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The potential use of novel techniques in plant breeding

B. J. MIFLIN

Biochemistry Department, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ, England

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The new technologies of molecular and cell biology have created a large interest in plant science. Some of the recent advances are reviewed. These techniques are of themselves advancing rapidly in terms of increasing sophistication; as they advance they allow even more detailed analysis of the plant's genome and the processes that it specifies. Even from today's standpoint it is possible to see some applications in terms of manipulating plants. These are particularly likely to come from enhancing (rather than replacing) the efficiency with which plant breeders carry out their tasks. However, in the long-term the dramatic gains will come as the technologies provide the plant scientists with a vastly increased knowledge of how plants work. The challenge will then be to turn that knowledge into improved cultivars.

Benjamin J. Miflin, Biochemistry Department, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ, England

There is much hope and excitement being generated throughout the world by the potential application of new approaches in molecular biology to crop plants. In addition, there has been steady progress in plant cell and tissue culture to the stage where further applications can be foreseen. It is my task to review briefly these advances and to suggest how they may affect the practice of plant breeding. However, before starting it may be helpful to define what I mean by this new technology, which is often termed 'genetic manipulation of crop plants'.

(1) Novel techniques for altering the genetic composition of plants and microorganisms associated with them in agriculture, including recombinant DNA techniques, limited gene transfer, partial genome transfer, somatic hybridization, somaclonning and allied tissue culture techniques, but excluding conventional plant breeding.
(2) Application of techniques for in vitro production, multiplication and selection of plants including tissue culture, protoplast culture, plant regeneration, and the selection of mutant cells or plants by novel techniques.

In assessing progress I would emphasize three aspects of the subject which although not mutually exclusive will help to focus the discussion; these are technical advances, analytical aspects, and manipulation.

Technical advances

Recombinant DNA techniques

Gene isolation is now well underway for plants. The majority of the work is still at the level of making banks of cDNA clones from mRNA from various tissues but has extended to a number of genomic libraries. Naturally, most of the cDNA libraries are for proteins present in reasonable abundance. Not surprisingly the first genes to be described in detail included those for the large and small subunits of RuBP carboxylase. Furthermore, cDNAs related to all of the groups of storage proteins present in the cereals of the Triticeae have been described, as have clones for legume storage proteins. As yet, few of the low abundance class of plant mRNAs appear to have been cloned and identified.

Genomic libraries have been obtained, after initial difficulties, from most major crop species. Of the genes characterized most have introns, e.g. phaseolin (SUN et al. 1981; SLIGHTOM et al. 1983) and other legume storage protein genes (BOULTER 1984; FISCHER and GOLDBERG 1982; NIELSEN 1984), the small subunit of RuBP carboxylase (CASHMORE 1983) and leghaemoglobin (BRISSON and VERMA 1982; WIBORG et al. 1982). In many cases gene identification has been achieved by first identifying a cDNA clone usually by hybrid-select translation (RICCIARDI et al. 1979; FORDE et al. 1981) although

also by immunopurification of mRNA (CULLIMORE and MIFLIN 1983) and by the use of synthetic DNA probes. Some use has been made of heterologous probes—that is genomic or cDNA clones for the protein from non-plant species. This has been done to find genomic clones for actin by means of a cloned probe from *Drosophila* (SHAW et al. 1982) or to identify clones corresponding to the mitochondrial gene for a subunit of cytochrome oxidase by means of a probe from yeast (FOX and LEAVER 1981). Since conservation of function has often resulted in conservation of sequence this approach may be applied more widely in the future as more cloned genes become generally available.

Another approach for the future is transposon mutagenesis. Transposable elements have been cloned from maize (COURAGE-TEBBE et al. 1983; DORING et al. 1984) and the phenomenon of transposition in maize involving single and multiple element systems has been well known for a long time due to the pioneering work of Barbara McClintock (see FINCHAM and SASTRY 1974). If a transposition event results in an inactivation of function, then the element may well have inserted itself into or near the gene responsible for the function. Given the availability of cloned DNA probes for the transposable element it is possible to sort out the particular piece of DNA containing the transposon that is related to the observed change in phenotype. From this it should then be possible to isolate the gene for the mutated function. This approach is particularly useful where the gene product causing the phenotype is not known—for example the *opaque*-2 mutation in maize, which causes the high-lysine phenotype. Transposon mutagenesis is not so likely to be fruitful if the function under investigation is coded for by more than one gene so that multiple inactivations would be required. Unfortunately, many attributes which might be expected to be coded for by single genes, such as given enzymes, turn out to be coded for by more than one gene. This has been revealed by genetic analysis, for example there are at least three genes for aspartate kinase activity (BRIGHT et al. 1982), or by genomic blotting and cloning for example in the genes for the small subunit of RuBP carboxylase (CASHMORE 1979; DUNSMUIR et al. 1983). However, there is at least one example where a transposable element has been used to distinguish a functional gene from copies which are either non-functional or involved with a different pathway (WEINAND et al. 1982).

A further method of gene isolation and identification, which has been used by Schröder and colleagues, is to transfer the 'unknown' gene into *E. coli* by means of a vector giving a high degree of expression and then studying either the altered metabolism of *E. coli* or the characteristics of the protein produced (SCHRÖDER et al. 1983, 1984).

Gene transfer and expression

Foreign genes have been transferred into regenerated plants by means of modified *Agrobacterium tumefaciens* tumour-inducing (Ti) plasmids (OTTEN et al. 1981; WULLEMS et al. 1981; DE GREVE et al. 1982; BARTON et al. 1983; for reviews see BINNS 1984; SHAW 1984). This plasmid has been modified in a number of ways, for example by mutating or removing one or more of the genes causing the disruption of plant growth (OOMS et al. 1981; GARFINKEL et al. 1981; BARTON et al. 1983), or by inserting selectable markers (FRALEY et al. 1983; BEVAN et al. 1983; HERRERA-ESTRELLA et al. 1983). Recently, it has proved possible to reduce markedly the size of the plasmid by removing large portions of the non-T-DNA region including the virulence genes to produce a mini-plasmid; the mini-plasmid is then complemented by another compatible plasmid containing the virulence region to produce a binary vector transformation system (HOEKEMA et al. 1983; DE FRAMOND et al. 1983). So far, the number of genes transferred and expressed has been small but include bacterial encoded genes other than T-DNA and higher plant genes. As yet, little is known about gene expression but this system will soon be exploited by many people to identify DNA sequences important in controlling gene expression. Already there is some evidence that sequences 5′ to the small coding sequence of the subunit of RuBP carboxylase, when joined to a bacterial gene, can be used to obtain light-stimulated expression of that gene transferred into higher plants (CAPLAN et al. 1983).

