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Managerial tools for seed regeneration[1]

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This report reviews some tools that curators of ex situ *plant germplasm collections can employ to manage seed regeneration. It examines the various roles of germplasm users as sources of technical expertise and advice about germplasm needs. Analysis of past demand for germplasm and forecasts of future demand trends are valuable guides to plan regeneration. Seed quantity and viability are key planning criteria, but regeneration planning should also weigh such factors as overall genetic diversity within collections, institutional duplication, and the relative quality and completeness of passport and characterization data. The North Central Regional Plant Introduction Station conducts applied research to develop effective techniques for seed multiplication of cross-pollinated crops and their wild relatives. An overview of the Station's experiences with insect pollination in field cages, high-density pot culture, and mating scheme evaluation for maize is presented. Optimal use of all these management tools relies on the development of a corps of crop-specific curators, who can gain the expertise needed to anticipate users' needs and understand the intricate patterns of genetic diversity and reproductive biology within their respective crops.*

INTRODUCTION

Any discussion of current practices and past experiences in seed regeneration for plant germplasm conservation should begin with a reflection on institutional contexts. While organizations may share many goals in the preservation of plant biodiversity, their practices and experiences are shaped by diverse institutional missions, cultures and goals. Any recommendations made herein should be viewed as constructive advice to be adapted to each institution's own mission and overall goals. For example, programmes with missions highly focused in support of specific crop-improvement projects may rightly view comments regarding relations with a broader user community as only marginally relevant.

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USERS AND DEMAND

As one examines factors to be considered when planning regeneration schemes and the resources that might be mobilized to overcome constraints to successful regeneration, one should first consider the potential and actual roles played by the germplasm user community. Users play critical roles as advisers to, and advocates for, *ex situ* germplasm conservation and as the drivers of demand for germplasm collections.

For many crops, there is a large body of expertise on plant culture and protection, genetics, systematics, breeding biology, seed production, and utilization. This expertise is multidisciplinary and diffused among many researchers, who individually may or may not be aware of pertinent germplasm collections. Some researchers with long-standing knowledge of collections and their curators regularly request germplasm from *ex situ* collections; many others have less contact with, or understanding of, such collections; whereas still others are totally ignorant of germplasm collections or how well-documented and evaluated germplasm can contribute to their research.

When curators plan regeneration programmes and confront the physical, financial, and political constraints that may impede such plans, they should be able to bring to bear the combined expertise and influence of researchers and others who request samples. By developing a network of 40 commodity-oriented Crop Germplasm Committees, the U.S. National Plant Germplasm System (NPGS) has organized a valuable mechanism for convening teams of experts to advise curators on a broad range of managerial issues (Anonymous, 1992), including aspects of seed regeneration. Well-crafted surveys of potential and actual germplasm users (McFerson *et al.*, 1996) can also provide advice for curators when such expert committees are not easily assembled. And, finally, curators may benefit by publicizing their work to those likely to be ignorant of germplasm collections and their significance.

Building strong and mutually beneficial relationships with the broadest possible range of germplasm users will help ensure the long-term success of *ex situ* conservation. Should resources or national priorities shift away from one discipline towards another, it would be wise for germplasm managers to remain flexible in meeting the needs of all pertinent users. To do so, managers of national and international germplasm programmes should be very interested in a disciplinary analysis of users and trends over time.

Such an analysis fits in well with a more comprehensive analysis of demand. Demand is a key criterion for setting regeneration priorities that deserves close scrutiny. A germplasm collection's value is entwined with its present and future uses. To maximize value, regeneration must be adequate for both long-term conservation and meeting users' requests.

Managerial decisions regarding regeneration can occur *ad hoc* in response to unmet requests, or, preferably, through more systematic long-term demand analyses (Bretting & Widrlechner, 1994; Widrlechner, 1995). An effective demand analysis should consider patterns of demand by taxon, accession, and end use, ideally by examining a period, perhaps five or more years, long enough to temper short-term fluctuations. Unmet requests should also be documented and quantified. From these analyses, the quantity of seed needed to meet past demand can be calculated and can serve as one predictor of future demand.

