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Improving the yield, processing quality and disease and pest resistance of potatoes by genotypic recurrent selection

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Abstract A potato (*Solanum tuberosum* Gp Tuberosum) breeding programme is described and analysed in which resistances to late blight [*Phytophthora infestans* (Mont.) de Bary] and the white potato cyst nematode (*Globodera pallida*) have been combined with a modest increase in yield and acceptable fry colour for processing. It began in 1991 and has involved cycles of crossing, selection between from 120 to 145 progenies (full-sib families), and clonal selection within the selected progenies. We have shown that the breeding scheme can operate on a 3-year cycle with limited within progeny selection, and on a 5- or 6-year cycle with more extensive within progeny selection. Six years are required when resistance to late blight is assessed in the tubers as well as the foliage. The more extensive within progeny selection is recommended once genes have been combined from sufficient parents to achieve one's objectives. The yield increase after three cycles of indirect selection through breeders' visual preference was only modest because it was operating against a decrease which would occur in the absence of selection. A practical improvement in the scheme would be to increase the number of progenies assessed to over 200, given the moderate to high heritabilities of the progeny and clonal tests. But this would require a considerable effort because the success rate achieved with the potato pollinations was typical at just over 30%. In the fourth cycle we showed how new breeding objectives and germplasm could be accommodated whilst continuing to maintain progress, something that is important in any long term breeding strategy.

Keywords Fry colour · Late blight · Potato breeding · Potato cyst nematodes · Progeny testing · Selection index

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Introduction

The potato (*Solanum tuberosum* Gp Tuberosum) breeding programmes at the Scottish Crop Research Institute (SCRI) date back to the foundation of the Scottish Plant Breeding Station (SPBS) in Edinburgh in 1920, since when 72 cultivars have been bred and released. Within these programmes it soon became apparent that the European potato lacked genes for resistance to what were, or became, major diseases and pests. Hence desirable genes were introgressed from the wild and cultivated tuber-bearing species of Latin America: first for late blight [*Phytophthora infestans* (Mont.) de Bary] resistance from 1932, then for resistance to viruses from 1941, and finally for resistance to potato cyst nematodes (PCN) from 1952

(Bradshaw and Ramsay 2005). By 1990 cultivars and clones were available with these disease and pest resistances; but no systematic attempt had been made to combine them in a single cultivar, although parents for crossing had been chosen to complement one another for desirable characteristics.

Therefore, in 1991, a multitrait (MT) breeding programme was started at SCRI to combine quantitative resistances to late blight and the white potato cyst nematode (*Globodera pallida*) with commercial worth as judged by breeders through a visual assessment of tubers (breeders' preference; Bradshaw et al. 2003). Quantitative resistances were used because major gene resistance to late blight had not proved durable and major gene resistance to pathotype Pa2/3 of *G. pallida* had not been found. The parents with resistance to pathotype Pa2/3 of *G. pallida* also had either major gene (*H1*) or quantitative resistance to pathotype Ro1 of *G. rostochiensis*, the golden potato cyst nematode, pathotypes Pa2/3 and Ro1 being the ones present in Britain. Parents were also included with resistance to *potato leafroll virus*, *potato virus Y* and *potato virus X*, but time and resources did not permit direct selection for virus resistance in each generation (Solomon-Blackburn and Bradshaw 2007). Such an overall combination of traits was, and still is, lacking in European potato cultivars, despite 50 years of breeding effort.

The breeding programme has made use of progeny tests developed at SCRI (Bradshaw et al. 2003) and has involved cycles of crossing, selection between progenies (full-sib families) and clonal selection within the selected progenies. We have shown that recurrent selection based on progeny testing, with limited within progeny selection, can operate on a 3-year cycle and full combined selection between and within progenies on a 6-year cycle. These cycle lengths are much shorter than the time from making a cross to releasing a new cultivar which has averaged 13 years since 1975, a year longer than the target of 12 years (Mackay 2005). This would be the cycle time if one waited for release of a cultivar before using it as a parent. In the fourth cycle of the programme we introduced processing quality as a new objective along with new germplasm for this trait and for late blight resistance. Furthermore, we have previously shown that progeny testing provides a solution to the common but ineffective practice in potato breeding of intense visual selection of quantitative traits between seedlings in a glasshouse and spaced plants at a seed site (Bradshaw and Mackay 1994; Bradshaw et al. 1998). The results from the new breeding programme are presented for the first three cycles followed by the changes made in the fourth cycle.

Materials and methods

Parents and crossing for first three cycles of breeding programme

Thirty-six parents were chosen to initiate the breeding programme in 1991. They comprised ten parents with field resistance to late blight assumed to be derived primarily from *S. demissum*; 12 with resistance to the white potato cyst nematode of which six had resistance derived from *S. vernei*, four from *S. tuberosum* Gp Andigena, and two from both these sources; and 14 with virus resistance derived from a number of sources including *S. acaule*, *S. chacoense*, *S. demissum*, *S. microdontum* and *S. stoloniferum* (Bradshaw and Ramsay 2005). After three cycles of selection, the pedigrees of the 15 selected clones could be traced back to 15 out of the 36 initial parents, four from the blight resisters, five from the PCN resisters and six from the virus resisters (Solomon-Blackburn and Bradshaw 2007).

