Breeding virus resistant potatoes (*Solanum tuberosum*): a review of traditional and molecular approaches

RUTH M. SOLOMON-BLACKBURN* & HUGH BARKER

Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, U.K.

Tetraploid cultivated potato (*Solanum tuberosum*) is the World's fourth most important crop and has been subjected to much breeding effort, including the incorporation of resistance to viruses. Several new approaches, ideas and technologies have emerged recently that could affect the future direction of virus resistance breeding. Thus, there are new opportunities to harness molecular techniques in the form of linked molecular markers to speed up and simplify selection of host resistance genes. The practical application of pathogen-derived transgenic resistance has arrived with the first release of GM potatoes engineered for virus resistance in the USA. Recently, a cloned host virus resistance gene from potato has been shown to be effective when inserted into a potato cultivar lacking the gene. These and other developments offer great opportunities for improving virus resistance, and it is timely to consider these advances and consider the future direction of resistance breeding in potato. We review the sources of available resistance, conventional breeding methods, marker-assisted selection, somaclonal variation, pathogen-derived and other transgenic resistance, and transformation with cloned host genes. The relative merits of the different methods are discussed, and the likely direction of future developments is considered.

Keywords: breeding, host gene, potato virus, resistance, review, transgenic.

The viruses

The potato viruses discussed in this paper (and their abbreviations) are listed in Table 1. For a more comprehensive list, including symptoms, see Jeffries (1998). Most of these viruses cause yield losses, which vary between cultivars. Some also cause tuber defects, which can render the tubers unsaleable, including: spraing (necrotic arcs or rings in or on the tubers) resulting from TRV or PMTV; potato tuber necrotic ringspot disease resulting from PVY^{NTN}; net necrosis resulting from PLRV in a few cultivars and deformed tubers resulting from PSTVd. Symptom severity also depends on

whether the infection is primary (current season infection) or secondary (tuber-borne).

PLRV is probably the most damaging and widespread virus of potato and is found wherever potato crops are grown. PVY is next in importance and although it tends to cause less damage than PLRV in some cultivars, it can cause severe damage in others (Beemster & Rozendaal, 1972), and isolates of the PVY^{NTN} subgroup cause severe symptoms. PVY is also widespread, and in some parts of Europe it is more common than PLRV. The viruses PVA and PVV are related to PVY, but are less commonly reported. PVA occurs worldwide (except for the Andes) and causes symptoms that range from very mild to fairly severe. PVV is relatively restricted in its distribution, and tends to cause few symptoms. PVX occurs worldwide and is also important, although its symptoms tend to be mild. However, mixed infections with PVX and other viruses are very damaging. Other viruses that we shall consider, such as PVS, PVM, TRV and PMTV, tend to be locally important. For example, PMTV seems to be restricted to areas with cooler climates such as the Andean region of South America, Canada, China, Japan and Northern Europe. TRV is prevalent in light sandy soils that favour the trichodroid nematode vectors.

*Correspondence. E-mail: rsolom@sari.sari.ac.uk

Abbreviations: AFLP, amplified fragment length polymorphism; [a/s], antisense; cDNA, complementary DNA; CP, coat protein; CPMR, coat protein-mediated resistance; CSIRO, Commonwealth Scientific and Industrial Research Organization, Australia; dsRNA, double-stranded RNA; ELISA, enzyme-linked immuno-sorbent assay; ER, extreme resistance; GM, genetically modified; HC-Pro, helper component proteinase; HR, hypersensitive resistance; ORF, open reading frame; PAP, pokeweed antiviral protein; PTGS, post-transcriptional gene silencing; QTL, quantitative trait locus; [s], sense; SSR, simple sequence repeat; TGB, triple gene block; wt, wild type.

Table 1 The potato viruses mentioned in this paper

Virus	Abbreviation	Genus	Means of transmission
<i>Potato leafroll virus</i>	PLRV	<i>Polerovirus</i>	aphid – persistent
<i>Potato virus Y</i>	PVY	<i>Potyvirus</i>	aphid – nonpersistent
<i>Potato virus X</i>	PVX	<i>Potexvirus</i>	contact
<i>Potato virus A</i>	PVA	<i>Potyvirus</i>	aphid – nonpersistent
<i>Potato virus V</i>	PVV	<i>Potyvirus</i>	aphid – nonpersistent
<i>Potato virus S</i>	PVS	<i>Carlavirus</i>	contact (aphid – nonpersistent*)
<i>Potato virus M</i>	PVM	<i>Carlavirus</i>	aphid – nonpersistent (contact†)
<i>Tobacco rattle virus</i>	TRV	<i>Tobravirus</i>	Trichodorid nematodes
<i>Potato mop-top virus</i>	PMTV	<i>Pomovirus</i>	<i>Spongospora subterranea</i>
<i>Potato spindle-tuber viroid</i>	PSTVd	<i>Pospiviroid</i>	contact (aphid‡)

* Some isolates of PVS are transmitted by aphids.

† Some isolates are transmitted by contact.

‡ PSTVd can be transmitted by aphids if encapsidated by PLRV (Querci *et al.*, 1997).

Note: PVY, PVA and PVV can also be transmitted by contact, but much less readily than PVX and PVS.

(Adams *et al.*, 1998)

PSTVd occurs in North and South America, China and parts of Eastern Europe but is considered to be non-indigenous in most of Western Europe.

The need for resistance

Potato is the world's fourth major food crop. Viruses are a serious problem, not only because of effects caused by primary infection, but also because the crop is vegetatively propagated and they are transmitted through the tubers to subsequent vegetative generations. Crop yield losses resulting from PLRV, PVY and PVX in the UK were estimated, using 1982 prices, at £30–50 million during a year of average infection (Hull, 1984). The potential net gain from using cultivars resistant to these three viruses has been estimated at 22% in Mexico (where only a quarter of ware hectareage uses certified seed) (Quaim, 1998). Yield losses attributable to PLRV worldwide have been estimated at 20 million tonnes per year (Kojima & Lapierre, 1988). Losses can take the form of reduced yield, downgrading of seed crops, and/or tuber blemishes (described above) but, in most countries, the true cost has also to be seen in terms of expensive control measures. These include seed potato production/certification schemes (with the attendant costs of roguing, crop inspections and diagnostics including the increasing use of postharvest tuber testing); the use of pesticides to kill vector aphids (for PLRV control) or nematodes (for TRV control) and other measures such as mineral oil sprays to prevent nonpersistent aphid transmission of PVY. Control of aphids and nematodes by pesticides is expensive, and likely to become more difficult with increasing insecticide resistance in aphid populations, any long-term climatic changes leading to changes in vector popula-

tions, concern about environmental effects of pesticides, and consumer concern about pesticide residues in food. The potential benefits of virus resistance are therefore great, because resistant cultivars are the most economic and environmentally acceptable way of controlling virus diseases of potato.

Nomenclature of virus resistance genes

This paper follows the conventions described in Solomon-Blackburn & Barker (2001) for resistance gene nomenclature, indicating the type and origin of resistance and the virus or strain resisted.

Types of resistance

The nomenclature for the responses of plants to virus infection was reviewed by Cooper & Jones (1983), and the types of host resistance in potato by Valkonen (1994) and Valkonen *et al.* (1996). The following are descriptions of different types of responses that occur in resistant potato plants. Most can be applied to host gene-mediated and transgenic resistance.

Extreme resistance (ER) and hypersensitive resistance (HR) are described in Solomon-Blackburn & Barker (2001). Host gene-mediated ER prevents virus multiplication at an early stage of infection and is not normally associated with cell death (Hämäläinen *et al.*, 1997; Gilbert *et al.*, 1998). HR is well studied and can be considered as a rapid defence response that results in death of a few cells (necrosis) at the site of infection which prevents the infection from spreading further (Dixon *et al.*, 1994).

Resistance to infection can be defined as when the likelihood of infection by natural means (i.e. from vector

inoculation) is reduced in resistant plants. Such resistance was called 'field resistance' by Cooper & Jones (1983). Infection resistance can also be manifested in plants that are resistant or unattractive to the vector (e.g. aphids) itself (Flanders *et al.*, 1992).

Plants with resistance to virus accumulation are infectible but the virus reaches only a relatively low concentration in the plant. The term 'moderate resistance', adopted by Valkonen *et al.* (1996) for this type of resistance, is not used here, as it could equally apply to several other types of resistance (including resistance to infection).

Resistance to virus movement can be expressed in a number of ways. For example, it has long been recognized with respect to PLRV (Hutton & Brock, 1953; Barker, 1987) that certain potato clones produce a lower percentage of virus-infected daughter tubers from an infected plant, than other clones do. This could be the result of impeded virus movement. HR could also be regarded as resistance to virus movement, because it limits spread within the plant following the initial infection.

Cooper & Jones (1983) defined tolerance as when there is little or no apparent effect on the infected plant, i.e. infected plants are symptomless or have reduced symptom expression in the presence of disease. Tolerance is not synonymous with resistance, but over the years breeders have sometimes used the two terms interchangeably and without definition which can lead to confusion. Tolerance can be better defined as resistance to disease (the effects of the pathogen), but tolerant plants are often susceptible to infection with the pathogen which multiplies extensively in the host.

Sources of resistance

Host resistance

Introduction. Potato breeding has been reviewed by Bradshaw & Mackay (1994) and potato breeding for virus resistance by Davidson (1980), Ross (1986) and Świeżyński, 1994. Sources of host resistance in *S. tuberosum* include existing cultivars and improved breeders' clones. Some clones contain multiple copies of a resistance gene (multiplex state, see below) and in others different virus resistances have been combined (Ross, 1978; Świeżyński, 1983, 1994; Dziołowska, 1986; Mendoza *et al.*, 1996; Solomon-Blackburn, 1998). These represent the efforts of a succession of breeders over many years and provide a large pool of parental material for future breeding. Known resistance genes are also available in wild species that exist in various collections around the world or are in the wild. New resistance genes are still being discovered (Barker, 1996;

Hämäläinen *et al.*, 1998) and there are, no doubt, many others as yet undiscovered.

Introduction of potato to Europe and its early decline in vigour. The history of the potato crop has been reviewed by Bradshaw & Mackay (1994) and Glendinning (1983), and by Davidson (1980) with respect to virus problems. The modern *Tuberosum* potato (*S. tuberosum* ssp. *tuberosum*) was derived from (probably two) introductions of cultivated *Andigena* (or *S. tuberosum* ssp. *andigena*) to Europe from South America in 1570. For many years, there were serious problems with 'degeneration' in the crop, which worsened with successive generations. This was finally recognized by Salaman (1921) to be the result of virus infection. This led to targeted breeding for virus resistance, the introduction of virus disease regulations into the UK Statutory Seed Certification Scheme, and then protected regions for seed production. Similar measures were adopted in many other countries.

Early resistance breeding programmes. The recognition of the virus problem was followed, from the 1930s onwards, by much research on the characterization of numerous virus resistance genes and phenotypes in *Solanum* species (e.g. Schultz & Raleigh, 1933; Salaman, 1938; Cadman, 1942; Cockerham, 1943a, b; Cockerham, 1945; Stelzner, 1950; Hutton, 1951; Cockerham, 1952; Hutton & Wark, 1952; Ross, 1952; Ross, 1954a, b; Cockerham, 1955; Cockerham, 1958; Easton *et al.*, 1958; Ross, 1958; Ross, 1961; Mills, 1965; Cockerham, 1970). Comprehensive lists of known genes for resistance to potyviruses and PVX, their known relationships and mapped locations, are presented in Solomon-Blackburn & Barker (2001). However, before the virus problem was recognized as such, there had no doubt been selection for virus resistance within the *Tuberosum* gene pool by selecting cultivars that withstood degeneration better than others. A number of HR genes were already present, including *Na_{tbr}*, *Nc_{tbr}*, *Nx_{tbr}* and *Nb_{tbr}* (Cockerham, 1943b). Wild species from South America had been used since 1851 for breeding, and species including the hexaploid *S. demissum* were used as sources of blight resistance in the UK by Salaman in 1909 (Bradshaw & Mackay, 1994) and by Wilson before 1920 (Davidson, 1980); this probably also introduced some virus resistance. Two of Wilson's cultivars, Templar and Crusader, were reputed to have resistance to PLRV (Davidson, 1980). Ross (1986) reported that PLRV resistance in some modern German cultivars could be traced back to *S. demissum* crosses. The HR gene *Ny_{tbr}* in the Australian clone 11-79 bred by Hutton from cvs Katahdin and Snowflake, was a later introduction to the U.K. (Davidson,

1980). As it was already in a *Tuberosum* background of cultivar quality, its use did not involve introgression from a wild species.