To date, regenerated and transformed plants of tobacco, petunia, diploid and tetraploid *Solanum tuberosum* have been obtained. In the first two the T-DNA has been shown to be inherited via the seed into the next generation (WULLEMS et al. 1981; OTTEN et al. 1981), and in the latter to be passed through the tuber to the ensuing vegetative generation (OOMS et al. 1983). Regenerated plants of carrot (CHILTON et al. 1982), and tetraploid potato (OOMS et al., pers. commun.) have also been obtained following transformation with *Agrobacterium rhizogenes* containing a root-inducing (Ri) plasmid. Again the T-DNA portion of the Ri plasmid appears to be transferred to subsequent seed generation of carrot (CHANTAL et al. 1984) and tuber

generation of potato (Ooms et al., pers. commun.).

So far, all transformation has been confined to a relatively narrow range of dicotyledonous species. Because the host range of *Agrobacterium* is wide there is no reason why the range should not eventually be extended. But, monocotyledons are not hosts and at present the Ti or Ri plasmids are not suitable vectors for the important cereal crop plants. However, another possibility is the use of transposable elements, which has been successful in *Drosophila*. Recently several transposable elements have been cloned from maize (Courage-Tebbe et al. 1983; Doring et al. 1984) and *Antirrhinum* (Bonas et al. 1984). Ways in which these might be useful in cereals have been discussed by Flavell and Mathias (1984).

Protoplast and tissue culture

Protoplasts have been obtained from a wide range of plants and have been cultured to obtain division and ultimately plant regeneration (Cocking 1981). However, techniques for the mass isolation, culture and regeneration have been developed for only relatively few species—chiefly members of the *Solanaceae*. Of the major crops only potato, tobacco, rape and alfalfa would be included in this category. Cereals are notable by their absence. In contrast, the number of crop species that can be readily regenerated from tissue cultures derived from various plant parts is considerable and includes most cereals, many forage legumes, all solanaceous species, most brassicas and several other crops. The one group that appears to be particularly difficult is grain legumes.

Protoplasts are generally considered of potential importance as:

(a) recipients for transformation, particularly by naked DNA or by co-cultivation with *Agrobacterium;*
(b) allowing fusion of whole or partial nuclear and/or organellar genomes from a donor to a recipient outside the barriers of sexual crossing;
(c) an efficient means, along with tissue culture, of obtaining a wide range of selected mutants.

Whilst some success has been achieved in each of these areas there is still much work to be done before they become routine and widely applicable techniques. Perhaps the most successful development has been the transformation of protoplasts, not by naked DNA but by 'co-cultivation' with *Agrobacterium*. This technique developed by Marton et al. (1979) has been exploited by Fraley et al. (1983) to give very high frequencies of transformation.

Total fusion of nuclear genomes followed by plant regeneration has been achieved between many species, particularly where sexual crossing can take place, albeit with difficulty (for reviews, see Schieder and Vasil 1980; Evans and Wilson 1984). Certain fusions have been obtained with either full or partial regeneration for sexually non-compatible species (e.g. Power et al. 1980; Melchers et al. 1978). Little work has been published so far on partial transfer of nuclear genes by this method.

The sorting of organellar genomes during regeneration after fusion has been studied in a few instances. Results with different fusions have shown that usually it is either one or the other parental plastid genome that is found in the regenerated plant (e.g., Chen et al. 1977; Menzcel et al. 1981) although in some regenerants both plastid types persist (see, e.g., Bonnett and Glimelius 1983). No examples of recombination of chloroplast genomes have yet been found. The situation with mitochondrial genomes is more complex and there is evidence that novel mitochondrial genomes may be formed in regenerants (see Hanson 1984 for further discussion).

In terms of mutant selection, studies with protoplasts or tissue cultures have been relatively disappointing. Although a number of variants and some mutants have been selected there have been numerous problems associated with the regeneration of fertile, cytologically normal plants. Perhaps the most successful series are the auxotrophs selected by King, Gebhardt and colleagues (Gebhardt et al. 1983; King 1984) and by Negrutiu (1984). However, few of these have been carried through to fertile plants and analysed genetically. Selection of mutants at protoplast or cell level also restricts the range of mutations to those whose phenotype is expressed in culture; conversely they are well suited to investigating the type of resistance to toxins or adverse environments that results from gene amplification mechanisms. Those variants and/or mutants obtained in culture will probably be of considerable use as selectable markers in fusion experiments (for further discussion, see Schieder and Vasil 1980; Evans and Wilson 1984) though so far they have not contributed greatly to the analysis of biochemical pathways important in crop producton. For detailed reviews of this area the reader is referred to King (1984); Somerville (1984); Miflin et al. (1983); Chaleff (1981); Maliga (1984).

Analytical aspects

Many of the techniques above—particularly gene isolation, characterization and transfer—are analytical techniques that will tell us a great deal about the way plants are organized. Much of this information will add to our store of knowledge without providing direct application and some will provide new insights into how to breed, select and develop our crops. The analytical nature of the techniques—particularly the ability to recognize defined pieces of DNA—are also of use directly in crop breeding.

Biochemical pathways

The use of biochemical mutants to analyse biochemical pathways was fundamental in establishing much of our understanding of bacterial metabolism; many of the pathways thus established have been shown subsequently to occur in plants, but even now several assumptions are made based on analogies with bacterial systems. There are obvious reasons, such as generation time, genome complexity and the inability to work easily with large populations, why a similar approach was not used in higher plants. Recently, a number of groups have begun to analyse pathways of nitrogen and carbon metabolism by means of mutants with defined genetical and biochemical properties. The overwhelming majority of these mutants have been obtained by selection at the whole plant or embryo level. The critical factors have been the devising of suitable selection screens and the willingness to undertake a considerable amount of routine work such as embryo dissection or measurements on each individual in a mutant population. Advances in this field have been specifically reviewed (SOMERVILLE and OGREN 1982; MIFLIN et al. 1983; SOMERVILLE 1984). These emphasize the amount of novel biochemical information made available by this approach.

Further progress is likely to come from mutant studies as other selection screens are devised. Progress is also likely as more genes for enzymes are cloned. The availability of these will allow very detailed biochemical analysis, for example in vitro mutagenesis allows questions to be asked and answered regarding the role of amino acid residues in the active site of an enzyme. Examples of this are the successfully induced change at the activation site of the RuBP carboxylase of *Rhodospirillum rubrum* (GUTTERIDGE et al. 1984) and the recent 100-fold increase in the affinity of tyrosyl tRNA synthetase for ATP induced by a single base change in the cloned gene (WILKINSON et al. 1984).

The regulation of metabolism will also slowly be uncovered by the use of recombinant DNA techniques. For example, a fascinating story is unfolding regarding the way in which a plant deals with ammonia assimilation. The enzyme responsible—glutamine synthetase—appears to be present in almost all tissues of the plant. However, it is clear from biochemical studies that different tissues have different forms of the enzyme (for a review, see MCNALLY et al. 1983; MIFLIN et al. 1981) and that these may change in activity in response to certain stimuli. For example, the nodules of *Phaseolus vulgaris* contain two forms of the enzyme. One form (GS_{n2}) resembles that present in uninoculated roots (GS_r) and does not change in activity as nodules develop. The other form (GS_{n1}) is physically separable (CULLIMORE et al. 1983) and immunologically distinct (CULLIMORE and MIFLIN 1984) from GS_{n2} and GS_r, and increases in activity in parallel with nitrogenase and leghaemoglobin; neither GS_{n1} or leghaemoglobin increase markedly in ineffective nodules induced by certain strains of *Rhizobium phaseoli* containing specific mutations (LARA et al. 1983; see also ROBERTSON et al. 1975). The availability of cloned probes for nodule GS (CULLIMORE et al. 1984) will allow the determination of the number of GS genes involved in higher plants and eventually their differential expression in response to tissue specific and external controls.

