

# Conservation of DNA: DNA Banking

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## 7.1. Introduction

The loss of native plant diversity also means a loss of genetic diversity present in and available to our current and potential crop species. Cultivated crops are extremely inbred for factors such as yield, uniform flowering and height, and cosmetic features of products. This narrow genetic base has resulted in several disastrous crop failures. Ireland's potato famine of 1846, which resulted in the emigration of a quarter of the country's human population, occurred because the potato crop had no resistance to the late blight fungus, *Phytophthora infestans* (Mont.) DeBary (Plucknett *et al.*, 1987). This can be traced to the lack of genetic diversity in Irish potatoes, which had been multiplied using clonal materials from just two separate South American introductions, to Spain in 1570 and to England in 1590 (Hawkes, 1983).

A more recent example is the epidemic of southern corn leaf blight caused by the fungus *Helminthosporium maydis* Nisik. & Miy., in 1970 in the USA. Because almost all of the corn (maize: *Zea mays* L.) in the USA was of hybrid origin and contained the Texas cytoplasmic male sterile line, the fields of corn presented an unlimited extremely narrow gene base habitat for the fungus. By the late summer, 1970, plant breeders were scouring corn germplasm collections in Argentina, Hungary, Yugoslavia and the USA for resistant sources (Plucknett *et al.*, 1987). Nurseries and seed fields were used in Hawaii, Florida, the Caribbean, and Central and South America to incorporate the resistance into hybrid corn in time for planting in the spring of 1971 (Ullstrup, 1972). Without these genetic resources this technological feat would not have been possible.

Since the first plant-to-plant gene transfer in 1983 (Murai *et al.*, 1983), genes have been transferred to plants from viruses (Nelson *et al.*, 1988), bacteria (Barton *et al.*, 1987; Della-Cioppa *et al.*, 1987; Fischhoff *et al.*, 1987), and even from mammals to plants (Lefebvre *et al.*, 1987; Maiti *et al.*, 1988). Genetic transfers are being performed in order to attain insect, bacterial, viral and fungal resistance, a more nutritionally balanced protein, more efficient photosynthesis, nitrogen fixation, and salt and heavy metal tolerance, to name a few. These kinds of gene transfers from one unrelated organism to another indicate that we must now view the world's genetic resources (i.e. genes, or DNA) from a horizontal perspective in which gene transfers will cut across species, genus and family boundaries.

For example, a strain of cowpea (*Vigna unguiculata* (L.) Walp.) discovered in a market in Ilorin, Nigeria, contains a protein that inhibits trypsin digestion by insects (Redden *et al.*, 1984). This gene has been moved to tobacco (*Nicotiana*) where the trypsin-inhibiting gene is expressed and offers tobacco the same resistance against insects as in cowpea (Newmark, 1987). It is interesting to note that although a very active form of the gene has been found in a Nigerian cowpea, scarcely 100 of the world's 13 000 legume species have been examined for this gene. Yet the tropical legumes, one of the most promising groups for the evolution of natural insecticides, will certainly be subject to considerable germplasm loss in the next decade.

The novel insecticides, biocides, medicines, etc. that may exist in nature are innumerable. Yet the principal areas of diversity among plants, the lowland tropical forests, will have been felled or severely damaged within the next 20 years (Raven, 1988). The Amazon River system, for example, contains eight times as many species as the Mississippi River system (Shulman, 1986). Raven (1988) estimated that as many as 1.2 million species would become extinct in the next 20 years. The loss of plant species will mean a loss of potential plant-derived pharmaceuticals, estimated at \$US2 billion/year in the USA alone (US Congress, 1987).

The National Cancer Institute (NCI) has spent \$US8 million in the last five years for a massive plant-collecting effort in the tropics to find anti-cancer and anti-AIDS virus compounds (Booth, 1987). The plant collectors will gather leaves and/or bark and air-dry the material for shipment to NCI where it will be extracted and assayed against 100 cancer cell lines and the AIDS virus. Yet, *no* genetic resources are being collected! When a promising compound is found, the plants will have to be recollected. For extensive testing (as well as commercial utilization), plantations will have to be established in the tropics to provide material.

