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Age-Related Resistance in Commercial Varieties of *Solanum tuberosum* to the Late Blight Pathogen, *Phytophthora infestans*

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Abstract: This study reports the occurrence of Age-Related Resistance (ARR) in the potato- *P. infestans* pathosystem. Six potato varieties with different degrees of resistance to *P. infestans* were evaluated on the basis of disease severity according to the widely-used Malcolmson's scoring scale at 4, 8 and 12 weeks after plant emergence. The results show that, out of the six varieties, those exhibiting high to moderate levels of resistance to late blight became consistently more resistant as they grew older, while the effect was less pronounced on the two remaining susceptible varieties. Those six varieties exhibited their maximum level of resistance at 12 weeks after plant emergence, when they were just at the bud stage, making the transition to flowering. It is possible that blight resistance in potato plants might be a developmentally regulated response and as the potato plant makes the transition from vegetative to reproductive growth, the re-wiring of certain developmental pathways induces the expression, or results in the accumulation of, certain genes which might also play an important part in defense responses. The data presented in this study emphasizes the importance of considering the age of potato plants when challenged in screening for resistance a well as in determining the most effective timing for use of chemical controls.

Key words: Age-related resistance, developmental acquired resistance, *Phytophthora infestans*, *Solanum tuberosum*

INTRODUCTION

Age-Related Resistance (ARR) refers to the phenomenon whereby, as plants mature, they become more resistant to normally virulent pathogens. ARR has been observed in a number of pathosystems involving bacterial, fungal and viral pathogens such as in the tomato-*Pseudomonas syringae* (Rusterucci *et al*., 2005), Arabidopsis-*Pseudomonas synringae* (Kus *et al*., 2002; Cameron and Zaton, 2004), pepper-*Phytophthora capsici* (Kim *et al*., 1989), tomato-*Phytophthora capsici* (Hwang and Hwang, 1993), grape-*Uncinula necator* (Ficke *et al*., 2002), pepper-*Cucumber mosaic virus* (Garcia-Ruiz and Murphy, 2001), tomato-*Tomato Yellow leaf curl virus* (Levy and Lapidot, 2007), Arabidopsis-*Pseudomonas viridiflava* (Goss and Bergelson, 2006) and cowpea-*Uromyces vignae* (Heath, 1993) pathosystems.

The exact biochemical and molecular mechanisms for the different forms of ARR in these various pathosystems remains yet to be established. In many cases, a positive correlation has been observed between the production of defense-associated compounds in leaves at older stages of development and plants displaying ARR. It has been proposed that ARR might be a developmentally-regulated response to pathogen infection (Kus *et al*., 2002; Hugot *et al*., 2004). A number of genes, known to be up-regulated in later stages of development, such as during the transition to flowering, have also been shown to be involved in plant defense. Examples are Pathogenesis-Related (PR) proteins (Herbers *et al*., 1996; Walter *et al*., 1996), transcription factors (Chen and Chen, 2002), as well as senescence-associated proteins (Butt *et al*., 1998; Quirino *et al*., 1999). Taken together, these observations imply that there are common steps in the signaling pathways that mediate pathogen-induced defense responses and plant ageing.

The oomycete *Phytophthora infestans* causes late blight disease on potato (*Solanum tuberosum*) and, although the disease managed to stay under reasonable control through the application of fungicides for a long time, the recent emergence of fungicide-resistant strains of the pathogen means that late blight has once again become a very serious economic threat in the vast majority of potato production systems, as well as many tomato production systems, worldwide. According to CIP Statistics, in developing countries alone, yield loss due to late blight is estimated to add up to US$ 2.75 billion each year, in addition to money spent on fungicides (http://www.ciotato.org/potato/pests diseases/late blight/index.asp). For this reason, the *Solanum tuberosum-Phytophthora infestans* pathosystem is one of the most extensively studied pathosystems in the world.

