User Manual for

QTL.gCIMapping

QTL genome-wide Composite Interval Mapping

(version 3.5)

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Disclaimer: While extensive testing has been performed by Yuan-Ming Zhang's Lab (Statistical Genomics Lab) at Huazhong Agricultural University, the results are, in general, reliable, correct or appropriate. However, results are not guaranteed for any specific datasets. We strongly recommend that users validate the results with other software packages, such as Windows QTL Cartographer V2.5_011 (https://brcwebportal.cos.ncsu.edu/qtlcart/WQTLCart.htm), QTL IciMapping V4.2 (https://www.isbreeding.net/software/?type=detail&id=28) and QTLNetwork 2.1 (http://ibi.zju.edu.cn/software/).

Download website:

https://github.com/YuanmingZhang65/GCIM

References

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Quantitative Trait Loci

Genome-wide Composite Interval Mapping

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Contents

1. INTRODUCTION

1.1 Why GCIM?

QTL.gCIMapping v3.5 (**QTL G**enome-wide **C**omposite **I**nterval **M**apping) is an R package, which is used to identify main-effect QTL and QTL-by-environment interaction (QEI) for quantitative traits in recombinant inbred lines (RIL), backcross (BC), doubled haploid (DH) lines, F_2, immortalized F_2 (IF_2), and $F_{2:3}$ design. QTL.gCIMapping v3.5 works well on the R environment on Windows.

1.2 Getting started

The software package QTL.gCIMapping v3.5 can be freely downloaded from https://github.com/YuanmingZhang65/GCIM, or request from the maintainer, Dr Yuan-Ming Zhang at Crop Information Center, College of Plant Science & Technology, Huazhong Agricultural University, Wuhan 430070, China (soyzhang@mail.hzau.edu.cn).

1.2.1 One-Click online installation

Within R environment, the QTL.gCIMapping v3.5 software can be installed as below.

First install the dependency packages:

```
install.packages(c("data.table","doParallel","foreach","glmnet","iterators","magrittr","openxlsx","qtl","Rcpp","shape","stringi","stringr","zip","MASS","lars","readxl","biglasso","progress","RcppEigen"))
```

Then install the QTL.gCIMapping_3.5 package from local files.

User Manual file (name: Instruction.pdf) can be downloaded from: https://github.com/YuanmingZhang65/GCIM

1.2.2 Run QTL.gCIMapping v3.5

Once the software package QTL.gCIMapping v3.5 is installed, users may run it using two commands:

```
library("QTL.gCIMapping")
```

QTL.gCIMapping (***) (***: please see the example of § 2.2~2.4)

If users re-use the software QTL.gCIMapping v3.5, users use the above two commands as well.

2. Parameter settings in QTL.gCIMapping

The red parameter is only used when method="Multi_env_RIL" (3vGCIM).

Parameter	Meaning	File format	Note
fileGen	Name & path of genotypic file in your computer, i.e., fileGen="D:/Users/fileGen_Multi_env_RIL.csv"	*.csv; *.txt	Table 1
filePhe	Name & path of trait phenotypic file in your computer, i.e., filePhe="D:/Users/filePhe_Multi_env_RIL.csv"	*.csv; *.txt	Table 2
fixedModel	Use fixed model or random model, i.e., fixedModel=FALSE		
Marker_Space_Type	Distance type in the map, i.e., Marker_Space_Type="Position", or Marker_Space_Type="Interval"		
Geno_Type	The type of markers, i.e., Geno_Type=c("A","H")		
insert_mkr	If the distance between two markers exceeds a certain range (cM), insert a virtual marker in the middle, i.e., insert_mkr =1, default value is FALSE.		
n.en	The number of environments for each trait in the filePhe, i.e., n.en=c(2,2,3) (The filePhe file contains the phenotypic values of three traits, and the environmental numbers of each trait are 2, 2, and 3, respectively.)		
sblgwas_t	A number between [-3,0] to control sparseness of the “sblgwas” function, default value is -0.6.		
ebayes_tau	A number between [-2,2] for “em_bayes” function, default value is 0.		
SearchRadius	i.e., SearchRadius=10, indicating the fact that only one potentially associated QTL or QEI is selected within 20 markers.		
file	File path & name in your computer, i.e., file="D:/Users/GCIM_Format_DH.csv"	*.csv; *.txt	Table 4
fileFormat	Format for input file: GCIM (QTL.gCIMapping), ICIM (QTL IciMapping), MCIM (QTLNetwork) and Cart (WinQTLCart)		
filecov	File that requires additional input due to the absence of covariates in the input information of ICIM and MCIM, i.e., filecov="D:/Users/cov_file.csv" or filecov=NULL	*.csv; *.txt (Covariate values: **Row:** individual**; Column**: covariate name)	Table 5