## 7.2. Initiation of DNA Bank-Net

Concurrent with the advancements in gene cloning and transfer has been the development of technology for the removal and analysis of DNA. DNAs from the nucleus, mitochondrion and chloroplast are now routinely extracted and immobilized onto nitrocellulose sheets where the DNA can be probed with numerous cloned genes. In addition, the rapid development of polymerase chain reaction (PCR) now means that one can routinely amplify specific oligonucleotides or genes from the entire mixture of genomic DNA. These advances, coupled with the prospect of the loss of significant plant genetic resources throughout the world, have led to the establishment of DNA Bank-Net, an international network of DNA repositories for the storage of genomic DNA.

The organizational meeting involved a group of 18 scientists who met at the Royal Botanic Gardens, Kew, London, in April 1991, to share national and institutional experiences using *in vitro* biotechnology and particularly cryostorage of DNA and DNA-rich materials (Adams and Adams, 1991). A second meeting was held at the Missouri Botanical Garden in 1993 (Adams *et al.*, 1994). Relatively few scientists were interested in a 'genetic insurance policy' when the idea of banking genomic DNA from plants was first proposed (Adams, 1988, 1990). However, currently there are 40 institutions, representing 25 nations and every inhabited continent, that have expressed interest in DNA Bank-Net (Fig. 7.1).

The conserved DNA will have numerous uses: molecular phylogenetics and systematics of extant and extinct taxa; production of previously characterized secondary compounds in transgenic cell cultures; production of transgenic plants using genes from gene families; *in vitro* expression

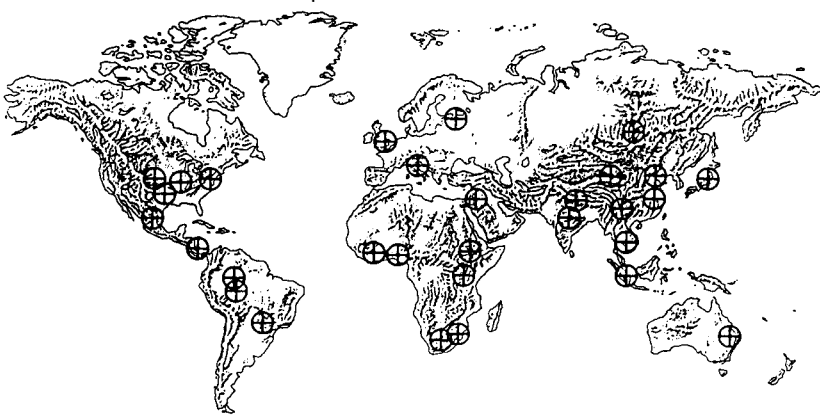


Fig. 7.1. Map of individuals/institutions currently interested in DNA Bank-Net.

and study of enzyme structure and function; and genomic probes for research laboratories.

### 7.3. Structure and Operation of DNA Bank-Net

At the organizational meeting of DNA Bank-Net, a task force was convened to define the functions of working (DNA-dispensing) and reserve (base) nodes in the DNA Bank network. The group recommended the following functions (Adams and Adams, 1991):

*Working (DNA-dispensing) nodes:*

1. Collection of plant material by taxonomists. This may be the primary function of a particular node or be in association with other organizations such as universities, botanic gardens, etc.
2. DNA extraction by molecular biologists or trained staff.
3. Long-term preservation of DNA-rich materials and/or extracted DNA in liquid nitrogen.
4. DNA analysis/gene replication by molecular biologists or trained staff.
5. Distribution of DNA (genes, gene segments, oligonucleotides, etc.).