Plant development

Most of the processes involved in plant development are a complete mystery. As yet we have no tenable hypothesis for the mechanisms of action of plant growth regulators. The molecular factors involved in switching the plant from vegetative to reproductive development in response to environmental changes are virtually unknown beyond the discovery of the light receptor phytochrome. Nevertheless, this switching and the subsequent transfer of the resources to the developing seed are one of the important processes in determining yield—in cereals this is generally considered to have been much more important than primary CO_2 assimilation in the development of modern high-yielding cultivars (see GIFFORD and EVANS 1981). I would predict that much of the efforts and rewards in the future will be concerned with the application of the new techniques to these problems. Some progress is already being made. The Ti and Ri plasmids show how plant morphology can be altered by

a small number of genes. Which genes are important (at least in the Ti plasmid) is now known, as is their complete DNA sequence (BARKER et al. 1983): their transcripts can be recognized and some have been characterized (see BINNS 1984). Some evidence as to the nature of the biochemical reactions catalysed by these genes is now available (SCHRÖDER et al. 1984). This system is likely to be extremely useful in analysing the role of plant growth regulators in the control of crop development.

The availability of cDNA and genomic clones of genes that are active during seed development in both cereals (LARKINS 1983; MIFLIN et al. 1984) and grain legumes (BOULTER 1984; NIELSEN 1984; SLIGHTOM et al. 1983) means that we are on the way to describing tissue specific genes important in seed storage. As sink (seed) storage capacity is one of the most important determinants of yield it is important to know some of the factors which determine this. For example, what is the relative importance of gene number and strength of promoters in determining the expression of the protein in the grain? Can we define the nature of the DNA sequences which determine that a gene is expressed in the seed?

Plant/micro-organism interactions

Crop plants interact with microorganisms in a whole range of associations from the wholly beneficial to the totally devastating. At the molecular level most attention has been given to the *Rhizobium*/legume interaction and its role in the fixation and subsequent assimilation of nitrogen (MIFLIN and CULLIMORE 1984). The genetic system of free-living and symbiotic nitrogen-fixing bacteria has been analysed intensively. We are now able to describe the genes involved in nitrogen fixation in *Klebsiella* in almost complete detail and, to a lesser extent, in *Rhizobium*. A promising start has been made in cloning the genes determining nodulation and host-specificity (JOHNSTON and DOWNIE 1984). Allied to this is the availability of cloned probes for defined and undefined plant genes involved in the plant's response (VERMA and LONG 1983; BISSELING et al. 1984). Thus the tools are being obtained for a detailed analysis of this particular interaction. Similarly, as mentioned before, the pathogenic relationship between *Agrobacterium* and their hosts is also being studied intensively. Although both of these systems have obvious direct applications they also provide model systems on which to base the study of other plant/microorganism interactions.

Plant defence mechanisms

Many aspects of the environment of crop plants besides microorganisms adversely affect crop plants. These include cold, heat, lack of moisture, salinity and vertebrate and invertebrate pests. Osmotic stress results in the synthesis of low molecular weight nitrogenous compounds such as glycine-betaine or proline (STEWART and LAHRER 1980; WYN JONES and GORHAM 1983). Valentine and colleagues have used a biochemical mutant approach to analyse the analogous response in bacteria (see STROM et al. 1983). KUEH and BRIGHT (1982) have selected proline overproducers in barley and tested their response to stress. The results showed only marginal differences and suggested that higher levels of proline accumulation might be required. Various other responses such as the synthesis of pathogenesis-related proteins (ANTONIW and WHITE 1983; CARR et al. 1982) of protease inhibitors in response to disease (RYAN 1983) and of hydroxy-rich glycoproteins in response to pathogens and ethylene (TOPPAN et al. 1982) are being studied at the molecular level.

Conventional genetic analysis has revealed and characterized a large number of genes in crop plants that confer resistance to pests and diseases. So far, there is little information as to the nature of the gene products. As far as I am aware the only one that can be tied down to a molecular event is that for resistance to *Alternaria alternata* f. sp. *lycopersici* in tomato. In this example Gilchrist and colleagues have shown that *Alternaria* produces a toxin which affects aspartate transcarbamylase by making the enzyme more sensitive to feedback regulation by UMP. Resistant genotypes contain an enzyme which is altered in its response to UMP in the presence of the toxin (MCFARLAND and GILCHRIST 1982; SILER and GILCHRIST 1983; GILCHRIST and MCFARLAND 1982). The use of recombinant DNA techniques may provide the tools to allow us to define many more resistance genes in detail.

Since our knowledge is so limited there is obviously no shortage of systems which need to be analysed, and much work requires to be done before we have any real understanding of the range of defence mechanisms that operate in our crop plants.

Manipulation

To a large extent manipulation is the other side of the coin from analysis. Once a system has been analysed then decisions can be made as to how it

might be manipulated. Conversely analysis often depends on manipulating a change (e.g., selecting a mutant) in the system and observing the results. In the sections that follow I want to make some suggestions as to how the techniques reviewed above might contribute to improving plant cultivars.

Plant breeding is of course the oldest form of genetic manipulation practised by man. In the current age it is an extremely sophisticated process, particularly in our important cereals. However, there are a number of new techniques which are or may be applied to aid breeders in their tasks. For example, FLAVELL et al. (1983) have described how recombinant DNA techniques, by producing probes for specific viruses, may speed up the assessment of resistance during a plant breeding programme or the recognition of source of cytoplasmic male sterility.

In the immediate future, though, most applications are likely to arise through the use of tissue culture techniques. There are many practical advantages to be gained by the use of micropropagation, and these techniques are already widely applied in commerce. Similarly tissue culture techniques can aid in producing plants of different ploidy levels. Embryo culture has also been exploited for a long time both in the production of haploids and in the rescuing of products of wide crosses (see YEUNG et al. 1981 for review). The area of protoplast and tissue culture work that has generated the greatest amount of discussion in relation to plant breeding is that which has been christened 'somaclonal variation'. Much of the current phase of interest stemmed from papers of Shepard (SHEPARD et al. 1980; SHEPARD 1981; SECOR and SHEPARD 1981; MATERN et al. 1978) showing a wide range of variation—some of it in agronomically important characters—that existed among regenerant plants from potato protoplasts. However, variation among regenerated plants is not new and has been described by many authors (e.g., see HEINZ and MEE 1971; D'AMATO 1977) and has been exploited in the sugar cane industry. Nevertheless, the work of Shepard stimulated many groups to explore this variation further. It has been established that it occurs in regenerants of potato, wheat, rice, sugar cane and brassicas (see BRIGHT et al. 1983; THOMAS et al. 1982; and LARKIN and SCOWCROFT 1981, 1983) from either protoplast and/or tissue culture.