Population	BC1 (F1×P1), BC2 (F1×P2), DH, RIL, F_2, i.e., Population="F_2"
method	Multi_env_RIL for QEI detection in RIL/DH/BC population, GCIM-QEI for QEI detection in F_2 population, and GCIM for main-effect QTL detection are available, i.e., method=" Multi_env_RIL", method="GCIM-QEI", or method="GCIM"
MultiEnv	This parameter is specific to GCIM-QEI. If multiple environment datasets are analyzed, MultiEnv=TRUE. If not, MultiEnv=FALSE
Model	Random (random model) or Fixed (fixed model) for QTL or QEI effects, i.e., Model="Random"
WalkSpeed	Walk speed for Genome-wide Scanning (centi-Morgan, cM), should be less than 5 cM when setting, i.e., WalkSpeed=1
CriLOD	The LOD score threshold for significant QTL or QEI. CriLOD=3: the LOD score threshold for significant QTL is set at 3
CriDis	This parameter is specific to GCIM-QEI. The default is CriDis=5, which means that the significant QTLs and QEIs are optimized within the range of ≤ 5cM.
Likelihood	This parameter is only for GCIM in F_2 population, including restricted maximum likelihood (REML) and maximum likelihood (ML). Likelihood="REML" or Likelihood="ML"
SetSeed	This parameter is only for GCIM in F_2 population, in which the cross-validation experiment is needed
flagrqtl	This parameter is only for GCIM in F_2 population, flagrqtl="FALSE" in the first running. If the other software detects only one QTL in a neighborhood but this software finds two linked QTLs (one with additive effect and another with dominant effect) in the region, let flagrqtl="TRUE"
DrawPlot	This parameter is for all the populations, including FALSE and TRUE. DrawPlot=FALSE indicates no figure output; DrawPlot=TRUE indicates the LOD score [or $-\log_{10}(P\text{-}value)$] figure against genome position.
Plotformat	*.jpeg, *.png, *.tiff and *.pdf. For example, Plotformat="jpeg" indicates the *.jpeg format of the figure file.
Resolution	Low or High. Resolution="Low" indicates the low resolution of the figure file.
Trait	Trait=1:3 indicates the analyses from the first trait to the third trait.
dir	Save path in your computer, i.e.,"D:/Users"
CLO	The number of CPU occupied by running. The default is the number of CPUs on the computer minus 1, and CLO≤10.
MCIMmap	If the format for input file is MCIM, please input the map file, i.e., MCIMmap="D:/Users/SimF2_MCIM_Format.map"

3. Dataset format

3.1 Dataset for QEI detection in RIL/DH/BC population (3vGCIM)

Format for the dataset “fileGen”

The type of genotypic file for complex trait is *.csv or *.txt, as shown below. In this file, all the columns are named as marker (marker name), chr (chromosome), pos (marker's position (cM) on the genome), and individual names (or IDs), such as B46.

Table 1. The format genotypic dataset

marker	chr	pos	B46	B52	…
a0	1	0	A	H	…
a1	1	1	A	H	…
a2	1	2	A	H	…
a3	1	3	A	H	…
a4	1	4	H	H	…
⋮	⋮	⋮	⋮	⋮	…

Format for the dataset “filePhe”

The type of phenotypic file for complex trait is *.csv or *.txt, as shown below. The first row in the first column: "<Phenotype>", while the second to nth rows in the first column: individual names (or IDs), such as B46. The first rows from the second column: trait names, such as “trait1Env1”, while the other rows from the second column: phenotypic values of complex traits. The phenotypic file is arranged by traits, each trait has at least two columns, and each column is the phenotypes measured in an environment. The missed phenotypes are represented by “NA”.

