THE RELEVANCE OF NEW APPROACHES TO BREEDING FOR DISEASE RESISTANCE — PROSPECTS AND PRIORITIES FOR APPLE AND PEAR BREEDING

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ABSTRACT

To justify the necessary long term programmes, resistance breeding should concentrate on the diseases of greatest economic importance. Priorities should be carefully assessed paying due regard to alternative methods of disease control.

High levels of stable monogenic resistance can be introduced to cultivated varieties from wild species if special care is taken to ensure that such genes are accompanied by background genes to ensure expression over a range of conditions.

Gene transfer and cell transformation techniques present further possibilities of introducing genes for resistance from a wider range of wild species. These techniques, together with mutation breeding and somaclonal selection, are invaluable means of improving the potential of crop breeding materials for disease resistance. However, only rarely will single step improvements remove all the limitations of established varieties. It is therefore important for plant breeding to proceed on a broad front using genetic manipulation techniques principally to improve the genetic resources of crop genera.

Recently developed greenhouse and detached shoot selection techniques promise to be supplemented by *in vitro* culture selection procedures, thus enabling rapid and efficient screening of plants as cell and tissue cultures.

The potential of these approaches for increasing the efficiency of plant breeding will be discussed using examples from apple and pear breeding.

KEYWORDS

Gene transfer, mutation, selection.

INTRODUCTION

Breeding for disease resistance is a prime means of reducing crop losses and production costs. The use of resistant varieties in the poorer regions of the world enables crops to be grown without fungicides. The rising costs of spray chemicals concern agriculturists in all parts of the world. Until now crops, such as fruit, which have a high unit value, have justified a relatively high expenditure on spray chemicals. For example, the total cost of fungicides and their application now amounts to 40% of the growing costs of apples in England (Steer, 1984). As consumers become increasingly concerned about spray residues in food any means of reducing residues will make an important contribution to food acceptability. Concern about residues is high in fruit crops, which although mainly consumed fresh, frequently need post-harvest dips of chemical 'cocktails' to maintain a long shelf and storage life.

Resistance to most if not all crop diseases is available amongst plant species. The incorporation of much resistance is restricted by intergeneric and interspecific fertility barriers, the long periods needed to introduce a new resistance trait into a commercial variety through several generations of backcrossing and selection, and the necessarily large scale of programmes needed to incorporate all available resistance into a crop.

When considering disease resistance in a breeding programme it is essential to assess, (a) the feasibility of achieving a satisfactory level of disease control, (b) the techniques which should be used to achieve reliability and stability efficiently and, (c) the suitability of alternative methods of disease control.

It is difficult to predict the reliability of a new source of resistance or the risk of new physiological races arising. Analogies between monogenically-determined resistance and instability and polygenically-determined resistance and stability can be very misleading (Russell, 1978). Both types of resistance may be durable but full assessments of durability can only be made after resistant varieties have been grown for long periods (Johnson, 1983). In long-lived perennial crops the breeder must provide for the possibility of resistance breakdown by incorporating more than one type of resistance.

Several new techniques present prospects for transferring genes for high resistance from distantly related species and the production of resistant clones from established varieties. These techniques are relevant to the production of disease resistant apple and pear varieties and must be considered in close association with established procedures.

TREE FRUIT BREEDING METHODS

In tree fruits, the random mating of superior phenotypes followed by mass selection might be considered the most effective means of increasing the number of favourable alleles since there is a relatively high additive variance governing the inheritance of most traits. However, several characters including disease resistance, are determined by single genes or small groups of genes. This requires careful matching of parents according to genotype.

It is customary to select individuals which are exceptionally outstanding members of a progeny, exhibiting an heterotic type of effect in the F_1 generation. Clonal propagation of such elite selections allows the full exploitation of all genetic variance and permits the establishment of uniform stands of the best selections. This largely off sets the slow pace of genetic gain — a consequence of the long juvenile phase of most tree crops (Alston and Spiegel-Roy, 1985).

BREEDING PRIORITIES

It is frequently impossible to include all available resistance in a breeding programme since due attention must be given to the main breeding aims, usually yield and quality. Present priorities for apple and pear were chosen after considering principles proposed for all fruit crops (Alson *et al.*, 1986) which have an economic base related to: cost of the breeding programme; potential for reducing growing costs; or increasing market value. The greatest gains should arise from the introduction of varieties with resistance to diseases which are, (a) expensive to control chemically, (b) difficult to control chemically, or, (c) for which chemical control presents spray residue problems. Fungal foliar diseases are expensive to control chemically, accounting for the major part of the fungicide outlay in apples whilst virus diseases cannot be controlled chemically and root and shoot diseases are difficult to control in that way. Many countries ban the use of post-harvest dips as a means of controlling fruit rotting.

