Stable and Extreme Resistance to Common Scab of Potato Obtained Through Somatic Cell Selection

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ABSTRACT

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Somatic cell selection with thaxtomin A as a positive selection agent was used to isolate variants of potato cv. Russet Burbank with strong to extreme resistance to common scab. Glasshouse and field trials identified 51 variants with significantly reduced disease incidence (frequency of infected tubers) and severity (tuber lesion coverage) compared with the parent cultivar. The most promising variants exhibited extreme disease resistance, rarely showing lesions, which were invariably superficial and shallower than those on the parent. Resistance traits were consistently expressed both in 10 glasshouse and two field trials at different locations, with varied inoculum and disease pressure. Disease-resistant variants differed in their response to thaxtomin A in tuber slice bioassays. Of 23

variants tested, 10 showed reduced thaxtomin A susceptibility, with the remaining 13 responding similar to that of the parent. Thus, toxin tolerance was not the only factor responsible for observed disease resistance; however, four of the five most disease-resistant variants had enhanced thaxtomin A tolerance, suggesting that this factor is important in the expression of strong disease resistance. Pathogenicity and toxin tolerance remained stable over a 6-year period, demonstrating that selected phenotypes were robust and genetic changes stable. The majority of disease-resistant variants had tuber yields equivalent to the parent cultivar in glasshouse trials. This suggests that selection for disease resistance was not associated with negative tuber attributes and that certain variants may have commercial merit, worthy of further agronomic testing.

Additional keywords: host resistance, potato, *Solanum tuberosum*, *Streptomyces scabiei*.

Common scab is one of the most economically important diseases affecting potato (*Solanum tuberosum* L.; 22). This disease seldom affects yield, but economic losses may be substantial, either through reduction in the value of fresh produce or by rejection of seed lots that fail to meet certification standards. Deep-pitted lesions, which are common for infected tubers in Australia (37,39), also affect processing quality of potatoes where the normal steam-peeling processes do not remove diseased material.

Streptomyces scabiei is the predominant pathogen species associated with this disease, although several distinct *Streptomyces* species have also been demonstrated to cause the disease (23). A common feature of all pathogenic strains and species of *Streptomyces* is their ability to synthesize the thaxtomin group of phytotoxins (of which thaxtomin A is the predominant compound produced), while nonpathogenic strains fail to produce the phytotoxin (3,18,19,23). Thaxtomin A is thought to inhibit cellulose biosynthesis and to trigger a programmed cell death response in plants (5,8,9), and evidence suggests that thaxtomin A has an essential role in disease (11,14,17). Due to its central importance in disease expression, selecting for tolerance to thaxtomin A may be an effective way of obtaining or identifying resistance.

Russet Burbank, one of the most commonly cropped potato cultivars in the world with tuber characteristics and cooking qualities that make it ideal for French fry processing, is generally regarded as possessing moderate resistance to common scab (10). Despite this, substantial epidemics of common scab, including occurrence of deep-pitted lesions, and associated losses can frequently occur in this cultivar, especially where disease pressure is high (1,37,39). There are numerous "strains" of Russet Burbank that have been selected over time as somaclonal variants. Limited variation within these strains in susceptibility to common scab has been demonstrated (36), but no existing strain of Russet Burbank, nor other commercial cultivar, possesses extreme resistance.

Potato breeding programs around the world strive to enhance resistance to common scab; however, selecting disease resistance traits whilst maintaining other essential agronomic and quality features is extremely difficult, particularly for the French fry sector where there are additional demands on tuber processing characteristics. An alternate approach utilizing tissue culture technologies and directed mutation to select somaclonal variants with specific traits of interest offers rapid production of clonal variants without the genetic segregation associated with breeding crosses. These variants therefore have a greater chance to retain the desirable agronomic characters and market acceptability of the original cultivar (16). Selection of mutations in potato through in vitro-derived genetic variation has been successfully attempted to improve resistance to several important potato pathogens including *Phytophthora infestans*, *Verticillium dahliae*, *Alternaria solani*, *Fusarium oxysporum*, and *S. scabiei* (4,12,24,29,31).