Table 2. The format of phenotypic dataset

<Phenotype>	trait1Env1	trait1Env2	trait2Env1	trait2Env2	…
B46	8.71	11.74	9.81	8.55	…
B52	20.30	27.61	21.13	19.30	…
B57	16.10	20.69	21.66	16.88	…
B64	2.50	12.55	21.43	18.50	…
B68	25.35	23.05	16.72	18.35	…
⋮	⋮	⋮	⋮	⋮	…

3.2 Dataset for QTL detection in RIL/DH/BC/F2 population or QEI detection in F2 population

3.2.1 GCIM format for Dataset

The first three columns, named "**marker**", "**chr**" and "**pos**", stand for marker name, chromosome and marker position (cM) on the chromosome, respectively. Among the remaining columns, each column lists all the genotypes of one individual or line, while the first row shows the name of one individual or line. For the genotypes of each marker, the coding criteria are shown as Table 3.

Table 3. Coding criteria for GCIM format

Genotype	Code	Meaning
AA	A	Homozygous genotype (P_1)
Aa	H	Heterozygous genotype (F_1)
aa	B	Homozygous genotype (P_2)
AA + Aa (Not aa)	D	Dominance to P_1
Aa + aa (Not AA)	C	Dominance to P_2
Missing	-	Missing or unclear genotype

The genotypic, phenotypic and covariate datasets are located on the upper, middle, lower sections, and each covariate or trait is presented on one row. On each row, the first column is empty (single environment analysis using GCIM) or "**Env1**" (multiple environment analysis using GCIM-QEI), followed by "**trait1**", "the name of quantitative trait", and "phenotypic values for all the individuals or lines". If there are multiple traits, these traits occupy multiple lines. **"NA" indicates the missing or unknown phenotypes.** If there are covariates, the content lies below the trait dataset. The format is seen in Table 4. If there is no covariate, users should delete the last row in Table 4.

3.2.2 The format of ICIM dataset

If users have the QTL IciMapping dataset, these files are also available in our software. Details can be found in the folder of "…/QTL.gCIMapping/inst/extdata", i.e.,

WheatDH_QTLIciMapping_Format.xlsx.

3.2.3 The format of MCIM dataset

If users adopted the QTLNetwork software to analyze the dataset, the files with QTLNetwork format are also available by GCIM-QEI in our software. See folder "…/QTL.gCIMapping/inst/extdata" for details, i.e., SimF2_MCIM_Format.txt and SimF2_MCIM_Format.map.

3.2.4 The format of WinQTLCart dataset

If users adopted the WinQTLCart software to analyze the dataset, its file with WinQTLCart format is also available in our software. Details can be found in the folder of "…/ QTL.gCIMapping/inst/extdata", i.e., env1-jun3_WinQTLCart_Format.mcd. It should be noted that WinQTLCart only treats multiple environments as multiple traits when analyzing multi-environment data. Therefore, when inputting this format, the trait name needs to be written as follows:

```
 -start traits
t1E1    1.370946    0.539544
t1E2    -0.326883   1.082551
t2E1    4.112838    -1.618632
t2E2    3.181120    -1.220501
 -stop traits
```

Note that "t1" is "the first trait" and "t2" is "the second trait", while "E1" is "the first environment" and "E2" is "the second environment".

Table 4. The GCIM format of the dataset

marker	**chr**	**pos**	**DH6-10**	**DH6-101**	**DH6-102**
RGA3(1)	1	0	B	-	B
wPt-6358	1	3.034	B	-	-
Hplc2	1	8.8291	A	A	B
abc156a	1	41.3408	A	A	B
⋮	⋮	⋮	⋮	⋮	⋮
gwm437	21	162.5218	A	B	-
wmc157	21	197.9196	A	B	A
*stm1actc	21	200.4216	-	-	-
Env1	**trait1**	T19	10.27	15.68	9.98

Env2	**trait1**	T19	11.55	18.63	5.66
Env1	**trait2**	T191	74	105.68	97.16
Env2	**trait2**	T191	75.37	104.67	95.55
Env3	**trait2**	T191	75.33	105	96.33
	Covar1	CovarName	A	B	B

3.2.5 The format of ICIM and MCIM covariate dataset

If users adopted the ICIM or MCIM methods to analyze the dataset, and the covariates exist, users should input a covariate file. In the file, the first column indicates individual name and the second column is the covariate information (Table 5). In Table 5, the covariate values are indicated by such as A, B, and C.

