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ASSESSING RESISTANCE TO LATE BLIGHT OF POTATO: METHODS USED AT THE SCOTTISH CROP RESEARCH INSTITUTE

ABSTRACT

At SCRI, wild species and clones of *Solanum tuberosum* are assessed for resistance to late blight in order to study the genetics and breed for resistance.

Glasshouse progeny tests for foliage and tuber blight resistance, using true seedlings, are described. These enable the rapid screening of accessions of wild species, and the selection of the most resistant progenies in a breeding programme within one year of crossing.

Assessment of the foliage resistance of clones is carried out in glasshouse tests of whole plants in flower–bud, and field trials using infector plants inoculated in the glasshouse with a complex race of *Phytophthora infestans*. The glasshouse test provides a reliable method of identifying *R*–genes and the virulence characteristics of blight isolates. The field trial gives the best estimate of field resistance and is being used to develop marker–assisted selection. Both tests are used to study the inheritance of resistance, to locate quantitative trait loci (QTL) and *R*–genes, and to select the most resistant clones.

The tuber resistance of clones is assessed by spray–inoculating whole, immature, field–grown tubers on the day of harvest. However when large numbers of clones are involved, e.g. in the location of QTL, this is impractical, so glasshouse–grown tubers are dip–inoculated.

Key words: late blight, *Phytophthora infestans*, potato, resistance breeding, resistance test, screening methods, *Solanum tuberosum*

INTRODUCTION

Late blight resistance is assessed with the objectives of producing potato cultivars with a higher level of durable resistance for use in an integrated control system, studying the inheritance of resistance and enabling the molecular study of resistance mechanisms. Laboratory tests were initiated by W. Black in the 1950s, when selection for field resistance began. They were developed and improved by J. Malcolmson, R. Wastie and H. Stewart.

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METHODS

Seedling progeny tests, glasshouse clonal tests and field trials for resistance to foliage blight and tuber blight are conducted as listed below:

Foliage blight

Glasshouse progeny test for resistance to foliage blight

Plants:	True seedlings, 5–7 weeks from sowing, 10 cm tall. Uniform growth is important: younger seedlings are more susceptible.
Samples:	2 pots of 25 seedlings per family. Control families covering a range of resistance are included.
Inoculation:	Damp plants are sprayed with a suspension of *Phytophthora infestans*, 5×10^4 zoospores per ml in an enclosed chamber.
Race used:	A single complex race, to overcome as many *R*–genes as possible.
Incubation:	6 days at 15°C (air–conditioned glasshouse, natural light); high humidity for the first 24 h.
Scoring:	The percentage of blight–infected foliage in each pot of 25 seedlings is assessed visually after 6 days (in 4 categories), to estimate the resistance of each family.

Clonal whole plants test for resistance to foliage blight (glasshouse)

Plants:	Sturdy plants grown from tubers in 10 cm pots in a cool glasshouse, reduced to a single stem and well spaced. Plants (2 per clone) are inoculated just before flowering (when resistance is expressed best).
Inoculation:	As for the glasshouse progeny test.
Race used:	A single complex race if for breeding purposes. Selected races to identify *R*–genes. Unknown race for identification.
Incubation:	7 days at 15°C (air–conditioned glasshouse, natural light); high humidity for the first 24 h.
Scoring:	The amount of blighted foliage on each plant is scored on Malcolmson's (1976) 1–9 scale (9 = no blight) after 7 days (illustrated by Cruickshank *et al.* 1982).

Field trial for resistance to foliage blight

Where: West Scotland (favourable climate).

Race used: A single complex race virulent against the *R*–genes present in the experiment (not a race mixture, which could overestimate the field resistance of genotypes with *R*–genes overcome by one of the races).

Inoculation: Glasshouse–grown infector plants (inoculated) are placed in "spreader" drills of susceptible cv. King Edward in mid–July.

Two drills of 2–plant plots of test clones between spreader drills.

R–gene differential genotypes are included, to monitor virulence.

Earlies and maincrop genotypes are grown in separate blocks: epidemic progress is more rapid in earlies.

Control cvs cover a range of resistance of each maturity.

Scoring: The amount of blighted foliage/stem on each plant is assessed on the 1–9 scale, at intervals of 4 or 5 days. Scores are used from the date that gives the best discrimination between control cvs.