Other factors to be considered for projecting future demand include (1) an awareness of new threats to crop production, such as recently discovered virulent pathogens or insect pests, (2) a realization that, as collections are better characterized and more thoroughly evaluated and as curators learn more about them, requests should become more highly focused, (3) an evaluation of the role that cores or other special subsets may have in directing and managing demand, and (4) informed forecasts of upcoming changes in germplasm use, such as developments in new crops, electronic communications, large-scale germplasm evaluation programmes, impending retirements of plant breeders, curators, or other significant users, and shifts in national disciplinary priorities.

SETTING PRIORITIES FOR GERMPLASM REGENERATION

Although projections of future demand should guide plans for germplasm regeneration, there are inherent risks in trying to plan for an uncertain future (Bretting & Widrlechner, 1994). Other factors must also be weighed. For example, those accessions that help maximize available genetic diversity may receive high priority. For collections containing core subsets carefully chosen to maximize genetic diversity [see Schoen & Brown (1993) for a discussion of strategies and Erskine & Muehlbauer (1991) and Tohme *et al.* (1995) for two examples], priority can be given to core accessions. Or, if those accessions have already been regenerated, others with novel genotypes or adaptations may be placed first in the regeneration queue. In collections organized by genus or family, diversity might be maximized by regenerating those species or genera most divergent from taxa presently available for distribution.

Another approach somewhat different from maximizing genetic diversity within a collection is to maximize the degree to which collections at various institutions are unique. Genebank holdings for many crops are extensively duplicated among institutions (Williams, 1989; van Hintum & Knüpffer, 1994). If duplicated accessions are readily available from other sources, perhaps they should receive lower priority for regeneration. Between the issues of outright duplication and genetic uniqueness lies a middle ground of institutional overlap in the historical, cultural, and geographical aspects of germplasm and its associated information. It is important to recognize that germplasm is more than just genes or gene products. Cultivated germplasm has a human cultural context, and, especially with traditional societies, so may many wild species. Finally, one must consider whether germplasm accessions with more complete or accurate passport, characterization, and/or evaluation data should be given priority for regeneration over those samples with complete documentation.

One of the most common challenges faced by curators was noted by Deputy Director Iwanaga in his invitation letter to the IPGRI meeting (ICRISAT, December 1995), 'Two key factors that determine the frequency of regeneration are the viability of the accession and quantity of seed held. Which factor predominates when deciding to regenerate the accessions in your genebank?' A small, unscientific poll of curators at five NPGS sites produced three replies that viability and quantity are equally important in a decision to regenerate. In contrast, another response suggested that viability would be the driving factor when low, but that otherwise quantity would be the key factor. From a very different perspective, a curator of genetic stocks indicated that more compelling than either quantity or viability was that regeneration should occur so that the curator 'can observe the mutant traits, otherwise there would be no institutional memory as to how a particular trait behaves.'

Probably there is no single best answer to Dr. Iwanaga's question. Rather, the optimal solution will vary according to the characteristics of the particular accessions managed. Breese (1989) reviewed many of the factors influencing the development of optimal solutions. For example, for crops with highly heterogeneous accessions (often the case with allogamous species), quantity becomes more important, both because of statistical sampling concerns and the need to conserve sufficient numbers of cross-compatible individuals. For crops in which seed deterioration is relatively rapid, unpredictable, or difficult to monitor, viability is more important. When the two factors are considered equally important, it may be useful for management purposes to express seed quantities on a live-seed basis, but I am unaware of any NPGS site that has adopted this approach.

One recurring problem for setting regeneration priorities for original samples by quantity and viability is that original samples are often so small that seeds cannot be sacrificed for viability tests. If viability tests are conducted and the resulting germination-test seedlings serve as plants for regeneration, then there is likely no prioritization. For such cases, non-destructive testing of small seedlots is a crucial topic for future research.

To end this overview of ways to set regeneration priorities, one must consider the challenges created by dynamic constraints and technologies. Curators must weigh the probability of successful regeneration under current protocols against the probable outcomes resulting from new regeneration technologies or by future access to controlled environments or other more optimal growing sites (either *ex situ* or through coordinated *in situ* conservation efforts). No curator should attempt regeneration when the probability of outright failure or drastic selective change is high, if better protocols can be followed in the near future and the seeds are viable and well stored. The success rates of current protocols should be monitored frequently and new protocols compared by their relative success rates standardized by input costs.

