

User Manual for

S E A

a **R** software package of **SE**gregation **A**nalysis

for quantitative traits in plants

(**version 1.0**)

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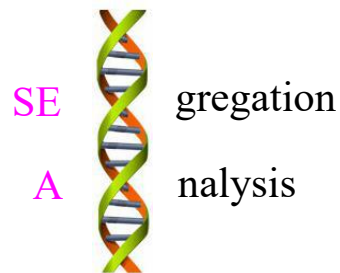
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Disclaimer: While extensive testing has been performed by Yuan-Ming Zhang's Lab (Statistical Genomics Lab) at the College of Plant Science and Technology, Huazhong Agricultural University, the results are, in general, reliable, correct or appropriate. However, results are not guaranteed for any specific datasets. You could consult us if you have any questions.

Download website:

<https://cran.r-project.org/web/packages/SEA/index.html>



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1 Introduction

1.1 Why SEA?

Quantitative traits are controlled by a few major genes and a series of polygenes. Major genes can be individually identified and polygenes are collectively detected. This is mixed major-gene plus polygenes inheritance model. This model for complex trait can be obtained from the comparison between frequent distribution in real data and theoretical distribution of mixed genetic model. This method, named “segregation analysis (SEA)”, has been widely used in China since 1990s. To popularize this approach, we open R software SEA with interactive graphic user interface (GUI) under the framework of the R Studio 1.1.383 platform. In the SEA, the packages **kolmim** and **KScorrect** were used to implement the Lilliefors-corrected Kolmogorov-Smirnoff test; **doParallel** is used to do parallel computation; **data.table** is used to read and write the file quickly; **MASS** is used to solve linear equations and to estimate the first-order genetic parameters.

The current software SEA includes fourteen types of populations: 1) SEA-F₂ (F₂); 2) SEA-F₃ (F_{2:3}); 3) SEA-DH (DH or RIL); 4) SEA-BIL (BIL); 5) SEA-BC (B₁ & B₂); 6) SEA-BCF (B_{1:2} & B_{2:2}); 7) SEA-G4F₂ (P₁, P₂, F₁ and F₂); 8) SEA-G4F₃ (P₁, P₂, F₁ and F_{2:3}); 9) SEA-G3DH (P₁, P₂ and DH); 10) SEA-G5BC (P₁, P₂, F₁, B₁ and B₂); 11) SEA-G5BCF (P₁, P₂, F₁, B_{1:2} and B_{2:2}); 12) SEA-G5 (P₁, P₂, F₁, F₂ and F_{2:3}); 13) SEA-G6 (P₁, P₂, F₁, F₂, B₁ and B₂); 14) SEA-G6F (P₁, P₂, F₁, F_{2:3}, B_{1:2} and B_{2:2}).

SEA is able to work on the Windows, Linux (desktop) and MacOS platforms.

1.2 Getting started

SEA is a package that runs in the R software environment, which can be freely downloaded from <https://cran.r-project.org/web/packages/SEA/index.html>, or request from the maintainer, Dr Yuan-Ming Zhang at the College of Plane Science and Technology, Huazhong Agricultural University (soy Zhang@mail.hzau.edu.cn or soy Zhang@hotmail.com).

1.2.1 One-Click installation

Within R environment, the SEA software can be installed directly using the below command:

```
install.packages(pkgs="SEA")
```

1.2.2 Step-by-step installation

1.2.2.1 Install the add-on packages

Online installation Within R environment on the internet, the SEA package can be installed online, using the below command:

```
install.packages(pkgs=c("shiny","MASS","KScorrect","kolmim","data.table","doParallel"))
```

Offline install The following R packages are needed: [bit64](#), [curl](#), [data.table](#), [digest](#), [doParallel](#), [foreach](#), [htmltools](#), [httpuv](#), [iterators](#), [jsonlite](#), [knitr](#), [kolmim](#), [KScorrect](#), [MASS](#), [mclust](#), [mime](#), [nanotime](#), [R6](#), [Rcpp](#), [reshape2](#), [shiny](#), [sourcetools](#), [testthat](#), [xtable](#), [xts](#), [zoo](#), which can be downloaded from CRAN (<https://cran.r-project.org/>) on your desktop. Open R GUI, select "Packages"—"Install package(s) from local files..." then find R packages (only [*.zip](#) and [*.tar.gz](#) files available) above and install them in order, as some depend on others.

1.2.2.2 Install SEA

Download the SEA package (only [*.zip](#) and [*.tar.gz](#) files available) on your desktop ahead, method of installing SEA is the same as §1.2.2.2. Within R environment, launch the SEA by command: [library\(SEA\)](#), To restart the GUI, the command [SEA\(\)](#) can be issued ([Figure 1.1](#)).

