Gene transfer in plants

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Abstract With the specter of dwindling biotic diversity, need is increased for preservation of available germplasm. Plant germplasm may be preserved in a variety of ways including seed storage, cryopreservation, and tissue culture. A new method of germplasm preservation may be through the storage of DNA libraries, which maintain in test tubes the genes that encode life's diversity. This new area of plant research germinated more than a decade ago and is beginning to bear fruit today. To be a viable alternative to traditional methods, however, ways must be devised to return cryopreserved genes to living cells, where they can be expressed and utilized to man's benefit. DNA libraries also can be used to increase genetic diversity greatly through methods involving molecular biology and gene transfer.

Numerous strategies have been developed to transfer genes into plants. Some of these techniques can be used to combine (entire) whole genomes by fusing two plant protoplasts. These somatic hybridization procedures have been refined to allow the combination of nuclear and organelle genomes from different plant species. Transfer procedures also have been developed for moving genes from chloroplasts, mitochondria, or nuclei into alien cytoplasms. Still other techniques can shuttle single genes from one plant species to another. These gene transfer techniques are being developed at a rapid pace to complement equally rapid developments in gene isolation, cloning, and recombination techniques.

A multitude of barriers inhibiting the transfer of genes to plants plagued the agricultural scientists of the 1970's, and many problems face these scientists today. But significant progress is being made, and further refinements in current plant gene transfer techniques will provide a strong base for future crop improvement using biotechnology.

Key words: protoplast fusion, electrofusion, electroporation, Ti-plasmid, pollen, transformation

Introduction

Gene transfer is no longer a vision of the future. Gene transfer is a technology of today. Scientists now can transfer genes from one plant to another. Genes isolated from animals and bacteria can even be transferred into plants. We are now learning how to control the regulation of genes after transfer, so that those genes are expressed at the proper time of plant development, in the correct tissues, and at the optimum levels. Through gene transfer, gene diversity of plants can be increased by inserting new genes and devising new
gene combinations, while bypassing sexual breeding barriers. Gene transfer may make it feasible to store germplasm and, at a future date, isolate desired genes from the stored germplasm and transfer it into commercial varieties. Perhaps someday rare germplasm may even be stored simply as DNA.

The new gene transfer techniques allow the possibility for the development of more productive crop plants at a pace much faster than before. Gene transfer and selection of the best plants has been the objective of breeding programs for years. Powerful new techniques for gene transfer recently have been developed for moving single genes and whole blocks of genes from one plant to another and even for moving genes from non-plants into plants. These new techniques can help mankind preserve the genetic diversity of plants by actually creating greater diversity through gene recombination. This is very important for future generations because, as human civilization advances and uninhabited areas are destroyed, natural genetic diversity dwindles. To preserve nature’s rich legacy and to ensure mankind’s continuing progress and well-being, scientists are challenged to find not only means to preserve existing germplasm but procedures to create new germplasm. Newly developed techniques for gene transfer can help mankind in this struggle. These new gene transfer techniques, when blended with conventional breeding methods, promise to revolutionize agriculture in the 21st century.

Rationale for gene transfer

The development of new plant varieties possessing superior agronomic traits is limited by conventional breeding because of sexual breeding barriers. When attempts are made to sexually breed two widely divergent plant species, often no seeds are formed or low seed numbers are produced. Sometimes these seeds are sterile or produce plants that are of little commercial value unless backcrossed for numerous generations to remove unwanted traits introduced during the initial cross. This process is very time-consuming, costly, and does not assure success. In some instances, detrimental genes are closely associated with agronomically valuable genes and are difficult or impossible to separate by breeding. In other instances, no viable seeds are produced from a cross between genetically dissimilar breeds. The breeder may resort to embryo rescue and artificially culture the zygote or may try a bridge hybridization, using a plant that is sexually compatible with both plants. This technique is even more difficult and time consuming. Finally, some crosses simply cannot be made through use of any technique known.

However, with the new tools of gene transfer we can combine single genes, multiple genes, organelles, and even whole cells to produce new
genetic combinations. These new gene transfer tools include protoplast fusion, electroporation, polyethylene glycol, and the use of *Agrobacterium tumefaciens* to introduce DNA into plants. These and other gene transfer tools will help the new plant breeder meet the agricultural demands of the 21st century.