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Few studies have been published about the changes in resistance against *P. infestans* with increasing plant age in potato in the past twenty years. A more recent study investigating the effects of plant age, leaf age and leaf position on the linear growth rate of lesions of *P. infestans* in laboratory-based experiments was carried out by Visker *et al*. (2003). Although leaf position proved to be the most significant of all considered factors and to have the largest effect on the linear lesion growth rate of *P. infestans* (apical leaves being more resistant than basal leaves), plant age also proved to be a significant factor and in general, it was reported that older plants were slightly more resistant than younger plants. As discussed by the authors, this finding differed slightly with what had been reported in other similar studies, where the more frequent observation was the result that very young plants were susceptible, plants of intermediate age were the most resistant and old plants (70 days after planting) became more susceptible again. The present study aims to investigate whether a positive correlation does in fact exist between plant age and resistance level, up to 84 days (12 weeks) after planting in the six local varieties that are planted in Mauritius.

Age-related changes in susceptibility of potato foliage to *P. infestans* has several implications for disease management strategies, most importantly in determination of the most effective timing for the application of chemical controls against late blight. Knowing at which physiological growth stage the plant is most susceptible to late blight will allow planters to concentrate their fungicide-spraying at those times when the plants are at their most susceptible stage, in this way avoiding multiple spraying schedules which consume more fungicide, are more time-consuming and which might be less efficient at controlling disease progress.

The main objective of this study was to investigate whether the mechanism of ARR occurred in the potato-*P. infestans* pathosystem by inoculating selected local commercial potato varieties with the late blight pathogen. Six commercial varieties of potato, namely Stirling, Belle Isle, Mondial, Delaware, Spunta and Up-to-Date, displaying differing levels of field resistance, were selected and inoculated with a virulent strain of *Phytophthora infestans* locally isolated from the field. The intensity of foliage blight in potato plants was measured by assessing the overall amount of necrotic tissue per plant on a scale of 1 (highly susceptible) to 9 (highly resistant) according to Malcolmson's original scale of assessment (Cruickshank *et al*., 1982).

MATERIALS AND METHODS

Fungal isolate and inoculum: The *Phytophthora infestans* isolate used in this study was kindly provided by Dr. S. Saumtally, from the Mauritius Sugar Industry Research Institute (MSIRI). The strain was isolated from infected potato plants from a local field in the region of Union Park in the South East of the island in the winter of 2006. The isolate was grown on pea agar (12.5% w/v peas, 1.5% w/v agar) containing the following antibiotics: 0.03 g L^{-1} Rifampicin, 0.10 g L^{-1} Ampicillin and 0.02 g L^{-1} Nystatin. The isolate was cultured and incubated at 16-18°C for 15 days to wait for sporulation.

Cultivation of potato varieties used: Cultivation of potato tubers and inoculation procedures were carried out in August-October 2006 at the University of Mauritius. Certified disease-free potato seeds of six commercial local varieties, Stirling, Belle-Isle, Mondial, Delaware, Spunta and Up-To-Date, were obtained from the MSIRI. The seeds were sterilized with 70% alcohol (2 min) and 1.5% sodium hypochlorite (15 min), washed with sterilized water (3×10 min) and sown *in vitro* in 8-inch diameter pots containing well-drained, light soil, which had been previously sterilized by oven-baking for 30 min at a temperature of 82-87°C to eliminate the risk of contaminants from the soil. Pots were filled with 5 cm thick layer of soil. Fifteen gram of the NPK fertilizer complex 13-13-20 was spread evenly on the soil surface before covering it with another layer of soil. Two seed tubers were placed in the middle and covered with a 10 cm layer of soil in each pot. Pots were irrigated with 5-10 cm^3 of water daily.