Breeding for HR and ER. With the identification of HR and ER genes, deliberate attempts have been made to breed for and select such genes in breeding programmes. Greater emphasis has been placed on utilizing sources of HR and ER rather than other forms of resistance to potyviruses and PVX, probably because selection for this type of resistance is relatively straightforward and can be carried out in the glasshouse following simple mechanical inoculation tests, and because HR and ER are effective, quite durable and simply inherited. Where the inheritance of ER or HR to viruses has been investigated in potato (reviewed for potyviruses and PVX in Solomon-Blackburn & Barker (2001)), it is monogenic, with dominance for resistance with one exception, the recessive gene *s* (or *ss_{ibr}* in the nomenclature used here), which confers resistance to PVS infection (Bagnall & Young, 1972). There are also dominant genes for HR or ER to PVS or PVM (Baerecke, 1967; Waś & Dziewońska, 1984; Świeżyński *et al.*, 1993).

ER can be comprehensive (effective against several strains of a virus or even several viruses), e.g. various *Rx* and *Ry* genes from *S. tuberosum* ssp. *andigena*, *S. acaule* and *S. stoloniferum* (reviewed in Solomon-Blackburn & Barker, 2001). Comprehensive ER has obvious advantages over specific HR for breeding purposes, but HR also provides useful protection and is more widely available in cultivars than ER.

Attempts to introgress ER to PVX have been made since about 1952 (Ross, 1954a, b, 1986) and to PVY since 1944 (Ross, 1978; Davidson, 1980). Relatively few cultivars have ER to PVX and PVY, probably for a variety of reasons. First, in comparison with the ubiquitous HR genes, ER genes are generally much more recent introductions to the *S. tuberosum* gene pool and were introduced from relatively few sources. *S. stoloniferum*, the source of genes *Ry_{sto}* and *Ry_{sto}^{na}*, does not intercross freely with *S. tuberosum* as the two species differ in endosperm balance number (EBN) (reviewed by Hawkes, 1994). One of the more frequently used *Ry* parents, the hybrid MPI 61.303/4, had *S. tuberosum* ssp. *andigena*, *S. acaule*, *S. demissum* and *S. spgazzini* as well as *S. stoloniferum* in its pedigree (Ross, 1986). The *Ry* cultivars listed by Ross were third to seventh backcross generations from *S. stoloniferum*. The *S. stoloniferum* source material was generally highly susceptible to PLRV; PLRV resistance and ER to PVX were introduced to *S. stoloniferum* breeding lines in the U.K. from other sources (Cockerham, unpubl. report). However, to date, no U.K.-bred cultivars are known to

have an *Ry* gene. This may reflect a higher priority given to cultivars resistant to PVY (including PVY^N strains) in the countries where *Ry* cultivars have been produced (Germany, Holland, Poland and Hungary) and possibly an association of *Ry* genes with wild characteristics. Furthermore, (Świeżyński, 1994) and Ross (1986) reported that most breeding lines with the gene *Ry_{sto}* were male-sterile. It is also difficult and time-consuming to select ER when HR is also present in the population. Cultivars with *Rx_{ibr}*, *Rx_{adg}* or *Rx_{act}* have been produced in several countries including USA, Germany, Argentina (Ross, 1986), UK and Ireland.

Major gene resistance to viruses in potato has proved quite durable, unlike major-gene resistance to late blight (*Phytophthora infestans*). Some evolution of resistance-breaking strains is apparent (Jones, 1982, 1985), but it is a relatively slow process and resistance-breaking strains often do not become prevalent (Harrison, 1981): the HR genes *Nx_{ibr}* (Cadman, 1942; Cockerham, 1970) and *Ny_{ibr}* (Davidson, 1980; Jones, 1990) still confer very useful resistance, though neither offers resistance to all strains.

Resistance to infection. Resistance to infection is, by its nature, somewhat difficult to select and there is a requirement for plants to be exposed to the viruliferous vector in order to assess this type of resistance. In most cases, this means that expensive and laborious field trials are necessary. Resistance to infection with PLRV appears to be inherited polygenically (Ross, 1958; Baerecke, 1961; Davidson, 1973). It could include several different mechanisms; this could contribute to the polygenic nature of this trait (or traits). Brown *et al.* (1997) suggested that the most resistant *S. tuberosum* parents might have major genes for resistance to PLRV infection. Quantitative resistance to PVY infection, from *S. phureja*, has been reported (Davidson, 1980), but it dissipated in outcrossing and would be difficult to assess accurately enough for selection. It had been thought that this quantitative resistance to PVY might be more durable than major gene resistance, but ER and HR have proved quite durable and offer a higher degree of protection.

Resistance to virus accumulation. Resistance to virus accumulation is readily assessed by measuring the virus titre using a quantitative ELISA of tissue from glasshouse- or field-grown plants. A high level of resistance to PLRV accumulation in *S. brevidens* was identified by Jones (1979). Gibson *et al.* (1990) also found a high level of resistance to accumulation of PVX and PVY, as well as of PLRV in *S. brevidens*. The results of attempts to transfer this resistance into a *S. tuberosum* background by somatic hybridization suggested that the gene

controlling resistance to PLRV is different from those controlling resistance to PVY and PVX, and that genes conferring resistance to PVY and PVX are linked (Valkonen *et al.*, 1994). The resistance to PVY and PVX in *S. brevidens* is thought to be associated with slow cell-to-cell spread of the viruses (Valkonen *et al.*, 1991).

Resistance to PLRV accumulation was found in *S. tuberosum* breeding lines with other species in their pedigrees (Barker & Harrison, 1985), and in a range of potato material by other authors (Gase *et al.*, 1988; Świeżyński *et al.*, 1988; Van Den Heuvel *et al.*, 1993; Wilson & Jones, 1993). The most resistant tetraploid clones had 1–5% of the PLRV concentration found in susceptible clones. Virus is less likely to be acquired (Barker & Harrison, 1986) and transmitted to other plants by aphids from plants with this type of resistance (Barker & Woodford, 1992), which is therefore useful for reducing virus spread within the crop. Barker & Harrison (1985) originally referred to this form of resistance as restricted virus multiplication, but the term 'resistance to virus accumulation' allows for the possibility that it may sometimes be a consequence in part, for example, of restricted movement (Wilson & Jones, 1992) rather than restricted virus multiplication *per se*. In inheritance studies on resistance to PLRV accumulation in *S. tuberosum*, a dominant major-gene effect has been reported, possibly involving two complementary genes both needed for resistance (Barker & Solomon, 1990; Barker *et al.*, 1994a). This hypothesis is currently under test.

Sources of PLRV resistance have been found in other *Solanum* species. Very strong resistance to PLRV accumulation found in *S. chacoense* appeared to be controlled by a single dominant major gene (Brown & Thomas, 1994) although the possibility of a tightly linked group of genes was not ruled out. This resistance may be useful as *S. chacoense* crosses readily with *S. tuberosum*, bears tubers and is diploid, which may facilitate the identification of linked molecular markers. Resistance to PLRV accumulation has also been found in *S. etuberosum* (Chavez *et al.*, 1988a). A similar resistance was found in *S. phureja* by Franco-Lara & Barker (1999), who suggested that this species may be the most valuable of these three diploid species because it is already cultivated and well recognized as a source of desirable characteristics for potato germplasm improvement.

Resistance to virus movement. Resistance to virus movement may underlie the mechanism of several forms of resistance. Resistance to accumulation of PVY and PVX in *S. brevidens* is thought to be the result of slow cell-to-cell spread (Valkonen *et al.*, 1991), and resistance to PLRV in *S. tuberosum* clones may be the result of

impaired movement of virus from sieve elements to companion cells in the external phloem bundles (Derrick & Barker, 1997).

A different form of resistance to virus movement has long been recognized with respect to PLRV (Hutton & Brock, 1953; Barker, 1987) in certain potato clones where infected plants transmit PLRV infection to fewer of the tuber-progeny plants than in susceptible clones. It is associated with phloem necrosis in cv. Bismark (Hutton & Brock, 1953) and cv. Apta (Golinowski *et al.*, 1987), but not in the breeding clone G7445(1) (Barker, 1987). Resistance to phloem transport has been demonstrated in cv. Bismark by Wilson & Jones (1992), who found it to be separate from resistance to accumulation and resistance to infection. Świeżyński *et al.* (1989) found a high level of PLRV resistance associated with limited virus spread in four diploid potato clones. Resistance to PLRV movement was reviewed in greater detail by Barker & Waterhouse (1999).

Tolerance. Resistance is obviously preferable to tolerance, where available, but where the choice of resistant cultivars is limited, a tolerant cultivar may be preferred for reasons other than virus resistance (e.g. yield, quality, maturity, other disease resistance). However, there are dangers in selecting tolerant lines, because of the risk of virus spread from infected symptomless stocks grown in proximity to healthy material, or of introducing a soil-borne virus to sites that were previously uncontaminated.

The tolerance problem can be illustrated with the types of reaction of potato clones to TRV. Some clones are susceptible but show no spraing symptoms, and infective virus is found in tubers. Other clones are hypersensitive to TRV ('susceptible' to spraing disease) and react by producing spraing symptoms (an HR response) in tubers. Although virus is detectable, infective virus often cannot easily be recovered from tubers of these clones (Xenophonos *et al.*, 1998). When clones with ER are planted in infested soil, tubers do not develop spraing symptoms and no virus can be recovered (Robinson & Dale, 1994). The tolerant clones that become infected but produce no symptoms provide a means by which new virus-free sites could become infected if the vector nematodes are present (Xenophonos *et al.*, 1998).

Summary of available host resistance to each virus. There is HR and ER to PVY, PVX, PVA, PVV, PVM and PVS in breeding lines, wild species and cultivars. Some of these resistances have been used for many years in cultivars to good effect. There is resistance to PLRV infection, accumulation or movement in a few cultivars and, to a greater extent, in breeding lines and wild

species. ER to TRV exists in some cultivars and tolerance exists in others. There are no reports of confirmed host resistance (as opposed to tolerance) to PMTV, and few of host resistance (in wild species) to PSTVd (Bagnall, 1972; Salazar, 1981).

Pathogen-derived transgenic resistance

Introduction. There are many examples of pathogen-derived resistance to potato viruses, especially coat protein-mediated, which was developed first. Genetic engineering of plants for virus resistance was reviewed by Harrison (1992), Fitchen & Beachy (1993), Wilson (1993), Baulcombe (1994b), Pierpoint (1996) and Fuchs *et al.* (1997), and specifically to PLRV by Barker & Waterhouse (1999).

Coat protein-mediated resistance. Transformation with viral coat protein genes was the first approach adopted for pathogen-derived resistance because it was thought it might work like cross-protection (Pierpoint, 1996). Transformation with the coat protein gene of PVX was one of the first attempts to obtain pathogen-derived resistance to a major potato virus (Hemenway *et al.*, 1988). Numerous other examples of coat protein-mediated resistance (CPMR) to potato viruses followed soon after. In many cases, the transgenes were tested in a model species, frequently tobacco, but in many other instances potato was transformed and tested. In some cases, but not all, the resistant transgenic plants produce coat protein. The resistance varies in degree and type but often acts against virus accumulation. Kawchuk *et al.* (1991) observed PLRV titres as low as 1% of the level found in control plants at the stage of primary infection. Either immunity or extreme resistance has also been reported in potatoes with CPMR, e.g. to PVY (Lawson *et al.*, 1990; Hassairi *et al.*, 1998). CPMR is often strain specific but sometimes not. For example, Okamoto *et al.* (1996) found that transformation of potato cv. Folva with a coat protein (CP) gene from a PVY strain of the PVY^N subgroup, conferred extreme resistance to isolates from the PVY^N, PVY^O and PVY^{NTN} subgroups. Sometimes it is effective against other viruses, at least within the same virus genus: potato cvs Spunta and Claustar transformed with a CP gene from *Lettuce mosaic virus* were immune to PVY (Hassairi *et al.*, 1998). However, broad-spectrum CPMR in tobacco (*Nicotiana tabacum*) was found in two cases to take the form of reduced or delayed symptom development (Anderson *et al.*, 1989; Stark & Beachy, 1989), thus conferring little useful benefit.

In some cases, CPMR can be overcome by a high concentration of inoculum, e.g. delayed accumulation of PVA in potato transformed with a CP gene from

PVY (Hefferon *et al.*, 1997). CPMR sometimes confers resistance to inoculation with viral RNA, e.g. in cases of CPMR to PVX (Hemenway *et al.*, 1988), PVS (MacKenzie & Tremaine, 1990) and PMTV (Barker *et al.*, 1998b). In some cases CPMR was proportional to the amount of CP produced in transformed tissue (Hemenway *et al.*, 1988) but not in other cases (Angenent *et al.*, 1990). In several cases the resistance is effective against manual inoculation and vector-transmission (Lindbo & Dougherty, 1992; Van Der Vlugt & Goldbach, 1993; Reavy *et al.*, 1995). However, there are examples in which CPMR was effective against manually inoculated virus but not against vector-borne virus, e.g. a case of PVY resistance (Lawson *et al.*, 1990), and one of TRV resistance (Ploeg *et al.*, 1993).

Resistance is conferred in some cases by nontranslatable CP genes (e.g. antisense sequences) or by truncated versions of the viral CP gene (Van Der Vlugt *et al.*, 1992). In many such cases it is presumed that resistance is the result of CP RNA transcript rather than CP itself.