**Protoplast fusion**

Many genes could contribute importantly to crop productivity if they could be incorporated into the desired crop plant. For example, genes encoding disease resistance, pest resistance, and tolerance to heat, salt, and drought could all improve crop yields. However, these genes often reside in diverse plant species, which make crosses difficult due to sexual incompatibility with the crop species susceptible to that biotic or abiotic stress. Protoplast fusion is one method that has been developed for the transfer of large numbers of genes, polygenic traits, and traits encoded by uncloned genes among sexually incompatible plants. Protoplast fusion has been the subject of numerous reviews [1–4]. In general, both the nuclear and mitochondrial genomes are combined, while the chloroplast genome oftentimes remains identical to that of one parent [5–8]. However, techniques recently have been developed that promote chloroplast genome recombination [9].

Protoplast fusion is now a routine laboratory technique, although it still requires considerable skill and attention. The walls of the cells to be combined are enzymatically removed, and the resultant protoplasts are merged by fusion (Fig. 1). The resultant fusion products must be separated from

![Diagram of protoplast fusion](https://example.com/protoplast-fusion-diagram.png)

**Figure 1.** Selection scheme for somatic hybrids. Protoplasts from aminoethyl cysteine (AEC) resistant cells of *Daucus carota* and from 5-methyltryptophan (5MT) resistant cells of *D. capillifolius* are fused. Somatic hybrids are identified by their growth in the presence of both AEC and 5 MT.
unfused protoplasts and from products of self-fusion. This separation can be achieved by tagging the parental protoplasts with different selectable markers (for reviews see 1, 2). One of the simplest methods used to identify somatic hybrids employs visual markers. One parental type of protoplast may be isolated from green mesophyll cells, while the other parental type may be derived from cell suspension cultures and would be translucent. Another system for the identification of fusion progeny uses different fluorescent dyes to identify each parent [10, 11]. In each of these examples, the fusion products can be selected and removed from background protoplasts with a micropipette or cell sorter and the fused single cell then can be cloned.

Other schemes for the selection of somatic hybrids include the use of compounds that selectively inhibit the growth of one parental type [12–14]. Thus, only somatic hybrids that express a double resistance will grow when in the presence of both inhibitory compounds. Numerous other methods also have been employed.

Similarly, several methods of protoplast fusion also have been developed. The most extensively used techniques employ chemical fusigen agents such as polyethylene glycol or changes in pH and calcium levels. A more recently developed technique is electrofusion, which is the fusion of two cells by an electric charge [3].

Protoplast fusion can be used to combine the nuclear genomes of two plants, exchange chloroplasts, and recombine mitochondrial DNA. The presence of portions of both nuclear genomes often can be demonstrated by examination of the karyotype of the somatic hybrid, which may contain all or most of the chromosomes of both parents. Our laboratory has successfully used isoenzymic patterns on electrophoretic gels to confirm the identity of *Daucus* somatic hybrids [15]. When an isoenzyme from one parent migrates on a polyacrylamide gel at a different rate than that of the other parent, the isoenzymic pattern of the somatic hybrid often shows the presence of both isoenzymes. In fact, we have shown new isoenzymic forms present in the hybrid as a result of mixing of subunits of the enzyme in the somatic hybrid. We also have examined mitochondrial DNA recombination in *Daucus* somatic hybrids by analyzing patterns of mitochondrial DNA endonuclease restriction fragments on agarose gels (Fig. 2). The somatic hybrid contained some of the mitochondrial DNA fragments specific to each of the parents and also contained new DNA fragments not present in either parent. Thus, protoplast fusion can be used to transfer large blocks of nuclear and cytoplasmic genes.

**Electrofusion**

The fusion of somatic cells by use of chemical fusogenic agents, initiated in the 1960's by Cocking [16], represented an important beginning in the
development of gene transfer techniques in plants. This was the first time that non-gametic cells from higher plants had been used to combine nuclear material from two distinct cells without the use of sexual reproduction. These resultant cell lines and later regenerated hybrid plants could be produced without the necessity of using gametic cells and confrontation with sexual incompatibility barriers. Although numerous research studies were to result from this important discovery, commercial application of this technique in major agronomic crops was scant over the next twenty years. In the early 1980’s, use of these chemical fusogens was replaced in many research laboratories by the simple yet elegant technique of electrofusion of protoplasts [3, 17]. This reversible procedure breaks down the cell membrane of adjacent cells by use of brief high voltage discharges. Upon resealing, many adjacent cells reanneal at the point of cell membrane dis-
ruption and fuse, creating a single cell with two nuclei. In plants, with the
subsequent nuclear fusion of these two nuclei, a hybrid cell line would be
formed. In some cases, this cell line could be induced through tissue culture
techniques to grow and regenerate a hybrid plant.