Priorities chosen for apples are resistance to mildew (*Podosphaera leucotricha*), scab (*Venturia inaequalis*) and storage diseases caused by *Gloeosporium* sp., *Phytophthora cactorum*, and *Nectria galligena*. In pears only one disease merits special treatment, fireblight (*Erwinia amylovora*).

THE POTENTIAL OF RESISTANCE

Most new appled varieties carry a higher level of mildew resistance than older established varieties. This has been achieved through stringent field selection as early as two or three years from germination. Such resistance, which is polygenically determined and found only in a small proportion of plants, is only rarely sufficient to provide full escape from the disease. However, recent work on mildew thresholds shows that this type of resistance can keep infection at a sufficiently low level to allow an economic crop in the absence of sprays (Jeger and Butt, 1983). Not all varieties showing low mildew levels produce economic crops without sprays, since varieties differ in their damage thresholds (Butt *et al.*, 1983a). The complete elimination of mildew by sprays can be very beneficial to susceptible varieties like Cox, but is very expensive and would normally require more than the 17 spray applications commonly applied during the English growing season. The same effect, the elimination of secondary mildew, has been achieved by transferring resistance determined by two dominant genes from *Malus* species (Alston, 1983a). This resistance has remained stable in unsprayed orchards for more than 20 years. Special care has been taken to combine this simply inherited resistance with polygenic resistance to improve the prospect of durability.

The combining of two types of resistance has also been successfully followed in breeding for scab resistance. Alone, the key gene V_f from *Malus floribunda* has only a moderate effect on fungal growth; full resistance only occurs with the addition of polygenes (Rouselle *et al.*, 1975). This resistance has remained stable for more than 40 years and is now available in several new varieties. The value of this resistance has been demonstrated in unsprayed orchards which have a high natural infection and in glasshouse tests on potted trees (Jeger and Alston, 1985).

The main approach with storage diseases of fruit is to select varieties where the fruit remains sound in the absence of pre-harvest sprays or post-harvest dips, criteria largely achieved by the new East Malling varieties.

Fireblight has destroyed commercial pear growing over wide areas of North America and is now threatening important production areas in Europe. Secondary blossoms are the prime infection sites. Selection for the absence of secondary blossoms is therefore an important aim; it can be achieved concurrently with selection for precocity and heavy regular cropping (Alston, 1983b). In spite of the elimination of varieties prone to secondary blossoms in England, locally disastrous outbreaks can occur thus emphasising the necessity for new varieties with a high level of tissue resistance. The prospects of breeding such varieties are good. Very high levels of resistance (possibly under simple genetic control) are available in *Pyrus* species, but equally high levels of resistance are available from the cultivated pear (polygenically inherited) combined with good fruit quality. The most promising results so far are the new varieties released in Canada, but more recently resistant pears have entered advanced trials from the East Malling breeding programme.

Strategically it is wise to regard disease resistance in long-lived perennial crops such as tree fruits, as a valuable bonus in new varieties bred primarily for improved yield and quality. If resistance eventually breaks down disease can be controlled by other methods but the grower and consumer will still benefit from improved high quality varieties.

GENE TRANSFERENCE

The principal genes for scab and mildew resistance in apple have been transferred from small-fruited *Malus* species by backcrossing. A different cultivated species parent is used in each generation to avoid excessive inbreeding and concurrently to introduce new commercial features. With mildew resistance, first crosses at East Malling were made in 1963 and by 1980 (at the third backcross) precocious heavy cropping selections were produced combining mildew resistance with good commercial quality (Alston, 1983a) During the same period, attempts to transfer the very high level of mildew resistance present in *M. hupehensis* (a triploid apomict) were not successful; all diploid derivatives showed susceptibility.

New techniques now being developed in fruit crops promise to improve the efficiency of transfer of single and multigenically-determined characters between distantly related species. Protoplast fusion and gene transformation techniques using specific DNA vectors are particularly promising. These techniques have the potential to introduce key genes controlling specific enzyme systems. At present plant resistance mechanisms are not sufficiently understood to distinguish significant gene products. There are possibilities that already identified alleles which govern enzyme systems controlling plant growth may, by *in vitro* manipulation, be used to modify plant disease response.