In potato the indications of MATERN et al. (1978) and SECOR and SHEPARD (1981) were that characters of agricultural importance varied in ways that could be exploited in the production of new cultivars. Similarly, work in the U.K. (BRIGHT et al. 1983; EVANS et al. 1984) has suggested that variation in agronomically useful characters is occurring. Currently none of the regenerants has yet been released as a cultivar but a several are likely to undergo detailed testing during the next few years. Similarly, large numbers of wheat regenerants are currently being evaluated in the field for agronomically useful characters. Thus it is possible that, even at a relatively early stage, some useful material might emerge from such a programme. This could be particularly valuable in potato where progress from conventional techniques has been relatively slow and where there are problems of sterility in certain cultivars.

In terms of technique, results have shown that to obtain somaclonal variation in potato or cereals there is no need to go through the technically demanding business of isolating protoplasts and inducing them to divide and regenerate plants but that a similar range of variation is available in plants derived from tissue culture (VAN HARTEN et al. 1981; BRIGHT et al. 1983; WHEELER et al. 1984). This simplification should make the technique much more attractive to breeders and thus of wider application.

The reasons for the release of variation after passage through tissue culture are not clear. Certainly, a significant proportion can arise from chromosome abnormalities in that regenerants can have a wide range of aneuploidy or, where euploid, may show evidence of chromosome translocations (KARP et al. 1982; SREE RAMULU et al. 1983). However, the majority of regenerants are euploid with no visible chromosome damage (WHEELER et al. 1984). It will be interesting to probe the DNA of these regenerants by means of Southern blotting using a range of cDNA or genomic DNA probes to see how much restriction fragment variation has been induced by passage through culture. Although it may be expected that agronomically useful variants will arise from the euploid regenerants, the ability of plant cells to lose chromosomes (or parts of them) in culture may be useful in certain circumstances. Thus such techniques may be useful for obtaining introgression of genetic material following wide crosses or protoplast fusion (see ORTON 1979).

Gene transfer by *A. tumefaciens* Ti plasmid derivatives has been achieved in dicotyledons, and the commercial potato cultivar Maris Bard has been transformed and T-DNA shown to persist through into the subsequent tuber generation (OOMS et al. 1983). Thus in principle there is no reason why defined gene transfer should not be rapidly applied to certain crops. The problem is which genes to trans-

fer and it is here that progress will halt until we have more knowledge of genes that are important for crop production. This in turn will require advances in our ability to analyse various crop processes. However, even this analysis may not always provide the answer as many processes which affect yield are likely to be polygenic (see SIMMONDS 1983 for discussion).

Polygenic characters, or traits whose molecular genetic basis is unknown, may be transferred by protoplast fusion, either by using entire protoplasts and hoping for a subsequent reassortment perhaps via 'somaclonal variation' or by using irradiated donors to attain partial chromosome transfer. Protoplast fusion can also be important in transferring traits encoded by organellar genomes. In many crops potential yield gains may be achieved by utilizing hybrid vigour. One of the problems in obtaining this is to have an effective system for producing hybrids; in practice this is often achieved by utilizing cytoplasmic male sterility (CMS) (e.g., in maize). The primary forms of CMS appear to be coded for by the mitochondrial genome (see HANSON 1984 for review). Recently, PELLETIER et al. (1983) have succeeded in transferring CMS from radish to *Brassica napus* by means of protoplast fusion.

Despite the importance of polygenic systems there are also single gene traits that are worth transferring. At the molecular level, if it ever proves possible to improve RuBP carboxylase by in vitro mutagenesis then these genes will have to be reinserted. Certain traits for herbicide resistance are likely to be single genes—for example a single base change in the chloroplast gene coding for a 32,000 M_r protein is thought to be responsible for atrazine (a herbicide) resistance. Again such resistance can be transferred between species by protoplast fusion (see BINDING et al. 1982; PELLETIER et al. 1983 for a description of such attempts). Perhaps in the future certain genes for high lysine proteins may be inserted into cereals (e.g., see SHEWRY et al. 1981) — and other quality traits may also be altered by single gene changes.

Finally, one should not be limited by our current view of agricultural crops, particularly as surpluses of certain of them accumulate in Europe and farmers in the USA are paid not to sow them. There is no reason why the ability of plants to harvest efficiently the sun's energy should not be used in situ for producing novel products. Where any protein—particularly, but not necessarily, one encoded by an eukaryote gene—is required in large amounts at low cost then plants in the long-term may be better producers than microbial fermentors. Both legume and cereal seeds have the ability to synthesize and package proteins into deposits within the seed. Thus the legume seed is well able to carry out cleavage of signal peptides, core glycosylation and terminal fucosylation (see review by CHRISPEELS 1983) and can even subsequently cleave storage proteins at sites that bear a striking resemblance to cleavage sites in the precursors of animal peptide hormones (BOULTER 1984; NIELSEN 1984). The technology for subsequently harvesting the seed and storing and transporting it is well developed. Thus one could envisage higher plants as efficient low cost fermentors, a development that could extend the use of agricultural crops and increase their value and provide the third world with access to biotechnological products without a massive chemical industry infrastructure.

Summary

Some devotees of the new technology have made startling pronouncements as to the replacement of plant breeding by a new era of gene cloners. This will not happen. My prediction is that we shall continue to rely on the skill of the conventional plant breeder for some considerable time. However, the breeder will increasingly be helped in his task by some of the techniques described above.

Recombinant DNA technology by virtue of its power—e.g. in theory and given time, it is technically possible to produce a complete DNA sequence for a cultivar—is bound to improve our knowledge of plant chromosomes. Since plant breeding is the art of reassorting genes and chromosomes into the most favourable combination, then any technique that provides more information of this raw material and allows it to be followed through successive crosses and subsequent segregations must be capable of improving the art. I am sure that as plant research workers become more familiar with the techniques, then various ingenious applications will follow (see, for example, the discussion by BURR et al. 1983 on the use of restriction fragment polymorphism in plant breeding). In the long-term, specific genes are likely to be transformed into a limited range of crop species—perhaps bacterial genes for herbicide resistance may be some of the first—in order to achieve precise tailoring of the crop to specific needs.

The techniques of tissue culture are and will increasingly affect plant breeders. Micro-propagation is widely used for horticultural crops and will be

used in the production of virus-tested propagules for vegetatively propagated field crops such as potato. Variants arising from somaclonal variation may be useful. The techniques may be applied to producing in-breds for crossing to produce hybrid seed (already one patent for this has been taken out). Protoplast fusion, particularly where the donor has had its chromosomes fragmented, will eventually allow transfer of complex (or genetically ill-defined) traits across species incompatibility barriers. Similarly, tissue culture techniques will probably speed up the introgression of desired genes into existing improved backgrounds.