In electrofusion experiments with plants, it has been necessary first to
isolate protoplasts by mechanically or enzymatically stripping off the cell
wall, which represents a substantial physical barrier for the fusion of the
internal cell membranes. This, of course, is much less of a problem in fusion
of animal cells than with plant cells because of the lack of the cell wall in
animal cells; however, animal systems lack the potential to regenerate into
mature organisms and therefore only can be induced to form hybrid cell lines
by use of this technique. Whether plant or animal cells are used for electro-
fusion, the cells must be brought into close proximity so that adjacent cell
membranes can fuse during the reannealing phase. Cells have been placed
in juxtaposition by a number of procedures, including cell-surface binding
agents [18] and packed cell densities [19]. In addition to these techniques,
one of the simplest procedures used to move nearby cells into close proximity
has been the process of dielectrophoresis [20]. This procedure exposes the
cells in a low ionic media to an alternating sine wave (A.C.) low-voltage
current. The dielectrophoretic current creates charged dipoles in the cells in
this field because of the inherent ionic salts present in individual cells. In a
non-homogenous field, the cells migrate to areas of highest field strength.
The induced attractive forces of the dielectrophoretic current are sufficient
to overcome the weaker repulsive forces on the surface of the membrane.
As the field strength of areas rich in cells is increased, due to conductivity
of the cells, additional cells are drawn into the region, and this further
enhances the probability of successful fusion events.

Although electrofusion techniques now are used commonly in many
laboratories, very few reports have appeared in the literature citing recovery
of stable regenerated hybrids from electrofused plant protoplasts. This may,
in part, be a result of the dynamic nature of this rapidly expanding field.
Alternatively, it may result from difficulties encountered in either the stability
of the somatic hybrid during subsequent tissue culture procedures or un-
explained modifications to the cell lines that present problems in the regener-
ation of mature plants. In cooperation with Dr. Steven Sinden of the
Vegetable Laboratory, USDA, Beltsville, Maryland, our laboratory has
successfully fused and regenerated protoplasts from two different potato
species by use of electrofusion techniques [Table 1]. In these experiments,
esophyll protoplasts isolated from *Solanum tuberosum* were electrofused
with leaf mesophyll protoplasts isolated from *Solanum chacoense* by a single
40 µsec D.C. pulse of 1.2 kvolts/cm. These fused cell lines, cultured and
regenerated on modified MS media, show unique biochemical characteristics
Table 1. List of characteristics of hybrid potato

<table>
<thead>
<tr>
<th>Solanum tuberosum</th>
<th>Solanum chacoense</th>
<th>Hybrid</th>
</tr>
</thead>
<tbody>
<tr>
<td>(USW)</td>
<td>55—1</td>
<td>Regenerates in tissue culture</td>
</tr>
<tr>
<td>Regenerates in tissue culture</td>
<td>Never regenerates in tissue culture</td>
<td>Grows rapidly in tissue culture</td>
</tr>
<tr>
<td>Grows slowly in culture</td>
<td>Grows slowly in culture</td>
<td></td>
</tr>
<tr>
<td>Contains tomatidinol</td>
<td>Contains acetylated solanidine</td>
<td>Contains both tomatidinol and acetylated solanidine</td>
</tr>
<tr>
<td>Pubescent leaves</td>
<td>Glabrous leaves</td>
<td>Pubescent leaves</td>
</tr>
<tr>
<td>No anthocyanidin</td>
<td>Anthocyanidins present in leaf and stem</td>
<td>Anthocyanidins very apparent in leaf and stem</td>
</tr>
<tr>
<td>Flowers white</td>
<td>Flowers blue</td>
<td>Flowers bluish</td>
</tr>
</tbody>
</table>

of both parental cell lines (Fig. 3). This particular hybrid was partially selected by the inability of *chacoense* to regenerate in tissue culture and the inability of *tuberosum* to produce the readily apparent red anthocyanin pigment on the leaves and stem of the regenerated plant.

