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Long-day-adapted *Solanum phureja* as a source of resistance to blackleg caused by *Erwinia carotovora* subsp. *atroseptica*

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Summary

Long-day-adapted *Solanum phureja* clones were assessed for resistance to blackleg caused by *Erwinia carotovora* subsp. *atroseptica* under field and controlled environmental conditions over two years. In the field, twenty-two of the twenty-three clones of *S. phureja* assessed were as resistant to blackleg as the commercial cultivar Ailsa, the most resistant control, and were significantly ($P<0.001$) more resistant than the intermediate and susceptible cultivars Wilja and Estima, respectively. Under controlled environmental conditions, resistance in commercial cultivars was more easily overcome. However, 18 of the 21 *S. phureja* clones assessed were significantly more resistant to blackleg than these cultivars.

Introduction

Blackleg of potato stems is mainly caused by *E. carotovora* subsp. *atroseptica* (van Hall) Dye under Scottish growing conditions, and can result in significant economic losses in the growing crop (Perombelon & Kelman, 1980) in addition to providing inoculum for the soft rotting of tubers in storage. Chemical control methods for blackleg are largely ineffective and several authors have discussed the possibilities for disease reduction offered by breeding for disease resistance (Wastie & Mackay, 1985; Huaman et al., 1988).

However, resistance to blackleg can be difficult to determine due to large effects of environmental factors on expression of resistance (Logan & Little, 1988) and breeding for disease resistance has been hindered by the lack of reliable test methods. There is evidence for blackleg resistance in potato cultivars from both glasshouse tests (Lapwood & Read, 1986b) and field tests (Hossain & Logan, 1983; Lapwood & Read, 1986a) using various inoculation methods. However, Gans et al. (1991) found that there was a poor correlation for cultivar resistance between inoculation methods and warned against drawing conclusions from single trials. Overall, the level of resistance in tetraploid cultivars is generally low and can be overcome as pathogen populations increase.

In addition to tetraploid resistance, numerous sources of resistance to blackleg have been described in wild diploid *Solanum* species, as detailed by Elphinstone (1994). For example, Łojkowska & Kelman (1989) found that accessions of *S. demissum* and *S. phureja* were highly resistant to stem rot and that, in resistant lines, disease severity was often low when high levels of bacteria were used.

The utilisation of such high levels of resistance found in wild *Solanum* spp. and primitive cultivars has been hindered by sexual incompatibility between tetraploid cultivars and these species. Even when hybridisation has been possible, the accompanying transfer of undesirable phenotypic characteristics has hindered breeding efforts (Elphinstone, 1994).

A population of diploid long-day-adapted *S. phureja* developed at SCRI using a process of mass selection and open pollination (Carroll, 1982) has been used as parental material to produce high yielding tetraploid *S. tuberosum* × *S. phureja* hybrids (Carroll & De,Maine, 1989). Recent work (De,Maine et al., 1998) has shown that clones of the diploid long-day-adapted *S. phureja* population have good levels of resistance to soft rot caused by *E. carotovora* subsp. *atroseptica*. However, the relationship between resistance to soft rot and blackleg has not been satisfactorily resolved.

This study investigates the levels of blackleg resistance in a range of long-day-adapted *S. phureja* clones in order to identify parents that will allow the rapid introduction of resistance into commercially acceptable tetraploid material.

Materials and methods

Field tests. Twenty-three clones of the SCRI long-day-adapted *S. phureja* collection were selected on the basis of disease resistance to other pathogens and good agronomic traits.

In April 1998 and 1999, two replicate plots of each of the *S. phureja* clones and the control cultivars, Ailsa, Estima and Wilja, were grown in a randomised complete block field trial at SCRI. Each plot consisted of five inoculated tubers and two uninoculated tubers. Planting was at 0.5 m spacing between plants, within drills 0.75 m apart, with a 2 m gap between plots in the same drill, and a 1 m path between uninoculated and inoculated tubers within a plot. Standard commercial rates of fertiliser and pesticides were applied and the crop was irrigated throughout the growing season. Tubers were inoculated with *E. carotovora* subsp. *atroseptica* (*Eca*) at a concentration of 4×10^6 colony forming units ml^{-1} using the vacuum infiltration method as described by Gans et al. (1991), two days before planting.

Assessments were made of non-emergence, blackleg symptoms (wilted plants and plants with stem lesions at soil level) and plant vigour, a subjective appreciation of height and number of stems of plants in relation to the healthiest plants in the uninoculated treatments, on a scale of 0–5 (0 = non-emerged; 5 = maximum vigour), according to Gans et al. (1991).

