



## **Users' Manual of Breeding Planner (BP)**

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# **Chapter 1. Introduction**

## **1.1 Breeding programs in Breeding Planner**

Molecular genetics and associated technologies have greatly contributed to our understanding of the inheritance of targeted traits in plant breeding, which in turn open new ways to improving the efficiency of breeding programs. Molecular marker techniques are influencing the breeding process from parental selection and cross prediction, to introgression of known genes and population enhancement. Breeding programs MARS (marker assisted recurrent selection), MABC (marker assisted backcrossing) and MAS (marker assisted selection) can be handled in Breeding Planner.

### **1.1.1 MARS breeding program**

Recurrent selection involves evaluating a population and selecting the best individuals, recombining these selected individuals to form the next generation or cycle of selection, and repeating the previous procedure. MARS (marker-assisted recurrent selection) was proposed to overcome the disadvantages when using markers in selecting complex traits. MARS procedures used to date have relied on ad hoc significance tests to identify markers associated with the trait, and subsequently to estimate the effect associated with each marker. QTL mapping is expected to uncover most, if not all, of the genes for important traits in crops (Figure 1.1). With its advantage to rapidly increase the frequency of favorable alleles, MARS has been commercially used for selecting complex traits in maize, sunflower and soybean breeding programs. MARS requires the embedding of a marker-based selection process within a phenotypic and marker-assisted breeding program.

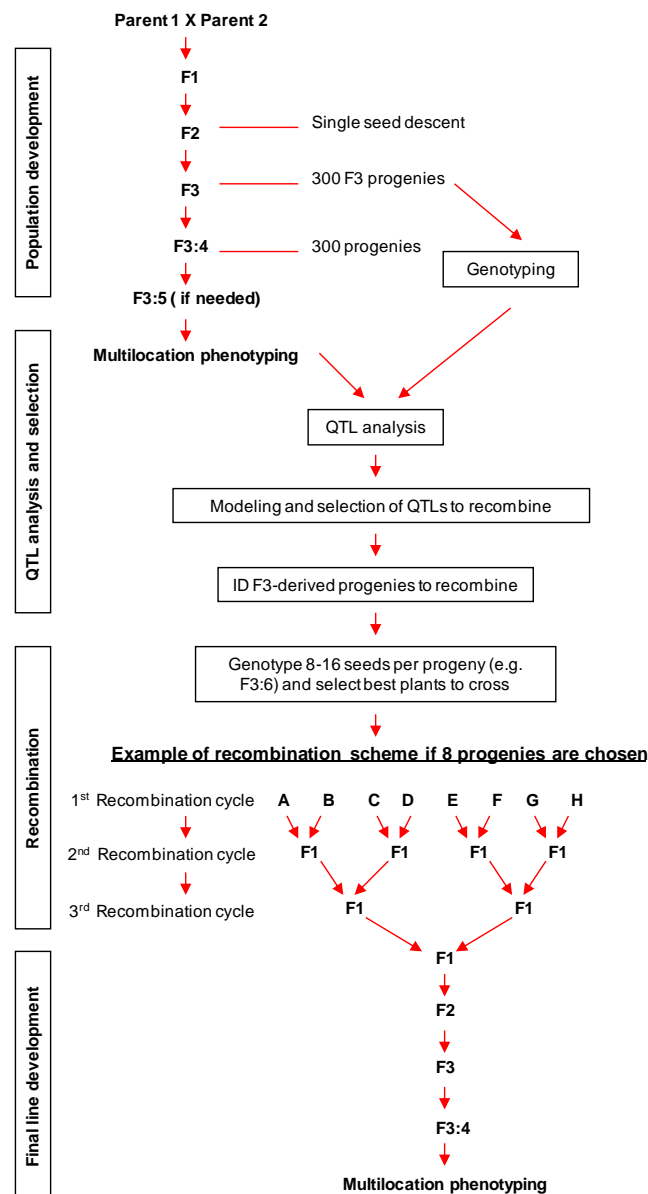


Figure 1.1 Diagram of a MARS breeding program in self-pollinated species

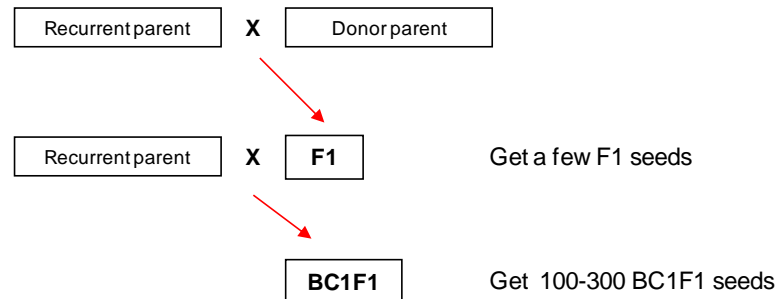
### 1.1.2 MABC breeding program

When the donor parent only has a few favorable alleles, backcrossing is commonly applied in breeding (Figure 1.2). However, breeders need to know times of backcrossing, population size in each generation, and how MABC is conducted etc. PMABC is designed to meet these requirements.

#### **Choose markers to use in MABC process**

- 2-4 well spread polymorphic markers per chromosome for background selection
- 2-3 flanking markers on each side of locus to introgress (2-5 cM apart if major gene , if QTL involved the whole confidence interval needs to be spanned (4-5 markers))

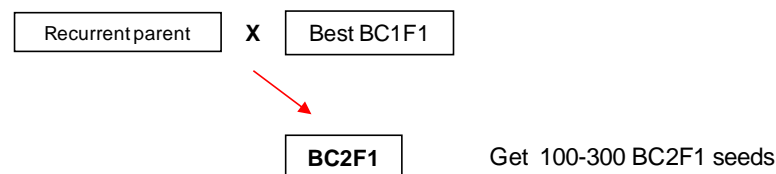
#### **Start crosses**



#### **Grow BC1F1 plants and genotype them with chosen markers**

Select best BC1F1 based on:

- close recombination on one side of target locus (between two flanking markers)
- best recovery of recurrent background at non-carrier chromosomes



#### **Grow BC2F1 plants and genotype them with chosen markers**

Select best BC2F1 based on:

- close recombination on the other side of target locus (between two flanking markers)
- best recovery of recurrent background at non-carrier chromosomes



#### **Select best BC3F1 plant based only on non-carrier chromosomes**

#### **Self and check BC3F2s for homozygosity at introgressed locus**

- Homozygous positive progenies can be bulked

#### **Seed increase and final testing**

**Note:** 2-3 loci can be backcrossed at the same time using a similar process but larger populations will be needed at each generation. For more loci, conduct parallel MABCs and combine the loci at the end (crossing final BC3F1s).