![Figure 3. Comparison of Solanum chacoense (A), fusion hybrid (B), and S. tuberosum (C). The fusion hybrid, with characteristics of each parental type, was formed through electrofusion of mesophyll protoplasts of the parent potato species shown.](image-url)
The increase in chromosomes or ploidy number of the resultant hybrid cell line is one inherent difficulty that must be addressed in somatic fusion procedures, whether they are produced through chemical fusogens or through electrofusion. In plant species known to be tolerant of variation in ploidy numbers (e.g., tobacco and potato), successful somatic hybrids can be obtained much more frequently than in other species less tolerant of modifications in chromosome numbers. This difficulty has been addressed partially by use of enucleated cells as one of the fusion parents. In this way, cytoplasmically encoded genes can be transferred to fully developed somatic cells through electrofusion procedures without an increase in the chromosome number. This procedure, however, is only useful for those traits that are expressed by chloroplast or mitochondrial genomes, which creates a severe limitation on the number of genes that can be transferred by cell enucleation.

Organelle transfer

In many cases, it is desirable to transfer only organellar traits. Several important genes are encoded within the organelles. Genes encoding resistance to the triazine herbicides [21, 22], such as atrazine, are good examples of chloroplast encoded traits. In addition, the large subunit of ribulose bisphosphate carboxylase, which fixes carbon in plants, also is located in the chloroplast. An important trait encoded within the mitochondrion is cytoplasmic male sterility [23, 24]. Cytoplasmic male sterility is quite important in breeding programs throughout the world and makes hybrid seed production substantially easier. The transfer of organelles containing these genes from one plant species to another has great commercial implications. The transfer of chloroplasts and mitochondria from a donor protoplast to a recipient protoplast without the transfer of nuclear DNA into the recipient protoplast allows much more specific transfer of only a subset of the total genetic material of a specific cell. It is desirable in some cases not to move nuclear DNA into the recipient protoplast, if possible, because the donor crop species may have nuclear traits that are totally undesirable. These traits could have a devastating effect on a breeding program. If inserted into a commercial crop species, it could decrease the commercial value of the newly developed hybrids. Some techniques recently have been refined so that organelles can be transferred without the nucleus. Several methods of organelle transfer have been shown to work. One such method (Fig. 4) involves the enucleation of the donor protoplasts, which produces a cytoplast containing only organelles [2–28]. The cytoplasts then are fused with the recipient protoplasts, which may be treated with iodoacetate to inactivate those organelles. In another method developed in Galun’s laboratory [29–32], the donor nucleus is disrupted by X-ray irradiation so that it is no
Figure 4. Enucleation of protoplasts by centrifugation yields cytoplasts containing organelles with selectable markers. These cytoplasts can be fused with protoplasts to transfer the organelles. Hybrids are recovered on selection medium.

longer viable (Fig. 5). The recipient protoplast is treated with iodoacetate to prevent these organelles from replicating. The protoplasts are fused and hybrids selected. These methods more precisely incorporate organelle-encoded traits than do techniques that fuse protoplasts with intact, viable nuclei.

**Single gene transfer**

As genes are identified and better understood, it has become apparent that the method of choice for most gene transfers is one that transfers only the specific DNA sequences desired and which inserts these sequences in the desired position on the chromosome or organelle genome. Thus only defined sequences of DNA would be inserted into the recipient genome. There would be no transfer of unknown genes or sequences and no transfer of undesirable genes or sequences. The appropriate promoters and regulators
Figure 5. Fusion of donor-recipient protoplasts is used to transfer organelles from one plant species to another without transferring and combining nuclei. The organelle donor is treated with X-rays to disrupt the nucleus, and the recipient is treated with iodoacetate to disrupt the organelles. After fusion, the hybrids are selected by means of the proper functioning of both nuclear and organellar genomes.

would be incorporated into the gene construct so the gene is translated at the proper time in the desired cells and in amounts suitable to the plant.

*Ti plasmid-mediated transfer*
Numerous investigators are making remarkable strides towards gene transfer mediated through *Agrobacterium tumefaciens*. The tumor inducing (T)-DNA of *A. tumefaciens* has been extensively studied and developed as a vector for the stable integration of foreign DNA into plant cells.

