

GENETIC ANALYSIS OF A REGION ASSOCIATED
WITH HEAT AND DROUGHT TOLERANCE ON
CHROMOSOME 3B OF HEXAPLOID WHEAT
(*Triticum aestivum*)

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Abbreviations

ABA	Abscisic acid
BAC	Bacterial artificial chromosome
BIC	Bayesian information criterion
Bp	Base pair
CIM	Composite interval mapping
cM	Centimorgan
CT	Canopy temperature
ctg	Contig
DArT	Diversity arrays technology
DH	Doubled haploid
FA	Factor analytic
GC	Growth chamber
ISBP	Insertion site-based polymorphism
Kb	Kilo base pair
LR	Leaf rolling
MARS	Marker assisted recurrent selection
MAS	Marker assisted selection
Mb	Mega base pair
MEIM	Multi-environment inferred marker
MET	Multi-environment trial
NDVI	Normalized difference vegetative index
NIL	Near isogenic line
PAV	Presence absence variation
PCR	Polymerase chain reaction
QTL	Quantitative trait locus
RIL	Recombinant inbred line
RING	Really interesting new gene
SIM	Single interval mapping
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
TGW	Thousand grain weight
TILLING	Targeting induced local lesions in genomes
WGAIM	Whole genome average interval mapping

Abstract

Drought and heat can occur during the growth cycle of crops and severely reduce yield. A QTL associated with yield and yield-related component was found in four wheat populations (*Triticum aestivum* L.) on the long arm of chromosome 3B “*qYDH.3BL*”. The four populations were grown under various climatic conditions including drought, heat and combinations of both in a number of different areas (Australia and Mexico). Linear mixed models that partition and account for genetic and non-genetic or extraneous variation were used to detect loci in single-environment and/or multi-environment QTL analysis using ASReml-R. The alleles carried by RAC875, Excalibur or Drysdale improved grain yield by between 5% and 12.5%. Two doubled haploid populations (RAC875/Kukri and Excalibur/Kukri) and two recombinant inbred line populations (RAC875/Kukri and Gladius/Drysdale) were used to fine map *qYDH.3BL* and identify candidate gene(s). A total of thirty-seven molecular markers were mapped on one or both genetic maps of chromosome 3B enabling development of a consensus genetic map of the *qYDH.3BL* region. The markers were selected based on comparisons with a published “neighbour map” of chromosome 3B or designed using either BAC-end, contig or gene sequences from the chromosome 3B sequencing project; 3BSEQ <http://urgi.versailles.inra.fr/> (cv. Chinese Spring).

A positional cloning approach was used to identify candidate genes for *qYDH.3BL*. Molecular markers from the targeted region were assigned to physical contigs by screening the chromosome 3B BAC library experimentally using PCR or *in silico* by sequence comparison. A total of eight physical contigs containing 85 genes, were anchored to the *qYDH.3BL* region. Public and in-house resources of wheat transcript sequences were used to restrict the gene list to 65 expressed genes.

Based on comparison of the 65 gene sequences to gene probes in a drought transcriptomic database, three genes were found to be differentially expressed between RAC875 and Kukri under drought conditions. Short genomic sequence reads (10× coverage) from each of the five parental lines (RAC875, Kukri, Excalibur, Gladius and Drysdale) were mapped against the 65 genes for polymorphism discovery. One gene exhibited sequence polymorphism between the drought tolerant parents (RAC875, Excalibur and Drysdale)

and the drought-sensitive parents (Gladius and Kukri). In addition, presence/absence polymorphisms were consistently detected throughout a region containing 12 genes, indicating that the drought tolerant parents may have a deletion (or alien introgression) in this region. Thus, in this work, we confirmed the genetic effect of *qYDH.3BL* in multiple environments and multiple populations, saturated the target region with new molecular markers and defined a preliminary list of genes located in the *qYDH.3BL* region and selected candidate genes for further investigations.

Résumé

Des épisodes climatiques de sécheresse et/ou de hautes températures peuvent engendrer de fortes pertes de rendement pour les cultures de céréales au champ. Un QTL associé au rendement et à ses composantes a été détecté dans quatre populations de blé (*Triticum aestivum* L.) sur le bras long du chromosome 3B « *qYDH.3BL* ».

Deux populations d'haploïdes doublés (RAC875/Kukri et Excalibur/Kukri) et deux populations de lignées recombinantes (RAC875/Kukri et Gladius/Drysdale) ont été utilisées pour cartographier finement le QTL, au même titre que l'identification de gènes candidats. Ces quatre populations ont été testées sous des conditions environnementales variées, incluant des périodes de sécheresse et/ou hautes températures en Australie et au Mexique. Des modèles statistiques mixtes et linéaires décomposant les variations génétiques et non-génétiques ont été utilisés pour la détection de QTL en considérant dans un premier temps chaque environnement unique, puis en considérant les environnements multiples dans une analyse commune. Les allèles de RAC875, Drysdale et Excalibur à ce locus ont montré une hausse du rendement de 5 à 12.5 % comparées à celles de Gladius ou Kukri.

Un total de trente-sept marqueurs moléculaires a été cartographié dans la région du QTL. Les marqueurs moléculaires ont été sélectionnés (i) par comparaison avec une carte génétique publiée du chromosome 3B, ou (ii) en désignant de nouveaux marqueurs moléculaires sur les séquences de BAC-end, de contig ou de gènes provenant du projet de séquençage du chromosome 3B (3BSEQ, <http://urgi.versailles.inra.fr/>, cv. Chinese Spring). Ceci a permis la construction d'une carte génétique consensus du locus *qYDH.3BL*.

A ce jour, aucun QTL associé au rendement ou ses composantes en condition de sécheresse et/ou de hautes températures n'a encore été cloné positionnellement chez le blé tendre. Les marqueurs moléculaires de la région d'intérêt ont été utilisés pour cartographier physiquement des contigs, soit par PCR, soit par comparaison de séquences *in silico*. La région du QTL inclus un total de huit contigs physiques comprenant 85 gènes annotés.

L'utilisation de base de données de transcrits biologiques publiques ou internes ont été utilisées pour détecter la présence de ces gènes, réduisant la liste à soixante-cinq gènes. Sur les contigs ayant une confiance élevée, aucun des vingt gènes n'a été exprimé différemment entre RAC875 et Kukri. Cependant, un gène présentant du polymorphisme dans sa séquence ainsi qu'une délétion/insertion d'un segment portant 12 gènes ont été découverts permettant ainsi de continuer à affiner la liste de gènes candidats. Les trois lignées parentales (RAC875, Drysdale et Excalibur) qui ont l'allèle liée au haut rendement ont le même haplotype pour ce gène, et la même délétion/insertion en opposition au deux autres lignées parentales Gladius et Kukri.

Ainsi, dans ce travail de thèse nous avons pu confirmer la présence d'un QTL répondant aux stress environnementaux sur le chromosome 3BL dans différentes populations et différents environnements, identifier des gènes candidats sous le QTL, et proposer une liste restreinte pour de futures analyses sur la base de données d'expression et de polymorphismes entre les parents des populations de cartographie.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Julien Bonneau and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Julien Bonneau

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