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Pasture Improvement Research in Eastern and Southern Africa

Proceedings of a workshop
held in Harare, Zimbabwe,
17-21 September 1984

Proceedings Series



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Pasture Improvement Research in Eastern and Southern Africa

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Abstract: The proceedings contains reviews by national scientists on pasture research done primarily in Eastern and Southern Africa (Ethiopia, Kenya, Tanzania, Burundi, Zambia, Zimbabwe, Swaziland, Lesotho, Botswana, Mozambique, and Madagascar). The application of the results obtained and lessons learned are highlighted and used in setting of national priorities for research areas for the future. Critical reviews on current pasture research methodologies are included in the proceedings. The research methods discussed are germ-plasm collection, storage, and dissemination; and germ-plasm introduction and evaluation, nutritive evaluation of pastures, grazing experiments, and range monitoring. Specific guidelines on methodologies are outlined and these are useful to pasture agronomists, animal nutritionists, and range-management scientists.

Two case studies of pasture-research regional networks in Asia and Latin America were presented and discussed. A strategy for future pasture research coordinated through a regional Pastures Network for Eastern and Southern Africa (PANESA) was discussed and agreed upon.

Résumé: Dans les actes ci-joints, des scientifiques de divers pays analysent la recherche entreprise sur les pâturages en Afrique orientale et australe (Éthiopie, Kenya, Tanzanie, Burundi, Zambie, Zimbabwe, Lesotho, Botswana, Mozambique et Madagascar). L'utilisation des résultats obtenus et les connaissances acquises sont mises en lumière, puis utilisées pour établir les priorités nationales en matière de recherche. Les actes comportent une analyse critique des méthodes de recherche actuelles sur les pâturages : rassemblement, entreposage et diffusion du matériel génétique; mise à l'essai et évaluation de ce matériel; expériences de pâturage; évaluation nutritive des pâturages et exploitation rationnelle de ceux-ci. On présente des lignes directrices précises sur les méthodes à suivre, qui seront utiles aux agronomes en charge des pâturages, aux spécialistes de la nutrition animale et aux scientifiques responsables de la gestion des pâturages.

Deux études de cas ont fait l'objet d'une présentation suivie d'une discussion : il s'agit des réseaux régionaux de recherche sur les pâturages en Asie et en Amérique latine. Après discussion, on a convenu d'une stratégie de la recherche sur les pâturages, dans les années à venir; la coordination de cette stratégie sera assurée par une section régionale du Pastures Network for Eastern and Southern Africa (PANESA).

Resumen: En las actas se recogen ponencias presentadas por científicos de diferentes países sobre las investigaciones en pastos que se han realizado principalmente en el África oriental y meridional (Etiopía, Kenia, Tanzania, Burundi, Zambia, Zimbabwe, Suazilandia, Lesotho, Botswana, Mozambique y Madagascar). Se destaca la aplicación de los resultados y experiencias obtenidos, muy útiles para determinar las prioridades de las investigaciones futuras en las diferentes naciones. En las actas se recogen también ponencias críticas sobre las metodologías empleadas actualmente en las investigaciones sobre pastos. Se analizan los siguientes métodos de investigación: recogida, almacenamiento, disseminación, introducción y evaluación de germoplasmas; evaluación del valor nutricional de los pastos; experimentos de pastoreo; y control de dehesas. Se resumen directrices y metodologías específicas de gran utilidad para agrónomos especializados en pastos, expertos en nutrición animal y científicos especializados en gestión de dehesas.

Se presentan y analizan dos estudios de casos de las redes regionales de investigación en Asia y Latinoamérica. Se discutió y aprobó una estrategia para realizar investigaciones sobre pastos en el futuro que serán coordinadas por la Red de Investigaciones sobre Pastos para África Oriental y Meridional (RIPAOM).

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GERM-PLASM STORAGE AND DISSEMINATION

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Abstract *An exemplary work procedure for orthodox seeds is given describing the handling of germ plasm entering a genebank until final storage under long-term conditions, i.e., at low-seed moisture content, subzero temperatures, and hermetically sealed in airtight containers.*

To enhance dissemination and utilization of germ-plasm description of the collection site, the morphological and agronomical characters as well as specific traits such as tolerance to abiotic or biotic stress are indispensable prerequisites. Only with this information can germ plasm be accurately channeled into breeding programs.