*Figure 1.2 Diagram of a MABC breeding program*

### 1.1.3 MAS breeding program

When gene information is known and associated markers are available, MAS can be applied to combine multiple favorable alleles into one genotype. However, breeders face many complex choices in the design of efficient crossing and selection strategies aimed at combining multiple desired alleles into a single target genotype. Under simplified conditions, population genetic theory could be used to establish general rules for the number of markers required, the best crossing strategies, and the level of inbreeding to maximize the efficiency of marker implementation. When the scenario is

extended to more genes and linked markers (see Figure 1.3 and 1.4 for an example in wheat), simulation analysis could be adopted to develop rules for crossing and selection.

Gene	<i>Rht-B1</i>	<i>Rht-D1</i>	<i>Rht8</i>	<i>Sr2</i>	<i>Cre1</i>	<i>VPM</i>	<i>Glu-B1</i>	<i>Glu-A3</i>	<i>tin</i>
Chr.	4BS	4DS	2DL	3BS	2BL	7DL	1BL	1AS	1AS
Marker	Codominant	Codominant	Codominant	Codominant	Dominant	Dominant	Codominant	Codominant	Codominant
MK-gene distance	0	0	0.6	1.1	0	0	0	0	0.8
HM14BS	<i>Rht-B1a</i>	<i>Rht-D1a</i>	<i>Rht8</i>	<i>sr2</i>	<i>cre1</i>	<i>vpm</i>	<i>Glu-B1a</i>	<i>Glu-A3e</i>	<i>Tin</i>
Sunstate	<i>Rht-B1a</i>	<i>Rht-D1b</i>	<i>rht8</i>	<i>Sr2</i>	<i>cre1</i>	<i>VPM</i>	<i>Glu-B1i</i>	<i>Glu-A3b</i>	<i>Tin</i>
Silverstar +tin	<i>Rht-B1b</i>	<i>Rht-D1a</i>	<i>rht8</i>	<i>sr2</i>	<i>Cre1</i>	<i>vpm</i>	<i>Glu-B1i</i>	<i>Glu-A3c</i>	<i>tin</i>
Target	<i>Rht-B1a</i>	<i>Rht-D1a</i>	<i>Rht8</i>	<i>Sr2</i>	<i>Cre1</i>	<i>VPM</i>	<i>Glu-B1i</i>	<i>Glu-A3b</i>	<i>tin</i>

Figure 1.3 Nine major genes and their distribution in three wheat parental lines

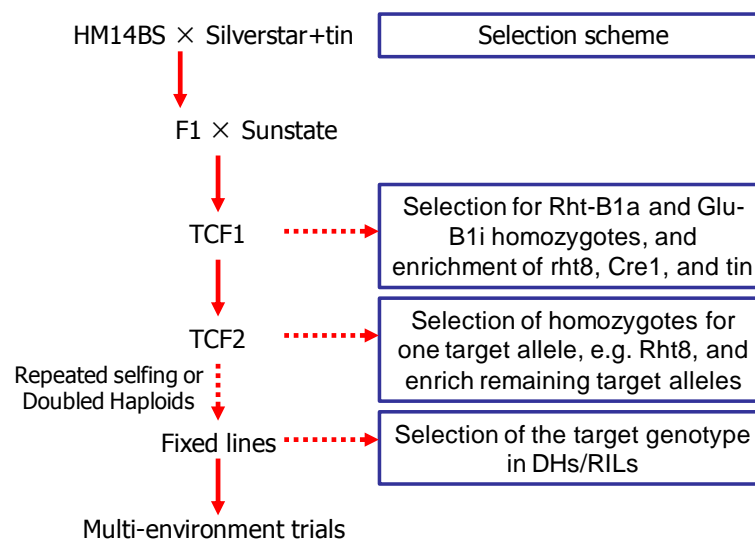


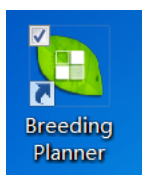
Figure 1.4 An optimum crossing and selection schemes in MAS identified by simulation

## 1.2 Development and application environments

In Breeding Planner, the interface was written by JAVA. The software runs on Windows XP/Vista/7/8.

## 1.3 User interface

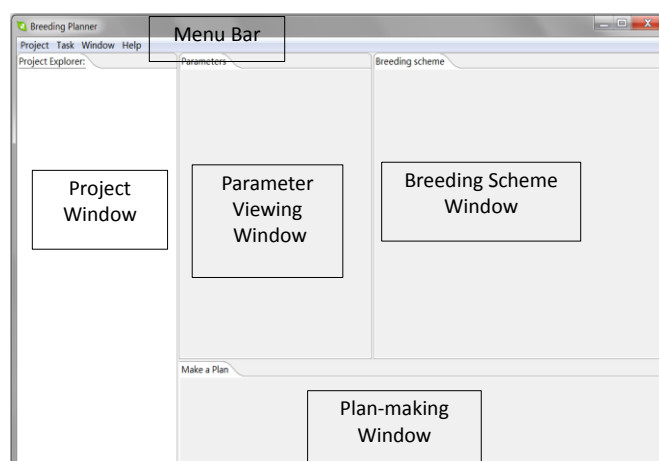
The Breeding Planner software can be opened by double click the software icon in your computer desktop (Figure 1.5).



*Figure 1.5 The Breeding Planner software icon on desktop after installment*

Figure 1.6 is the interface of BP. The interface includes Menu Bar, Project Window, Parameter Viewing Window, Breeding Scheme Window and Plan-making Window.