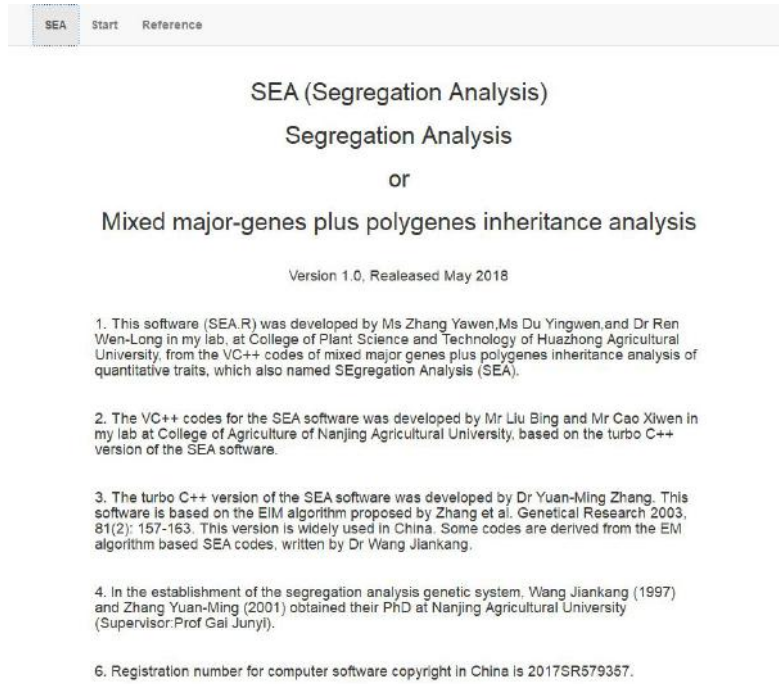


Figure 1.1 Screenshot of SEA package GUI

2 Phenotypic datasets and genetic models

2.1 Population types

Bi-parental segregation populations in the software package of SEA

Population	Abbreviation	Population	Abbreviation
F ₂	SEA-F2	P ₁ , F ₁ , P ₂ and F _{2:3}	SEA-G4F3
F _{2:3}	SEA-F3	P ₁ , P ₂ and DH	SEA-G3DH
DH or RIL	SEA-DH	P ₁ , F ₁ , P ₂ , B ₁ and B ₂	SEA-G5BC
BIL	SEA-BIL	P ₁ , F ₁ , P ₂ , B _{1:2} and B _{2:2}	SEA-G5BCF
B ₁ and B ₂	SEA-BC	P ₁ , F ₁ , P ₂ , F ₂ and F _{2:3}	SEA-G5
B _{1:2} and B _{2:2}	SEA-BCF	P ₁ , F ₁ , P ₂ , F ₂ , B ₁ and B ₂	SEA-G6
P ₁ , F ₁ , P ₂ and F ₂	SEA-G4F2	P ₁ , F ₁ , P ₂ , F _{2:3} , B _{1:2} and B _{2:2}	SEA-G6F

Note: DH: doubled haploid; RIL: recombinant inbred line; BIL: backcross inbred line; B₁ = F₁ × P₁; B₂ = F₁ × P₂; B_{1:2} and B_{2:2}: families derived from B₁ and B₂, respectively.

Citation:

Populations	References
SEA-F ₂	Wang et al. <i>Acta Genetica Sinica</i> 1997, 24(5):432-440 ^[4] Zhang et al. <i>Journal of Biomathematics</i> 2000, 15(3):358-366 ^[7]
SEA-F ₃	Zhang et al. <i>Hereditas (Beijing)</i> 2001, 23(4):329-776 ^[10] Zhang et al. <i>Genetical Research</i> 2003, 81(2): 157-163 ^[8]
SEA-DH	Zhang et al. <i>Hereditas (Beijing)</i> 2001, 23(5):467-470 ^[12]
SEA-BIL	Wang et al. <i>Acta Agron Sin</i> 2013, 39(2):198-206 ^[5]
SEA-BC	Zhang et al. <i>Journal of Biomathematics</i> 2000, 15(3):358-366 ^[7]
SEA-BCF	Zhang et al. <i>Hereditas (Beijing)</i> 2001, 23(4):329-776 ^[5] Zhang et al. <i>Genetical Research</i> 2003, 81(2): 157-163 ^[8]
SEA-G4F2	Zhang et al. <i>Journal of Southwest Agricultural University</i> 2000, 42(1):6-9 ^[11]
SEA-G4F3	Zhang et al. <i>Journal of Southwest Agricultural University</i> 2000, 42(1):6-9 ^[11] Zhang et al. <i>Genetical Research</i> 2003, 81(2): 157-163 ^[8]
SEA-G3DH	Zhang et al. <i>Hereditas (Beijing)</i> 2001, 23(5):467-470 ^[12]
SEA-G5BC	Zhang et al. <i>Acta Agron Sin</i> 2000, 26(6):699-706 ^[13]
SEA-G5BCF	Zhang et al. <i>Acta Agron Sin</i> 2000, 26(6):699-706 ^[13] Zhang et al. <i>Genetical Research</i> 2003, 81(2): 157-163 ^[8]
SEA-G5	Wang et al. <i>Acta Agron Sin</i> 1998, 24(6):651-659 ^[6] Zhang et al. <i>Genetical Research</i> 2003, 81(2): 157-163 ^[8]
SEA-G6	Gai et al. <i>Theor Appl Genet</i> 1998, 97(7): 1162-1168 ^[2] Gai et al. <i>Acta Agron Sin</i> 2000, 26(4):385-391 ^[3]
SEA-G6F	Zhang et al. <i>Acta Agron Sin</i> 2001, 27(6):787-793 ^[14] Zhang et al. <i>Genetical Research</i> 2003, 81(2): 157-163 ^[8]

Note: The above references are listed in **References**.

2.2 Phenotypic dataset format

The **Phenotypic** file should be a *.csv format file, the phenotypic observations for all the populations are included in one file, and all the observations for each population are listed in one same column (**Table 2.1**). In each column, the first element must be header, and stands for population type, such as “F2”, “P1” (**Table 2.2**). This dataset can be uploaded into the software SEA by clicking the button “Browse” (**Figure 2.1**).

Table 2.1. The phenotypic file (*.csv)

P1	P2	F1	F2	B1	B2
56.33	74.21	62.2	55.19	60.83	58.79
58.96	85.3	54.02	57.64	57.84	64.58
62.93	79.95	52.72	53.24	56.45	62.86
55.97	82.56	55.47	50.58	55.06	60.96
59.28	81.12	51.4	47.03	53.64	70.42
59.45	75.86	55.69	58.71	56.89	56.8
65.56	82.47	53.08	54.78	67.26	51.23
57.67	77.58	58.81	48.04	57.72	74.88
62.68	76.81	51.85	53.71	54.9	67.86
61.5	76.31	48.26	69.15	60.2	60.66
64.64	79.99	51.25	67.5	57.77	60.72

Table 2.2. The column name in phenotypic file (*.csv)

Population	Column name		Population	Column name
F ₂	F2		G4F3	P1, F1, P2, F23
F _{2;3}	F23		G3DH	P1, P2, DH
DH or RIL	DH		G5BC	P1, F1, P2, B1, B2
BIL	BIL		G5BCF	P1, F1, P2, B12, B22
BC	B1, B2		G5	P1, F1, P2, F2, F23
BCF	B12, B22		G6	P1, F1, P2, F2, B1, B2
G4F2	P1, F1, P2, F2		G6F	P1, F1, P2, F23, B12, B22

SEA
Start
Reference

SEA (Segregation Analysis)

Dataset
Result
Posterior Probability
Distribution curves

Select population:
F2

Input dataset
Browse... No file selected

Model Selection
All models

Run

Posterior Probability

Distribution curves

User manual

Figure 2.1. The interface of data input

2.3 Genetic models and meanings of model codes

Table 2.3. Genetic models in the joint segregation analysis of the five generations of P₁, F₁, P₂, F₂ and F_{2:3}