Because of the high cost of collecting, maintaining, describing, and conserving germ plasm, regional genebanks are required to serve a large area that spans political borders and necessitates the unrestricted global exchange of material and information.

The strategy of a genebank to conserve germ plasm is determined by the type of propagation of its mandate crops. Asexual propagated plant species such as banana are maintained in living collections, either in nurseries or arborata, or as meristeme culture as it is introduced in potatoes, for example. In sexually propagated plant species, the seeds are categorized according to their storage physiology into "orthodox seeds" and "recalcitrant seeds." The first group comprises most of

the agricultural plants including forage and pasture crops. Recalcitrant seeds belong mainly to tropical fruit trees such as mango, avocado, cacao, or coconut, and forest trees like oak or chestnut. Orthodox seeds can be dehydrated without being damaged, but, in contrast, the viability of recalcitrant seeds depends on a relative high-water content. However, dehydration is one of the prerequisites to extend the longevity of seeds, in particular when stored at subzero temperatures, otherwise freezing water in the seed tissue leads to lethal injuries. Therefore, germplasm of plant species with recalcitrant seeds are also maintained in living collections.

The type of conservation, as a living collection or as seeds, also determines the dissemination of germ plasm. In living collections, the prospective user can compare and select the appropriate germ plasm from a permanent display of the material, however, a genebank conserving seeds can offer only either a one-season display of a portion of the whole collection (during multiplication) or usually only seed samples with the description of the corresponding plant. In the latter, the user has to rely on the accuracy and comprehensiveness of the information when requesting germ plasm. In the paper, the conservation and dissemination of germ plasm from plants with "orthodox seeds" will be discussed using the Plant Genetic Resources Center of Ethiopia (PGRC/E) as an example.

SEED CONSERVATION

The reduction of the seed moisture content (SMC) extends the longevity of orthodox seeds. Harrington's (1963) "rule of thumb" expects a doubling of longevity if the SMC is reduced by 1%. Therefore, seeds with 4% SMC are expected to retain viability approximately 1,000 times longer than seeds with 14% SMC.

A second determining factor for extending the life expectancy of seeds is temperature. Another "rule of thumb" indicates that longevity of seeds is also doubled by reducing the storage temperature by 5 °C (within a range of 50 to 0 °C). Because both factors, moisture content (MC) and temperature, are obviously acting independently (Harrington 1970), seeds with an SMC of

4% and stored at 0 °C have a $1,000 \times 10^3$ times longer life expectancy than stored with 14% SMC at 50 °C.

Apart from the relative rough estimation of the storage life time of seeds with Harrington's rules of thumb, many attempts have been made to predict more precisely the storage duration of seeds. In this respect Roberts and Abdalla (1968) have developed crop-specifically advanced equations and nomographs to calculate and determine the survival probability of a certain seed lot (Ellis and Roberts 1980).