- **Project Window:** List all molecular breeding programs you have planned. Three distinct breeding programs can be considered: MARS, MABC and MAS.
- **Parameter Viewing Window:** You can view your breeding parameters in this window.
- **Breeding Scheme Window:** Once the breeding parameters are specified, a breeding flowchart will be demonstrated in this window.
- **Plan-making Window:** You can select the current stage/generation of your breeding programs in this window. A detailed plan for the near future will be made by the BP system.

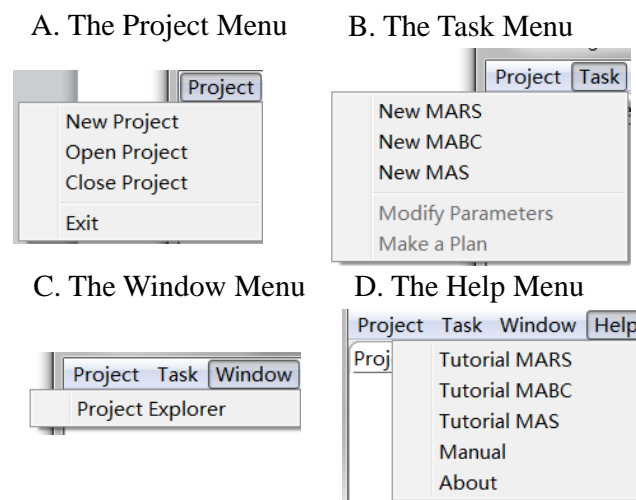


*Figure 1.6 Interface of BP*

## 1.4 Menu bar

- Project: open and close projects (Figure 1.7A)
  - New Project: To create a new project
  - Open Project: To open an existing project
  - Close Project: To close the current project
  - Exit: To exit the BP software
- Task: To manage a batch of jobs (Figure 1.7B)
  - New MARS: To create a new MARS breeding program
  - New MABC: To create a new MABC breeding program
  - New MAS: To create a new MAS breeding program

- Modify Parameters: To modify the parameters of the breeding program
- Make a Plan: To make a plan for the breeding program
- Window: To manage the interface windows (Figure 1.7C)
  - Project Explorer: To open or close Project Window
- Help: To access help information (Figure 1.7D)
  - Tutorial MARS: Open the tutorial for PMARS
  - Tutorial MABC: Open the tutorial for PMABC
  - Tutorial MAS: Open the tutorial for PMAS
  - Manual: To view the Users' Manual of Breeding Planner
  - About: To view the version information of Breeding Planner

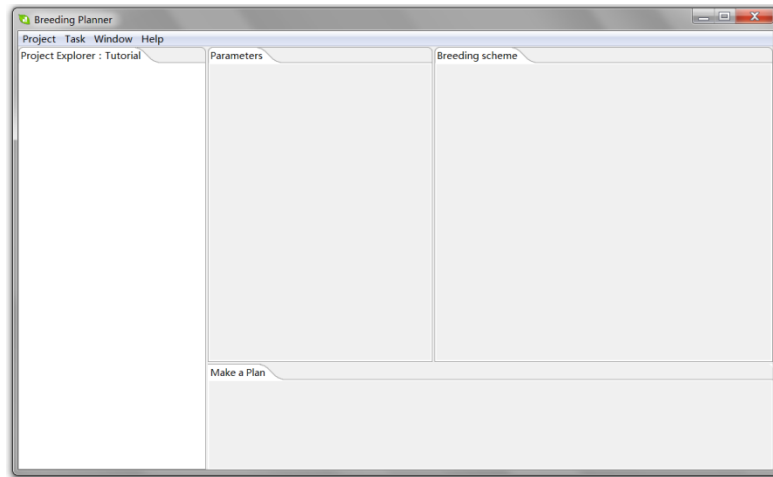


*Figure 1.7 Menu bars in BP*

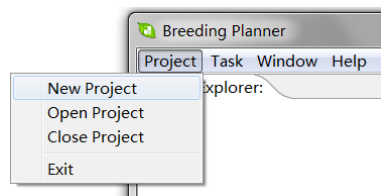
## 1.5 The project concept in Breeding Planner

The BP system is project-based. When you first use the system, you must build a new project first. Then you can make various breeding programs. When you leave the system, the system automatically saves the jobs you have done. The next time, you can start from a new project, or from an existing project. Figure 1.8 is the interface of an open project given the name "...\\Tutorial.ibp". Click **New Project** in **Project menu** (Figure 1.9) and then specify Project Name (Tutorial for example) and Project Path (D:\\ for example) (Figure 1.10) to build a new project.

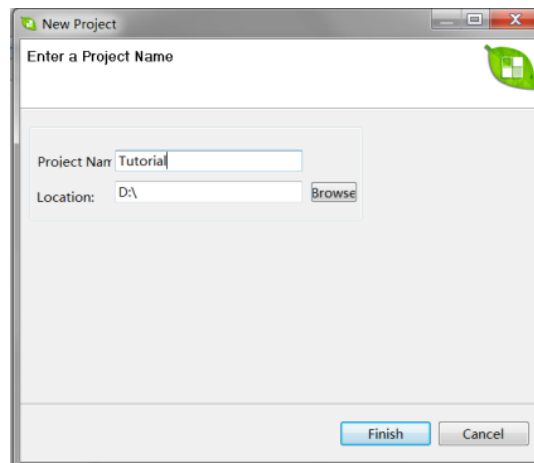




*Figure 1.8 Interface of BP with an open project “Tutorial”*

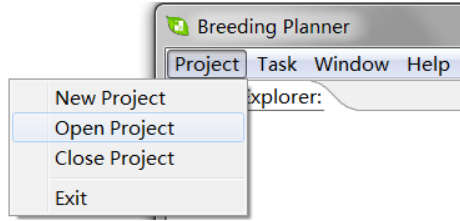


*Figure 1.9 New Project button Project Menu*

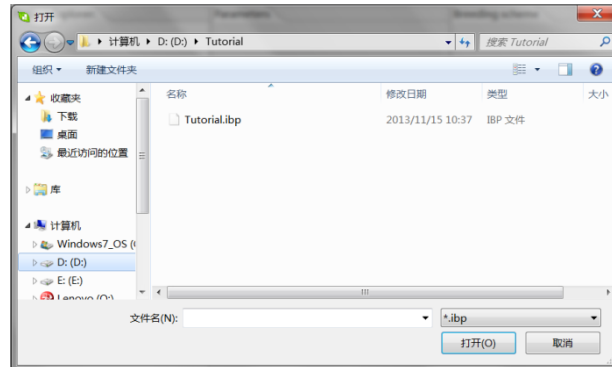


*Figure 1.10 Window for specifying Project Name (Tutorial for example) and Project Path*