Class	Major gene	Polygenes	Model code	
			Only major gene	Mixed major gene & polygenes
Polygenes	-	Additive-dominant-epistasis, $[d]$, $[h]$, $[i]$, $[j]$, $[l]$	-	PG-ADI
	-	Additive-dominant, $[d]$, $[h]$	-	PG-AD
A major gene	Additive-dominant, d , h	Additive-dominant-epistasis, $[d]$, $[h]$, $[i]$, $[j]$, $[l]$	1MG-AD	MX1-AD-ADI
	Additive-dominant, d , h	Additive-dominant, $[d]$, $[h]$	1MG-AD	MX1-AD-AD
	Additive, d ($h=0$)	Additive-dominant, $[d]$, $[h]$	1MG-A	MX1-A-AD
	Completely dominant, d ($h=d$)	Additive-dominant, $[d]$, $[h]$	1MG-CD	MX1-CD-AD
	Completely negative dominant, d ($h=-d$)	Additive-dominant, $[d]$, $[h]$	1MG-NCD	MX1-NCD-AD
Two major genes	Additive-dominant-epistasis, d_a , d_b , h_a , h_b , i , j_{ab} , j_{ba} , l	Additive-dominant-epistasis, $[d]$, $[h]$, $[i]$, $[j]$, $[l]$	2MG-ADI	MX2-ADI-ADI
	Additive-dominant-epistasis, d_a , d_b , h_a , h_b , i , j_{ab} , j_{ba} , l	Additive-dominant, $[d]$, $[h]$	2MG-ADI	MX2-ADI-AD
	Additive-dominant, d_a , d_b , h_a , h_b , $i=j_{ab}=j_{ba}$, l	Additive-dominant, $[d]$, $[h]$	2MG-AD	MX2-AD-AD
	Additive, d_a , d_b , $h_a=h_b=0$	Additive-dominant, $[d]$, $[h]$	2MG-A	MX2-A-AD
	Equally additive, d ($=d_a=d_b$, $h_a=h_b=0$)	Additive-dominant, $[d]$, $[h]$	2MG-EA	MX2-EA-AD
	Completely dominant, $d_a=h_a$, $d_b=h_b$	Additive-dominant, $[d]$, $[h]$	2MG-CD	MX2-CD-AD
	Equally dominant, $d=d_a=h_a=d_b=h_b$	Additive-dominant, $[d]$, $[h]$	2MG-EAD	MX2-EAD-AD

Meanings of model codes:

- 1) **1MG ~ 4MG**: One to four major genes
- 2) **MX1 ~ MX3**: One to three major genes plus polygenes
- 3) **A**: additive
- 4) **AD**: additive-dominance
- 5) **ADI**: additive-dominance-epistasis
- 6) **CD**: $d = h$, completely dominance
- 7) **NCD**: $d = -h$, negatively completely dominance
- 8) **EA**: $d_a = d_b$, equally additive
- 9) **EAD**: $d_a = d_b = h_a = h_b = d$, equally additive-dominance
- 10) **AI**: additive-epistasis
- 11) **CEA**: $d_a = d_b = d_c = d$, completely equally additive
- 12) **PEA**: $d_a = d_b = d_1, d_c = d_2$, partially equally additive
- 13) **CE**: $9A_B_ : 7 (3A_bb + 3aaB_ + 1aabb)$, complementary effect.
- 14) **AE**: $9A_B_ : 6 (3A_bb + 3aaB_) : 1aabb$, additive effect.
- 15) **DE**: $15 (9A_B_ + 3A_bb + 3aaB_) : 1aabb$, duplicate effect (The effect of A or B allele on the trait phenotype).
- 16) **ED**: $12 (9A_B_ + 3A_bb) : 3aaB_ : 1aabb$, epistatic dominance between two pairs of major genes (The effect of A allele on Bb locus).
- 17) **RE**: $9A_B_ : 3A_bb : 4 (3aaB_ + 1aabb)$, epistatic recessiveness of aa on Bb locus.
- 18) **IE**: $13 (9A_B_ : 3aaB + 1aabb) : 3A_bb$, inhibiting effect of B allele on Aa locus.

3 Running and Results

3.1 Start the software

Select the option “**Start**” (for example), the following interface will appear.

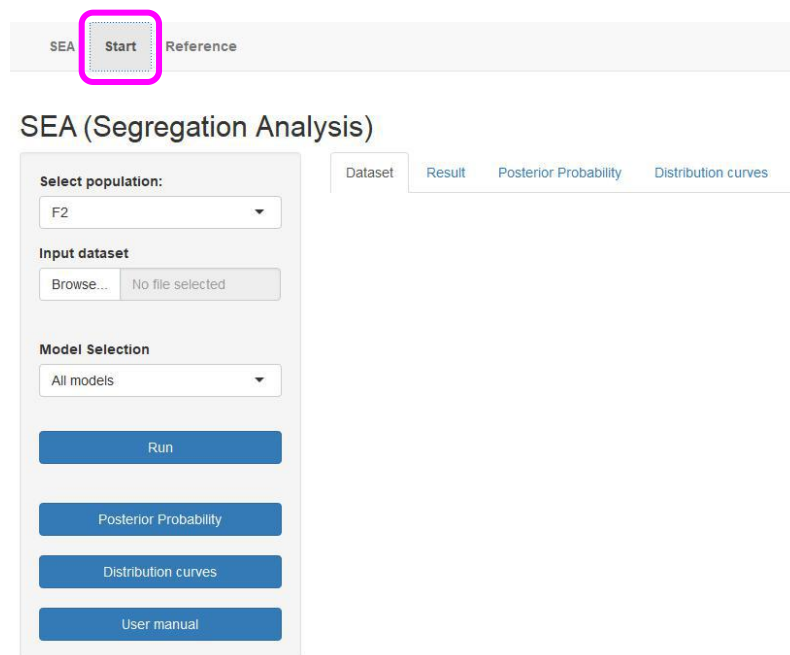


Figure 3.1. Screenshot of SEA GUI

3.2 Select population

Use the drop down menu to select which population will be analyzed **Figure 3.2**.

