apples, e.g. Gala, continues.

Plantibodies. Animal genes encoding antibodies to several bacteria, viruses and fungi have been successfully expressed in plants (Hiatt *et al.*,1989). Since bacteria have a very wide range of epitopes for antibody production, many of which are common to non-pathogens, selection of the epitope is critical for successful development of resistance. Specific enzymes produced by pathogens which are essential for pathogenicity are good targets for plantibodies. It is necessary, however, to consider that the plantibody must be transported to the site of infection on the surface of cells so that the pathogenic enzyme can be inactivated before it can carry out its function. Antibodies to host pathogen- receptor molecules also have the potential to induce resistance.

Antisense genes. There is one record (Walter, 1991) of an attempt to engineer resistance in grapevine to pathogenic agrobacteria, *A. tumefaciens* and *A. vitis*, by introducing *A. tumefaciens* oncogenes in an antisense form. The results are not recorded.

9.2.5   Gene transfer with *Agrobacterium* spp

One basic assumption when using plant pathogenic bacteria such as *Agrobacterium* spp. to introduce genes into plant tissue is that once the plant genome has been transformed, the vector bacterium can be eradicated. This is necessary not only to prevent disease (although such strains have usually been disarmed) but also to prevent the binary vector meeting wild type agrobacteria and transfering genetic information thus allowing gene escape. Recent but as yet unpublished work indicates that conventional antibiotics often fail to eradicate *Agrobacerium spp* from genetically engineered plants, and that the binary vector itself can survive for many months. Although the bacterium is unlikely to be transmitted through seed from one generation of plants to the next, this is an aspect of genetic modification that must be studied more closely and the risks eliminated if such modifications are to be acceptable. Vector elimination should not simply be judged by lack of tumorigenic or rhizogenic activity, but tested for by using appropriate genetic probes. Little epidemiological work on agrobacteria, their latency and disease expression has been done as they are not often of economic importance, and are ususaly only seen as causing quality problems in ornamental plants.

9.3   DISCUSSION

The development of the R gene- and avr gene-engineered resistance has occurred as a result of painstaking research on the genetics of the host-pathogen interaction. Further development in this area could be limited unless more is known of the gene products and the way they interact to cause or prevent the hypersensitive reaction. Likewise, it will be difficult to incorporate genetic resistance mechanisms other than those involved in the gene-for-gene mechanism, unless more is known about the way they work in the plant. Hence there is currently much research on the types and rates of accumulation of pathogenesis-related proteins, peroxidases and hydrolytic enzymes in resistant and susceptible cultivars. Implicit in this research is the elucidation of the regulatory factors involved. One potential "growth" area is in the investigation of the sources of active oxygen production and membrane-bound oxidase activity which appear to be involved in the response of the plant to pathogens.

Another way to identify the essential components of resistance is to produce susceptible mutants in plants which are resistant to a specific pathogen (Salmeron *et al.*,1994). However, it must be recognised that success will be most likely in plants with smaller genomes such as *Arabidopsis* (Dangl *et al.*, 1992; Dangl *et al.*, 1993; Kunkel *et al.*, 1993), or with plants whose host-pathogen genetics is well known such as tomato (Martin *et al.*, 1993; Salmeron *et al.*, 1994).

Another key feature that will undoubtedly continue to be at the forefront of engineering resistance is to ensure that the resistance factor is made available at the site where it is best needed. Genes are now being inserted in potato, for example under the control of the 35S promoter of Cauliflower mosaic virus. Tissue specific promoters and sequences for signal peptides can be combined with "resistance" genes to give chimeric genes. These will ensure that gene products, e.g. lysozyme, will be produced in appropriate tissues and directed into cellular compartments or intercellular spaces where they are required.

Methods of insertion of genes of interest continue to be improved. For most current bacterial resistance work, transformation is usually based on *Agrobacterium tumefaciens* or *A. rhizogenes* but the risks of using this system may be more tightly regulated and researchers may choose alternative methods such as particle bombardment, or electroporation.

Durability of resistance is seen as a potential problem, more so for some methods than others. For example, a very minor mutation in a plantibody could render the resistance useless. R/avr gene resistance mechanisms could relatively easily be overcome. Now that engineering is more straightforward, it will be possible to incorporate two or more very different resistance mechanisms, for example a specific and a general mechanism. Durability would almost certainly be improved.

