work should also be in touch with any existing networks among plant breeders for screening for current breeding aims, so that genetic resources samples can be moved into the networks as the next stage in their evaluation.

Finally, the characterization of samples of industrial crops should be mentioned. This work needs to be catalyzed by the IBPGR but the respective industries should play a large role.

Dealing with the backlog for characterization with a need for information exchange

The information on the majority of stored genetic resources samples is currently limited to an accession number and a seed source. This material must be characterized, but who will undertake such a massive task?

The seed for evaluation by plant breeders is currently given freely by genebanks, usually with a rarely-heeded plea to report back on the performance of the samples. In an effort to gain more information on undescribed samples, the genebanks could be firmer and only give out seed if the cooperating breeder first agrees to do some characterization in addition to screening for his current breeding aim. Seed could be sent out with the appropriate evaluation forms to the breeder, who knows that ultimately, his access to further seed samples from the genebank will rely upon his cooperation in returning the data from characterization.

Recommendations

- (i) A systematic characterization of genetic resources samples is necessary to provide information suited to the future requirements of plant breeders
- (ii) The systematic characterization should be undertaken at the initial planting of samples at genetic resources centres. The other aims of the initial planting are (a) the multiplication of seed and (b) the maintenance of the genetic composition of samples
- (iii) The principles and methodology of this initial planting are outlined herein and should be followed
- (iv) The traits to be studied in systematic characterization should be visible in any such planting and be of potential use in agronomic or taxonomic work. This restricts the choice of traits to morpho-agronomic characters with a reasonably high heritability
- (v) A representative group of plant breeders should be convened to agree on the traits for use in systematic characterization. Then the descriptors and the descriptor states should be defined. The responsibilities of the various genetic resources centres in regard to the initial planting, rejuvenation and storage should also be defined.

It is suggested that the IBPGR could be responsible for all the above. However, some help in describing the backlog of samples for characterization can be expected from plant breeders on the basis of a seed for information exchange.

The Board's possible support for further evaluation for the current aims of plant breeders is not warranted. But the Board should be aware of any existing networks for screening germplasm for particular aims, so that genetic resources samples can be exploited through such networks.

References

- Blixt, S. The gene symbols of *Pisum*. *Pisum* Newsl. Suppl., 9:2-59.
1977
- Blixt, S. Descriptive list of genes for *Pisum*. *Pisum* Newsl. 10:80-101.
1978
- Brown, A.H.D. Isozymes, plant population genetic structure and genetic
1978 conservation. *Theor. Appl. Genet.*, 52:145-157.
- Chang, T.T. Manual of Genetic Conservation of Rice Germ Plasm for Evaluation
1976 and Utilization. IRRI, Los Baños, Philippines.
- Chang, T.T. and E.A. Bardenas. The morphology and varietal characteristics
1965 of the rice plant. IRRI, Tech. Bull., 4. Los Baños, Philippines.
- Chang, T.T. *et al.* IRRI's role as a genetic resources centre, II. Proc.
1977 South Asian Workshop on Plant Genetic Resources, IBPGR/PCARR:
66-85.
- Erskine, W. The exploitation of genetic diversity in the winged bean
1979 (*Psophocarpus tetragonolobus* (L.) D.C.) for grain yield.
Ph.D. thesis, University of Cambridge, UK.
- IBPGR Priorities among Crops and Regions.
1976
- IBPGR Descriptors for Winged Bean.
1979
- IRRI Standard Evaluation System for Rice. IRRI, Los Baños, Philippines.
1975
- IRRI Proceedings Workshop on the Genetic Conservation of Rice. IRRI,
1978 Los Baños, Philippines.
- Shevchuk, T. Evaluation of plant collections. *Plant Genetic Res. Newsl.*
1973 *FAO*, 29:2-6.
- Zadoks, J.C., T.T. Chang and C.F. Konzak. A decimal code for the growth
1974 stages of cereals. *EUCARPIA Bull.*, 7:42-52.

