Biotechnology and Plant Productivity[1]

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ABSTRACT. There is considerable potential for the application of biotechnology and genetic engineering to plant productivity. The basic manipulations for the isolation of genes and their transfer between species are well documented in model systems. However, more information is required concerning the basic processes governing plant productivity at the molecular level before practical applications can be achieved. In this paper, detailed consideration is given to (1) the use of restriction fragment polymorphisms as genetic markers, and (2) the molecular basis of hybrid vigor. In both cases it is clear that these techniques will be adjuncts to the already established methods for plant improvement but with far reaching potential for the future.

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INTRODUCTION

Broadly defined, biotechnology is the application of biological science for practical or commercial purposes. Thus, biotechnology began with the domestication of plants and animals. The word has taken on a new meaning since the revolution in molecular biology and biochemistry, and is now associated with the applied aspects of molecular genetics. Modern biotechnology includes a wide diversity of activities such as gene splicing (Murray 1987), cell and tissue culture (Sharp and Evans 1987, Sahai and Smith 1985), immobilized cell and enzyme technology, and fermentation technology (Strohl and Pfister 1987). The use of recombinant DNA methodology has raised the possibility of the isolation, manipulation, and transfer of genes from any living system into bacteria, fungi, animals, and plants. The applications of this technology have developed at different rates in the various systems, with the practical advances being most limited in higher plants although there are great potential benefits in this area.

Grain yields of the major U.S. crops have increased continually since about 1930 (Duvick 1984b). This increase has been due to genetic improvements in yield and to other factors not directly related to the breeding gains. Non-breeding inputs such as nitrogen fertilizer, timeliness of planting, better pest control, and improved harvesting machinery have been important causes of increased yield. However, variety improvement, interacting with changes in agronomic practices, has probably been the most important cause of higher yields (Duvick 1984b).

Genetic improvements in yield have increased linearly for the past 25 years and have shown no signs of levelling off. Plant breeders intuitively agree with the conclusion that the gains in genetic yield can continue with present-day breeding methods (Duvick 1984a). Much time, work, and ingenuity will be needed, however, to bring about these changes which will be at the expense of ever increasing research inputs. It has been estimated that maize yield potential (the genetic component) has increased at an average rate of 1.4% per year during the period of 1930-1980. For the same period the number of maize breeders has increased at an average rate of 4% per year (Dubick 1984b).

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With this as background, what roles can the new technologies play in plant breeding? It is important to identify the genetic needs for crop improvement as judged by plant breeders. A list of the most important factors has been drawn up (Goodman et al. 1987). These include the basic physiology and genetics of durable pest resistance, the large number of years and locations needed to evaluate and identify stress tolerance, and the long time (in generations) needed to break up undesirable genetic linkages or, conversely, to assemble desirable traits. The last factor is particularly important in the utilization of new germ plasm. Where hybrid crops are concerned, the inability to predict accurately the amounts of heterosis among crosses of inbred lines, and the complete failure of genetics and physiology to explain, except in very general, essentially descriptive terms, the basis for heterosis constrains the selection of base material in breeding programs (Kaushik and Sharma 1986, Crossa et al. 1987).

The basis for genetic engineering has been established in model systems (Wilke-Douglas et al. 1986, Perani et al. 1986). This includes the ability to isolate genes, tailor them, transfer them between species, and subsequently recover them in mature plants that can then be used directly or incorporated into an established breeding program. However, the present methods are designed to transfer single genes which can be identified and isolated (Goodman et al. 1987). Many traits that are connected with the final productivity are controlled by multiple genes that are only recognized in the context of the ability to manipulate them in a breeding program, but with little or no knowledge of the biochemical basis of their action. Under these circumstances it is not possible to devise a strategy to isolate and transfer the complexes of genes responsible for these quantitative traits in a single step. Thus, it must be emphasized that biotechnology will have to be used in conjunction with 'conventional' plant breeding to manipulate these gene complexes. While the new technology is very powerful, it does depend on the amount and quality of the biological knowledge that has already been accumulated (Helentjaris et al. 1986).

The potential applications of biotechnology to plant productivity cover a wide range of subjects. Two areas in which advances have been made recently are in the introduction of novel genes to control virus susceptibility (Abel et al. 1986) and pest resistance (Vaeck et al. 1987). In this paper, I will consider two aspects of recombinant

DNA technology related to identifying new genetic markers for aiding in the selection of material to be evaluated and the detection of desirable combinations. These are the potential use of restriction fragment length polymorphisms to increase the number of genetic markers and an attempt to understand the basis of heterosis.