Some clones with CP genes have been tested in field trials, which have confirmed the resistance identified in glasshouse tests, and that some clones retain the intrinsic properties of the cultivar. These include trials for resistance to PLRV in Australia (Graham *et al.*, 1995), the USA (Thomas *et al.*, 1997) and Canada (Kawchuk *et al.*, 1997), to PVY in Switzerland (Malnoe *et al.*, 1994) and to PVX in the Netherlands (Van Den Elzen *et al.*, 1993).

Movement protein-mediated resistance. Virus-encoded movement proteins, which facilitate spread of virus through the plant, have also been targeted as potential sources of resistance transgenes. Tacke *et al.* (1996) transformed potato with a sequence from PLRV open reading frame (ORF) 4, which encodes a protein (pr17), believed to be a phloem-specific movement protein. They used mutant versions and wild-type (wt) genes. PLRV accumulation was reduced in transgenic plants with secondary infection. Lines expressing mutant pr17 were also resistant to PVY and PVX accumulation. Transgenic lines that did not express mutant protein but in which its transgene mRNA accumulated were resistant to PLRV but not to PVY or PVX. Transgenic plants expressing wt pr17 were also resistant to PLRV only. Tacke *et al.* (1996) concluded that PLRV resistance was operating at the RNA level and that PVX and PVY resistance was protein-mediated. They suggested that mutant pr17 proteins might be binding to plasmodesmata and inhibiting cell-to-cell movement of unrelated viruses, possibly by blocking binding sites for their movement protein. Herbers *et al.* (1997) found plasmodesmatal

alterations in phloem but not mesophyll tissue in tobacco plants transformed with the same construct. They also found altered carbohydrate metabolism in these plants. Seppänen *et al.* (1997) transformed potato with mutant versions of two genes from the triple gene block (TGB) of PVX (the TGB proteins provide the movement function in PVX and some other viruses). In inoculation tests, transgenic plants were resistant to PVX, *Potato aucuba mosaic virus*, PVS and PVM (which have a TGB), but not to PVY (which does not). The resistance was to virus movement and accumulation, and was dependent on inoculum concentration. If these forms of resistance can be shown to be effective in other cultivars and in the field, they have the potential to provide a valuable type of broad-spectrum resistance.

Polymerase-mediated resistance. Expression of sequences from a replicase gene was first attempted by Golemboski *et al.* (1990), with *Tobacco mosaic virus*. Similar approaches have been tried subsequently with other RNA viruses using both mutant and functional forms of RNA polymerases. In general, resistance generated by this route is characterized by strong inhibition of virus replication and can be very effective against high inoculum levels (Braun & Hemenway, 1992; Mueller *et al.*, 1995). It operates in protoplasts and against viral RNA, but tends to be highly strain-specific. In some cases, e.g. with PVX resistance in tobacco, the most resistant plants showed the lowest levels of replicase (polymerase) protein synthesis (Longstaff *et al.*, 1993), suggesting that resistance may be mediated by mRNA rather than protein, at least in these cases (Baulcombe, 1994a). This was subsequently confirmed by Mueller *et al.*, 1995 (see below).

Resistance to PLRV mediated by a region of the PLRV polymerase gene has been produced in potato by Monsanto, USA (Monsanto, 1994; Shah *et al.*, 1994, 1995) and CSIRO, Australia (Waterhouse, see Barker & Waterhouse, 1999). Monsanto produced a line of cv. Russet Burbank, expressing the whole polymerase (ORFs 2a and 2b), that is highly resistant or immune to PLRV infection, and potato lines (NatureMark's NewLeaf Plus) that contain the PLRV replicase gene and also express the *Bacillus thuringiensis* insecticidal protein (Thomas *et al.*, 1995).

Ribozyme-mediated resistance. Ribozymes are small RNA molecules derived from the satellite RNA of *Tobacco ringspot virus*, certain viroids and viroid-like satellite RNAs that are capable of cleaving specific RNA molecules *in trans*. One particular success is the use of a ribozyme designed to target minus-strand PSTVd RNA to transform potato cv. Désirée (Yang *et al.*, 1997). In

inoculated plants of most transgenic lines, PSTVd was not detectable; in other lines viroid accumulation was reduced. Vegetative progeny stably inherited the resistance. This was the first example of a ribozyme suppressing a viroid to an undetectable level *in planta*.

Post-transcriptional gene silencing. In a number of cases, resistance conferred by various virus-derived transgenes is thought to be mediated by RNA transcript rather than by the encoded protein, as mentioned above under the CPMR heading. Another example is the strain-specific (homology-dependent) PVX resistance conferred in tobacco by the RNA polymerase gene of PVX (Mueller *et al.*, 1995), where the highest resistance is associated with low polymerase accumulation and multiple copies of the transgene, and resistance is also conferred by untranslatable versions of the transgene. The mechanism underlying this form of resistance appears to be a form of post-transcriptional gene silencing (PTGS) (Mueller *et al.*, 1995; Baulcombe, 1996; Van Den Boogart *et al.*, 1998). PTGS is characterized by the highly specific degradation of the transgene mRNA and target RNA. For PTGS to operate, the sequences of these two RNAs must be the same or highly homologous (English *et al.*, 1996). The mechanisms underlying the induction and control of the degradation pathway are not yet understood, although they are much discussed (for reviews see: Bruening, 1998; Grant, 1999).

Recent work by Waterhouse *et al.* (1998) using the PVY protease gene in sense [s] and antisense [a/s] forms has produced some very intriguing results that may go a long way to explain PTGS and also indicate how the frequency of obtaining PTGS (and strong resistance) in transgenic plants can be improved. Waterhouse *et al.* (1998) found that the expression of both [s] and [a/s] homologous transcripts leads to much higher frequencies of silencing than in plants transformed with the same gene expressed in only one orientation, thus 44–54% of [s] + [a/s] transgenic lines were resistant or immune, compared with less than 15% of lines expressing only [s] or [a/s]. The immunity conferred was not correlated with multiple loci or multiple transgenes at a single locus. This immunity is stable and inherited in a Mendelian way. Waterhouse *et al.* (1998) concluded that the use of RNAs with the potential to form duplexes ([s] + [a/s]) may be an important new strategy for virus resistance in transgenic plants.

It is intriguing that PTGS may have parallels with natural general antiviral defence in plants where virus-induced gene silencing is believed to have a role (Dawson, 1996; Carrington & Whitham, 1998; Baulcombe, 1999): it has been suggested that virus-induced gene silencing is part of a defence mechanism mediated by viral RNA in plants, which slows down and stops

virus accumulation at a certain stage of infection. It has also been suggested that viruses evolve strategies that overcome this (Carrington & Whitham, 1998), e.g. the helper component proteinase HC-Pro in potyviruses (Kasschau *et al.*, 1997), which mediates synergistic viral disease (Shi *et al.*, 1997).

In research with transgenes other than those for virus resistance, potyviruses including PVY produced strong suppressors of PTGS, whereas PVX was not able to reverse PTGS in transgenic plants (reviewed by Baulcombe, 1999). Béclin *et al.* (1998) suggested that potyviral infection could reverse RNA-mediated resistance to other viruses in a transgenic variety. Hence, PVY resistance could be important for the effectiveness of RNA-mediated resistance to other viruses, and it would be a wise precaution to ensure that viral genes from potyviruses used for transformation do not include the sequence for HC-Pro, which suppresses gene silencing (Anandalakshmi *et al.*, 1998), in translatable form.

Ecological impact of transgenic potatoes. It is not our intention to discuss the pros and cons of GM crops, but it is relevant to consider potential risks that may arise from recombination between transgene RNA and RNA of a challenge virus. It is generally acknowledged that this is the most important risk area with the use of viral genes. Thus, it is proposed by some that new viruses or strains could arise by recombination between RNA of infecting viruses and virus-derived transgene RNA. The likelihood of such recombination relative to that of natural recombination between viruses is difficult to determine (Aaziz & Tepfer, 1999), although one report suggested it is common (Malnoe *et al.*, 1997) and likely to result in the survival of natural mutant viruses. However, mutants and recombinant viruses with transgene RNA are likely to be less fit than wild-type viruses (Falk & Bruening, 1994), which have been naturally selected (after natural recombination). Indeed, new variant strains have been detected in plants with host resistance (Dawson & Hilf, 1992), although these are generally less fit than the original virus in susceptible plants. Aaziz & Tepfer (1999) suggested that RNA-mediated resistance based on PTGS could present certain advantages from the biosafety perspective because little or no transgene-derived RNA accumulates, and hence the likelihood of its recombination with the RNA of infecting viruses is expected to be extremely low.

Nonpathogen-derived forms of novel resistance

Nonpathogen-derived transgenic resistance. A number of alternative means by which transgenic resistance can be induced using nonpathogen-derived sequences have

been reported. Several of these are reported to induce broad-spectrum (nonspecific) resistance which may have distinct advantages, particularly if combined with other more specific forms of transgenic resistance.

Pokeweed antiviral protein (PAP) is a ribosome-inhibiting protein found in the cell walls of pokeweed (*Phytolacca americana*). Plants of potato cv. Russet Burbank transformed with cDNA to the PAP gene showed partial resistance to PVY and PVX infection by mechanical inoculation and to PVY infection by aphids (Lodge *et al.*, 1993).

Another nonpathogen form of transgenic resistance has been obtained using the gene for mammalian 2'-5' oligo-adenylate synthetase. This gene is activated by dsRNA and it, in turn, activates a latent endoribonuclease which degrades viral RNAs (Truve *et al.*, 1993). Potato cv. Pito was transformed with a copy of a rat gene encoding the 2'-5' oligoadenylate synthetase, and some transformants showed some resistance to PVX infection and/or accumulation.

PSTVd infection and accumulation was suppressed in transgenic potato cv. Russet Burbank expressing the yeast-derived RNA-specific ribonuclease *pac1* (Sano *et al.*, 1997). All the progeny tubers were viroid-free. It was suggested that the *pac1* gene product digested double-stranded regions in the PSTVd, formed transiently during replication. This could be a useful form of resistance if it proves to be stable and effective in field conditions.

Plants transformed with an antibody gene can synthesize protein chains of mammalian antibodies to a plant virus (Hiatt *et al.*, 1989) and assemble them into functional complexes. Plant expressed antibodies to virus particles have been shown to induce resistance (Tavladoraki *et al.*, 1993; Zimmermann *et al.*, 1998). However, this approach is in its infancy and it remains to be seen whether plant-produced antibodies can provide useful resistance.

Somaclonal variation. Somaclonal variants (produced by regenerating plants from isolated protoplasts) were investigated as a means of generating novel phenotypes in potato (Shepard *et al.*, 1980). It has been found that somaclonal variants may be a common by-product of attempts to generate transgenic lines of potato by transformation (Jongedijk *et al.*, 1992). Presting *et al.* (1995) transformed potato cultivars Russet Burbank and Ranger Russet with the native PLRV CP gene, a modified form to optimize protein expression, and a control consisting of a vector plasmid only. Resistant lines were obtained from transformations with all three constructs, the most resistant resulting from transformation with the control construct. This resistance was dominant and simply inherited and Presting *et al.* (1995)

suggested it could be the result of somaclonal variation. The value of deliberate selection of somaclonal variants to obtain improved clones has been proven by Kawchuk *et al.* (1997), who obtained a somaclonal variant of Russet Burbank with resistance to PLRV accumulation and to tuber symptoms. It remains to be seen whether such an approach will be beneficial for obtaining resistance to other viruses.

Breeding techniques and strategies

Methods of screening

Introduction. In order to select for virus resistance genes in a breeding programme, it is necessary to assess resistance in plants. Although linked molecular markers present the possibility of selection on the basis of genotype rather than phenotype in the future (discussed later), traditional forms of screening for resistance are still essential in general where selection is needed.

Field exposure trials. For many purposes a field exposure trial to determine virus resistance is the only way one can be sure of obtaining results that are relevant to the crop situation. For some forms of resistance, glasshouse or screenhouse testing might be used in the initial stages, but the final confirmation and proof that the resistance is operating satisfactorily, is likely to come from a field trial. Some forms of resistance can only be assessed in the field. Thus, resistance to PLRV infection is usually assessed by field exposure trials (Davidson, 1973), where the virus is spread from infector plants to exposed plants by aphids (predominantly *Myzus persicae*), and the frequency of secondary infection in tuber progeny plants is assessed by symptoms, ELISA or a combination of the two, in comparison with standard cultivars. For selection purposes, it is convenient to expose the plants to PVY at the same time (Davidson, 1973), because it is also aphid-transmitted. In general, field trials are grown in areas of high natural aphid infestation, but are more successful in some years than others, because the levels of aphid infestation vary (though natural infestation can be supplemented by applying cultured aphids to infector plants). Thus, estimates of quantitative resistance to PLRV infection can only be rough. However, all exposed plants of the most resistant clones regularly remain PLRV-free, at least in U.K. conditions (Solomon-Blackburn & Barker, 1993).