Table 5. The covariate file format

Individual ID	Covariate
DH6-10	A
DH6-101	A
DH6-102	A
DH6-104	A
DH6-164	B
DH6-165	B
DH6-166	C
DH7-124	C

4. RIL/DH/BC population

4.1 QTL identification

The full codes

QTL.gCIMapping(file="D:/Users/GCIM_Format_DH.csv",fileFormat="GCIM",filecov=NULL,Population="DH",method="GCIM",Model="Random",WalkSpeed=1,CriLOD=2.5,Likelihood="REML",SetSeed=11001,flagrqtl="FALSE",DrawPlot="TRUE",PlotFormat="png",Trait=1:1,dir="D:/Users")

The reduced codes

QTL.gCIMapping(file="D:/Users/GCIM_Format_DH.csv",Population="DH",method ="GCIM",WalkSpeed=1,CriLOD=2.5,Trait=1,dir="D:/Users")

Note

It should be noted that users must set "file", "Population", "method", "WalkSpeed", "CriLOD", "Trait" and "dir", and the other parameters may be defaulted, including Likelihood="REML"; SetSeed=11001 and flagrqtl="FALSE" only for F2 population; DrawPlot=TRUE; Plotformat= "jpeg"; Resolution= "Low". Generally speaking, the random seed in the cross-validation experiment was set as 11001. If some known genes aren't identified by the seed, users may try to use some new random seeds. At this case, one better result may be obtained.

4.2 QEI identification (3vGCIM)

The full codes:

QTL.gCIMapping(fileGen="D:/Users/fileGen_Multi_env_RIL.csv",filePhe="D:/Users/filePhe_Multi_env_RIL.csv",filecov=NULL,method="Multi_env_RIL",fixedModel=FALSE,Marker_Space_Type="Position",Geno_Type=c("A","H"),insert_mkr=FALSE, Trait=1,n.en=3,sblgwas_t=-0.6,ebayes_tau=0,SearchRadius=10,CriLOD=2.5, DrawPlot=TRUE,PlotFormat="tiff",Resolution="Low",dir="D:/Users/")

Users must set "fileGen", "filePhe", "method", "fixedModel", "Marker_Space_Type", "Geno_Type", "Trait", "n.en", and "dir", while the other parameters may be default in function QTL.gCIMapping, including filecov=NULL; insert_mkr=FALSE; sblgwas_t=-0.6; ebayes_tau=0; SearchRadius=10; CriLOD=2.5; DrawPlot=TRUE; PlotFormat="tiff"; Resolution="Low".

The reduced codes:

QTL.gCIMapping(fileGen="D:/Users/fileGen_Multi_env_RIL.csv",filePhe="D:/Users/filePhe_Multi_env_RIL.csv",method="Multi_env_RIL",fixedModel=FALSE,Marker_Space_Type="Position",Geno_Type=c("A","H"), Trait=1,n.en=3,dir="D:/Users/")

5. F2 population

5.1 QTL identification

The full codes

QTL.gCIMapping(file="D:/Users/GCIM_Format_F2.csv",fileFormat="GCIM",filecov=NULL,Population="F2",method="GCIM",Model="Random",WalkSpeed=1,CriLOD=2.5,Likelihood="REML",SetSeed=11001,flagrqtl="FALSE",DrawPlot="TRUE",PlotFormat="png",Trait=1:1,dir="D:/Users")

The reduced codes

QTL.gCIMapping(file="D:/Users/GCIM_Format_F2.csv",Population="F2",method="GCIM",WalkSpeed=1,CriLOD=2.5,Trait=1,dir="D:/Users")

Note

It should be noted that users must set "file", "Population", "method", "WalkSpeed", "CriLOD", "Trait" and "dir", and the other parameters may be defaulted, including Likelihood="REML"; SetSeed=11001 and flagrqtl="FALSE" only for F2 population; DrawPlot=TRUE; Plotformat= "jpeg"; Resolution= "Low". Generally speaking, the random seed in the cross-validation experiment was set as 11001. If some known genes aren't identified by the seed, users may try to use some new random seeds. At this case, one better result may be obtained.