APPLICATION OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

When the genomic DNAs from two genetically distinct individuals are digested with a restriction enzyme, electrophoresed, blotted onto a nitrocellulose membrane, and probed with a radioactively-labelled DNA clone, polymorphisms in the hybridization patterns sometimes result due to differences between the individuals. The term restriction fragment length polymorphism (RFLP) has been coined to describe this variation. The appearance of these polymorphisms is not surprising in view of the number of supposedly neutral mutations encountered at the protein level in recent years. Randomly dispersed RFLPs were first proposed as a new source of genetic markers in humans, where the low amount of genomic variability, as well as other factors, complicate the application of genetic analyses (Botstein et al. 1980). The actual feasibility of using RFLPs as genetic markers for the diagnosis of human diseases/disorders has since been demonstrated (Little et al. 1980, Phillips et al. 1980; Gusella et al. 1983).

The potential usefulness of RFLP markers in basic plant genetic studies, as well as in plant improvement programs, has been reviewed by Tanksley (1983) and Beckmann and Soller (1983). In many cases the RFLP markers would essentially replace the existing isozyme variants as the molecular markers. Although isozyme loci have proved useful in several instances (Conkle 1981), their application is limited by insufficient numbers and general lack of informativeness. In contrast, a potentially unlimited number of RFLPs exist, which should allow a much wider use of the molecular marker approach.

The degree of genetic variability detectable by RFLPs in plants has been examined. Rivin et al. (1983), Burr et al. (1983) and Helentjaris et al. (1985) have reported significant variability in maize using either repetitive or single-copy DNA sequences. Restriction fragment length polymorphisms have also been detected in the tomato (Helentjaris et al. 1986), barley (Saghai-Maroof et al. 1984), and peas (Polans et al. 1985).

An example of an RFLP is shown in Figure 1. The DNAs from two flax (*Linum usitatissimum*) lines were digested with a restriction endonuclease, separated on an agarose gel, transferred to nitrocellulose, and hybridized with a labeled ribosomal DNA probe. The variant fragments are identified with an arrow and are due to a variation in the length of the spacer region in the ribosomal DNA repeat unit (Cullis 1983). In a cross between the two lines, the F1 contained equimolar amounts of the two variants, and they segregated out in selfed F2 (Cullis, unpublished data). Thus, they can be used as genetic markers since they behave as normal Mendelian markers.

The molecular basis for the generation of RFLPs is best demonstrated in genes where the complete sequence is known for what is considered the wild-type allele, as well as for a number of variants. One such case is the alcohol dehydrogenase gene isolated from maize (Dennis et al. 1984). In this case, many of the changes appear to be due to the insertion and excision of transposable elements. However, single base substitutions that alter the restriction site can also be the cause of RFLPs. Thus, any change that alters the length of a restriction fragment effectively creates a new allele. The only requirement to define this "locus" is an appropriate DNA probe. The pervasiveness of RFLPs in the genomes of higher organisms means that the number of loci that can be ultimately obtained is limited only by the number of informative probes which can be generated.

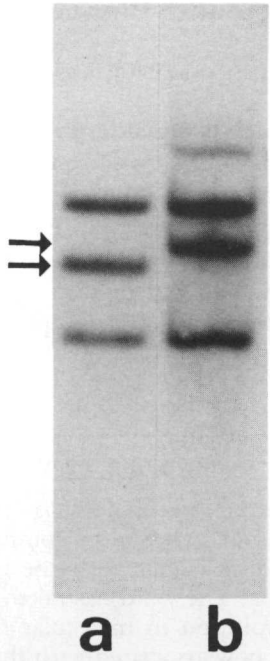

FIGURE 1. DNA from two individual flax plants, digested with the restriction endonuclease EcoR1, separated on a 1% agarose gel by electrophoresis, transferred to a nitrocellulose membrane, and hybridized with 32-P-labelled, cloned flax ribosomal DNA. The three bands make up the repeat unit of the rDNA in the genome and have length variants between individuals. The bands varying in length are indicated with arrows. The extra band in lane b is due to an incomplete digest of the DNA in that lane. Data from Cullis (1983).

The use of RFLPs as genetic markers will greatly increase the precision of genetic maps. They also have a number of characteristics which increase their potential usefulness (Helentjaris et al. 1986). They vastly increase the number of potential markers, are unaffected by the developmental state of the organism, as are many proteins that are expressed in some form of developmental time scale; and can be characterized using a small amount of material obtained from seedlings.