Click **Open Project** in **Project menu** (Figure 1.11) to open an existing project in the given path in your system (Figure 1.12).



*Figure 1.11 Open Project button Project Menu*



*Figure 1.12 Window for specifying the path of an existing project*

## 1.6 Functionalities of Breeding Planner

Breeding Planner is designed to facilitate population development and testing in MARS, MABC, and MAS breeding programs. It is a computing system for planning Molecular Breeding, which consists of three components: (1) Planning tool of MARS (PMARS); (2) Planning tool of MABC (PMABC); and (3) Planning tool of MAS (PMAS). The system developed in this project assist the ongoing molecular breeding programs on track. These tools will be able to identify ‘robust’ breeding schemes, and enable breeders to develop design-led breeding schemes that will great improve the efficiency of their breeding efforts both in terms of genetic gain and economic benefit.

## Chapter 2. Planning tool of MARS (PMARS)

The MARS program consists of four stages. **1. Training population development:** Given that the MARS inter-mating will take place in 2014, PMARS will tell breeders when to do what from now to 2014 to reach the target, i.e. completion of the training population, and completion of genotyping and phenotyping of the training population. **2. QTL analysis and selection:** PMARS will tell breeders when to product QTL analysis, then do modeling and selection of QTLs to recombine. **3. Recombination:** Selection in the training population and inter-mating among selected lines will be conducted in this step. PMARS will conduct the selection to tell which lines in the training population should be selected for inter-mating (selection criteria provided by breeders), and propose a crossing plan for inter-mating. **4. Fixed line development:** repeated selfing will be conducted in this step to derive fixed lines. PMARS will propose optimum selection scheme for each generation of selfing, and predict the homozygosity of the breeding population. In conclusion, PMARS will provide breeders when to do what, and predict the recovery of the recurrent parent.

### 2.1 Make a new MARS program to the newly-built project

Click **Task-> New MARS** (Figure 2.1A) or right click in the blank area of project explorer, then choose **New MARS** (Figure 2.1B).

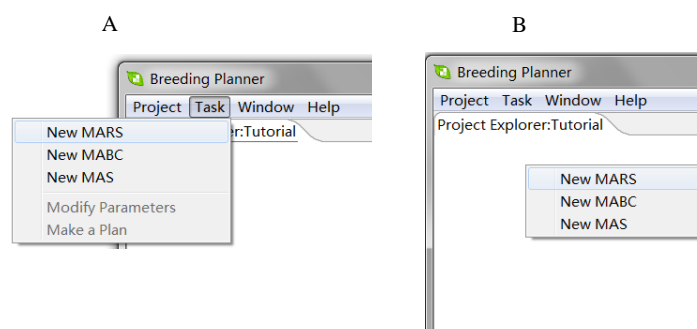


Figure 2.1 Window for making a new MARS program

### 2.2 Parameters required to define a MARS program

Users can use either way to set parameters for a MARS program (Figure 2.2):

- Import parameters from an external file
- Specify the initial parameters by hand

*Figure 2.2 Window for setting parameters for a new MARS program*

Below are more details on the required parameters.

## 1. Researcher information

- Country;
- Researcher's name;

## 2. Species information

- Crop species: Select one crop species from Cowpea, Rice, Wheat, Maize, Groundnuts, and Cassava
- Expected seeds per plant (or propagation rate) (the default values for different crops are given in Table 2.1)

*Table 2.1 Default numbers of expected seeds per plant for different crops*

Crop	Minimum number	Maximum number	Median (used as default in Breeding Planner)
Cowpea	10	300	50
Rice	50	300	200
Wheat	50	250	150
Maize	50	500	200
Groundnuts	30	200	80
Cassava	10	100	50

### **3. Greenhouse/offseason**

- Length of each season (months).

**Note:** We assume the crop can be grown across the whole year under the Greenhouse condition. That is, the next season can start in the same month when the previous season is harvested. So the planting time for each season is not needed.

### **4. Field condition**

- Seasons per year: the number of seasons per year, select from 1-3
- 1st season starts in: select a month
- 2nd season starts in: select a month, after the end of the 1st season
- 3rd season starts in: select a month, after the end of the 2nd season

**Note:** The crop cannot be grown across the whole year under the Field condition. So if multiple seasons are possible, the system asks for the planting time for each season.

### **5. Population development**

- Parental generation starts in: select the start time of the parental generation
- Generation for genotyping: select from F2 or F3
- Generation for phenotyping: select from F3-F6 and after the generation for genotyping
- Early generation growing condition: select either “Greenhouse/offseason” or “Field condition”

### **6. Multi-location phenotyping**

- Number of locations
- Replicates in each location: select from 1-4
- Plot length (m)
- Number of rows
- Individual plants per plot

### **7. Rounds of selfing:** select from 1-5

When all parameters are set, the parameters will appear in the Parameter Viewing Window and the flowchart will appear in the Breeding Scheme Window (Figure 2.3).