*Reserve (base) nodes:*

1. Long-term DNA preservation in liquid nitrogen and monitoring of potential DNA degradation.
2. Act as genetic reserve buffer for working nodes.
3. Replenishment of DNA if a working node experiences the catastrophic loss of storage parameters and DNA.

Figure 7.2 depicts the relationship between working and reserve nodes. Note the projected flow of plant materials and DNA through the working (DNA-dispensing) node. It is likely that some of the working nodes would be actively acquiring and/or dispensing DNA from some geographic area (e.g. Africa), yet maintain separate cryovats, functioning as a reserve (base) node for another area (e.g. South America).

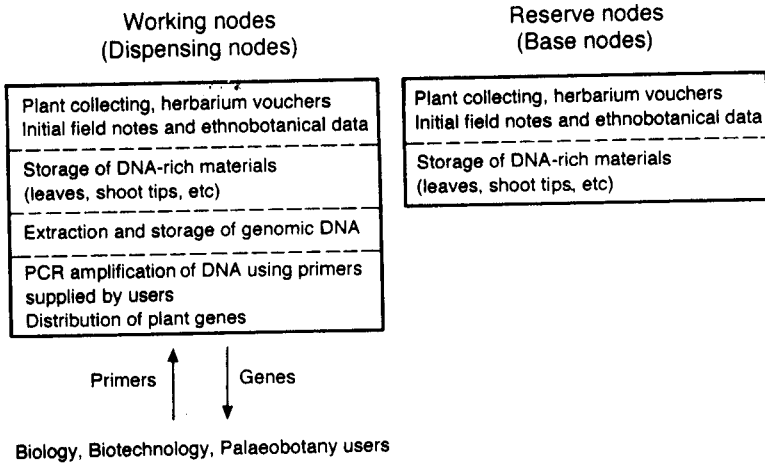
### 7.4. General Requirements for Nodes in the DNA Bank-Net

The task group recommended (Adams and Adams, 1991) that the following were the minimum requirements for nodes:

*Working (DNA dispensing) nodes:*

- Personnel: taxonomists/collectors, biochemists/molecular biologists, technicians for practical work, capable administration.
- Equipment: storage facilities (liquid nitrogen, cryovats), extraction

## DNA Bank-Net nodes



**Fig. 7.2.** Schematic representation of the flow of materials and the relationship between working (DNA-dispensing), reserve (base) nodes and users.

facilities (centrifuges, gel electrophoresis, UV spectrophotometer, etc.), DNA analyses and PCR duplication (PCR thermal cycler, microcentrifuges, etc.), distribution system (packaging and mailing supplies), computer (database for inventory and correspondence).

*Reserve (base) nodes:*

- Personnel: technicians, capable administration.
- Equipment: storage facilities (liquid nitrogen, cryovats), computer (data base for inventory and correspondence).

Each DNA collection should be split initially into at least two or three portions. One sample (DNA-rich material or extracted DNA) should be stored at a working (DNA-dispensing) node and another portion(s) should be stored in one, but preferably two, back-up reserve (base) nodes. The reserve nodes should be in different countries and if possible on different continents to safeguard the DNA samples against various natural and man-made catastrophes. An example is shown in Fig. 7.3, where three replicate samples are collected and taken to Medellin, with replicates then sent to the Vavilov Institute and the Missouri Botanical Garden. Plant materials (in silica gel) could be stored in a freezer until the identification and other documentation have been accomplished and then shipped in quantity with other samples in off-season periods. No doubt other strategies will be developed with experience.



**Fig. 7.3.** Hypothetical example of a triplicate collection in Colombia. The DNA-rich materials are placed in silica gel or Drierite for interim preservation and taken to the working node (A, Medellin). From Medellin (A), two replicates would be sent to reserve nodes at (B) Vavilov Institute and (C) the Missouri Botanical Garden.

