Variation of flour colour in Western Australia adapted wheat: comparative genomics, molecular markers and QTL analysis

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Declaration & List of Papers Published

I declare that this thesis is my own account of my research and contains as its main content, work that has not previously been submitted for a degree at any tertiary education institution.

Papers that have been published from the research described in this thesis are:

Francki M, Carter M, Ryan K, Hunter A, Bellgard M and Appels R (2004). Comparative organization of wheat homoeologous groups 3S and 7L using wheat-rice synteny and identification of potential markers for genes controlling xanthophyll content in wheat. Functional & Integrative Genomics 4, 118-130.

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Abstract

The yellowness of flour colour ranges is an important quality trait in wheat for end-use products and is determined by the accumulation of carotenoids in the endosperm. The aims of this study were to develop EST-based molecular markers for genes encoding enzymes of the carotenoid biosynthetic pathway leading to xanthophyll accumulation and identify quantitative trait loci for flour colour (b*) and xanthophyll content in Western Australian adapted germplasm.

A novel bioinformatic strategy was developed to identify rice genes encoding key enzymes of the carotenoid biosynthetic pathway and to predict wheat orthologues on the short arm of chromosome 3 or long arm of chromosome 7. The bioinformatic strategy involved the identification of rice carotenoid genes on BAC/PAC contigs aligned to wheat mapped ESTs. Rice genes predicted to have wheat orthologues were selected based on ESTs mapping to regions on wheat homoeologous chromosomes 3 and 7 known to be involved in flour colour. The rice genes predicted to have wheat orthologues were *Geranylgeranyltransferase* I *β*–subunit (*GGT*-I*beta*) and *Rab geranylgeranyltransferase* component A (*RGGT*-A) on the short arm of chromosome 3, *Lycopene β–cylcase* (*LBC*) on the long arm of chromosome 3 and *Lycopene ε–cylcase* (*LEC*) on the long arm of chromosome 7.

The prediction of these wheat orthologues provided the basis for development of EST-based molecular markers for detecting variation in xanthophyll content. Wheat ESTs with unknown chromosomal locations and having the highest similarity to *GGT*-I*beta*, *RGGT*-A and *LBC* were selected for the development of molecular markers. No EST homologues were identified for LEC and therefore this gene was not further considered. Orthology was confirmed by sequencing and deletion lines were used to confirm chromosomal locations. Two partial orthologues of *GGT*-I*beta* were identified on the short arms of chromosomes 3B and 3D. A partial orthologue of *RGGT*-A was mapped to the proximal regions of the short and long arms of chromosome 3B. At least two or more orthologues of *LBC* were identified from nullisomic-tetrasomic lines. An EST-based molecular marker for *GGT*-I*beta* was found to be involved in minor variation of xanthophyll content in a Westonia*2/Janz doubled haploid population.

QTL analysis from three doubled haploid populations indicated variation in WA-adapted germplasm may be due to different alleles controlling flour colour. QTLs for

b* and xanthophyll content were found to coincide on the short arms of chromosomes 3A, 4D, and 7B and the long arm of chromosomes 7A and 7B in WA-adapted germplasm. Homoeologous expression of regions controlling variation in b* and xanthophyll content on the long arm of chromosomes 7A and 7B suggests the shut-down of genes in the same region on chromosome 7D. The main outcome of this study is flour colour and identification of gene orthologues in wheat controlling variation in xanthophyll content is complex most likely because of the interaction of the carotenoid biosynthetic pathway with other pathways.

Acknowledgements

There are so many people that I must thank who have contributed throughout my PhD candidature which has been a rewarding experience, both professionally and personally. I sincerely thank my supervisors, Dr Michael Francki and Prof Rudi Appels, for their constant guidance and support over the years. I am especially grateful to Michael for his endless patience and constant encouragement during the last four months because I would not have got through it without him. I am also grateful to Rudi for his invaluable advice and infinite wisdom on molecular genetics. He has been a wealth of knowledge for me. Michael and Rudi, through their kindness and belief in me, gave me the courage and strength to continue and I will always appreciate what both have done for me.

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Thank you to Paula Moolhuijzen and Adam Hunter from CBBC for their bioinformatic support. I also thank Dr Rob Trengove for the HPLC work in association with Prof Rudi Appels. Thank you to Dr Wujun Ma for kindly providing technical advice on QTL Cartographer. I especially thank everyone from the DAWA lab in the SABC at Murdoch for their endless support and help over the years. I particularly thank Danielle for listening to me and making me laugh. I also thank my fellow PhD students, Dan and Yumi, and I wish them all the best for their remainder of their candidature.

I have been so lucky to have the best support from my family and all my friends. I am grateful to them all because they gave me the emotional strength to see it through. I especially thank my Mum, my brother Shaun and sister-in-law Kerry for their guidance and understanding. To my friends Debs and Shane, I wouldn't have made it without your help and I will always be truly grateful.

Glossary

ABA abscisic acid.

ABA abscisic acid locus.

ADR Australian Durum wheat.

AFLP Amplified fragment length polymorphisms.

allele One of the different forms of a gene that can exist a single locus.

ASW Australian Standard White.

AWB Australian Wheat Board

BAC Bacterial artificial chromosome; an F plasmid engineered to act as a cloning vector that can carry large inserts.

BCH *β-Carotene hydroxylase*.

BLAST Basic Local Alignment Search Tool.

BLASTN comparison of nucleotide sequence databases.

BLASTP comparison of protein sequence databases.

BLASTX comparison of nucleotide query sequence translated in all reading frames against a protein sequence database.

BSA Bulked segregant analysis.

candidate gene A sequenced gene of previously unknown function that, because of its chromosomal position or some other property, becomes a candidate for a particular function such as disease determination.

Ccs *Capsanthin-capsorubin synthase* gene.

cDNA (complementary DNA) Synthetic DNA transcribed from a specific RNA through the action of the enzyme reverse transcriptase.

CIE b* Commission Internationale l'Eclairage whiteness/yellowness value.

CIE L* Commission Internationale I'Eclairage L* (brightness) value.

CIELAB Commission Internationale I'Eclairage L* a* b* colour space.

CO_2 Carbon dioxide

crtE *Lycopene ε-cyclase* gene from *Erwinia uredovora.*

CrtL-b *Lycopene β-cyclase* gene.

CrtL-e *Lycopene ε-cyclase* gene.

CrtR *β-Carotene hydroxylase* gene.

DAWA Department of Agriculture Western Australia.

dbEST Expressed sequence tag database.

deletion Removal of a chromosomal segment from a chromosome set.

DH Doubled haploid.

DMADP dimethylallyl diphosphate.

DNA (deoxyribonucleic acid) A double chain of linked nucleotides (having deoxyribose as their sugars); the fundamental substance of which genes are composed.

DPA Days post anthesis.

environment The combination of all the conditions external to the genome that potentially affect its expression and its structure.

EST Expressed sequence tag(s).

FAD Flavin adenine dinucleotide.

FAO Food and Agricultural Organization of the United Nations

FDP Farnesyl diphosphate.

ha Major locus controlling the recessive grain hardness trait.

GDP Geranyl diphosphate.

GDPS Rab Geranylgeranyl transferase-like protein.

gene The fundamental physical and functional unit of heredity, which carries information from one generation to the next; a segment of DNA, composed of a transcribed region and a regulatory sequence that makes possible transcription.

gene family A set of genes in one genome all descended from the same ancestral gene.

genome The entire complement of genetic material in a chromosome set.

genotype The specific allelic composition for a certain gene or set of genes.

GGH Geranylgeranyl hydrogenase (reductase).

GGPP Geranylgeranyl pyrophosphate.

GGPP Geranylgeranyl pyrophosphate synthase.

GGT-Ibeta Geranylgeranyltransferase I β–subunit.

GRDC Grains Research and Development Corporation of Australia

HIF heterogenous inbred families.

HMW-GS high-molecular-weight glutenin subunit.

IDP Isopentyl diphosphate.

ITMI International Triticeae Mapping Initiative.

L Long arm of chromosome.

LBC *Lycopene β–cyclase*.

LEC *Lycopene ε–cyclase*.

LHC light harvesting complex.

library A collection of DNA clones obtained from one DNA donor.

linkage The association of genes on the same chromosome.

linkage group A group of genes known to be linked; a chromosome.

linkage map A chromosome map; an abstract map of chromosome loci, based on recombinant frequencies.

LMW-GS low-molecular-weight glutenin subunit.

locus (plural **loci**) The specific place on a chromosome where a gene is located.

MAS Marker assisted selection.

molecular markers genetic markers; alleles used as experimental probes to keep track of an individual, a tissue, a cell, a nucleus, a chromosome, a gene or a trait.

NIL Near isogenic line.

NS *Neoxanthin synthase*.

Nulli-tetra Nullisomic-tetrasomic line.

NWMMP National Wheat Molecular Marker Project.

PAC Phage P1 artificial chromosome.

PCR *See* **Polymerase chain reaction**.

PDS *Phytoene desaturase*.

phenotype (1) The form taken by some character (or group of characters) in a specific individual. (2) The detectable outward manifestations of a specific genotype.

Polymerase chain reaction A method for amplifying specific DNA segments which exploits certain features of DNA replication.

polymorphism The occurrence in a population (or among populations) of several phenotypic forms associated with alleles of one gene or homologs of one chromosome.

PPDP Prephytoene diphosphate.

PPO Polyphenol oxidase.

PHST Pre-harvest sprouting tolerance.

PSII Photosystem II.

PYS *Phytoene synthase*.

Quantitative trait loci loci controlling genetic variation of a quantitative trait.

QTL *See* **Quantitative trait loci**.

RAPD Randomly amplified polymorphic DNA.

RFLP Restriction fragment length polymorphisms.

***RGGT*-A** *Rab geranylgeranyltransferase* component A.

Rht Semi-dwarfing (restricted height) gene.

RIL Recombinant inbred line.

S Short arm of chromosome.

SNP Single nucleotide polymorphisms.

SSR Simple sequence repeats (or microsatellites).

STS Sequence tagged site.

TBLASTN comparison of a protein query sequence against a nucleotide sequence database translated in all reading frames.

TBLASTX comparison of six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.

TIGR The Institute for Genomic Research

VDE *Violaxanthin deepoxidase*.

Vp14 *Violaxanthin-cleavage* gene (*Zea mays*).

WA Western Australia.

WSN White salted noodles.

YAC yeast artificial chromosome.

YAN Yellow alkaline noodles.

ZDS *ζ-Carotene desaturase*.

ZE *Zeaxanthin epoxidase*.

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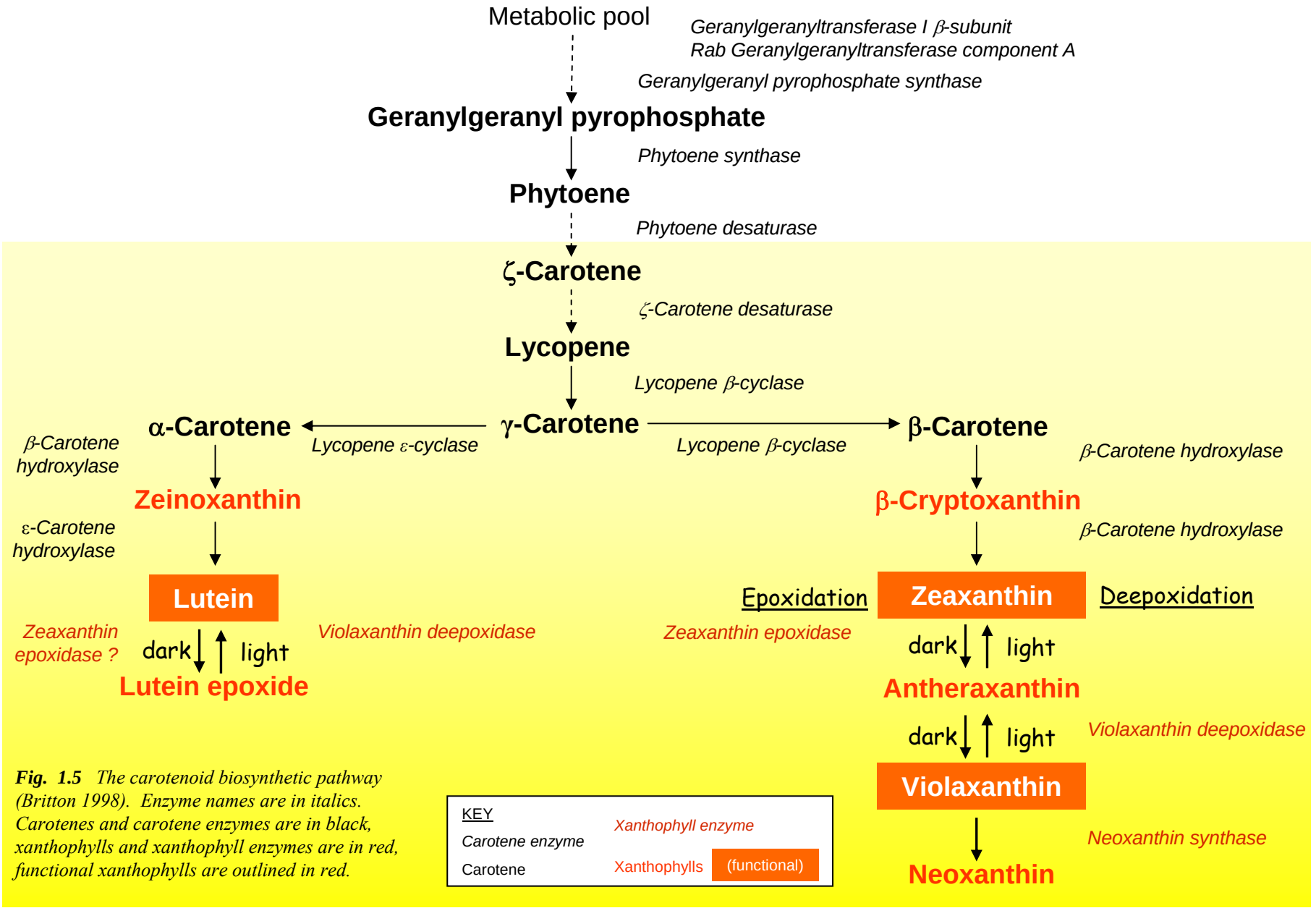


Fig. 1.5 *The carotenoid biosynthetic pathway (Britton 1998). Enzyme names are in italics. Carotenes and carotene enzymes are in black, xanthophylls and xanthophyll enzymes are in red, functional xanthophylls are outlined in red.*