RESUME

Cet article décrit les problèmes principaux et annexes relatifs à l'évaluation des échantillons de ressources génétiques végétales. Il est recommandé:

1. Une caractérisation systématique des échantillons de germoplasme est nécessaire afin de fournir des informations convenant aux besoins futurs des sélectionneurs.
2. Cette caractérisation systématique devrait être entreprise dès les plantations initiales des échantillons dans des centres de ressources génétiques. Les autres buts de ces plantations initiales sont (a) la multiplication des graines et (b) l'entretien de l'intégrité génétique des échantillons.
3. Les principes et la méthodologie des ces plantations initiales sont explicités ici et devraient être appliqués.
4. Les caractères à étudier en caractérisation systématique devraient être visibles au cours de la culture et être d'une utilité potentielle pour tout travail agronomique et taxonomique. Ceci restreint le choix des caractères aux caractères morfo-agronomiques à héritabilité raisonnablement élevée.
5. Un groupe représentatif de sélectionneurs devrait convenir des caractères à considérer en caractérisation systématique. Alors, les descripteurs et leurs états possibles devraient être définis. Il en est de même des responsabilités des divers centres de ressources génétiques concernant les plantations initiales, le rajeunissement et la conservation.

RESUMEN

En este artículo se describen los principales problemas y los anexos relativos a la evaluación de muestras de recursos genéticos vegetales. Se recomienda lo siguiente:

1. Es necesaria una caracterización sistemática de las muestras de recursos genéticos a fin de facilitar información que ayude a atender las necesidades futuras de los fitogenetistas.
2. La caracterización sistemática debe emprenderse durante la plantación inicial de muestras en los centros de recursos genéticos. Los otros objetivos de la plantación inicial son (a) la multiplicación de semillas y (b) la conservación de la composición genética de las muestras.
3. Se enuncian los principios y la metodología que debe seguirse en relación con esta plantación inicial.
4. Los caracteres que han de estudiarse en la caracterización sistemática deben ser observables en toda plantación de esa clase y han de tener aplicación potencial en trabajos agronómicos o taxonómicos. Esto circunscribe la elección de caracteres a aquellos morfo-agronómicos de hereditabilidad razonablemente alta.
5. Conviene seleccionar un grupo representativo de fitogenetistas para que disutan y lleguen a un acuerdo sobre los caracteres que han de utilizarse en la caracterización sistemática. Deberán definirse después los descriptores y los correspondientes estados. Conviene definir también la competencia y funciones de los diversos centros de recursos genéticos en lo que se refiere a la plantación inicial, al rejuvenecimiento y al almacenamiento.

LIST OF OFFICIAL CORRESPONDENTS TO THE PLANT GENETIC RESOURCES NEWSLETTER

AFGHANISTAN

Mr. Mohammad Asef Rashid
Plant Research & Soil Science
Department
Ministry of Agriculture and
Land Reform
Kabul

BOLIVIA

Ing. Julio Rea
Instituto Boliviano de Tecnología
Agropecuaria
La Paz

BURMA

Dr. U. Ohn Kyaw
Deputy General Manager
Agriculture Corporation
72 Shwedagon Pagoda Street
Rangoon

COLOMBIA

Dr. Luis E. Lopez Jaramillo
Programa Tuberosas
Instituto Colombiano Agropecuario
ICA - Tibaitatá

CUBA

Ing. Tomás Rivera Amarán
Especialista
Division de Ciencias y Tecnología
Ministerio de Agricultura
La Habana

EL SALVADOR

Ing. Félix Rodolfo Cristalo
Centro Nacional de Tecnología
Agropecuaria
(CENTA)
San Salvador

ETHIOPIA

Dr. Melaku Werede
Head
Genetic Resources Centre
Institute of Agricultural
Research
P.O. Box 30726
Addis Ababa

FIJI

Mr. Param Sivan
Principal Research Officer (Agronomy)
c/o Koronivia Research Station
Department of Agriculture
Nausori

GHANA

Mr. M.A. Adansi
Chief Research Officer of the Oil Palm
Research Centre
Crops Research Institute
Council for Scientific and Industrial
Research
Kusi/Kade