Experimental design: Six potato seed tubers of each variety were planted at a one-week interval (batch 1 and batch 2, respectively) so as to obtain plants of the same age when repeating the experiment. At each inoculation time-point (4, 8 and 12 weeks, respectively after plant emergence), four plants from each variety were inoculated and two plants were left uninoculated and kept as control. The inoculation of these four plants from each variety was carried out on the same day, using the same inoculum and under the same conditions so that data obtained was comparable between varieties. Each inoculation, for each of the three different time-points, was first carried out using batch 1 plants, then the inoculation was repeated after one-week, using batch 2 plants, thereby ensuring that plants were of the same physiological age during the repeat experiment.

Inoculation procedures: The inoculum was prepared from pure agar cultures by adding 10-15 mL of cold, sterile distilled water onto each agar plate and swirling the plate around gently so as to collect the maximum amount of sporangia from the culture. This sporangial suspension was then adjusted to a concentration of 2.5×10^4 spores mL^{-1} using a standard haemocytometer and then placed at 4°C and checked every 30 min under the microscope for zoospore release. When zoospores were released, the liquid inoculum was poured into a hand sprayer and sprayed evenly on the plant foliage. Both the inoculated plants and the uninoculated control plants were left for 7 days at a temperature of 20-22°C and Relative Humidity (RH) of about 75-85%, for disease progress and scoring.

Disease evaluation and data analysis: Scoring was based on Malcolmson's scoring scale, where the intensity of foliage blight caused by *P. infestans* is measured by assessing the overall amount of necrotic tissue per plant as follows: score 1-percentage of necrotic tissue >90%, score 2: 81-90%, score 3: 71-80%, score 4: 61-70%, score 5: 41-60%, score 6: 26-40%, score 7: 11-25%, score 8: <10%, score 9: complete immunity to the disease (no disease symptoms). A mean score was calculated for each of the four plants at each inoculation experiment and the mean score was then calculated for each of the two separate experiments (batch 1 and batch 2 plants) at each of the three different time-points.

RESULTS

The resistance levels of the six local varieties ranged from full susceptibility to partial resistance (Table 1). Susceptible plants showed spreading, sporulating lesions throughout plant foliage, moderately resistant plants showed expanding lesions on the older leaves and fairly restricted lesions on the younger leaves and plants with high levels of resistance showed small, restricted lesions on both younger and older leaves. Results obtained were consistent with what was already known about the resistance level of the four different varieties when evaluated in local field trials (data not published); Stirling and Belle Isle-high level of resistance, Delaware and Mondial-moderate levels of resistance, Spunta and Up-To-Date-low level of resistance. The mean scores for resistance at the three different time-points for the six potato varieties are shown in Fig. 1.

In general, all cultivars became more resistant as plants grew older. Based on the Malcolmson's scoring scheme, ranking of resistance (in order from greatest to least) was as follows: Stirling > Belle-Isle > Delaware > Spunta > Mondial > Up-To-Date. The increase in resistance was most significant in the highly-resistant

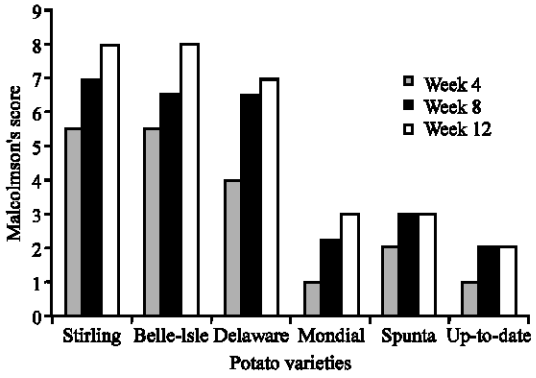

Fig. 1: Disease severity ratings for six local varieties of potato, based on Malcolmson's scoring scheme, shown at three different time-points: 4, 8 and 12 week-old plants. Values are the Means±SD of four replicates of two different experiments

Table 1: Mean scores (based on Malcolmson's scoring scheme where a score of 1 represents >90% necrotic tissue and 8 represents <10% necrotic tissue, 9 is reserved for healthy plants) and standard deviations showing resistance levels of six local commercial varieties of potato to the late blight pathogen, *Phytophthora infestans*. Raw data is presented for the two inoculation experiments (batch 1 and 2) as well as the mean score from the two separate experiments