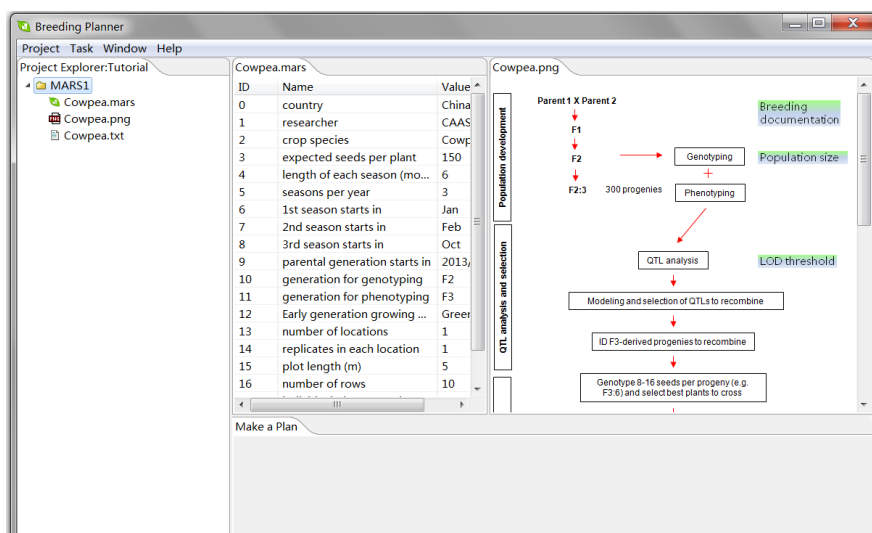


Figure 2.3 Interface of PMARS

It should be noted that:

- Expected seeds per plant in Breeding Planner will be used to calculate if there are enough seeds for phenotyping. If not, additional seed increase (by selfing) will be requested.
- The user input must fall into the min-max range for the selected crop! Otherwise, when the input number is smaller than the minimum number, the minimum number will be assumed. When the input number is greater than the maximum number, the maximum number will be assumed.
- The number of seeds required is calculated from settings for “Multi-locational phenotyping”. Say, when genotyping is conducted in F2, multi-locational phenotyping is only possible when each F2 plant can give enough seeds. Otherwise, phenotyping will be delayed until the required seeds are produced.

## 2.3 Modify parameters

Right click the MARS1 folder (Figure 2.4A) then choose **Modify Parameters**, or click **Task-> Modify Parameters** (Figure 2.4B).

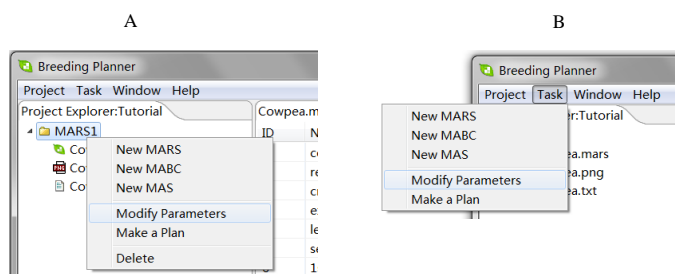


Figure 2.4 Button for modifying parameters

Window for modifying parameters is similar to the window for setting parameters

(Figure 2.5).

**Modify Parameters**

**Input Information**

Import file

**Researcher information**  
Country:   
Researcher's name:

**Greenhouse/offseason**  
Length of each season (months):

**Species information**  
Crop species:   
Expected seeds per plant:

**Field condition**  
Seasons per year:   
1st season starts in:   
2nd season starts in:   
3rd season starts in:

**Population development**  
Parental generation starts in:   
Generation for genotyping:   
Generation for phenotyping:   
Early generation growing condition:  
☒ Greenhouse/offseason ☐ Field condition

**Multi-location phenotyping**  
Number of locations:   
Replicates in each location:   
Plot length (m):   
Number of rows:   
Individual plants per plot:

Rounds of selfing after inter-mating:

*Figure 2.5 Window for modifying parameters*

## 2.4 Breeding Scheme Window

The flowchart of MARS is shown in the Breeding Scheme Window (Figure 2.6).

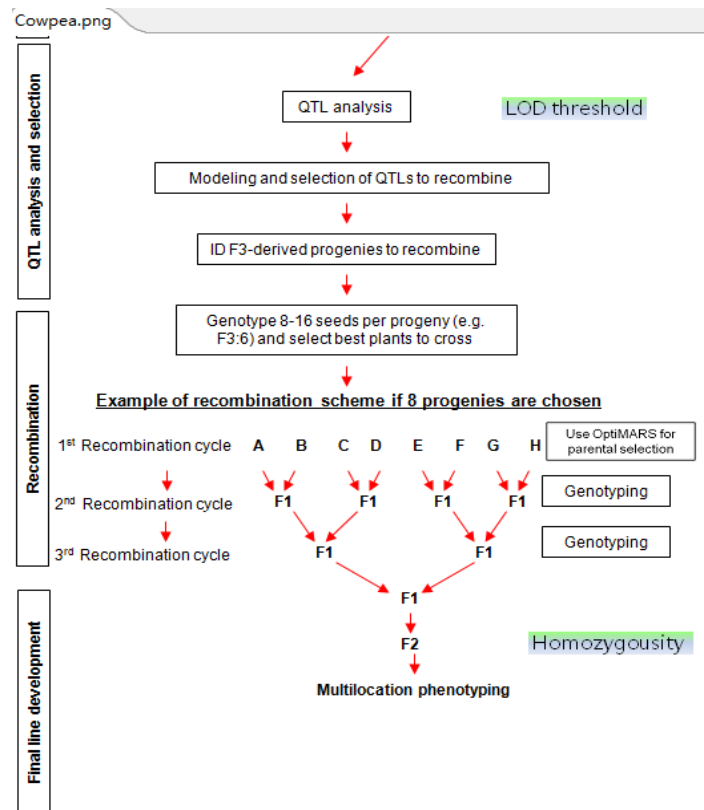
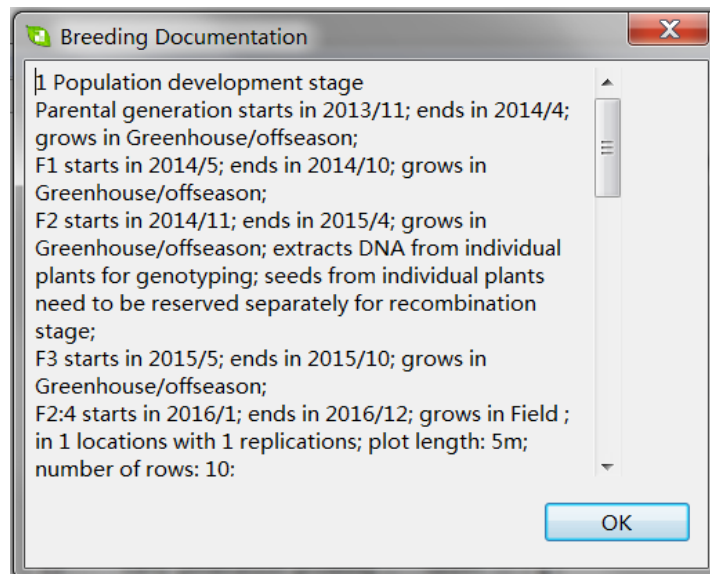