The transfer of fragments of DNA from one genotype to another (Pandey, 1978; Power *et al.*, 1983) following pollination by irradiated pollen, presents a prospect of dispensing with lengthy backcrossing programmes for gene transference, and of transferring genes between inter-sterile species. This technique is being used in another attempt to transfer the mildew resistance of *M. hupehensis* to the cultivated apple. First results in 1985 at East Malling were disappointing; fruit set from irradiated pollen contained only seeds without embryos and were shed during mid-summer. Although there is some doubt about the value of this approach to plant breeding (Chyl and Sanford, 1985) there is insufficient evidence to justify the cessation of work at this stage. Satisfactory irradiation doses for apple pollen have not yet been determined; the first experiments were on a small scale. Pollination needs to be on a large scale, because transformed embryos may only be expected in very small proportions of which much smaller proportions might be expected to carry resistance.

CLONAL SELECTION

The selection of improved clones resulting from natural mutations has had an important role in improving and maintaining established varieties of apple and pear in cultivation. This has encouraged work on inducing more mutations by irradiation and chemical means. The mutants produced are mainly chimaeric and are usually the result of indiscriminate chromosome breakage rather than point mutations. Such mutations can be accompanied by many deleterious effects, thus emphasising the need for long selection and stabilisation periods after irradiation. Mutagenic agents usually alter genes already present by exchanging base pairs or by altering the base pair sequence. Thus the primary gene product is changed resulting in a modification of the phenotypic expression of the gene concerned.

The development of somaclonal selection techniques presents further possibilities for introducing disease resistant clones. Clones of potato resistant to *Alternaria solani* have been isolated by this method (Matern *et al.*, 1978). Success depends on efficient *in vitro* selection techniques and, a satisfactory callus culture system, since much of the variation appearing amongst micropropagated shoots is cryptic and reverts to normal after one or two season's growth. These techniques are labour intensive and require a high degree of sophistication in the laboratory and nursery for effective application. Thus, this approach should be confined to the development of disease resistance in high value, vegetatively propagated crops which owe their place in commerce to a unique genotype, as may be the case with some apple and pear varieties.

Specific morphological features can have secondary effects on disease development in selected clones. In apples for example the extra layer of leaf palisade cells present in spur-type mutations (Arasu, 1968) provides resistance to mildew.

SELECTION TECHNIQUES

A number of rapid early selection techniques have been developed for apples and pears, including seed-tray tests using spore suspension sprays for scab and mildew, and flooding for crown rot (*Phytophthora cactorum*). Results from these tests correlate well with subsequent field performance. In the field, mildew resistant derivatives of *Malus robusta* show varying degrees of necrosis on the underside of leaves. The amount of necrosis is related to the degree of polygenic resistant carried along with the key resistant genes. Selection for a low incidence of necrosis maximises the polygenic resistance component.

Due to the risk of transferring infected plant material to disease-free orchard sites, it is often disadvantageous to infect test plants in the field or glasshouse. Detached shoot tests have been developed to overcome such problems with fireblight and crown rot. Toxin-based selection techniques are also promising in this respect, notably in selection for resistance to *Alternaria mali* in apple, where there is a very strong correlation between plant reaction to AM toxin I and to the pathogen (Kohmoto *et al.*, 1977).

The fireblight toxin amylovorin produces similar symptoms in pear to those produced by live inoculum and indeed progeny differences can be observed. However, other evidence suggests that this toxin is non-selective in apple (Beer and Aldwinkle, 1976). This approach to fireblight resistance selection needs further study using a range of toxin dose rates. Toxins have an important potential role in *in vitro* selection of the products of somaclonal variation and mutagenisis.

Isozymic analytical techniques promise to be useful in resistance screening, where resistance genes are linked to isozymic alleles (Risk and Fobes, 1974). Success depends on locating very close linkages, otherwise large complements of deleterious genes may also be retained, linkages which will need to be broken in later generations. In apple, where many isozymic loci are known (Chevreau, 1984; Maganaris, 1985), there is potential for early identification of commercial-type genotypes in backcrossing programmes, resulting in a shortening of the breeding cycle. At East Malling attempts are also being made to relate these loci to those known to control disease resistance. Such developments towards linkage maps in apple are of fundamental significance for future genetic manipulation work.