Field exposure is necessary if quantitative field resistance to PVY is being assessed. It is also useful for confirming the resistance of clones already selected for major-gene resistance (or the unscreened progeny of multiplex parents, see below); most clones with ER or HR genes to PVY remain healthy in field exposure

conditions, or with a low frequency of infection, e.g. 4% in clones with the HR gene *Ny_{ibr}*, but some develop systemic necrosis.

Screening in the glasshouse. Field trials for virus resistance are expensive, and therefore substantial cost savings can be made if satisfactory or preliminary tests can be performed on glasshouse-grown plants. Thus, for major gene resistance to manually transmissible viruses, such as PVX and PVY, glasshouse-grown plants can be inoculated with infective leaf extracts and the response observed over the following weeks. Local or systemic necrosis (indicating HR) or mosaic symptoms are recorded, and noninoculated leaves tested for infection using serological or biological methods, e.g. *S. demissum* detached leaflet tests for PVY and PVA (Cockerham, 1970). However, a null response to sap-inoculation could be attributable either to ER or to failure of inoculation. Alternatively, plants can be graft-inoculated using infected scions, but although this is a more reliable method, it is more laborious.

Large numbers of young seedlings at the cotyledon stage in seed pans can be inoculated with PVX or PVY, using infective plant extracts mixed with an abrasive in a spray gun (Wiersema, 1972). Susceptible seedlings (showing mosaic symptoms) can be discarded from about two weeks after inoculation. This rapid inoculation method can be used for early selection or for progeny tests (see below). However, to minimize the possibility of 'escapes' from inoculation, an additional (manual) inoculation two weeks later is advisable.

Resistance to PLRV accumulation can be assessed by first graft-inoculating glasshouse-grown plants and then determining the virus titre in the leaves of glasshouse-grown daughter plants the following year, using a quantitative ELISA technique, and comparing with resistant and susceptible controls (Barker & Harrison, 1985).

Resistance and tolerance to PMTV and TRV can be assessed by field trials on infective land (with viruliferous *Spongospora subterranea* or trichodoriid nematodes), and scoring the tubers for severity and frequency of spraing symptoms, but problems of uneven distribution of the viruliferous vector in the soil and variable weather conditions make such trials difficult (Dale & Solomon, 1988). A glasshouse test was developed to overcome some of the problems with TRV field trials (Dale & Solomon, 1988). Tubers are grown in pots of tested field soil containing viruliferous nematodes, and daughter tubers scored for the severity of spraing symptoms (but not frequency). In order to distinguish between resistance and tolerance, it is necessary to test symptomless tubers for infection. Transgenic resistance to PMTV was

evaluated in a screen house trial by Barker *et al.* (1998a) using infested field soil and irrigation. Such glasshouse tests can overcome the problems of weather and patchy distribution of viruliferous vectors, and hence need less replication.

Progeny testing. Progeny tests are used to determine the relative breeding value of parents or crosses, the gene dosage in a resistant parent (see multiplex parents below), or to select the best progenies. This type of screening is carried out on glasshouse-grown plants.

To determine the number of copies of a major gene for resistance to PVY or PVX in a potato clone, it is test-crossed with a susceptible parent. A sample of each progeny is sown, then spray-inoculated as described above. The gene dosage in the resistant parent is deduced from the segregation ratio of resistant to susceptible seedlings in the progeny (Solomon-Blackburn & Mackay, 1993).

Another application of progeny tests was developed by Chuquillanqui & Jones (1980), where families of true seedlings were assessed by aphid inoculation in a screen house in Peru, to identify progenies with resistance to PLRV infection. This method was adapted for experimental use in Scotland, using caged aphids in a glasshouse, and compared with a field trial (described above). The seedling progeny test did distinguish between resistant and susceptible progenies, but was not effective for selecting the more resistant individuals within progenies (Solomon-Blackburn *et al.*, 1994).

Breeding strategies

Development of parents with multiplex resistance genes. One of the difficulties with potato breeding is that so many different characteristics are desirable in a cultivar. High yield and good quality characteristics are now a prerequisite in many countries, and breeders have the task of combining these with resistance not only to virus diseases but also to a range of other diseases and pests. Added to this is the complication of tetrasomic inheritance, because *S. tuberosum* is autotetraploid.

One approach to these problems has been the production of multiplex resistant parents (Wastie *et al.*, 1992; Bradshaw & Mackay, 1994). The term multiplex is used here to mean triplex or quadruplex, i.e. with three or four copies of the (dominant) resistance gene. When a multiplex resistant parent is crossed, all of the progeny, or (in the triplex case) nearly all, allowing for some double reduction (Mendoza *et al.*, 1996), will be resistant, even when the other parent is susceptible (Cadman, 1942). By using these multiplex parents, screening and selection of the progeny for resistance is avoided, resources are saved by not having to raise seedlings

that will be discarded as susceptible, and the chances of combining the virus resistance with the many other desirable attributes for potato cultivars are increased, because these parents can be crossed with parents that have other attributes but not virus resistance, and still produce all resistant progeny.

By cycles of crossing, test-crossing and progeny tests, parent clones with multiplex PVY resistance and with multiplex PVX resistance have been produced (Mendoza *et al.*, 1996; Solomon-Blackburn, 1998).

Combining host resistances. There would be great value in obtaining a potato clone with resistance to all the major potato viruses. However, we know of no cultivars with such multiple virus resistance, probably because the cost, time and effort required to achieve this is great and many other features have higher priority. Nevertheless, there are some notable examples of combining different types of host resistance. For example, resistance to PLRV accumulation is not necessarily associated with resistance to PLRV infection, but clones containing both types of resistance have been identified (Solomon-Blackburn & Barker, 1993). These include cv. Pentland Crown and some breeding clones that have greater resistance of both types. These should have great benefit in diminishing PLRV spread. They also have genes for resistance to PVY, and also PVX in some cases. Dziewońska & Waś (1994) have reported that a diploid clone has been selected that has excellent resistance to PLRV infection and accumulation, and in addition has extreme resistance to PVX and high resistance to PVM. Crosses have been made to combine multiplex PVY resistance with both sorts of PLRV resistance (Solomon-Blackburn, 1998). Potato cultivars with ER to PVY, PVA and PVV are known, though this combination is the consequence either of a comprehensive gene or possibly a tight linkage group (Barker, 1997).

Introgression of host resistance genes from wild species

Introgression usually involves hybridization of the donor species and *S. tuberosum*, followed by repeated backcrossing to *Tuberosum* and selection for the desired genes. However, not all potato species inter-cross freely (if at all): species differ in ploidy and endosperm balance number (Bradshaw & Mackay, 1994; Hawkes, 1994; Hermesen, 1994). This has posed problems for the introgression of resistance genes from host species.

One solution has been through bridging crosses and ploidy manipulation using intermediate species. This method has been used to introgress resistance to PLRV, PVY and PVX from the nontuberosous *S. brevidens* into a

S. tuberosum background (Johnston & Hanneman, 1982; Ehlenfeldt & Hanneman, 1984), and PLRV resistance from the nontuberous *S. etuberosum* into a tuber-bearing *Solanum* gene pool (Hermesen *et al.*, 1981; Chavez *et al.*, 1988a, b). The diploid *S. sparsipilum* has been used as a link between diploid and tetraploid species for introducing virus resistance (Cockerham, 1970). However, this approach is time-consuming, demanding of resources and limited in genetic efficiency (Hermesen, 1987).

Dihaploids have also been used for introgressing genes for virus resistance from diploid species (Cockerham, 1970). Dihaploids are diploids produced from tetraploids (e.g. *S. tuberosum*) by pseudogamy. Their use for potato breeding was reviewed by Ross (1986) and Bradshaw & Mackay (1994). Most of the more evolutionarily advanced tuber-bearing wild species are diploid and cross readily with dihaploids of *S. tuberosum* (Bradshaw & Mackay, 1994). The resulting hybrids are usually long-day adapted and produce tubers. Improved diploid material produced from these can then be hybridized with tetraploid *Tuberosum*. Because genetics is simpler with diploids than with tetraploids, rapid progress can be made in breeding at the diploid level. Furthermore, the capacity of diploids to produce unreduced gametes means that tetraploid offspring with all the genes of the diploid parent can be produced from $4x \times 2x$ crosses (De Maine, 1982). The use of dihaploids for breeding purposes is limited by the difficulty of producing enough fully fertile true dihaploids of *Tuberosum*.

An alternative approach is somatic hybridization *via* protoplast fusion (Barsby *et al.*, 1984). This has been used to transfer resistance to PLRV, PVY and PVX from *S. brevidens* into *S. tuberosum* hybrids (Austin *et al.*, 1985; Gibson *et al.*, 1988; Valkonen *et al.*, 1994). This method can produce many hybrids, but their identification and genetic instability have been a problem (Helgeson *et al.*, 1988; Fehér *et al.*, 1992).

Applications of molecular marker technology

Introduction. Molecular markers linked to resistance genes can be used for marker-assisted selection or for mapping, and thence gene cloning. Prospects for applying these techniques to a tetraploid and heterozygous outbreeding species such as potato have been increased by improvements in marker technology including the use of bulked segregant analysis (BSA) to identify markers (Michelmore *et al.*, 1991), and use of simple sequence repeats (SSRs) together with amplified fragment length polymorphisms (AFLPs) (Milbourne *et al.*, 1997, 1998). Those authors published a map of a large (and expanding) set of SSRs in potato, which should

speed up mapping and provide common points of reference between mapping studies in different laboratories.

In the longer term, large-scale sequencing may lead to the development of a complete gene map of potato, provide a short cut to cloning genes, and provide improved markers, single nucleotide polymorphisms (SNPs), which are likely to supersede SSRs (W. De Jong, pers. comm.).

Marker-assisted selection. Screening by molecular markers (linked to resistance genes) is quick and accurate (Watanabe, 1994). Marker-assisted selection may be useful where phenotypic selection is difficult, or where it is not possible or convenient to use the virus for direct screening. It can also be useful for backcross breeding, for the introgression of resistance genes from wild species whilst selecting against the undesirable characteristics of the wild parent (Young & Tanksley, 1989). Small leaf samples can be used (Deragon & Landry, 1992), so marker-assisted selection could save some years in a potato breeding programme by selecting true seedlings for several unlinked traits at the same time. It may be used on quantitative trait loci (QTLs), with the advantage of selecting pairs of parents with genes at different QTLs for the same trait, provided that genes with large enough effects can be found (Bradshaw *et al.*, 1998) and a large enough population (150–250) is used to map markers (Hackett *et al.*, 1998). The success of marker-assisted selection will depend on close linkage between the resistance genes and (preferably flanking) markers.

Marker-assisted selection for virus resistance in potato has been applied to the gene *Ry_{adg}* (Hämäläinen *et al.*, 1997) but, so far, no research on its effectiveness in a breeding programme has been reported. The applicability of marker-assisted selection may increase with the identification of SSR markers (for mapping) because they may overcome the problem of population specificity found with AFLPs (Milbourne *et al.*, 1998). The most likely practical use for marker-assisted selection for virus resistance in potato seems to be the introgression of resistance genes from wild species or otherwise poor parents, or possibly for selecting seedlings for several traits simultaneously. In the short term, marker-assisted selection does not seem a cheap or easy option for most practical purposes (except where suitable markers have been identified for other purposes, e.g. mapping), but is likely to become a more viable proposition with further improvements in the technology in the long term.

Molecular mapping and host gene isolation. To date, eight virus-resistance genes have been mapped in potato

(summarized in Solomon-Blackburn & Barker, 2001). Disease resistance gene clusters (covering a diverse range of pathogens) have been found in many plant species, including potato (see Solomon-Blackburn & Barker, 2001). These linkage groups would afford the advantage of efficient selection for several disease resistances at once without screening for them all, whether by marker-assisted or by phenotypic selection. Throughout the history of potato breeding and natural selection for disease resistance, it is probable that some advantage has already accrued from the fact that these resistances are linked.

Following mapping, several virus resistance genes in potato have been or are being cloned (reviewed in Solomon-Blackburn & Barker, 2001). The *Rx_{adg}* transgene was inserted into the susceptible potato cv. Maris Bard and found to be effective, conferring the same phenotype as in the source cv. Cara, both in protoplasts and whole plants in seven out of eight transgenic lines (Bendahmane *et al.*, 1999). It also conferred ER when inserted into *Nicotiana* species. The *Ry_{sto}* gene is potentially particularly useful, as this gene (or set of closely linked genes) confers extreme resistance to all known PVY strains including PVY^{NTN} (Barker, 1996; Le Romancer & Nedellec, 1997) and also to PVA (Ross, 1961; Cockerham, 1970) and PVV (Barker, 1997). *Ry_{adg}* would also be a useful transgene as it also confers extreme resistance to isolates from all PVY subgroups, including PVY^{NTN} (Le Romancer & Nedellec, 1997).