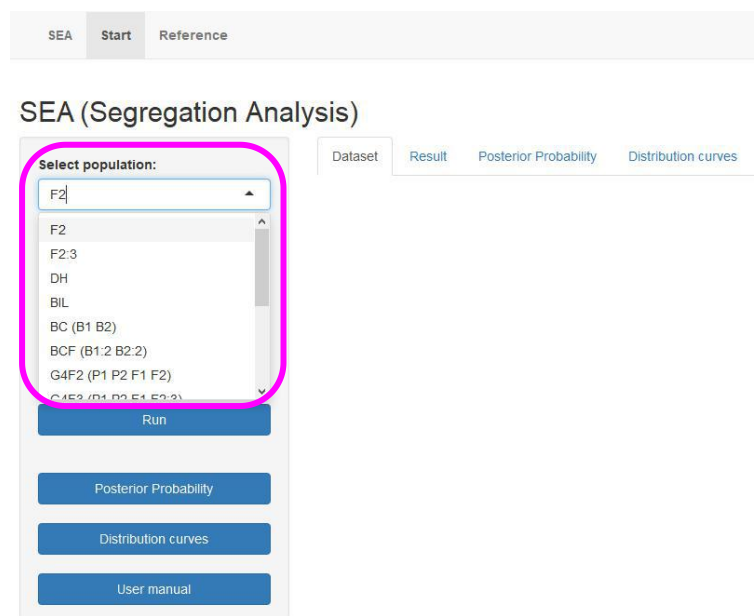


Figure 3.2. Select population

3.3 Input dataset

Use the **Browse** button to input dataset files. Once one file is successfully uploaded,

the result will be shown as **Figure 3.3**

SEA (Segregation Analysis)

Select population: F2

Input dataset

Browse... F2.csv

Upload complete

Model Selection: All models

Run

Posterior Probability

Distribution curves

User manual

Dataset Result Posterior Probability Distribution curves

Show 25 entries Search:

F2

66.82
65.07
54.53
36.32
50.89
58.63
59.22
68.22
49.68
44.19
62.54

Figure 3.3. The Input Dataset module for SEA

3.4 Parameter settings

Use the Model Selection option menu to choose model before running the program,

“All models” which means to run all the models under this population (**Figure 3.4**).

In such population like: F3 (F2:3), BCF (B1:2, B2:2), G4F3 (P1, P2, F1, F2:3), G3DH (P1, P2, DH), G5BCF (P1, P2, F1, B1:2, B2:2), G5 (P1, P2, F1, F2, F2:3), G6F (P1, P2, F1, F2:3, B1:2, B2:2), you need to set the parameter of **No. of plants in each family** (1 is default value) as well (**Figure 3.5**). In BIL population, you also need to select The **BIL type**: BIL1 ($F1 \times P1$) and BIL2 ($F1 \times P2$) (**Figure 3.6**).

SEA
Start
Reference

SEA (Segregation Analysis)

Select population:
F2

Input dataset
Browse...
F2.csv
Upload complete

Model Selection
All models

Run

Posterior Probability

Distribution curves

User manual

Dataset
Result
Posterior Probability
Distribution curves

Show
25
entries
Search:

F2
66.82
65.07
54.53
36.32
50.89
58.63
59.22
68.22
49.68
44.19
62.54

Figure 3.4. Model selection

SEA
Start
Reference

SEA (Segregation Analysis)

Select population:
F2:3

Input dataset
Browse...
F23.csv
Upload complete

Model Selection
All models

No. of plants measured in each family
1

Run

Posterior Probability

Distribution curves

User manual

Dataset
Result
Posterior Probability
Distribution curves

Show
25
entries
Search:

F23
0.16
0.17
0.18
0.19
0.17
0.19
0.19
0.19
0.16
0.16
0.17
0.18
0.18

Figure 3.5. Set “No. of plants in each family”

SEA Start Reference

SEA (Segregation Analysis)

Select population:

BIL

Input dataset

Browse... BIL.csv

Upload complete

Model Selection

All models

The BIL type

BIL1(F1xP1)