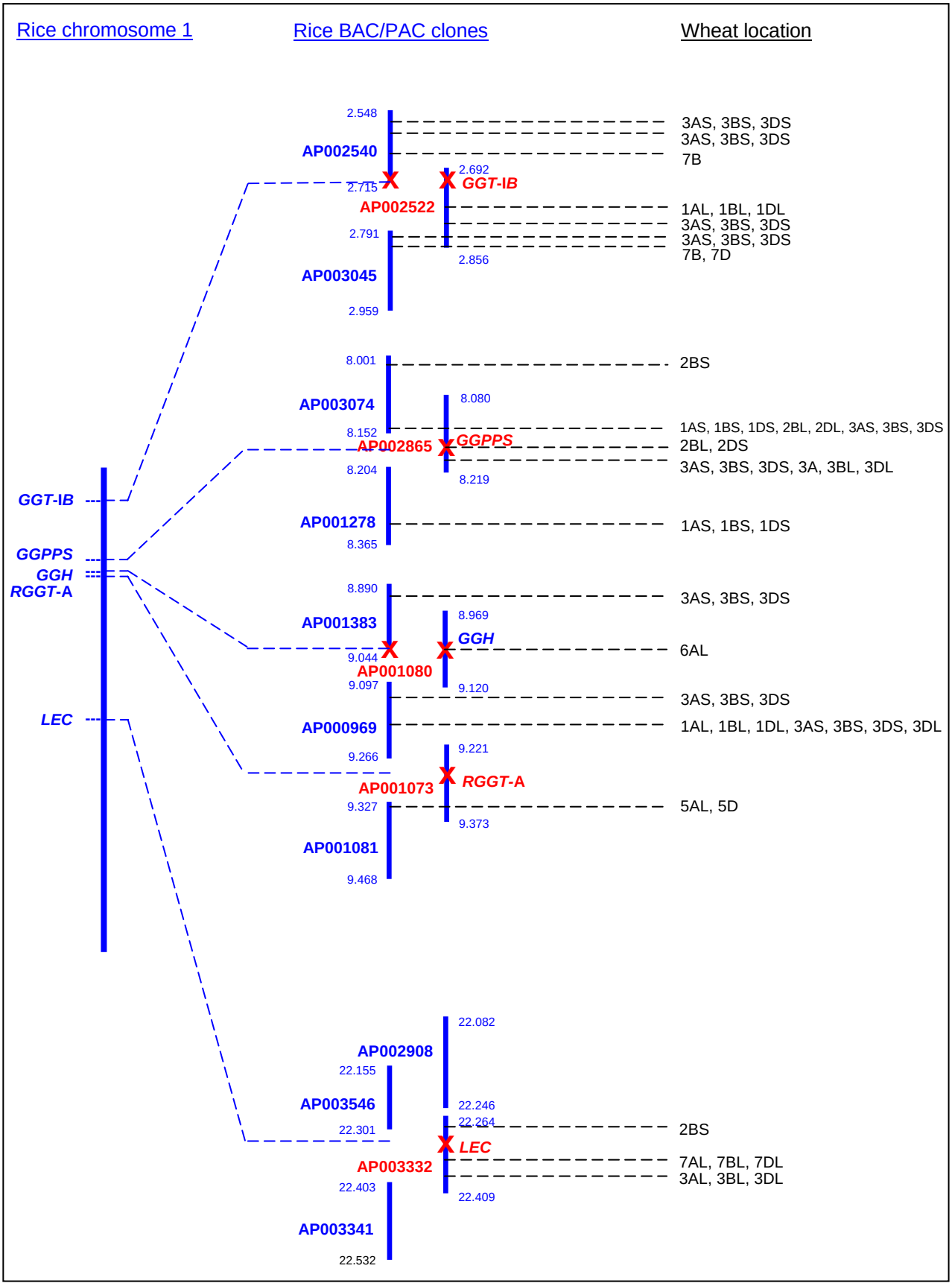
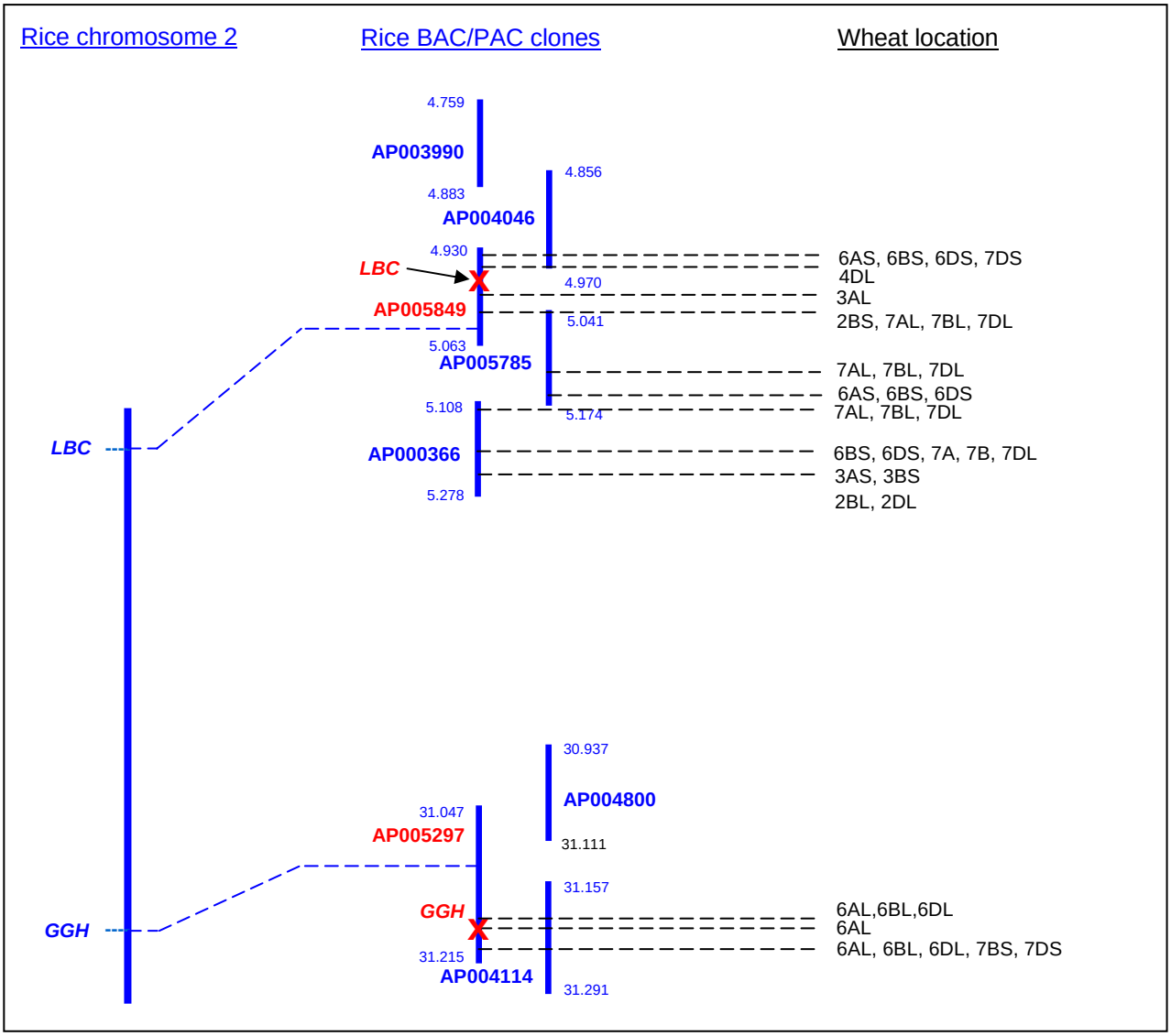


Fig. 2.3 *Summary of chromosomal locations of carotenoid genes in rice and alignment of mapped wheat ESTs to flanking BAC/PAC clones.*



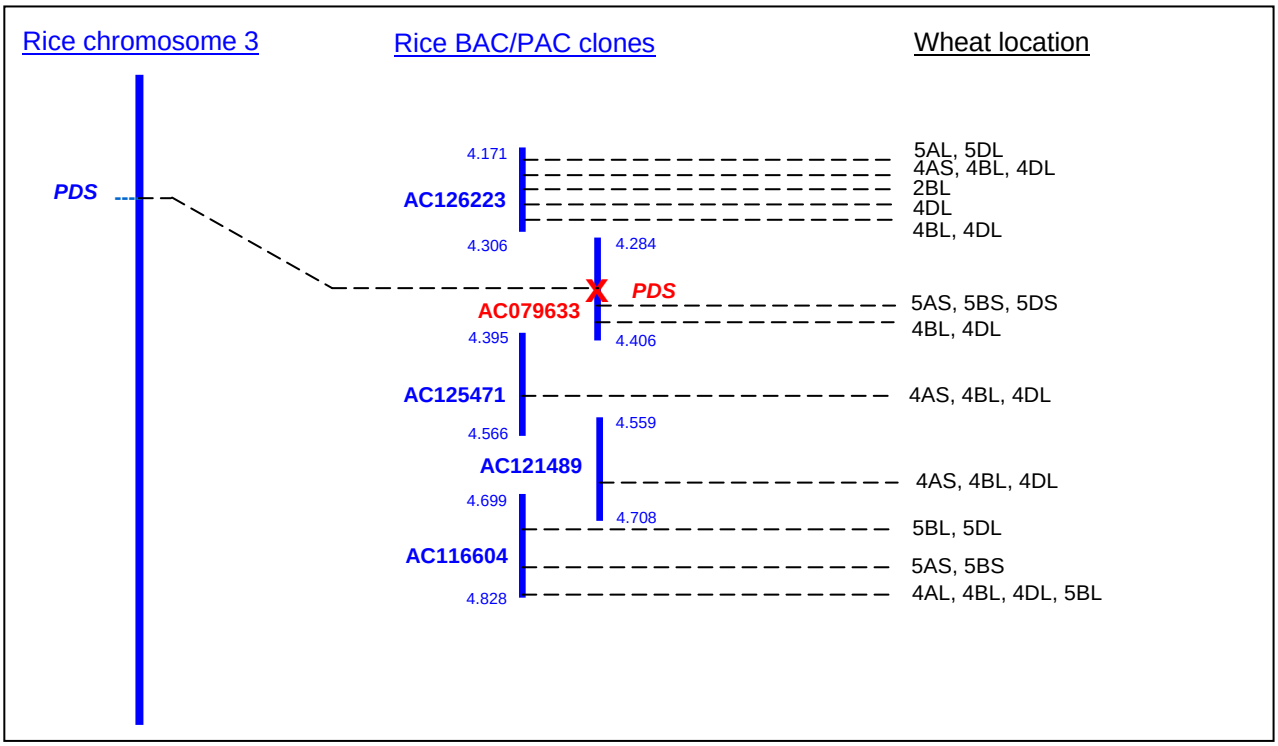
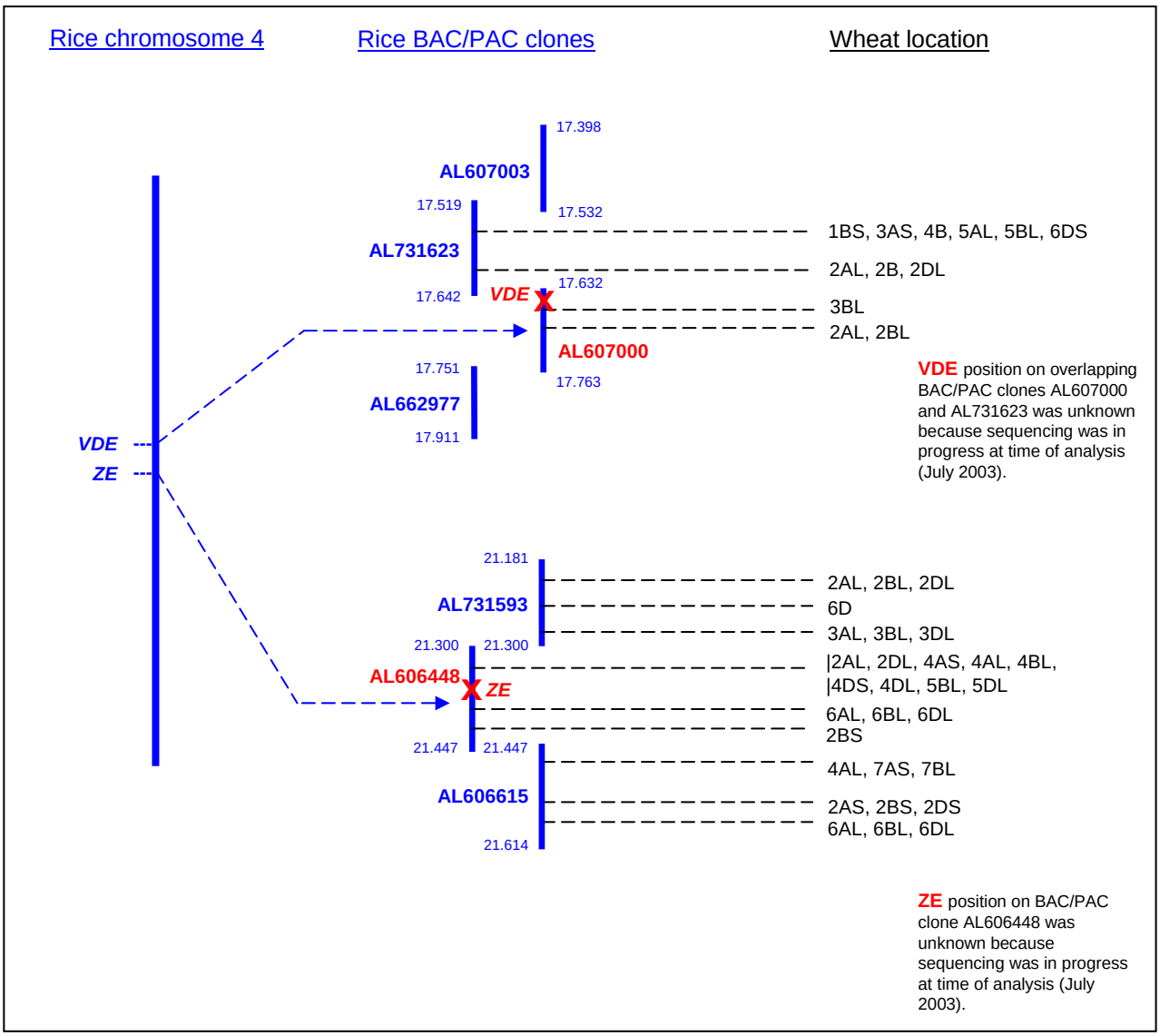