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In a prior study, we developed a novel somaclonal cell selection technique that utilized thaxtomin A as a positive selection agent to efficiently select variant cells likely to possess tolerance to the toxin and resistance to the disease (38). This work provided proof of concept for this strategy by successful selection of significant levels of resistance in variants of the susceptible potato cultivar Iwa. Although this cultivar is no longer grown commercially, it provided a model system for potato due to its high cell culture aptitude and shoot regeneration potential. Resistance in variants was expressed as reduced lesion presence (with less mean tuber surface coverage and a greater proportion of disease-free tubers), but mean lesion depth was not significantly affected across all trials. However, this study did not demonstrate efficacy under field conditions (with natural and varied inocula) or evaluate the stability of resistance obtained, a critical parameter given that phenotypic changes observed in some somaclonal variants are known to be transient (2,20). In the present study, we address these two critical issues in larger scale somatic cell selection experiments. Furthermore, here we use a cultivar of global commercial importance (Russet Burbank) that naturally possesses moderate resistance to common scab disease and test the hypothesis that our cell selection system can augment such natural resistance to obtain very high to extreme disease resistance with both reduced lesion incidence and depth, something not found in our cv. Iwa variants, nor to our knowledge present within any current commercial potato cultivars.

MATERIALS AND METHODS

Bacterial cultures and plant material. *S. scabiei* isolate G#20 was obtained from common scab-diseased potato tubers (26) from Tasmania, Australia and maintained on ISP2 medium (32).

In vitro plants of potato cv. Russet Burbank (Vancouver strain) were micropropagated on potato multiplication (PM) medium comprising MS salts and vitamins (25), plus sucrose (30 g/liter), ascorbic acid (40 mg/liter), casein hydrolysate (500 mg/liter), and 0.8% agar (Sigma-Aldrich, St Louis), with pH adjusted to 5.8. Plants were routinely subcultured as two-node segments every 3 to 4 weeks and incubated at 22°C with a 16 h photoperiod under cool white fluorescent lamps (65 $\mu\text{mol m}^{-2}\text{s}^{-1}$).

Somatic cell selection and screening with thaxtomin A. Production and purification of the toxin thaxtomin A was as previously described and the cell selection process used was a minor variation on that previously reported (38). In brief, potato callus cultures were initiated from 2- to 3-week-old stem internodes of tissue culture plants of cv. Russet Burbank incubated on potato callus induction (CI) medium (MS salts and vitamins, plus sucrose, 30 g/liter; ascorbic acid, 40 mg/liter; casein hydrolysate, 500 mg/liter; BAP, 2 mg/liter; NAA, 0.2 mg/liter; GA₃, 5 mg/liter; and 0.8% agar (Sigma-Aldrich) with pH adjusted to 5.8) for 7 to 14 days at 22°C under a 16 h photoperiod (cool white fluorescent lamps; 30 to 40 $\mu\text{mol m}^{-2}\text{s}^{-1}$). Friable callus cells were isolated by agitating calloused stems in CI liquid medium on an orbital shaker (110 rpm) for 5 days followed by removal of stem pieces. Callus cells were suspended in 4 volumes of fresh CI medium containing thaxtomin A at either 2 mg/liter (4.57 μM) or 3 mg/liter (6.86 μM). The cell suspensions were gently agitated on an orbital shaker (110 rpm) for 1 to 8 days.