5.2 QEI identification

The full codes

QTL.gCIMapping(file="D:/Users/GCIM_Format_F2.csv",fileFormat="GCIM",filecov=NULL,Population="F2",method="GCIM-QEI",MultiEnv=TRUE,Model="Random",WalkSpeed=1,CriLOD=3,CriDis=5,DrawPlot=TRUE,PlotFormat="tiff",Resolution="Low",Trait=1:2,dir="D:/Users",CLO=NULL,MCIMmap=NULL)

The reduced codes

QTL.gCIMapping(file="D:/Users/GCIM_Format_F2.csv",Population="F2",MultiEn

v=TRUE,dir="D:/Users")

Note

It should be noted that users must set "file", "Population", "MultiEnv", and "dir" for GCIM-QEI method, and the other parameters can be default in this function, including method="GCIM-QEI", Model="Random", WalkSpeed=1, CriLOD=3, CriDis=5, DrawPlot=TRUE, PlotFormat="tiff", Resolution="Low",CLO=NULL. If Population="F2", the parameter of "method" may be defaulted, and the default is method="GCIM-QEI". If the parameter of "Trait" is defaulted, all traits are analyzed by default, and this default is only available in the GCIM-QEI method.

6. Result

6.1 Result for method="Multi_denv_RIL" (3vGCIM)

The result file (result-QEI_detection) includes three files: *_K.csv (Kinship matrix calculated by QTL.gCIMapping), *_midresult.csv (intermediate results), and *_result.xlsx (final result, including two sheets, significant main-effect QTLs (result.Q) and significant QEIs (result.QEI)), and two plots (if DrawPlot=TRUE), one for Main-effect QTLs and one for QEIs.

*_midresult.csv: This is the results of single marker scanning on the genome in the first step. In this file, all the columns are named as Chromosome, Position (marker's position (cM) on the genome), LeftMarker, RightMarker, pvalue.Q (the *P*-value for main-effect QTLs), and pvalue.QE (the *P*-value for QEIs).

Chromosome	Position	LeftMarker	RightMarker	pvalue.Q	pvalue.QE
1	0	a0	a0	0.987923275	0.998687441
1	1	a1	a1	0.989963789	0.99839232
1	2	a2	a2	0.924002891	0.520218151
1	3	a3	a3	0.998429654	0.998993246
1	4	a4	a4	0.992253645	0.998614293
⋮	⋮	⋮	⋮	⋮	⋮

result.Q: The results are for significant main-effect QTLs (LOD ≥ 2.5). In this sheet, all the columns are named as Trait ID, Trait name, Chromosome, Position (marker's position (cM) on the genome), LeftMarker, RightMarker, LOD (Q) (LOD score for main-effect QTLs), add (additive effect), and r^2 (%) (the proportion of total phenotypic variance explained by each QTL).

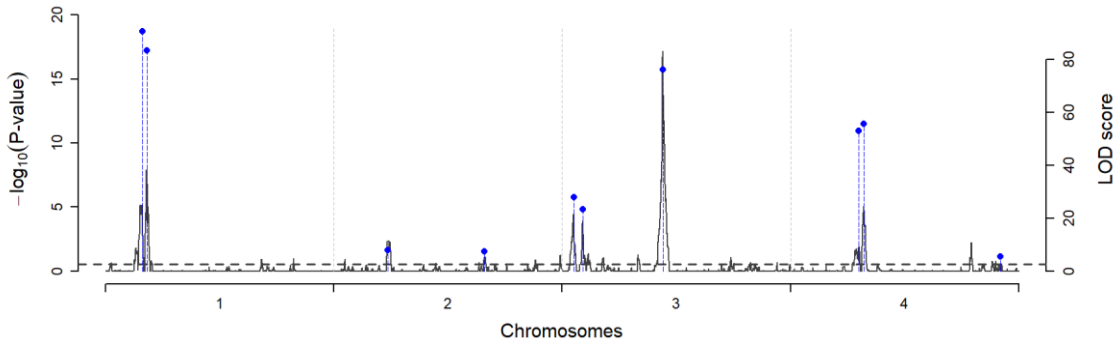
Trait ID	Trait name	Chromosome	Position	LeftMarker	RightMarker	LOD (Q)	add	r2(%)
1	trait_1	1	76	a76	a76	81.2469	2.2438	8.4576
1	trait_1	1	90	a90	a90	75.1029	-2.1393	7.688
1	trait_1	2	116	b116	b116	6.7068	0.584	0.5728
1	trait_1	2	318	b318	b318	8.7146	0.6674	0.7482
1	trait_1	3	25	c25	c25	27.6907	-1.2193	2.4975
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮

result.QEI: This is the results for significant QELs (LOD ≥ 2.5). In this sheet, all the columns are named as Trait ID, Trait name, Chromosome, Position (marker's position (cM) on the genome), LeftMarker, RightMarker, LOD (QE) (LOD score for QEIs). add*env*k* (additive effect in environment *k*), and r2 (%) (r2 (%) is the proportion of total phenotypic variance explained by each QEI).