In order to combine desirable genes from the three sets of parents, the following procedure was followed. Pair crosses were made in 1991 between the blight and PCN resisters (set A), the blight and virus resisters (set B), and the PCN and virus resisters (set C), and 42/120, 54/140 and 24/168 combinations, respectively, secured with adequate seed for progeny testing (120/428). This was also the number of progenies achieved as one was secured for each combination. From these three sets of crosses, 14, 10, and 12 progenies were selected. Then in 1994 crosses were made between progenies from different sets (A $\times$ B, A $\times$ C and B $\times$ C) and 43/140, 48/168 and 34/120 combinations secured (125/428). The number of progenies achieved was greater than 125, namely 137, because two progenies were secured for each of 12 combinations. Finally, in 1997, crosses were attempted between 27/29 selected progenies and 130/351 combinations were achieved with adequate seed for progeny testing. Again the number of progenies (145) was greater than the number of combinations.

Table 1 Summary of first three cycles of breeding programme

Cycle	Year	Germplasm	Assessed	Selected	Criteria
1	1991	Crosses			
	1992	Progenies	120	36	Preference, PCN (Pa2/3), blight
	1993	Clones	1620	108	Preference
2	1994	Crosses			
	1995	Progenies	137	29	Preference, PCN (Pa2/3), blight
	1996	Clones	870	108	Preference, PCN (Pa2/3, Ro1)
3	1997	Crosses			
	1998	Progenies	145	31	Preference, PCN (Pa2/3), blight
	1999	Clones	930	144	Preference

The essence of the breeding programme is summarised in Table 1.

In each cycle between progeny (full-sib family) selection was practised. The progeny tests were done as described by Bradshaw et al. (1995) for breeders' preference, *G. pallida* (pathotype Pa2/3) and foliage blight and as described by Bradshaw et al. (2003) for tuber blight. Complex isolates of *P. infestans* were used in order to overcome the *S. demissum*-derived R-genes known to be present. All progeny tests had two replicates apart from breeders' preference and PCN in 1992 which both had four. Each replicate in each progeny test required 25 seeds to be sown. Clonal selection within the selected progenies took place as described for each cycle.

Cycle 1

In the 1992 seedling progeny tests, any of the 120 progenies that were below average for breeders' preference were eliminated, together with any that were below average for foliage blight and *G. pallida* resistance in set A, foliage blight resistance in set B, and *G. pallida* resistance in set C; leaving 48 progenies. These 48 progenies were grown at our seed site in 1993 as an RCB design with four replicates and single-drill plots of 15 spaced plants (singles). However, seven progenies (from A and B) were eliminated as too susceptible to tuber blight, two as too susceptible to powdery scab and three on breeders' preference at seed site, leaving 36 progenies (Table 1). At harvest, two breeders (JEB and MFBD) visually selected (breeders' preference) the most attractive looking clone out of 15 in each of three replicates for each of the 36 progenies to provide 108 parents for crossing in 1994. Thus 108 clones were selected out of 1,620 (36 progenies × 3 replicates × 15 spaced plants; Table 1).

Cycle 2

In the 1995 progeny tests, 36 out of 137 progenies were selected on the basis of a selection index in which the breeders' preference, *G. pallida* and foliage blight scores in standard deviation units from their overall means were weighted by their broad-sense heritabilities. The 36 progenies were grown at our seed site in 1996 as an RCB design with two replicates and single-drill plots of 15 spaced plants (singles). However, seven progenies were rejected as too susceptible to tuber blight, leaving 29 progenies (Table 1). At harvest, two breeders (JEB and MFBD) visually selected (breeders' preference) the six most attractive looking clones out of 15 in both replicates for each of the 29 progenies, making a total of 348 clones. Thus 348 clones were selected out of 870 (29 progenies × 2 replicates × 15 spaced plants; Table 1). Of the 348 clones, 108 (from 27 of the 29 progenies) were selected for use as parents in 1997 on the basis of PCN canister tests (Phillips et al. 1980), with three replicates for *G. pallida* and two for *G. rostochiensis*, done from 30 January to 16 April 1997. The selected clones had cyst counts <40% of susceptible control cultivar Desiree with *G. pallida* Pa2/3 (78/108 were as good as or better than partially resistant cultivar Eden on 26%) and <35% of the same control with *G. rostochiensis* Ro1 (34/108 had *H1* gene, as did Eden, i.e. 0%). Pair crosses with the 108 parents were attempted between but not within the 27 progenies.

Cycle 3: progeny tests

In the 1998 progeny tests, 36 out of 145 progenies were selected on the basis of a selection index in which the breeders' preference, *G. pallida* and foliage blight scores in standard deviation units from their overall means were weighted by their broad-sense heritabilities. The 36 progenies were grown at our seed site in 1999 as an RCB design with two replicates and single-drill plots of 15 spaced plants (singles). However, five progenies were rejected as too susceptible to tuber blight, leaving 31 progenies (Table 1) and a total of 930 spaced plants (31 progenies × 2 replicates × 15 plants). At harvest, two breeders (JEB and MFBD) visually selected (breeders' preference) at least one attractive looking clone from each plot. They selected 144 clones in total and these were grown as four-plant plots (fours) at the seed site in 2000 (Table 1).