Toxin-treated cells were allowed to settle, cell density was adjusted to approximately 10^5 plating units per ml, and 0.5 ml was plated onto a sterile 7-cm-diameter Whatman No. 1 filter paper that had been placed on top of a nurse culture of *Nicotiana glauca* grown on CI medium (6). After 3 to 4 weeks, rescued potato cell colonies were transferred to callus regeneration (CR) medium (MS salts and vitamins, plus sucrose, 5 g/liter; ascorbic acid, 40 mg/liter; casein hydrolysate, 500 mg/liter; zeatin, 1 mg/liter; NAA, 0.2 mg/liter; and GA₃, 5 mg/liter, with pH adjusted to 5.8) and incubated under reduced light intensity

(7 $\mu\text{mol m}^{-2}\text{s}^{-1}$) at 22°C for 2 to 3 months during which calli were transferred to fresh media every 5 weeks. All regenerated shoots were then transferred to PM medium for micropropagation and subsequent screening. A total of 29 selection experiments were conducted between 2002 and 2003.

Glasshouse screening. Variants of cv. Russet Burbank were initially screened for common scab resistance in 10 glasshouse pot trials (GH1 to GH10) conducted between 2003 and 2008. A total of 21, 60, 40, 21, 14, 15, 47, 46, 51, and 64 variants were tested in each of the trials, respectively, over the 5-year period. The unselected parent cv. Russet Burbank was included in each trial.

Inoculum was prepared using spores harvested from a 2-week-old culture of *S. scabiei* (isolate G#20), suspended in 5 ml of sterile water, added to a sterilized mixture of 100 g of vermiculite and 500 ml of SAY solution (20 g of sucrose, 1.2 g of L-asparagine, 0.6 g of K₂HPO₄, 10 g of yeast extract in 1 liter of water, adjusted to pH 7.2), and incubated for 2 weeks at 24°C. Inoculum (10 g) was mixed into the top one-third of the soil (1:1:8 mix of peat, coarse sand, and composted pine bark; premixed with 6 kg/m³ fertilizer [16:3.5:10 NPK]; pH 6.0) of each pot (20 cm in diameter). Five tissue cultured plantlets of each variant and the unselected parent were directly transplanted into individual pots. Overhead irrigation providing even distribution of water to all pots was supplied every second day taking care to maintain low soil moisture content allowing the soil to periodically dry, and no pesticides were applied. Glasshouse temperatures were maintained between 18 and 24°C. In each experiment, treatment replicates were arranged in randomized blocks and all care was taken to ensure equivalent environmental conditions across all treatments. Plants were grown to senescence. All tubers were harvested, washed, counted, and weighed. Those with a mass greater than 4 g were assessed for common scab disease incidence and severity using the rating scales previously described (36,38). Incidence was measured by determining the proportion of tubers with visible lesions. Severity was measured by two assessments; firstly the tuber surface cover score was estimated visually (0 = no visible disease on tuber surface, 0.5 = 0 to 1%, 1 = 1 to 5%, 2 = 5 to 10%, 3 = 10 to 30%, 4 = 30 to 50%, 5 = 50 to 70%, and 6 = >70% tuber surface affected) and a percentage tuber coverage calculated from the mid-values of the percentage range of each score, and secondly, where lesions were present on a tuber, the depth of the deepest lesion was scored (1 = superficial lesions, 2 = lesions of 0 to 1 mm deep, 3 = lesions 1 to 3 mm deep, and 4 = lesions >3 mm deep).

Field screening. Selected Russet Burbank variants that showed consistently enhanced disease resistance from glasshouse screening and tuber yields approximating that of the unselected parent were further screened for common scab resistance in two field trials. Both field trials (F1 and F2) were planted in November 2007 within commercial farms in NW Tasmania, Australia (Bishopsbourne and West Pine, respectively) on distinct soil types typical of the region with a known history of problems with common scab (21). No additional inoculum was introduced, but lime was applied (1.0 kg/ha) at the time of planting to both field sites to increase disease severity. The two trials assessed 44 and 31 variants, respectively, with the unselected parent cv. Russet Burbank included in each trial.

Field trials were arranged in a randomized block design comprising five (F1) or four (F2) replicated plots, each containing 8 to 10 plants for each variant and control (unselected parent). Fungicide application and irrigation were applied following standard commercial practice. Plants were grown until senescence. All tubers were harvested and a representative sample of 20 tubers per plot was selected for disease assessment using the same scales and ratings as for the glasshouse trials.