Run

Posterior Probability

Distribution curves

User manual

Dataset Result Posterior Probability Distribution curves

Show 25 entries Search:

BIL

22.32

22.49

22.74

20.39

22.78

23.25

21.76

23.22

21.95

23.26

23.05

23.34

23.74

Figure 3.6. Select population types for BIL

3.5 Run the program

Use the [Run](#) Button to run the program, the result will be shown in the result module, and then it can be download use the [Download result](#) Button ([Figure 3.7](#))

SEA Start Reference

SEA (Segregation Analysis)

Select population:

BIL

Input dataset

Browse... BIL.csv

Upload complete

Model Selection

All models

The BIL type

BIL1(F1xP1)

Run

Posterior Probability

Distribution curves

User manual

Dataset Result Posterior Probability Distribution curves

Download result

Show 25 entries Search:

Model	Log_Max_likelihoood_Value	AIC	mean[1]	mean[2]
0MG	-370.7047	745.4094	33.176	
1MG-A	-321.1667	648.3334	35.812	24.1059
2MG-AI	-320.0589	650.1178	36.5101	33.5724
2MG-A	-320.9535	649.907	36.3574	34.1605
2MG-EA	-324.2827	654.5654	36.2329	29.7619
2MG-ED	-321.1672	650.3343	35.812	24.1296
2MG-ER	-320.0893	648.1785	36.4996	33.5854

Figure 3.7. Run the program

Descriptions in Results file

Descriptions in Results interface in §3.6 are as follows:

- 1) **Model:** genetic model
- 2) **Log_Max_likelihood_Value** $L(Y|\Theta)$: $\log_{10}(\text{maximum likelihood function value})$.
- 3) **AIC:** $AIC = -2L(Y|\Theta) + 2k$; $L(Y|\Theta)$: logarithm likelihood function, Θ : parameter in the logarithm likelihood function, k : the number of independent parameters in the model
- 4) **mean:** mean for each component distribution in one segregation population
- 5) **Proportion:** genotypic proportions or proportions of component distributions in one segregation population, which are calculated from posterior probability
- 6) **m, d, h :** total average, additive effect, dominant effect for major genes.
- 7) **i, j_{ab}, j_{ba}, l :** additive \times additive, additive \times dominance, dominance \times additive, and dominance \times dominance epistatic effects between two major genes
- 8) **$[d], [h], [i], [j], [l]$:** additive effect, dominance effect, additive \times additive, additive \times dominance (or dominance \times additive) and dominance \times dominance effects for polygenes
- 9) **Major-Gene Var, Polygenes Var:** major gene genotypic variance, polygenic variance (second-order genetic parameter)
- 10) **Heritability (%):** the proportion of genetic variance in phenotypic variance
- 11) **U* square-**** (U_1^2, U_2^2 and U_3^2 ; or $U_1^2 - P_1$), **nW square-**** (${}_nW^2$ or ${}_nW^2 - F_1$), **Dn-**** (D_n or $D_n - F_2$): uniform, Smirnov and Kolmogorov statistics. *: numbers 1 to 3; **: population notion, i.e., P_1, F_1, P_2, F_2 and F_3
- 12) **P(U1 square-*), P(U2 square-*), P(U3 square-*), P(nW square-*), P(Dn-*):** P -value of the above corresponding statistics.

3.6 Posterior probability

Posterior probability module shows the posterior probability of j th major gene

genotype for the i th individual (line) in the optimal model, implemented by the **Calculate Posterior Probability** button, and downloaded the results by the **Save Posterior Probability** button (Figure 3.8). If there is only one major-gene genotype in this model, **Warning** notice “No posterior probability!” will be appeared (Figure 3.9).