Cycle 3: resown progenies

In 1998, a total of 122/145 progenies were assessed in all of the progeny tests, including the one for tuber blight (Table 2). Twelve of these progenies were selected using an index based on Smith's (1936) discriminant function for plant selection. The relative economic weights in phenotypic standard deviation units were 1:1:½:½ for breeders' preference, *G. pallida*, foliage blight and tuber blight, respectively, so that the two blight scores together were given a similar weight to each of the other two traits. The index also took into account the small correlation between foliage and tuber resistance ($r = -0.35$, with negative sign as the resistance scales were in opposite directions). Two out of the 12 progenies were in addition to the 31 selected out of the 145 (Table 1), making a total of 33 (Fig. 1). More seedlings of each progeny (140 to 213) were raised in a glasshouse in 1999 and grown as four-plant plots at the seed site in 2000 (total 2,178 clones: 402 from 2 progenies + 1,776 from 10

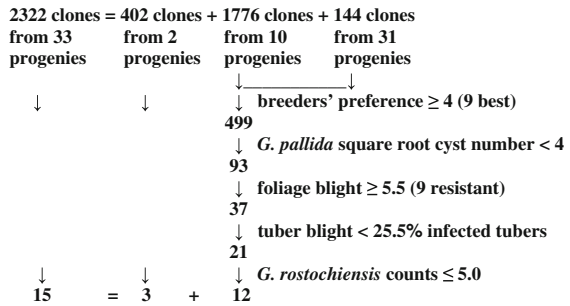

Fig. 1 Sequential clonal selection from selected third cycle progenies

Table 2 Progeny tests: selection differentials (selected minus assessed means), best progeny and heritability of progeny means over two replicates; preference was measured on a 1–9 scale of increasing preference, PCN as the square root of cyst counts, foliage blight on a 1–4 scale of increasing resistance, and tuber blight as the percentage of infected tubers transformed to angles

1995 Progenies	137 Assessed	29 Selected	Best for trait	Heritability
Preference	4.44	4.68	5.04	0.63
PCN	8.72	8.34	5.03	0.60
Foliage blight	1.78	2.28	3.50	0.63
1998 Progenies	122 Assessed	12 Selected	Best for trait	Heritability
Preference	4.00	4.44	4.85	0.63
PCN	7.92	6.40	3.91	0.72
Foliage blight	1.76	1.98	3.00	0.39
Tuber blight	31.9	18.9	0.00	0.82
2004 Progenies	122 Assessed	25 Selected	Best for trait	Heritability
Preference	3.70	4.07	4.66	0.68
PCN	3.68	3.07	1.00	0.55
Foliage blight	2.95	3.48	4.00	0.53
Tuber blight	20.6	18.0	0.00	0.63

progenies). Thus a total of 2,322 (2,178 + 144) clones were grown as four-plant plots at seed site in 2000 (Fig. 1).

The 2,322 clones were reduced to 15 for use as parents in 2003 as follows (Fig. 1). All clones (402) of two resown progenies were maintained for research purposes and assessed separately for all traits. Out of the remaining 1,920 clones (1,776 from 10 progenies + 144 from 31 progenies), 499 were visually selected at harvest at the seed site (breeders' preference $\geq$ 4, on a 1–9 scale of increasing preference which took account of visual assessment of tuber yield, size, regularity of shape and freedom from growth cracks). During 2001 and 2002 the 499 clones were reduced to 93 after assessment for resistance to *G. pallida* (square root of cyst number $<$ 4) using a closed container test with four replicates (Phillips et al. 1980); to 37 after assessment for foliage blight resistance (third assessment date score $\geq$ 5.5 on a 1–9 scale of increasing resistance) in a field test in Ayrshire with two replicates (Bradshaw et al. 2004); to 21 after assessment for tuber blight resistance (percentage of infected tubers $<$25.5%) in a glasshouse trial with two replicates (Bradshaw et al. 2004); and finally to 12 after nine clones were eliminated as too susceptible to *G. rostochiensis* Ro1 [mean counts of 4.3–35.6 in a three replicate container test (Phillips et al. 1980) where ten clones were clearly resistant ($\leq$2.0) and two borderline ones (4.3 and 5.0) were accepted]. The nine eliminated clones either lacked quantitative resistance or the *H1* gene for resistance. Three clones from one of the two progenies (97MT168) maintained for research purposes also met all of these criteria, making a total of 15 parents for the next cycle of crosses (Fig. 1).

The 15 parents were subsequently retested for their foliage blight resistance in a field trial in Ayrshire in 2004 and in 2007, for their tuber blight resistance in a glasshouse trial in 2004 and in 2008, and for their *G. pallida* resistance in 2007. The only differences to the first set of tests were the isolates of *P. infestans*: 99/23 (race 1,2,3,4,6,7) in 2001 and 2002, 01/29 (race 1,2,3,4,6,7,8,10,11) in 2004, and genotype 'blue' 13_A2 (race 1,2,3,4,5,6,7,10,11) in 2007 and 2008. Six of the 15 clones were subsequently used as parents in commercially funded breeding programmes, three extensively, but three were dropped based on the 2004 foliage blight results (Table 5).

New breeding objectives and germplasm for cycle 4

It was decided to improve the processing quality of the population by introducing progeny testing and clonal selection for fry colour after storage at 10°C for 3 months (Bradshaw et al. 2003). It was also decided to introduce new germplasm for this trait and for late blight resistance.

