Inheritance of resistance to potato viruses Y and A in progeny obtained from potato cultivars containing gene \( R_y \): evidence for a new gene for extreme resistance to PVA

Abstract Extreme resistance in cultivated potato (Solanum tuberosum) to potato viruses Y and A (PVY and PVA) conditioned by the presence of \( R_y \) genes introduced from Solanum stoloniferum was described by Cockerham (1970). Cockerham detailed a number of genes which controlled a variety of reactions, including extreme resistance to both viruses (i.e. little or no visible reaction of plants and no viral replication following graft and manual inoculation) controlled by gene \( R_y_{ss} \). In the present study, cvs ‘Pirola’ and ‘Barbara’, which contain a \( R_y \) gene, were found to have extreme resistance to PVY isolates from the ordinary (PVY\textsuperscript{O}) vein necrosis (PVYN) and potato tuber necrotic ringspot (PVYN\textsuperscript{T}) subgroups, and PVA. The inheritance of this phenotype was examined in seedling progenies obtained by crossing ‘Barbara’ and ‘Pirola’ with susceptible cultivars. Segregation data for resistance to PVY and PVA in a progeny involving cv ‘Pirola’ best fitted a genetic model of one gene controlling extreme resistance to both PVY and PVA, although the possibility that there are two genes, each controlling resistance to one virus but closely linked, cannot be excluded. Segregation data from progenies involving cv ‘Barbara’ best fitted a genetic model in which there are two independent genes, one controlling extreme resistance to PVA and PVY and a second gene controlling extreme resistance to PVA but not to PVY. This previously unrecognised gene conferring extreme resistance to PVA only, should be given the notation \( R_a \) in keeping with nomenclature used for other resistance genes.

Key words Extreme virus resistance · Potyviruses · Genetics · Genes \( R_y \) and \( R_a \) · New gene

Communicated by G. Wenzel

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Introduction

Two distinct potyviruses, potato viruses Y (PVY) and A (PVA), were recognised many years ago to infect cultivated potato (Solanum tuberosum). Control of these aphid-borne viruses can be difficult, and the most effective means of preventing their spread in potato crops is by the use of durable resistance genes. A range of primitive Solanum species have resistance to PVY and PVA controlled by major genes (reviewed by Valkonen 1994), several of which have been transferred to S. tuberosum cultivars. The resistance can be classified into two main types depending on the reaction of plants following inoculation: necrotic (hypersensitive reaction), conferred by the \( N \) genes; and extreme resistance (very little or no visible effect) (Delhey 1975), conferred by the \( R \) genes. Following inoculation, virus can usually be recovered from plants with \( N \) genes but not from those with \( R \) genes. \( N_y \) and \( N_a \) genes conferring hypersensitive resistance to PVY and PVA, respectively, occur individually in several cultivars, while a few cultivars contain both genes. S. tuberosum ssp. andigena and S. stoloniferum are sources of \( R \) genes to potyviruses. Clones containing \( R_y \) genes from S. tuberosum ssp. andigena are resistant to PVY but susceptible to PVA (Ross 1986). The extreme resistance to PVY in the polymorphic Mexican species S. stoloniferum was first reported by Cockerham (1943), Hawkes (1945) and Ross and Baerecke (1950). This extreme resistance is expressed to a range of PVY strains and also to PVA. However, comprehensive extreme resistance to both PVY and PVA was only one of seven phenotypic responses to be recognised by Cockerham (1970) in plants containing \( R_y \) genes from S. stoloniferum. Other phenotypes included necrotic and susceptible reactions to inoculation with one or both of the viruses. Cockerham (1970) found that the phenotypes observed in reaction to PVY and PVA are determined by five genes at three loci. The most valuable of these is a gene controlling extreme resistance to both viruses, which Cockerham (1970) designated as...
Materials and methods

Plant material

All plant material was grown in soil-less potting compost in an aphid-proof glasshouse at approximately 20°C. Plants of virus-free potato cultivars were grown from tubers propagated in the glasshouse. Seedling progenies produced from crosses between 'Barbara' (Ry) and either 'Flourball' or 'Dr Macintosh' (susceptible cultivars) and between 'Pirola' (Ry) and 'Dr Macintosh' were sown, and each seedling plant (genotype) was grown until it was large enough for propagation by stem cuttings. Virus resistance tests on progenies were made with plants grown from stem cuttings.

Virus isolates and inoculation

An isolate of PVY\textsuperscript{N} was obtained from field-grown plants of potato cv. 'Record' (Barker 1994); PVY\textsuperscript{O} and PVA were SCR\textsuperscript{I} stock isolates. Isolates of PVY\textsuperscript{N}, PVY\textsuperscript{O} and PVA were maintained in potato by vegetative propagation in cv 'Record'. 'Craugs Snow-White' and 'Majestic', respectively, and transmitted by manual inoculation as necessary to Nicotiana tabacum. Two Spanish isolates from the potato tuber necrotic ringspot disease subgroup (PVY\textsuperscript{TN}) from potato cvs 'Hermes' and 'Picasso' were obtained from Dr J. Legorburu (Vitoria-Gasteiz, Spain) and maintained in N. tabacum.