The transfer of T-DNA into plant cells relies upon the presence of two border sequences, which are 24-bp imperfect direct repeats flanking the T-DNA. The left-hand border repeat can be deleted without significantly decreasing pathogenicity; however, if the right-hand border is deleted, pathogenicity is totally eliminated [33, 34]. This border sequence is also polar, because alteration of the orientation of the right-hand sequence with respect to the Ti plasmid attenuates the effectiveness of transfer and integration. The T-DNA is processed by nicking at unique, identical positions of the border sequences [34]. The major intermediate of T-DNA transfer is believed to be a linear, single-stranded T-DNA molecule [34, 35]. In binary vectors, only one copy of the 25-nucleotide T-DNA border sequence is necessary to direct and allow stable integration of T-DNA in plants [33]. In some cases the target DNA of the plant is rearranged upon
the integration of the T-DNA [36]. The variety of insertion, deletion, and duplication of the target DNA indicates that T-DNA integration is a multistep process that includes replication and repair processes conducted by enzymes of the recipient plant cell.

Regenerated plants transformed by Ti plasmid T-DNA transmit the T-DNA in a Mendelian manner. In most cases, the trait is inherited as a single locus [37, 38]; however, some do segregate as two independent loci [37]. Although molecular and genetic analysis of transgenic plants is still limited, it appears that the T-DNA integrates at different sites. Wallroth et al. [39] demonstrated that six transgenic Petunia hybrida plants were independently transformed at six different chromosome loci. Similarly, Ambros et al. [40] showed that the T-DNA integrated into the chromosomes of transgenic Crepis capillars at different sites. Two transformed lines of C. capillars had one T-DNA insertion, while two other lines had two copies of T-DNA. In one case the T-DNAs were on separate chromosomes, whereas in the other case the T-DNAs were integrated into the same chromosome close together, although not in tandem.

Much new knowledge is being gained about the sequences controlling the developmental regulation of specific genes through the use of gene transfer and stable insertion of foreign genes into plant cells via the Ti plasmid system. The gene encoding the small subunit of ribulose-1,5-bisphosphate carboxylase (Rubisco) has been examined extensively [41, 42]. Mutations in the putative regulatory regions of the Rubisco gene have been constructed, and the effects of these alterations on gene expression have been examined. Broglie et al. [43] have inserted different constructs of the gene for the small subunit of pea Rubisco into petunia protoplasts. The resulting transformed Petunia calli synthesized pea Rubisco small subunit protein. This synthesis was light-regulated, and there was a 50-100 x increase in the transcript level of the small subunit of pea Rubisco.

Two different pea genes encoding the small subunit of Rubisco were examined by Fluhr and Chua [44] to determine the effects of light quality on the light-induced expression of the two genes. Different responses were obtained from the two genes depending upon the developmental stages of the leaves examined. Phytochrome mediated the response of the two genes in etiolated leaves, while phytochrome and a blue-light receptor mediated the responses of the two genes in mature leaves. Through gene construction and transfer experiments such as these, it now is possible to dissect the gene to determine which sequences are important in the regulation of transcription of that gene.

Because of the limited host range of A. tumefaciens, it has been much more difficult to transfer genes to monocots than to dicots by use of the Ti plasmid. Several new approaches may allow genes to be transferred readily.
to monocots as well as to dicots. One general method, which holds great promise, electrically induces the uptake of DNA into plant protoplasts by a process very similar to electrofusion. This method, called electroporation, avoids the use of *A. tumefaciens* and potentially can be used on a wider range of crop plants.

