# Inheritance and Molecular Studies of Durable Resistance to Stripe Rust (*Puccinia striiformis* West.) in Wheat (*Triticum aestivum* L.)

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A Thesis Submitted in Fulfilment of the
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Doctor of Philosophy

At

Lincoln University

By

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# ABSTRACT

Abstract of a thesis submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy at Lincoln University

**Inheritance and Molecular Studies of Durable Resistance to Stripe Rust (*Puccinia striiformis* West.) in Wheat (*Triticum aestivum* L.)**

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To study the mode of inheritance of durable resistance to stripe rust (*Puccinia striiformis* West.), and design a molecular approach to determine the contribution of genomic region(s) to the durability of this resistance, two double haploid (DH) wheat (*Triticum aestivum* L.) populations each of 140 DH lines, the first derived from a cross between a susceptible cultivar Tiritea and a durably resistant cultivar Otane, and the second from a cross between cv. Otane and cv. Karamu (reported to have the durable gene $Yr_{18}$) were tested in four sets of glasshouse and field experiments after inoculation with a defined single pathotype of *Puccinia striiformis* 106E139A$^{+}$. For both crosses, seedlings and adult plants were tested in the glasshouse for four components of resistance, namely infection type (IT), latent period (LP), pustule density (PD) and pustule length (PL). Data for only IT at the seedling stage, and for three measures of resistance namely IT, final disease severity (FDS) and area under the disease progress curve (AUDPC) at the adult plant stage were recorded in the field. Based on the phenotypes exhibited by the progenies of the cv. Tiritea x Otane cross, three bulks of DH lines named as resistant (R), moderately resistant (MR) and susceptible (S) were genotyped with 139 microsatellite markers (4 to 6 per chromosome) and 60 AFLP primer combinations. From these results 11 polymorphic microsatellite markers and six AFLP primer combinations were tested on a representative sample of 50 DH lines to construct linkage maps and identify markers linked to stripe rust disease.

In the seedling stage cv. Tiritea, cv. Otane and cv. Karamu were all susceptible, and the observed phenotypic ratio of progeny fitted a three-locus model; three recessive genes for R and two recessive genes for MR (cv. Tiritea x Otane). No segregation of resistance in the

case of cv. Otane and Karamu was recorded at this stage. In the adult plant stage the observed ratio fitted a two gene model in the glasshouse with reversal of dominance in the $F_1$ heterozygotes, and a three gene model in the field (cv. Tiritea x Otane); data from both the glasshouse and field for cv. Otane x Karamu fitted a three gene model, with all genes being recessive. For FDS and AUDPC, the observed data fitted the three gene model for both measures of resistance in the case of cv. Tiritea x Otane, and the two gene model for FDS and three gene model for AUDPC in the case of cv. Otane x Karamu.

This first study of the inheritance of components of stripe rust resistance revealed that although the three parents did not differ in their LP, PD or PL at the seedling stage, transgressive segregations had occurred. For the cv. Tiritea x Otane cross three to four genes for LP and three genes each for PD and PL were estimated, while for the cv. Otane x Karamu cross four genes, two each for PD and PL were estimated; no significant segregation for LP was observed in progenies of this cross. The short LP, higher PD and long PL of the $F_1$ progenies showed that the genes involved were recessive. At the adult plant stage the three parents also did not differ in their LP, but did differ in their PD. For PL cv. Otane differed from cv. Tiritea but did not differ from cv. Karamu. However, transgressive segregation for all of the three components was observed. The genes estimation, based on the probability of either parental types, or significant transgressants, gave two independently segregating genes in each cross for LP. For PD two genes, one gene each for cv. Tiritea and cv. Otane and five genes, three from cv. Otane and two from cv. Karamu were estimated. Similarly the estimate was three genes (cv. Tiritea x Otane) and two genes (cv. Otane x Karamu) for PL at this stage. In both crosses the segregating genes appeared to be recessive as the population distribution was positively skewed for LP with a reversal of the dominance effect in the case of cv. Tiritea x Otane, and with higher PD and longer PL of the $F_1$ progenies in the case of cv. Otane x Karamu. Transgressive segregation occurred in both crosses for all measures of resistance, suggesting that the resistant genes in the three parental cultivars are all different.

Quantitative trait locus (QTL) analysis tools were used to identify significant determinants of stripe rust resistance assessed through different measures of this resistance such as IT, FDS, AUDPC, LP, PD, and to provide support to the hypothesized genetic models inferred from the phenotypes of the cv. Tiritea x Otane cross. Two markers, gwm 611 and gwm 44, located on chromosome arms 7BL and 7DS respectively were associated with IT, LP, PD at the seedling stage and IT, FDS, LP and PD at the adult plant stage. The highest

association was observed for marker alleles (gwm 611) of cv. Tiritea which accounted for 49% of the total variation in seedling IT, while marker alleles (gwm 44) of cv. Otane explained 24% of the total variation in seedling IT. At the adult plant stage gwm 611 also accounted for 42% of the total variation and gwm 44 accounted for 23% of the total variation in the IT. These associations were therefore stable irrespective of plant development stage or experimental method (glasshouse and field). Simple interval mapping placed the resistant factor of cv. Tiritea at a distance of 13 cM from marker gwm 611 with a likelihood ratio statistics (LRS) value of 34, and the resistant factor of cv. Otane at a distance of 6 cM from marker 44 with a LRS of 10. The presence of $yr_6$ in cv. Tiritea and $yr_{18}$ in cv. Otane was established in this study. Two other regions from cv. Otane on chromosome arms 5DL and 3DL were also associated with FDS, AUDPC and PL at the adult plant stage only. Two genomic regions of cv. Tiritea on chromosome arms 3BL and 5BS contributed to the seedling stage IT, but only the region on chromosome 3BL was associated with LP at the adult plant stage.

AFLP markers were also associated with different measures of resistance but the genome location for most of them is not known, as there was no linkage with genome specific microsatellite markers. Among these markers, loci eACCmCTA16 accounted for 42% of the variation in AUDPC and 22% of the variation in PD at the adult plant stage. Overall the microsatellite and AFLP markers data confirmed the multi-locus control of stripe rust in cv. Otane, provided evidence for resistant factors in cv. Tiritea and in general agreed with the proposed phenotypic models. Furthermore the molecular data and parental lineages of cv. Otane and cv. Karamu supported the presence of the durable resistance gene $yr_{18}$ in cv. Otane rather than in cv. Karamu. Thus cv. Otane could serve as a source of this gene for use in future breeding programmes. The results suggest that resistance to stripe rust in these populations is quantitative in nature, and is controlled by a few recessive genes.

**Key words**: *Triticum aestivum*, *Puccinia striiformis*, genetic models, disease resistance, resistance components, molecular markers, QTL mapping, representative genotyping (RG), bulk segregant analysis.