Several general recommendations came from the task groups (Adams and Adams, 1991) and these include:

1. DNA should be extracted from cryopreserved DNA-rich materials only when the DNA is needed. Delaying the extraction has the advantage of letting technology catch up, so advanced techniques can be used as they become available.
2. Working nodes should generally be an existing organization with adequate biochemical expertise and an associated herbarium. Although on-site herbarium is not required, a very close, local (same city) association with a recognized herbarium (Holmgren *et al.*, 1990) is required.
3. For the working as well as reserve nodes, it is necessary to have a strong institutional commitment, not just a personal commitment, in order that the collection be maintained in perpetuity and not just for the lifetime of one person who has committed himself/herself to the idea.
4. Consideration should be given to the availability of dependable supplies of electricity and liquid nitrogen in determining the feasibility of establishing a node.
5. Considerable interest was shown in the concept of storing composite DNA samples (e.g. a composite of DNA from all the legumes in a region, to be used for screening or retrieval of unusual genes).
6. The need for computer and database compatibility was expressed. Given the number of flat file and relational databases that are compatible with dBASE, it would seem that dBASE compatibility would be desirable. No consensus was reached in regard to this or on the use of a flat file

versus relational database. It was felt that the critical issue at present was to begin collecting DNA-rich materials.

## 7.5. Scope of Plant Collections

The task group given this assignment felt that there is a need for an initial focus rather than random collections and that economically useful plants should be given some priority (Adams and Adams, 1991). However, this priority would not include the major crop plants of commercial usage that are widely cultivated (e.g. maize, rice, wheat, etc.), but rather those indigenous species that are tended and/or otherwise used by local people.

One problem with giving a priority to species is that field collecting then becomes 'plant hunting' trips, which tend to be very expensive. It would seem that the cheapest and most practical way to preserve the largest percentage of plant genes would be to utilize the current (and additional) floristic collectors (such as those of the Missouri Botanical Garden, Royal Botanic Gardens, etc.), who are already in the field and are familiar with vegetation of the region. The collections of DNA-rich material (leaves) could be done with little additional effort when specimens are collected.

## 7.6. Collecting Procedures

DNA collectors should be considered the same as all other plant collectors. Consequently they should (Adams and Adams, 1991):

1. Voucher all collections in recognized herbaria (i.e. ones listed in Index Herbariorum, 8th edn; see Holmgren *et al.*, 1990).
2. Provide proper label information as to the locality, habitat, etc. for each plant collected.
3. Follow all procedures concerning permits, convenios, and deposition of duplicate vouchers in the country of origin.
4. Collect leaf samples and pack them in desiccants (see Adams *et al.*, 1991) immediately (the same day). Leaves themselves provide simple long-term storage.
5. In the case of legumes, samples of root nodules should be taken if possible, but kept as a separate accession.
6. If a chemical treatment is used in the field, information should be provided concerning the method, and some untreated leaves must be stored in desiccant (see no. 4 above).
7. When possible, fossil material should be included in DNA Bank-Net. In this case, when destruction of the source material occurs, documentation via photographs and fragments is necessary.

8. Some material may be accessioned from herbarium specimens under control of local curators using current methods of DNA extraction. Herbarium sheets should be marked if sampled for DNA. Herbarium specimens are limited in supply and their utility appears to be limited to material collected without chemical preservation. Material may be sampled directly from the sheet or the attached specimen envelope if it contains sufficient leaf material ( $\approx 0.1\text{--}0.5$  g dry wt.) for DNA extraction.

### 7.7. Interim Field Storage of Specimens

The problems associated with transporting fresh or frozen materials can generally be overcome by specialists (e.g. the worldwide collections of fresh foliage of *Juniperus* for analysis of essential oils and DNA undertaken by the author). However, botanists doing floristic research will likely collect many of the specimens from rare and endangered tropical species. They often collect specimens from scores of different species in a single day. The sheer bulk of the materials that they have to process and ship requires that any protocol for the collection of samples for specialized needs (e.g. DNA storage/analyses) must be quick, simple and trouble-free. The generalist collector, working in tropical areas, cannot be expected to preserve hundreds or thousands of samples for months under tropical conditions and then arrange transport through customs, all the while keeping the individual specimens frozen.

