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Molecular Conservation Technologies For Germplasm Conservation

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ABSTRACT

Molecular biology provides a scientific framework that describes the elements of the genetic system as sequences of four nucleotide bases that make up DNA. Knowledge of how these DNA sequences are expressed and how expression is regulated and coordinated during development is growing rapidly. It is now commonplace to introduce foreign gene constructs into an organism, and the ability to add regulatory sequences that determine when and how strongly the introduced genes will be expressed to alter the phenotype of the recipient is often possible. The numbers of genes that have been isolated,cloned, and sequenced increase daily, and the information thus obtained already represents a genetic resource of considerable and growing scientific and commercial value.

INTRODUCTION

Within the past decade rapid progress has been made in developing and applying methods to identify, isolate, and characterize individual genes at the molecular level. At the larger scale of the genome, the DNA sequence can be cut up into fragments and reassembled in the form of a linkage map. While the task of characterizing germplasm at the molecular level has just begun, these molecular technologies can offer new approaches for both preserving and evaluating germplasm resources.

DNA as a Genetic Resource

The DNA sequences in the genomes of germplasm accessions are the sources of the genes required in breeding programs. When identified and isolated by cloning, these genes may be used to prepare transgenic organisms that express them. Transformation may avoid much of the genetic disruption that accompanies sexual hybridization and, since it is not limited by sexual compatibility, can make use of genes from other life forms. The genes used to breed crop plants that are resistant to various herbicides or insect larvae or to increase the growth rate of transgenic fish, are most conveniently conserved as cloned DNA. The extent of this kind of conservation is limited by the technical problems of identifying and cloning the commercially important genes that breeders would like to have and obtaining high rates of stable transformation in a broad range of recipient genera. Genes of this kind that have been cloned so far are also protected by patents and so are not freely available to anyone who wishes to exploit them commercially.

For economic and technical reasons it is unlikely, in the foreseeable future, that gene synthesis will make physical storage of germplasm in the form of seeds, whole plants, or tissue cultures obsolete. The conservation of DNA and the assembly of sequence data bases are not alternatives to conventional germplasm conservation because they are not coordinated in a genome (Peacock, 1984). Recent progress in the synthesis of artificial chromosomes in yeast raises the possibility of conserving coordinated assemblies of genes that would allow more complex phenotypic changes to be engineered. To be of practical use, however, DNA and artificial chromosomes require the conservation of recipient organism. Their genetic information is incomplete. Unlike a seed or an embryo in a surrogate mother, a DNA sequence cannot yet be used to regenerate a whole organism. Nevertheless, cloned genes, genomic libraries, and sequence data bases have significant potential uses in germplasm conservation and management. They may be more compatible with genetic stock collections, rather than national and international germplasm collections because of the specialty nature of the material and data.

In the broader context of conserving global genetic diversity, some (Adams et al., 1992; Adams, 1993) have proposed the collection of small samples of plant material (leaf tissue, seeds, and so on) for conservation as sources of DNA. Tissue samples from all taxa threatened with extinction would be collected and preserved in liquid nitrogen. These samples would, in theory, be available indefinitely. DNA extraction would be deferred until needed. At that time, the DNA could be isolated, immobilized on membranes, used as a source of specific genes or DNA sequences (see polymerase chain reaction technique below), and then returned to liquid nitrogen storage. Several conservation groups are now establishing a network of such DNA banks with duplicate samples as a safeguard against loss through extinction (Adams et al., 1992; Adams, 1993). This technique may be useful for the many underscribed or unstudied plant species with seeds that cannot be stored, but it is unlikely to be of direct value for those of recognized importance that are already conserved in germplasm banks.

DNA Sequence Data Banks

There is now much DNA sequencing carried out in various laboratories worldwide that the ability to compare new sequences with those that have already been described and well characterized is of considerable importance. Comparisons may suggest unsuspected functions or may reveal useful homologies between unrelated organisms. Several organizations support the central storage and coordination of DNA sequence information. The two major data banks are the European Molecular Biology Laboratory and, in the United States, GenBank, which is operated by IntelliGenetics and the U.S. Department of Energy's Los Alamos National Laboratory.

