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Molecular Markers for Bacterial Ring Rot Immunity and Resistance Genes

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Introduction

Bacterial ring rot (BRR) is an economically important disease affecting seed and table stock production of potatoes in Colorado. The disease is managed primarily by the use of disease-free seed. However, while this approach has reduced economic losses from BRR, it cannot eradicate the pathogen, *Clavibacter sepedonicum* (Cms), which is often present in the absence of disease symptoms. Currently, there is no effective means to control BRR. There are no chemical control measures for eliminating the pathogen in seed used for table stock production cr for preventing certified seed from becoming contaminated by any of several possible sources.

Resistance to BRR has been identified in a number of potato cultivars. However, these cultivars are generally omitted from potato breeding programs, because these often delay rather than preclude symptom expression and serve as latent carriers of Cms. We recently identified accessions of related *Solanum* species that are immune to BRR (Ishimaru et al. 1994). In an accession of *S. acaule* and *S. phureja*, cells of the pathogen were not detected in inoculated plants grown under controlled environmental conditions. Immunity is a highly desirable form of resistance, because immune plants would not be expected to carry latent infections of Cms.

To expedite the transfer of immunity genes from *S. acaule* or *S. phureja* into cultivated potato, we proposed to develop molecular markers for immunity genes. Molecular markers, such as restriction fragment length polymorphism (RFLP) markers, can then be used as visual tags for the genes to enable their efficient transfer to susceptible potato cultivars. In this report, we describe the identification of an RFLP marker that is associated with BRR immunity genes in *S. acaule*.

Results and Discussion

An immune *S. acaule* accession (#7-8) was crossed to a susceptible accession of *S. infundibuliforme* to generate an F1 population that is segregating for immunity. One hundred and one F1 individuals from the cross were germinated, grown in the greenhouse, and established in tissue culture.

Tissue cultured F1 plantlets were inoculated with Cms and grown in the

greenhouse. The immune *S. acaule* parent, susceptible parent *S. infundibuliforme*, and susceptible internal control *S. tuberosum* cv. Sangre were also inoculated with Cms and grown in the greenhouse. After 60 days, foliar symptoms of BRR, leaf morphology, and necrosis were assessed for each plant in each of three replications. Stem sections were also collected and assessed for presence or absence of Cms cells using an immunoflurescent antibody staining (IFAS) assay.

Nine plants exhibited symptoms of BRR, which included interveinal chlorosis and wilting. In IFAS assays, these plants were also observed to show the presence of Cms cells. These plants were therefore classified as non-immune. Some asymptomatic plants were found to have Cms cells, and were also classified as non-immune. Twenty-one percent of the F1 plants were found to be immune while 77% were non-immune. A Chi-square test supported the hypothesis that immunity segregates in a ratio of 3 non-immune:1 immune in this F1 population. This ratio indicates that there are two dominant genes at different loci controlling immunity to bacterial ring rot in *S. acaule*. It cannot be concluded from these results whether the two genes are located in two different genomes or in just one genome of *S. acaule*.

To identify RFLP markers linked to the immunity genes, the F1 plants were screened with a total of 49 RFLP markers that are distributed throughout the 12 chromosomes of potato. A grand total of 147 marker/enzyme combinations was used in this study. Since the immunity genes are in *S. acaule*, markers that were segregating in this species, rather than in the non-immune parent *S. infundibuliforme*, were identified. Five markers, TG18, CD14, TG285, GP38 and CP105 produced bands in *S. acaule* that segregated in the population. Association of these markers with immunity was tested using Chi-square analysis. Only CP105 showed an association with immunity. CP105 has five bands (Figure 1), one of which (CP105-3) was found to be loosely associated with BRR immunity (Table 1). The association between CP105-3 and immunity was indicated by a calculated chi square value of 3.84 which was statistically significant at $p=0.05$ (Table 1). CP105 was previously mapped to potato chromosome 10. To check if CP105-3 was also on chromosome 10, a marker near CP105, TG63, was surveyed for polymorphisms in *S. acaule*. No bands produced in TG63 segregated in the F1 population. This result indicates that CP105-3 and the immunity genes are not likely to be located on chromosome 10.

Conclusion

The results of this study showed that immunity in *S. acaule* is controlled by two dominant genes. An RFLP marker, CP105-3, was found to be associated with BRR immunity. The next step in this study is to determine the genetic distance between CP105-3 and the immunity genes. Other markers that are nearer the genes will be identified.