Fortunately, at least as far as DNA preservation is concerned, interim preservation in silica gel or Drierite is an effective way to keep plant materials in the field and/or in transit for several months at ambient temperatures (Adams *et al.*, 1991).

### 7.8. Protocol for Field Preservation of Foliage

Drierite has a water capacity of 10 to 14%, but above 6.6% the capacity varies inversely with temperature (W.A. Hammond, Drierite Co., personal communication). One would not want to risk possible rehydration of leaves, so storage ratios should be based on the 6.6% capacity. In laboratory tests, silica gel absorbed 8.85% of its weight of water after exposure to 100% humidity for 16 h at 22°C (Adams *et al.*, 1991). We have found that plant materials contain as much as 92% moisture (Adams *et al.*, 1991), so a useful approximation would be to assume the plant is mostly water and use 16 to 20 times the fresh leaf weight for the Drierite or silica gel component.

Now that inexpensive (\$US100) battery-powered, portable balances are available, one could take a supply of jars that hold, e.g., 100 g of silica



gel and then weigh out 5 g of fresh leaf material and add it to the jar along with silica gel (or Drierite). We have found that air-dried leaves (suitable for herbarium vouchers) generally contain 10 to 15% water. Using a robust value of 20% water for air-dried leaves, one can weigh out 5 g of these leaves ( $5 \text{ g} \times 20\% = 1 \text{ g water}$ ) or 1 g fresh leaves per 20 g of silica gel. This procedure may seem time consuming, but in practice we merely do a quick check on the leaf area needed to give approximately 1 g (fresh leaves) or 5 g (dried leaves) and then just use that amount of leaf area. For example, for spinach, a 2 cm  $\times$  4 cm fresh leaf area weighs about 1 g. So, one can just cut the leaves into roughly 2 cm  $\times$  4 cm squares and add one square to 20 g of silica gel. For succulent leaves, a slightly different protocol may be used. Liston *et al.* (1990) removed succulent leaf material after 24 h in Drierite and placed it in fresh Drierite.

A note of caution is necessary concerning field drying of specimens for subsequent silica gel/Drierite storage. We have experienced difficulty obtaining DNA from leaves dried at temperatures higher than about 55°C. In very rainy conditions where high drying temperatures (from butane stoves, for example) are used to dry specimens, it would seem advisable to merely blot leaves free of surface moisture and then place the fresh leaf material directly into silica gel or Drierite. Liston *et al.* (1990) took 2–5 g of plant tissue and wrapped it in tissue paper to prevent it from fragmenting, then placed it in a 125 ml Nalgene bottle, one-third prefilled with Drierite (with blue indicator crystals), and then filled the bottle (two-thirds) with additional Drierite.

Clear plastic bottles are probably preferable to glass, to avoid breakage in transit. Using clear jars allows one to check the indicating crystals without opening the jar. The lids should be sealed with vinyl tape to prevent moisture leakage. The use of parafilm to seal containers is not recommended, as we have found it to come loose at 37°C (and of course, at tropical temperatures!).

Silica gel and Drierite do differ in one characteristic that may be a consideration. We have found that silica gel can be dried (recharged) at 100°C for 24 h, but Drierite must be dried at much a higher temperature (200°C). In addition, we could easily dry (recharge) silica gel, but were unable to dry (recharge) Drierite in a microwave oven. If the desiccant gets wet before use, silica gel appears to be much easier to dry. Silica gel is used in large quantities for flower drying and, thus, may be cheaper, depending on the source. Both Drierite and silica gel could be recharged for reuse on subsequent trips, but one should be very careful to remove any leaf fragments. If materials are to be checked through customs, it is useful to have a small container of silica gel/Drierite that you can open and show customs agents. A demonstration that the blue indicator crystals will turn pink when you breathe on or moisten them is helpful in convincing the customs officials *not* to open your sealed specimen jars.