However, the aspect that excites me most is that plant scientists now have an entirely new range of techniques available to them. These will enable us to probe plant metabolism and the control of plant development to a far greater degree than previously possible. What this will reveal cannot be predicted; however I am convinced that it will be this knowledge that will open up our ability to manipulate our crop plants in entirely novel ways. The challenge will be to apply that ability to increase crop productivity, largely through the production of new cultivars. Since the rapidly increasing human population is entirely dependent, directly or indirectly, on the products of plant metabolism it is essential that we succeed.

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Literature cited

ANTONIW, J. F. and WHITE, R. F. 1983. Biochemical properties of pathogenesis-related proteins from tobacco. — *Neth. J. Plant Pathol. 89:* 255–264

BARKER, R. F., IDLER, K. D., THOMPSON, D. V. and KEMP, J. 1983. Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octopine Ti plasmid 15955. — *Plant Mol. Biol.* (in press)

BARTON, K. A., BINNS, A. N., MATZKE, A. J. M. and CHILTON, M.-D. 1983. Regeneration of intact tobacco plants containing full length copies of genetically engineered T-DNa, and transmission of T-DNA to R_1 progeny. — *Cell 32:* 1033–1043

BAULCOMBE, D. 1983. Wheat α-amylase genes: Cloning of a developmentally regulated gene family. — In *Genetic Engineering: Principles and Methods. Vol. V (Eds.* J. SETLOW *and* A. HOLLAENDER), *Plenum Press, N.Y.*, p. 93–108

BEVAN, M. W., FLAVELL, R. B. and CHILTON, M.-D. 1983. A chimaeric antibiotic resistance gene as a selectable marker for plant cell transformation. — *Nature 304:* 184–187

BINDING, H., JAIN, S. M., FINGER, J., MORDHORST, G., NEHLS, R. and GRESSEL, J. 1982. Somatic hybridization of an atrazine-resistance biotype of *Solanum nigrum* with *Solanum tuberosum.* — *Theor. Appl. Genet. 63:* 273–277

BINNS, A. N. 1984. The biology and molecular biology of plant cells infected by *Agrobacterium tumefaciens.* — *Oxford Surv. Plant Mol. Cell Biol. 1:* 133–160

BISSELING, T., GOVERS, F. and STIEKMA, W. 1984. The identification of proteins and their mRNAs involved in the establishment of effective symbiosis. — *Oxford Surv. Plant Cell Mol. Biol. 1:* 53–83

BONAS, U., SOMMER, H., HARRISON, B. J. and SAEDLER, H. 1984. The transposable element of Tam 1 of *Antirrhinum majus* is 17 kb long. — *Mol. Gen. Genet. 194:* 138–143

BONNETT, H. T. and GLIMELIUS, K. 1983. Somatic hybridization in *Nicotiana:* behaviour of organelles after fusion of protoplasts from male-fertile and male-sterile cultivars. — *Theor. Appl. Genet. 65:* 213–217

BOULTER, D. 1984. Cloning of pea storage protein genes. — *Philos. Trans. R. Soc. Lond. B. 304:* 323–332

BRIGHT, S. W. J., KUEH, J. S. H., FRANKLIN, J., ROGNES, S. E. and MIFLIN, B. J. 1982. Two genes for threonine accumulation in barley seeds. — *Nature 299:* 278–279

BRIGHT, S., JARRETT, V., NELSON, R., CREISSEN, G., KARP, A., FRANKLIN, J., NORBURY, P., KUEH, J., ROGNES, S. and MIFLIN, B. 1983. Modification of agronomic traits using in vitro technology. — In *Plant Biotechnology, SEB Seminar Series 18 (Eds.* S. H. MANTELL *and* H. SMITH), *Cambridge University Press, Cambridge, U.K.*, p. 251–265

BRISSON, N. and VERMA, D. P. S. 1982. Soybean leghaemoglobin gene family: Normal, pseudo and truncated genes. — *Proc. Natl. Acad. Sci. USA 79:* 4055–4059

BURR, B., EVOLA, S. V., BURR, F. A. and BECKMANN, J. S. 1983. The application of restriction fragment length polymorphism to plant breeding. — In *Genetic Engineering: Principles and Methods. Vol. V (Eds.* J. SETLOW *and* A. HOLLAENDER), *Plenum Press, N.Y.*, p. 45–59

CAPLAN, A., HERRERA-ESTRELLA, L., INZÉ, D., HAUTE, E. VAN, MONTAGU, M. VAN, SCHELL, J. and ZAMBRYSKI, P. 1983. Introduction of genetic material into plant cells. — *Science 222:* 815–821

CARR, J. P., ANTONIW, J. F., WHITE, R. F. and WILSON, T. M. A. 1982. Latent mRNA in tobacco (*Nicotiana tabacum*). — *Biochem. Soc. Trans. 10:* 353–354

CASHMORE, A. R. 1979. Reiteration frequency of the gene coding for the small subunits of ribulose-1,5-bisphosphate carboxylase. — *Cell 17:* 383–388

CASHMORE, A. R. 1983. Nuclear genes encoding the small subunit of ribulose-1,5-bisphosphate carboxylase. — In *Genetic Engineering of Plants (Eds.* T. KOSUGE, C. P. MEREDITH *and* A. HOLLAENDER), *Plenum, N.Y.*, p. 29–38

CHALEFF, R. S. 1981. Genetics of Higher Plants—Applications of Cell and Culture. — *Cambridge University Press, U.K.*

CHANTAL, D., CHILTON, M. D. and TEMPE, J. 1984. Conservation of T-DNA in plants regenerated from hairy root cultures. — *Biotechnology 2:* 73–76

CHEN, K., WILDMAN, S. G. and SMITH, H. H. 1977. Chloroplast DNA distribution in parasexual hybrids as shown by polypeptide composition of fraction 1 protein. — *Proc. Natl. Acad. Sci. USA 74:* 5109–5112

CHILTON, M.-D., TEPFER, D. A., PETIT, A., DAVID, C., CASSE-DELBART, F. and TEMPE, J. 1982. *Agrobacterium rhizogenes* inserts T-DNA into the genomes of the host plant root cells. — *Nature 295:* 432–434

CHRISPEELS, M. J. 1983. Biosynthesis, processing and transport of storage proteins and lectins in cotyledons of developing seeds. — *Philos. Trans. R. Soc. Lond. B. 304:* 287–296

COCKING, E. C. 1981. Opportunities for the use of protoplasts. — *Philos. Trans. R. Soc. Lond. B. 22:* 557–568

COURAGE-TEBBE, U., DORING, H. P., FEDEROFF, N. and STARLINGER, P. 1983. The controlling element *Ds* and the

Shrunken locus in *Zea mays*: structure of the unstable *sh-m* 5933 allele and several revertants. — *Cell 34:* 383–393

CULLIMORE, J. V. and MIFLIN, B. J. 1983. Glutamine synthetase from the plant function of *Phaseolus* root nodules: purification of the mRNA and *in vitro* synthesis of the enzyme. — *FEBS Lett. 158:* 107–112