Figure 2.6 Flowchart in PMARS

There are also some documents and tables in this window:

- **Breeding Documentation:** The pure text describing the whole flow of the MARS program (Figure 2.7). There are four major stages, each consisting of several steps.
- **Population Size:** Help to decide on a suitable population size (Figure 2.8)
- **LOD Threshold:** Help to decide on a suitable threshold value in QTL mapping (Figure 2.9)
- **Homozygosity:** Help to learn the rate of homozygosity during repeated selfing (Figure 2.10)





*Figure 2.7 Breeding Documentation window*

**Population size**

Population sizes required to identify QTL at a detection power of P

PVE (%) of QTL	Marker density (MD) and detection power (P)					
	MD=5 cM		MD=10 cM		MD=20 cM	
	P=0.8	P=0.9	P=0.8	P=0.9	P=0.8	P=0.9
1	300	560	540	>600	>600	>600
2	160	300	280	320	360	460
3	110	200	180	200	220	280
4	100	160	140	180	200	240
5	80	140	120	140	160	200
10	50	80	70	80	80	100
20	40	60	50	60	60	80
30	40	40	40	40	40	60

OK

*Figure 2.8 Population Size window*

LOD threshold												
LOD threshold required to achieve an overall significance of $\alpha$ for two marker densities in three biparental populations												
Total genome size in cM	Marker density = 1 cM						Marker density = 20 cM					
	BC		RIL		F2		BC		RIL		F2	
	$\alpha=0.05$	$\alpha=0.01$	$\alpha=0.05$	$\alpha=0.01$	$\alpha=0.05$	$\alpha=0.01$	$\alpha=0.05$	$\alpha=0.01$	$\alpha=0.05$	$\alpha=0.01$	$\alpha=0.05$	$\alpha=0.01$
250	2.24	3.02	2.44	3.22	3.06	3.88	1.86	2.64	1.96	2.63	2.59	3.35
500	2.51	3.31	2.72	3.5	3.36	4.18	2.13	2.92	2.24	2.91	2.89	3.65
750	2.68	3.47	2.89	3.67	3.54	4.36	2.3	3.09	2.4	3.07	3.07	3.82
1000	2.8	3.59	3	3.79	3.66	4.49	2.41	3.21	2.51	3.19	3.19	3.95
1250	2.89	3.68	3.09	3.88	3.76	4.58	2.5	3.3	2.6	3.28	3.29	4.05
1500	2.96	3.76	3.17	3.95	3.84	4.66	2.57	3.37	2.68	3.36	3.37	4.13
1750	3.02	3.82	3.23	4.02	3.91	4.73	2.64	3.44	2.74	3.42	3.44	4.19
2000	3.08	3.88	3.29	4.07	3.96	4.79	2.69	3.49	2.8	3.47	3.49	4.25
2250	3.13	3.93	3.34	4.12	4.01	4.84	2.74	3.54	2.84	3.52	3.55	4.3
2500	3.17	3.97	3.38	4.17	4.06	4.88	2.78	3.58	2.89	3.57	3.59	4.35
2750	3.21	4.01	3.42	4.21	4.1	4.93	2.82	3.62	2.92	3.61	3.63	4.39
3000	3.24	4.05	3.45	4.24	4.14	4.96	2.86	3.66	2.96	3.64	3.67	4.43
3250	3.28	4.08	3.49	4.27	4.17	5	2.89	3.69	2.99	3.67	3.71	4.46
3500	3.31	4.11	3.52	4.31	4.21	5.03	2.92	3.72	3.02	3.71	3.74	4.49
3750	3.34	4.14	3.55	4.33	4.24	5.06	2.95	3.75	3.05	3.73	3.77	4.52
4000	3.36	4.16	3.57	4.36	4.26	5.09	2.97	3.78	3.08	3.76	3.8	4.55