Controlled environment tests. Small (40–50 mm) tubers of twenty-one clones of *S. phureja*, of which 19 were the same clones tested in the field, were grown on two separate occasions in 1999 in an individually randomised block design having 4 replicates. Cultivars Ailsa, Morene and Wilja were included as controls. Tubers were chitted for one month under constant light. Plants were grown in 10 cm square pots containing SCRI mix compost in a controlled environment cabinet at 15 °C, 16 hours

daylight and 10 °C, 8 hours darkness for four weeks before inoculation. Inoculum was prepared by overnight culture of an isolate of *Eca* in Nutrient Broth shaken continuously at 24 °C. The inoculum concentration was adjusted to 4×10^8 colony forming units ml^{-1} using a spectrophotometer, and then diluted 1:100 with water to produce a final inoculum concentration of 4×10^6 c.f.u. ml^{-1}. Wooden toothpicks were soaked in this suspension for 1 hour. Plants were inoculated by inserting a toothpick into the potato stem at the first node. The toothpick was left in the plant and the plants were sprayed with a fine mist of water and covered with plastic bags to maintain humidity for the first 24 hours following inoculation. Plants were incubated at 24 °C, 14.5 hours light and 9.5 hours dark for 10–15 days until blackleg symptoms were visible. Each plant was then scored for blackleg disease severity on a 1–5 scale where 1 = total collapse of the plant and 5 = no visible disease symptoms.

Statistical analysis. Analyses of variance were carried out separately for the field tests over two years and for the controlled environment tests over two occasions using Genstat V (Lawes Agricultural Trust).

A Spearman rank correlation was carried out to compare the two field tests, the two controlled environment tests and the field tests with the controlled environment tests.

Results

Field tests. The results are from two years of experiments. There were differences between clones that were statistically significant ($P<0.001$) when tested against the clone × year interaction, which was also significant ($P<0.05$). When compared with uninoculated controls, clones of *S. phureja* showed very few blackleg symptoms or any reduction in vigour in either year. Twenty two of the twenty three clones of *S. phureja* assessed for disease in the field following vacuum infiltration of tubers, were as resistant to blackleg as the commercial cultivar Ailsa, the most resistant control. These clones were also significantly ($P<0.05$) more resistant than the intermediate and susceptible cultivars Wilja and Estima, respectively (Table 1). The remaining clone [DB 337(37)] was resistant in 1999, but susceptible in 1998. This reduced the correlation ($r=0.43$, $P<0.05$) between clones for their ranking in the field tests in 1998 and 1999 (see Table 3).

Controlled environment tests. Twenty one clones of *S. phureja*, of which 19 were also assessed in the field, were tested for blackleg resistance under controlled environmental conditions using a stab-inoculation method (Table 2). Plants showed more severe blackleg symptoms than in the field, and the resistance of the commercial control cultivars was more easily overcome. There were differences between clones which were statistically significant ($P<0.001$) when tested against the clone × test interaction, which was also significant ($P<0.05$). However, 15 of the 21 *S. phureja* clones assessed were significantly ($P=0.05$) more resistant than the commercial controls. Ailsa and Wilja were more resistant than the susceptible control Morene. There was a good correlation ($r=0.62$, $P< 0.01$) between the tests

Table 1. Mean blackleg disease score of selected *S. phureja* clones and control cultivars in the field. Scores are based on a 0–5 scale of increasing resistance.

Clone	Field score 1998	Field score 1999	Mean
DB358(24)	5.00	5.00	5.00
81 S 66	5.00	5.00	5.00
DB244(37)	5.00	5.00	5.00
DB226(70)	5.00	5.00	5.00
DB358(23)	5.00	5.00	5.00
DB375(2)	5.00	5.00	5.00
DB384(88)	4.90	5.00	4.95
71 P 10	4.90	5.00	4.95
DB384(112)	4.90	5.00	4.95
DB384(85)	4.90	5.00	4.95
DB384(4)	4.90	4.94	4.92
71 T 6	4.80	5.00	4.90
DB333(16)	4.90	4.90	4.90
DB375(1)	4.90	4.90	4.90
DB358(30)	4.80	5.00	4.90
DB384(76)	4.70	5.00	4.85
DB168(11)	4.93	4.70	4.82
71 T 46	4.50	5.00	4.75
DB378(1)	4.30	4.88	4.59
DB384(18)	4.10	5.00	4.55
DB161(10)	4.00	5.00	4.50
DB337(37)	2.90	5.00	3.95
Ailsa	4.90	4.90	4.90
Wilja	3.70	3.40	3.55
Estima	2.40	3.00	2.70
LSD (5%)	0.71	0.49	0.72

carried out under controlled environmental conditions on two occasions (Table 3), but not between field and controlled environment tests (r=-0.14 for the mean of the two tests compared).

Discussion

Results show that high levels of resistance to blackleg caused by *Eca* can be identified in clones of SCRI's long-day-adapted *S. phureja* population. Clones were found to be highly resistant in comparison with the well-characterised commercial cultivars used as controls.