**Electroporation**

In the process of electrofusion research, it was noted that the reversible breakdown of the cell membrane occurred at localized regions on the cell so that the disruption caused a small hole or pore in the membrane [45]. It was in these regions that adjacent membranes would reanneal to form a common cell or hybrid. In 1982, Neuman [46] reported that cloned DNA could be taken up through these pores if the cloned DNA in the cell suspension media was at a relatively high concentration. This process, termed electroporation, represents an effective modification of the electrofusion procedures by eliminating some of the drawbacks associated with the increases in chromosome numbers during electrofusion. The electroporation process is somewhat less complex than electrofusion because cells or protoplasts need not be brought into close contact to effect gene transfer. Thus the need for the dielectrophoresis step of the fusion process is eliminated. This modification of the electrofusion process also enables the researcher to employ a fundamentally simpler and less expensive electroporation device. Two different electroporation machines currently are in use. The first uses a square-wave D.C. pulse to provide a specified pulse of known voltage over a controlled time. The second approximates this square-wave pulse by using an exponentially decaying capacitor-discharge wave. The capacitor pulse decays over a much longer time period than the duration of the square-wave pulse. A great deal of confusion has been introduced into the literature because of the use of these two different types of wave forms for the electroporation of cells. Using *Nicotiana tabacum* protoplasts, we found that viral RNA was optimally incorporated with a square-wave pulse of 60–80 uSec and a field strength of approximately 2 kvolts/cm. These conditions maintain viability of the protoplasts at greater than 95% and yield incorporation rates for the uptake and expression of cloned RNA at 50%. Using a capacitor-discharge D.C. pulse generator, incorporation of RNA into an aliquot of the same protoplasts was 25% at an optimal field strength of 1 kvolts/cm (maximum peak). By use of a minimum capacitance of 50 uF, the viability of these protoplasts was decreased to a far greater extent with the exponential pulse than the square-wave pulse (36% of the controls) [Saunders et al. in press].

One disadvantage inherent in both protoplast fusion and protoplast electroporation is the necessity to culture and regenerate the genetically
modified germplasm into mature plants. Although the regeneration of many plants from protoplast culture has been reported, important groups of economic crops are recalcitrant to regeneration. At best the procedure is an arduous, time-consuming task that may be prone to difficulties associated with contamination of the tissue cultures or to unstable germplasm lines.

Other methods of direct gene transfer
Although the use of the Ti plasmid is the most common method used to transfer single genes into plants, electroporation and other methods are being developed as alternatives. These methods may be most useful with certain plants, such as monocots, which are not readily infected by *A. tumefaciens*. These methods also may prove to be simpler and easier, but they also must be refined into highly efficient and reliable methods.

One new method for direct gene transfer involves the incubation of naked DNA with plant protoplasts. Polyethylene glycol is added as a fusigen to yield transformants [47, 48]. Protoplasts of several plant species, such as tobacco [47, 49], soybean [50], and petunia [51], have been successfully transformed by direct gene transfer. This method may be useful in transferring genes into plants that are not susceptible to *A. tumefaciens* infection, but the regeneration of intact plants from protoplasts does pose a major problem with some species.

Our laboratory is currently developing a possible alternative to the elec-

![Figure 6. Germinating tobacco pollen for transformation studies. The pollen is incubated in a germination medium to promote the initiation of the pollen tube, which has a thin cell wall.](image)
troporation of protoplasts and subsequent tissue culture steps to introduce foreign genes into plants. We are exploring the possibility of electroporating cloned DNA directly into germinating pollen grains and then fertilizing developing ovules with the genetically modified pollen. This technique may completely circumvent tissue culture procedures required for current procedures. We have used germinating pollen with an extremely thin cell wall as the recipient of the electroporated DNA (Fig. 6). Naked DNA is then added to the pollen, which is treated with polyethylene glycol or by electroporation to insert the DNA into the germinating pollen tube containing the generative nucleus. After the DNA has been incorporated, the pollen is placed on the stigma of a receptive plant species. After seed set, the seeds are collected and germinated on selective media allowing the identification of transformants. The transformants are rescued and grown to full plants, selfed, and the resulting seed is examined for the presence of the marker gene and other incorporated genes. If this method proves successful and reliable, regeneration of plants from protoplasts normally will not be a problem in direct gene transfer experiments.

Summary
Several reproducible methods are now available for transferring genes into plants. Large blocks of genes may be moved among closely related plants species, and whole plants may be obtained from this process. As the parents of choice are more distantly related, the problem of obtaining regenerated plants becomes dominant.

The transfer of single or multiple traits encoded on cloned, defined DNA is also possible and will become the method of choice as more and more genes are identified and characterized. Several methods are now available for the transfer of this DNA and may include the use of the Ti-plasmid or the use of electroporation or polyethylene glycol. Some techniques being developed may eliminate host range barriers of A. tumefaciens and may eliminate the need to regenerate plants by use of tissue culture. The real challenge of the future will be to clone genes of high agronomic value and insert them into crop plants, where they function properly at the correct time and place, without compromising other traits of the plant. However, we must have the genes available to transfer. Germplasm preservation is important for the ultimate success of gene transfer. The loss of one species is the loss of tens of thousands of genes, many of which are unique, encoding that species. Can we afford that loss?
References