The use of transgenes in breeding programmes

One use of transgenes (host- or pathogen-derived) is to insert genes for attributes that are lacking in an existing cultivar. This has already been applied in the U.S.A.: NatureMark's 'NewLeaf' series of cultivars are transformed versions of established cultivars such as Russet Burbank and Shepody. They have transgenic resistance to insects (Colorado beetle) conferred by the Bt gene, and pathogen-derived transgenic resistance to PLRV and PVY (Monsanto, 1997). This resistance to PLRV gives protection against the net necrosis symptoms seen in tubers of the original cv. Russet Burbank when PLRV-infected (Thomas *et al.*, 1995). In the U.K., a change in regulations might be a prerequisite to the use of such cultivars differing in only few genes from existing cultivars: at present, new cultivars must be visibly distinct from existing cultivars (or never grown on the same seed farm).

Transforming clones at an early stage in a breeding programme would be inefficient, as large numbers of clones would have to be transformed and then most of them rejected. Another disadvantage would be that the transgenes might be superseded by better ones before

ever reaching cultivar release. Transformation of parent clones would have the same disadvantage, and also only a proportion of the progeny would inherit the transgenes. The most efficient way to use transgenes in a breeding programme might be to insert a series of genes for different disease resistances/attributes in selected advanced clones nearing cultivar selection, allowing time for trialling after transformation. Resistance to PVY (transgenic or natural) may be important for the effectiveness of PTGS-based transgenic resistance to other viruses, as discussed above.

Conclusions

Viruses are relatively easily controlled in temperate potato crops and there is much input into virus control measures in temperate regions, notably in seed certification schemes and application of pesticides to control aphid vectors. However, such measures can have high financial and environmental costs. Other diseases (e.g. late blight and PCN) are more important in temperate regions. For these and other reasons, potato breeding in such areas puts less emphasis on selection of virus resistance than other characteristics. Furthermore, there has been a traditional view that virus resistance is not in the best interest of seed producers, as they would lose trade through the use of 'home-saved' seed. However, although virus problems are the main force behind the seed industry, seed producers have also benefited from virus-resistant cultivars by reduced rejection or downgrading of seed crops (Howell, 1977). The situation is different in tropical areas with a higher inoculum potential and where production and use of certified seed is less practical: the need for resistance is greater in these regions. However, there is an increasing need for resistance wherever potatoes are grown as pesticide use comes under close scrutiny and restrictions may be imposed, as has already happened in Denmark (Jørgensen & Secher, 1996). In future, changes in regulations governing pesticide use, or the imposition of an environmental tax on agrochemical use, could quickly change growers' attitudes to the desirability of virus-resistant cultivars.

Although there is very good host-gene-mediated resistance to several viruses, traditional breeding has produced cultivars with resistance to only two or three viruses, and many cultivars have little or no virus resistance. Furthermore, in many cases the resistance is not fully effective. For example, it may be strain-specific, or confer only partial resistance that breaks down under high inoculum pressure or only reduces the incidence of infection. Partial resistance to PLRV has been enhanced by combining different types of host resistance (Solomon-Blackburn & Barker, 1993). Combining resistances to several viruses and other diseases with

desirable agronomic features in a single cultivar is difficult and time-consuming by conventional means. However, progress has been made, for example by the production of multiplex resistant parents (in UK and Peru) and by combining resistance to different viruses in some parent clones (Ross, 1978; Świeżyński, 1983, 1994; Dzięwońska, 1986; Solomon-Blackburn, 1998).

New molecular technologies seem likely to benefit resistance breeding in future, as they promise to shorten the breeding process through the more rapid and cost-effective introgression of desirable characteristics, including virus resistance. As discussed above, marker-assisted selection may, in future, prove to be particularly useful for the introgression of resistance genes from wild species or parents with poor agronomic characteristics. The ability to select seedlings for several traits simultaneously will also be valuable, as more and better markers are developed. Marker-assisted selection may also prove useful for selecting ER genes in populations containing genes for HR to the same virus. However, even with the advent of marker-assisted selection, it is unrealistic to expect that conventional breeding could succeed in producing potato cultivars with resistance to the 10 most common viruses, as well as all the other attributes required in a cultivar. This is where transgenic resistance offers great potential.

As research on mapping progresses, more will become known about the locations and relationships of host genes, and more will probably be identified which might be exploited. The cloning of host genes is at present slow and expensive, but likely to become easier as knowledge expands. In future, use of the sequences of cloned host genes to make transgenes, possibly with improved characteristics, for reinsertion into breeding clones is an attractive possibility. However, transgenes produced in this manner seem likely to raise concerns in the minds of the public in some of the same ways that transgenes using pathogen-derived sequences have done, and this could inhibit their exploitation.

A frequently occurring problem with pathogen-derived resistance is that many of the transgenic lines in a transformation do not confer adequate resistance. Screening lines for resistance is expensive and time-consuming, so measures to increase the success rate by deliberate targeting of PTGS through transformation with sense and antisense sequences may be a useful approach. Cases where transgenic resistance has been developed to viruses for which there are no host resistance genes, or where they are only partially effective or improvable by combination (e.g. for PMTV and PLRV, respectively) (Barker *et al.*, 1994b, 1998a) are very promising, as are the two cases of transgenic PSTVd resistance. If such forms of resistance are stable

and effective in the field, and if there are no side effects, they should have great potential.

Future trends in virus resistance breeding are very difficult to predict, and will increasingly depend on politics, the consumer and the manner of science funding. Considerable scope remains for conventional breeding and the use of host genes, but new opportunities are emerging with molecular techniques for gene identification and selection. Thus far, the main interest in pathogen-derived resistance at a commercial level has been for the transformation of special-purpose cultivars that lack resistance to a particular virus (already taken up in the U.S.A.). In future, however, pathogen-derived resistance is likely to be much more useful for constructing transgenes that confer resistance to a range of viruses (for example, the 6–10 most widespread viruses in potato). Such resistance could be developed quite soon and could provide a practical means of obtaining multiple virus resistance much faster and more economically than by any other route, including use of cloned host gene sequences in transgenes and marker assisted selection in conventional breeding programmes. Several broad-spectrum (nonspecific) approaches to obtaining multiple virus resistance using nonvirus sequences in transgenes have been tested. However, questions remain over the validity of these approaches in terms of effectiveness and their public acceptability.

Outstanding problems of how multiple virus resistance transgenes based on virus sequences should be made, and the determination of their stability and durability in a range of field conditions, need to be examined. Nevertheless, for the short-term goal of producing broad-spectrum virus resistance, effort in this area is likely to be more fruitful than the pursuit of alternative approaches. However, uptake of many of these possibilities will depend upon the willingness of consumers to eat GM potatoes. They will need to be convinced that it is not dangerous but instead beneficial to both consumer and environment.

Acknowledgements

We thank Drs W. De Jong, J.E. Bradshaw, D.J. Robinson, D.C. Baulcombe and Mr M.J. De Maine for helpful discussions.

The Scottish Crop Research Institute receives grant-in-aid from the Scottish Executive Rural Affairs Department.

References

- AAZIZ, R. AND TEPFER, M. 1999. Recombination in RNA viruses and in virus-resistant transgenic plants. *J. Gen. Virol.*, **80**, 1339–1346.

- ADAMS, M. J., ANTONIW, J. F., BARKER, H., JONES, A. T., MURANT, A. F. AND ROBINSON, D. J. 1998. *Descriptions of Plant Viruses on CD-ROM*. AAB, Wellesbourne, U.K.
- ANANDALAKSHMI, R., PRUSS, G. J., GE, X., MARATHE, R., MALLORY, A. C., SMITH, T. H. AND VANCE, V. B. 1998. A viral suppressor of gene silencing in plants. *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 13079–13084.
- ANDERSON, E. J., STARK, D. M., NELSON, R. S., POWELL, P. A., TUMER, N. E. AND BEACHY, R. N. 1989. Transgenic plants that express the coat protein genes of tobacco mosaic virus or alfalfa mosaic virus interfere with disease development of some non-related viruses. *Phytopathology*, **79**, 1284–1290.
- ANGENENT, G. C., VAN DEN OUWELAND, J. M. W. AND BOL, J. F. 1990. Susceptibility to virus infection of transgenic plants expressing structural and non-structural genes of tobacco rattle virus. *Virology*, **175**, 191–198.
- AUSTIN, S., BAER, M. A. AND HELGESON, J. P. 1985. Transfer of resistance to potato leafroll virus from *Solanum brevidens* to *Solanum tuberosum* by somatic fusion. *Plant Sci.*, **39**, 75–82.
- BAERECHE, M.-L. 1961. Ehrfahrungen mit einjährigen Kartoffelabbaubauversuchen unter starken Blattrollinfektionsbedingungen. *Z. PflZücht.*, **45**, 225–253.
- BAERECHE, M.-L. 1967. Überempfindlichkeit gegen das S-Virus in der Kartoffel in einem bolivianischen *Andigena*-Klon. *Züchter*, **37**, 281–286.
- BAGNALL, R. H. 1972. Resistance to potato viruses M, S, X and the Spindle tuber virus in tuber-bearing *Solanum* species. *Am. Potato J.*, **49**, 342–348.
- BAGNALL, R. H. AND YOUNG, D. A. 1972. Resistance to virus S in the potato. *Am. Potato J.*, **49**, 196–201.
- BARKER, H. 1987. Multiple components of the resistance of potatoes to potato leafroll virus. *Ann. appl. Biol.*, **111**, 641–648.
- BARKER, H. 1996. Inheritance of resistance to potato viruses Y and A in progeny obtained from potato cultivars containing gene Ry, evidence for a new gene for extreme resistance to PVA. *Theor. Appl. Genet.*, **93**, 710–716.
- BARKER, H. 1997. Extreme resistance to potato virus V in clones of *Solanum tuberosum* that are also resistant to potato viruses Y and A, evidence for a locus conferring broad-spectrum resistance. *Theor. Appl. Genet.*, **95**, 1258–1262.
- BARKER, H. AND HARRISON, B. D. 1985. Restricted multiplication of potato leafroll virus in resistant potato genotypes. *Ann. appl. Biol.*, **107**, 205–212.
- BARKER, H. AND HARRISON, B. D. 1986. Restricted distribution of potato leafroll virus antigen in resistant potato genotypes and its effect on transmission of the virus by aphids. *Ann. appl. Biol.*, **109**, 595–604.
- BARKER, H., REAVY, B. AND MCGEACHY, K. D. 1998a. High level resistance in potato to potato mop-top virus induced by transformation with the coat protein gene. *Eur. J. Pl. Pathol.*, **104**, 737–740.
- BARKER, H., REAVY, B., MCGEACHY, K. D. AND DAWSON, S. 1998b. Transformation of *Nicotiana benthamiana* with the potato mop-top virus coat protein gene produces a novel resistance phenotype mediated by the coat protein. *Mol. Plant-Microbe Interact.*, **11**, 626–633.
- BARKER, H. AND SOLOMON, R. M. 1990. Evidence of simple genetic control in potato of ability to restrict potato leaf roll virus concentration in leaves. *Theor. Appl. Genet.*, **80**, 188–192.
- BARKER, H., SOLOMON-BLACKBURN, R. M., MCNICOL, J. W. AND BRADSHAW, J. E. 1994a. Resistance to potato leaf roll virus multiplication in potato is under major gene control. *Theor. Appl. Genet.*, **88**, 754–758.
- BARKER, H. AND WATERHOUSE, P. M. 1999. The development of resistance to luteoviruses mediated by host genes and pathogen-derived transgenes. In: Smith, H. G. and Barker, H. (eds) *The Luteoviridae*. CAB International, Wallingford.
- BARKER, H., WEBSTER, K. D., JOLLY, C. A., REAVY, B., KUMAR, A. AND MAYO, M. A. 1994b. Enhancement of resistance to potato leafroll virus multiplication in potato by combining the effects of host genes and transgenes. *Mol. Plant-Microbe Interact.*, **7**, 528–530.
- BARKER, H. AND WOODFORD, J. A. T. 1992. Spread of potato leafroll virus is decreased from plants of potato clones in which virus accumulation is restricted. *Ann. appl. Biol.*, **121**, 345–354.
- BARSBY, T. L., SHEPARD, J. F., KEMBLE, R. J. AND WONG, R. 1984. Somatic hybridization in the genus *Solanum*, *S. tuberosum* and *S. brevidens*. *Pl. Cell Rep.*, **3**, 165–167.
- BAULCOMBE, D. 1994a. Replicase-mediated resistance, a novel type of virus resistance in transgenic plants. *Trends Microbiol.*, **2**, 60–63.
- BAULCOMBE, D. 1994b. Novel strategies for engineering virus resistance in plants. *Curr. Opin. Biotechnol.*, **5**, 117–124.
- BAULCOMBE, D. C. 1996. Mechanisms of pathogen-derived resistance to viruses in transgenic plants. *Pl. Cell*, **8**, 1833–1844.
- BAULCOMBE, D. C. 1999. Fast forward genetics based on virus-induced gene silencing. *Curr. Opin. Pl. Biol.*, **2**, 109–113.
- BÉCLIN, C., BERTHOMÉ, R., PALAUQUI, J.-C., TEPFER, M. AND VAUCHERET, H. 1998. Infection of tobacco or *Arabidopsis* plants by CMV counteracts systemic post-transcriptional silencing of non-viral (trans) genes. *Virology*, **252**, 313–317.
- BEEMSTER, A. B. R. AND ROZENDAAL, A. 1972. Potato viruses: properties and symptoms. In: de Bokx, J. A. (ed.) *Viruses of Potatoes and Seed-Potato Production*, pp. 115–142. PUDOC, Wageningen.
- BENDAHMANE, A., KANYUKA, K. AND BAULCOMBE, D. C. 1999. The Rx gene from potato controls separate virus resistance and cell death responses. *Pl. Cell*, **11**, 781–791.
- BRADSHAW, J. E., HACKETT, C. A., MEYER, R. C., MILBOURNE, D., MCNICOL, J. W., PHILLIPS, M. S. ET AL. 1998. Identification of AFLP and SSR markers associated with quantitative resistance to *Globodera pallida* (Stone) in tetraploid potato (*Solanum tuberosum* subsp. *tuberosum*) with a view to marker-assisted selection. *Theor. Appl. Genet.*, **97**, 202–210.
- BRADSHAW, J. E. AND MACKAY, G. R. 1994. Breeding strategies for clonally propagated potatoes. In: Bradshaw, J. E. and Mackay, G. R. (eds) *Potato Genetics*, pp. 467–497. CAB International, Wallingford.
- BRAUN, C. J. AND HEMENWAY, C. L. 1992. Expression of amino-terminal portions of full length viral replicase genes in transgenic plants confers resistance to potato virus X infection. *Pl. Cell*, **4**, 735–744.