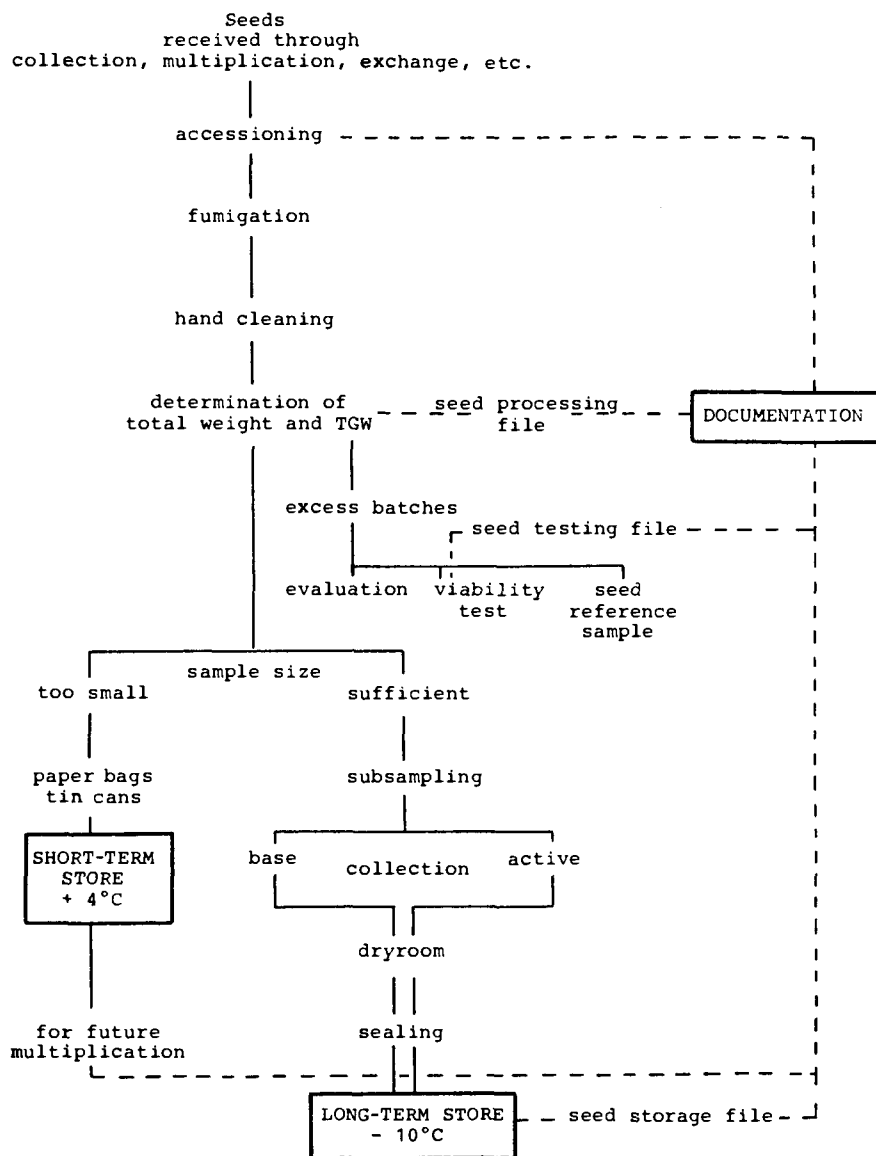
Knowledge of the rate of ageing during storage is indispensable to determine the interval of rejuvenation of the stored seeds. According to the negative correlation between storage temperature/SMC and longevity, the lower the temperature (and SMC) the longer the rejuvenation interval. As shown by Roberts (1975) wheat stored at +4 °C with 9% MC has to be stored up 9 years, whereas wheat stored at -20 °C and 5% SMC can be stored for 390 years. Corresponding figures for Hordeum distichum are 109 and 33,500 years and for horsebean 20 and 1,600 years, respectively.

Apart from the interaction between storage conditions and longevity the time interval for rejuvenation is also dictated by the initial viability of the seeds. According to Ellis et al. (1980), barley stored at -18 °C with an initial viability of 100% requires rejuvenation only after 8,050 years; with 95% viability, 1,560 years; and with only 90% initial viability, 620 years. In this context, the environmental conditions before harvest can influence considerably the viability (Austin 1972), which in turn will also have an influence on the rejuvenation interval.

A third factor determining the storage durability of seeds is the oxygen pressure (Roberts and Abdalla 1968). Oxygen enhances respiration, which reduces seed viability, and it is noteworthy that with sealing as it is normally done oxygen pressure is reduced.

When considering the storage lifetime of seeds, not only the change of the viability in the course of time should be kept in mind but also changes in the genetic integrity (Abdalla and Roberts 1968).

Based on the experience of other genebanks, the recommendations of IBPGR and the specific conditions of the host country PGRC/E has developed the following procedure for long-term storage of orthodox seeds. (Fig. 1).



_____ = flow of material; - - - - = flow of information

Fig. 1. Scheme of seed processing for long-term storage at PGRC/E.