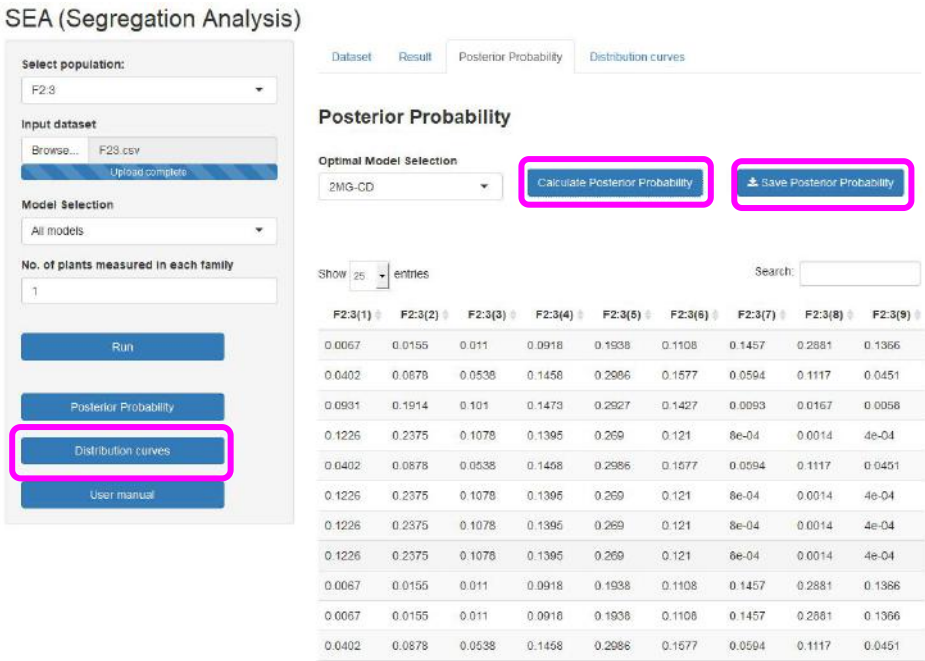


Figure 3.8. Posterior Probability module

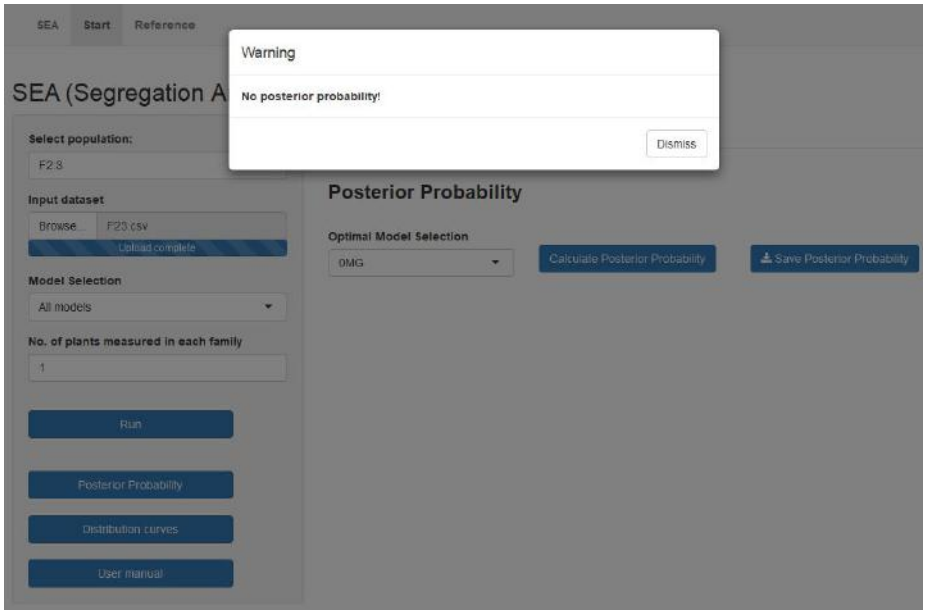


Figure 3.9. The model with only one genotype of major gene

3.7 Distribution curve

In Parameter Settings module, users can use the **Draw Distribution curves** button to preview the plot (**Figure 3.10**). In the download plot module, users can select general or high resolution plot, and use the **Save Distribution Curves** button to save the results as *.png, *.tiff, *.jpeg, and *.pdf files in your selected pathway (**Figure 3.11**).

SEA (Segregation Analysis)

