GENETIC ENGINEERING LUPINS (*LUPINUS* SP.) FOR RESISTANCE TO ANTHRACNOSE

FINAL REPORT

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Genetic engineering of lupins (*Lupinus* sp.) for resistance to anthracnose

Executive Summary

Colletotrichum acutatum, the causal agent of anthracnose, is the major worldwide constraint to lupin production. In South Africa the disease amounts to losses of sixty million rand per annum. In an effort to combat lupin anthracnose, CSIR Bio/Chemtek and ARC Roodeplaat embarked on a project to introduce antifungal genes into a white lupin (*Lupinus albus*) and a narrow leafed lupin (*Lupinus angustifolius*) cultivar.

In order to obtain long term durable resistance against *C. acutatum*, we decided to combine antifungal genes with different modes of action in an effort to genetic engineer lupins for resistance to anthracnose. Although *in vitro* assays indicated that the apple polygalacturonase inhibiting protein (MdPGIP) was effective in inhibiting *C. acutatum* growth, an exo-β-1,3-glucanase was not able to inhibit *C. acutatum* spore germination and mycelial growth sufficiently. Therefore work was undertaken to isolate the *Shpx6a* peroxidase gene from *Stylosanthes humilis* as an alternative antifungal gene to introduce into lupin plants for resistance to anthracnose. Experiments are presently underway to clone a novel, pathogen inducible promoter upstream of the *Shpx6a* peroxidase gene.

Like most legume plants, *Lupinus* sp. is extremely recalcitrant to transformation. In an effort to improve transformation frequencies, a binary vector was constructed that contained an additional copy of the *vir*G gene, and an Agrobacterium-mediated vacuum infiltration technique was employed to transform germinating lupin seedlings. More than 3000 lupin seeds were exposed to *A. tumefaciens*, and developing plants were subjected to molecular analyses to assess for the presence of the *gus* and *bar* marker gene, and the apple *pgip1* (*Mdpgip1*) antifungal gene. Although some T_0 generation plants exhibited *gus* activity, no progeny were found to harbour the transgenes, suggesting that the transformation technology employed produced chimeric transgenics. For this reason, no further transformation experiments have been undertaken. We are currently negotiating with an international laboratory involved in lupin transformation work to introduce the antifungal genes in *L. albus* by means of particle bombardment.