(a) Accessioning of the Samples. All samples entering the genebank, either through collection or seed exchange, receive an accession number before further handling. The accession numbers are assigned consecutively regardless of the crop type. It is questionable whether a crop index number or letter should be added to the accession number to assist crop identification, especially when a genebank deals with many different crop types.

(b) Fumigation. As a phytosanitary measure, all seed samples coming from the field are fumigated for 72 hours with Phosphin to control further damage of the seeds and to avoid infestation of the genebank with insects carried by the samples.

(c) Hand Cleaning. Because of the enormous heterogeneity of Ethiopian germ plasm, the threshed samples are hand cleaned to avoid mechanical selection according to the seed size. The efficiency of handcleaning declines with the seed size. In cereals, to clean 200-250 samples a week, 6-8 workers are necessary. At the same time, cleaning samples composed of different crop types are separated into "one-crop samples."

(d) Determination of Sample and Thousand-Grain Weight. Thousand-grain weight (TWG) is one of the characteristics describing an accession. Together with the total sample weight, TGW is also necessary to determine the sample size based on the seed number. At PGRC/E, the standard sample size for long-term storage is 8,000 seeds in heterogeneous material and 3,200 seeds in genetically uniform (homogeneous) samples. The standard sample size applies to crops with a TGW of 5-200 g. For practical and economical reasons, in large-seeded crops (TGW above 200 g), such as horse-bean or maize, the corresponding sample sizes are 4,000 and 1,600 seeds, and in small seeded crops with a TGW below 5 g, such as millet or teff, one takes samples weighing 80 and 32 g, respectively. TGW is determined by taking two samples of 500 seeds each, weighing them, and calculating the mean weight. One batch of the seeds used is kept apart for the consecutive viability test, provided the sample size fulfills the above mentioned standard requirements. The second 500 seed batch used is kept as a seed reference sample.

As seen in Fig. 1, samples smaller than the required limit are stored in paper bags or tin cans under short-term conditions (+4 °C and 35-40% relative humidity (RH)). Large samples exceeding the limit are adjusted to the indicated standard sizes, the excess material is used for immediate further evaluation.

At PGRC/E, sample size and TGW are determined by a two-person team equipped with an electrical balance, an electrical seed counter and a calculator. The data are recorded manually in a crop-specific "seed-processing file." The data are then transferred after compilation into the computerized documentation system. The handling capacity is approximately 200-250 samples/week.

(e) Viability Test. The test procedures strictly follow rules developed by the International Seed Testing Association (ISTA) (1976) for both the number of seeds per replicate and the time interval for the first and second counting. The results are also recorded manually using a "seed testing file" and after compilation they are transferred to the documentation unit. For the viability test, PGRC/E is equipped with the standard tools such as a water deionizer, autoclave, germination room (20 °C), and an incubator for alternating temperatures. During viability testing, care must be taken not to mix up dormancy with low viability.

(f) Subsampling for Long-Term Storage. Heterogeneous standard samples (8,000 seeds) are subdivided into 3,000 seeds for "base" and 5,000 seeds for "active collection." The latter is subsampled into five equal portions. Homogeneous material (e.g., ear-to-row progenies) is equally subsampled in eight batches of 400 seeds, one subsample is kept in the base and the remaining seven subsamples in the active collection. The corresponding quantities for small-seeded crops are 30 g for base and five samples of 10 g each for the active collection in heterogeneous accessions and eight samples of 4 grams each in uniform material, and in large-seeded crops half of the quantity of a standard sample is used.

All samples, the base and the active collection are stored at the same temperature (at present -10 °C, in future -20 °C) but in separate coldrooms. The base

collection is used for undisturbed long-term conservation, the active collection serves for seed exchange, monitoring the viability, research, breeding stock, etc. The quantities shown differ in some respect from the recommendation given by IBPGR (Cromarty et al. 1982). According to IBPGR a genebank should conserve not less than 12,000, 3,000, and 5,000 seeds for base, duplicate, and active collection in heterogeneous accessions, and 4,000, 1,000 and 3,000 seeds in genetically uniform material, respectively. Furthermore, only the base collection (and the duplicate samples) are stored under long-term conditions but not the active collection.