CULLIMORE, J. V. and MIFLIN, B. J. 1984. Immunological studies on glutamine synthetase using antisera raised to two forms of the enzyme from *Phaseolus* root nodules. — *J. Exp. Bot. 35:* 581–587

CULLIMORE, J. V., LARA, M., LEA, P. J. and MIFLIN, B. J. 1983. Purification and properties of the two forms of glutamine synthetase from the plant fraction of *Phaseolus* root nodules. — *Planta 157:* 245–253

CULLIMORE, J. V., GEBHARDT, C., SAARELAINEN, R., MIFLIN, B. J., IDLER, K. B. and BARKER, R. F. 1984. Glutamine synthetase of *Phaseolus vulgaris*—organ-specific expression of a multigene family. — *J. Mol. Gen. Genet.* (in press)

D'AMATO, F. 1977. Cytogenetics of differentiation in tissue and cell cultures. — In *Applied and Fundamental Aspects of Plant Cell Tissue and Organ Culture* (*Eds.* J. REINERT *and* Y. P. S. BAJAJ), *Springer-Verlag, Berlin,* p. 343–357

DE FRAMOND, A. J., BARTON, K. A. and CHILTON, M.-D. 1983. Mini-Ti: A new vector strategy for plant genetic engineering. — *Biotechnology 1:* 262–269

DORING, H. P., TILLMANN, E. and STARLINGER, P. 1984. DNA sequence of transposable element *Dissociation* (*Ds*) in maize. — *Nature 307:* 127–131

DUNSMUIR, P., SMITH, S. and BEDBROOK, J. 1983. A number of different nuclear genes for the small subunit are transcribed in *Petunia*. — *Nucleic Acid Res. 11:* 4177–4183

EVANS, N. E., FOULGER, D., FARRER, L., RISIOTT, R. and BRIGHT, S. W. J. 1984. Variation in tissue culture derived clones of potato over three generations. — *Euphytica* (submitted)

FINCHAM, J. R. S. and SASTRY, G. R. K. 1974. Controlling elements in maize. — *Annu. Rev. Genet. 8:* 15–36

FISCHER, R. and GOLDBERG, R. 1982. Structure and flanking regions of soyabean seed protein genes. — *Cell 29:* 651–660

FLAVELL, R. B. and MATHIAS, R. 1984. Prospects for transforming monocot crop plants. — *Nature 307:* 108–109

FLAVELL, R. B., KEMBLE, R. J., GUNN, R. E. and BAULCOMBE, D. 1983. Applications of molecular biology in plant breeding: the detection of genetic variation and viral pathogens. — In *Better Crops for Food: CIBA Symposium 97* (*Eds.* J. NUGENT *and* M. O'CONNER), *Pitman*, p. 198–209

FORDE, B. G., KREIS, M., BAHRAMIAN, M. B., MATTHEWS, J. A., MIFLIN, B. J., THOMPSON, R. D., BARTELS, D. and FLAVELL, R. B. 1981. Molecular cloning and analysis of cDNA sequences derived from poly A^+ RNA from barley endosperm: identification of B hordein related clones. — *Nucl. Acid Res. 9:* 6689–6707

FOX, T. D. and LEAVER, C. J. 1981. The mitochondrial gene coding cytochrome oxidase subunit II has an intervening sequence and does not contain TGA codons. — *Cell 26:* 315–324

FRALEY, R. T., ROGERS, S. G., HORSCH, R. B., SANDERS, P. R., FLICK, J. S., ADAMS, S. P., BITTNER, M. L., BRAND, L. A., FINK, C. C., FRY, J. S., GALLUPI, G. R., GOLDBERG, S. B., HOFFMAN, N. L. and WOO, S. L. 1983. Expression of bacterial genes in plant cells. — *Proc. Natl. Acad. Sci. USA 80:* 4803–4807

GARFINKEL, D. J., SIMPSON, R. B., REAM, L. W., WHITE, C. F., GORDON, M. P. and NESTER, E. W. 1981. Genetic analysis of crown gall: fine structure map of the T-DNA by site-directed mutagenesis. — *Cell 27:* 143–153

GEBHARDT, C., SHIMAMOTO, K., LAZAR, G., SCHNEBLI, J. and KING, P. J. 1983. Isolation of biochemical mutants using haploid mesophyll protoplasts of *Hyoscyamus muticus*. — *Planta 159:* 18–24

GIFFORD, R. M. and EVANS, L. T. 1981. Photosynthesis carbon partitioning and yield. — *Annu. Rev. Plant. Physiol. 32:* 485–509

GILCHRIST, D. G. and MCFARLAND, B. L. 1982. Isolation and partial characterization of aspartate transcarbamylase from tomato seedlings. — *Plant Physiol. 69, Suppl. 20*

GREVE, H. DE, LEEMANS, J., HERNALSTEENS, J-P., TOONG, L. T., BUUCKELEER, M. DE, WILLMITZER, L., OTTEN, L., MONTAGU, M. VAN, and SCHELL, J. 1982. Regeneration of normal and fertile plants that express octopine synthase from tobacco crown galls after deletion of tumour-controlling functions. — *Nature 300:* 752–755

GUTTERIDGE, S., SIGNAL, I., THOMS, B., ARENTZEN, R., CORDOVA, A. and LORIMER, G. H. 1984. A site-specific mutation within the active-site of ribulose 1,5-bisphosphate carboxylase of *Rhodospirillum rubrum*. — *EMBO J. 3:* 2737–2743

HANSON, M. 1984. Stability, variation and recombination in plant mitochondria genomes via tissue culture and somatic hybridization. — *Oxford Surv. Plant Mol. Cell Biol. 1:* 33–52

HARTEN, A. M. VAN, BOUTER, H. and BROERTJES, C. 1981. In vitro adventitious bud techniques for vegetative propagation and mutation breeding of potato (*Solanum tuberosum* L.). — *Euphytica 30:* 1–8

HEINZ, D. J. and MEE, G. W. P. 1971. Morphologic, cytogenetic and enzymic variation in *Saccharum* species hybrid clones derived from callus tissue. — *Am. J. Bot. 58:* 257–262

HERRERA-ESTRELLA, L., DEPICKER, A., MONTAGU, M. VAN and SCHELL, J. 1983. Expression of chimaeric genes transferred into plant cells using a Ti plasmid derived vector. — *Nature 303:* 209–213

HOEKEMA, A., HIRSCH, P. R., HOOYKAAS, P. J. J. and SCHILPEROORT, R. A. 1983. T-region and Vir-region of the *Agrobacterium tumefaciens* Ti plasmid separated on two different plasmids are functional: possibilities for a binary plant vector system. — *Nature 303:* 179–180

JOHNSTON, A. W. B. and DOWNIE, J. A. 1984. Molecular genetics of *Rhizobium*—three recent advances. — *Oxford Surv. Plant Mol. Cell Biol. 1:* 221–224

KARP, A., NELSON, R. S., THOMAS, E. and BRIGHT, S. W. J. 1982. Chromosome variation in protoplast-derived potato plants. — *Theor. Appl. Genet. 62:* 265–272

