



**Manganese nutrition status and resistance in barley (*Hordeum vulgare* L.)
to take-all (*Gaeumannomyces graminis* var. *tritici*)**

by

Julia M Lloyd

BSc. (Hons)

Flinders University, South Australia

Thesis submitted to Adelaide University for the degree of Doctor of Philosophy

Department of Plant Science

Waite Agricultural Research Institute

Adelaide University

South Australia

July, 2000

Thesis summary

Wilhelm *et al.*, (1990) had shown that wheat genotypes with increased Mn efficiency when grown under Mn-deficient soil conditions, showed less *Gaeumannomyces graminis* var. *tritici* (*Ggt*) infection compared to Mn-inefficient wheat genotypes. This result suggested the hypothesis that take-all resistance in wheat may be genetically linked to Mn efficiency. In this thesis I set out to test that hypothesis but chose to use barley, a simpler diploid crop species, in which traits such as Mn efficiency are likely to be more simply inherited than in hexaploid wheat. Using both physiological and genetic studies, no causal connection has been found between enhanced resistance to *Ggt* in barley and the presence of manganese efficiency alleles at two loci.

To begin to test if Mn efficiency is associated with resistance to *Ggt*, infection levels were compared between the Mn-efficient Amagi Nijo and the Mn-inefficient breeder's line, WI 2585, when grown in Mn-deficient soil. Amagi Nijo was found to have a higher shoot Mn concentration and low root infection measured as a short root average length of stelar lesion (Av.LSL) (mm/plant), compared to WI 2585.

Comparison of WI 2585 and Amagi Nijo grown over a range of Mn additions with no *Ggt* inoculum found them to have the same critical shoot Mn concentration. It was therefore reasonable to assume that there is equal Mn stress in WI 2585 and Amagi Nijo when they have the same shoot Mn concentrations. When WI 2585 and Amagi Nijo infected with *Ggt* were grown under soil conditions of equally high Mn stress (similar and low shoot Mn concentration), the Av.LSL in WI 2585 was significantly longer than in Amagi Nijo. But the susceptibility of WI 2585 to *Ggt* was not reduced by the elimination of Mn stress.

A major Mn efficiency locus, *Mel 1*, had been mapped using 4 RFLP markers to chromosome 4HS in an F₂ population of a cross between WI 2585 and Amagi Nijo (Pallotta *et al.*, 1999). Further, a WI 2585 x Amagi Nijo doubled haploid (DH) population had been generated by Dr. P. Davies (SARDI). A field trial was conducted using 62 entries from this DH population to search for more loci linked to Mn efficiency. A new locus controlling shoot Mn concentration showed significant linkage with RFLP marker, *Xwg645* on chromosome 2HL.

An investigation of the genetics of Mn efficiency in Amagi Nijo was used to determine if the take-all resistance was an Mn-independent varietal trait, unlinked to Mn-efficient alleles at both the *Mel 1* and *Xwg645* loci on chromosomes 4HS and 2HL respectively. Neither Mn-

efficient allele at *Mel 1* or *Xwg645* showed linkage with a short Av.LSL in a controlled-environment experiment on 29 DH lines grown in Mn-deficient soil.

Chapter 1 Literature Review 1

1.1 Introduction..... 1
1.1.1 Scope of research..... 1
1.1.2 Adding Mn fertiliser to the soil..... 1
1.1.3 Mn status and *Ggt* infection 2
1.1.4 Aim of this research..... 2
1.1.5 Benefits of this research 3
1.2 *Mn* in plants..... 4
1.2.1 Symptoms of Mn deficiency in barley 4
1.2.2 Genotypic differences in Mn efficiency in barley..... 5
1.3 The role of *Mn* in biosynthetic processes..... 5
1.3.1 Photosynthesis 6
1.3.2 Superoxide dismutase and Mn..... 6
1.3.3 Mn and the lignin biosynthetic pathway..... 7
1.3.4 Mn and auxin biosynthesis 7
1.4 *Mn* in soils..... 8
1.4.1 Forms of Mn in soils..... 8
1.4.2 Factors affecting Mn availability in soil..... 8
1.4.3 Biotic and environmental factors 9
1.4.4 Fertilisers and Mn availability in soil 10
1.5 Plant micronutrient uptake, absorption and translocation..... 11
1.5.1 Mn efficiency and root system size..... 11
1.5.2 Mn efficiency and root Mn uptake..... 12
1.5.3 Mn absorption by roots..... 12
1.5.4 Vesicular-arbuscular mycorrhiza (VAM)..... 13
1.5.5 Mn translocation in plants 14
1.6 Molecular studies of *Mn* efficiency..... 15
1.6.1 Plant Mn status and Mn efficiency..... 15
1.6.2 Screening for Mn efficiency..... 15
1.6.3 The identification of genes for plant nutrition..... 16
1.6.4 Molecular markers for nutritional traits 17
1.6.5 Genetic strategies used 17
1.6.6 Summary..... 18
1.7 Disease resistance in plants..... 18
1.7.1 Host disease resistance 18
1.7.2 Host disease tolerance 19
1.7.3 Host disease resistance or tolerance? 19
1.8 *Gaeumannomyces graminis* var. *tritici* (*Ggt*) 19
1.8.1 *Ggt* disease 20
1.8.2 The biology of *Ggt*..... 20
1.8.3 Pathogenic variation 21
1.8.4 *Ggt* survival and crop rotation 21
1.8.5 Attenuation and restoration of *Ggt* pathogenicity 22
1.8.6 Environmental conditions and take-all disease 22
1.9 *Mn* plant defence mechanisms against take-all 23
1.9.1 Micronutrient stress, and disease resistance and tolerance 23
1.9.2 Lignin biosynthetic pathway and take-all..... 23
1.9.3 Mn and aminopeptidase..... 24
1.9.4 Mn and pectin methylesterase 24
1.9.5 Mn and *Pseudomonas fluorescens* 24
1.9.6 The role of micronutrients in susceptibility to *Ggt* 25
1.10 Thesis prologue..... 25

