THE ROLE OF AGROCIN 434 AND OTHER FACTORS IN THE BIOLOGICAL CONTROL OF CROWN GALL DISEASE.

ALI-REZA AHMADI

M.Sc. University of Tarbiat Modarress (Tehran-Iran)

A thesis submitted for the degree of Doctor of Philosophy in the Faculty of Agricultural and Natural Resource Sciences at the University of Adelaide.

Department of Crop Protection

Waite Campus

University of Adelaide

February 1996

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Summary

Crown gall, caused by the soil-borne bacterium *Agrobacterium spp.*, is a common disease of a wide variety of dicotyledonous plants such as peaches, grape vines, almond, cherry, *Rubus* species and various other nut-bearing trees. The biological control agents *A. rhizogenes* strain K84 and its genetically engineered derivative, K1026 have been used successfully for a number of years to control crown gall in stone fruits and ornamentals. Strain K84 produces a potent inhibitory agent agrocin 84. A number of researchers have suggested that other mechanisms of control other than agrocin 84 production may be involved in the biocontrol process of crown gall by strains K84 and K1026. One of these mechanisms may be the effect of another antibiotic, agrocin 434, which is produced by *A. rhizogenes* strains K84, K1026 and K434. This thesis studies the role of agrocin 434 and other factors in the biological control process.

Initial studies have shown that genes involved in the biosynthesis of agrocin 434 are located on a large cryptic plasmid (300-400 kb) of strain K84 and derivatives (Donner *et al.*, 1993). The results of this study indicate that genes involved in the immunity/resistance to agrocin 434 are also carried by pAgK434. This has been demonstrated by transferring the plasmid pAgK434 to the agrocin 434 sensitive strain, K27. The resulting strain became resistant to agrocin 434 and had acquired the ability to produce agrocin 434 as well. Another derivative of K84, strain K1318 with pAgK1318, carries a deleted version of pAgK434. This strain is unable to produce agrocin 434 but produces a modified agrocin 434 (nucleoside 4176) which has no inhibitory activity. The kanamycin resistance transposon Tn*5* was introduced into derivatives of pAgK1318 marked with antibiotic resistance markers and resulting plasmids transferred to an agrocin 434 sensitive strain. The resulting transconjugants

were able to produce modified agrocin 434 as did K1143 pAgK1318. The results of this study show that resistance/immunity functions are also carried on pAgK1318, as transconjugant strains were resistant to agrocin 434.

To understand the role of each of the K84 plasmids, pAgK84, pAtK84b and pAgK434 in the biocontrol process, a range of derivatives of strain K84 harbouring all combinations of these three resident plasmids in the same host background were constructed.

A rapid efficient method for testing pathogenicity and/or the efficacy of biocontrol strains was developed by using a leaf disc tumorigenesis assay. A range of tobacco and tomato cultivars were tested to determine which plant cultivars gave the most rapid and reproducible callus formation with different concentrations of pathogen. The results of these experiments indicated that tobacco cultivar White Burley and *cv.* Virgie are the best host plants for plant transformation because these cultivars produced more calluses than other cultivars. The results of stem inoculation bioassays determined that tomato *cv.* Quick Pick is the best plant for stem inoculation, because gall formation by stem inoculation of tobacco requires a longer incubation period than tomato and this cultivar produced more galls than other tomato cultivars.

The efficacy of all derivatives of strain K84 in controlling the pathogenic biovar 2 strain K27 was assessed using root inoculation of almond seedlings, tomato stem inoculation and leaf disc culture bioassays. Results from stem inoculation and leaf disc culture assays showed that all the derivatives of K84, including the plasmid free strain, K1347, significantly reduced galling by the pathogen *A. rhizogenes* K27. Strains carrying one or more of the three plasmids of K84 showed a significantly greater biocontrol ability than strain K1347. Results from root inoculation assays indicated that strains harbouring pAgK84, pAtK84b or pAgK434 significantly reduced gall formation by the pathogen K27. Gall formation following treatment with strain K1347 was not significantly different from that with the pathogen alone.

The insertion of Tn*5* into pAgK434 stopped agrocin 434 production by strains K1356 and K1357. The biological activity of these new strains was tested using the different bioassays and indicated that these strains can significantly reduce gall formation or callus induction in leaf disc culture and/or stem inoculation bioassays, but they do not produce significant control of the pathogen in almond root bioassays.

The efficacy of agrocin 434 producer strains to control pathogenic *Agrobacterium* strains from different species was assessed using rapid bioassay methods, leaf disc culture and stem inoculation. The results of this study determined that strain K434 is not quite efficient in controlling crown gall by biovar 1 and 3 and *A.rubi* in the stem inoculation and/or leaf disc tumorigenesis bioassays as wel as controlling crown gall induction by a biovar 2 pathogen.