Fig. 2.3 *continued.*



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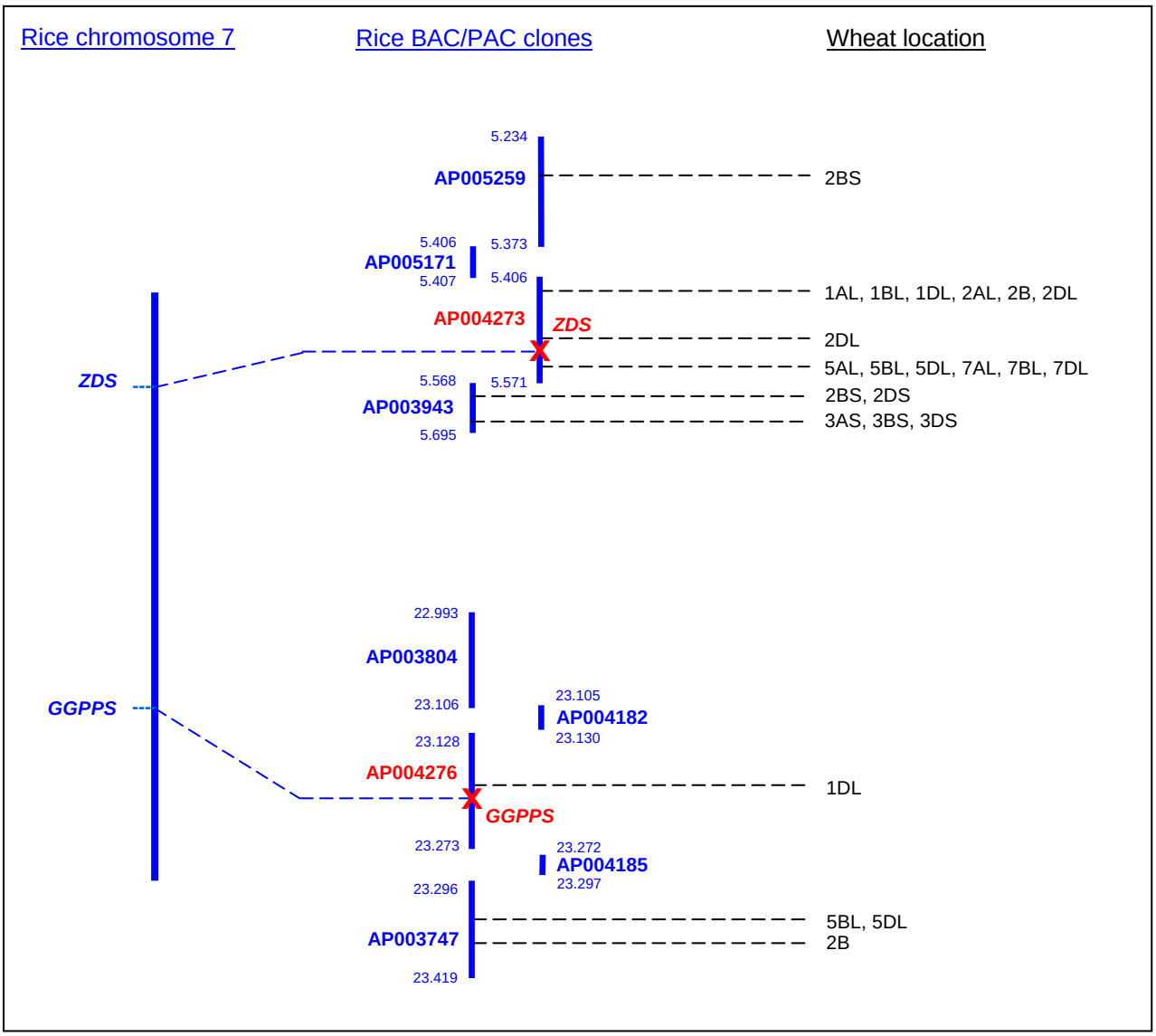

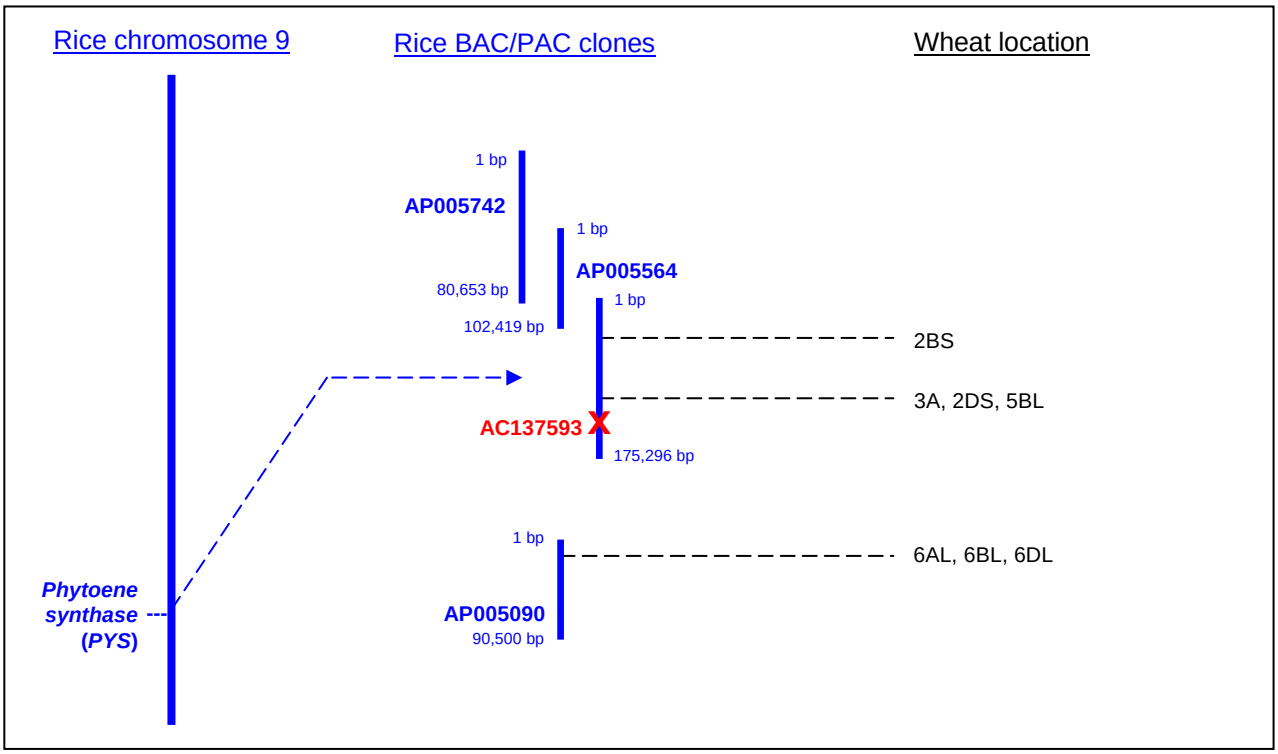

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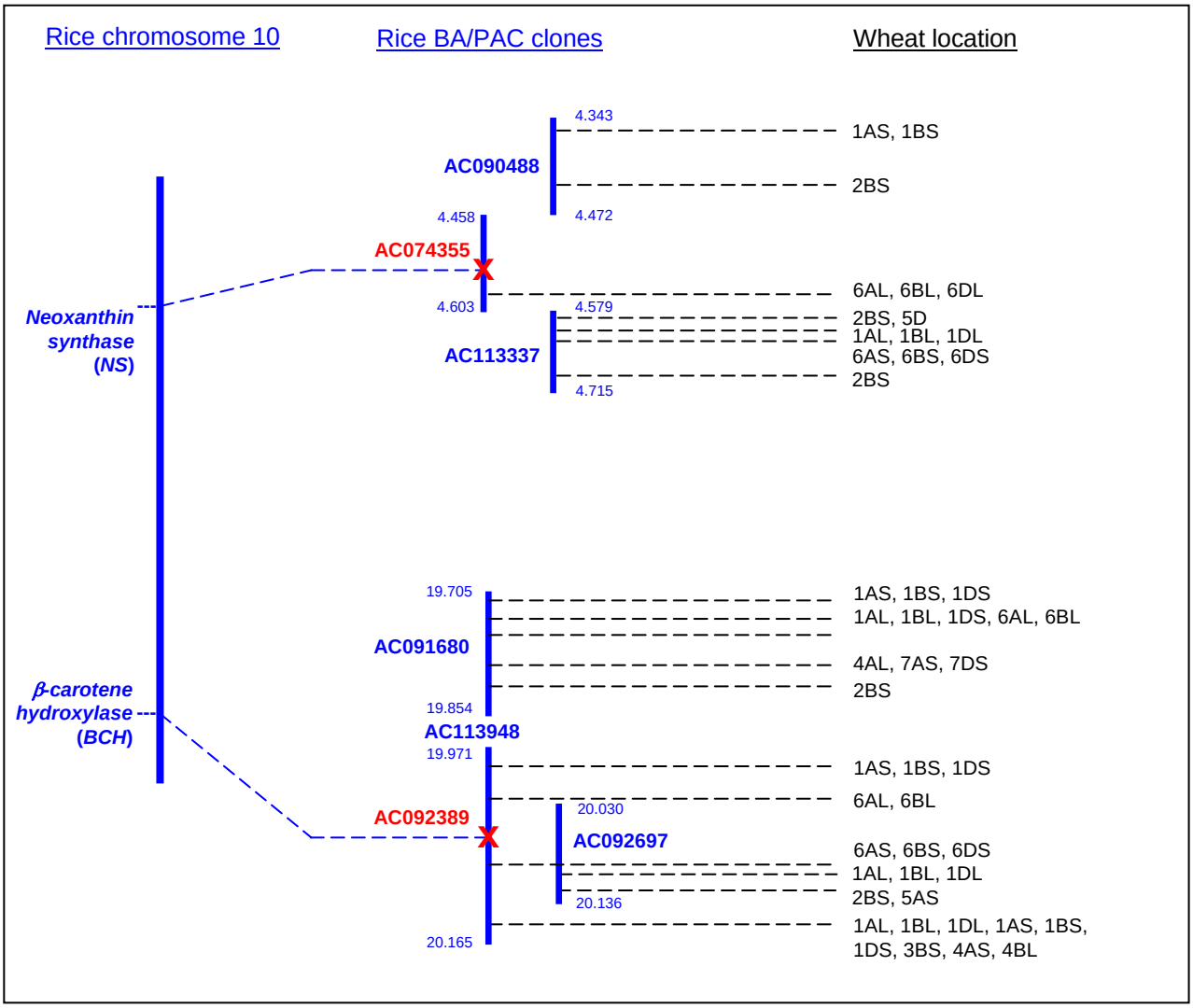


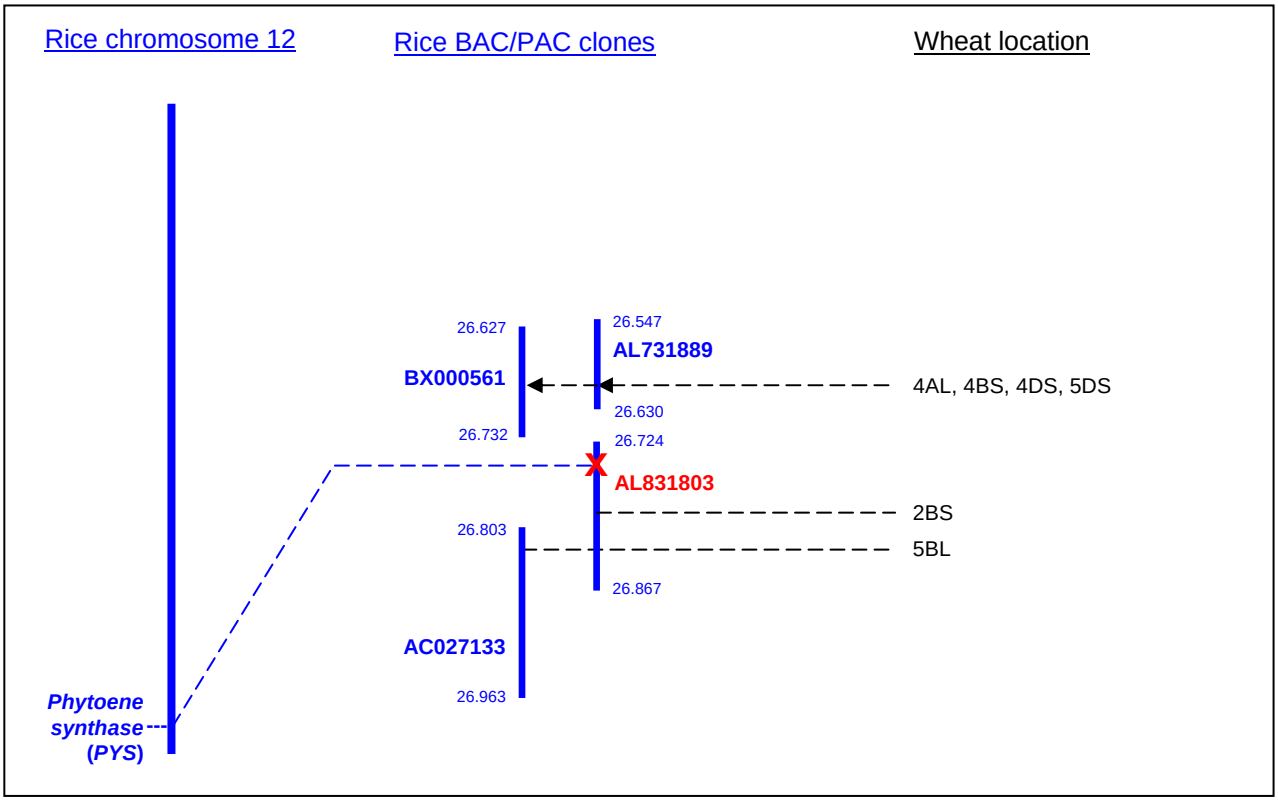
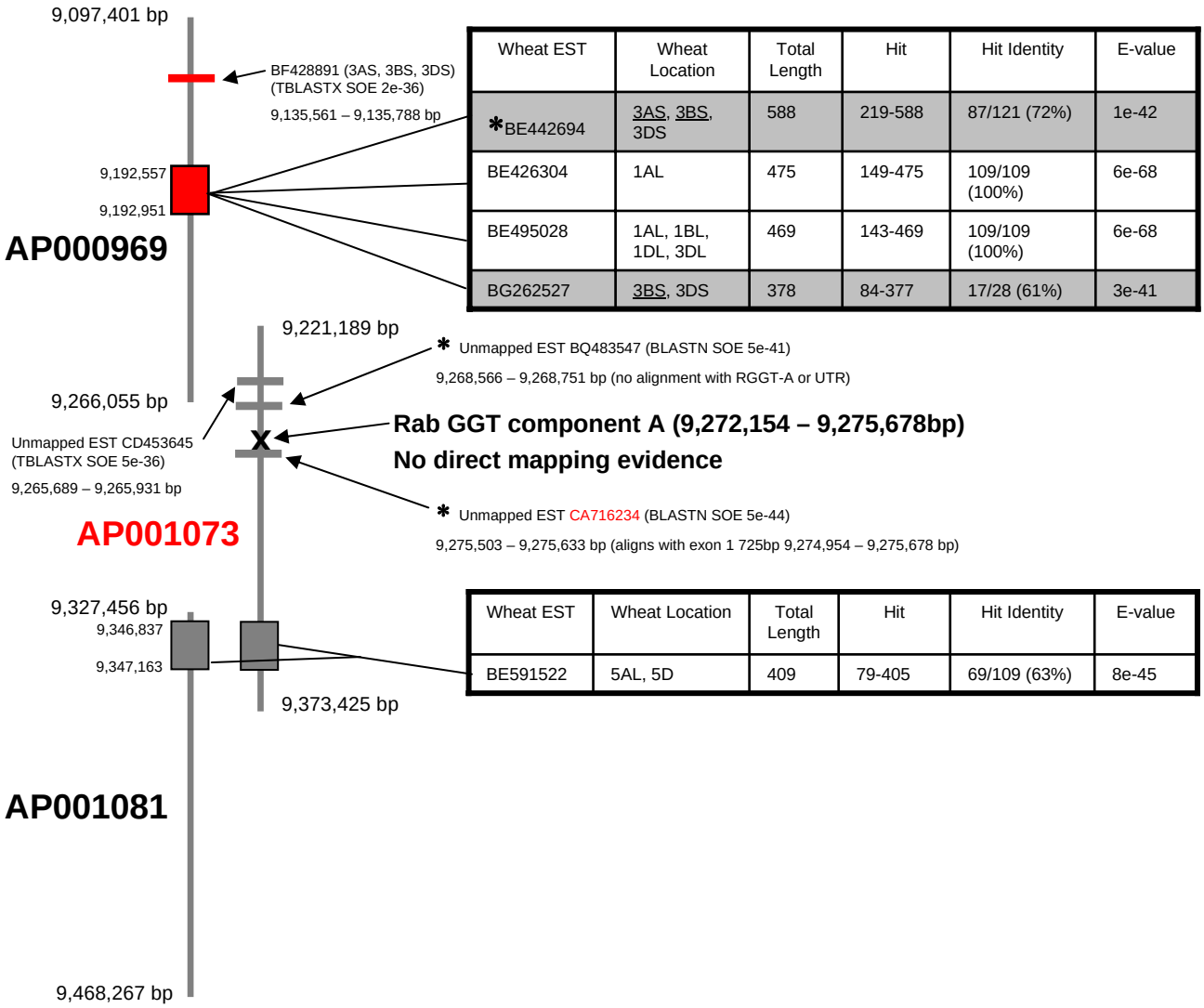