The rate at which data on DNA sequences is accumulating is likely to continue to increase exponentially in the near term as aresult of such major initiatives as sequencing the entire human genome and the genomes of rice and Arabidopsis. This rapidly growing body of

data raises important problems of storage and access to facilitate rapid comparisons with newly obtained information.

Restriction Fragment length Polymorphisms

When DNA is extracted from an organism and digested with one or more restriction enzymes and the products (called restriction fragments) are separated by electrophoresis on a gel, the result is a smear of many DNA fragments with different lengths. The longest fragments move slowly in the gel, staying near the origin; the smallest move faster and are farthest from the origin. If the smear is transferred to a membrane by blotting, it may be hybridized with a radiolabelled DNA probe. After autoradiography, the regions of hybridization are revealed as one or more distinct bands at constant and characteristic distances from the origin of the gel. Each band identifies a restriction fragment that contains a stretch of DNA complementary to the sequence of nucleotide bases in the DNA probe. The same digest tested with different DNA probes shows different band patterns that are also constant and characteristic for each probe.

When the DNA digests from separate organisms are compared, the differences in banding patterns are called restriction fragment length polymorphisms (RFLPs) and correspond to points, or very small regions, of physical difference on the chromosomes. The number of polymorphisms (RFLPs) provides some indication of the number of differences that exist between the genomes of the organisms being compared. The RFLPs segregate as allelic differences and may be tested for linkage with each other and with other characters of agronomic importance. RFLPs, in theory, provide an almost inexhaustible number of markers and should make possible the rapid construction of linkage maps even in species in which this has been hampered because of the lack of morphological markers or long generation times.

There are several practical limitations, however. The DNA extracts, digests, gel separation, blots, hybridizations, auto radiography, and pattern interpretation are expensive and time-consuming and require trained personnel. It may be necessary to test several hundred, or even thousand, different DNA probes to find RFLPs suitably spaced throughout the genome. The ideal is to locate one at about every 10 map units. Some of these difficulties may be overcome by automation of the procedures. The analysis of the resulting data can be complex. In some cases the results obtained for onesegregating F2 population cannot be directly applied to an F2 generation from another, different cross because of major duplication at the DNA level that may even obscure the chromosomal assignments obtained with the first set of probes. This difficulty is proving to be an obstacle to the use of RFLPs as an aid to selection in maize breeding, because maize contains an excessive number of polymorphisms in the noncoding regions of its DNA that complicate the banding patterns and often make them difficult to decipher. In contrast, among some inbreeders, such as the tomato, there are not enough RFLPs among commercial cultivars, evidently reflecting their genetic similarity.

Recent research indicates that RFLPs can be used to map quantitative trait loci (Paterson et al., 1988). Linked RFLPs that identify relevant portions of chromosome arms may potentially be used to select characters that are inherently difficult to assay, such as resistance to insects that cannot be reared, drought tolerance, and processing quality of harvested product. In germplasm enhancement programs, linked RFLPs could be used to mark genes for resistance to pests or pathogens that do not occur when or where the work is being done and that cannot be introduced because of quarantine regulations. An example might be breeding U.S. maize for resistance to African streak virus.

A barrier to using RFLPs is the investment of effort needed to find useful probes. Probes could be used to screen germplasm accessions for alternative alleles for important disease resistance loci.