Screening for thaxtomin A tolerance. Twenty-three disease-resistant variants and the unselected parent were tested for thaxtomin A tolerance using a tuber slice bioassay (34). Disease-

free tubers produced from glasshouse-grown plants of each variant were cut into 0.5 cm slices and placed in petri dishes with moist filter paper. Three tuber slices were used for each tested variant. Filter paper disks of 6 mm diameter were immersed in a 1% acetone solution containing 7 μ M thaxtomin A for 1 h and air-dried. Three disks were placed on each potato slices. Disks immersed in 1% acetone solution were used as controls. Tuber slices were incubated at 24°C in the dark. Assessments were made after 5 to 10 days for necrosis in the defined area under the filter paper disk: 0 = no necrosis, 0.5 = very sparse flecks, 1 = few light brown flecks, 1.5 = few dark brown flecks, 2 = light brown flecks in determined necrotic area, 2.5 = dark brown flecks in determined necrotic area, 3 = light brown necrosis, 3.5 = dark brown necrosis, and 4 = black necrosis. Nine tuber slice bioassay trials were conducted with each variant tested in at least four trials with data for each variant again directly compared with the control cv. Russet Burbank prior to analysis.

In a further trial, we compared the relative thaxtomin A tolerance of the parent cultivars Iwa and Russet Burbank using both the tuber slice bioassay (as described above) and detached leaflets (38). Tests were repeated nine times.

Data analysis. Disease data for all tubers per replicate in both glasshouse and field trials were averaged prior to analysis. Data for pathology trials and toxin bioassays were analyzed by one-way analysis of variance (ANOVA) using GENSTAT 9.1 software (VSN International Ltd., Hemel Hempstead, UK) after ensuring an approximate normal distribution for each variable. Data for selected disease-resistant variants were normalized for comparison

of results among trials by dividing the mean value of each variant by the mean value of the control. Relative data were further compared by one-way ANOVA.

RESULTS

Selection of regenerated Russet Burbank variants. Exposure to 4.75 or 6.86 μ M thaxtomin A for 1 to 8 days successfully inhibited much of the growth of wild-type cells that would have been expected in absence of toxin treatment. From the 29 selection experiments between 2002 and 2003, a total of 253 regenerated plants were recovered from 212 cell colonies (Table 1).

Glasshouse and field resistance screening. Initial glasshouse trials examined all 253 variants for disease response, with those showing enhanced disease resistance rescreened in subsequent glasshouse and field trials. Thus, each putative disease-resistant variant was represented in at least two trials (most in four or more). A total of 51 variants (20%) consistently demonstrated enhanced resistance to common scab in both incidence and severity assessments (Tables 1 and 2).

The relative disease pressure across the 10 glasshouse trials varied markedly evidenced by the resultant disease expressed in the unselected parent cultivar (Table 2), despite all best efforts to provide consistent inoculum content and growth conditions to optimize disease expression. Disease severity was much higher in the F1 field trial (100% of control tubers with lesions, an estimated 20.4% mean surface coverage and a mean lesion depth

TABLE 1. Summary of variants of potato cultivar Russet Burbank rescued following exposure of somatic cells to thaxtomin A

Exposure to thaxtomin A (days) ^a	Number of selection experiments	Number of recovered callus colonies producing shoots	Number of regenerant plants (variants) ^b	Number of variants exhibiting disease resistance ^c
1	1	5	10	4
2	1	4	4	1
4	4	31	54	15
4*	1	10	10	0
5	1	4	4	1
6	7	46	46	7
7	8	106	111	20
8	5	13	13	3
8*	1	1	1	0
Totals	29	212	253	51

^a Days of incubating potato cell suspension cultures in the presence of 4.75 μ M (or 6.86 μ M where marked with an asterisk) thaxtomin A.

^b For most colonies only a single shoot was selected and maintained.