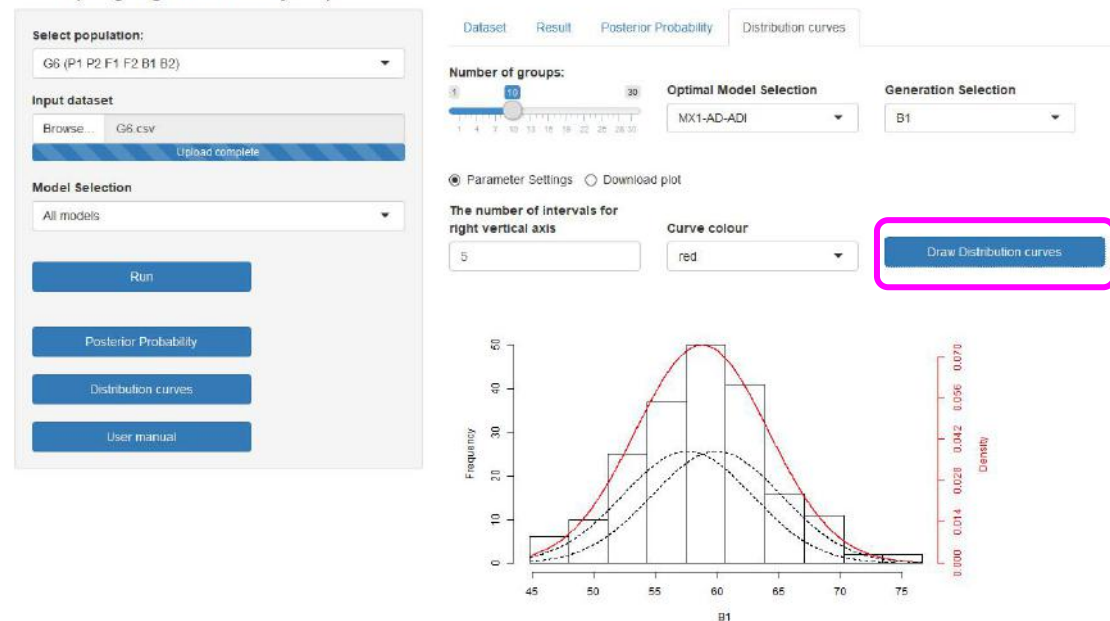


Figure 3.10. Draw Distribution curves

SEA (Segregation Analysis)

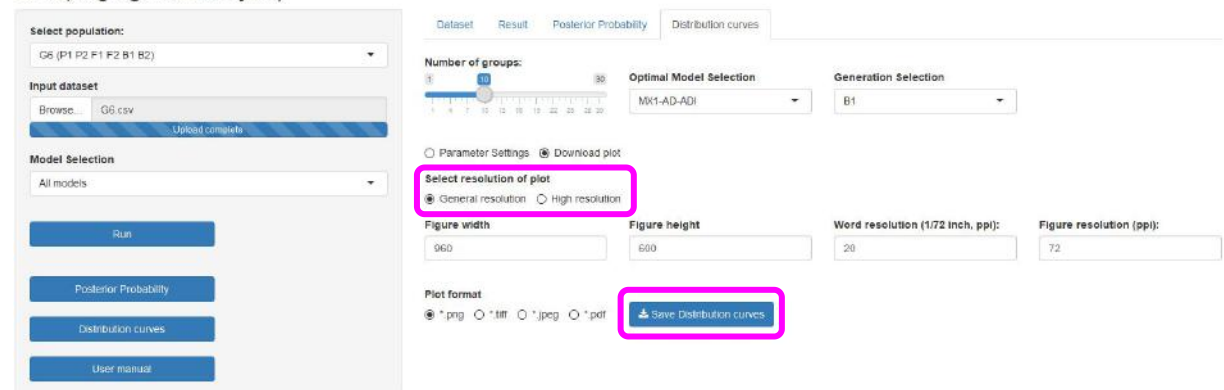


Figure 3.11. Download Distribution curves

Parameter settings

- 1) **Number of groups:** Frequent distribution for quantitative traits can be indicated as the ideal figure that users want, if users change the number of groups.

- 2) **Optimal Model Selection:** the optimal model will be list in the drop down menu after calculation. Users can choose one model to draw the picture.
- 3) **Generation selection:** For the single segregating population, users could draw the distribution curves directly. For the multi-generation populations, users need to first select the populations, and then users can see the corresponding plots.
- 4) **Curve color:** The colors of the density curve can be changed via the combo box, with a drop-down option.
- 5) **The width and height of the Figure,** with the unit of pixel (px).
- 6) **The word resolution in the Figure,** with the unit of 1/72 inch, being pixels per inch (ppi).
- 7) **The figure resolution in the Figure,** with the unit of pixels per inch (ppi).

4 References

- 1 Gai Junyi, Zhang Yuan-Ming, Wang Jiankang. Genetic system of quantitative traits in plants. Beijing: Science Press, 2003.
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- 3 Gai Junyi, Zhang Yuan-Ming, Wang Jiankang. A joint analysis of multiple generations for QTL models extended to mixed two major genes plus polygene. *Acta Agron Sin* 2000, 26(4): 385-391.
- 4 Wang Jiankang, Gai Junyi. Identification of major gene and polygene mixed inheritance model and estimation of genetic parameters of a quantitative trait from F₂ Progeny. *Acta Genetica Sinica* 1997, 24(5): 432-440.
- 5 Wang Jinshe, Zhao Tuanjie, Gai Junyi. Establishment of segregation analysis of mixed inheritance model with four major genes plus polygenes in backcross inbred lines (BIL) populations. *Acta Agron Sin* 2013, 39(2): 198-206.
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