- BROWN, C. R., CORSINI, D., PAVEK, J. AND THOMAS, P. E. 1997. Heritability of field resistance to potato leafroll virus in cultivated potato. *Pl. Breed.*, **116**, 585–588.
- BROWN, C. R. AND THOMAS, P. E. 1994. Resistance to potato leafroll virus derived from *Solanum chacoense*, characterization and inheritance. *Euphytica*, **74**, 51–57.
- BRUENING, G. 1998. Plant gene silencing regularized. *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 13349–13351.
- CADMAN, C. H. 1942. Autotetraploid inheritance in the potato, some new evidence. *J. Genet.*, **44**, 33–52.
- CARRINGTON, J. C. AND WHITHAM, S. A. 1998. Viral invasion and host defense, strategies and counter-strategies. *Curr. Opin. Plant Biol.*, **1**, 336–341.
- CHAVEZ, R., BROWN, C. R. AND IWANAGA, M. 1988a. Transfer of resistance to PLRV titer buildup from *Solanum tuberosum* to a tuber-bearing *Solanum* gene pool. *Theor. Appl. Genet.*, **76**, 129–135.
- CHAVEZ, R., BROWN, C. R. AND IWANGA, M. 1988b. Application of interspecific sesquiploidy to introgression of PLRV resistance from non-tuber-bearing *Solanum tuberosum* to cultivated potato germplasm. *Theor. Appl. Genet.*, **76**, 497–500.
- CHUQUILLANQUI, C. AND JONES, R. A. C. 1980. A rapid technique for assessing the resistance of families of potato seedlings to potato leaf roll virus. *Potato Res.*, **23**, 121–128.
- COCKERHAM, G. 1943a. Potato breeding for virus resistance. *Ann. Appl. Biol.*, **30**, 105–108.
- COCKERHAM, G. 1943b. The reactions of potato varieties to viruses X, A, B and C. *Ann. appl. Biol.*, **30**, 338–344.
- COCKERHAM, G. 1945. Some genetical aspects of resistance to potato viruses. *Ann. appl. Biol.*, **32**, 280–281.
- COCKERHAM, G. 1952. Breeding for resistance to potato viruses. In: Streutgers, E., Beemster, A. B. R., Walgrave, J. and van der Want, J. P. H. (eds) *Proceedings of the Conference on Potato Virus Diseases, Wageningen-Lisse, 1951*, pp. 37–39. Staatsdrukkerij.
- COCKERHAM, G. 1955. Strains of potato virus X. In: Streutgers, E., Beemster, A. B. R., Walgrave, J. and van der Want, J. P. H. (eds) *Proceedings of the 2nd Conference Potato Virus Diseases, Lisse-Wageningen, 1954*, pp. 89–92. H. Veenman & Zonen, Wageningen.
- COCKERHAM, G. 1958. Experimental breeding in relation to virus resistance. In: Streutgers, E., Beemster, A. B. R., Walgrave, J. and van der Want, J. P. H. (eds) *Proceedings of the 3rd Conference Potato Virus Diseases, Lisse-Wageningen, 1957*, pp. 199–203. H. Veenman & Zonen, Wageningen.
- COCKERHAM, G. 1970. Genetical studies on resistance to potato viruses X and Y. *Heredity*, **25**, 309–348.
- COOPER, J. I. AND JONES, A. T. 1983. Responses of plants to viruses, proposals for the use of terms. *Phytopathology*, **73**, 127–128.
- DALE, M. F. B. AND SOLOMON, R. M. 1988. A glasshouse test to assess the sensitivity of cultivars to tobacco rattle virus. *Ann. appl. Biol.*, **112**, 225–229.
- DAVIDSON, T. M. W. 1973. Assessing resistance to leafroll in potato seedlings. *Potato Res.*, **16**, 99–108.
- DAVIDSON, T. M. W. 1980. Breeding for resistance to virus disease of the potato (*Solanum tuberosum*) at the Scottish Plant Breeding Station. In: *Scottish Plant Breeding Station 59th Annual Report*, pp. 100–108.
- DAWSON, W. O. 1996. Gene silencing and virus resistance, a common mechanism. *Trends Pl. Sci.*, **1**, 107–108.
- DAWSON, W. O. AND HILF, M. E. 1992. Host-range determinants of plant viruses. *Ann. Rev. Pl. Physiol./ Pl. Mol. Biol.*, **43**, 527–555.
- DE MAINE, M. J. 1982. An evaluation of the use of dihaploids and unreduced gametes in breeding for quantitative resistance to potato pathogens. *J. Agric. Sci., Camb.*, **99**, 79–83.
- DERAGON, J. M. AND LANDRY, B. S. 1992. RAPD and other PCR-based analyses of plant genomes using DNA extracted from small leaf disks. *PCR Meth. Appl.*, **1**, 175–180.
- DERRICK, P. M. AND BARKER, H. 1997. Short and long distance spread of potato leafroll luteovirus, effects of host genes and transgenes conferring resistance to virus accumulation in potato. *J. Gen. Virol.*, **78**, 243–251.
- DIXON, R. A., HARRISON, M. J. AND LAMB, C. J. 1994. Early events in the activation of plant defense responses. *Ann. Rev. Phytopathol.*, **32**, 479–501.
- DZIEWOŃSKA, M. A. 1986. Development of parental lines for breeding of potatoes resistant to viruses and associated research. In: Beckman, A. G. B., Louwes, K. M., Dellaert, L. M. W. and Neele, A. E. F. (eds) *Potato Research for Tomorrow*, pp. 96–100. PUDOC, Wageningen.
- DZIEWOŃSKA, M. A. AND WAE, M. 1994. Diploid genotype DW.84-1457, highly resistant to potato leafroll virus (PLRV). *Potato Res.*, **37**, 217–224.
- EASTON, G. D., LARSON, R. H. AND HOUGAS, R. W. 1958. Immunity to virus Y in the genus *Solanum*. *Univ. Wisc. Res. Bull.*, **205**, 1–31.
- EHLENFELDT, M. K. AND HANNEMAN, R. E. 1984. The use of endosperm balance number and 2n gametes to transfer exotic germplasm in potato. *Theor. Appl. Genet.*, **68**, 155–161.
- ENGLISH, J. J., MUELLER, E. AND BAULCOMBE, D. C. 1996. Suppression of virus accumulation in transgenic plant exhibiting silencing of nuclear genes. *Pl. Cell*, **8**, 179–188.
- FALK, B. W. AND BRUENING, G. 1994. Will transgenic crops generate new viruses and new diseases? *Science*, **263**, 1395–1396.
- FEHÉR, A., PREISZNER, J., LITKEY, Z., CASANÁDI, G. AND DUDITS, D. 1992. Characterization of chromosome instability in interspecific somatic hybrids obtained by X-ray fusion between potato (*Solanum tuberosum* L.) and *S. brevidens* Phil. *Theor. Appl. Genet.*, **84**, 880–890.
- FITCHEN, J. H. AND BEACHY, R. N. 1993. Genetically engineering protection against viruses in transgenic plants. *Ann. Rev. Microbiol.*, **47**, 739–763.
- FLANDERS, K. L., HAWKES, J. G., RADCLIFFE, E. B. AND LAUER, F. I. 1992. Insect resistance in potatoes, sources, evolutionary relationships, morphological and chemical defenses, and ecogeographical associations. *Euphytica*, **61**, 83–111.
- FRANCO-LARA, L. AND BARKER, H. 1999. Characterisation of resistance to potato leafroll virus accumulation in *Solanum phureja*. *Euphytica*, **108**, 137–144.
- FUCHS, M., FERREIRA, S. AND GONSALVES, D. 1997. Management of virus diseases by classical and engineered protection. *Mol.*

- Pl. Pathol. On-Line*, 1997, 0116fuchs at <http://www.bspp.org.uk/mpopol>.
- GASE, G., MÖLLER, K.-H. AND SCHENK, G. 1988. Die Bestimmung der relativen Viruskonzentration zur Einschätzung der quantitativen Resistenz von Kartoffelgenotypen gegen das Kartoffelblattroll-Virus. *Arch. Phytopathol. Pflanzenschutz*, **24**, 163–165.
- GIBSON, R. W., JONES, M. G. K. AND FISH, N. 1988. Resistance to potato leaf roll virus and potato virus Y in somatic hybrids between dihaploid *Solanum tuberosum* and *brevidentis*. *Theor. Appl. Genet.*, **76**, 113–117.
- GIBSON, R. W., PEHU, E., WOODS, R. D. AND JONES, M. G. K. 1990. Resistance to potato virus Y and potato virus X in *Solanum brevidentis*. *Ann. appl. Biol.*, **116**, 151–156.
- GILBERT, J., SPILLANE, C., KAVANAGH, T. A. AND BAULCOMBE, D. C. 1998. Elicitation of Rx-mediated resistance to PVX in potato does not require new RNA synthesis and may involve a latent hypersensitive response. *Mol. Pl.-Microbe Interact.*, **8**, 833–835.
- GLENDINNING, D. R. 1983. Potato introductions and breeding up to the early 20th century. *New Phytol.*, **94**, 479–505.
- GOLEMBOSKI, D. B., LOMONOSOFF, G. P. AND ZAITLIN, M. 1990. Plants transformed with a tobacco mosaic virus nonstructural gene sequence are resistant to the virus. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 6311–6315.
- GOLINOWSKI, W., TOMENIUS, K. AND OXELFELT, P. 1987. Ultrastructural studies on potato phloem cells infected with potato leaf roll virus – comparison of two potato varieties. *Acta Agric. Scand.*, **37**, 3–19.
- GRAHAM, M. W., KEESE, P. AND WATERHOUSE, P. M. 1995. The search for the perfect potato. *Today's Life Sci.*, **7**, 34–41.
- GRANT, S. R. 1999. Dissecting the mechanisms of posttranscriptional gene silencing, divide and conquer. *Cell*, **96**, 303–306.
- HACKETT, C. A., BRADSHAW, J. E., MEYER, R. C., McNICOL, J. W., MILBOURNE, D. AND WAUGH, R. 1998. Linkage analysis in tetraploid species, a simulation study. *Genet. Res.*, **17**, 143–154.
- HÄMÄLÄINEN, J. H., SORRI, V. A., WATANABE, K. N., GEBHARDT, C. AND VALKONEN, J. P. T. 1998. Molecular examination of a chromosome region that controls resistance to potato Y and A potyviruses in potato. *Theor. Appl. Genet.*, **96**, 1036–1043.
- HÄMÄLÄINEN, J. H., WATANABE, K. N., VALKONEN, J. P. T., ARIHARA, A., PLAISTED, R. L., PEHU, E. ET AL. 1997. Mapping and marker-assisted selection for a gene for extreme resistance to potato virus Y. *Theor. Appl. Genet.*, **94**, 192–197.
- HARRISON, B. D. 1981. Plant virus ecology, ingredients, interactions and environmental influences. *Ann. appl. Biol.*, **99**, 195–209.
- HARRISON, B. D. 1992. Genetic engineering of virus resistance, a successful genetical alchemy. *Proc. R. Soc. Edinb.*, **99B** (3/4), 61–77.
- HASSAIRI, A., MASMOUDI, K., ALBOUY, J., ROBAGLIA, C., JULLIEN, M. AND ELLOUZ, R. 1998. Transformation of two potato cultivars 'Spunta' and 'Claustar' (*Solanum tuberosum*) with lettuce mosaic virus coat protein gene and heterologous immunity to potato virus Y. *Plant Sci., Limerick*, **136**, 31–42.
- HAWKES, J. G. 1994. Origins of cultivated potatoes and species relationships. In: Bradshaw, J. E. and Mackay, G. R. (eds) *Potato Genetics*, pp. 3–42, CAB International, Wallingford.
- HEFFERON, K. L., KHALILIAN, H. AND ABOUHAIKAR, M. G. 1997. Expression of the PVY^o coat protein (CP) under the control of the PVX CP gene leader sequence, protection under greenhouse and field conditions against PVY^o and PVY^N infection in three potato cultivars. *Theor. Appl. Genet.*, **94**, 287–292.
- HELGESON, J. P., HABERLACH, G. T., PHOLMAN, J. AND AUSTIN, S. 1988. Somatic fusions of *Solanum* species. *Pl. Cell Tissue Organ Cult.*, **12**, 185–187.
- HEMENWAY, C., FANG, R. F., KANIEWSKI, W. K., CHUA, N. H. AND TUMER, N. E. 1988. Analysis of the mechanism of protection in transgenic plants expressing the potato virus X coat protein or its antisense RNA. *EMBO J.*, **7**, 1273–1280.
- HERBERS, K., TACKE, E., HAZIREZAEI, M., KRAUSE, K.-P., MELZER, M., ROHDE, W. AND SONNEWALD, U. 1997. Expression of a luteoviral movement protein in transgenic plants leads to carbohydrate accumulation and reduced photosynthetic capacity in source leaves. *Plant J.*, **12**, 1045–1056.
- HERMSEN, T. J. G. 1987. Efficient utilization of wild and primitive species in potato breeding. In: Jellis, G. J. and Richardson, D. E. (eds) *The Production of New Potato Varieties. Technological advances*, pp. 172–185. Cambridge University Press, London.
- HERMSEN, J. G. T. 1994. Introgression of genes from wild species, including molecular and cellular approaches. In: Bradshaw, J. E. and Mackay, G. R. (eds) *Potato Genetics*, pp. 515–538. CAB International, Wallingford.
- HERMSEN, T. J. G., RAMANNA, M. S. AND SAWOR, Z. 1981. The effect of chromosome doubling on fertility, meiotic behaviour and crossability of *Solanum etuberosum* x *S. pinnatisectum*. *Euphytica*, **30**, 33–39.
- HIATT, A., CAFFERKEY, R. AND BOWDISH, K. 1989. Production of antibodies in plants. *Nature*, **342**, 76–78.
- HOWELL, P. J. 1977. Recent trends in the incidence of aphid-borne viruses in Scotland. In: Fox, R. A., (ed.) *Problems of Pest and Disease Control in Northern Britain*, pp. 26–28. Scottish Horticultural Research Institute, Dundee.
- HULL, R. 1984. Rapid diagnosis of plant virus infections by spot hybridisation. *Trends Biotechnol.*, **2**, 2.
- HUTTON, E. M. 1951. Possible genotypes conditioning virus resistance in the potato and tomato. *J. Aust. Inst. Agric. Sci.*, **17** (3), 132–138.
- HUTTON, E. M. AND BROCK, R. D. 1953. Reactions and field resistance of some potato varieties and hybrids to the leaf-roll virus. *Aust. J. Agric. Res.*, **4**, 256–263.
- HUTTON, E. M. AND WARK, D. C. 1952. A relationship between immunity and localised reaction to virus X in the potato (*Solanum tuberosum*). *Austr. J. Sci. Res.*, **5**, 237–243.
- JEFFRIES, C. J. 1998. *FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm, No. 19. Potato*. Food and Agriculture Organization of the United Nations/International Plant Genetic Resources Institute, Rome.
- JOHNSTON, S. A. AND HANNEMAN, R. E. 1982. Manipulations of endosperm balance number overcome crossing barriers between diploid *Solanum* species. *Science*, **217**, 446–448.