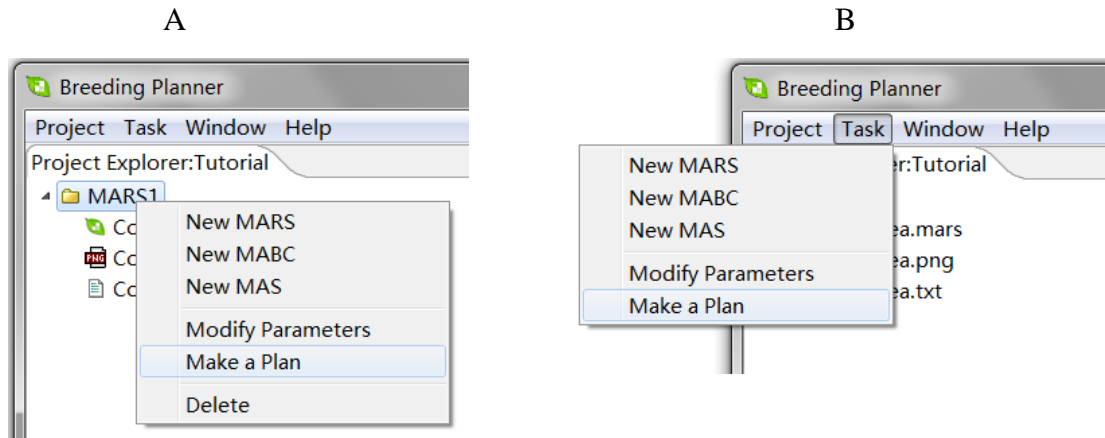
Figure 2.9 LOD Threshold window

Homzygosity								
Rate (%) of homozygosity during repeated selfing								
Generation	Number of independent loci (higher value for linkage)							
	1	2	3	4	5	10	20	30
F2	50.00	25.00	12.50	6.25	3.13	0.10	0.00	0.00
F3	75.00	56.25	42.19	31.64	23.73	5.63	0.32	0.02
F4	87.50	76.56	66.99	58.62	51.29	26.31	6.92	1.82
F5	93.75	87.89	82.40	77.25	72.42	52.45	27.51	14.43
F6	96.88	93.85	90.91	88.07	85.32	72.80	52.99	38.58
F7	98.44	96.90	95.39	93.89	92.43	85.43	72.98	62.35
F8	99.22	98.44	97.67	96.91	96.15	92.46	85.48	79.03
F9	99.61	99.22	98.83	98.45	98.06	96.16	92.47	88.92
F10	99.80	99.61	99.42	99.22	99.03	98.06	96.17	94.30

Figure 2.10 Homozygosity window

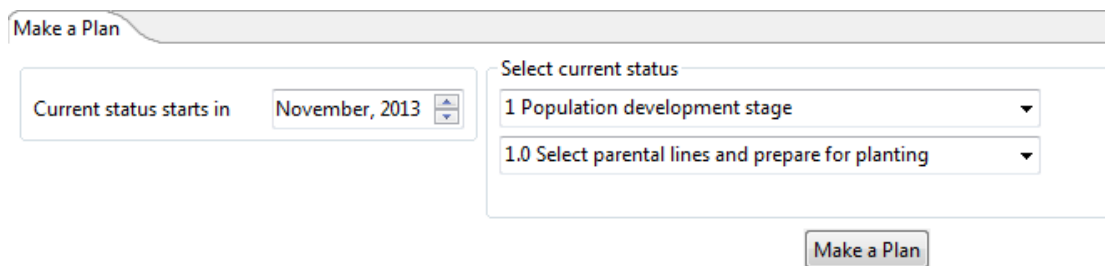
## 2.5 Plan-making Window

Right click the **MARS** folder in the **Project Window** (Figure 2.11A), then choose **Make a Plan**, or click **Task-> Make a Plan** (Figure 2.11B).



*Figure 2.11 Button for Make a Plan*

The window for making a plan will be initialized. Some dialog information will appear in the Plan-making Window (Figure 2.12).



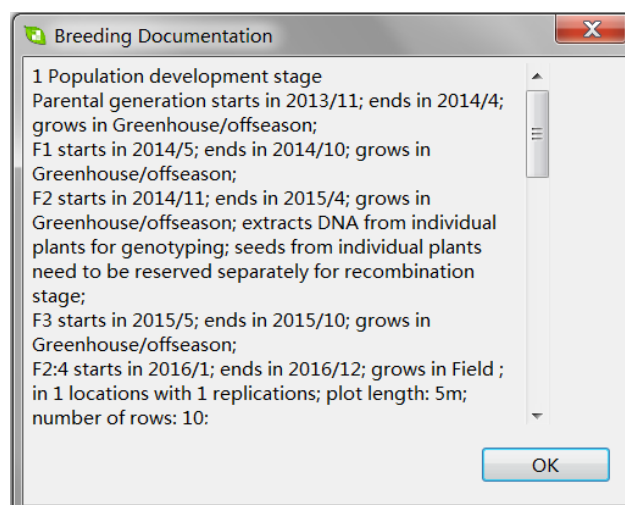
*Figure 2.12 Plan-making Window*

Two inputs are needed in this window:

- Tell the system when the current season starts. The system will know the current season is grown in Greenhouse or in Field from the breeding parameters you specified.
- Tell the system where you are by selecting:
  1. Population development stage
    - 1.0 Select parental lines and prepare for planting
      - 1.1 Parental lines is growing
      - 1.2 F1 generation is growing
      - 1.3 F2 generation is growing
      - 1.4 F3 generation is growing
      - 1.5 F4 generation is growing
    2. QTL analysis and selection stage
      - 2.1 Genotypic and phenotypic data collection
      - 2.2 QTL analysis
      - 2.3 Selecting parental lines for inter-mating

- 3. Recombination stage
  - 3.1 Two-way inter-mating (or single cross)
  - 3.2 Four-way inter-mating (or double cross)
  - 3.3 Eight-way inter-mating (or double double cross)
- 4. Final line development stage
  - 4.0 Completion of inter-mating
  - 4.1 Self pollination F2 is growing
  - 4.2 Self pollination F3 is growing

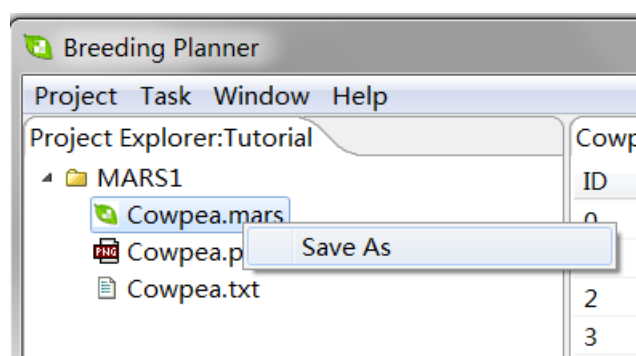
Then click the **Make a Plan** button to complete the on-going MARS breeding program. New window will show the **Breeding Documentation** for the remaining status, and the time to complete the MARS breeding program (Figure 2.13).



*Figure 2.13 Breeding Documentation for Plan-making*

## 2.6 Save the results

Right click the file name, and then select **Save As** to save the breeding documentation on your computer (Figure 2.14), and then specify the path and rename of the output file.