RFLP markers linked to the immunity genes can be immediately applied in breeding to incorporate immunity into susceptible potato cultivars. The use of markers to monitor the genes can speed up breeding by enabling the quick identification of plants that have incorporated the genes using very young seedlings or tubers. The need to conduct greenhouse or field screening and IFAS assays can be eliminated. The use

of markers will allow the identification of plants that contain the immunity genes but not other unwanted DNA from the immune *Solanum* parent that may produce deleterious traits in potato. The results of this project will also lay the foundation for isolation of immunity genes based on their genetic map positions. Cloning of immunity genes will provide the fastest and most efficient approach of producing immune potato cultivars. Onced cloned, immunity genes can be incorporated into susceptible potato by means of transformation. With this approach, an immune potato cultivar can be produced in less than two years compared to approximately 15 years required for breeding. This project will therefore expedite the incorporation of a desirable form of resistance to bacterial ring rot into cultivated potato.

Publication acknowledging the Potato Administration of Colorado:

Ishimaru, C.A., N.L.V.Lapitan, A. VanBuren, and K. Pedas. 1994. Identification of parents suitable for molecular mapping of immunity and resistance genes in Solanum species. Amer. Pot. J. 71:517-533.

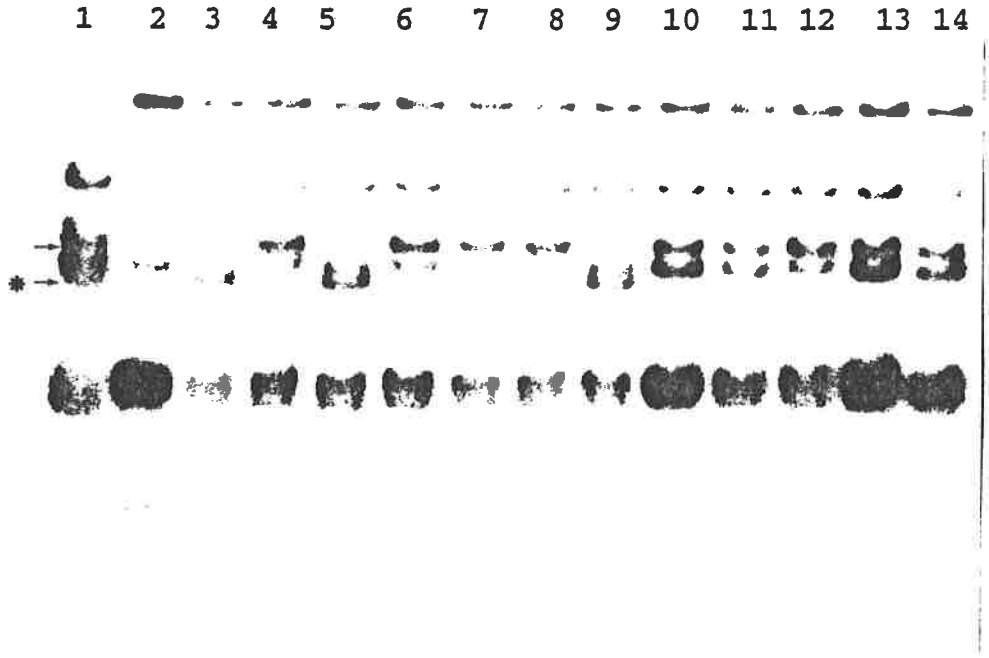

Figure 1. Autoradiogram showing hybridization of *Eco*RV digested DNA to potato clone CP105. Lane 1: *S. acaule*; lane 2: *S. infundibuliforme*; lanes 3-14: F1 individuals. Arrows indicate segregating bands. * indicates a 3 Kb band loosely associated with bacterial ring rot immunity.

Table 1. Contingency table and Chi square analysis to test the independent segregation of CP105-3 and bacterial ring rot immunity.

CLASS	OBSERVED	EXPECTED	X^2
CP105-3 present/immune	11	6.84	2.5
CP105-3 present/non-immune	21	25.10	0.53
CP105-3 absent/immune	11	13.70	0.67
CP105-3 absent/non-immune	53	50.30	0.14
TOTAL			3.84*

* Statistically significant at $p = 0.05$.