The lower quantities adopted by PGRC/E are based on PGRC's collection strategy that states that in areas with a high degree of diversity instead of collecting one or few large samples more smaller samples should be taken. This also takes the relative small plot sizes of the Ethiopian farmer's field into account. By that, a sample composed of, e.g., 20-30 spikes of self-pollinating crop might, after multiplication, be very well represented by a 1,000 seed subsample. The deposition of a safety duplicate from each of the accessions (active collection) in one of the corresponding genebanks outside of Ethiopia is under operation.

As indicated, the active collection is stored as individually sealed subsamples and not as a bulked large sample. With this procedure, unnecessary exposure of the whole sample to the atmosphere outside of the coldroom and, therefore, the possible absorption of moisture is avoided if a subsample is taken. As is already known, bodies colder than the dewpoint temperature of the ambient atmosphere will condense water from the air. To illustrate the fact, at +25 °C air temperature and 80, 60, or 40% relative humidity the corresponding dewpoint temperatures are +21, 17, and 11° C, respectively. This means, seeds taken from a -10 °C coldroom and exposed immediately to the outside atmosphere will, because it is colder, definitely condense and absorb moisture unless the seed temperature is in equilibrium with the air temperature first before the container is opened.

(g) Drying of Seeds. To reduce the seed MC before sealing and storing, the samples are placed in a dryroom operating at 10-15% RH at 20 °C. In the

dryroom, the equilibrium seed MC at a given temperature depends on the RH of the ambient air and the oil content of the seeds as shown in Table 1.

Because of the hydrophobic nature of lipids, the higher the oil content the lower is the equilibrium seed MC. The figures reveal, furthermore, that to reach, e.g., in wheat or barley seeds, an MC of at least 6% (dry base) the RH in the dryroom has to be at least 15%. Because of the interdependency between air temperature and RH, increasing the dryroom temperature would also lower the equilibrium seed MC. However, care must be taken not to overheat the seeds.

The time requirement to reduce the seed MC is determined, in addition to the parameters already mentioned, by the difference between initial and final SMC, the wind velocity in the dryroom, and the seed size. Millet seeds, for instance, with an oil content similar to sorghum but with a 10 times smaller TGW (2 compared to 26 g in sorghum), would need only 3 hours to

Table 1. Equilibrium MC of seeds that differ in oil content as affected by the humidity of the atmosphere at 25°C.^a

Crop type	Oil content (% DM) ^b	Seed MC (% DM)	
		20% RH	15% RH
Horsebean, field pea, lentil	1.5-1.9	7.1	6.0
Barley, wheat	1.7-1.9	7.1	6.0
Maize	4-5	6.9	5.9
Sunflower	32	4.8	4.1
Rapeseed	45	3.9	3.4

^a Source: Data derived from equation 3.5 (Cromarty et al. 1982).

^b DM = dry matter, MC = moisture content, RH = relative humidity (air temperature 25°C).

reduce the MC from 10 to 6%; sorghum, in contrast, requires 16 hours under the same dryroom conditions (25 °C, 15% RH, and 1 m/s wind speed).

Although the figures in Table 1 provide valuable basic information, the frequent determination of the actual MC of the seeds during the drying process remains as a necessary measurement to safeguard the germ plasm. To determine SMC, two methods can be employed, either gravimetrically or by following the weight loss of reference samples in the course of exposure time. In the latter the final weight is given by the equation:

$$W_f = W_o(100 - M_o)/(100 - M_f)$$

where W_o , and W_f , M_o , and M_f are the initial and final weight and moisture content, respectively (Cromarty et al. 1982). If the gravimetrical method is used, ISTA rules should be followed.

(h) Sealing of the Samples. At PGRC/E, the samples are sealed in laminated aluminium foil bags, heavy quality of approximately 110 g/m². Alternatives are the storage in air- and moisture-tight plastic bottles (e.g., at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)), glass jars (Gatersleben), or corrosion- and leakage-proof tin cans (Izmir). To monitor leakages the International Rice Research Institute (IRRI) is using evacuated aluminium cans for long-term storage.