- JONES, R. A. C. 1979. Resistance to potato leaf roll virus in *Solanum brevidens*. *Potato Res.*, **22**, 149–152.
- JONES, R. A. C. 1982. Breakdown of potato virus X resistance gene Nx, selection of a group four strain from strain group three. *Pl. Pathol.*, **31**, 325–331.
- JONES, R. A. C. 1985. Further studies on resistance-breaking strains of potato virus X. *Pl. Pathol.*, **34**, 182–189.
- JONES, R. A. C. 1990. Strain group specific and virus specific hypersensitive reactions to infection with potyviruses in potato cultivars. *Ann. appl. Biol.*, **177**, 93–105.
- JONGEDIJK, E., DE SCHUTTER, A. A. J. M., STOLTE, T., VAN DEN ELZEN, P. J. M. AND CORNELISSEN, B. J. C. 1992. Increased resistance to potato virus X and preservation of cultivar properties in transgenic potato under field conditions. *Bio/Technology*, **10**, 422–429.
- JØRGENSEN, L. N. AND SECHER, B. J. M. 1996. The Danish Pesticide Action Plan – ways of reducing inputs. In: *Crop Protection in Northern Britain 1996 (Proceedings of the Conference)*, pp. 63–70. Scottish Crop Research Institute, Dundee.
- KASSCHAU, K. D., CRONIN, S. AND CARRINGTON, J. C. 1997. Genome amplification and long-distance movement functions associated with the central domain of *Tobacco etch potyvirus* helper component-protease. *Virology*, **228**, 251–262.
- KAWCHUK, L. M., LYNCH, D. R., MARTIN, R. R., KOZUB, G. C. AND FARRIES, B. 1997. Field resistance to the potato leafroll luteovirus in transgenic and somaclone potato plants reduces tuber disease symptoms. *Can. J. Plant Pathol.*, **19**, 260–266.
- KAWCHUK, L. M., MARTIN, R. R. AND MACPHERSON, J. 1991. Sense and antisense RNA-mediated resistance to potato leafroll virus in Russet Burbank potato plants. *Mol. Pl.-Microbe Interact.*, **4**, 247–253.
- KOJIMA, R. AND LAPIERRE, H. 1988. Potato leafroll virus. In: Smith, I. M., Dunez, V., Philips, D. H., Leliot, R. A. and Archer, S. A. (eds) *European Handbook of Plant Diseases*, pp. 23–24. Blackwell Scientific Publications, Oxford.
- LAWSON, C., KANIEWSKI, W., HALEY, L., ROZMAN, R., NEWELL, C., SANDERS, P. ET AL. 1990. Engineering resistance to mixed virus infection in a commercial potato cultivar, resistance to potato virus X and potato virus Y in transgenic Russet Burbank potato. *Bio/Technology*, **8**, 127–134.
- LE ROMANCER, M. AND NEDELLEC, M. 1997. Effect of plant genotype, virus isolate and temperature on expression of the potato tuber necrotic ringspot disease (PTNRD). *Pl. Pathol.*, **46**, 104–111.
- LINDBO, J. A. AND DOUGHERTY, W. G. 1992. Pathogen-derived resistance to a potyvirus, immune and resistant phenotypes in transgenic tobacco expressing other forms of a potyvirus coat protein nucleotide sequence. *Mol. Pl.-Microbe Interact.*, **5**, 144–153.
- LODGE, J. K., KANIEWSKI, W. K. AND TUMER, N. E. 1993. Broad-spectrum virus resistance in transgenic plants expressing pokeweed antiviral protein. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 7089–7093.
- LONGSTAFF, M., BRIGNETI, G., BOCCARD, F., CHAPMAN, S. AND BAULCOMBE, D. 1993. Extreme resistance to potato virus X infection in plants expressing a modified component of the putative viral replicase. *EMBO J.*, **12**, 379–386.
- MACKENZIE, D. J. AND TREMAINE, J. H. 1990. Transgenic *Nicotiana debneyi* expressing viral coat protein are resistant to potato virus S infection. *J. Gen. Virol.*, **71**, 2167–2170.
- MALNOE, P., FARINELLI, L., COLLET, G. F. AND REUST, W. 1994. Small-scale field tests with transgenic potato, cv. Bintje, to test resistance to primary and secondary infections with potato virus Y. *Pl. Mol. Biol.*, **25**, 963–975.
- MALNOE, P., JAKAB, G., DROZ, E. AND VAISTIJ, F. 1997. Risk assessment, the evolution of a viral population in transgenic plants. In: Wirz, J. (ed) *Dialogue on Risk Assessment of Transgenic Plants, Scientific, Technological and Societal Perspectives. Proceedings of the 3rd Ifgene Workshop, Dornach, Switzerland, October, 1997*, pp. 4–9. Ifgene: International Forum for Genetic Engineering, Criccieth, UK.
- MENDOZA, H. A., MIHOVLOVICH, E. J. AND SAGUMA, F. 1996. Identification of triplex (YYYy) potato virus Y (PVY) immune progenitors derived from *Solanum tuberosum* ssp. *andigena*. *Am. Potato J.*, **73**, 13–19.
- MICHELMORE, R. W., PARAN, I. AND KESSELI, R. V. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis, a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 9828–9832.
- MILBOURNE, D., MEYER, R., BRADSHAW, J. E., BAIRD, E., BONAR, N., PROVAN, J., POWELL, W. AND WAUGH, R. 1997. Comparison of PCR-based marker systems for the analysis of genetic relationships in cultivated potato. *Mol. Breed.*, **3**, 127–136.
- MILBOURNE, D., MEYER, R. C., COLLINS, A. J., RAMSAY, L. D., GEBHARDT, C. AND WAUGH, R. 1998. Isolation, characterisation and mapping of simple sequence repeat loci in potato. *Mol. Gen. Genet.*, **259**, 233–245.
- MILLS, W. R. 1965. Inheritance of immunity to potato virus X. *Am. Potato J.*, **42**, 294–295.
- MONSANTO 1994. Plants resistant to infection by PLRV. *International patent application WO 94/18336*.
- MONSANTO 1997. NewLeaf Potatoes. In: *Biotechnology Resource Guide. Naturemark Potatoes*. www.naturemark.com. Monsanto, 250 Bobwhite Court, Suite 300, Boise, ID 83706, U.S.A.).
- MUELLER, E., GILBERT, J., DAVENPORT, G., BRIGNETI, G. AND BAULCOMBE, D. C. 1995. Homology-dependent resistance, transgenic virus resistance in plants related to homology-dependent gene silencing. *Plant J.*, **7**, 1001–1013.
- OKAMOTO, D., NIELSEN, S. V. S., ALBRECHTSEN, M. AND BORKHARDT, B. 1996. General resistance against potato virus Y introduced into a commercial potato cultivar by genetic transformation with PVY^N coat protein gene. *Potato Res.*, **39**, 271–282.
- PIERPPOINT, W. S. 1996. Modifying resistance to plant viruses. In: Pierpoint, W. S. and Shewry, P. R. (eds) *Genetic Engineering of Crop Plants for Resistance to Pests and Diseases*, pp. 16–37. British Crop Protection Council, Farnham, U.K.