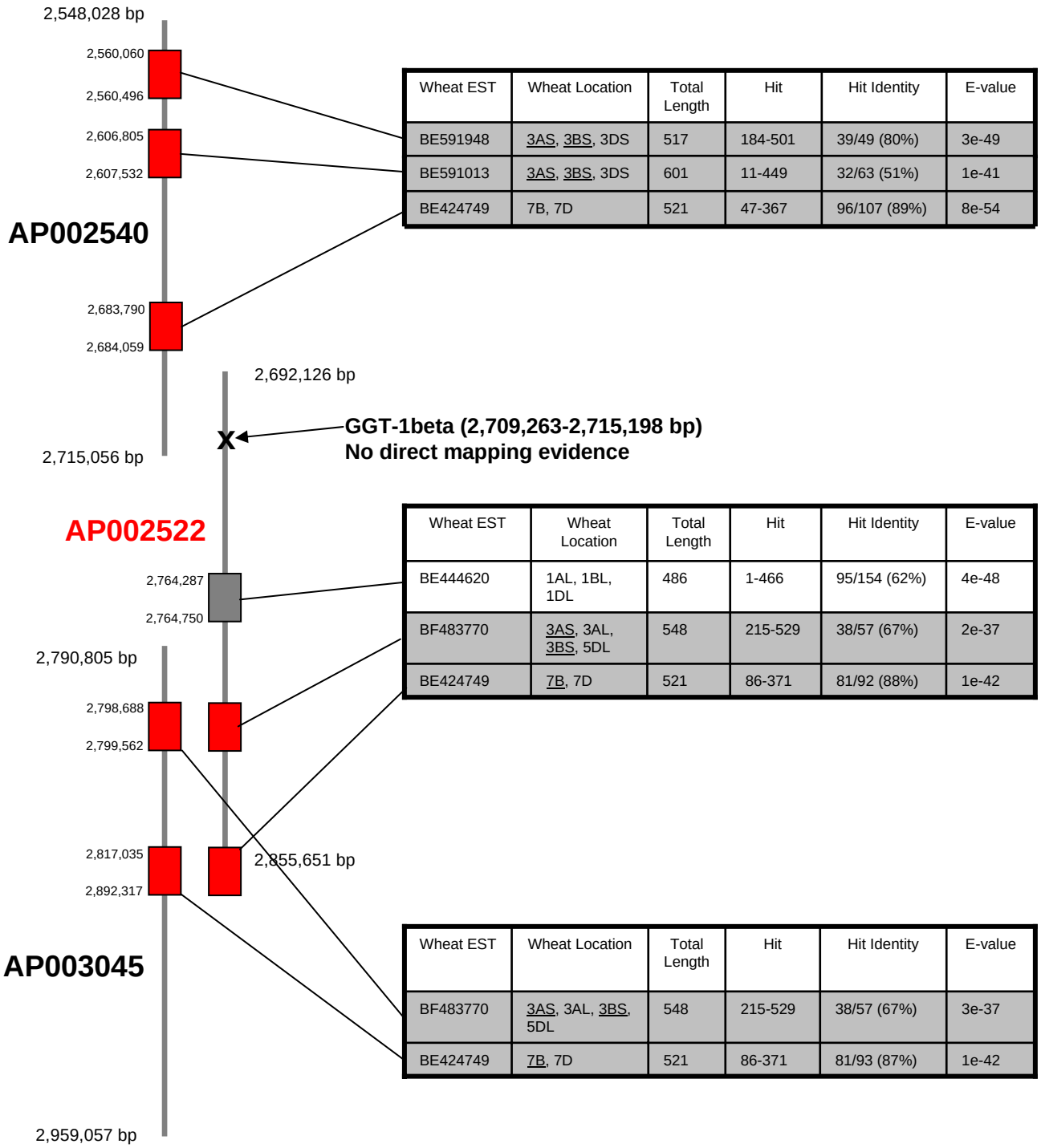
Fig. 2.3 *continued.*



Wheat EST	Wheat Location	Total Length	Hit	Hit Identity	E-value
*BE442694	3AS, 3BS, 3DS	588	219-588	87/121 (72%)	1e-42
BE426304	1AL	475	149-475	109/109 (100%)	6e-68
BE495028	1AL, 1BL, 1DL, 3DL	469	143-469	109/109 (100%)	6e-68
BG262527	3BS, 3DS	378	84-377	17/28 (61%)	3e-41

Wheat EST	Wheat Location	Total Length	Hit	Hit Identity	E-value
BE591522	5AL, 5D	409	79-405	69/109 (63%)	8e-45

Fig. 2.4 *Alignment of mapped wheat ESTs to rice* Rab Geranylgeranyltransferase *component A (AP001073) and flanking BAC/PAC clones on chromosome 1. The homology of the rice BAC/PAC clone and the aligned EST is detailed. Wheat ESTs mapped to groups 3 or 7 are shown in red.*

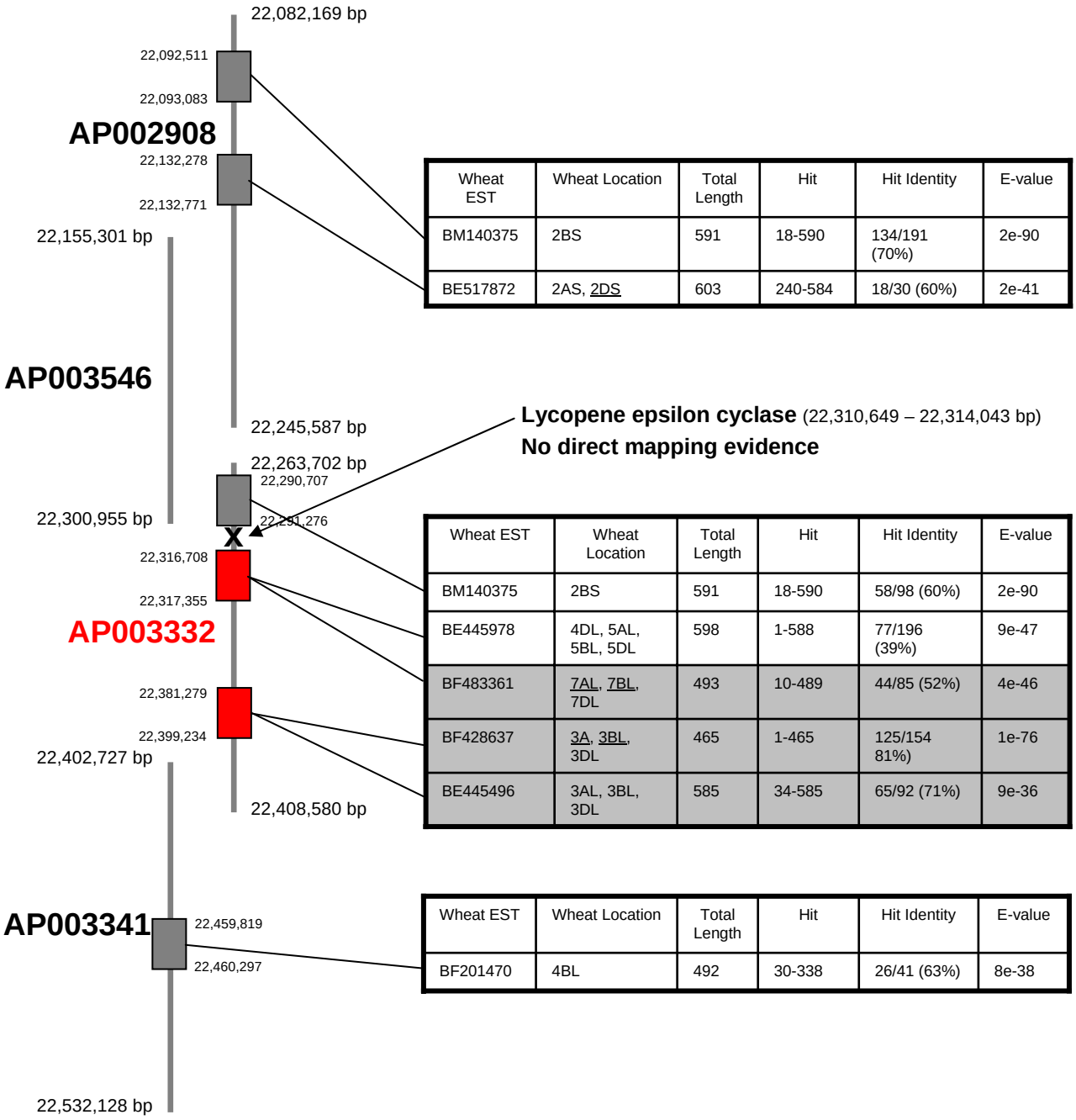


Wheat EST	Wheat Location	Total Length	Hit	Hit Identity	E-value
BE591948	3AS, 3BS, 3DS	517	184-501	39/49 (80%)	3e-49
BE591013	3AS, 3BS, 3DS	601	11-449	32/63 (51%)	1e-41
BE424749	7B, 7D	521	47-367	96/107 (89%)	8e-54

Wheat EST	Wheat Location	Total Length	Hit	Hit Identity	E-value
BE444620	1AL, 1BL, 1DL	486	1-466	95/154 (62%)	4e-48
BF483770	3AS, 3AL, 3BS, 5DL	548	215-529	38/57 (67%)	2e-37
BE424749	7B, 7D	521	86-371	81/92 (88%)	1e-42

Wheat EST	Wheat Location	Total Length	Hit	Hit Identity	E-value
BF483770	3AS, 3AL, 3BS, 5DL	548	215-529	38/57 (67%)	3e-37
BE424749	7B, 7D	521	86-371	81/93 (87%)	1e-42

Fig. 2.5 *Alignment of mapped wheat ESTs to rice* Geranylgeranyltransferase *I β-subunit (AP002522) and flanking BAC/PAC clones on chromosome 1. The homology of the rice BAC/PAC clone and the aligned EST is detailed. Wheat ESTs mapped to groups 3 or 7 are shown in red.*



