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HERBICIDE RESISTANCE OF TRANSGENIC PLANTS

A thesis presented
in partial fulfilment of the requirements for
the degree of Doctor of Philosophy in
Plant Breeding and Genetics
Department of Plant Science
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New Zealand

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1994

**In the name of God the most compassionate
and the most merciful**

In memory of my brother Hokm Ali

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Abstract

A cloned dehalogenase gene, conferring the ability to degrade the herbicide dalapon, was introduced into white clover and tobacco using *Agrobacterium*-mediated transformation. The objectives of this study can be divided into three parts. The first part consists of the evaluation of genetically transformed white clover and tobacco plants for their level of resistance to dalapon, determination of the heritability of the introduced transgene at different stages of growth, and identification of the segregation pattern of the transgene. The second part consists of the study of the quantitative inheritance of the transgene in transgenic tobacco plants. The third part consists of a determination of the number of copies of the transgene integrated into the genome of a transgenic tobacco line, inheritance of the transgene over successive generations and analysis of steady state levels of mRNA of the transgene in leaf tissue. Relationships between the levels of transgene mRNA and the degree of resistance of these plants to dalapon were also assessed.

The resistance of genetically transformed white clover and tobacco plants to dalapon was studied under both *in vitro* and greenhouse conditions using different experimental designs. In the *in vitro* studies, both white clover callus lines and tobacco seedlings showed resistance to high concentrations of dalapon. The level of resistance of the tobacco plants to dalapon was studied under greenhouse conditions using six transgenic lines and one non-transgenic control line. The non-transgenic line was unable to grow at dalapon levels greater than 6.0 kg ha⁻¹, while the majority of the transgenic lines were able to grow at a herbicide level of 48.0 kg ha⁻¹. There were significant differences between the transgenic tobacco lines in their resistance to the dalapon.

The heritability of necrosis, leaf length, leaf width and stem height characters were estimated at various levels of dalapon. The heritability of dalapon resistance for developed transgenic tobacco plants at various levels of dalapon was high. The heritability of dalapon resistance for the characters under study decreased with increasing dalapon levels, with the lowest values of heritability occurring at the highest level of dalapon (48 kg ha⁻¹). The leaf length and leaf width characters had the highest heritabilities, while the necrosis and stem height characters had the lowest heritabilities. The effect of time and the interaction between time and herbicide concentration as environmental factors were lowest for the leaf length

and leaf width characters, while the time effect was highest for the stem height character. The interaction between time and the effects of dalapon were highest for the necrosis character. The heritability of dalapon resistance in transgenic tobacco seedlings grown *in vitro* was significantly lower than in plants, indicating either a low expression of the transgene or a high effect of environmental factors for plants at an early stage of growth. The segregation ratio (resistant:susceptible phenotype) for the transgenic lines was 3:1, and χ^2 test results demonstrated the involvement of single gene inheritance for the lines.

Quantitative inheritance studies of the transgene in tobacco plants using generation means with six generations and 9x9 full diallel mating designs revealed that the additive component of variation was greater than the dominance (hemizygoty) component of variation. The hemizygoty effect was partial and towards the dalapon resistant phenotype. There was significant inter-allelic interaction (epistasis), either between the host plant allele(s) and the dehalogenase transgene or between copies of the transgene. The non-significance of reciprocal effects in the diallel table analysis revealed a lack of maternal or cytoplasmic effects. The analysis of general combining ability and specific combining ability in the diallel table indicated that the majority of transgenic parents had significant general combining ability effect (g.c.a. effects) towards the resistant phenotype, while the non-transgenic parents showed significant g.c.a. effects towards the susceptible phenotype. The progeny derived from crosses between resistant transgenic parents and susceptible, non-transgenic parents showed significant s.c.a. effects towards the resistant phenotype. In contrast, progeny derived from crosses between the susceptible, transgenic parent and non-transgenic parents, as well as progeny derived from crosses between non-transgenic parents showed significant s.c.a. effects towards the susceptible phenotype.

In molecular studies of the copy number of the transgene at different generations of one transgenic tobacco line, the transgenic plants were shown to contain two closely linked copies of the transgene at a single locus, whereas the non-transgenic plants were shown to lack the transgene. It was also shown that the transgene was stably integrated into the plant genome in successive generations and that rearrangement of the integrated transgene did not occur. A dehalogenase-specific mRNA was detected in total RNA extracted from leaves of the transgenic plants. Although all of the transgenic plants contained the same

gene, they showed significant variation in the accumulation of dehalogenase-specific mRNA. In control, non-transgenic plants no dehalogenase-specific mRNA was detected. Although the level of the dehalogenase-specific mRNA in transformed plants varied considerably between the lines, was no significant differences between the individual plants within the lines.

In a two phase selection experiment, some transgenic callus lines exhibited a dissimilarity in expression of the dehalogenase gene and the neomycin phosphotransferase II gene, conferring kanamycin resistance, used in these experiments as a second selectable marker. Some of the genetically transformed cells selected on medium containing kanamycin, when transferred onto medium containing dalapon, did not show resistance to dalapon. Similarly, when transformed cells selected on medium containing dalapon were transferred onto medium containing kanamycin, some of the callus lines did not show resistance to kanamycin. These results show that in some cases selection for one of the transferred genes does not result in expression of the other, non-selected, transferred gene.

Acknowledgement

I would like to thank my chief supervisor Dr. I.L. Gordon, of the Seed and Crop Group, Plant Science Department, Massey University, for guidance and assistance during this course of study and for computer software. I would also like to thank my co-supervisor, Dr. D.W.R. White, of the Molecular Genetics Laboratory, New Zealand Pastoral Agricultural Research Institute, Palmerston North for supplying the plasmid pAS501 and transgenic tobacco seed material and his guidance, particularly for the molecular genetics aspects and for providing excellent research facilities as well as other research materials.

I would like to thank my friends in the Molecular Genetic Laboratory, AgResearch, both staff and postgraduate students for their kindness and invaluable assistance in various ways. I would especially like to thank Dr. N. Ellison for his advice, guidance, comments and assistance during this course of study, Dr. R. Appleby, A. Griffiths, D. Kerr, R. Meeking, and A. Lambert for their advice and assistance in some experiments, and Dr. P. Ealing for providing the E3 primer.

I would like to express my gratitude to all staff of the Seed and Crop Group and the Pasture Group, Plant Science Department especially the head of the former Agronomy Department Professor J. Hodgson and the head of the Seed and Crop Group, Professor M. Hill, for their help and assistance during the course of this study. Thanks also to Mr. D. Sollite, Mrs K. McKenzie of the Plant Science Department for providing material and assistance, and Dr. K. Harrington for providing herbicide and for designing the herbicide sprayer which was used in the greenhouse experiments. I also wish to thank Dr. M. Behboudian of the Horticulture Group for his invaluable guidance and advice.

I am grateful to the Ministry of Jehade Sazandegi of the Islamic Republic of Iran for allowing me to take study leave and for providing financial support during the course of this study.

My special thanks are offered to my mother, Nimtaj, my sister, Zahra, and my brothers, Ibrahim, Abbas and Bahram, for their support and encouragement.

Finally I would like to thank my wife Masoumeh, and children Marzieh and Tahereh, for their encouragement, patience and kindness.

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