In 2003, a half diallel set of crosses plus selfs was attempted with the 15 clones selected in the previous cycle. Seed was secured for progeny tests from 60 crosses and eight selfs. In addition, seed was secured from 24 crosses out of 75 attempted between the 15 clones and five SCRI cultivars with good processing quality (Golden Millennium, Harborough Harvest, Montrose, Scarborough and Tay); from 15 crosses between three blight resistant parents (Chota Nawi, Cordillera and Robusta) and five parents with PCN resistance and processing quality (clones 12601ab1, 12636a2 and 12674ab1 and cultivars Eden and Maris Piper; all with *H1* and all except Maris Piper with both resistance to *G. pallida* from *S. tuberosum* Gp Andigena and resistance to cold sweetening); and from 15 crosses involving parents with processing quality and PCN, late blight and virus resistance. Hence 122 progenies with sufficient seed were available for testing in 2004 (Table 2).

Cycle 4

In the 2004 progeny tests, 36 out of 122 progenies were selected on the basis of a selection index in which the breeders' preference, *G. pallida* and foliage blight scores in standard deviation units from their overall means were weighted by their broad-sense heritabilities. None were rejected as too susceptible to tuber blight. In both the foliage and tuber blight tests the isolate of *P. infestans* was 01/29 (race 1,2,3,4,6,7,8,10,11). All 36 progenies progressed to the seed site in 2005 as an RCB progeny trial for fry colour with two replicates, as done by Bradshaw et al. (2003). After harvest and storage at 10°C for 3 months, the 11 worst progenies for fry colour were eliminated (score $<$ 3.7 compared with mean of 3.9 for all 36 progenies). A total of 108 clones out of 900 had been visually selected (breeders' preference) by two breeders (JEB and MFBD) from the remaining 25 progenies and were used as parents to start cycle 5 in 2006. Of these 25 progenies, 18 were from the diallel

crosses and one was a diallel self. In other words, just six out of 54 new crosses were good enough to enter the breeding programme.

Assessment of the 108 clones selected from cycle 4

The 108 clones selected from cycle 4 were grown at a high-grade seed site (Derachie farm) in 2006 as four-plant plots and again in 2007 as six-plant plots to provide seed for further evaluations. In 2007, they were assessed for foliage blight resistance in the field trial in Ayrshire, for PCN (Pa2/3) resistance in the closed container test, and for yield, agronomic and quality traits at Gourdie farm, Dundee. The yield trial had an alpha-design with two complete replicates, incomplete blocks of size 8, and single-drill plots of five tubers spaced 45 cm apart, with adjacent drills 90 cm apart. It was planted on 20 April, scored for maturity on 20 August on 1–9 scale of increasing lateness, and harvested on 28 September. Fertilizer, herbicide, aphicide and fungicide (for control of late blight) applications were standard for a ware crop in S.E. Scotland. Harvested plots were scored as described by Bradshaw et al. (2008) for trials done in 2000 and 2001. Plots were given an overall (Over) score on a 1–9 scale of increasing preference which included internal condition of tubers but not yield as plots were weighed. For regularity of tuber shape (Shape), tubers with deep eyes would get a low score, but so would ones that showed the more common occurrence of departures from a regular round, oval or long oval shape. In 2008, the 108 clones were assessed for tuber blight resistance in the glasshouse trial, as described by Bradshaw et al. (2004). Included in all of these assessments were the original 36 parents from 1991, as well as the 15 clones selected from cycle 3 and nine other controls, thus making a total of 168 entries. Based on all of these results, eight out of the 108 fourth cycle clones were used as parents in commercially funded breeding programmes in 2008 (Table 6).

Results

Parents

The first opportunity to assess the blight and PCN resistance of the original 36 parents came in the clonal tests undertaken in 2001 and 2002. Of the ten parents selected for blight resistance, cultivars Draga and Cramond proved susceptible in both their foliage and tubers, and Shelagh had susceptible tubers. The other parents had varying degrees of resistance with clone 8204a4 (a parent of Stirling) and cultivars Stirling and Torridon the best. The 12 PCN resisters were susceptible to foliage blight with the exception of clone 11234ab4 and to tuber blight with the exception of clone 12636a2. Three of the virus resisters had moderate foliage blight resistance but susceptible tubers. When all 36 parents were reassessed for foliage blight resistance in 2007, with a different isolate of *P. infestans*, there was good agreement with the previous results with two exceptions, cultivar Torridon was now susceptible (2.5 compared with 6.5) and Stirling was not as resistant (5.5 compared with 7.0). For tuber blight, use of the new isolate resulted in many quantitative differences from the previous results, with Stirling, Torridon and clone 12636a2 now clearly susceptible with 55, 50 and 78%, respectively, of their tubers infected. However, Shelagh (14%) was now one of the more resistant clones along with clones 8204a4 (10%) and 14697a1 (19%). Four of the virus resisters were now also among the most resistant clones.

Assessment of the parents for PCN resistance in 2001 and 2007 revealed that those with good resistance were nine out of the 12 included for their reputed resistance. Clone 11234ab4 proved susceptible and clone 14016a7 and cultivar Eden proved only moderately resistant. Taken together, the results confirmed that none of the original 36 parents used in 1991 combined PCN resistance with blight resistance in both foliage and tubers.

Progeny tests

The results shown in Table 2 are for those sets where all progenies were assessed for all of the traits shown. Standard errors are not given because the aim is to give a feel for the magnitudes of effects, not to accurately predict responses to selection. The heritabilities, based on the mean of two replicates, vary from 0.39 to 0.82 with most in the range 0.53–0.72.