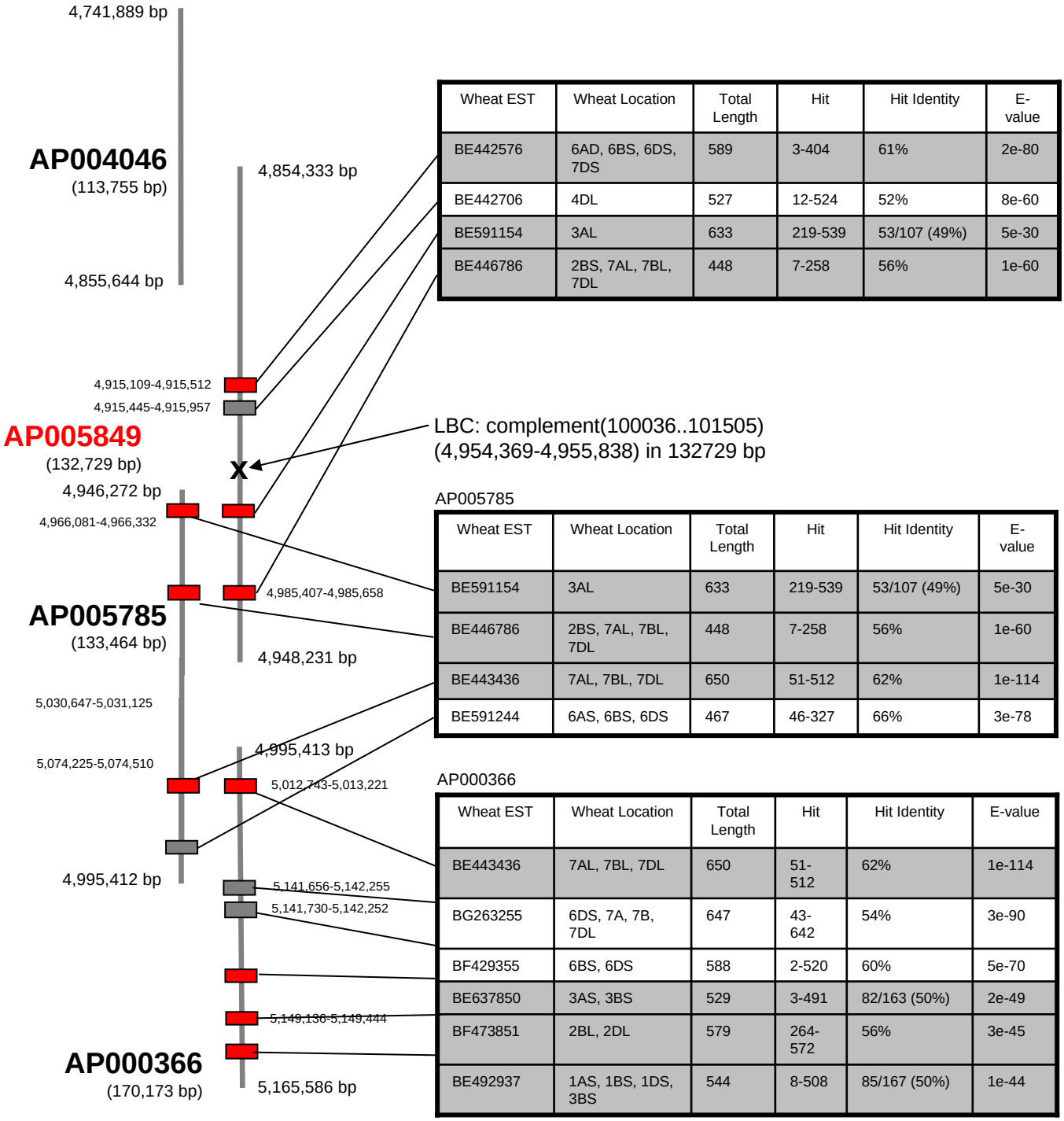
Wheat EST	Wheat Location	Total Length	Hit	Hit Identity	E-value
BM140375	2BS	591	18-590	134/191 (70%)	2e-90
BE517872	2AS, 2DS	603	240-584	18/30 (60%)	2e-41

Wheat EST	Wheat Location	Total Length	Hit	Hit Identity	E-value
BM140375	2BS	591	18-590	58/98 (60%)	2e-90
BE445978	4DL, 5AL, 5BL, 5DL	598	1-588	77/196 (39%)	9e-47
BF483361	7AL, 7BL, 7DL	493	10-489	44/85 (52%)	4e-46
BF428637	3A, 3BL, 3DL	465	1-465	125/154 81%)	1e-76
BE445496	3AL, 3BL, 3DL	585	34-585	65/92 (71%)	9e-36

Wheat EST	Wheat Location	Total Length	Hit	Hit Identity	E-value
BF201470	4BL	492	30-338	26/41 (63%)	8e-38

Fig. 2.6 *Alignment of mapped wheat ESTs to rice* Lycopene ε-cyclase *(AP003332) and flanking BAC/PAC clones on chromosome 1. The homology of the rice BAC/PAC clone and the aligned EST is detailed. Wheat ESTs mapped to groups 3 or 7 are shown in red.*

Lycopene beta-cyclase (rice 2)



Wheat EST	Wheat Location	Total Length	Hit	Hit Identity	E-value
BE442576	6AD, 6BS, 6DS, 7DS	589	3-404	61%	2e-80
BE442706	4DL	527	12-524	52%	8e-60
BE591154	3AL	633	219-539	53/107 (49%)	5e-30
BE446786	2BS, 7AL, 7BL, 7DL	448	7-258	56%	1e-60

AP005785

Wheat EST	Wheat Location	Total Length	Hit	Hit Identity	E-value
BE591154	3AL	633	219-539	53/107 (49%)	5e-30
BE446786	2BS, 7AL, 7BL, 7DL	448	7-258	56%	1e-60
BE443436	7AL, 7BL, 7DL	650	51-512	62%	1e-114
BE591244	6AS, 6BS, 6DS	467	46-327	66%	3e-78

AP000366

Wheat EST	Wheat Location	Total Length	Hit	Hit Identity	E-value
BE443436	7AL, 7BL, 7DL	650	51-512	62%	1e-114
BG263255	6DS, 7A, 7B, 7DL	647	43-642	54%	3e-90
BF429355	6BS, 6DS	588	2-520	60%	5e-70
BE637850	3AS, 3BS	529	3-491	82/163 (50%)	2e-49
BF473851	2BL, 2DL	579	264-572	56%	3e-45
BE492937	1AS, 1BS, 1DS, 3BS	544	8-508	85/167 (50%)	1e-44

Fig. 2.7 *Alignment of mapped wheat ESTs to rice* Lycopene β-cyclase *(AP005849) and flanking BAC/PAC clones on chromosome 2. The homology of the rice BAC/PAC clone and the aligned EST is detailed. Wheat ESTs mapped to groups 3 or 7 are shown in red.*

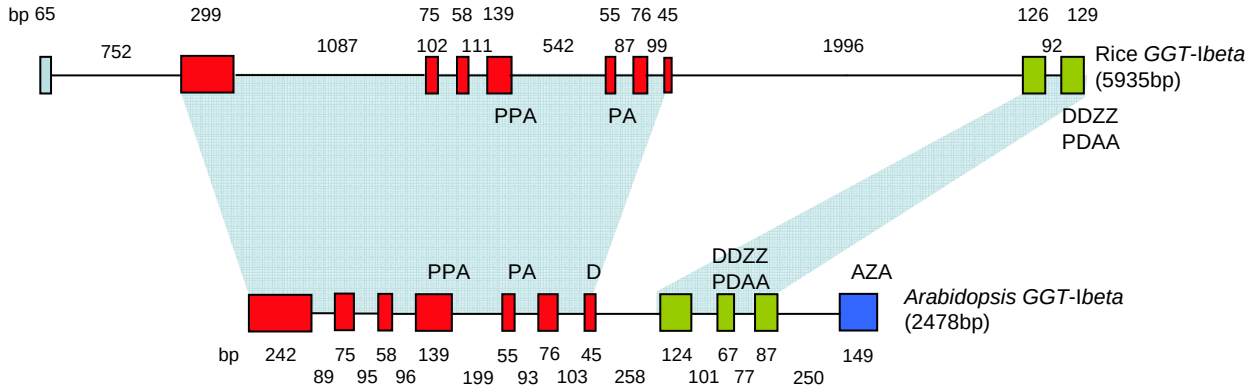


Fig. 2.8 *Intron-exon structure of rice (AP002522) and* Arabidopsis GGT-*I*beta *(AC004218) orthologues from cDNAs. Exons coloured in red are conserved in each species. Green exons represent a variable region between rice and Arabidopsis. Exons that are not conserved are shown in blue. Symbols indicated are A, residues in hydrophobic pocket; P, residues that interact with the CAAL peptide; D, residues that interact with the diphosphate group of GGPP; and Z, ligands of the catalytic zinc atom.*

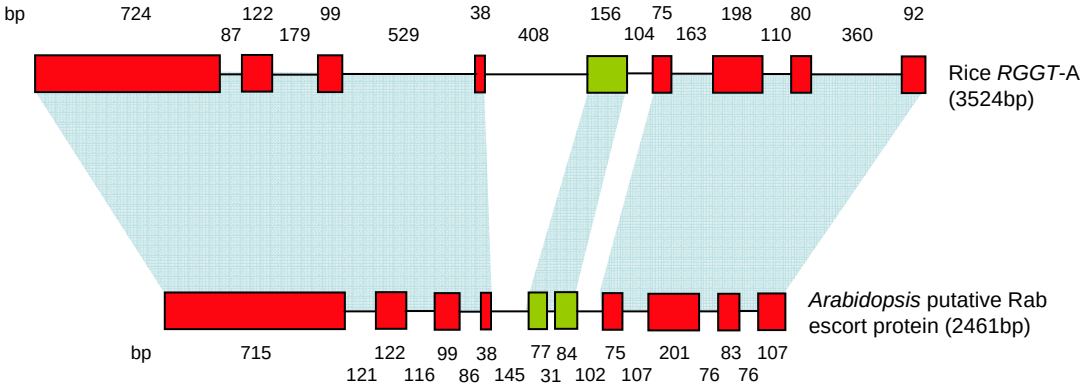


Fig. 2.9 *Intron-exon structure of rice* RGGT-*A (AP001073) and* Arabidopsis *putative Rab escort protein (AC020580) from cDNAs. Exons coloured in red are conserved in each species. Green exons represent a variable region between rice and Arabidopsis.*

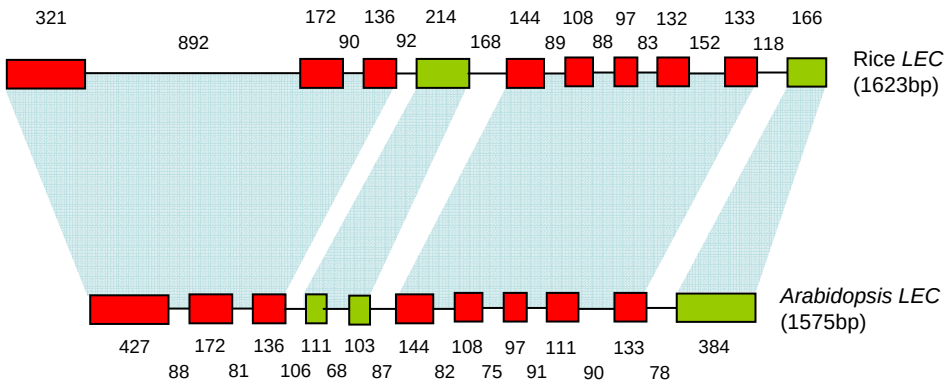


Fig. 2.10 *Intron-exon structure of rice (AP003332) and* Arabidopsis LEC *(At5g57030) orthologues from cDNAs. Exons coloured in red are conserved in each species. Green exons represent a variable region between rice and* Arabidopsis.

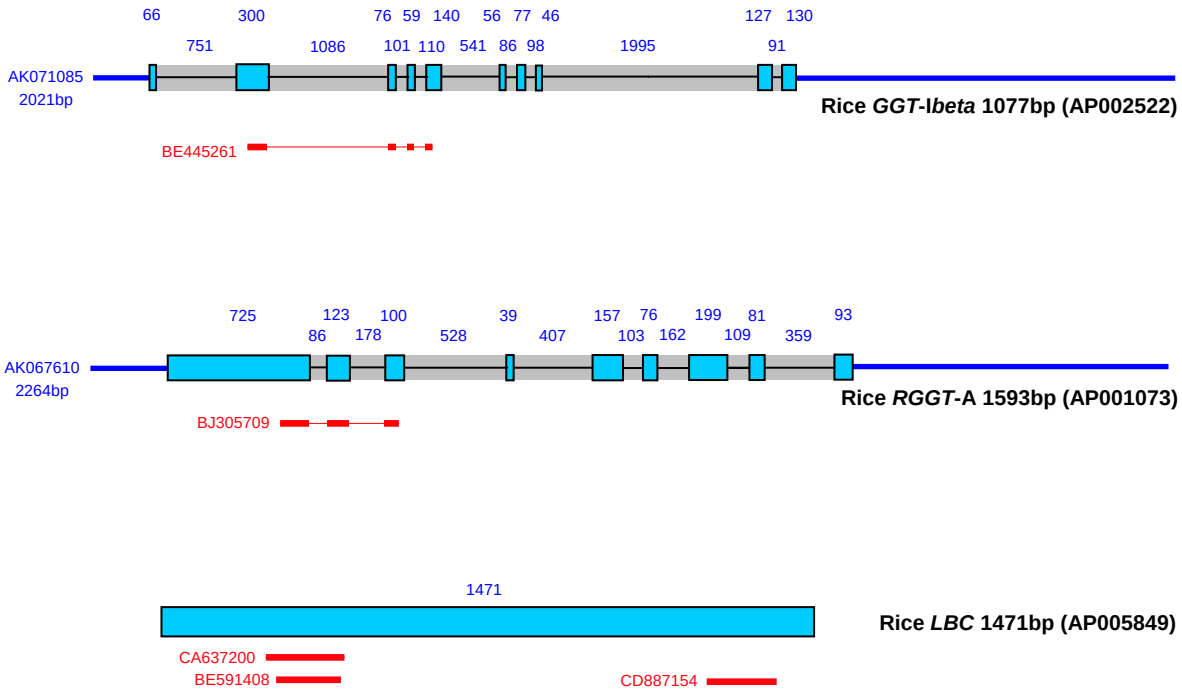


Fig. 3.2 *Summary of wheat ESTs that showed highest homology to the four candidate genes from rice. The wheat ESTs (red) are shown relative to the gene structure with thin red line indicating the EST spans an intron of the rice gene sequence. Exons are shown in cyan, introns are black lines and full-length cDNA clones are shown as dark blue lines.*