KING, P. J. 1984. From single cells to mutant plants. — *Oxford Surv. Plant Mol. Cell Biol. 1:* 7–32

KUEH, J. S. H. and BRIGHT, S. W. J. 1982. Biochemical and genetical analysis of three proline accumulating barley mutants. — *Plant Sci. Lett. 27:* 233–241

LARA, M., CULLIMORE, J. V., LEA, P. J., MIFLIN, B. J. JOHNSTON, A. W. B. and LAMB, J. W. 1983. Appearance of a novel form of plant glutamine synthetase during nodule development in *Phaseolus vulgaris* L. — *Planta 157:* 254–258

LARKIN, P. J. and SCOWCROFT, W. R. 1981. Somaclonal variation—a novel source of variability from cell cultures for plant improvement. — *Theor. Appl. Genet. 60:* 197–214

LARKIN, P. J. and SCOWCROFT, W. R. 1983. Somaclonal variation and crop improvement. — In *Genetic Engineering of Plants* (*Eds.* T. KOSUGE, C. P. MEREDITH *and* A. HOLLAENDER), *Plenum, N.Y.*, p. 355–370

LARKINS, B. A. 1983. Genetic engineering of seed storage proteins. — In *Genetic Engineering of Plants* (*Eds.* T. KOSUGE, C. P. MEREDITH *and* A. HOLLAENDER), *Plenum, N.Y.*, p. 93–118

MALIGA, P. 1984. Isolation and characterization of mutants in plant cell culture. — *Annu. Rev. Plant Physiol. 35:* 519–542

MARTON, L., WULLEMS, G. J., MOLENDIJK, L. and SCHILPEROORT, R. A. 1979. In vitro transformation of cultured cells from *Nicotiana tabacum* by *Agrobacterium tumefaciens*. — *Nature 277:* 129–131

MATERN, U., STROBEL, G. and SHEPARD, J. 1978. Reaction to

phytotoxins in a potato population derived from mesophyll protoplasts. — *Proc. Natl. Acad. Sci. USA 75:* 4935–4939

MCFARLAND, B. L. and GILCHRIST, D. G. 1982. Synergistic inhibition of tomato aspartate transcarbamylase by UMP and host selective toxins produced by *Alternaria alternata* F. sp. *lycopersici* in culture and in tomato plants. — *Physiol. Plant Pathol. 23:* 265–274

MCNALLY, S. F., HIREL, B., GADAL, P., MANN, F. and STEWART, G. R. 1983. Glutamine synthetase of higher plants. Evidence for specific isoform content related to their possible physiological role and their compartmentation within the leaf. — *Plant Physiol. 72:* 22–25

MELCHERS, G., SACRISTÁN, M. D. and HOLDER, A. A. 1978. Somatic hybrid plants of potato and tomato regenerated from fused protoplasts. — *Carlsberg Res. Commun. 43:* 203–218

MENZCEL, L., NAGY, F., KISS, Z. and MALIGA, P. 1981. Streptomycin resistant and sensitive somatic hybrids of *Nicotiana tabacum* and *Nicotiana knightiana:* Correlation of resistance of *N. tabacum* plastids. — *Theor. Appl. Genet. 59:* 191–195

MIFLIN, B. J. and CULLIMORE, J. V. 1984. Nitrogen assimilation in the legume—*Rhizobium* symbiosis: a joint endeavour. — In *Advances in Plant Gene Research. Vol. I* (*Eds.* D. P. S. VERMA *and* T. HOHN), *Springer-Verlag, Vienna,* p. 129–178

MIFLIN, B. J., WALLSGROVE, R. M. and LEA, P. J. 1981. Glutamine metabolism in higher plants. — *Curr. Top. Cell Regul. 20:* 1–43

MIFLIN, B. J., BRIGHT, S. W. J., ROGNES, S. E. and KUEH, J. S. H. 1983. Amino acids, nutrition and stress. The role of biochemical mutants in solving problems of corn quality. — In *Genetic Engineering of Plants* (*Eds.* T. KOSUGE, C. P. MEREDITH *and* A. HOLLAENDER), *Plenum, N.Y.,* p. 391–414

MIFLIN, B. J., FORDE, B. G., KREIS, M., RAHMAN, S., FORDE, J. and SHEWRY, P. R. 1984. Molecular biology of the grain proteins of the Triticeae. — *Philos. Trans. R. Soc. Lond. B. 304:* 333–339

NEGRUTIU, I. 1984. Isolation of amino acid requiring lines by negative selection in haploid protoplast of *Nicotiana plumbaginifolia (viviani).* — *Experientia Suppl. 45:* 158–159

NIELSEN, N. C. 1984. The chemistry of legume storage proteins. — *Philos. Trans. R. Soc. Lond. B. 304:* 287–296

OOMS, G., HOOYKAAS, P. J., MOLEMAN, G. and SCHILPEROORT, R. A. 1981. Crown gall plant tumors of abnormal morphology, induced by *Agrobacterium tumefaciens* carrying mutated octopine Ti plasmids; analysis of T-DNA functions. — *Gene 14:* 33–50

OOMS, G., KARP, A. and ROBERTS, J. 1983. From tumour to tuber; tumour cell characteristics and chromosome numbers of crown-gall derived tetraploid potato plants (*Solanum tuberosum*) or Maris Bard. — *Theor. Appl. Genet. 66:* 169–172

ORTON, T. J. 1979. Haploid barley regenerated from callus cultures of *Hordeum vulgare* × *H. jubatum.* — *J. Hered. 71:* 780–782

OTTEN, L., GREVE, H. DE, HERNALSTEENS, J. P., MONTAGUE, M. VAN, SCHIEDER, O., STRAUB, J., and SCHELL, J. 1981. Mendelian transmission of genes introduced in plants by the Ti plasmids of *Agrobacterium tumefaciens.* — *Mol. Gen. Genet. 183:* 209–213

PELLETIER, G., PRIMARD, C., VEDEL, F., CHETRIT, P., REMY, R., ROUSELLE, P. and RENARD, M. 1983. Intergeneric cytoplasmic hybridization in Cruciferae by protoplast fusion. — *Mol. Gen. Genet. 191:* 244–250

POWER, J. B., BERRY, S. F., CHAPMAN, J. V. and COCKING, E. C. 1980. Somatic hybridization of sexually incompatible petunias: *Petunia parodii, Petunia parviflora.* — *Theor. Appl. Genet. 57:* 1–5

RICCIARDI, R. P., MILLER, J. S. and ROBERTS, B. E. 1979. Purification and mapping of specific mRNAs by hybridization-selection and cell-free translation. — *Proc. Natl. Acad. Sci. USA 76:* 4927–4931

ROBERTSON, J. G., FARNDEN, K. J. F., WARBURTON, M. P. and BANKS, J. M. 1975. Induction of glutamine synthetase during nodule development in lupin. — *Aust. J. Plant Physiol. 2:* 265–272