Chapter 2 Pilot studies..... 28

2.1 Introduction..... 28
2.1.1 Pilot studies..... 28
2.2 Method..... 28
2.2.1 Experiment 1: *Ggt* tolerance to Mn..... 28

2.2.2 Experiment 2: Increased <i>Ggt</i> pathogenicity.....	29
2.2.3 Experiment 3: Calculation of sample size.....	31
2.2.4 Experiment 4: Confirmation of an adequate sample size.....	31
2.2.5 Seed Mn.....	32
2.3 Results.....	32
2.3.1 Experiment 1: <i>Ggt</i> tolerance to Mn.....	32
2.3.2 Experiment 2: Increased <i>Ggt</i> pathogenicity.....	33
2.3.3 Experiment 4: Confirmation of an adequate sample size.....	34
2.4 Discussion.....	38
Chapter 3 Critical shoot Mn concentration.....	40
3.1 Introduction.....	40
3.2 Method.....	40
3.2.1 The critical shoot Mn concentration experiment.....	40
3.3 Results.....	40
3.3.1 Leaf symptoms	40
3.3.2 Shoot and root DM.....	41
3.3.3 Shoot Mn concentration	42
3.3.4 Critical shoot Mn concentration of Amagi Nijo and WI 2585.....	43
3.3.5 Determining the rate of soil Mn addition as the basis of comparing the genotypes with equal Mn status.....	44
3.3.6 Shoot Mn content	44
3.3.7 Root Mn concentration.....	45
3.4 Discussion.....	46
3.4.1 Critical shoot Mn concentration.....	46
Chapter 4 Physiological studies	47
4.1 Introduction.....	47
4.2 Method.....	47
4.2.1 The <i>Ggt</i> experiment.....	47
4.2.2 The temporal study	47
4.3 Results.....	48
4.3.1 The <i>Ggt</i> experiment.....	48
4.3.2 The temporal study	55
4.4 Discussion.....	59
4.4.1 The <i>Ggt</i> experiment.....	59
4.4.2 The temporal study	60
Chapter 5 Microscopy investigation.....	61
5.1 Introduction.....	61
5.1.1 Lignin and <i>Ggt</i>	61
5.1.2 The morphology and pathology of <i>Ggt</i> infected roots.....	61
5.1.3 SEM and EASEM.....	62
5.2 Method.....	62
5.2.1 UV fluorescence microscopy.....	62
5.2.2 Fluorescent staining for phenols, DNA and proteins.....	62
5.2.3 SEM and EASEM.....	63
5.3 Results.....	64
5.3.1. Control stain for autofluorescence, 80 % glycerol.....	64
5.3.2 Acid fuchsin stain for proteins and micro-organisms.....	64
5.3.3 Ethidium bromide stain for phenols and cell nuclei DNA.....	65
5.3.4 DAPI staining for cell nuclei DNA	65
5.3.5 Double staining: DAPI for DNA and ethidium bromide for phenols and DNA	65
5.3.2 SEM EASEM	66
5.4 Discussion.....	66
5.4.1 UV microscopy.....	66
5.4.2 SEM and EASEM.....	66