REFINING REGENERATION PROTOCOLS

To examine the development of new regeneration protocols, I will cite some examples gleaned from personal experience as Horticulturist at the North Central Regional Plant Introduction Station (NCRPIS). These examples fit into three general areas of applied research: insect pollinators, high-density pot culture, and mating-scheme evaluation, and a fourth area just now emerging: Geographic Information Systems (GIS). These experiences in developing and refining protocols can be applied to many crops and generally rely on widely available technologies.

The NCRPIS focuses on seed regeneration of allogamous crops and their wild relatives; consequently, most of our accessions are highly heterogeneous and heterozygous. Conserving the genetic diversity within such accessions presents challenges more complex than those associated with more homogeneous germplasm. During the late 1970s, the NCRPIS developed a regeneration system primarily for vegetable crops, employing screened cages with specially designed small hives of honey bees (Ellis *et al.*, 1981). Later larger cages were constructed to accommodate wild *Helianthus*. In addition to reducing net cost per regenerated seed relative to those produced by hand pollinations, the cages protect *Cucumis* from beetle-transmitted bacterial wilt. Technical descriptions of these systems can be found in Widrlechner *et al.* (1997). NCRPIS staff and collaborators have tested the system's ability to restrict gene flow (Wilson, 1989), compared seeds produced by various races of honey bees (Wilson & Collison, 1988), and documented improvements in regeneration quantity and quality (Wilson *et al.*, 1991; Widrlechner *et al.*, 1992).

From modest beginnings, the insect-cage regeneration programme has expanded to its present size of about 800 cages per year. During this expansion, NCRPIS staff developed expertise in beekeeping, with particular emphasis on increasing our self-sufficiency in maintaining honey bee colonies (Cox *et al.*, 1996). In recent years, so many honey bee colonies were located on the NCRPIS research farm that local nectar and pollen resources could not maintain the hives, necessitating labour- intensive artificial feeding and offsite bee yards. This has given impetus to a small research project on plants native to the region that produce large quantities of nectar (Ayers &

Widrlechner, 1994). Beyond the inadequacy of local bee forage, other very important limitations to honey bee survival in field cages should be noted. Honey bees are social insects; more than 5000 worker bees are needed for ongoing colony maintenance. This number is much greater than that needed to effect pollination among the 100 or fewer plants in a cage. In addition, many plants with floral morphologies are more suited for pollination by insects other than honey bees.

For all these reasons, other insects, such as flies, bumble bees, and solitary bees, are undergoing evaluation as pollinators in cages (Cox *et al.*, 1996; Widrlechner *et al.*, 1997). In some cases, these may be used in combination with honey bees (Wilson *et al.*, 1991); in others, they may be more efficient substitutes for honey bees (Wilson & Roath, 1992).

The NCRPIS's location at 42°N latitude in a region with a continental climate greatly reduces success rates for field regeneration of plants requiring photoperiods shorter than 12.5 hours to induce flowering. Accordingly, the NCRPIS cooperates with a low-latitude site in Puerto Rico (18°N) to regenerate short-day maize. For short-day amaranths, a protocol has been developed for cultivating large populations at high density in containers under plastic tents in a greenhouse during the short days of winter (Brenner, 1993; Williams & Brenner, 1995). The advantages of pot culture in germplasm regeneration and evaluation are often overlooked (Spoor & Simmonds, 1993). This protocol's applicability to small, rapidly flowering plants with autogamous or mixed mating systems is now being tested. Such a greenhouse regeneration programme can facilitate more complete seasonal use of structures primarily designed for other purposes, e.g. starting seedlings for field plots or conducting experiments under longer photoperiods.

Many of the maize accessions that can be regenerated under field conditions in Iowa are heterogeneous landraces that require large populations and well-designed mating schemes for hand pollination. Various mating schemes have been proposed and their genetic consequences theoretically tested (Crossa *et al.*, 1994). A doctoral candidate at Iowa State University has been deploying isozymes to track changes in gene frequency and population structure in maize accessions after they had been subjected to various mating schemes. When combined with practical information about time and labour investments, it may be feasible to apply his results to determine the most cost-effective protocols for conserving diversity in maize landraces.