	Potato varieties					
Scores	Stirling	Belle-Isle	Delaware	Mondial	Spunta	Up-to-date
4-week-old plants						
Batch 1	5.5±0.41	5.6±0.85	3.9±0.48	1.0±0.0	1.9±0.63	1.00±0.0
Batch 2	5.5±0.71	5.4±0.48	4.1±0.48	1.0±0.0	2.1±0.25	1.00±0.0
Mean score	5.5±0.53	5.5±0.65	4.0±0.46	1.0±0.0	2.0±0.46	1.00±0.0
8-week-old plants						
Batch 1	7.0±0.41	6.4±0.48	5.9±0.85	2.5±0.41	3.0±0.71	1.75±0.29
Batch 2	7.0±0.41	6.6±0.48	6.1±0.85	2.5±0.41	3.1±0.85	2.38±0.48
Mean score	7.0±0.38	6.5±0.46	6.0±0.80	2.5±0.38	3.0±0.73	2.00±0.50
12-week-old plants						
Batch 1	7.9±0.63	7.8±0.50	7.0±0.71	2.8±0.65	3.0±0.00	2.00±0.71
Batch 2	8.1±0.48	8.3±0.29	7.1±0.63	3.3±0.65	3.0±0.71	2.10±0.25
Mean score	8.0±0.53	8.0±0.46	7.0±0.62	3.0±0.65	3.0±0.46	2.00±0.50

(Stirling and Belle-Isle) and moderately-resistant (Delaware and Mondial) varieties. The susceptible varieties (Spunta and Up-To-Date) did not show any significant increase in resistance between weeks 8 and 12. Although Mondial scored lower than Spunta at week 4, it showed more steady increase in resistance over the three time-points than Spunta or Up-To-Date. For these two susceptible varieties, the plants at all three time-points showed extensive, water-soaked, spreading lesions throughout both stem and foliage and it was difficult to discriminate whether resistance level had effectively increased or not.

DISCUSSION

In general, resistance responses of the various varieties observed in this study were consistent with data previously collected. Late blight trials carried out in the early 1990's by the MSIRI and the International Potato Centre (CIP) as part of the Global Initiative on Late Blight (GILB) program classified variety Stirling as highly resistant, varieties Delaware and Up-To-Date as highly susceptible and variety Mondial as susceptible. Belle-Isle is a high-yielding variety released by the MSIRI in 2005 and has also proved to be highly resistant to late blight. The variety Spunta, which is the most widely grown and most marketable variety locally, has exhibited variable response to late blight during the last eighteen years. When it was tested as part of the MSIRI/CIP trial, it proved tolerant to late blight but since 1995 onwards, this resistance was broken, possibly due to new emerging races of the pathogen. Spunta also exhibits variable response to different uncharacterized strains of *P. infestans* collected from the field (data not published), thereby suggesting that there might be a specific cultivar x race interaction operating in Spunta.

Among the 6 potato varieties tested in this study, those exhibiting moderate to high levels of resistance clearly showed an increase in resistance at the three different developmental stages at which inoculation was carried out. This observation was not apparent in the two susceptible varieties tested. A whole-plant spray inoculation technique was used rather than a detached-leaf assay so that the effect of leaf detachment from the whole plant does not interfere with the true physiological age of the tested plants. Also, it has been reported that expression of age-related resistance to blight caused by *Phytophthora capsici* on pepper (Kim and Hwang, 1989) and tomato (Hwang and Hwang, 1993) was more apparent in stems and/or roots rather than on foliage, suggesting perhaps that distinct mechanisms of ARR might operate in different parts of the plant. No distinct differences between disease severity in stems compared to foliage at the three different time-points were made in this study. A specific stem-wound inoculation method would have to be utilized to investigate whether stem blight is also affected by plant age.