The means of the selected progenies compared with the means of all those assessed (Table 2) show the selection differentials that were achieved in practice for individual traits when selecting for more

than one trait. It was not possible to achieve a high selection differential for individual traits with the numbers of progenies being assessed and selected, and the means of the selected progenies were always notably lower than the best progeny. It should be pointed out that in 1998 the 12 progenies were all selected using an index based on Smith's discriminant function, whereas in 1995 and 2004 the situation was more complicated. In 1995, seven progenies were eliminated as too susceptible to tuber blight whereas in 2004, none were eliminated for this trait but there was a correlation of $r = 0.49$ between foliage and tuber resistance once sign was taken into account. However, 11 progenies were eliminated as below average for fry colour. Nevertheless, it is still revealing to compare the selected progenies with all of those assessed and also with the best one.

Clonal tests

The results shown in Table 3 are for the sequential clonal selection from the ten third cycle progenies as this was the most extensive within progeny selection done. As mentioned earlier, selection was sequential due to differing numbers of clones that could be handled in each test, and a target criterion was set for each trait rather than selecting a fixed proportion of clones. Over one quarter of the clones (26%) met the target for breeders' preference whereas only 19% of the remaining clones met the target for PCN (Pa2/3). This left 93 clones for assessment for foliage then tuber blight, which combined resulted in 23% being selected. Hence moderate selection differentials were achieved for all traits. Furthermore, heritabilities of clone means were high, particularly for foliage blight.

Progress at end of cycle 3

The inclusion in the clonal tests of a random sample of clones from the ten best progenies which were resown in 1999 allows an assessment of progress up until 2003 (Table 4). It was possible to score breeders' preference in 2002 at the seed site because all clones were grown from common origin seed tubers and were included in the same block. The score took account of visual assessment of tuber yield, size, regularity of shape and freedom from growth cracks.

The random samples were more resistant to PCN and foliage blight than the assessed samples (Table 3) which means that selection for breeders' preference in 2000 eliminated a disproportionate number of

Table 3 Sequentional clonal selection from selected third cycle progenies showing means of assessed and selected clones, the best clone, and the heritability of clone means in the replicated assessment trials; the numbers assessed are less than the numbers previously selected when there were not sufficient tubers of all clones for the assessment

Clones	Assessed number	Assessed mean	Selected number	Selected mean	Best clone	Heritability
Preference	1,920	3.31	499	4.82	8.00	Unreplicated
PCN Pa2/3	498	6.41	93	2.37	0.00	0.80
Foliage blight	92	4.37	37	6.34	7.50	0.95
Tuber blight	36	23.6%	21	10.1%	2.5%	0.70

Table 4 Comparison of random and selected third cycle clones with original 36 parents from 1991, in sequential clonal tests from 1999 to 2002, where means of 36 parents are weighted by their contributions to successful crosses and randomly chosen clones came from the four-plant plots of the ten progenies; preference was measured on a 1–9 scale of increasing preference, PCN as the square root of cyst counts, foliage blight on a 1–9 scale of increasing resistance, and tuber blight as the percentage of infected tubers

Clones	36 Original parents	Random clones (numbers in brackets)	12 Selected clones
Preference	6.15	5.51 (35)	7.17
PCN Pa2/3	8.26	5.61 (37)	2.72
Foliage blight	3.89	5.67 (35)	6.46
Tuber blight	38.5%	22.6% (33)	11.4%

resistant clones. Comparison of the random samples with the original 36 parents gives an estimate of the combined effect of progeny and clonal selection for breeders' preference and PCN up to 1998 but of progeny selection alone for foliage and tuber blight. There was a distinct lack of progress with breeders' preference, but it should be borne in mind that breeders' preference takes account of more traits in plots at a seed site than in pots in a glasshouse. In contrast, there was a good reduction in susceptibility to PCN, from 8.26 to 5.61. The blight results indicate that modest selection differentials and heritabilities in the seedling progeny tests have translated into worthwhile gains for whole plants (this was particularly true for foliage blight where there were just four scoring categories). Thus foliage resistance increased from 3.89 to 5.67 on a 1–9 scale of increasing resistance, and tuber susceptibility decreased from 38.5 to 22.6% infected tubers. Comparison of the 12 selected clones with the random clones gives the further progress from clonal selection for breeders' preference and the selection differentials for PCN and blight resistance, most of which should be realised given their high heritabilites (Table 3).

Independent assessments of progress with breeders' preference and with blight and PCN resistance were made after 2002 when the 15 selected clones were included in tests along with cultivar Maris Piper, currently the most widely grown cultivar in Britain, but one that is susceptible to blight and *G. pallida* (Table 5). There was no evidence of improvement in breeders' preference when the 15 clones were compared with the original 36 parents in

Table 5 Repeatability of tests on 15 clones selected from 1997 crosses ('a' clones are from tuber progeny test at seed site and 'b' clones from resowings): foliage blight in Ayrshire in 2004 and 2007 on third scoring date on 1–9 scale of increasing resistance, tuber blight in glasshouse in 2004 and 2008 as percentage of infected tubers, PCN in container test in 2007 as square root of number of cysts, breeders' visual preference score on tubers from yield trial in 2007 on 1–9 scale of increasing preference, yield in kg/plot and regularity of tuber shape on 1–9 scale of increasing regularity