IRAQ

Mr. Adil Towfiq Sharif
Officer-in-Charge of Conservation
of Germplasm Project
Plants Division
State Board for Applied Agricultural
Research
Abu Ghraib

JAMAICA

Mr. Noel Singh
Acting Deputy Director
Research and Development Department
Ministry of Agriculture
Hope Gardens
Kingston 6

KOREA

Mr. Young-Man Lee
Researcher
Genetics Division
Institute of Agricultural Sciences
Office of Rural Development
Seoul

LIBERIA

Mr. Francis Sumo
Rice Breeder
Suakoko Agriculture Research Center
Monrovia

MALAWI

Mr. P. Sibale
Chitdze Agricultural Research
Station
Box 158
Lilongwe

PAKISTAN

Dr. M. Akbar
Coordinator (Rice)
Pakistan Agricultural Research
Council
P.O. Box 1031
L.13, F.7/2
Elmarkaz
College Road
Islamabad

PAPUA NEW GUINEA

Mr. Kana Aburu ^{1/}
Lowlands Agricultural Experiment
Station
Keravat
East New Britain Province

PERU

Ing. Flaminio Villavicencio Camacho
Dirección de Investigación Agrícola
y Criazas
Instituto Nacional de Investigación
Agraria
Sinchi Roca No. 2728
Lince
Lima

PHILIPPINES

Mrs. Lolita Nuñez Ragus
Senior Subject Matter Specialist
Crops Research Division
Philippine Council for Agriculture
and Resources Research
Los Baños
Laguna

SRI LANKA

Dr. P. Ganeshan
Geneticist
Agricultural Research Centre
Thinnaveli
Jaffna

SUDAN

Dr. Mahmoud A. Mahmoud
Head
Plant Breeding Section
Gezira Research Station
Wad Medani

URUGUAY

Sr. Gastón Navarro
Facultad de Agronomía
Avda. Eugenio Garzón 780
Montevideo

YEMEN, PEOPLE'S DEMOCRATIC REPUBLIC

Mr. A.B.S. El-Muallem
Head
Agronomy Section
UNDP
P.O. Box 1188
Aden

1/ Member of the IBPGR Regional Committee for Southeast Asia

The Plant Genetic Resources Newsletter will be pleased to consider reports, notes and news from anyone working with genetic resources.

Please send manuscripts, typed in double space. Relevant photographs too, will be greatly appreciated, but only if high quality black and white prints rather than colour prints or slides. Colour photographs will reproduce, but not without a considerable loss of quality.

The Newsletter will also review salient books, scientific papers and other publications.

All contributions should be sent to the Editor, AGP, FAO, 00100 Rome, Italy.

Le Bulletin des Ressources Génétiques Végétales apprécierait de connaître les rapports, notes et nouvelles écrits par quiconque travaillant en relation avec les ressources génétiques.

Prière d'envoyer les manuscrits tapés en double interligne, accompagnés le cas échéant de photographies s'y référant, mais de préférence sous forme d'excellents tirages en blanc et noir plutôt que de tirages en couleurs ou de diapositives. Les photographies en couleurs peuvent être reproduites mais sans garantie de perte de qualité.

Le Bulletin passera en revue les livres les plus en vue, les notes scientifiques et autres publications.

Toute contribution sera envoyée à l'Editeur, AGP, FAO, 00100 Rome, Italie.

El Noticiario de Recursos Genéticos Vegetales considerará con gusto informes, notas y noticias provenientes de aquellas personas trabajando en recursos genéticos.

Agradecemos el envío de los trabajos mecanografiados a doble espacio. También apreciaremos el envío de fotografías relacionadas con la materia, pero deberán ser impresiones de buena calidad preferiblemente en blanco y negro. Fotografías en color pueden ser reproducidas, pero con una apreciable pérdida de calidad.

El Noticiario también hará revisiones de libros, trabajos científicos y otras publicaciones.

Todas las contribuciones deben ser enviadas al Editor, AGP, FAO, 00100 Roma, Italia.