In general, RFLPs are associated with random sequences, so that their mapping requires considerable effort. However, they would be most useful in material where a preliminary genetic linkage map has already been established and can be used to provide a more comprehensive map. Many hundreds of new markers have been added to the already extensive maps of maize and tomato (Helentjaris et al. 1986, Helentjaris et al. 1986). Forest tree species are examples where RFLPs will be used to generate a much more extensive map. In some of these species, linkage maps have been established on the basis of isozymes and secondary products (Conkle 1981), and the original material is still available. From this material it would be possible to construct a more detailed map

from the RFLPs. There is already evidence for considerable genetic heterogeneity among conifers (Ledig and Conkle 1983), in particular, so that RFLPs should abound.

There are many potential applications of RFLPs in plant breeding (Burr et al. 1983). Examples of these are strain identification, mapping and monitoring of quantitatively inherited traits, measurement of genetic diversity, and controlling the level of heterozygosity/homozygosity.

Strain identification is likely to be most useful in non-truebreeding plants that are propagated asexually. These are the plants that are most frequently covered by plant patents. In many cases, the sequences used to generate RFLPs are those which are of low copy number in the genome (Burr et al. 1983). In one case, however, it has been shown that a dispersed, repeated sequence shows many RFLPs. Hence, the overall pattern shown is equivalent to a fingerprint, and a small number of such sequences may be sufficient to identify an individual unambiguously (Jeffreys et al. 1985). These repetitive sequences may be very useful for the identification of individuals, but their complex patterns make interpretation in crosses difficult. For genetic markers, low copy number sequences are likely to be the ones of choice.

The evaluation of selections in a plant breeding program requires considerable effort. Field testing limits the size of breeding programs, and in the case of fruit or forest trees, which do not come to maturity in a single growing season, the field evaluation becomes even more important. If linkages between marker loci and factors for quantitative traits could be tight enough, then meaningful correlations could be established between the two. Since it appears possible to saturate the genome with RFLP markers, these relationships could be established for a chosen population. If the linkage was sufficiently close, then it would also be possible to use the RFLPs in the selection of material to include in the construction of an elite population as a source for future breeding programs. A start on the correlation of quantitative traits and RFLPs has been made in maize (Figdore 1987). Using the large number of RFLPs mapped and the wealth of genetic information in maize, a number of loci have been identified, which have major effects on quantitative traits such as plant height.

Widespread epidemics, such as the southern corn leaf blight in the U.S. in 1970 and the potato leaf blight in Ireland in the 1800s, can be traced to the use of a limited genetic base. Genetic diversity does not necessarily overcome the vulnerability to disease, but the maintenance of a wide variety of germplasms is increasingly recognized as important for future breeding. However, natural habitats are being lost, and the resources for maintaining extensive collections are finite. Thus, the use of RFLPs may give an indication of the extent of diversity among collections. In addition, since they are based on random sequences, they should not be biased for any particular trait. This is important as the genetic trait next in demand cannot be known.

HYBRID VIGOR

The general phenomenon of hybrid vigor (heterosis) is manifested in the improved performance of F1 hybrids as compared with the inbred parents. However, there is no adequate theoretical, genetic, or physiological explanation for this phenomenon. It is clear that the new technologies could help to unravel this phenomenon.

The extent of heterozygosity in an F1 is one of the suggestions for the heterotic effect observed in hybrids (Fincham 1985). The use of RFLPs, since they can measure directly the extent of heterozygosity, will be ideal for testing this hypothesis. A study of quantitative traits in maize has started to approach this question (Figdore 1987). In this study, the regression of single cross yield on the percentage heterozygosity at 34 RFLP loci was highly significant. The relationship appeared to break down at levels of heterozygosity of more than fifty percent. Thus, the combining ability of maize inbreds may be predicted by levels of heterozygosity at RFLP loci to a point, after which other factors may become an important consideration. The use of this RFLP data base grouped the maize inbreds into heterotic groups, based on whether or not they were derived from a common ancestor. The predominant allele at a given RFLP locus differed among the heterotic groups for most of the loci studied, thus providing a basis for further testing of the association between particular RFLP loci and heterotic effects.

Another phenomenon shown to occur in F1 hybrids is that of genomic instability. In certain instances it has been shown that the nuclear DNA in the F1 of a cross between two inbred parents is not necessarily equal to the sum of half of each of the two parents. The observations were that the amount of DNA in the F1 for either a specific sequence, or for the total DNA amount, was not equal to the expected value. It could be either more or less than the expected value. This effect has been demonstrated in three different systems, namely maize (McClintock 1978, Rivin and Cullis 1983), flax (Cullis 1979), and *Microseris* species (Price et al. 1983).

In maize, it was shown that wide crosses could activate previously quiescent, transposable elements. These elements can cause the restructuring of the genome and appear to be normal components of the genome (Dellaporta and Chomet 1985). The net effect of the activation of these transposable elements is an increased mutation rate, particularly with respect to the generation of unstable alleles (one of the characteristics of transposable, element-induced mutations). It has been shown recently (Rivin and Cullis 1983, Price et al. 1983) that wide crosses may not be necessary to get genomic alterations, but may occur in the combinations of more closely related inbreds without the concomitant induction of transposon activity. Thus, genomic restructuring is not the sole preserve of transposable elements, although they may play a prominent role in many species.