^c Variants consistently exhibiting reduced disease incidence (frequency of diseased tubers) and severity (lesion coverage of tuber surface).

TABLE 2. Summary of resistance responses of potato cultivar Russet Burbank (RBK) and somatic cell variants to common scab (*Streptomyces scabiei*) in glasshouse (GH1 to GH10) and field (F1 to F2) trials

Trial	Diseased tubers (%)				Coverage score (% tuber coverage) ^a				Lesion depth score (1-4) ^b			
	RBK parent	Variants	Ratio ^c	P (LSD) ^d	RBK parent	Variants	Ratio ^c	P (LSD) ^d	RBK parent	Variants	Ratio ^c	P (LSD) ^d
GH1	37.9	7.7–57.3	3/15	0.001 (23.9)	0.76 (4.1%)	0.11–1.1 (0.5–4.5%)	3/15	0.004 (0.45)	1.1	1.0–1.5	0/15	0.33 (0.41)
GH2	84.2	26.7–100	3/60	<0.001 (32.4)	2.15 (16.2%)	0.6–3.7 (3.5–38.5%)	5/60	<0.001 (1.41)	2.6	1.0–3.6	8/60	<0.001 (0.97)
GH3	55.8	0.0–61.4	8/40	<0.001 (31.8)	0.96 (4.8%)	0.0–1.3 (0.0–5.9%)	11/40	<0.001 (0.56)	1.7	1.0–1.8	12/39	0.015 (0.55)
GH4	73.9	0.0–73.5	5/19	<0.001 (35.5)	1.11 (6.5%)	0.0–1.6 (0.0–9.7%)	4/19	<0.001 (0.82)	1.6	1.0–2.1	0/17	0.20 (0.71)
GH5	46.1	0.0–72.1	2/13	0.013 (41.6)	0.74 (2.9%)	0.0–1.3 (0.0–6.5%)	6/13	<0.001 (0.49)	1.9	1.0–2.7	0/13	0.34 (1.13)
GH6	4.60	3.4–6.0	0/15	0.001 (2.14)	0.16 (0.36%)	0.0–0.22 (0.0–0.4%)	4/15	0.030 (0.15)	1.5	1.0–2.0	9/10	0.006 (0.38)
GH7	29.5	0.0–29.5	28/46	0.050 (20.7)	0.28 (0.78%)	0.0–0.32 (0.0–1.0%)	33/46	0.080 (0.18)	1.4	1.0–3.0	0/31	0.15 (1.3)
GH8	50.0	0.0–66.7	11/46	<0.001 (38.6)	0.45 (1.2%)	0.0–0.78 (0.0–3.0%)	12/46	<0.001 (0.39)	1.5	1.0–3.8	0/42	<0.001 (1.2)
GH9	59.2	0.0–50.1	23/51	0.120 (28.6)	0.70 (2.41%)	0.0–0.55 (0.0–1.8%)	41/51	0.021 (0.31)	1.4	1.0–1.4	41/49	0.05 (0.22)
GH10	34.4	0.0–44.4	23/62	<0.001 (23.9)	0.24 (0.3%)	0.0–0.40 (0.0–0.9%)	27/62	<0.001 (0.16)	1.2	1.0–1.5	0/49	0.49 (0.39)
F1	100.0	92.0–100	6/42	<0.001 (3.2)	2.80 (20.4)	1.3–2.70 (4.6–18.2)	34/42	<0.001 (0.38)	2.58	1.6–2.5	35/42	<0.001 (0.27)
F2	45.0	10.0–45.0	13/28	<0.001 (14.8)	0.33 (0.78)	0.07–0.28 (0.13–0.51)	21/28	<0.001 (0.11)	1.87	1.1–1.71	13/28	0.005 (0.38)

^a Mean lesion coverage of tuber surface: 0 = no disease, 0.5 = 0 to 1%, 1 = 1 to 5%, 2 = 5 to 10%, 3 = 10 to 30%, 4 = 30 to 50%, 5 = 50 to 70%, and 6 = >70%. Derived % tuber coverage was calculated from disease cover score utilizing median percentile scores within the allocated range.

