Assessing potato germplasm for resistance to late blight

H.E. Stewart, J.E. Bradshaw & G.R. Mackay

Introduction Late blight, caused by the oomycete *Phytophthora infestans*, is the most important disease of potatoes, and occurs world-wide wherever they are grown. Black or brown lesions develop on the leaves, and in warm, humid weather, these can expand and increase in number to destroy the foliage of the whole crop very rapidly (Fig.1). Spores are washed from the leaves into the soil by rain, and the tubers become infected whilst in the ground or during lifting. This can result in severe or total loss of the crop, as happened in Ireland in the 1840's, causing the famine and subsequent emigration. Today, control of the disease depends on the use of costly fungicides and the world-wide economic cost of protection and crop loss is estimated at $3 billion per annum. However, in the developing world, where potato production is increasing, the cost of protection is unaffordable for many farmers. In Europe, on the other hand, there is an increasing demand for reduced use of agro-chemicals because of concern for the environment. The way to overcome these problems is to develop varieties with a higher level of durable resistance for use as part of an integrated control system. There are two kinds of resistance; one is race specific, governed by one or more major (R) genes, and is inherited in a simple Mendelian manner. It confers immunity to the disease or a very high level of resistance. The other is effective against all races and reduces lesion size, frequency and the amount of sporulation. It is more complex in inheritance and is termed field resistance. Major gene resistance has proved short lived, for example cv. Pentland Dell, bearing three R-genes, was immune to blight when it was released as a commercial cultivar in 1961, but succumbed to extensive infection by new races 6 years later. Consequently, for some time potato breeders have opted for field resistance. SCRI has a long history of breeding for resistance to blight, beginning at the Scottish Plant Breeding Station in the 1920s. Several cultivars with good levels of resistance have been released, and three more recent ones, cvs Stirling, Torridon and Teena, have performed well in blight trials in Europe, South America, and the United States[1]. In order to become commercially successful, resistant cultivars also need to possess other desirable agronomic traits and recent research has aimed to combine resistance to blight with resistance to potato cyst nematodes and high yield, and the quality demanded by processors and supermarkets. Moreover, in order to widen the genetic base of the resistance as insurance for the future, novel sources have been sought amongst accessions of the wild species of the Commonwealth Potato Collection (CPC), which is maintained at SCRI. Success in achieving such aims depends on reliable methods of selecting the best parents and the most resistant genotypes from crosses between them. The tests need to enable assessment of large numbers of genotypes, to ensure as far as possible that durable field resistance is selected and not short-lived R-gene resistance, and laboratory or glasshouse tests need to reflect actual performance in the field. Laboratory tests were initiated by William Black in the 1950s, when selection for field resistance began. Since then they have been developed and improved by SCRI pathologists, in particular Jean Malcolmson, Roger Wastie and Helen Stewart, and a field site established which has given reliable epidemics every year for over 20 years. This brief review records the most relevant factors for achieving success in assessing potato germplasm for resistance of both foliage and tubers to this devastating disease.

Figure 1 Potato crop infected with late blight.

Foliage resistance *Progeny test* True seedlings, 5 to 7 weeks from sowing and about 10 cm tall, are sprayed with a suspension of *P. infestans* containing 5 x 10^4 zoospores per ml. A complex race capable of overcoming any R-genes present is used, and the amount of

Figure 2 Progeny test for foliage resistance.

blight recorded 6 days later[2] (Fig. 2). The test has been shown to consistently rank the seedling progenies with their subsequent performance in the field, although it is not a very effective means of identifying resistant individual genotypes within progenies. It has been successfully used in breeding programmes at SCRI to select the best progenies within a year of making crosses, to estimate breeding value of parental genotypes, and to identify resistant accessions of wild species of the CPC for genetic studies as well as introgression into the cultivated potato (*S. tuberosum*).

Glasshouse Whole plant test Plants are raised in the glasshouse until just prior to flowering, the stage of growth at which resistance is best expressed. They are sprayed to the point of run-off with a zoospore suspension (5 x 10^4 spores/ml) of a complex race of *P. infestans,* incubated at 15°C, and kept at high humidity for the first 24 hrs to keep the leaves wet to enable infection. The amount of blighted foliage is recorded 7 days after inoculation on a 1-9 scale of increasing resistance[3] (Fig. 3). The test broadly agrees with field assessment, but the difference in score between the most resistant and most susceptible genotypes is smaller in the glasshouse than in the field trial, leading to underestimation of both resistance and susceptibility. It is therefore important that the glasshouse plants are reduced to a single stem and raised well spaced out in a cool glasshouse in order to produce sturdy plants that most closely reflect field behaviour. The test enables assessment of several hundred individual