Trait ID	Trait name	Chromosome	Position	LeftMarker	RightMarker	LOD (QE)	add*env1	add*env2	add*env3	r2(%)
1	trait_1	1	10	a10	a10	58.1226	2.3098	-0.1191	-2.1907	5.6829
1	trait_1	1	21	a21	a21	60.2527	-2.3558	0.1158	2.2401	5.9249
1	trait_1	1	342	a342	a342	9.8824	-0.8971	0.0533	0.8439	0.851
1	trait_1	2	200	b200	b200	25.1393	0.3219	-1.5514	1.2295	2.2523
1	trait_1	2	220	b220	b220	21.7154	-0.2103	1.4046	-1.1943	1.9282
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮

*_Q_plot (*_QE_plot): Plot for QTLs (QEIs). Y-axis on the left side reports -$\log_{10}$ *P*-values of QTLs (QEIs), which are obtained from single-marker genome-wide scanning for all the markers in the first step of QTL.gCIMapping, while Y-axis on the right side

reports LOD scores, which are obtained from likelihood ratio test for significant QTLs (QEIs), with the threshold of LOD = 2.5 (dashed line), in the second step of QTL.gCIMapping. These LOD scores are shown in points with straight lines.



6.2 Result for method=" GCIM " or method=" GCIM-QEI "

Once the software running is finished, “results” files are appeared on the Directory, which was set up by users before running the software. When GCIM is adopted, three files are outputted, including “*_GCIM result.csv”, “*_resultforplot.csv”, and a plot. When GCIM-QEI is adopted, three files are outputted, including “*_GCIM-QEI result.csv”, “*_resultforplot.csv”, and one GCIM-QEI plot.

In the *_GCIM result.csv file, there are ten columns for BC1, BC2, DH, and RIL populations, as shown below.

Trait: The name of trait analyzed

Chr: Chromosome, represented by an integer number

Position (cM): The QTL position (cM) on the chromosome

Additive Effect: Additive effect for significant QTL

LOD: LOD score for significant QTL

Left_Marker: the name of Left flanking marker around significant QTL

Right_Marker: the name of Right flanking marker around significant QTL

Var_Genet: Genetic variance for each significant QTL

r^2 (%): Proportion of phenotypic variance explained by single QTL

Var_Error: residual variance under the full model

Var_Phen (total): Phenotypic variance in the analyzed population

In main-effect QTL detection in F_2, the **Results** file includes twelve columns. Trait, Chr, Position (cM), Left_Marker, Right_Marker, Var_Genet, LOD, r^2 (%), Var_Error, and Var_phen are same as those in the above populations, while QTL effects include additive (**Effect.a**) and dominant (**Effect.d**) effects.

In the GCIM plot of main-effect QTL detection, the $-\log_{10}(P)$ values are indicated by a curve. If the genetic populations analyzed are F_2, there will be two curves in the GCIM plot, one is for additive effect and another is for dominant effect. All the significant QTLs identified are indicated by the vertical lines (**Figure 1**).