GIS are rapidly gaining prominence as tools to manipulate complex, site-specific datasets. Wild plants, weeds, and landraces have all evolved in response to ecogeographic variables and such accessions can be linked to pertinent environmental data through GIS. Evaluation data from modern varieties are also collected under well-characterized environments at defined locations. Some applications of GIS for refining plant exploration and increasing the potential value of collections have recently been outlined by Guarino (1995). Knowledge about the climatic and edaphic determinants of plant performance can also refine targets for future exploration (Widrlechner, 1994) and help match germplasm more appropriately to requests from geographically diverse areas (Pollak & Corbett, 1993). Perhaps curators will soon use GIS to develop models for co-ordinating field regenerations among multiple locations in national or international networks. In 1995, the NPGS formed an *ad hoc* committee to examine how GIS could assist germplasm managers and to design prototype applications. Applications of GIS to regeneration management will probably be unimportant until a greater proportion of verified accession locality data are incorporated into our national database, the Germplasm Resources Information Network (GRIN).

REFINING POST-HARVEST SEED-MANAGEMENT PROTOCOLS

The timing of harvest, the interval between harvest and storage, and the methods for cleaning and preparing seeds for storage can all influence seed quality and longevity (Clark *et al.*, 1997). Protocols for seed drying and vigour testing are widely studied within the discipline of seed science. But many of these studies examine only the seeds of modern commercial varieties of the world's major crops. The seeds with orthodox storage characteristics that present the greatest managerial difficulties are those which often have received the least attention in seed science research. Heterogeneous landraces and semi-domesticated taxa pose special impediments for seed science research and for developing post-harvest protocols that produce high quality samples without decreasing genetic variation. Landraces may vary widely within populations for seed size, shape, density, and dormancy characteristics. Seeds of wild taxa may be even more problematic. Curators at the NCRPIS manage genera, such as *Chamaebatiaria*, *Holodiscus*, and *Spiraea* (Rosaceae), *Jamesia* (Saxifragaceae), and *Tridens* (Poaceae), in which the visual recognition of individual seeds can be very difficult even under 10 × magnification, and other genera in the Lamiaceae and Caryophyllaceae with seeds so small that they pass through the finest seed-cleaning screens.

These limitations to basic research and more applied post-harvest protocols also apply to published seed testing standards, which are often based on experiments with commercial seedlots. The Handbook of Seed Technology for Genebanks (Ellis *et al.*, 1985), a rich assemblage of data and general advice, presents strategies for both post-harvest handling and viability testing. But for many taxa, Ellis *et al.* (1985) rely heavily on national and international standards and present perhaps too little information or advice on ways to cope with variability within and among accessions.

CROP-SPECIFIC CURATORS AND SOME CRITICAL MANAGERIAL ISSUES

Many of the accomplishments of the NCRPIS result from actions begun about 15 years ago by Dr. Raymond Clark, at that time the Station's Research Leader/ Coordinator, with the support of the NC-7 Regional Technical Advisory Committee, to develop a team of crop-specific curators. Today the NCRPIS's team includes six full-time curators and myself, with part-time curatorial responsibility for certain ornamental genera, collectively comprising over 60 years of curatorial experience. Curatorial responsibilities are organized by genera grouped into crop categories, such as vegetables, pseudocereals, and forage legumes. This is consistent with a national system that divides responsibility taxonomically among sites and that receives advice from a network of 40 Crop Germplasm Committees organized by a combination of end-use and taxonomic groupings (Clark *et al.*, 1997). In this way, the subtleties of diversity within particular crops and their user communities can be learned and harnessed to produce better seeds and to meet the needs of those who request samples. Without a crop-specific focus, it is difficult to imagine how this plethora of information could be organized or how managers could develop a high degree of specialized expertise, especially related to the intricacies of regeneration.

Regenerations, post-harvest processing, and initial viability testing may best be entrusted to crop-specific curators, who, with experience, are best qualified to recognize differences among accessions and to work with other experts to develop or refine suitable protocols. Crop-specific curators removed from day-to-day regeneration management are likely to have less understanding of practical constraints. And, conversely, regeneration experts without a crop focus would be unlikely to relate their experiences to patterns of genetic variation or adaptation within taxa or to communicate as effectively with the user community. Ideally, networks of crop-specific curators should form to foster rapid and frequent exchange of curatorial observations and strategies. Perhaps they could be organized like the working groups of the European Cooperative Programme for Crop Genetic Resources Networks, or more informal groups linked by the Internet.