Resistance should not be considered an absolute requirement. Initial results show that tolerance is probably a more achievable goal, in which symptom expression is delayed, and severity is reduced. For diseases of quarantine significance such tolerance may not be so acceptable. Engineered disease resistance is likely to boom over the next decade and plant health authorities will almost certainly be under pressure from the international trade to review the status of certain diseases and the statutory regulations by which they are currently controlled.

One major area of work which must not be forgotten is the rigorous testing for *Agrobacterium* in engineered plant material. In the early period of release, it may be necessary for independent screening to be carried out rather than leave responsibility for this with the developers especially where they may be under commercial pressures. The seemingly relatively frequent accidental release of viable binary vector agrobacteria which appear to be free to exchange genetic information with wild type agrobacteria should prove a timely lesson.

One very recent critique of the gene revolution (Schmidt, 1995) indicates that no transgenic crops resistant to bacterial diseases are currently on their way to the market place. Of seven cases cited, three involve pest/disease resistance. Further commercial

development may well depend on the success of these examples which, subject to USDA approval, will reach American markets in 1996.

9.4 CONCLUSIONS AND RECOMMENDATIONS

1) Bacterial pathogens are probably less destructive to UK agricultural crops than are fungi, viruses or insects. Nevertheless they cause serious losses in both growing and stored potatoes, and these would certainly be larger if statutory regulations and inspections did not prevent the introduction of bacterial diseases that are not yet established in the UK.

2) Research on the genetic manipulation of plants to produce resistance to bacterial pathogens has progressed far enough to indicate its feasibility. Some degree of resistance to specific bacteria has been induced by introducing R genes, and genes for enzymes resistant to bacterial toxins and genes for proteins toxic to bacteria derived from plants and other sources. The successful development of this work will need a better understanding of the natural processes of resistance, and of the expression and activity of introduced genes.

3) Most of the research work done on engineering resistance to bacteria has been done overseas. However, there are strong basic research programmes on pathogenic bacteria and their interactions with plants in UK Institutes (notably the Sainsbury Laboratory) and Universities.

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10.   POTENTIAL MANIPULATION OF CROP RHIZOSPHERE

10.1   BACKGROUND

That distinctive rhizosphere microflora are associated with particular crops have been known for many years (Phillips & Streit, 1994), although its significance is not clear. Different soil types, and the age of the plant have an effect. However, rhizodeposition from plants, particularly the soluble root exudates, must be the major factor which influences the soil microbiota (Lynch, 1990). It is well established that signal molecules in root exudate play an important part in the association between symbiotic rhizobia and their leguminous plant hosts, and there is some preliminary evidence that plant pathogens may be stimulated by specific components of root exudates (Nelson, 1990, Phillips & Streit, 1994). The signal molecules from susceptible plants that act as nematode hatching factors are another well-known example. Whether the normal commensal rhizoflora of a healthy plant is attracted by specific signals or by the distinctive combination of nutrients in exudates needs further research. In the plants that have been studied, the major components of exudate are sugars, organic acids, amino acids and vitamins, with lower levels of more unusual molecules such as phenolics and flavonoids which are potential signal molecules.

10.2   CROPS AND TARGETS

At present there is insufficient information on the basis of particular plant-soil pathogen interactions for detailed discussion on an individual basis. Crops and their major soil pathogens are listed elsewhere, therefore the topic will be treated as a general case.

10.3   PROSPECTS

Manipulation of plant physiology to alter root exudation could take two paths. Firstly, the synthesis and exudation of specific signal molecules which is known to attract pathogens and pests could be reduced or eliminated by genetic manipulation. However, this may not be feasible because signal compounds may also have a dual role and also attract beneficial organisms. The second approach would be to stimulate exudation of the factors that attract beneficial and commensal organisms that compete with pathogens.

Beneficial organisms include those that improve plant growth, e.g. though improving plant nutrition (rhizobia, Vesicular arbuscular mycorrhizal fungi), and those which stimulate or modify root growth by production of phytohormones (Gareth Jones, 1993). A wide variety of plant growth-promoting rhizobacteria (PGPRs) have been reported including rhizobia, azospirilla and pseudomonads (Schippers *et al.,* 1990). Another group of beneficial organisms is those with biocontrol potential, although their efficacy in controlling major diseases in the field is often disputed. This may be because when they are applied to crops as seed or soil inoculants they do not survive well or compete with the native microflora to colonise roots, and do not have the efficacy demonstrated in laboratory and glasshouse trials. (Gareth Jones, 1993).