Viruses were transmitted to potato test plants by graft inoculation using scions (shoot apices) from infected potato plants which were cleft-grafted onto stems of test plants from which the shoot apex had been removed. Foliage of shoots which subsequently developed from the axillary meristems was used for resistance testing. Potato test plants were manually inoculated using freshly extracted sap from infected N. tabacum plants (1 g leaf/5 ml water) rubbed onto corundum-dusted leaves.

Resistance tests

A standard test was used to assess resistance of the cultivars and genotypes derived from the progeny of the crosses. Plants were grafted or manually inoculated when they were approximately 300 mm tall. Young foliage of each inoculated plant was tested twice by ELISA between 20 and 35 days after inoculation. Viral replication was also assessed by an infectivity assay in which indicator plants of N. benthamiana and N. clevelandii were manually inoculated with sap extracted from foliage of test plants. In addition, attempts were made to recover virus from selected test plants by 'return grafting' in which scions were grafted into virus-free susceptible indicator plants of potato (for PVY) or tomato (for PVA). Infection in indicator plants was assessed by ELISA 3–4 weeks after inoculation. In all cases when virus was not detected by ELISA of leaf tissue from potato test plants, neither was it possible to recover it by infectivity assay or 'return grafting' to virus-free susceptible plants. However, infectious virus was always recovered from plants in which it was detected by ELISA. Failure to detect virus by ELISA and to recover infectivity from inoculated plants was taken as an indication of resistance (immunity) to virus replication. Plants in which necrotic symptoms developed were classified as susceptible or resistant on the basis of whether virus was recoverable and not on the appearance of symptoms.

Enzyme-linked immunosorbent assay

PVY was assayed by the antibody-trapped antigen form of indirect enzyme-linked immunosorbent assay (ELISA) as described by Barker et al. (1993). Microtitre plates were coated with anti-PVY\textsuperscript{O}-globulin prepared from a polyclonal antiserum to PVY\textsuperscript{O} coat protein, which traps particles of PVY\textsuperscript{O}, PVY\textsuperscript{N} and PVY\textsuperscript{TN}. This polyclonal antiserum and a detecting monoclonal antibody that reacts with the coat protein of PVY\textsuperscript{O}, PVY\textsuperscript{N} and PVY\textsuperscript{TN} was obtained from the Scottish Agricultural Research Institute. Leaf tissue was disrupted in a Polyliner roller press (1 g leaf/10 ml extraction buffer). Samples of each extract were tested in duplicate wells. PVA was detected by double-antibody sandwich ELISA as described by Singh and Barker (1991) using a polyclonal antibody kindly donated by the Department of Agriculture, Belfast, Northern Ireland.

Results

Reaction of potato cultivars to inoculation with PVY\textsuperscript{O} and PVY\textsuperscript{N}

At least four plants of each cultivar, were graft-inoculated and four were manually inoculated with each of the isolates (PVY\textsuperscript{O} and PVY\textsuperscript{N}). Plants of cultivars not containing gene Ry occasionally developed systemic mosaic symptoms following manual or graft inoculation, although such symptoms could be difficult to identify. Virus was readily detected in these plants by ELISA and recovered by the infectivity assay (Table 1). No symptoms associated with virus multiplication developed in plants containing Ry genes following manual inoculation with either PVY\textsuperscript{O} or PVY\textsuperscript{N}. However, plants of all cultivars, particularly 'Barbara', occasionally developed fine necrotic streaks on the veins of the abaxial leaf surface following virus inoculation or 'mock' inoculation with virus-free sap. It is concluded that such symptoms were a response to stress. Following graft inoculation with PVY\textsuperscript{O} or PVY\textsuperscript{N}, plants of cvs 'Barbara' and 'Pirola' developed a few necrotic streaks in the stems, and occasionally a few leaves of apical shoots became necrotic and died, but virus was not detected in these plants by ELISA, the infectivity assay or 'return grafting' (Table 1). Barker and Harrison (1984) and Jones (1990) described similar localised necrotic reactions in cv 'Pirola' inoculated with PVY. Such necrotic symptoms did not develop on all occasions, and it seems likely that their development may be subject to differences between isolates and environmental conditions. Plants of cvs 'Fanal' and 'Corine' remained symptom-free after graft inoculation. It was not possible to detect PVY in inoculated plants of Ry-containing cultivars, by ELISA, infectivity assay or 'return grafting' (Table 1).