Finally, brief mention should be made of some critical research areas directly related to regeneration management, along with a few other important issues raised by NPGS colleagues. Germplasm demand and germplasm regeneration should be linked. It is obvious that patterns of demand among collections vary widely, and they can be expected to be dynamic. But very few analytical tools for assessing demand or projecting future demand have been widely disseminated or empirically tested. Can IPGRI help develop such analytical tools and/or convene working groups of curators and others best able to forecast future trends in plant science research and crop improvement?

Because protocols to balance factors, such as seed quantity and quality or the number of accessions regenerated and population size, are greatly influenced by patterns of genetic diversity, breeding systems, seed longevity, and regeneration conditions, any such protocols must be crop-specific. It is unlikely that much progress can be made on these topics by following general prescriptions, but perhaps, just as the International Board for Plant Genetic Resources (IBPGR) sponsored the development of descriptor lists, IPGRI might consider similar crop-specific examinations of regeneration issues. As mentioned earlier, related to the development of crop-specific protocols is the need for non-destructive viability testing of small samples.

On so many levels, from breeding biology to seed physiology, lack of information about the inherent characteristics of wild and weedy taxa is reducing the efficacy of regeneration programmes. The potential value of secondary and tertiary gene pools for crop improvement is increasing through developments in genetic transformation, somatic hybridization, and other biotechnologies. Thus, wild and weedy crop relatives deserve increased attention for basic and applied research into optimal seed propagation.

Biotechnological advances have made many classes of molecular genetic markers increasingly available. Genetic markers are proven tools for documenting trueness to type and other changes in populations during the course of regeneration (Bretting & Widrlechner, 1995). As new classes of markers are characterized and as the relative costs of deploying various markers change, who will translate these developments to the best advantage of curators? Before leaving the subject of biotechnology, one might also speculate as to its role in rescuing low-viability samples, either through regenerating intact plants or by capturing genetic information without direct regeneration.

All of these lines of research will have greater influence if institutions can collaborate to foster the discipline of germplasm conservation by educating an expanding corps of crop-specific curators. Ultimately, the investment in curators should produce the highest returns, for it is through their practical experience and scientific judgement that research results can best be applied.

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REFERENCES

Anonymous. (1992) Crop Advisory Committee chairs meet at Beltsville research center. *Diversity* **8**(3), 26.

Ayers, G.S. & Widrlechner, M.P. (1994) The genus *Agastache* as bee forage: A historical perspective. *American Bee Journal* **134,** 341–348.

Breese, E.L. (1989) *Regeneration and multiplication of germplasm resources in seed genebanks: The scientific background.* IBPGR, Rome.

Brenner, D. (1993) Amaranth seed regeneration in a greenhouse. *NPGS Research Workshop Abstracts.* p. 20. Fort Collins, CO.

Bretting, P.K. & Widrlechner, M.P. (1994) Managing risk and change for plant germplasm management. Unpublished presentation given at the 1994 annual meeting of the Crop Science Society of America. *Agronomy Abstracts* **1994,** 220.

Bretting, P.K. & Widrlechner, M.P. (1995) Genetic markers and plant genetic resource management. *Plant Breeding Reviews* **13,** 11–86.

Clark, R.L., Shands, H.L., Bretting, P.K., & Eberhart, S.A. (1997) Germplasm regeneration: Developments in population genetics and their implications. *Crop Science* **37,** 1–6.

Cox, R.L., Abel, C., & Gustafson, E. (1996) A novel use of bees: Controlled pollination of germplasm collections. *American Bee Journal* **136,** 709–712.

Crossa, J., Taba, S., Eberhart, S.A., Bretting, P., & Vencovsky, R. (1994) Practical considerations for maintaining germplasm in maize. *Theoretical & Applied Genetics* **89,** 89–95.

Ellis, M.D., Jackson, G.S., Skrdla, W.H., & Spencer, H.C. (1981) Use of honey bees for controlled interpollination of plant germplasm collections. *HortScience* **16**, 488–491.

Ellis, R.H., Hong, T.D., & Roberts, E.H. (1985) *Handbook of Seed Technology for Genebanks.* Volumes 1 and 2. IBPGR, Rome.