A well-known example is the ability of some rhizosphere fluorescent pseudomonads to produce phenazine antibiotics which strongly inhibit the take-all fungus of cereals, *Gaeumannomyces graminis* in laboratory culture (Thomashow & Weller, 1990). The application of such strains as biocontrol agents has not been proven in field trials and remains controversial. However, the phenomenon of take-all decline may be due to the build-up of bacterial populations with the ability to inhibit *G. graminis* in soils with long-term cereal cultivation. Fluorescent pseudomonads isolated from soil have also been shown to produce 2,4-diacetylphloroglucinol, an antibiotic that inhibits both damping-off fungi and *G. graminis* (Bangera *et al.*, 1994). Other pseudomonads appear to generate hydrogen cyanide which can control other microbes in the rhizosphere, and bacteria which secrete chitinases that have anti-fungal activity have also been described (Schippers *et al.*, 1990).

Other beneficial rhizosphere bacteria have been found to produce siderophores that chelate iron (Chet *et al.*, 1990). This is essential for microbial growth but is present in relatively low amounts in the rhizosphere. If the commensal rhizoflora removes iron from the rhizosphere, whether by siderophore production or by less efficient methods, it is not available for opportunist pathogens. This represents one mechanism by which the normal rhizoflora of a crop protect roots from pathogens by "niche exclusion". Vesicular arbuscular mycorrhizal fungi may play a similar role (in addition to improving phosphate nutrition) by colonizing and penetrating the regions of the root susceptible to infection, excluding other fungi. The protection may be purely physical, but there is some evidence that a degree of immunity to further fungal infection is induced in the plant (Gareth Jones *et al.*, 1993).

Seeds may also produce signal molecules or specific attractants during germination. Several volatile compounds from germinating seeds and root tips which stimulate the germination of pathogenic fungal spores have been described (Nelson 1990). Betaines such as trigonelline are found in many seeds where they protect against dessication, and induction of rhizobial symbiotic genes by trigonelline has been shown (Phillips & Streit, 1994).

If mechanisms by which beneficial soil microbes are attracted to crop roots can be manipulated, inefficient inoculation of seeds or soil will be unnecessary. Instead, the indigenous microflora which is well-adapted to local soil conditions should provide a rhizoflora that protects the plants from soil pathogens.

10.4   CONCLUSIONS AND RECOMMENDATIONS

1) Factors in root exudates influence the rhizoflora of different crops. Both beneficial and pathogenic organisms may be attracted by factors in exudate but if the roots are colonised initially by the numerous innocuous and beneficial soil microbes, opportunities for subsequent invasion by opportunist root pathogens will be reduced.

2) Very little is known at present about which specific factors are responsible for attracting soil microbes to roots. However, there is a possibility that plants could be manipulated to modify exudate composition, to enhance colonization by beneficial and innocuous microbes, or to cease to attract undesirable organisms. A prerequisite is identification of key exudate components which implies prior identification of innocuous and beneficial rhizosphere microorganisms which are competitive root colonisers.

3) There is an attractive long-term prospect of manipulating plants so that their root exudates create a more favourable rhizosphere either by not attracting pathogens, or by encouraging beneficial or competing microorganisms.

4) Before this prospect is possible and the problems of genetic manipulation of plants are faced, much more basic information needs to be known about the microflora which characterize crops, the beneficial and damaging microorganisms identified, as well as the components of root exudates to which they respond.

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APPENDIX I

QUESTIONNAIRE ON TARGETS FOR THE INTRODUCTION OF PEST AND DISEASE RESISTANCE INTO CROPS BY GENETIC MANIPULATION

1. Do you think that it will be possible to make agricultural plants "field resistant" to important pests and pathogens by the techniques of genetic manipulation?

2. If so, which plant/pathogen combinations ought for agricultural reasons to be the prime subjects of research?

3. Which plant/pathogen combinations do you think can be manipulated; a) currently b) within 2 years c) within 5 years?

4. By what means do you think resistance can best be achieved in these systems? (e.g. Tobacco plants can be made resistant to TMV by the insertion of part of the TMV genome that codes for viral coat protein.) Please indicate which, if any, of these systems are objects of study in your laboratory?

4. continuation space

5. Are you unable to answer these questions openly because of commercial-restrictions or agreements?

6. Do you think that resistance conferred by genetic manipulation will be quantitatively or qualitatively different in field conditions from the "natural" resistance manipulated by traditional plant breeding techniques?

7. Do you think that genetically-manipulated, resistant plants will present any sort of agricultural or environmental hazard?