Tuber blight

Tuber blight progeny test (glasshouse)

Tubers: From true seedlings raised in individual 10 cm pots, harvested at flowering.

One tuber per seedling is put into one of two bulk samples of 25 per family.

Inoculation: Washed tubers are dipped in a suspension of *P. infestans*, 2.5×10^4 zoospores per ml, on the day of harvest.

Race used: Complex race.

Incubation: High humidity for 24 h, ambient temperature (approx. 15–22˚C) for 10–14 days.

Scoring: The percentage of tubers (by number) with blight symptoms is recorded for each family sample after 10–14 days, ignoring infections through wounds or stolon scars.

Tuber blight clonal test on glasshouse–grown tubers

Tubers: Replicated samples of 20 tubers harvested from flowering plants grown from tubers in 10 cm pots.

Inoculation:
Race used:
Incubation:
Scoring:
} As for the tuber blight progeny test.

Tuber blight clonal test on field–grown tubers

Tubers: Immature, hand–dug in early to mid–August, before the skins set.
First earlies are lifted first; second earlies 1 week later; maincrop another week later.

Inoculation: Replicated samples of 20–40 damage–free tubers are placed rose–end–up in boxes and sprayed with zoospore suspension (5×10^4 spores per ml) on the day of harvest to mimic natural infection.

Race used: Complex race.

Incubation: As for the tuber blight progeny test.

Scoring: 1–9 scale of increasing resistance, by comparison with standard control cvs covering a range of reesistance; based on estimated percentage cover of blight symptoms per box of 20–40 tuber after 10–14 days, ignoring infections due to wounds and through the stolon scar.

DISCUSSION

Glasshouse progeny tests (on true seedling progeny samples) for resistance to foliage blight identify resistance within a year of crossing (Stewart *et al.* 1983c); they identify the best (most resistant) families (which are also the best in field clonal trials), so that further samples of the most resistant families can be raised healthy (they are not used for selecting resistant seedlings within families.) These tests are also used for estimating the breeding value of parents, and for identifying resistant accessions of wild species for genetic studies and to widen the genetic base of resistance.

Glasshouse clonal whole plant tests for resistance to foliage blight can assess several hundred genotypes, so can be used for selection of resistant clones moderately early in a breeding programme. These tests reflect field performance well (Stewart *et al.* 1983a), and have the advantage that non–indigenous isolates can be used in containment facilities. In addition to selection of resistant genotypes, they are used for

genetic studies, for mapping resistance genes (*R*–genes or quantitative trait loci – QTL), and for identifying the virulence characteristics of *P. infestans* isolates. By inoculating replicate plants with different races, these tests can be used for identifying *R*–genes. However *R*–gene resistance is not always easy to distinguish from a high level of field resistance; an inheritance test to confirm it takes 3 years.

Field clonal trials for resistance to foliage blight (Stewart *et al.* 1983a) show little variation between plots of the same clone within a trial. However year effects are found, especially in some cultivars, so repeated testing is needed to assess small differences accurately. These trials provide the best estimate of non–race–specific resistance. Only indigenous isolates should be used. Foliage blight field trials are used for assessing advanced selections, genetic studies and the development of marker–assisted selection.

Glasshouse seedling progeny tests for resistance to tuber blight are an easy and reliable way to assess large numbers of families, and give good agreement with tests on field–grown tubers (Wastie *et al.* 1987). They are used for selecting families with tuber blight resistance in combination with other traits.

Glasshouse clonal tests for tuber blight resistance also give close agreement with tests on field–grown tubers. They are less labour–intensive than field tests, so larger numbers of clones can be tested (allowing larger genetics experiments or earlier selection). Results suggest that they should prove more consistent over years than tests on field–grown tubers (Stewart *et al.* 1996).

Clonal tests on field–grown tubers are the closest method to natural conditions for tuber blight, but are not practical for large numbers of clones. Harvest date and inoculating freshly–harvested tubers are important (Stewart *et al.* 1983b). Clones are best tested in more than one year for a reliable estimate of resistance.

The methods described here are being used at SCRI to develop and evaluate molecular tests for assessment of resistance. Molecular tests, if successful, will be quicker and less labour–intensive. Marker–assisted selection should obviate the repeated testing needed because of environmental effects on phenotypic assessment.

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