(i) Coldroom. The coldrooms at PGRC/E are equipped with mobile shelves, subdivided into compartments. i.e, the intersections of the vertical shelf column with the horizontal rack. For identification of the position of an accession, the shelf compartments are labeled with four-digit numbers indicating sequentially the number of the coldroom, shelf, column, and rack. For recording the position and stock control, the "storage file" is used.

For long-term storage the coldroom operates at -10 °C without humidity control, and for short-term storage at +4 °C and 35-40% RH. Power supply is provided by two independent electrical power lines; an additional stand-by generator will be fixed soon.

To economize the available coldroom space and to reduce running costs, the more expensive mobile shelves should be given preference over the cheaper static shelves. Taking PGRC's new 100 m³ long-term storage into consideration, with a floor area of 37 m² the eight mobile and two static shelves provide a shelf area of 248 m². If the same coldroom is equipped only with static shelves, the same floor area would provide only 140 m² of shelf area, i.e., 57% of that of the mobile shelves. In other terms, providing the same shelf area with static shelves, the floor area of the coldroom has to be increased to 66 m² leading to a total coldroom volume of 177 m³. Due to enlarging the coldroom volume and, therefore, extending the coldroom surface, not only the initial costs would be increased but also the annual power costs. The annual power costs for a 100 m³ coldroom as installed at PGRC/E can be estimated by US\$750, if enlarged to 177 m³, US\$1,200 (calculations based on formulas given by Cromarty et al 1982). The initial cost for a 100 m³ coldroom including mobile shelves can be calculated at about US\$60,000 (FOB), increasing the volume to 177 m³ would increase the initial costs by 50-70%.

The storage capacity of the coldroom as described above (100 m³ volume and 248 m² shelf area) is approximately 80,000 standard samples of base collection (e.g., barley, 3,000 seeds sealed in Al-foil bag) or approximately 36,000 accessions as active collection with five subsamples (1,000 seeds) each. At PGRC/E, the samples in the Al-foil bags are kept together in cartons of two different sizes (30 x 24 x 8 cm and 30 x 12 x 8 cm) storing either 25 (12) samples of base collection or 11 (6) accession as active collection (in brackets the capacity of the smaller carton). In each compartment (90 x 60 cm) six large and two small cartons can be placed. Each carton is labeled with the compartment number and -- compartmentwise -- with a carton serial number.

DISSEMINATION OF GERM PLASM

It is foreseeable that a genebank failing to disseminate germ plasm would be degraded and considered merely as a "seed museum." It is also true that plant breeders often hesitate to introduce germ plasm

istics, topography, etc. With this base information, accessions can be earmarked for desirable characteristics, e.g., tolerant to extreme soil reactions or water regimes, according to the environmental conditions of the collection site.

Characterization describes mainly highly heritable morphological characters such as spike density, glume colour, or kernal colour. The information gathered during characterization assists in creating an image of a certain genotype. The accuracy, however, depends on the degree of heterogeneity of the accession, the more uniform the material the more accurate the description.

Evaluation can be subdivided into "preliminary" and "further" evaluation. The first describes mostly less-heritable characters, such as days to flower, plant height, number of tillers, or TGW. Although those results are based usually on only one season, one location, without replicates, the information helps to narrow the number of genotypes suitable for a certain breeding program. Like characterization, the reliability of the data is also determined by the degree of heterogeneity of the accessions.

Characterization and preliminary evaluation are usually combined in descriptor lists. Common for both is that collection and recording of the data are normally done during multiplication as indicated in Fig. 2.

Further evaluation considers mainly specific traits, such as quantity and quality of yield components or constituents, or tolerance to abiotic and/or biotic stress. This demands a relatively high input of sophisticated methods and equipment, the necessity of specialized personnel as well as highly homogenous germ plasm to yield reliable and reproducible results. To achieve the latter request, subsamples of the original population as collected should be split into single lines even to the expense of invading the genebank with large numbers of single lines. However, before procuring sophisticated and expensive equipment and employing specially trained personnel a genebank should compare whether further evaluation of germ plasm would be done better in well-equipped and experienced institutions outside of the genebank or sending the material

to areas that have certain abiotic or biotic stress situations (hot spots).