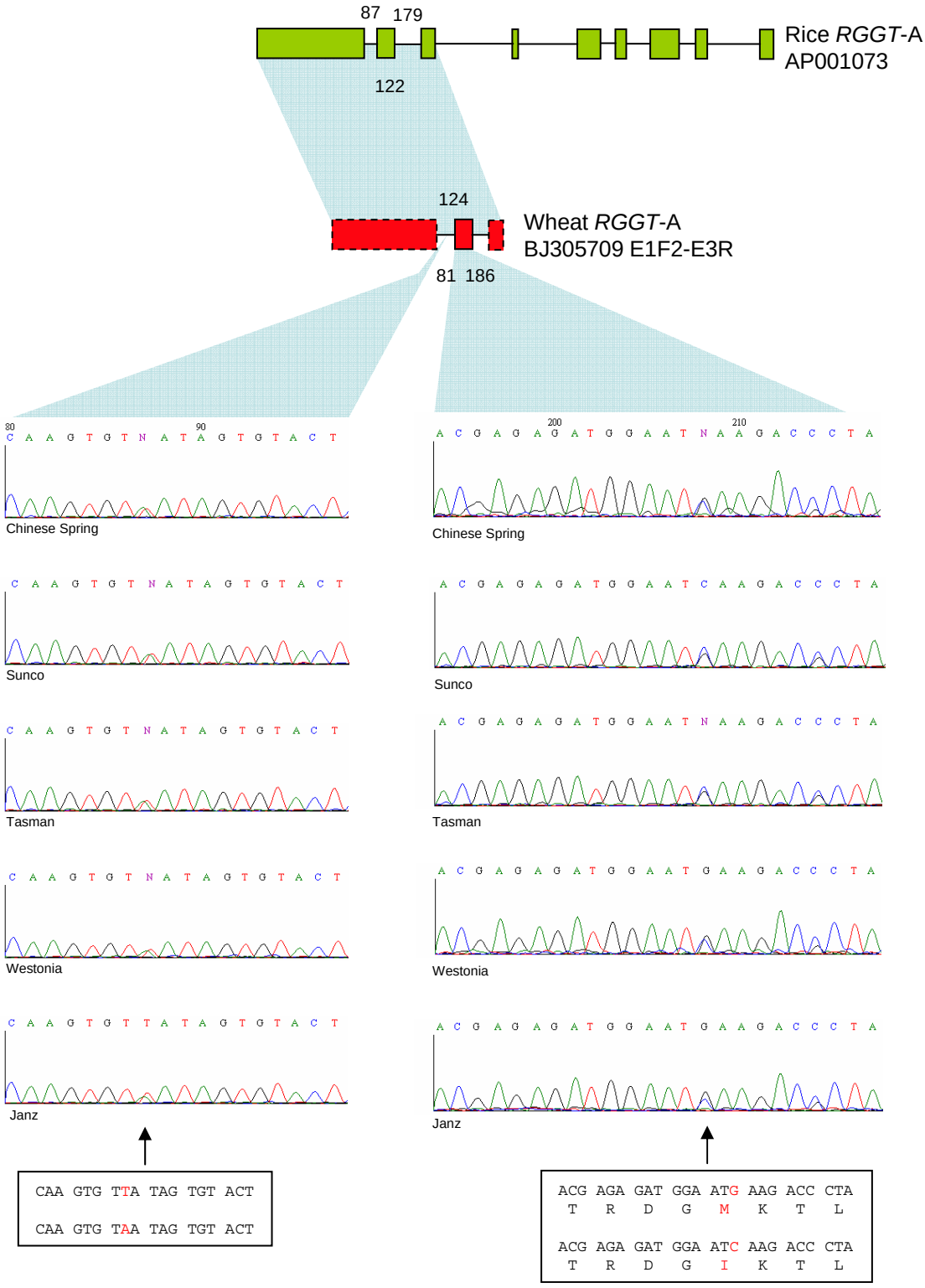


Fig 3.10 *Sequencing of wheat* RGGT-*A (intron 1-2, exon 2, intron 2-3) in cultivars Chinese Spring, Sunco, Tasman, Westonia and Janz using BJ305709 E1F-E3R. The gene structure of rice* RGGT-*A is shown at the top of the figure and below is EST BJ305709. Chromatograms of each cultivar are shown, arrows indicate genome-specific SNPs with translated protein sequence below.*

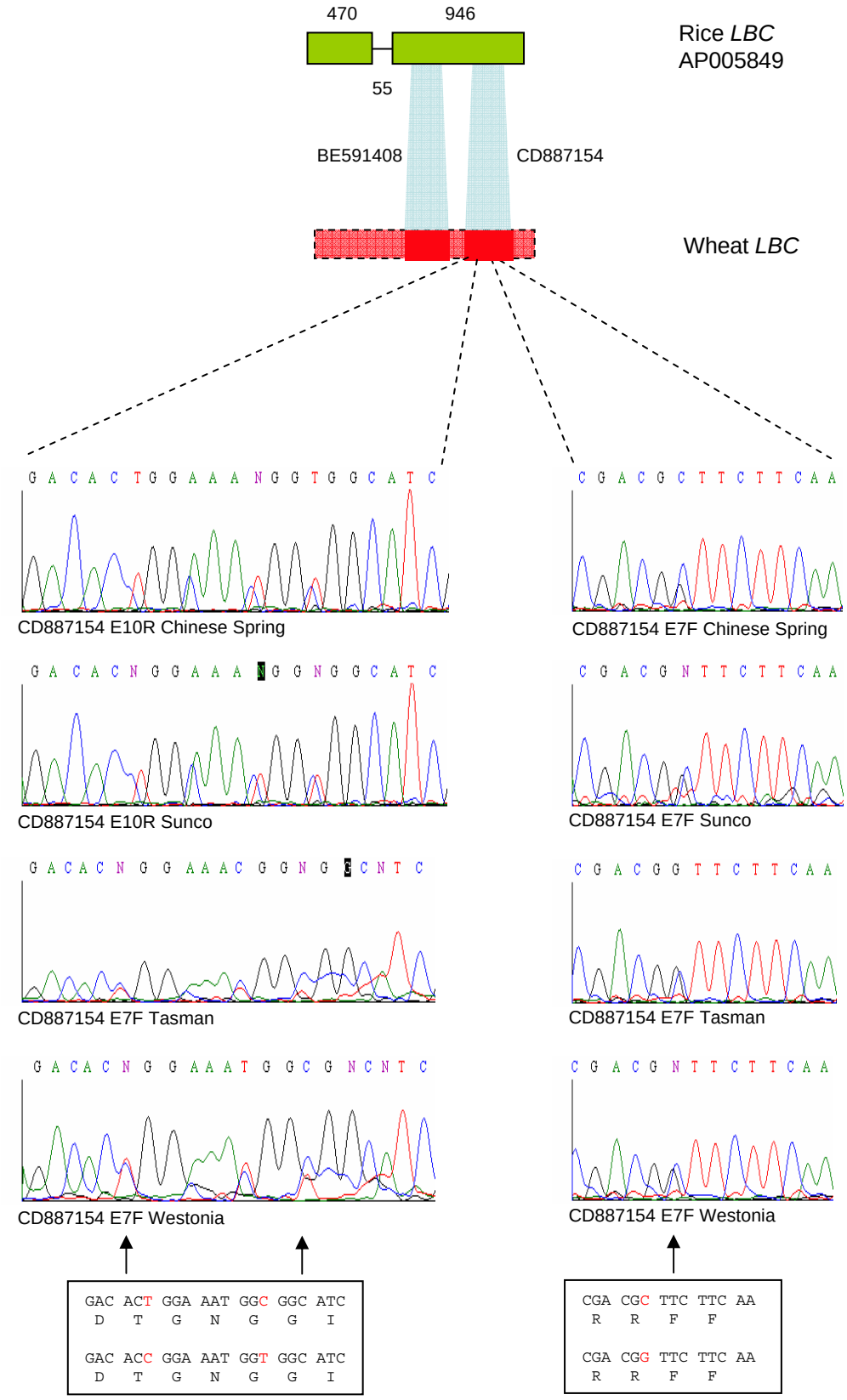


Fig 3.17 *Sequencing of wheat* LBC *in cultivars Chinese Spring Sunco, Tasman, Westonia and Janz using BE591408 E1F-E2R and CD887154 E7F-E10R. The intronless* LBC *gene of rice is shown at the top of the figure and below is the predicted location of the wheat orthologs BE591408 and CD887154 within the wheat gene sequence. Chromatograms of each cultivar are shown, arrows indicate genome-specific SNPs with translated protein sequence below.*

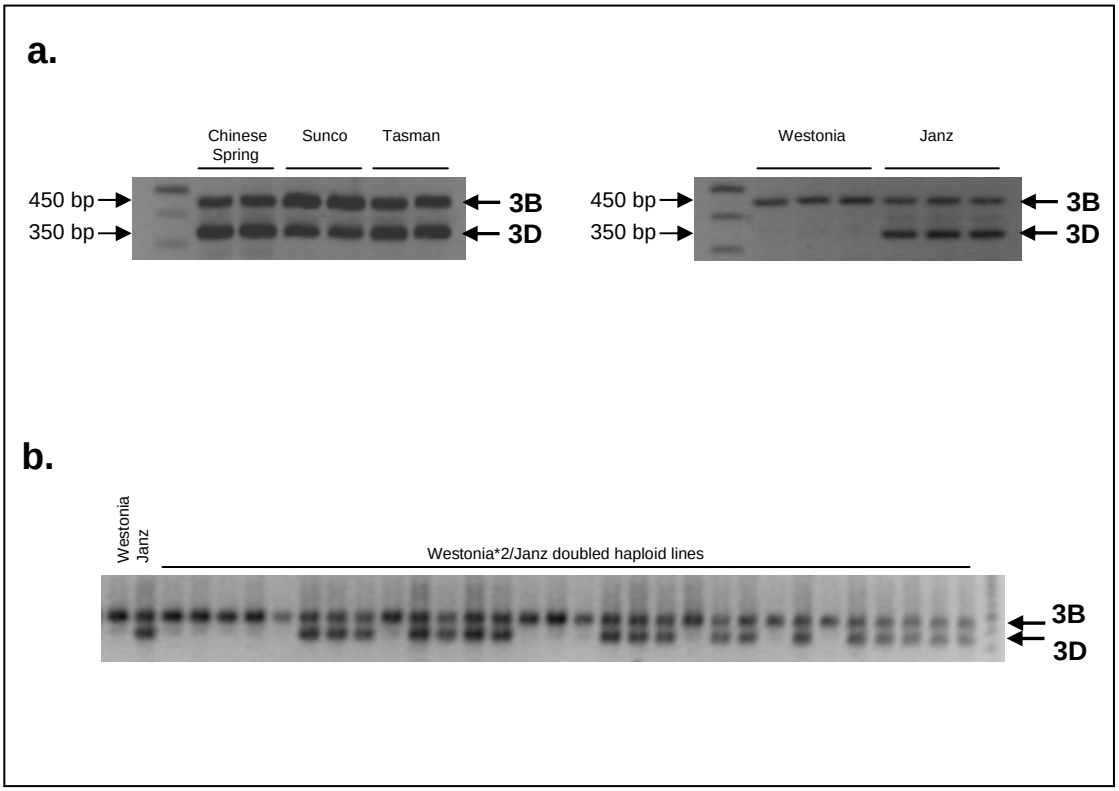


Fig. 3.18 *Polymorphic marker of* GGT-*I*beta *using primers E3F-E5R designed from wheat EST BE445261. (a.) Cultivars Chinese Spring, Sunco, Tasman and Janz contained both copies of GGT-Ibeta previously mapped to 3B and 3D. Westonia only contained the 3D copy of* GGT-*I*beta *as indicated by the amplification of the 450 bp band only. (b.) Screening of the Westonia*2/Janz doubled haploid population showing segregation of the EST-based molecular marker. A subset of the population containing 30 of a total of 180 doubled haploid lines is shown.*

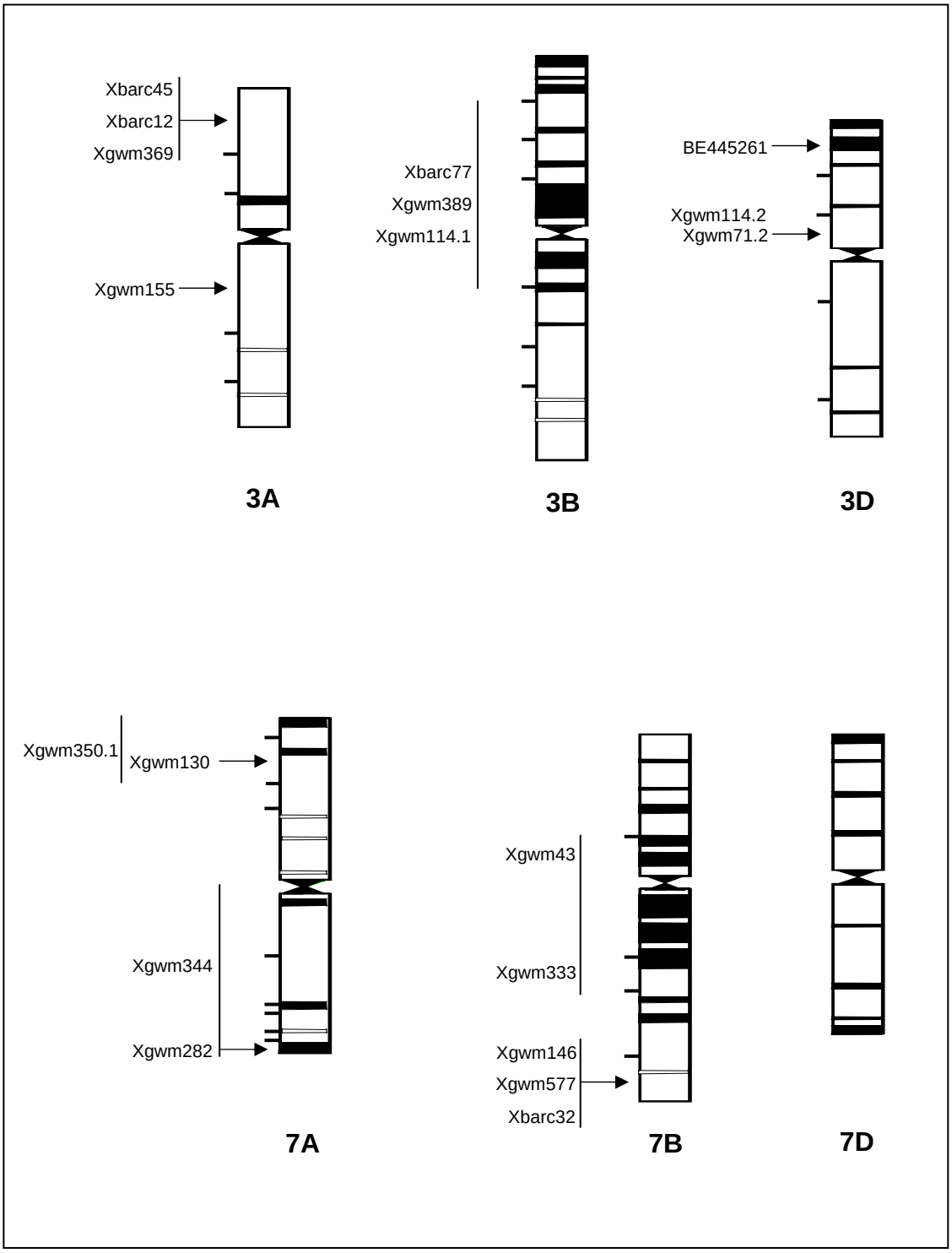
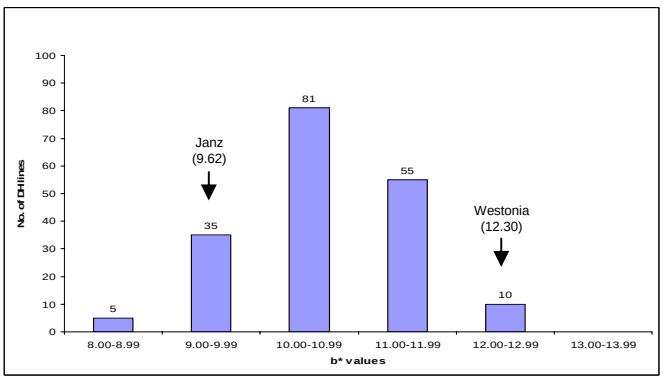
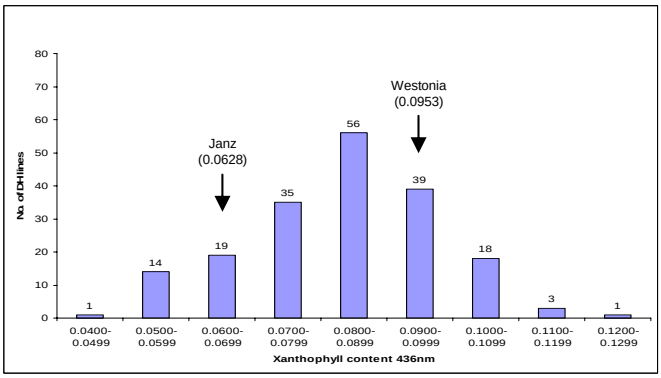


