CHARACTERISATION OF A 4BS.4BL-5RL WHEAT RYE TRANSLOCATION TO IMPROVE COPPER EFFICIENCY OF BREAD WHEAT

Thesis submitted for the degree of

Doctor of Philosophy

by

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STATEMENT

I here by certify that this thesis contains no material, to the best of my knowledge, which has been previously published by another person, except were due reference has been given.

I consent to this thesis being made available for photocopying and loan.

Richard Leach
August, 2004

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- Specific individuals are acknowledged in the chapters detailing work for which they provided equipment/materials and/or technical support for the experiments described in this thesis.

MATERIALS

Materials used in this study are listed below, together with the suppliers' names. All chemicals used for *in vitro* use were at least analytical grade in standard. Solutions were prepared under sterile conditions using Nanopure H_2O, and autoclaved when appropriate. Descriptions of RFLP clones and genetic material used in this study can be found in the individual chapters.

Chemicals: • bovine serum albumen (BSA) fraction V, spermidine, ampicillin, kanomycin, salmon sperm DNA, N-(2-hydroxyethyl) piperazine-N′-(2-ethane-sulfonic acid (HEPES), Tris (hydroxymethyl) amino-methane (trizma base), ethidium bromide, polyvinyl pyrrolidone (PVP, 40,000 molecular weight), *E. coli* t-RNA, salmon sperm DNA, dithiothreitol (DTT), 1,4-Piperazinediethanesulfonic acid (PIPES), polyoxyethylenesorbitan monolaurate (TWEEN), N,N,N',N'-tetramethylethylenediamine (TEMED), 4',6-diamidino-2-phenylindole dihydrochloride (DAPI), colchicine: Sigma Chemicals (USA). • dextran sulphate, Ficoll 400: Pharmacia (USA) • phenol: Wako Industries (Japan). • NaCl, NaOH, Na_2EDTA, $MgCl_2$, potassium acetate (KAc), sodium acetate (NaAc), urea, sucrose, glucose, ethanol (EtOH), iso-propyl alcohol, iso-amyl alcohol, chloroform, bromophenol blue, HCl, glacial acetic acid, sodium dodecyl sulphate (SDS): BDH. • xylene cyanol: Ajax Chemicals

Enzymes: • pancreatic RNase A: Sigma (USA). • *Taq* DNA polymerase, Klenow fragment (large fragment of *E. coli* DNA polymerase I): Bresatec (Australia). • restriction enzymes: Bresatec (Australia), Boehringer Mannheim (Germany), New England Biolabs and Promega (USA) • PNK T4 kinase: Geneworks (Australia).

Oligodeoxyribonucleotides: Synthetic oligodeoxyribonucleotides were made on an Applied Biosystems (USA) Model 380B DNA synthesizer by Neil Shirley in the Department of Plant Science, University of Adelaide. Oligonucleotides were purified by ion exchange HPLC using a MonoQ column (Pharmacia, USA).

Nucleotides and Radionucleotides: Ultrapure nucleotide triphosphates (NTPs) and deoxynuclotide triphosphates (dNTPs) were obtained from Pharmacia.
α-^{32}P-dCTP (10 μCi/μl) was obtained from Bresatec (Australia).

Molecular weight markers, and cloning vectors: • SPPI DNA cut with *Eco*RI, λ DNA cut with *Hind*III, and pUC19 DNA cut with *Hpa*II, 100 bp ladder: Bresatec (Australia).

- pBluescipt SK-: Stratagene (USA).
- pGem®-T Vector Kit: Promega (USA).

Bacterial media ingredients: bacto-agar, bacto-tryptone and yeast extract: Difco Laboratories (USA).

Agaroses: • low melting point agarose: BRL (USA). •LE agarose, Analytical Grade: Promega (USA). • NuSeive GTG grade: FMC Bioproducts (USA). •SeaKem® LE agarose: FMC Bioproducts (USA).

Bacterial strains: *Escherichia coli* DH5α: Stratagene (USA).

Kits: • Bresa-Clean: Bresatec (Australia). • pGem®-T Vector Kit: Promega (USA).• Quiagen tip-20: Quiagen (Germany).

SUMMARY

Copper deficiency causes significant annual losses in grain yield due to poor grain set. Cereals such as wheat and barley are particularly susceptible to low copper soils whereas, crops such as rye and triticale are better able to grow and yield under such conditions of nutrient stress. The ability of rye and triticale, which carries a complete set of rye chromosomes, to tolerate low copper conditions has been attributed to a gene on rye chromosome 5R.

Wheat-rye translocation lines have previously been produced carrying segments of the long arm of chromosome 5 of rye (5RL). Although these lines have expressed copper efficiency in University of Adelaide trials, until now they have been considered agronomically inferior and so have not been used as commercial cultivars.

The physical size of rye segment of the 4BS.4BL-5RL translocation in a Chinese Spring background derived from the Cornell Wheat Selection 82a1-2-4-7 was measured using GISH (genomic *in situ* hybridization) and found to be 16% of the long arm. The size of this translocation was similar to GISH measurements of another 4BS.4BL-5RL translocation in Viking wheat background, although both these lines arose spontaneously and at different times.

Molecular maps of both 4BS.4BL-5RL translocations in the two different wheat backgrounds were developed and used to screen for rare recombinants between wheat and rye in a background homozygous for the Sears' *ph1b* mutant. The maps revealed the approximate genetic location of the translocation breakpoint involved in these two 4BS.4BL-5RL translocations to be similar even though they are known to have arisen at different times and in different experimental populations. The similarity of these translocations suggests a unique property of the region at or near the translocation breakpoint that could be responsible for their similarity and spontaneous formation. After screening 703 critical seedlings for evidence of recombination between the 5RL segment and wheat homoeologues, no confirmed recombinants were identified.

Lines containing the 4BS.4BL-5RL translocation were shown to yield equally as well as their recurrent parent under normal field conditions. In addition the presence of the 4BS.4BL-5RL had no adverse effects on a range of grain quality characteristics measured in these lines.

A pot trial using lines derived from a cross between the CSHN translocation and the wheat cultivar Warigal (five backcrosses) revealed that they provided copper-efficiency even under the severest of deficiency conditions. While the results of this pot trial did not show the outstanding copper efficiency previously observed in these lines, the translocation did consistently out yield the recurrent parent under severe copper deficiency conditions.

Finally, a reliable PCR marker was developed for the rapid identification of lines containing the distal portion of the 5RL chromosome.