To assist the potential user in selecting the appropriate germ plasm, local varieties or standards should be included in the characterization and evaluation program. At the same time, the genebank can estimate the potential of the conserved germ plasm if compared with known standards as shown in the following few examples.

In a field experiment, conducted in 1982 at Kulumsa/Ethiopia, 61 accessions of PGRC's breadwheat collection (BW) and 142 accessions of the durumwheat collection (DW) have been compared with three widely used standard varieties in Ethiopia; Romany BC, Enkoy (BW), and Cocorit (DW). The results revealed that none of the breadwheat accessions but nearly 33% of the durumwheat accessions significantly outyielded the standards. Consequently, if an Ethiopian plant breeder is looking for yield increases in wheat, the breadwheat collection of PGRC/E might not be as promising as the durumwheat collection.

In a second example, the protein content in seeds of 222 accessions of Ethiopian sorghum germ plasm was compared with that of four local lines (Table 2). Astonishingly, more than 33% of the tested accessions (single lines) had a seed protein content higher than

Table 2. Comparison of the protein content in the dry matter of seeds of 222 Ethiopian sorghum accessions with four local varieties (protein = N x 6.25).

	Protein %
Germ plasm	
Mean	10.5
Range	5.1-15.3
Varieties	
Didessa	8.1
Melakmesh	9.3
Bakomesh	10.6
Gambella	10.9

the standard varieties. It can be expected that with testing all of PGRC's nearly 7,000 sorghum accessions, the number of high-protein genotypes will be high.

With a simple soil test, 654 wheat lines selected from populations have been tested for tolerance to acid soil. Nearly 9% of the tested material appeared to behave similarly to the known tolerant varieties of Chinese Spring, Vila Velha, and Trintani. Although breeding for tolerance to soil acidity in Ethiopia is only a minor priority, the results might be interesting for other countries suffering from soil acidity, and it might also be relevant to the land-use plan for Ethiopia. It is understood that other crop types such as pasture and forage crops may demand screening of different traits.

A second step to overcome the hesitation of plant breeders to use even primitive germ plasm is to involve plant breeders directly in the different aspects and activities of the genebank. Thus, with the permanent contact with germ plasm, the plant breeders may lose their suspicions and accept germ plasm. In this respect, an ideal combination is, of course, setting up the genebank under the same roof with plant breeding as at ICRISAT. In Ethiopia, although PGRC/E is located separately from plant breeding stations, PGRC/E is incorporated into the national breeding program in activities such as multiplication/rejuvenation being done within the plant breeding stations. Through this arrangement the Ethiopian plant breeders are automatically involved in those activities, and they also have the unique opportunities (a) to earmark promising genotypes, (b) to influence the development of descriptor lists, and (c) to encourage the genebank to look for specific traits the breeders are interested in. In this context, the cooperation between genebank and breeder is already in existence in planning and collection missions.

Irrespective of the method of improving dissemination before despatching germ plasm, the genebank has to perform appropriate seed health control measurements. The control of seed health is, however, not only restricted to checking outgoing material (as indicated in Fig. 2), but it also includes phytosanitary and plant protection measures during multiplication and processing

of the seeds as well as plant quarantine for material coming from abroad.

CONCLUSION

Dissemination and utilization of germ plasm depend on appropriate maintenance of the material and the accuracy and comprehensiveness of the description and evaluation. The extent of dissemination will increase by strengthening the involvement of plant breeders in the activities of the genebank. However, both conservation as well as characterization and evaluation are connected with relatively high costs (a) to install, run, and maintain the technical setup; (b) to maintain the labour-intensive fieldwork; and (c) to finance characterization and evaluation. It is still an open question whether the costs should rest exclusively on the host country, to be covered by international funds, or through licensing the germ plasm to determine continued, undisturbed long-term operation of the genebank. Considering the high operational cost, a genebank, regardless of its mandate crops, should not restrict its activity to the national level but should serve regional areas as the mandates for the international agricultural research centres such as ICRISAT or ILCA cover. It is also necessary to ensure the unrestricted global exchange of seeds and information to spread the dissemination and utilization of germ plasm.

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