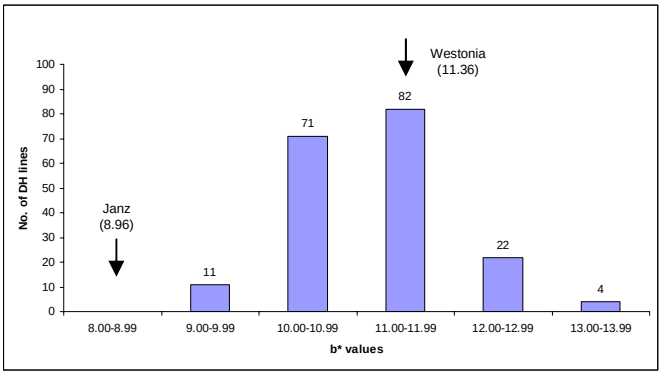
Fig. 3.19 *Polymorphic markers on groups 3 and 7 of Westonia*2/Janz doubled haploid population. Vertical lines indicate linked markers and the location of the linkage groups. Arrows indicate the chromosomal bin location of a molecular marker.*



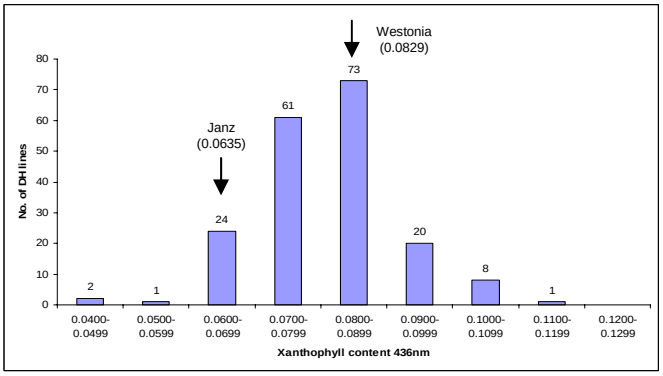
2002 Wongan Hills



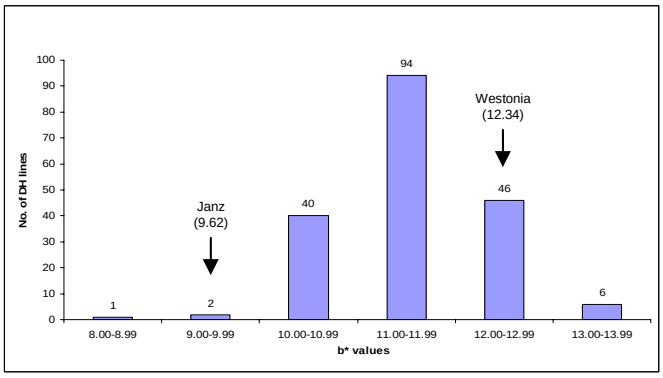
2002 Wongan Hills



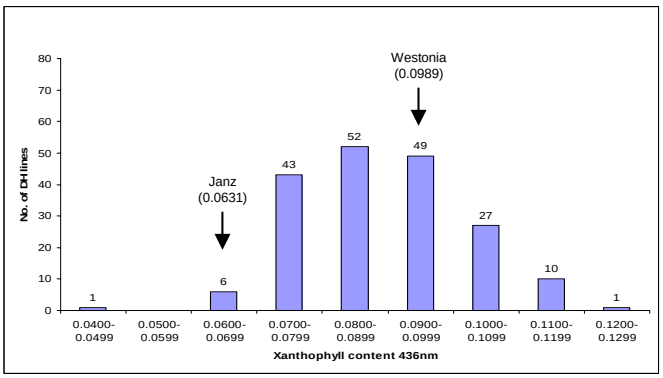
2003 Wongan Hills



2003 Wongan Hills



2003 Merridan



2003 Merridan

Fig. 3.20 *Histograms of frequency distribution of b* values and xanthophyll in Westonia*2/Janz doubled haploid population from the 2002 and 2003 sites of Wongan Hills and Merridan. The b* and xanthophyll values for Westonia and Janz at each site are shown and arrows indicate their distribution within the population.*

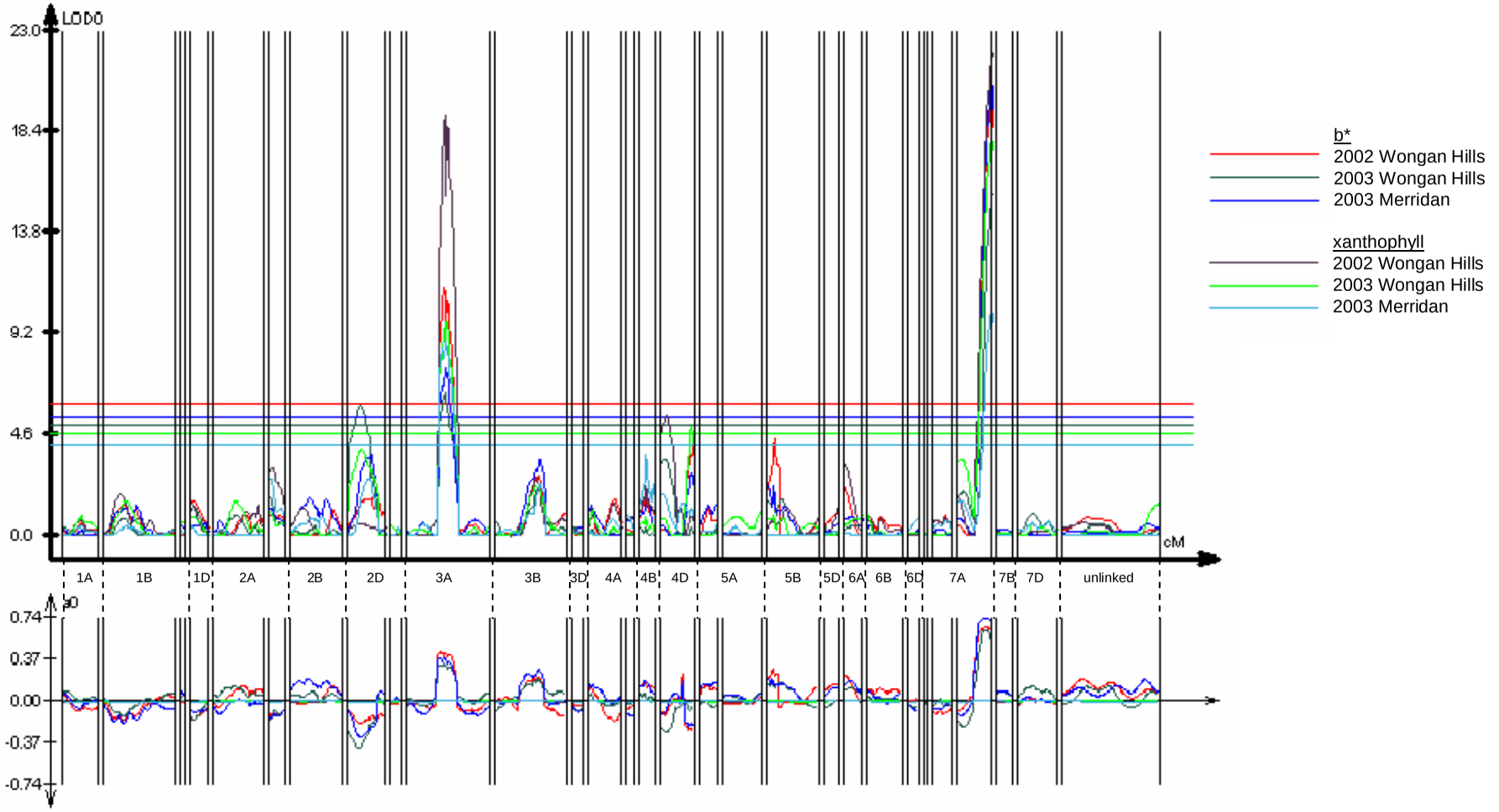


Fig. 4.3 *Summary of composite interval mapping for all chromosomes of b* values and xanthophyll in Ajana/WAWHT2074 doubled haploid population from the 2002 and 2003 sites of Wongan Hills and Merridan. Walk speed 2cM, 1000 permutations and significance level of P>0.001. The additive effect is shown below.*

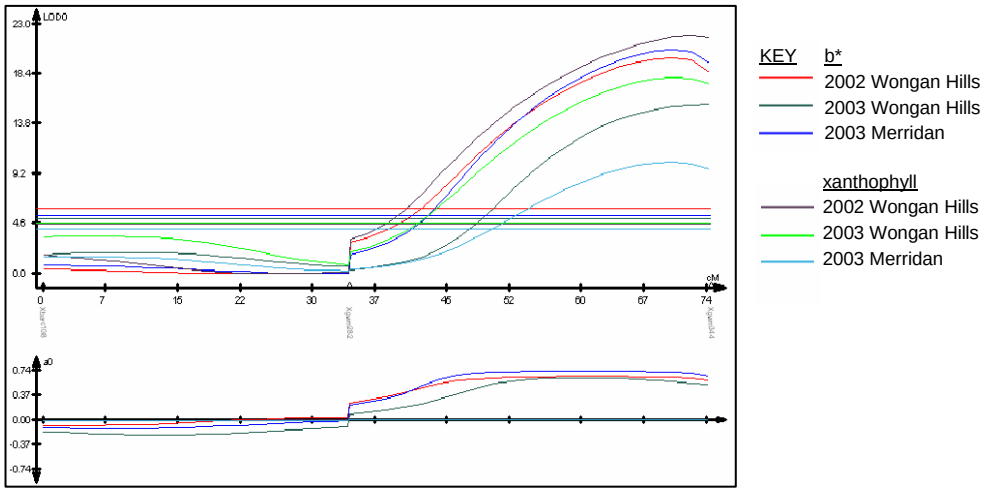


Fig. 4.4 *QTL of b* and xanthophyll content on chromosome 7A in Ajana/WAWHT2074 doubled haploid population from the 2002 and 2003 sites of Wongan Hills and Merridan. Composite interval mapping: Walk speed 2cM, 1000 permutations and significance level of P>0.001, LOD thresholds shown as horizontal line. The additive effect is shown below.*

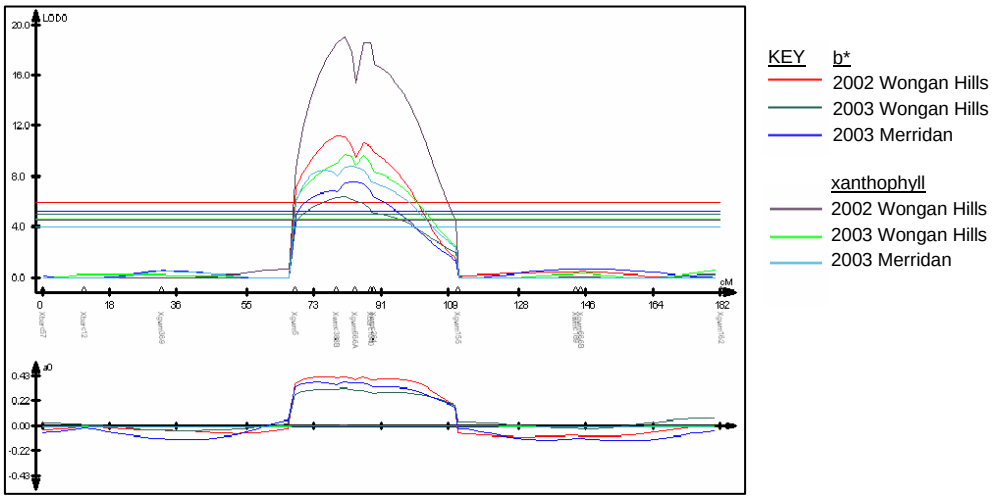


Fig. 4.5 *QTL of b* and xanthophyll content on chromosome 3A in Ajana/WAWHT2074 doubled haploid population from the 2002 and 2003 sites of Wongan Hills and Merridan. Composite interval mapping: Walk speed 2cM, 1000 permutations and significance level of P>0.001, LOD thresholds shown as horizontal lines. The additive effect is shown below.*

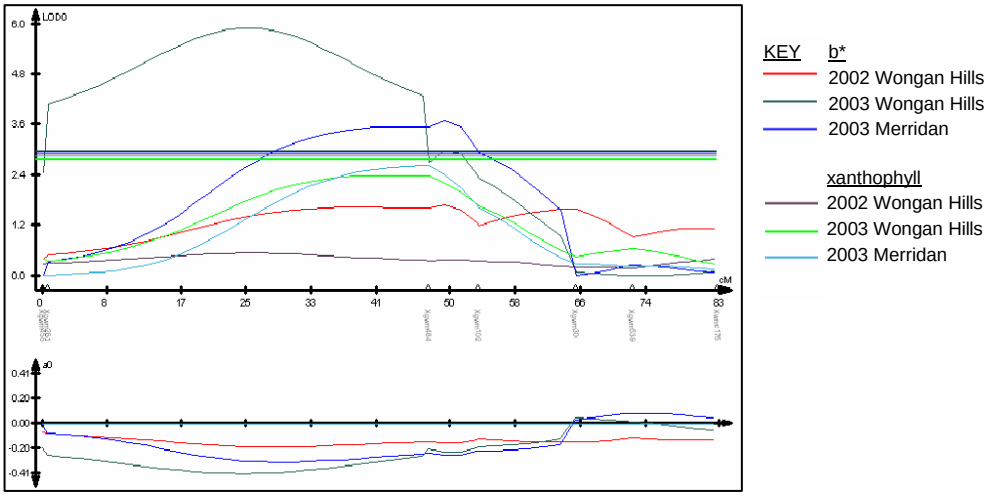


Fig. 4.6 *QTL of b* and xanthophyll content on chromosome 2D in Ajana/WAWHT2074 doubled haploid population from the 2002 and 2003 sites of Wongan Hills and Merridan. Composite interval mapping: Walk speed 2cM, 1000 permutations and significance level of P>0.05, LOD thresholds shown as horizontal line. The additive effect is shown below.*