^b Mean depth of deepest lesion per tuber (excluding disease free tubers): 1 = <1 mm deep, 2 = 1 to 2 mm deep, 3 = 2 to 3 mm deep, and 4 = >3 mm deep.

^c Ratio of variants to total showing significantly less disease than the unselected Russet Burbank parent cultivar.

^d P = probability that variant means differed for disease incidence (diseased tubers %) or severity (coverage score and lesion depth score) within trial; LSD = least significant difference (α = 0.05).

score of 2.6) than in the F2 field trial (45.0% of control tubers with lesions, an estimated 0.6% mean surface coverage and a mean lesion depth score of 1.8; Table 2). In spite of this variation, each trial successfully identified variants with enhanced resistance to common scab disease. Importantly, the 25 most resistant variants expressed consistency in disease response across all the trials (glasshouse and field) in which they were tested (Fig. 1).

Visual symptoms were rare in the most resistant variants, while tubers of the unselected parent frequently had severe disease symptoms (Fig. 2). Variant A260 had the greatest disease resistance. Rare superficial lesions were observed in only one of three trials in which it was tested. In comparison to the control, A260 had a mean relative reduction in disease incidence (number of infected tubers) of 97% and in disease severity (tuber surface coverage) of 98% (Fig. 1). Of the variants considered suitable for

field testing based on both resistance and tuber qualities, A380 had the greatest disease resistance. This variant showed a mean relative reduction in disease incidence of 82 and 78% (in glasshouse and field trials respectively) and severity of 86% and 66% (glasshouse and field) compared to the control. Again where rare lesions formed these were invariably superficial with 32 and 31% lower relative depth score in glasshouse and field trials (Figs. 1 and 2).

Estimated tuber yields. Mean tuber number and tuber weight per pot also significantly varied for all 10 glasshouse trials. Resistance to common scab appeared not to be associated with mean tuber number or weight per plant. For the 23 disease-resistant variants described here, two (A358 and TC9-T4) produced more tubers than the parent cultivar, eight variants (TC9-S7; NZ-17A, NZ-22C, A145; A313, A144, A289, and A153) had fewer tubers than the parent while all others had equivalent numbers. In terms

Fig. 1. Mean disease incidence (proportion of infected tubers) and severity (lesion coverage of tuber surface and lesion depth) rating of selected disease resistant-variants relative to the unselected parent (cv. Russet Burbank set at 1.0 for each parameter) in glasshouse and field pathology trials (each variant was tested in two to eight glasshouse and/or field trials). Error bars represent relative means $\pm$ standard error.

of total tuber weight, six variants had significantly less tuber mass (TC9-S7, NZ-17A, NZ-22C, NZ-23A, TC9-T4, and A260) with the remaining 17 showing no significant difference to the parent (Fig. 3).

Screening for thaxtomin A tolerance. Response to exposure to 7 μ M thaxtomin A varied significant among variants and the control cultivar across all tuber slice bioassay trials (Fig. 3). Ten of the 23 variants that were tested (A184b, TC9-T4, A260, A313, A145, A144, TC10-C1, A153, NZ-17A, and TC9-M4-7) had significantly less relative necrosis than the unselected parent following exposure to thaxtomin A with the remaining 13 variants having a mean relative necrosis score equivalent to the control. The variants showing the greatest thaxtomin A tolerance (A184b and TC9-T4) had relative necrosis scores that were 50% that of the parent control cultivar. Of note, four of the five most disease-resistant variants (A260, NZ-17a, TC9-T4, and A145) were toxin-tolerant variants.

Iwa had significantly greater tolerance to thaxtomin A than Russet Burbank in both tuber slice and leaflet assays (Fig. 4).