RYAN, C. A. 1983. Wound-regulation synthesis and compartmentation of proteinase inhibitors in plant leaves. — In *Structure and Function of Plant Genomes NATO ASI Series A Vol. 63* (*Eds.* O. CIFFERI *and* L. DURE), *Plenum, N.Y.,* p. 337–346

SCHIEDER, O. and VASIL, I. K. 1980. Fusion and somatic hybridization. — In *Perspectives in Plant Cell and Tissue Culture. Int. Rev. Cytol. Suppl. 11 B* (*Ed.* I. K. VASIL), *Academic Press, N.Y.,* p. 21–46

SCHRÖDER, G., KLIPP, W., HILLBRAND, A., EHRING, R., KONCZ, C. and SCHRÖDER, J. 1983. The conserved part of the T region in Ti plasmids expresses four proteins in bacteria. — *EMBO J. 2:* 403–409

SCHRÖDER, G., WAFFENSCHMIDT, S., WEILER, E. W. and SCHRÖDER, J. 1984. The T-region of Ti plasmids codes for an enzyme synthesizing indole-3-acetic acid. — *Eur. J. Biochem.* (in press)

SECOR, G. A. and SHEPARD, J. F. 1981. Variability of protoplast derived potato clones. — *Crop Sci. 21:* 102–105

SHAW, C. H. 1984. Ti-plasmid-derived plant gene vectors. — *Oxford Surv. Plant Mol. Cell Biol. 1:* 211–216

SHAW, D. M., HIGHTOWER, R. C. and MEAGHER, R. B. 1982. Complete nucleotide sequence of a soybean actin gene. — *Proc. Natl. Acad. Sci. USA 79:* 1022–1026

SHEPARD, J. F. 1981. Protoplasts as sources of disease resistance in plants. — *Annu. Rev. Phytopathol. 19:* 145–166

SHEPARD, J. F., BIDNEY, D. and SHAHIN, E. A. 1980. Potato protoplast in crop improvement. — *Science 208:* 17–24

SHEWRY, P. R., MIFLIN, B. J., FORDE, B. G. and BRIGHT, S. W. J. 1981. Conventional and novel approaches to the improvement of the nutritional quality of cereal and legume seeds. — *Sci. Prog. 67:* 575–600

SILER, D. J. and GILCHRIST, D. G. 1983. Properties of host-specific toxins produced by *Alternaria alternata* F. sp. *lycopersici* in culture and in tomato plants. — *Physiol. Plant Pathol. 23:* 265–274

SIMMONDS, N. W. 1983. Plant breeding: the state of the art. — In *Genetic Engineering of Plants* (*Eds.* T. KOSUGE, C. P. MEREDITH *and* A. HOLLAENDER), *Plenum, N.Y.,* p. 355–370

SLIGHTOM, J. L., SUN, S. M. and HALL, T. C. 1983. Complete nucleotide sequence of a French bean storage protein gene: Phaseolin. — *Proc. Natl. Acad. Sci. USA 80:* 1897–1901

SOMERVILLE, C. R. 1984. The analysis of photosynthetic carbon dioxide fixation and photorespiration by mutant selection. — *Oxford Surv. Plant Mol. Cell Biol. 1:* 103–131

SOMERVILLE, C. R. and OGREN, W. L. 1982. Genetic modification of photorespiration. — *Trends Biochem. Sci. 7:* 171–174

SREE RAMULU, K., DIJKHUIS, P. and ROEST, S. 1983. Phenotypic variation and ploidy levels of plants regenerated from protoplasts of tetraploid potato. — *Theor. Appl. Genet. 65:* 329–338

STEWART, G. S. and LAHRER, F. 1980. Accumulation of amino acids and related compounds in relation to environmental stress. — In *The Biochemistry of Plants Vol. V. Amino Acids and Derivatives* (*Ed.* B. J. MIFLIN), *Academic Press. N.Y.,* p. 609–636

STROM, A. R., LERUDULIER, D., JAKOWEC, M. W., BUNNELL, R. C. and VALENTINE, R. C. 1983. Osmoregulatory (osm) genes and osmoprotective compounds. In *Genetic Engineering of Plants* (*Eds.* T. KOSUGE, C. P. MEREDITH *and* A. HOLLAENDER), *Plenum, N.Y.,* p. 39–60

SUN, S. M., SLIGHTOM, J. L. and HALL, T. C. 1981. Intervening sequences in a plant gene: comparison of the partial sequence of

cDNA and genomic DNA of French bean phaseolin. — *Nature 293:* 37–41

THOMAS, E., BRIGHT, S. W. J., FRANKLIN, J., GIBSON, R., LANCASTER, V. and MIFLIN, B. J. 1982. Variation amongst protoplast-derived potato plants (*Solanum tuberosum* cv. Maris Bard). — *Theor. Appl. Genet. 62:* 65–68

TOPPAN, A., ROBY, D. and ESQUERRE-TUGAYE, M.-T. 1982. Cell surfaces in plant-microorganism interactions. — *Plant Physiol. 70:* 82–86

VERMA, D. P. S. and LONG, S. 1983. The molecular biology of *Rhizobium*-legume symbiosis. — *Int. Rev. Cytol. Suppl. 14:* 211–245

WEINAND, U., SOMMER, H., SCHWARZ, Zs., SHEPHERD, N., SAEDLER, H., KREUZALER, F., RAGG, H., FAUTZ, E., HAHLBROCK, K., HARRISON, B. and PETERSON, P. A. 1982. A general method to identify plant structural genes among genomic DNA clones using transposable element induced mutations. — *Mol. Gen. Genet. 187:* 195–201

WHEELER, V. A., EVANS, N. E., FOULGER, D., WEBB, K. J. KARP, A. and BRIGHT, S. W. J. 1984. Shoot formation from explant cultures of potato and an examination of chromosomal and morphological variation in the regenerated plants. — *Ann. Bot.* (submitted)

WIBORG, O., HYLDIG-NIELSEN, J. J., JENSEN, E. O., PALUDAN, K. and MARCKER, K. A. 1982. The nucleotide sequence of two leghaemoglobin genes from soybean. — *Nucl. Acid Res. 10:* 3487–3494

WILKINSON, A. J., FERSHT, A. R., BLOW, D. M., CARTER, P. and WINTER, G. 1984. A large increase in enzyme affinity by protein engineering. — *Nature 307:* 187–188

WULLEMS, G. J., MOLENDIJK, L., OOMS, G. and SCHILPEROORT, R. A. 1981. Retention of tumor markers in F_1 progeny plants from in vitro induced octopine and nopaline tumor tissues. — *Cell 24:* 719–727

WYN JONES, R. G. and GORHAM, J. 1983. Aspects of salt and drought tolerance in higher plants. — In *Genetic Engineering of Plants* (*Eds.* T. KOSUGE, C. P. MEREDITH *and* A. HOLLAENDER), *Plenum, N.Y.,* p. 355–370

YEUNG, E. C., THORPE, T. A. and JENSEN, C. J. 1981. In vitro fertilization and embryo culture. — In *Plant Tissue Culture* (*Ed.* T. A. THORPE), *Academic Press, N.Y.,* p. 253–271