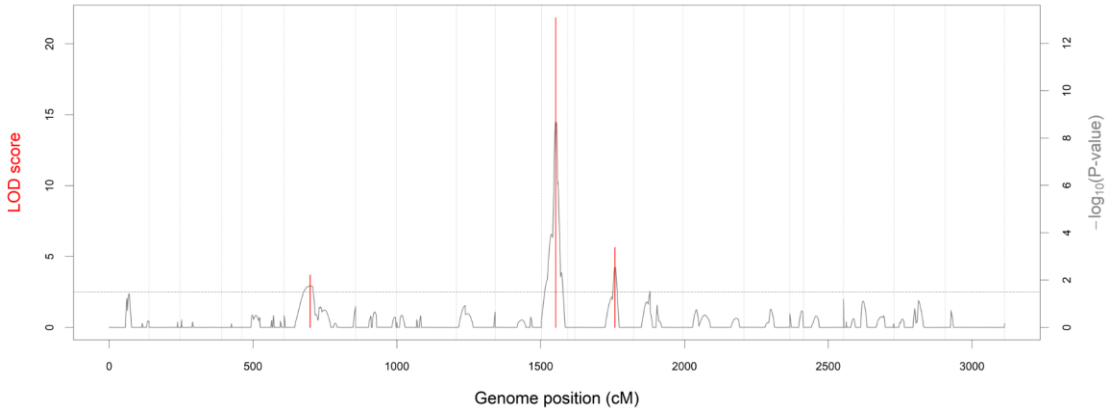


Figure 1. Genome-wide composite interval mapping plot

In QEI detection in IMF_2 or $F_{2:3}$ design with multi-environment datasets, QEI effects include additive-by-environment interaction effects (**Effect.aE1, Effect.aE2, ⋯**), and dominant-by-environment interaction effect (**Effect.dE1, Effect.dE2, ⋯**). There are three Var_Genet columns: Var_Genet (total), Var_Genet_QTL (main-effect QTL) and

Var_Genet_QEI (QEI); three LOD score columns: LOD (total), LOD_QTL (main-effect QTL) and LOD_QEI (QEI); three r2(%) columns: r2(%) (total), r2_QTL(%) (main-effect QTL), r2_QEI(%) (QEI).

In the GCIM-QEI plot, if single environment analysis of the GCIM-QEI method is selected, the parameter is set as "MultiEnv=FALSE", the -$\log_{10}$(P) values of main-effect QTL detection are indicated by a gray curve (Figure 2), and all the significant main-effect QTLs (red) identified are indicated by the red vertical lines.

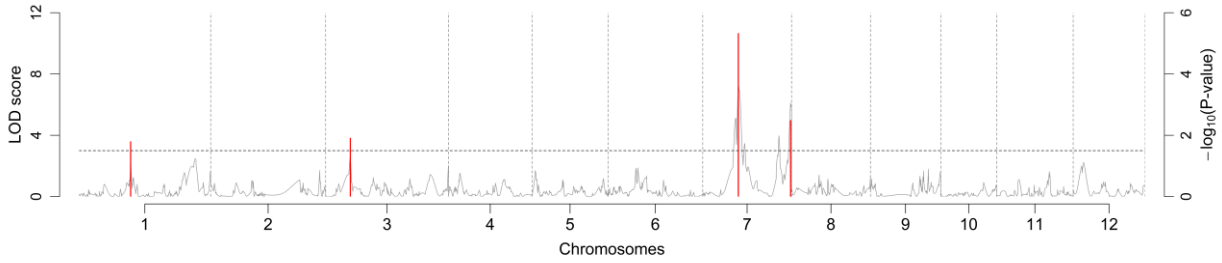


Figure 2. Main-effect QTL detection in single environment analysis using the GCIM-QEI method

If multiple environment analysis of the GCIM-QEI method is selected, the parameter is set as "MultiEnv=TRUE", the -$\log_{10}$(P) values of main-effect QTL and QEI detection are indicated by a gray curve and a light blue curve, respectively (Figure 3), and all the significant QTLs (red) and QEIs (pink) identified are indicated by the red vertical and pink vertical lines, respectively.

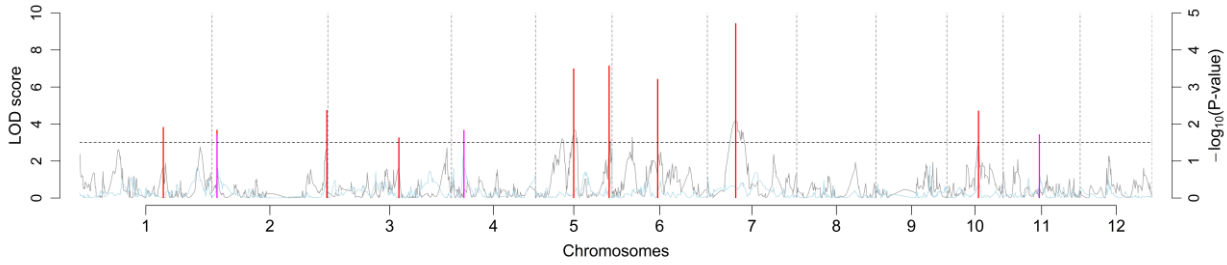


Figure 3. Main-effect QTL and QEI detection in multiple environment analysis using GCIM-QEI method