Erskine, W. & Muehlbauer, F.J. (1991) Allozyme and morphological variability: outcrossing rate and core collection formation in lentil germplasm. *Theoretical & Applied Genetics* **83,** 119–125.

Guarino, L. (1995) Geographic information systems and remote sensing for plant germplasm collectors. In: (Eds. L. Guarino, V.R. Rao, & R. Reid) *Collecting Plant Genetic Diversity Technical Guidelines.* pp. 315–328. CAB International, Wallingford, United Kingdom.

McFerson, J.R., Lamboy, W.F., & Kresovich, S. (1996) Assessing user perceptions of genetic resource collections in crucifer crops. *Crop Science* **36,** 831–838.

Pollak, L.M. & Corbett, J.D. (1993) Using GIS datasets to classify maize-growing regions in Mexico and Central America. *Agronomy Journal* **85,** 1133–1139.

Schoen, D.J. & Brown, A.H.D. (1993) Conservation of allelic richness in wild crop relatives is aided by assessment of genetic markers. *Proceedings of the National Academy of Science (USA)* **90,** 10623–10627.

Spoor, W. & Simmonds, N.W. (1993) Pot trials as an adjunct to cereal breeding and evaluation of genetic resources. *Field Crops Research* **35,** 205–213.

Tohme, J., Jones, P., Beebe, S., & Iwanaga, M. (1995) The combined use of agroecological and characterisation data to establish the CIAT *Phaseolus vulgaris* core collection. In: (Eds. T. Hodgkin, A.H.D. Brown, T.J.L. van Hintum, & E.A.V. Morales) *Core Collections of Plant Genetic Resources.* pp. 95–108. John Wiley & Sons, Chichester, United Kingdom.

van Hintum, T.J.L. & Knüpffer, H. (1994) Duplication within and between germplasm collections. I. Identification of duplication on the basis of passport data. *Genetic Resources & Crop Evolution* **42,** 127–133.

Widrlechner, M.P. (1994) Environmental analogs in the search for stress- tolerant landscape plants. *Journal of Arboriculture* **20,** 114–119.

Widrlechner, M.P. (1995) A new look at prairie plant germplasm. In: (Ed. D.C. Hartnett) *Prairie Biodiversity: Proceedings of the 14th North American Prairie Conference.* pp. 207–210. Kansas State Univ. Press, Manhattan, KS.

Widrlechner, M.P., Knerr, L.D., Staub, J.E., & Reitsma, K.R. (1992) Biochemical evaluation of germplasm regeneration methods for cucumber, *Cucumis sativus* L. *FAO/IBPGR Plant Genetic Resources Newsletter* **88/89,** 1–4.

Widrlechner, M.P., Abel, C.A., & Wilson, R.L. (1997) Ornamental seed production in field cages with insect pollinators. *Combined Proceedings of the International Plant Propagators' Society* **46**(1996), 512–516.

Williams, J.T. (1989) Plant germplasm preservation: A global perspective. In: (Eds. L. Knutson and A.K. Stoner) *Biotic Diversity and Germplasm Preservation, Global Imperatives.* pp. 81–96. Kluwer Academic, Dordrecht, Netherlands.

Williams, J.T. & Brenner, D. (1995) Grain amaranth (*Amaranthus* sp.). In: (Ed. J.T. Williams) *Cereals and Pseudocereals.* pp. 129–186. Chapman & Hall, London.

Wilson, R.L. (1989) Minimizing extraneous transfer of sunflower pollen by honey bees (Hymenoptera:

Apidae) in field cages. *Journal of the Kansas Entomological Society* **62**, 387–391.

Wilson, R.L. & Collison, V.L. (1988) Field cage study of the effects of four honey bee strains and hand pollination on the seed of a wild sunflower. *Seed Science & Technology* **16**, 471–475.

Wilson, R.L. & Roath, W.W. (1992) Potential of three lepidopteran species as pests and three hymenopteran species as pollinators of *Cuphea*. *Journal of the Kansas Entomological Society* **65**, 316–320.

Wilson, R.L., Widrlechner, M.P., & Reitsma, K.R. (1991) Pollination methods for maintaining carrot germplasm collections. *FAO/IBPGR Plant Genetic Resources Newsletter* **85**, 1–3.