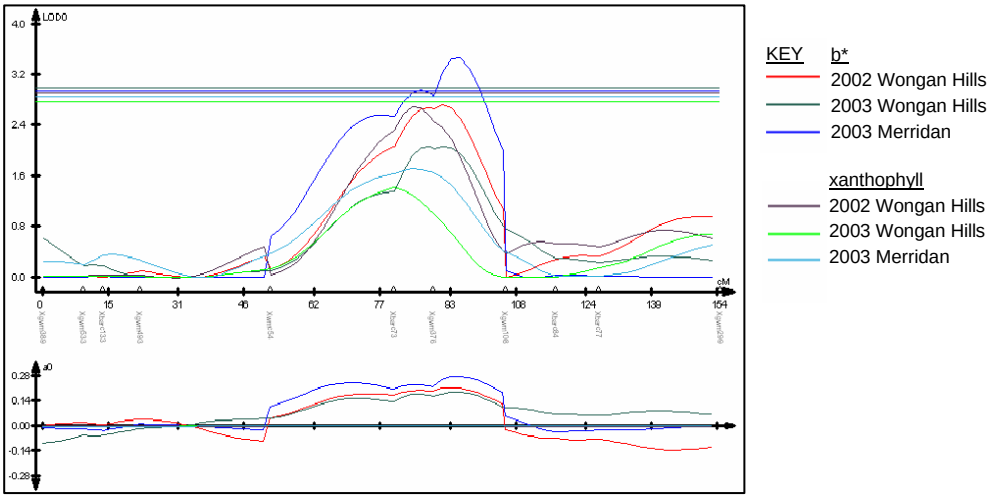


Fig. 4.7 *QTL of b* and xanthophyll content on chromosome 3B in Ajana/WAWHT2074 doubled haploid population from the 2002 and 2003 sites of Wongan Hills and Merridan. Composite interval mapping: Walk speed 2cM, 1000 permutations and significance level of P>0.05, LOD thresholds shown as horizontal line. The additive effect is shown below.*

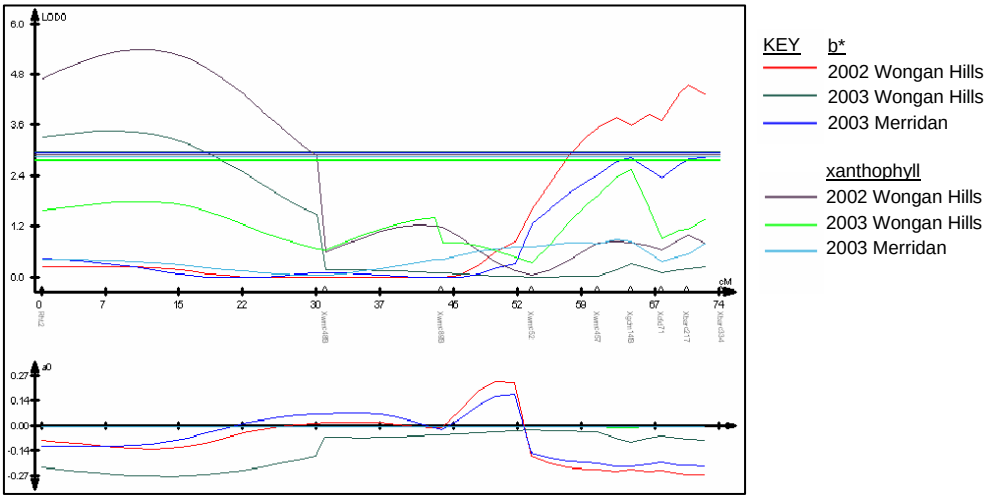


Fig. 4.8 *QTL of b* and xanthophyll content on chromosome 4D in Ajana/WAWHT2074 doubled haploid population from the 2002 and 2003 sites of Wongan Hills and Merridan. Composite interval mapping: Walk speed 2cM, 1000 permutations and significance level of P>0.05, LOD thresholds shown as horizontal lines. The additive effect is shown below.*

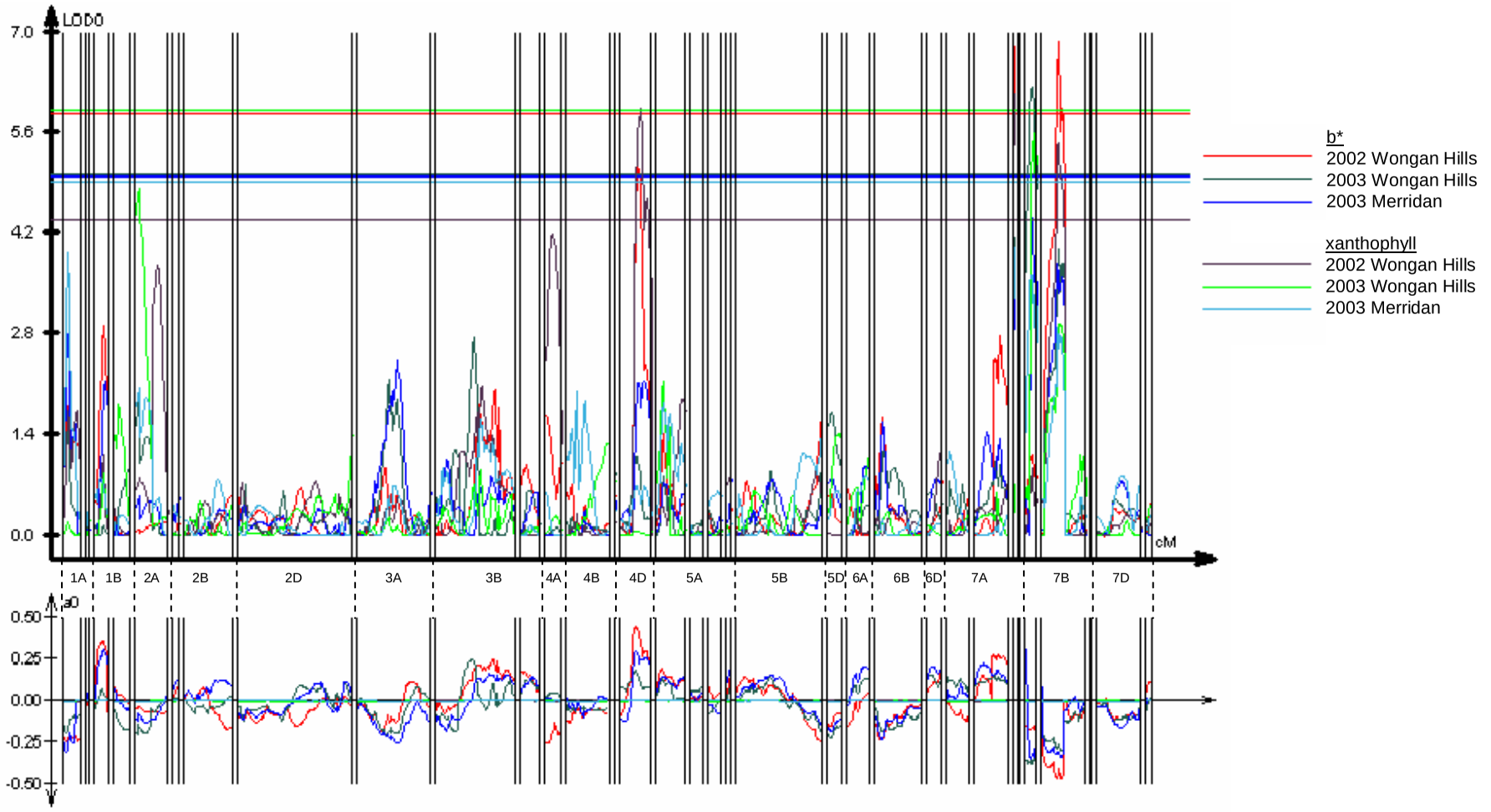


Fig. 4.9 *Summary of composite interval mapping for all chromosomes of b* values and xanthophyll in Carnamah/WAWHT2046 doubled haploid population from the 2002 and 2003 sites of Wongan Hills and Merridan. Walk speed 2cM, 1000 permutations and significance level of P>0.001. The additive effect is shown below.*

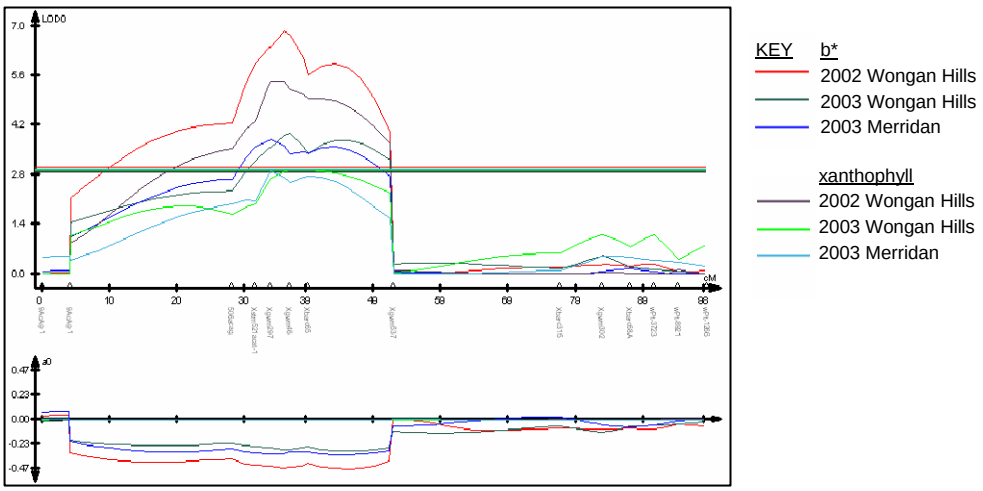


Fig. 4.10 *QTL of b* and xanthophyll content on the short arm of chromosome 7B in Carnamah/WAWHT2046 doubled haploid population from the 2002 and 2003 sites of Wongan Hills and Merridan. Composite interval mapping: Walk speed 2cM, 1000 permutations and significance level of P>0.05, LOD thresholds shown as horizontal lines. The additive effect is shown below.*

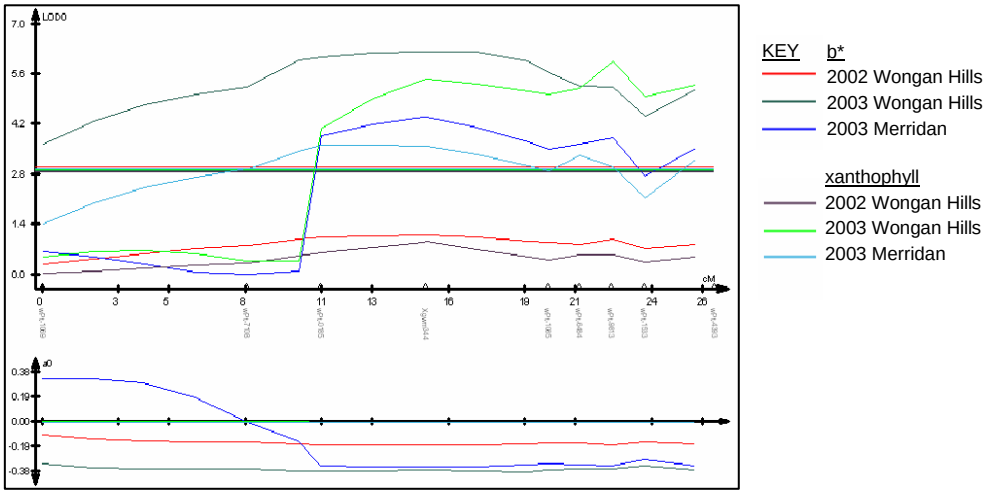


Fig. 4.11 *QTL of b* and xanthophyll content on the long arm of chromosome 7B in Carnamah/WAWHT2046 doubled haploid population from the 2002 and 2003 sites of Wongan Hills and Merridan. Composite interval mapping: Walk speed 2cM, 1000 permutations and significance level of P>0.05, LOD thresholds shown as horizontal line. The additive effect is shown below.*

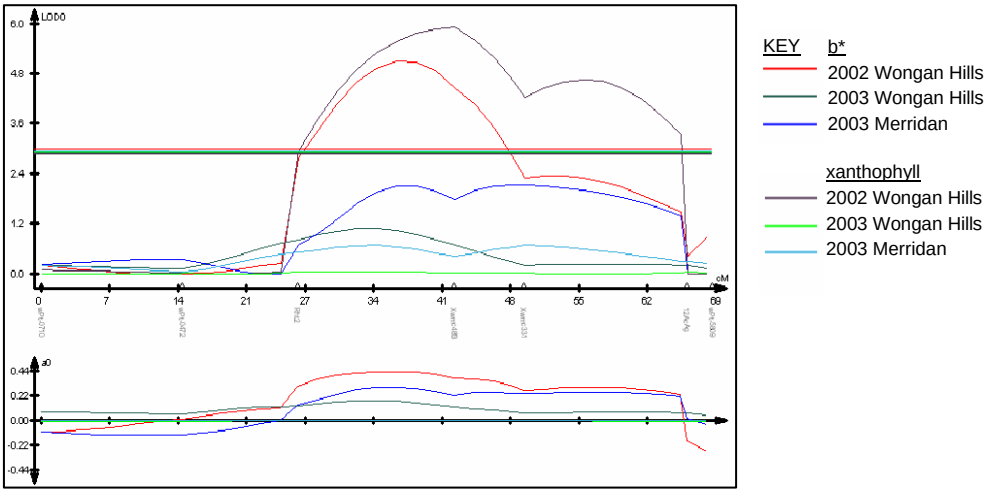


Fig. 4.12 *QTL of b* and xanthophyll content on chromosome 4D in Carnamah/WAWHT2046 doubled haploid population from the 2002 and 2003 sites of Wongan Hills and Merridan. Composite interval mapping: Walk speed 2cM, 1000 permutations and significance level of P>0.05, LOD thresholds shown as horizontal lines. The additive effect is shown below.*