Stability of selected phenotypes. All variants were selected between 2002 and 2003 with the pathogenicity and toxin tolerance trials conducted between 2003 and 2008. Expression of disease resistance and toxin response of disease resistant variants was consistent across all trials demonstrating stability of phenotypes (and genetic change) across 5 to 6 years (Fig. 1). This period included an estimated 20 to 24 successive subculture cycles in tissue culture (for glasshouse trials) and three tuber generations (for field trials). A few (5.7%) of the variants obtained initially showed enhanced resistance, but the trait was lost in subsequent years and probably reflected unstable variants. These were regarded as susceptible and discarded and are not within the 51 resistant variants indentified.

DISCUSSION

In this study, we confirmed the ability of our somatic cell selection approach with thaxtomin A as a positive selection agent evaluated against cv. Iwa (38) to select resistant variants of a second cultivar, Russet Burbank. We recovered a total of 253 variants of which 51 (20%) showed significantly enhanced disease resistance. Disease-resistant variants were screened in multiple glasshouse and field trials and maintained consistent resistant responses to disease challenge over a period exceeding 5 years, including multiple tissue culture and tuber generations. While glasshouse testing challenged variants with a single (highly virulent) pathogen isolate, subsequent field testing at two distinct sites exposed selected variants to a diversity of naturally occurring pathogenic strains. Moreover, independent testing of a few variants (A155, A168A, A380, and TC-RB8) at a third field site in the State of Victoria, Australia verified disease resistance status under further divergent pathogen population and environmental conditions (A Slater, DPI-Victoria, *personal communication*). These data demonstrate that the selected genetic changes and disease resistance phenotypes are stable. In other studies of somaclonal variation in potato, consistency of phenotype expression over a 3-year period was suggested as sufficient to demonstrate stability of the selected trait (20). With cv. Iwa, we were able to increase relative disease resistance from a previously susceptible cultivar to obtain variants with moderate disease resistance (up to 86% less tuber surface cover; 38). In using cv. Russet Burbank we have successfully enhanced its moderate disease resistance to obtain variants with extreme (near immunity) to common scab disease (up to 97% disease reduction; Fig. 2, Table 2).

Our somatic cell screening trials demonstrated that varying the exposure period to 4.75 μ M thaxtomin A did not greatly impact

Fig. 2. Typical incidence of common scab on resistant variants (NZ-17A, NZ-22C, TC9-T4, A380, and TC9-M4) and unselected parent cv. Russet Burbank (tubers from trial GH2).

the efficiency of selection of variants with enhanced disease resistance. The proportion of disease-resistant variants obtained ranged from 15% (6 days) to 40% (1 day), although the latter figure was from limited data. Conversely, exposure to a greater selection concentration of 6.86 μM thaxtomin A (for 4 and 8 days) resulted in recovery of no disease-resistant variants. Failure of the other 80% of variants to exhibit disease resistance can be attributed to a range of factors including escapes through the initial thaxtomin A selection, unstable resistance or other epigenetic factors, and/or the expression of the variants' traits only in cell culture where they were initially selected (8). The recovery of 51 variants with improved disease resistance from about 60 plates, each seeded with approximately 50,000 cells, represents one event in about 1.7×10^5 somatic cells plated, a frequency consistent with effective somatic cell selection in plants (7).

In our previous study none of the disease-resistant cv. Iwa variants showed any consistent change in lesion depth score compared to the unselected parent cultivar (38). In contrast, the mean depth of the worst lesion per tuber of the Russet Burbank disease-resistant variants was almost always less than the parent cultivar (23 of 25 variants). For most disease-resistant Russet Burbank variants, any rare lesions formed were invariably superficial, even under conditions of strong disease pressure. This has importance for the processing potato industry as the peeling process can effectively remove superficial (but not deep pitted) tuber lesions.