Figure 3 Glasshouse whole plant test.

genotypes, and is effective in identifying resistant ones and eliminating the most susceptible in the intermediate stages of a breeding programme. It has been used to study the genetics of resistance in the wild species *Solanum verrucosum* and *S. papita*, and in progenies of cv. Stirling. It has also provided phenotypic data to enable molecular work to locate a QTL (quantitative trait locus) responsible for a significant proportion of the high level of field resistance of cv. Stirling and the mapping of its R-gene. Furthermore, it provides a reliable method of identifying R-genes and the virulence characteristics of *P. infestans* isolates. Detached leaflet tests traditionally used for this purpose can give variable results depending on factors such as the age of the plant and inoculum concentration. These factors have less influence on the whole plant test, which is therefore preferable as clear, repeatable results are achieved. Genotypes can be assessed using different races, including non-indigenous ones which can be safely used in the enclosed incubation facility.

The field trial This is carried out on a farm near the west coast of Scotland, where the climate favours blight[3] (Fig. 4). It is inoculated with a single race of

Figure 4 Field trial.

Figure 5 Test for resistance of field grown tubers.

P.infestans, virulent against any R-genes present in the germplasm being assessed. Infected glasshouse-grown plants are spaced at metre intervals along drills of a susceptible cultivar situated on either side of pairs of drills of the genotypes being assessed. One complex race is used because mixed inoculum of complementary races can lead to overestimation of the resistance of genotypes bearing R-genes that only one component of the inoculum can overcome. The R-gene differentials, 11 genotypes each bearing one of the *S. demissum* derived resistance genes, are grown in the trial to monitor the virulence of the pathogen and hence confirm its ability to defeat R-genes borne by the genotypes being tested. The epidemic progresses more rapidly in early maturing genotypes (because they tend to be more susceptible) than in maincrops and so the two maturity groups are planted in separate blocks, each with control cultivars of appropriate maturity and covering a range of resistance. This enables the genotypes of each group to be assigned a 1-9 score, based on the amount of blighted foliage on the date which shows best discrimination between the control cultivars. The resistance score of a genotype is affected by the resistance of its neighbour, but variation between plots of the same genotype within the trial is low, probably because the test plants are all adjacent to the highly susceptible plants of the spreader drills. However, some differences in resistance between years occur which cannot be attributed to the presence of R-genes, particularly in some cultivars, and repeated testing is needed for accurate assessment of small differences. Severe epidemics have developed every year since the site was first used in the mid 1970's and the trial provides the best ultimate assessment of field resistance. Advanced selections from breeding programmes are thus assessed, and data collected for genetic studies and to develop marker-assisted selection (detecting resistance through testing for the presence of molecular markers associated with it).

Tuber resistance Although resistance of foliage and tubers is correlated, genotypes with resistant foliage do not always bear resistant tubers and selection for resistance of both has been SCRI policy and is advocated. Both are important because whereas susceptible genotypes are soon dead, partially resistant ones provide a source of inoculum for tuber infection over a prolonged length of time and hence with a greater chance of coinciding with weather conditions favourable for tuber infection.

Glasshouse progeny test for resistance of tubers True seedlings are sown, raised in individual pots in the glasshouse until flowering, and two samples of single tubers of 25 seedlings of each progeny dip-inoculated in a suspension of a complex race of *P. infestans* containing 2.5 x 10^4 spores/ml. The tubers are kept at high humidity for 24 hrs and the percentage of blighted tubers recorded 10 to 14 days later, ignoring infections through the artificial wound of the stolon scar[4]. The test has been shown to predict successfully the susceptibility of the same progenies assessed using field grown tubers, and hence is an easy and reliable way of assessing large numbers of progenies. It has been used to select progenies with tuber as well as foliage resistance, and to combine these with other agronomic traits.