	Foliage blight 2004	Foliage blight 2007	Tuber blight 2004	Tuber blight 2008	PCN 2007	Pref 2007	Yield 2007	Shape 2007
97MT30b69	2.5	3.0	2.5	40.2	5.35	1.0	4.33	5.5
97MT34b118[b]	8.0	6.0	0.0	24.2	2.73	3.1	19.51	4.5
97MT36b31	7.0	5.5	61.1	31.9	3.40	3.9	14.82	6.0
97MT36b110	3.5	6.0	21.8	0.0	4.50	2.6	16.55	4.5
97MT106a1	6.5	6.5	20.5	0.0	6.17	2.9	14.88	4.5
97MT106b84[c]	5.0	6.5	2.6	0.0	1.57	3.0	15.36	6.0
97MT106b182[c]	3.5	5.5	2.9	50.0	1.91	4.0	11.53	7.0
97MT146b101	8.0	7.0	26.8	–	6.84	2.3	12.28	4.5
97MT147a3	7.5	6.5	18.7	0.0	3.38	2.5	21.02	4.5
97MT187b146[b]	7.5	7.0	17.1	0.0	5.06	4.6	22.44	5.5
97MT187b191	4.5	5.0	8.5	27.5	5.15	2.7	18.09	4.5
97MT190b3[c]	4.0	4.0	5.6	26.3	7.58	2.8	15.61	7.0
97MT168b66[b]	7.0	7.0	4.6	0.0	1.14	2.4	14.78	4.5
97MT168b166	8.0	7.0	14.2	15.9	3.32	1.9	24.83	4.5
97MT168b203	7.0	3.5	0.0	44.8	2.40	4.0	24.17	6.0
Mean	5.96	5.73	13.8	18.63	4.03	2.91	16.68	5.27
Mean 36 parents[a]	–	4.41	–	33.94	5.07	3.36	14.66	5.43
Maris Piper	4.5	2.5	63.3	45.0	6.40	3.6	19.41	6.0
SED for clones	0.46	0.65	8.72	14.71	1.129	0.81	2.265	0.95

[a] Original 36 parents weighted by contribution to successful crosses in 1991

[b] Used extensively as parents in commercial contracts

[c] Used less extensively (dropped after 2004)

the 2007 yield trial. However, the preference score was an overall score on a 1–9 scale of increasing preference which included internal condition of tubers but not yield as plots were weighed. When two of the major components of breeders' preference in glasshouse trials were examined, a 14% increase in yield was found, despite the very low yield of 97MT30b69, but no improvement in regularity of tuber shape (Shape; Table 5). Whilst individual clones were superior to Maris Piper for these traits, the means of the 15 clones were inferior.

The blight tests used new isolates of *P.infestans* and in 2004 five out of the 15 clones were as susceptible as Maris Piper for foliage blight and one was as susceptible for tuber blight. Two were again susceptible to foliage blight in 2007, together with clone 97MT168b203 which was also susceptible to tuber blight in 2008, along with clones 97MT30b69 and 97MT106b182. As a consequence, the difference between the 15 clones and the original 36 parents in 2007 was not as great as might have been expected from the 2001 results. Surprisingly, with the same pathotype of *G. pallida* in 2007, only eight out of the 15 clones were significantly more resistant than Maris Piper, the susceptible control, with similar consequences for the difference between the 15 clones and the original 36 parents. Nevertheless, two (97MT106b84 and 97MT168b66) out of the 15 clones still combined blight and *G. pallida* on the criteria used to assess progress at the end of cycle 4.

Progress at end of cycle 4

The assessments done in 2007 and 2008 confirmed that none of the original 36 parents combined blight and *G. pallida* resistance whereas this was achieved with two of the 15 clones selected from the third cycle and 23 of the 108 clones selected from the fourth cycle. They had foliage blight scores greater than or equal to 5.5 (≤40% necrotic tissue) compared with a score of 2.5 (>80% necrotic tissue) for susceptible cultivar Maris Piper, 25 days after infectors were placed in the spreader rows of the field trial. They had <22.5% of their tubers infected with tuber blight, compared with 45% for Maris Piper. They also had *G. pallida* scores of ≤3.08 (square root of cyst number) which was taken as the cut-off point for resistance because it was the highest score of the nine original parents with resistance. Among the 23 clones were 16 with an acceptable fry colour after storage at 10°C (score ≥ 4.5), and of these, five were not significantly ($P > 0.05$) lower yielding than Maris Piper (yields > 15.66 kg/plot compared with 19.41). Three of these (03B15a4, 03MT78a2 and 03MT98a1) were used as parents in commercially funded breeding programmes in 2008, together with 03MT23a2 that was lower yielding but which molecular markers indicated had *S. tuberosum* Gp Andigena derived *G. pallida* resistance (Table 6). Also used were four clones that proved more susceptible to tuber blight once these results were known in 2008 (Table 6). Three of these clones (03MT39a4, 03MT76a2 and 03MT92a1) were relatively high yielding and one (03C4a3) had good fry colour after storage at 4°C. The pedigree of clone 03C4a3 is shown in Fig. 2.