As in the prior study, we have shown that not all selected disease-resistant variants possess enhanced tolerance to thaxtomin A. However, in contrast to cv. Iwa disease-resistant variants, we found evidence to suggest that tolerance to thaxtomin A was linked to the expression of strong resistance in cv. Russet Bur-

bank. The majority of the variants showing the greatest disease resistance (four of the top five or six of the top ten) expressed enhanced toxin-tolerance. While cv. Russet Burbank is regarded as moderately resistant to common scab disease, we have demonstrated here that this cultivar is significantly more sensitive to thaxtomin A than cv. Iwa. The natural resistance to common scab disease expressed by Russet Burbank must therefore rely on other features such as stronger, more robust tuber skin etc., and selection for thaxtomin A tolerance may add an additional resistance factor to this cultivars' defenses against common scab. The multigenic nature of resistance to common scab in potato has

Fig. 4. Comparison of cvs. Iwa and Russet Burbank for thaxtomin A response in detached leaflet and tuber slice assays. Error bars represent means $\pm$ standard error.

Fig. 3. Mean tuber yields (tuber weight and number per pot tested in two to six glasshouse trials) and mean necrosis rating following exposure of cut tuber surface to 7 μM thaxtomin A (tested in two to five tuber slice bioassays) of selected disease-resistant variants relative to the unselected parent (cv. Russet Burbank set at 1.0). Each variant tested in two to six glasshouse trials. Error bars represent relative means $\pm$ standard error.

been previously described (13). The relative susceptibility of Russet Burbank to thaxtomin A toxicity may explain why the cell selection process was highly effective with this cultivar. This may also explain why enhanced toxin tolerance is important in the most resistant variants, and why most variants show reduced mean lesion depth (which has been associated with toxin production by the pathogen) compared with the parent cultivar.

The use of thaxtomin A as a screening agent for seedlings within conventional breeding programs to quickly select putative resistant lines has been trialled (15). This approach has merit to screen within the genetically similar sibling populations that result from a sexual cross. However, the results comparing disease susceptible Iwa and moderately disease-resistant Russet Burbank demonstrate the lack of efficacy of this approach to identify useful disease resistance across genetically diverse cultivars.

Certain thaxtomin A-tolerant variants had reduced tuber yields (NZ17A, TC9-T4, and A260). Thaxtomin A-tolerant *Arabidopsis thaliana* mutants in the gene *txr-1* also grow poorly (30). We have demonstrated a competitive association between thaxtomin A and auxins, suggesting a common cell target (34,35). Thus, mutations in a putative thaxtomin A reception site may interfere with normal auxin cellular activities, including hormonal influence on regeneration resulting in observed detrimental growth effects. A homolog to TXR-1 has been identified in potato (33). Future studies will aim to determine whether mutations in this homolog are associated with the thaxtomin A tolerance in some of these potato variants.

The mechanism of enhanced disease resistance is not necessarily obvious in the absence of apparent increased tolerance to thaxtomin A. Variants NZ-22C and NZ-17A have a distinctive strongly russeted skin type (Fig. 2). This resembles descriptions of potato mutants produced in the former Soviet Union, selected following ionizing radiation and/or chemical mutagens that exhibited thick corky skin, were genetically stable and conferred resistance to *S. scabiei* infection (27,28). As with NZ-22C and NZ-17A, the mutants from the Soviet Union yielded poorly and had little commercial value. Furthermore, a preliminary study comparing disease-resistant and nontoxin tolerant variants A380 and A386 with the unselected parent and a disease-susceptible variant revealed a possible enhancement of an inducible resistance response. An increased rate of suberization in the phellem layer beneath tuber lenticels following exposure to the pathogen was shown for the disease-resistant variants when compared to the parent cultivar and susceptible variant (B. Khatri, *personal communication*). Further studies to examine this association between putative defense response and resistant variants will be undertaken.

Tuber yield data were only collected from the glasshouse experiments in this study, which showed most variant lines produced tuber mass and numbers not significantly different from the unselected parent. Subsequent evaluation of tuber yields and cooking qualities of a selection of disease-resistant variants have been undertaken in a series of agronomic field trials which will be reported elsewhere. As with the glasshouse results, several disease-resistant lines have yield and cooking qualities equivalent to the parent cultivar and are being considered for commercial development.

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