- PLOEG, A. T., MATHIS, A., BOL, J. F., BROWN, D. J. F. AND ROBINSON, D. J. 1993. Susceptibility of transgenic tobacco plants expressing tobacco rattle virus coat protein to nematode-transmitted and mechanically inoculated tobacco rattle virus. *J. Gen. Virol.*, **74**, 2709–2715.
- PRESTING, G. G., SMITH, O. P. AND BROWN, C. R. 1995. Resistance to potato leafroll virus in potato plants transformed with the coat protein gene or with vector control constructs. *Phytopathology*, **85**, 436–441.
- QUAIM, M. 1998. *Transgenic Virus Resistant Potatoes in Mexico, Potential Socioeconomic Implications of North-south Biotechnology Transfer*. ISAAA Briefs no. 7–1998 ISAAA/ZEF, Bonn.
- QUERCI, M., OWENS, R. A., BARTOLINI, I., LAZARTE, V. AND SALAZAR, L. F. 1997. Evidence for heterologous encapsidation of potato spindle tuber viroid in particles of potato leafroll virus. *J. Gen. Virol.*, **78**, 1207–1211.
- REAVY, B., ARIF, M., KASHIWAZAKI, S., WEBSTER, K. D. AND BARKER, H. 1995. Immunity to potato mop-top virus in *Nicotiana benthamiana* plants expressing the coat protein gene is effective against fungal inoculations of the virus. *Mol. Pl.-Microbe Interact.*, **8**, 286–291.
- ROBINSON, D. J. AND DALE, M. F. B. 1994. Susceptibility, resistance and tolerance of potato cultivars to tobacco rattle virus infection and spraing disease. *Aspects Appl. Biol.*, **39**, 61–66.
- ROSS, H. 1952. Studies on mosaic resistance in the potato. In: Streutgers, E., Beemster, A. B. R., Walgrave, J. and van der Want, J. P. H. (eds) *Proceedings of the Conference on Potato Virus Diseases, Wageningen, 1951*, pp. 40–47. Staatsdrukkerij.
- ROSS, H. 1954a. Über die extreme Resistenz von *Solanum acaule* gegen das X-virus. *Mitt. Biol. Bundes. Land- u. Forstwirts. Berlin*, **80**, 144–145.
- ROSS, H. 1954b. Die Vererbung der 'Imunität' gegen das X-virus in tetraploidem *Solanum acaule*. In: *Proceedings of the 9th International Congress of Genetics, Bellagio 1953*. *Caryologia*, **6** (Suppl.), 1128–1132.
- ROSS, H. 1958. Virusresistenzzüchtung an der Kartoffel. *Eur. Potato J.*, **1**, 1–19.
- ROSS, H. 1961. Über die Vererbung von Eigenschaften für Resistenz gegen das Y- und A- virus in *Solanum stoloniferum* und die mögliche Bedeutung für eine allgemeine Genetik der Virusresistenz in *Solanum* sect. *Tuberosum*. In: Brandes, J., Bartels, R., Völk, J. and Wetter, C. (eds) *Proceedings of the 4th Conference Potato Virus Diseases, Braunschweig, 1960*, pp. 40–49. H. Veenman & Zonen N. V., Wageningen.
- ROSS, H. 1978. Methods for breeding virus resistant potatoes. In: *Report of the Planning Conference on Developments in the Control of Potato Virus Diseases, International Potato Center, Lima, 1977*, pp. 93–114. International Potato Center, Lima.
- ROSS, H. 1986. Potato breeding – problems and perspectives. In: *Advances in Plant Breeding. J. Plant Breed.*, **13** (suppl.). Paul Parey, Berlin.
- SALAMAN, R. N. 1921. Degeneration of potatoes. In: *Report on the International Potato Conference*, pp. 79–91. Royal Horticultural Society, London.
- SALAMAN, R. N. 1938. The potato virus X, its strains and reactions. *Phil. Trans. R. Soc. B.*, **299**, 137–217.
- SALAZAR, L. F. 1981. Progress in PSTV research. In: *Report of the Planning Conference on Strategy for Virus Management, International Potato Center, Lima, 1980*, pp. 44–49. International Potato Center, Lima.
- SANO, T., NAGAYAMA, A., OGAWA, T., ISHIDA, I. AND OKADA, Y. 1997. Transgenic potato expressing a double-stranded RNA-specific ribonuclease is resistant to potato spindle tuber viroid. *Nature Biotechnol.*, **15**, 1290–1294.
- SCHULTZ, E. S. AND RALEIGH, W. P. 1933. Resistance of potato to latent mosaic. (Abstract). *Phytopathology*, **23**, 32.
- SEPPÄNEN, P., PUSKA, R., HONKANEN, J., TYULKINA, L. G., FEDORKIN, O., MOROZOV, S. Y. ET AL. 1997. Movement protein-derived resistance to triple gene block-containing plant viruses. *J. Gen. Virol.*, **78**, 1241–1246.
- SHAH, D., LAWSON, C. AND KANIEWSKI, W. 1994. Russet Burbank genetically improved for resistance to potato leaf roll virus and late blight. In: *Seventh International Symposium on Molecular Plant Microbe Interactions, Edinburgh (Abstracts)*, **S48**, p. 14.
- SHAH, D. M., ROMMENS, C. T. M. AND BEACHY, R. N. 1995. Resistance to diseases and insects in transgenic plants; progress and applications to agriculture. *Trends Biotechnol.*, **13**, 362–368.
- SHEPARD, J. F., BIDNEY, D. AND SHAHIN, E. 1980. Potato protoplasts in crop improvement. *Science*, **208**, 17–24.
- SHI, X. M., MILLER, H., VERCHOT, J., CARRINGTON, J. C. AND VANCE, V. B. 1997. Mutations in the region encoding the central domain of helper component-proteinase (HC-Pro) eliminate Potato virus X/potyviral synergism. *Virology*, **231**, 35–42.
- SOLOMON-BLACKBURN, R. 1998. Progress in breeding potatoes for resistance to virus diseases. *Aspects Appl. Biol.*, **52**, 299–304.
- SOLOMON-BLACKBURN, R. M. AND BARKER, H. 1993. Resistance to potato leafroll luteovirus can be greatly improved by combining two independent types of heritable resistance. *Ann. appl. Biol.*, **122**, 329–336.
- SOLOMON-BLACKBURN, R. M. AND BARKER, H. 2001. A review of host major-gene resistance to potato viruses X, Y, A and V in potato, genes, genetics and mapped locations. *Heredity*, **86**, 8–16.
- SOLOMON-BLACKBURN, R. M. AND MACKAY, G. R. 1993. Progeny testing for resistance to potato virus Y, a comparison of susceptible potato cultivars for use in test crosses with resistant parents. *Potato Res.*, **36**, 327–333.
- SOLOMON-BLACKBURN, R. M., MACKAY, G. R. AND BROWN, J. 1994. An evaluation of seedling progeny tests for resistance to potato leafroll virus in potato. *J. Agric. Sci.*, **122**, 231–239.
- STARK, D. M. AND BEACHY, R. N. 1989. Protection against potyvirus infection in transgenic plants, evidence for broad spectrum resistance. *Bio/Technology*, **7**, 1257–1262.
- STELZNER, G. 1950. Virusresistenz der Wildkartoffeln. *Z. PflZücht.*, **29**, 135–158.
- ŚWIEŻYŃSKI, K. M. 1983. Parental line breeding in potatoes. *Genetica (Yugoslavia)*, **15**, 243–256.
- ŚWIEŻYŃSKI, K. M. 1994. Inheritance of resistance to viruses. In: Bradshaw, J. E. and Mackay, G. R. (eds) *Potato Genetics*, pp. 339–363. CAB International, Wallingford.

- ŚWIEŻYŃSKI, K. M., CHRZANOWSKA, M., DZIEWOŃSKA, M. A. AND WAŚ, M. 1993. Durability of resistance to viruses in the potato. In: Jacobs, T. and Parlevliet, J. E. (eds) *Durability of Disease Resistance*, p. 350. Kluwer, Dordrecht.
- ŚWIEŻYŃSKI, K. M., DZIEWOŃSKA, M. A. AND OSTROVSKA, K. 1988. Reaction to the potato leafroll virus (PLRV) in diploid potatoes. *Potato Res.*, **31**, 289–296.
- ŚWIEŻYŃSKI, K. M., DZIEWOŃSKA, M. A. AND OSTROWSKA, K. 1989. Resistance to the potato leafroll virus (PLRV) in diploid potatoes. *Pl. Breed.*, **103**, 221–227.
- TACKE, E., SALAMINI, F. AND ROHDE, W. 1996. Genetic engineering of potato for broad-spectrum protection against virus infection. *Nature Biotechnol.*, **14**, 1597–1601.
- TAVLADORAKI, P., BENVENUTO, E., TRINCA, S., DE MARTINIS, D., CATTANEO, A. AND GALEFFI, P. 1993. Transgenic plants expressing a functional single-chain Fv antibody are specifically protected from virus attack. *Nature*, **366**, 469–472.
- THOMAS, P. E., KANIEWSKI, W. K. AND LAWSON, E. C. 1997. Reduced field spread of potato leafroll virus in potatoes transformed with the potato leafroll virus coat protein gene. *Plant Dis.*, **81**, 1447–1453.
- THOMAS, P. E., KANIEWSKI, W. K., REED, G. L. AND LAWSON, E. C. 1995. Transgenic resistance to potato leafroll virus in Russet Burbank potatoes. In: Manka, M. (ed.) *Environmental Biotic Factors in Integrated Plant Disease Control*, pp. 551–554. Polish Phytopathological Society, Poznan, Poland.
- TRUVE, E., AASPÖLLU, A., HONKANEN, J., PUSKA, R., MEHTO, M., HASSI, A. ET AL. 1993. Transgenic potato plants expressing mammalian 2'-5' oligoadenylate synthetase are protected from potato virus X infection under field conditions. *Bio/Technology*, **11**, 1048–1052.
- VALKONEN, J. P. T. 1994. Natural genes and mechanisms for resistance to viruses in cultivated and wild potato species (*Solanum* spp.). *Pl. Breed.*, **112**, 1–16.
- VALKONEN, J. P. T., JONES, M. K. AND GIBSON, R. W. 1991. Resistance in *Solanum brevidens* to both potato virus Y and potato virus X may be associated with slow cell-to-cell spread. *J. Gen. Virol.*, **72**, 231–236.
- VALKONEN, J. P. T., JONES, R. A. C., SLACK, S. A. AND WATANABE, K. N. 1996. Resistance specificities to virus in potato, standardisation of nomenclature. *Pl. Breed.*, **115**, 433–438.
- VALKONEN, J. P. T., XU, Y.-S., ROKKA, V.-M., PULLI, S. AND PEHU, E. 1994. Transfer of resistance to potato leafroll virus, potato virus Y and potato virus X from *Solanum brevidens* to *S. tuberosum* through symmetric and designed asymmetric somatic hybridisation. *Ann. appl. Biol.*, **124**, 351–362.
- VAN DEN BOOGART, T., LOMONOSOFF, G. P. AND DAVIES, J. W. 1998. Can we explain RNA-mediated resistance by homology-dependent gene silencing? *Mol. Pl.-Microbe Interact.*, **11**, 717–723.
- VAN DEN ELZEN, P. J. M., JONGEDIJK, E., MELCHERS, L. S. AND CORNELISSEN, B. J. C. 1993. Virus and fungal resistance, from laboratory to field. *Trans. R. Soc. B*, **342**, 271–278.
- VAN DEN HEUVEL, J. F. J. M., DIRVEN, J. A. A. M., VAN OS, G. J. AND PETERS, D. 1993. Acquisition of potato leafroll virus by *Myzus persicae* from secondarily-infected potato plants of different genotypes. *Potato Res.*, **36**, 89–96.
- VAN DER VLUGT, R. A. A. AND GOLDBACH, R. W. 1993. Tobacco plants transformed with the potato virus Y^N coat protein gene are protected against different PVY isolates and against aphid-mediated infection. *Transgenic Res.*, **2**, 109–114.
- VAN DER VLUGT, R. A. A., RUITER, R. K. AND GOLDBACH, R. 1992. Evidence for sense RNA-mediated protection to PVY^N in tobacco plants transformed with the viral coat protein cistron. *Plant Mol. Biol.*, **20**, 631–639.
- WAŚ, M. AND DZIEWOŃSKA, M. A. 1984. Reaction to PVM and PVS in potato clones with the genes Gm and Ns. In: Winiger, F. A. and Stöckli, A. (eds) *9th Triennial Conference of the European Association of Potato Researchers, Interlaken*, pp. 245–246. European Association for potato Research.
- WASTIE, R. L., BRADSHAW, J. E., DALE, M. F. B., MACKAY, G. R., PHILLIPS, M. S. AND SOLOMON-BLACKBURN, R. M. 1992. Progeny testing for resistance to diseases and pests of potatoes. In: *Annual Report of the Scottish Crop Research Institute, 1991*, pp. 13–16. Scottish Crop Research Institute, Dundee.
- WATANABE, K. N. 1994. Molecular genetics. In: Bradshaw, J. E. and Mackay, G. R. (eds) *Potato Genetics*, pp. 213–235. CAB International, Wallingford, UK.
- WATERHOUSE, P. M., GRAHAM, M. W. AND WANG, M.-B. 1998. Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 13,959–13,964.
- WIERSEMA, H. T. 1972. Breeding for resistance. In: de Bokx, J. A. (ed.) *Viruses of Potato and Seed Potato Production*, pp. 174–187. PUDOC, Wageningen.
- WILSON, T. M. A. 1993. Strategies to protect crop plants against viruses, Pathogen-derived resistance blossoms. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 3134–3141.
- WILSON, C. R. AND JONES, R. A. C. 1992. Resistance to phloem transport of potato leafroll virus in potato plants. *J. Gen. Virol.*, **73**, 3219–3224.
- WILSON, C. R. AND JONES, R. A. C. 1993. Resistance to potato leafroll virus infection and accumulation in potato cultivars, and the effects of previous infection with other viruses on expression of resistance. *Aust. J. Agric. Res.*, **44**, 1891–1904.
- XENOPHONTOS, S., ROBINSON, D. J., DALE, M. F. B. AND BROWN, D. J. F. 1998. Evidence for persistent, symptomless infection in some potato cultivars with tobacco rattle virus. *Potato Res.*, **41**, 255–265.
- YANG, X. C., YIE, Y., ZHU, F., LIU, Y., KANG, L. Y., WANG, X. F. ET AL. 1997. Ribozyme-mediated high resistance against potato spindle tuber viroid in transgenic potatoes. *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 4861–4865.
- YOUNG, N. D. AND TANKSLEY, S. D. 1989. RFLP analysis of the size of chromosomal segments retained around the *Tm-2* locus of tomato during backcross breeding. *Theor. Appl. Genet.*, **77**, 353–359.
- ZIMMERMANN, S., SCHILLBERG, S., LIAO, Y. C. AND FISHER, R. 1998. Intracellular expression of TMV-specific single-chain Fv fragments leads to improved virus resistance in *Nicotiana tabacum*. *Mol. Breed.*, **4** (4), 369–379.