## 7.9. Future Considerations

The vast resources of dried specimens in the world's herbaria may hold considerable DNA that would be suitable for polymerase chain reaction (PCR). It seems likely that the integrity of DNA would decrease with the age of specimens. Because there are many types of herbarium storage environments, preservation and collections, there is a need for systematic investigations of the effect of modes of preparation, collection and storage on the integrity of DNA in the world's major holdings.

One of the major concerns in storing DNA from extinct species is the limited amount of DNA available for distribution. A general process is needed by which the DNA could be immobilized, and then specific genes or oligonucleotides amplified. Genomic DNA immobilized onto nylon might be used, as described by Kadokami and Lewis (1990) for cDNA from spiders. Amplification would then involve removing the membrane with the bound DNA from cryostorage and amplifying the desired gene, washing away the primers and placing the bound DNA back into cryostorage. Although Kadokami and Lewis (1990) reported successful PCR amplification of membrane-bound cDNA, we have not been able to extend their work to genomic plant DNA. Additional research is needed in this area.

Research is also needed to amplify the entire genomic DNA of a species. Some modification of the GAWTS type (Genomic Amplification with Transcript Sequencing; Sommer *et al.*, 1990) protocol needs to be developed for eventual supplementation of DNA reserve stocks and to obviate the need for replenishment from outside sources.

Technical workshops need to be conducted in order to bring experts together and develop specific techniques and protocols for DNA extraction, amplification and storage.

Institutions in developing countries continue to report difficulties in obtaining liquid nitrogen (both supplies and sustained funds). There is a need for the development of DNA storage at ambient temperatures. These procedures would also be of great benefit when a freezer is lost or liquid nitrogen is not available for brief periods.

Funding for initial start-up is modest for the reserve nodes but would require substantial funds for working nodes. Working nodes will need to be added to an existing molecular biology laboratory. Operating funds would be modest for a reserve node, depending on the cost of liquid nitrogen. Several international funding organizations may consider support for the establishment of nodes, but funding for operations will likely need to be borne by the institution or national government.

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## References

- Adams, R.P. (1988) The preservation of genomic DNA: DNA Bank-Net. *American Journal of Botany* 75, 156 (abst., suppl.).
- Adams, R.P. (1990) The preservation of Chihuahuan plant genomes through *in vitro* biotechnology: DNA Bank-Net, a genetic insurance policy. In: Powell, A.M., Hollander, R.R., Barlow, J.C., McGillivray, W.B. and Schmidly, D.J. (eds) *Third Symposium on Resources of the Chihuahuan Desert Region*. Printech Press, Lubbock, TX, pp. 1–9.
- Adams, R.P. and Adams, J.E. (eds) (1991) *Conservation of Plant Genes: DNA Banking and In Vitro Biotechnology*. Academic Press, New York.
- Adams, R.P., Do, N. and Chu Ge-lin (1991) Preservation of DNA in plant specimens by desiccation. In: Adams, R.P. and Adams, J.E. (eds) *Conservation of Plant Genes: DNA Banking and In Vitro Biotechnology*. Academic Press, New York, pp. 135–152.
- Adams, R.P., Miller, J.S., Golenberg, E.M. and Adams, J.E. (1994) *Conservation of Plant Genes II: Utilization of Ancient and Modern DNA*. Missouri Botanical Garden Press, St Louis, MI, 276 pp.
- Barton, K.A., Whiteley, H.R. and Yang, N. (1987) *Bacillus thuringiensis*-endotoxin expressed in transgenic *Nicotiana tabacum* provides resistance to Lepidopteran insects. *Plant Physiology* 85, 1103–1109.
- Booth, W. (1987) Combining the earth for cures to cancer, AIDS. *Science* 237, 969–970.
- Della-Cioppa, G., Bauer, S.C., Taylor, M.L., Rochester, D.E., Klein, B.K., Shah, D.M., Fraley, R.T. and Kishore, G.M. (1987) Targeting a herbicide resistant enzyme from *Escherichia coli* to chloroplasts of higher plants. *Bio/Technology* 5, 578–584.
- Fischhoff, D.A., Bowdish, K.S., Perlak, F.J., Marrone, P.G., McCormick, S. M., Niedermeyer, J.G., Dean, D.A., Kusano-Kretzmer, K., Mayer, E.J., Rochester, D.E., Rogers, S.G. and Fraley, R.T. (1987) Insect tolerant transgenic tomato plants. *Bio/Technology* 5, 807–813.
- Hawkes, J.G. (1983) *The Diversity of Crop Plants*. Harvard University Press, Cambridge, MA, 184 pp.
- Holmgren, P.K., Holmgren, N.H. and Barnett, L.C. (1990) Index herbariorum. Part I: The herbaria of the world, 8th edn. *Regnum Vegetabile* 120, 1–693.
- Kadokami, Y. and Lewis, R.V. (1990) Membrane bound PCR. *Nucleic Acids Research* 18, 3082.
- Lefebvre, D.D., Miki, B.L. and Laiberte, J.F. (1987) Mammalian metallothionein functions in plants. *Bio/Technology* 5, 1054–1056.
- Liston, A., Rieseberg, L.H., Adams, R.P., Do, N. and Zhu, Ge-lin. (1990) A method for collecting dried plant specimens for DNA and isozymes analyses, and the