Polymerase Chain Reaction

The polymerase chain reaction (PCR) is an extremely sensitive and accurate method for recovering microgram amounts of single specific DNA sequences present in biological samples at very low concentrations. The reaction requires pairs of single-stranded primers (DNA template molecules) of 20 to 30 bases in length found on opposite strands at either end of the double-stranded DNA sequence of interest. These primers are added to a solution of DNA extracted from the sample, together with the four nucleotide bases that make up DNA and a DNA-polymerizing enzyme from the bacterium Thermophilusaquaticus (Taq), which is stable at high temperatures. The reaction mixture is heated to 92° C for 30 seconds to denature the DNA into single strands, cooled to 50° C for 1 minute to allow annealing to occur, and then heated to 72° C for 2 minutes to allow DNA synthesis to fill in the missing bases from one primer toward the other, using the single strands of sample DNA as templates for thecomplementary strand copies. The preparation is then heated again to denature the newly formed double-stranded DNA molecules, and another cycle of cooling-incubation-heating is begun. Since the primers are present in excess at each cycle, the amount of double-stranded DNA selected by the primers steadily builds up until after 40 cycles the preparation is sufficiently pure for that segment of DNA that it can be readily sequenced.

The ability to use PCR to select and amplify specific DNA sequences from desiccated dead seeds and inviable frozen semen and embryos raises the question of whether these "dead" materials are useful genetic resources. Depending on their scientific interest, commercial value, or rarity, there may be a case for keeping them as a source of particular DNA sequences. Already collected, stored, inventoried, and evaluated, there would be little more to do with them until they needed for DNA isolation. However, the fact that they had become inviable could mean that the original storage conditions were unsatisfactory and that, if left in place, further changes to the DNA might eventually reduce their usefulness. The DNA banks discussed above avoid these difficulties.

The use of PCR to recover sequences from herbarium specimens (Rogers and Bendich, 1985) may mean that specimens should be regarded as a form of germplasm. PCR

analyses will be useful for comparative genetic studies, which, until now, have depended on morphological or chemical comparisons rather than functional genetic analyses using the tools of molecular biology.

As with isolated DNA, cloned DNA, and DNA sequence information stored in data banks, the DNA of inviable specimens and cryogenically stored tissues cannot be used directly to reconstitute an organism. These forms of DNA are presently only valuable as a germplasm resource to the extent that they can be incorporated into other living organisms or for research. For the long-term preservation of genetic variability, they are only useful to the extent that viable hosts in which they are readily expressed can also be maintained.

PCR is a useful technique, but the requirement for primers means that it can only be used to find sequences that are already known in some detail. PCR is useful, however, for recovering allelic forms of well-characterized genes from any source, whether or not it is viable.

Randomly Amplified Polymorphic DNA Markers

PCR can also be used with single, arbitrary, 9- or 10-base primers to generate probes for detecting RFLPs. These polymorphisms arealled randomly amplified polymorphic DNA or RAPD markers (Welsh and McClelland, 1990; Williams et al., 1990). It is expected that any arbitrary 9- or 10-base sequence will occur at a sufficient frequency at points on opposite strands of DNA so that synthesis of the intervening segments will occur in PCR from points where the primers has hybridized. The optimum size range of these fragments can be controlled by prior digestion of the extracted DNA with one or more restriction enzymes. The several kinds of DNA fragments amplified in the PCR step can then be used as probes to detect RFLPs. The advantage of the method is that the original primers are relatively inexpensive and easy to make and that many probes are available that should cover all parts of the genome (Anderson and Fairbanks, 1990).

CONCLUSION

Recent advances in biotechnology provide powerful tools conserving, evaluating, and using genetic resources. Cloned DNA fragments synthetic DNA are unlikely to replace conventionally stored seeds other germplasm in the foreseeable future. In any case whole organisms must be conserved as recipients for the expression of introduced DNA.olecular techniques for characterizing genetic material, such as restriction fragment length polymorphism analysis, appear likely to provide the breeder with greater efficiency in selecting and developing new breeding lines and varieties.DNA sequences used as probes can detect viruses in germplasm bank materials and also reveal RFLPs that can be used to construct linkage maps and, as linked markers, to select desirable traits. Linked RFLP markers might be used to detect genes for resistance to pests and pathogens that themselves cannot be used because of quarantine restrictions. Information from RFLPs could potentially assist in the selection of core subsets of larger germplasm collections by

providing another measure of genetic diversity, but only if the technology becomes much less expensive.

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