More complex metabolic profiling techniques, using mass spectrometry and component analysis, have shown promise as aids to identifying pest resistant blackcurrant plants at an early stage (Austin *et al.*, 1983). Initial studies suggest that the technique could be developed to use conventional gas liquid chromatography equipment, which would allow rapid analysis of large populations.

CONCLUSIONS

A wide range of new breeding approaches is available and apple and pear breeders must decide the order of priority on the basis of feasibility and commercial need.

The durability and stability of resistance is difficult to assess without long-term orchard experience, it is therefore important to combine more than one type of resistance, as with mildew and scab in apple.

Many of the genetic manipulation techniques, mutation breeding, gene transference with DNA vectors, and somaclonal selection, are aimed essentially at introducing single genes or parts of genes into a genotype which can be substantially damaged and, as a result cause deleterious effects on other characters, including fruit quality. This is also a significant problem amongst naturally occurring mutants (Smith and Stow, 1985), and necessitates extensive selection periods before commercial introduction. Genetic improvement should be maximised by incorporating resistance in a crossing programme aimed at achieving improvements on a wide front, including quality and yield. In England, apples need such improvements even more than disease resistance, thus a genetic manipulation programme with resistance as the sole aim would be of limited value. With fireblight resistance in pears however, where the future of the crop in Southern Europe is imminently threatened, there is a strong case for using somaclonal and mutation breeding techniques to produce resistant clones of the established varieties.

Isozymic identification and tagging of specific loci has potential as a means of distinguishing plants carrying the universal scab resistance genes from those that carry resistance to specific strains only; at present progeny tests are necessary to confirm the presence of these genes.

Many technical problems remain unsolved before the most promising genetic manipulation techniques find a place in apple and pear breeding. The most significant contribution of these sophisticated techniques will be to improve the range of genetic resources available for hybridisation breeding programmes; it is envisaged that in most cases the primary products would benefit from at least one generation of crossing and selection before being introduced to commerce. Advanced techniques, such as attempts to modify plant enzyme systems by introducing bacterial DNA, should always be applied on the latest releases from breeding programmes (carrying much improved gene packages for a range of economic characters) and not on the older commercial varieties (which inevitably carry a greater number of inherent agronomic and cultural defects).

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SYMPOSIUM DISCUSSION

Dr W.J.R. Boyd, University of Western Australia

What makes you think the disease resistances will break down, and are you doing anything to develop more virulent strains to test and seek for resistance.

Alston

We are not doing anything to create more virulent strains of the pathogens.

With apple powdery mildew there have not yet been any indications of physiologic races anywhere in the world. However, there are physiologic races known of apple scab. Five that have been located so far by work dating back about 20 years. So knowing the experience with other crops, it must be wise to provide for the possibility, of resistance break down, especially considering that we are working with relatively long-lived perennials which are in the ground for 15-20 years in the modern intensive orchard.

An aspect which I have not mentioned so far is that we are first and foremost breeding varieties for improved quality and yield. Quality characteristics such as storage quality, size and skin finish and yield are the characters for which a variety will be chosen in the plantations of the future. Scab and mildew resistance will be bonuses, but important bonuses. In the event of a weakening or breakdown of resistance, orchardists will still have means of control. Control will be facilitated if there is some background resistance as well as the resistance determined by the dominant genes.

Dr W. Bushuk, University of Manitoba

What tissue did you use to analyse for the isozymes.

Alston

The cotyledons.

Mr L. Decourtye, INRA

The toxin that you mention, is it a filtrate of the culture or is it a known chemical compound?

Alston

I used the high molecular weight component of the filtrate, which I believe includes the compound amylovrin which other people have worked with on apples. I'm not sure if the chemical formula of amylovrin is known.

Dr P.J. Brook, Plant Disease Division, DSIR

What were the storage disorders you referred to?

Alston

The storage disorders that you would expect to get would be caused by *Gloeosporium, Phytophthora* and possibly *Nectria;* occasionally brown rot, but that would usually be visible before the fruit goes into store. This is an area where we are hoping to extend our work, and be more precise and scientific. All we are doing at the moment is not dipping our fruit and storing it in conditions which encourage the development of disease if inoculum is present. We are able to demonstrate, for example the contrast between storing Cox and Malling-Kent without dipping until the end of January, or between Fiesta and Cox. Without a full dipping programme, it would be almost impossible to store Cox after January or February in England — it is impossible to rely on a marketable product without a programme of dipping the fruit before it goes into the store.