Wastie & Mackay (1985) discussed the evidence for differences in severity of blackleg symptom expression between cultivars and concluded that genetically stable differences in resistance needed to be confirmed in field trials. Indeed, in this work, resistance levels did vary between tests, with higher susceptibility being evident amongst the control cultivars under controlled conditions. For example, the commercial cultivar Ailsa has been shown to be consistently more resistant to

Table 2. Blackleg disease scores of selected *S. phureja* clones and control cultivars under controlled environmental conditions in 2 tests. Scores are based on a 1–5 scale of increasing resistance.

Clone	Test 1	Test 2	Mean
DB384(4)	5.00	5.00	5.00
DB384(88)	5.00	5.00	5.00
71 P 10	4.75	4.75	4.75
DB161(10)	4.50	5.00	4.75
DB384(112)	4.50	5.00	4.75
DB384(26)	4.75	4.73	4.74
DB384(85)	4.50	4.57	4.53
71 T 46	4.25	4.75	4.50
80 CP 23	4.00	4.94	4.47
DB337(37)	3.75	5.06	4.41
DB168(11)	4.50	4.25	4.38
DB333(16)	4.25	4.50	4.38
DB384(18)	3.75	5.00	4.38
DB384(73)	4.36	4.40	4.38
DB384(76)	4.00	4.68	4.34
DB358(30)	4.25	3.50	3.88
DB226(70)	4.00	3.57	3.78
81 S 66	3.25	4.25	3.75
DB358(24)	3.50	3.65	3.57
DB244(37)	2.50	4.50	3.50
DB358(23)	3.75	2.83	3.29
Ailsa	1.69	3.50	2.60
Wilja	2.65	3.25	2.95
Morene	1.00	2.25	1.63
LSD (5%)	1.24	0.95	1.09

Table 3. Correlation coefficients between methods of assessing the resistance of clones to blackleg in 2 years (field tests) and 2 tests (controlled environment cabinet (CEC)).

	Field 1998	Field 1999	CEC1	CEC2	mean field score (1998/1999)
Field 1998	-				
Field 1999	0.43*	-			
CEC1	-0.13	0.39*	-		
CEC2	-0.29	0.48*	0.62**	-	
Mean CEC score (2 tests)	-	-	-	-	-0.14

Significance levels: * $P<0.05$; ** $P<0.01$

blackleg than other commercial cultivars in the field over 7 years (Lees & Wastie, unpublished data), but no significant differences in resistance between it and the more susceptible cultivars Wilja and Morene were noted under controlled conditions.

This may be due to the inoculation of high levels of bacteria directly into the stem being more effective at causing disease symptoms than the vacuum infiltration method employed in the field. In addition, constant environmental conditions are likely to have favoured the rapid development of disease, in contrast to the very variable physical and environmental conditions encountered in the field.

There was no correlation (Table 3) between the 1998 field test and either of the controlled environment tests, although there were significant correlations between the 1999 field test and both of the controlled environment tests.

However, the high levels of resistance identified under controlled environment conditions were found to be comparable to those in the field test and, in general, the controlled environment test was efficient at distinguishing the very high levels of resistance seen in the *S. phureja* clones.

Although the mode of resistance to blackleg has not been investigated in this work, it is thought that the rate of multiplication of *Eca* is much lower in *S. phureja* than in susceptible *S. tuberosum* cultivars (A. Avrova, personal communication).

These results are in accordance with those of several workers who have identified wild species and primitive cultivated species as source of resistance to *Erwinia*. For example, Koromyslova (1972) noted resistance to *Erwinia* in *S. phureja*, and Bains et al. (1999) showed that some accessions of *S. boliviense, S. chacoense* and *S. sanctarosae* are resistant and highly resistant. Corsini & Pavek (1986) concluded that although variation for resistance to *Erwinia* existed in *S. tuberosum* subsp. *tuberosum*, non-tuberosum germplasm was a better source of resistance. In addition, previous work has shown that clones of the SCRI long-day-adapted *S. phureja* population also had high levels of resistance to tuber soft rot, also caused by *Eca* (De,Maine et al., 1998).

In the past, these sources of resistance have been under-utilised due to difficulties in hybridising wild species with *S. tuberosum*, and the resulting problems of poor adaptation when such hybridisation was possible (Rousselle-Bourgeois & Priou, 1995). The advantage of the long-day-adapted *S. phureja* clones as a source of resistance is the opportunity that it presents for the incorporation of resistance in combination with other agronomically important characters which exist at a more commercially acceptable level than in short day native *S. phureja*. In particular, the clones selected for investigation in this case were those having desirable agronomic traits such as high tuber yield, tuber size greater than 80 g, good tuber regularity and good cooking qualities. Further details are given in De,Maine et al. (1998).

Clones that were identified as having high levels of resistance to blackleg in these experiments have been hybridised with susceptible commercial cultivars in order to study the inheritance of resistance.

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