As previously mentioned, ARR is a mechanism that has been described in a wide range of pathosystems but has been poorly documented in the potato-*P. infestans* pathosystem. An early report dates back to 1982, when Carnegie and Colhoun (1982) described the higher susceptibility of foliage from young potato plants to *P. infestans*. Stewart (1990) reported observations consistent with the occurrence of ARR on the expression of major gene resistance to *P. infestans* in detached potato leaflets. The effect of plant age was investigated using plants 4, 5, 6, 7, 8, 9 and 10 week-old of ten different genotypes and plants showed more immune or hypersensitive reactions just before or at flowering, at the 6-week and 9-week-old stages, respectively. Plants that were either younger or older exhibited poorer resistance. This suggests that blight resistance in potato plants might be a developmentally regulated response, as has been observed in the Arabidopsis-*Pseudomonas syringae* pathosystem (Kus *et al*., 2002) and as the potato plant makes the transition from vegetative to reproductive growth, the activation or re-wiring of certain developmental pathways necessary for the commitment to floral organ formation induces the expression, or results in the accumulation of, certain genes which might also play an important part in defense responses.

In this study, the 12 week-old plants were just at the bud stage and therefore making the transition to flowering. A number of genes with possible defense functions are expressed late in plant development, at the time of normal floral development and might be potentially involved in ARR. Among those, Pathogenesis-related (PR) and PR-like genes such as β-1,3-glucanase, chitinase and peroxidase are among the most widely reported (Wyatt *et al*., 1991; Coté *et al*., 1991). Another developmental stage of the plant at which potential defense-related gene expression might be up-regulated is at senescence (Quirino *et al*., 1999; Hensel *et al*., 1999; Capelli *et al*., 1997; Buchanan-Wollaston and Ainsworth, 1997). In fact, one of the reasons why this study was carried out on whole plants rather than on detached leaves is because of existing reports that leaf detachment is likely to induce early senescence of leaves (Quirino *et al*., 2000). Because of a possible overlap in the molecular mechanisms of pathogen response and senescence programs, it is necessary to set clear experimental distinctions between the two processes.

The potato-*P. infestans* transcriptome has been extensively studied and several genes with diverse functions, including genes known to be involved in stress

responses, Programmed Cell Death (PCD), primary or secondary metabolism (housekeeping genes) and genes involved in signaling pathways and regulation have been found to be specifically involved in incompatible interactions (Beyer *et al*., 2001; Evers *et al*., 2003; Birch *et al*., 2003; Avrova *et al*., 2004). It is not unlikely that some of those genes are also developmentally regulated and are possibly involved in mediating the molecular basis of ARR in the potato-*P. infestans* pathosystem. This study sets the stage for further gene expression analyses of selected defense genes to help elucidate the molecular pathways involved in ARR in potato. Gene expression analyses can be performed with potato genes that have already been documented as being developmentally regulated, such as genes involved in the transition to flowering or during senescence, to investigate whether they are also up-regulated as part of the defense response when young potato plants are challenged with *P. infestans*. Alternatively, the expression of selected defense-related genes that have been isolated from the potato transcriptome challenged with *P. infestans*, can also be analyzed with respect to the developmental stage of the plant. These types of analyses would reveal whether the potato plant possesses an innate built-in mechanism that allows it to better fight against late blight disease during the course of its growth, or at key developmental stages.

The data presented here can be further supported by testing the selected potato varieties with a range of *P. infestans* isolates displaying different virulence characteristics to investigate whether ARR is consistently expressed and is independent of the pathogen genotype. As of yet, no genotyping has been performed on local isolates of *P. infestans* and the strains prevalent in Mauritius remain unknown. It is essential that local strains be characterized at the molecular level in order to better understand and more effectively monitor the epidemiology of late blight outbreaks on local cultivation of potato. The experiments described in this study will also have be performed under the natural environmental conditions prevailing during the potato-growing season and repeated for at least two growing seasons using a larger number of replicates to confirm the data reported here.

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