This non-Mendelian inheritance of DNA amount does not occur in all crosses, but is dependent on the particular parents involved. The genetic basis for the control of the change in DNA is not known. However, it appears that the system may have at least two separate parts, one controlling whether or not there is any alteration in the F1 and the other controlling the extent of that alteration. It is not clear what effect this form of genomic rearrangement has on the phenotype. One possible way it could alter the phenotype would be by some form of variegated position effect (Cullis 1986).

Part of the heterosis observed in some crosses could be the result of an interaction at the genomic level with the restructuring of specific parts of the genome being responsible for the altered growth pattern. If this is the case, then the appearance of non-Mendelian effects at the DNA level in F1s may be diagnostic of heterotic crosses. This would be a direct determination of which inbred combinations would show heterosis. Such a factor would be extremely valuable in selecting the initial base population for a breeding program, especially in species where there is insufficient genetic data to identify the loci directly responsible for heterosis.

Another alternative way of approaching the molecular basis of heterosis would be to characterize the genes expressed in the heterotic hybrids and compare them to those expressed in their inbred parents. The technology to isolate the genes that are expressed in particular organs and tissues exists (e.g., the preparation of cDNA libraries and expression libraries) (Murray 1987). The characterization of those genes that differ in their expression in the hybrids compared to their parents will be the genes involved in the metabolic pathways important in the expression of hybrid vigor. Eventually it should be possible to isolate the genes responsible for the control of hybrid vigor. With the gene transfer technology already in place, a further advance would be the transfer of these genes into species where natural hybrid vigor is limited. New insights into the processes contributing to the phenomenon of heterosis and an ability to predict which individuals would be good combiners and show extensive heterosis would be extremely important. This information would alter the basis for selecting the parents for hybrid crops, and perhaps alter the range of crops in which hybrids are considerably superior to inbred varieties.

Two agronomically important crops in which heterosis is highly important are maize and *Pinus* species (Franklin 1970, Ledig et al. 1983). The life cycle of the former is short and the plants are relatively small, so that effective screening can be carried out to determine which lines will be useful parents in the production of hybrids. However, in the case of conifers, the time scales are very different; thus any method of predicting heterosis between individuals would dramatically affect the selection of elite material.

CONCLUSIONS

All of the technologies (i.e., gene isolation, transfer, and, to some extent, the regulation of transferred genes) used to modify higher plants are available in model systems and can clearly be made to work. Over the past few years the techniques have been extended to some of the agronomically important species. It is likely that we should be in a position soon to introduce novel traits into a number of species. However, the limiting factor may well be the ability to identify and isolate the genes for the desirable traits that the plant breeder is interested in.

It is undeniable that our understanding of the detailed basis for crop performance is very limited, and that most traits manipulated by plant breeders are genetically complex. However, the new technologies should facilitate the understanding of these characteristics and, at the same time, generate material that can be used subsequently for further manipulations.

The present search for disease resistance genes will also generate more information about the basic processes by which these genes act. A series of such genes will give insight into the interactions between host and parasite and allow the design of novel combinations that may have a more widespread applicability for durable resistances. There is also the demonstration that genes from other organisms can be useful sources of resistance (Vaek et al. 1987, Abel et al. 1986). These may appear to be a panacea, but there is a danger that if too much reliance is placed on too few resistance genes (whether they are from a normal higher plant origin or introduced from foreign species) problems will develop in the future.

There are few, if any, applications of biotechnology to the problems of plant productivity in which all the appropriate technology is at hand and the commercial success of the application can be anticipated. Rather, there are major scientific and technical barriers that will require an intensive research effort. At this time an appropriate goal is to acquire the basic information needed to assess and direct practical programs for genetic engineering for increased productivity. Until more is known about the genes involved in processes such as disease resistance, tolerance of environmental stress, growth control, and hybrid vigor, the chances for improvement in these properties by genetic engineering are somewhat limited.

The success of recombinant DNA technology in medical science required an exceptional research effort in molecular biology. This was needed to provide the fundamental information about the processes related to metabolism and disease. Because of these efforts the significant genes for many processes were defined and isolated. The advent of recombinant DNA technology can greatly accelerate the rate at which this essential information can be gathered for plant systems. It is reasonable to predict that this fund of basic information derived from the application of molecular genetics to plant productivity will surely lead to practical applications, the extent of which are likely to be underestimated in any projection from the present.

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