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ATTACHMENT C

FINAL REPORT FOR PROJECT CS61L

GENETIC ENGINEERING OF PLANTS FOR 2,4-D HERBICIDE RESISTANCE

2,4-D is rapidly broken down in soil by the bacterium *Alcaligenes eutrophus* which possesses a plasmid-encoded pathway for the degradation of this herbicide. The first enzyme in the pathway, 2,4-D monooxygenase, cleaves off an important functional group to produce 2,4-dichlorophenol, a compound which we have shown to be up to 100 times less toxic to plant tissue *in vitro* than 2,4-D. We expected that plants expressing this enzyme would detoxify the herbicide and therefore display tolerance.

The 2,4-D monooxygenase gene (*tfdA*) was isolated from *A. eutrophus* and manipulated for increased expression in plants before being cloned into vectors which would enable the gene to be introduced into plants by *Agrobacterium*-mediated transformation. Transgenic tobacco plants transformed with *tfdA* have been cultured ~~in~~ sterile pots and leaf tissue was assayed for expression of 2,4-D monooxygenase activity using a chromatographic assay that we have developed. Enzyme activity was detected in many of the plants and those plants demonstrating high levels of enzyme were transferred to soil to be propagated for seed.

In vitro tests, involving the plating of tobacco leaf pieces on varying concentrations of 2,4-D, were conducted to correlate the presence of 2,4-D monooxygenase with actual tolerance to the herbicide. Leaf pieces from plants expressing high levels of enzyme were able to form callus and shoots on levels of 2,4-D up to 30 times higher than tissue from untransformed plants. Seed harvested from transgenic and untransformed tobacco plants was germinated on varying levels of 2,4-D and once again the transgenic plants were shown to be up to 30-fold more tolerant in the presence of the herbicide than the untransformed plants. Spraying of tobacco seedlings with formulations of 2,4-D confirmed the increased tolerance exhibited by the transgenic plants to the herbicide. At levels of 300ppm, the transgenic plants were unaffected while untransformed plants were badly stunted if not killed, and at 1000ppm, a concentration roughly equivalent to 2-4 times the normal field application, some of the transgenic plants remained unaffected.

The *tfdA* gene constructs have now been used to transform cotton explants and tissue which is believed to have incorporated this gene is being cultured prior to assay for the expression of 2,4-D monooxygenase.

This project has achieved its stated goals in that a gene which confers tolerance to 2,4-D has been isolated and engineered for expression in plants. Good tolerance levels were observed in a model plant species and efforts have now turned to cotton. The project will therefore be funded through our genetic engineering of cotton project for which a separate application will be made in the 1990/91 financial year.