Discussion

Length of each breeding cycle

We have shown that recurrent selection based on progeny testing, with limited within progeny selection, can operate on a 3-year cycle and full combined selection between and within progenies on a 5- or 6-year cycle, depending on whether or not clonal tuber blight assessment is included as well as foliage

Table 6 Eight clones chosen for use as parents in commercial breeding programmes in 2008; foliage blight on third scoring date (FB) in Ayrshire is on 1–9 scale of increasing resistance, tuber blight (TB) in glasshouse test is percentage of infected tubers, *G. pallida* Pa2/3 (PCN) in container test is square root of number of cysts, fry colours of tubers from yield trial after storage at 4°C (FRY4) and 10°C (FRY10) are on 1–9 scale of increasing lightness and plot yield (YIELD) is kg/plot

	FB	TB	PCN	FRY4	FRY10	YIELD
03B15a4	8.0	16.3	2.37	1.4	5.1	18.27
03C4a3	7.0	28.1	1.41	4.5	6.8	11.09
03MT23a2	6.5	0.0	1.22	1.0	5.0	12.45
03MT39a4	6.5	42.9	2.57	1.0	5.1	17.43
03MT76a2	6.0	30.0	2.41	1.2	5.3	17.35
03MT78a2	7.0	21.9	2.14	1.3	4.5	17.65
03MT92a1	7.0	29.2	1.37	1.4	6.4	18.08
03MT98a1	6.5	19.1	0.43	2.0	8.2	17.99
Maris Piper	2.5	45.0	6.40	1.0	4.9	19.41
SED for clones	0.65	14.71	1.129	0.47	0.87	2.265

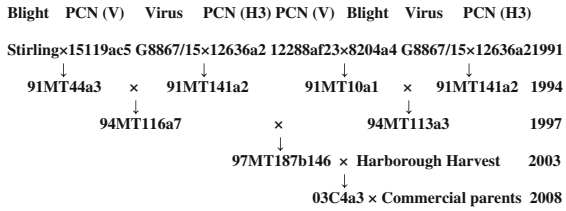

Fig. 2 The pedigree of fourth cycle clone 03C4a3 which is being used as a parent in commercial breeding programmes in 2008. The two sources of PCN resistance are *S. vernei* (V) and *S. tuberosum* Gp Andigena (H3)

blight assessment. Thus in Fig. 2, 6 years elapsed before 97MT187b146 was crossed with Harborough Harvest but only 5 years for 03C4a3 to be crossed with commercial parents. Where one wishes to combine genes from more than two parents, this can only be done through more than one cycle of crosses and a short cycle time with limited within progeny selection would seem desirable until that objective is achieved. We opted for three such cycles rather than the two which were required to bring together blight resistance, two sources of PCN resistance and virus resistance. This meant that in theory the third cycle clones could have eight different great grandparents, but the necessary constraints on crossing were not imposed in 1997, and from six (Fig. 2) to eight was achieved in practice with the 15 clones selected from cycle 3 (Table 5). In cycle 3 we opted to practise clonal selection within the most promising progenies and found that 15 out of 2,322 clones met all of our selection criteria (Fig. 1). The advantages of such selection have to be offset against the extra 3 years required before making further crosses, something that is difficult to quantify given the complexity and logistics of the recurrent selection programme. Nevertheless, the comparison of random and selected third cycle clones with the original 36 parents from 1991 (Table 4) provides some useful information. One round of clonal selection for PCN resistance appeared to have produced as much change as three rounds of between progeny selection, with limited or no within progeny selection, while clonal selection for foliage and tuber blight produced about 30 and 40%, respectively, of the change from three rounds of between progeny selection. These changes depended on the selection differentials and heritabilities actually achieved (Tables 2, 3), which are subject to manipulation by the breeder because they in turn depend on the number of families assessed and selected and the amount of replication chosen. The conclusion is that clonal selection for disease and pest resistance was worthwhile, and as a consequence, in our commercially funded breeding programmes we now practice combined between and within progeny selection on a 5-year cycle, rather than waiting to obtain more information on potential parents over further clonal generations.

Breeders' visual preference and yield

Despite selection for breeders' visual preference in each set of progeny tests and when selecting clones at the seed site (Table 1), no progress appears to have been made. In Table 4, the random clones have a lower preference score than the 36 original parents, and likewise in Table 5, the 15 selected clones have a lower score. However, the overall preference score for the produce of ware trials includes internal condition but not yield as plots are weighed. Hence it is more meaningful in Table 5 to consider tuber yield and regularity of shape as these are the traits that dominate the variation in preference scores in unreplicated plots at a seed site (Bradshaw et al. 1998), and they are also the most obvious differences between seedling tubers in the glasshouse and spaced plants in the tuber progeny trials at the seed site. The 15 clones were 14% higher yielding than the 36 original parents, but there was no improvement in regularity of tuber shape. The former improvement was expected as previous research had shown that between progeny selection for visual preference in seedling progeny tests and within progeny selection in four-plant plots at a seed site resulted in correlated responses in yield in replicated trials (Bradshaw et al. 1998). The same research also showed that such selection for visual preference resulted in improvements in visual preference scores and regularity of shape in the second (unreplicated plots at seed site) and third clonal generations (replicated yield trial at ware site). An explanation for the apparent lack of progress can be found in the genetic analysis done at the start of the programme when 15 of the original 36 parents were used in a diallel set of crosses and one cross was chosen for linkage and QTL analysis (Bradshaw et al. 2000, 2008). For breeders' preference, yield and regularity of tuber shape, the mean of

the offspring means was less than the mean of the mid-parent values (15, 11 and 14% less, respectively), and all three traits displayed inbreeding depression on selfing the parents. Hence without selection, the average scores for these traits would be expected to fall over sexual generations as favourable combinations of genes were broken up to a greater extent than new ones assembled in the offspring and some inbreeding depression occurred in the closed system operated for the first three cycles. For yield then, the net outcome was an increase, whereas for breeders' preference and regularity of tuber shape it was not possible to improve on the original parents. Nevertheless, it is important to practise selection in order to maintain tuber appearance as well as improve yield. These results also demonstrate the complexities of the genetic changes that can occur during population improvement under tetrasomic inheritance and hence the difficulty in trying to accurately predict responses to selection.