- results of a field test in Xinjiang, China. *Annals of the Missouri Botanical Garden* 77, 859-863.
- Maiti, I.B., Hunt, A.G. and Wagner, G.J. (1988) Seed-transmissible expression of mammalian metallothionein in transgenic tobacco. *Biochemical and Biophysical Research Communications* 150, 640-647.
- Murai, N., Sutton, D.W., Murray, M.G., Slightom, J.L., Merlo, D.J., Reichert, N.A., Sengupta-Gopalan, C., Stock, S.A., Barker, R.J., Kemp, J.D. and Hall, T.C. (1983) Phaseolin gene from bean is expressed after transfer to sunflower via tumor-inducing plasmid vectors. *Science* 222, 476-482.
- Nelson, R.S., McCormick, S.M., Delannay, X., Dube, P., Layton, J., Anderson, E.J., Kaniewska, M., Proksch, R.K., Horsch, R.B., Rogers, S. G., Farley, R.T. and Beachy, R.N. (1988) Virus tolerance, plant growth, and field performance of transgenic tomato plants expressing coat protein from tobacco mosaic virus. *Bio/Technology* 6, 403-409.
- Newmark, P. (1987) Trypsin inhibitor confers pest resistance. *Bio/Technology* 5, 426.
- Plucknett, D.L., Smith, N.J.H., Williams, J.T. and Anishetty, N.M. (1987) *Gene Banks and the World's Food*. Princeton University Press, Princeton, NJ.
- Raven, P.H. (1988) Tropical floristics tomorrow. *Taxon* 37, 549-560.
- Redden, R.J., Singh, S.R. and Luckefahr, M.J. (1984) Breeding for cowpea resistance to Bruchids at IITA. *Protection Ecology* 7, 291-303.
- Shulman, S. (1986) Seeds of controversy. *BioScience* 36, 647-651.
- Sommer, S.S., Sarkar, G., Koeberl, D.D., Bottema, C.D.K., Buerstedde, J-M., Schowalter, D.B. and Cassady, J.D. (1990) Direct sequencing with the aid of phage promoters. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White, T.J. (eds) *PCR Protocols*. Academic Press, San Diego, CA, pp. 197-205.
- Ullstrup, A.J. (1972) The impacts of the southern corn leaf blight epidemics of 1970-1971. *Annual Review of Phytopathology* 10, 37-50.
- US Congress (1987) *Technologies to Maintain Biological Diversity*. Office of Technology Assessment, Washington DC.