Chapter 6 Genetic studies	68
6.1 Introduction.....	68
6.2 Method.....	69
6.2.1 RFLP analysis to map <i>Mel 1</i> precisely.....	69
6.2.2 The DH <i>Ggt</i> experiment.....	69
6.2.3 Outdoor <i>Ggt</i> bioassay.....	69
6.2.4 Field trial.....	70
6.2.5 Field trial design.....	70
6.2.6 Analysis of 1999 field trial.....	71
6.3 Results.....	72
6.3.1 RFLP mapping of <i>Mel 1</i>	72
6.3.2 AFLP mapping of <i>Mel 1</i>	72
6.3.3 The DH <i>Ggt</i> experiment.....	73
6.3.4 Outdoor <i>Ggt</i> bioassay.....	77
6.3.5 QTL analysis of 1999 Marion Bay field trial.....	78
6.3.6 Allele combinations analysis of Av.LSL in DH <i>Ggt</i> experiment.....	81
6.4 Discussion.....	82
6.4.1 RFLP partial map of 140 DH lines.....	84
6.4.2 Loci contributing to Mn efficiency and linkage with resistance to <i>Ggt</i>	84
Chapter 7 Discussion	85
7.1 Introduction.....	85
7.1.1 The physiological and genetic experiments.....	85
7.2 Future research directions.....	86
7.2.1 Field solutions.....	86
7.2.2 Plant-pathogen interactions.....	87
7.2.3 Transformation of plant biochemical defence mechanisms.....	87
Appendix 1 Seed Mn concentration	90
1.1 Seed Mn concentration.....	90
Table 2.1 WI 2585 and Amagi Nijo.....	90
Table 2.2 DH lines.....	91
Appendix 2 Statistical analysis tables for 1-way and 2-way ANOVAs	92
Chapter 2 Pilot Studies.....	92
2.2 Experiment 2: Increased <i>Ggt</i> pathogenicity.....	92
2.2.3 Fig. 2.3 Av.LSL (mm).....	92
2.2.4 Fig. 2.4 PIR.....	92
2.3 Experiment 4: Confirmation of an adequate sample size.....	92
2.3.1 Fig. 2.5 Shoot Mn concentration (mg/kg DM).....	92
2.3.2 Table 2.3 Shoot Mn content ($\mu\text{g}/\text{pot}$).....	93
2.3.3 Table 2.4 Shoot DM (g/pot).....	93
2.3.4 Table 2.4 Root DM (g/pot).....	93
Chapter 3 Critical shoot Mn concentration experiment.....	94
3.1 Fig. 3.3 A Shoot DM (g/pot).....	94
3.2 Fig. 3.3 B Root DM (g/pot).....	94
3.3 Fig. 3.4 Shoot Mn concentration (mg/kg DM).....	94
3.4 Fig. 3.6 Shoot Mn content ($\mu\text{g}/\text{pot}$).....	95
3.5 Fig. 3.7 Root Mn concentration (mg/kg DM).....	95
Chapter 4 Physiological Studies.....	96
4.1 The <i>Ggt</i> experiment.....	96
4.1.1 Fig. 4.1 Nil <i>Ggt</i> shoot Mn concentration (mg/kg DM).....	96
4.1.2 Fig. 4.2 <i>Ggt</i> infected shoot Mn concentration (mg/kg DM).....	96
4.1.3 Fig. 4.3 Av.LSL (mm/plant).....	96
4.1.4 Fig. 4.4 PIR.....	97
4.1.5 Fig. 4.5 Nil <i>Ggt</i> root Mn concentration (mg/kg DM).....	97
4.1.6 Fig. 4.6 <i>Ggt</i> infected root Mn concentration (mg/kg DM).....	97

4.1.7 Table 4.1 Nil <i>Ggt</i> shoot DM (g/pot).....	97
4.1.8 Table 4.1 Nil <i>Ggt</i> root DM (g/pot).....	98
4.1.9 Table 4.2 <i>Ggt</i> infected shoot DM (g/pot).....	98
4.1.10 Table 4.2 <i>Ggt</i> infected root DM (g/pot).....	98
4.2 <i>The temporal study</i>	99
4.2.1 Fig. 4.7 Shoot Mn concentration (mg/kg DM).....	99
4.2.2 Fig. 4.8 Shoot Mn content ($\mu\text{g}/\text{pot}$).....	99
4.2.3 Fig. 4.9 Root Mn concentration (mg/kg DM).....	99
4.2.4 Fig. 4.10 Av.LSL (mm).....	100
4.2.5 Fig. 4.11 PIR.....	100
Chapter 6 Genetic Studies	101
6.1 <i>The DH <i>Ggt</i> experiment</i>	101
6.1.1 Fig. 6.6 Shoot Mn concentration (mg/kg DM).....	101
6.1.2 Fig. 6.7 Shoot Mn content ($\mu\text{g}/\text{plant}$).....	101
6.1.3 Fig. 6.8 Root Mn concentration (mg/kg DM).....	101
6.1.4 Fig. 6.9 Av.LSL (mm/6 plants).....	101
6.1.5 Fig. 6.10 PIR.....	101
6.2 <i>Marion Bay field trial</i>	102
6.2.1 Fig. 6.14 Field trial shoot Mn concentration (mg/kg DM).....	102
6.3 <i>Linkage of <i>Mel 1</i> and <i>Xwg645</i> with resistance to <i>Ggt</i></i>	102
6.3.1 Fig. 6.15 Linkage of Mn-efficient alleles at <i>Mel 1</i> and <i>Xwg645</i> with Av.LSL (mm/6 plants) between the four allele group combinations.....	102
6.3.2 Fig. 6.15 Linkage of Mn-efficient alleles at <i>Mel 1</i> and <i>Xwg645</i> with Av.LSL (mm/6 plants) between group 1 and group 4.....	102
Appendix 3 Materials and methods for molecular biology	103
3.1 <i>Small scale genomic DNA extraction</i>	103
3.2 <i>RFLP analysis and construction of a partial map</i>	103
3.3 <i>Linkage analysis for <i>Mel 1</i></i>	106
3.4 <i>AFLP analysis</i>	106
References	109