New breeding objectives and germplasm

We also showed in cycle 4 that the recurrent selection programme can accommodate new breeding objectives and germplasm, something that is important in any long term breeding strategy. It was decided that clones emerging from the programme for use as parents in commercially funded breeding programmes would be of more value if they had improved processing quality, given that 50% of the British potato crop is now processed. The genetic analysis of 15 of the original 36 parents had shown that prospects were good for improving fry colour and that progeny selection could be done at the seed site without lengthening the cycle time (Bradshaw et al. 2000). This was confirmed on selected progenies from cycle 3 which had a broad-sense heritability of 0.765 (Bradshaw et al. 2003), but not implemented until cycle 4 when five SCRI cultivars with processing quality were added as parents. Although these cultivars were resistant to cold sweetening and produced a good fry colour after storage at 4°C, it was decided to first improve the population for fry colour after storage at 10°C before introducing the more challenging objective of resistance to cold sweetening. This decision was justified by the results of the assessment of the 108 clones from cycle 4. Whilst 16 out of the 23 clones with blight and *G. pallida* resistance also had an acceptable fry colour after storage at 10°C, just one was resistant to cold sweetening (Table 6), and this came from the cross 97MT187b146 with one of the five SCRI cultivars, Harborough Harvester (Fig. 2). Three new blight resistant parents were also included in cycle 4 as they were available from the ECOPAPA project which was funded under the EU-INCO scheme and was completed in 2002. Its goal was the enrichment of potato breeding programmes in Latin America and Europe with resistance to late blight. Blight resistance in cycle 4 could be classified as a new breeding objective because a new isolate of *P. infestans*, 01/29 (race 1,2,3,4,6,7,8,10,11), was considered more relevant to the situation in Britain than previously used ones. Although quantitative field resistance was being used in the programme, the results in 2004 and again in 2007 and 2008 (Table 5) revealed that some of this resistance was isolate (race) specific, and previously resistant clones were now susceptible. The isolate used in 2007 and 2008 was genotype 'blue' 13_A2 of *P. infestans* (race 1,2,3,4,5,6,7,10,11) which had dramatically increased in Britain over the 3 years 2005–2007 to account for more than 70% of the *P. infestans* population (Cooke et al. 2008). It is an aggressive metalaxyl resistant A2 mating type isolate which has overcome the resistance in cultivars Stirling and Torridon, two out of the best three parents used for blight resistance in 1991. Hence further new sources of resistance are desirable despite two of the 15 clones selected from cycle 3 and 23 of the 108 clones selected from cycle 4 having useful resistance to genotype 'blue' 13_A2 as well as to *G. pallida*.

The new germplasm only entered the breeding scheme if it was selected from the progeny tests which included existing germplasm. Thus, 54 successful crosses were made in 2003 in addition to the 68 from the 15 MT parents (Table 5), but just six progenies were good enough to enter the breeding programme along with 19 from the 68 progenies. When the recurrent selection operates on a 5- or 6-year cycle to include clonal selection, potential new parents could be included for assessment, and these could include new cultivars selected from earlier cycles. Again, however, their progenies would survive only if superior to those from the most recent cycle. If, as a result, germplasm containing desirable genes failed to enter the breeding programme, then consideration should be given to introgression of the

genes or improvement of the germplasm in a separate population improvement programme.

Practical considerations

The complexities of population genetics under tetrasomic inheritance (Bradshaw 2007) mean that it is not realistic to try to accurately predict responses to selection in an actual breeding programme. Nevertheless, practical conclusions can be drawn from the results for the parents, progeny tests and clonal tests. First, it is important to phenotypically assess the parents as accurately as possible in order to make sensible crosses. Three out of the ten parents used for blight resistance in 1991 and three out of the 12 used for PCN resistance subsequently proved more susceptible than expected, and nothing was known about the susceptibility of the virus resisters to late blight and PCN. Consequently crosses were made which otherwise might have been avoided. Likewise, when the 15 clones from cycle 3 were subsequently retested for their PCN resistance, some proved more susceptible than expected. In order not to delay making the next cycle of crosses, a compromise would be to reassess the parents whilst making crosses and then not sow seed from any that no longer seemed sensible.

The heritabilities in the progeny (Table 2) and clonal (Table 3) tests were moderate to high. Hence increasing the number of replicates from the minimum of two to three or four is not desirable if done at the expense of assessing more progenies and using seed that could be kept for resowings of the best progenies. Furthermore, assessing more progenies is the only realistic way of increasing the intensity of selection as reducing the number of progenies selected could narrow the genetic base and result in inbreeding depression. However, increasing the number of progenies with sufficient seed for testing to over 200, from the 120 to 145 achieved, would require a considerable effort because potato pollinations can have a low success rate. Thus in cycles 1–3, 120/428, 125/428 and 130/351 combinations were achieved, a typical success rate of just over 30%.

Conclusions

In conclusion, good progress has been made in producing clones which combine resistances to late blight and potato cyst nematodes with improved yield and processing quality. This has been done through the use of cycles of recurrent selection based on progeny testing with selection between progenies and within the selected progenies. The extent of within progeny selection determined whether the breeding scheme operated on a 3-year cycle or required 5 or 6 years to complete a cycle. Finally, we have shown that the recurrent selection programme can accommodate new breeding objectives and germplasm, something that is important in any long term breeding strategy.

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