Test of field grown tubers Immature tubers are hand dug in early August before the skins set, as this is when natural infection is most likely to occur and because they become more resistant as they mature. First-early maturing genotypes are generally lifted a week before second-earlies and two weeks before the maincrop genotypes. The rose-end of replicate samples of 20 to 40 damage free tubers of each genotype is sprayed with a zoospore suspension (5 x 10^4 spores/ml) of a complex race of *P.infestans* on the day of lifting and incubated at high humidity for 24 hrs. The percentage of blighted tubers is recorded 10-14 days after inoculation[5] (Fig. 5). Inoculation on the day of lifting mimics natural infection and ensures meaningful discrimination between genotypes. Nevertheless, differences in resistance of the same genotypes within and between years of test do occur, and hence more than one year's results are required for a reliable estimate of resistance. Glasshouse grown tubers were found to be more susceptible when harvested from dry compost than from wet or moist compost, and a further study attributed this to the

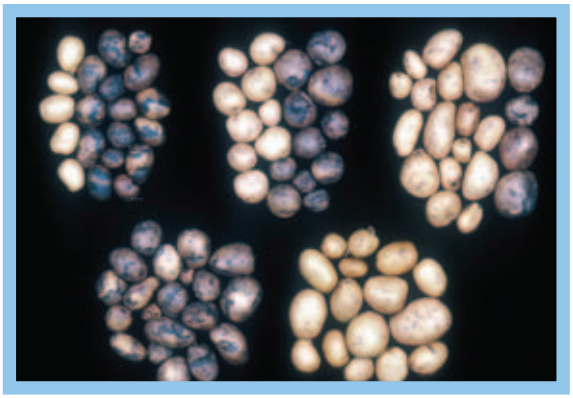

Figure 6 Glasshouse test for tuber resistance.

presence of a higher number of bacteria antagonistic to *P.infestans* on the surface of tubers grown in wet compost. Environmental factors such as this may account for some of the variation found in the field test results.

Glasshouse test of genotypes for resistance of tubers The test of field grown tubers is highly labour intensive and assessing large numbers of genotypes in this way is impracticable. So a test of glasshouse-grown tubers was developed, in which replicate samples of 20 tubers harvested from flowering plants were inoculated the same day with a zoospore suspension (2.5×10^4 spores/ml) of a complex race of *P. infestans*. They are inoculated, incubated and scored as described for the progeny test[6] (Fig. 6). This glasshouse test shows close agreement with the test of field grown tubers; indeed comparison of the two suggested that results of the glasshouse test would be more consistent over years than those of the field test. The glasshouse test is particularly valuable for assessing the hundreds of genotypes under evaluation during the intermediate stages of a typical breeding programme and for providing phenotypic data for genetical studies on tuber resistance.

Remaining challenges and prospects for the future

Ensuring that the race of *P.infestans* used is capable of overcoming all the R-genes present is not always possible. Although blight populations are becoming more virulent (able to overcome more R-genes) in some parts of the world, races able to overcome all of the recognised eleven R-genes from the wild species *S. demissum* are still often unavailable, especially for use in field trials for which indigenous races are essential. Other R-genes also exist, and since R-gene resistance does not always confer immunity, it can be phenotypically indistinguishable from a high level of field resistance. Therefore ensuring that the resistance selected is field resistance and not race specific R-gene resistance is difficult. At present, the only way of distinguishing them apart with confidence is to examine the way that the resistance is inherited. This can take at least three years. However, both R-gene and field resistance interactions with *P. infestans* are now being studied at the molecular level at SCRI by Paul Birch, and the potato genes up-regulated in the two processes identified.

The expression of resistance is influenced by differences in the environment as well as the physiology of the plant. Therefore the susceptibility of a genotype to blight can differ from test to test and year to year, to a greater extent in some tests and in some genotypes than in others. This means that genotypes have to be tested in several successive years in order to obtain an accurate estimate of resistance. Development of molecular marker assisted selection should eliminate the need for repeated testing since it will be unaffected by environmental differences. The tests described here are being used to develop the molecular techniques and will be used to verify them. Should this prove successful, assessing potato germplasm in future will be much more rapid, and less labour intensive.

Acknowledgement

This work was funded by the Scottish Executive Environment and Rural Affairs Department.

References

[1] Forbes, G.A. (1999). International Potato Center Program Report for 1997-1998, 57-66.

[2] Stewart, H.E., Taylor, K. & Wastie, R.L. (1983). *Potato Research* 26, 363-366.

[3] Stewart, H.E., Flavelle, P.H., McCalmont, D.C. & Wastie, R.L. (1983). *Potato Research* 26, 41-48.

[4] Wastie, R.L., Caligari, P.D.S., Stewart, H.E. & Mackay, G.R. (1987). *Potato Research* 30, 533-538.

[5] Stewart, H.E. McCalmont, D.C. & Wastie, R.L. (1983). Potato Research 26, 101-107.

[6] Stewart, H.E., Wastie, R.L. & Bradshaw, J.E. (1996). *Potato Research* 39, 283-288.