*Figure 2.14 Button for Save As*

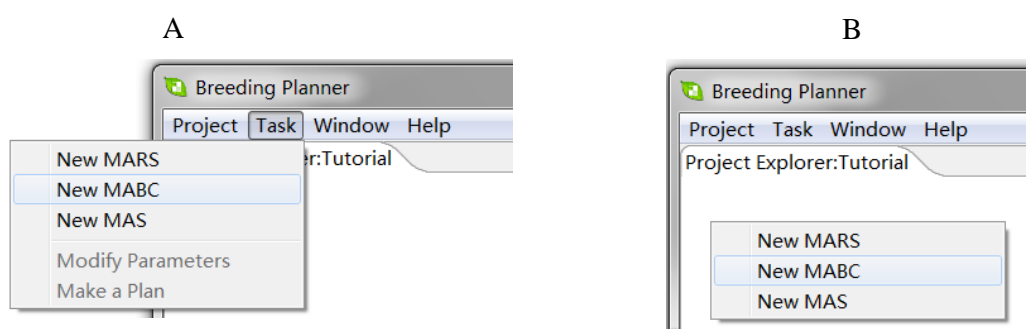


## Chapter 3. Planning tool of MABC (PMABC)

PMABC tool provides breeders times of backcrossing, population size in each generation, and how MABC is conducted etc, and predict the recovery of the recurrent parent. Given the genes in pyramiding, and their distribution in parental lines, the tool will provide the optimum number of backcrossing and population size in each generation.

### 3.1 Make a new MABC program to the newly-built project

Click **Task-> New MABC** (Figure 3.1A) or right click in the blank area of project explorer, then choose **New MABC** (Figure 3.1B).



*Figure 3.1 Window for making a new MABC program*

### 3.2 Parameters required to define a MABC program

Users can use either way to set parameters for a MABC program (Figure 3.2):

- Import parameters from an external file
- Specify the initial parameters by hand

*Figure 3.2 Window for setting parameters for a new MABC program*

Below are more details on the required parameters.

### 1. Researcher information

- Country;
- Researcher's name;

### 2. Species information

- Crop species: Select one crop species from Cowpea, Rice, Wheat, Maize, Groundnuts, and Cassava
- Expected seeds per plant (or propagation rate) (the suggested values for different crops are given in Table 2.1)

### 3. Greenhouse/offseason

- Length of each season (months).

**Note:** We assume the crop can be grown across the whole year under the Greenhouse condition. That is, the next season can start in the same month when the previous season is harvested. So the planting time for each season is not needed.

### 4. Field condition

- Seasons per year: the number of seasons per year, select from 1-3
- 1st season starts in: select a month
- 2nd season starts in: select a month, after the end of the 1st season
- 3rd season starts in: select a month, after the end of the 2nd season

**Note:** The crop cannot be grown across the whole year under the Field condition. So if multiple seasons are possible, the system asks for the planting time for each season.

## 5. Growing condition

- Parental generation starts in: select the start time of the parental generation
- Early generation growing condition: select one case “Greenhouse/offseason” or “Field condition”

## 6. Backcross and self generations

- Backcrossing generations: 1-3
- Repeated selfing generations: 1-3

When all parameters are set, the parameters will appear in the Parameter Viewing Window and the flowchart will appear in the Breeding Scheme Window (Figure 3.3).

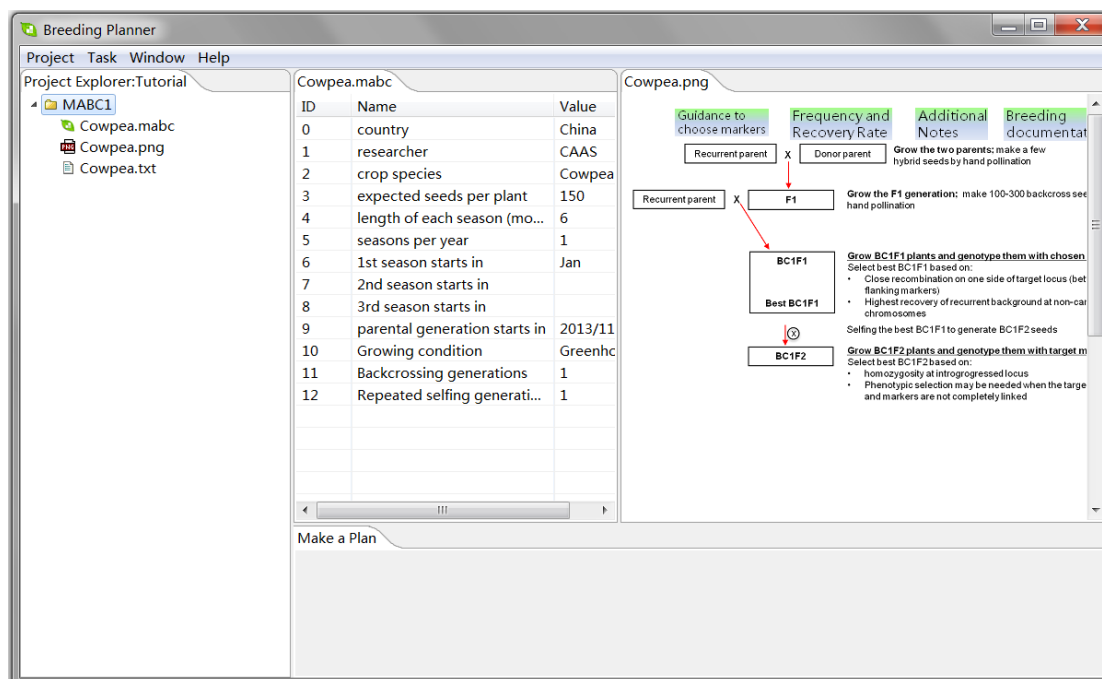


Figure 3.3 Interface of PMABC

It should be noted that:

- Expected seeds per plant in Breeding Planner will be used to calculate if there are enough seeds for phenotyping. If not, additional seed increase (by selfing) will be requested.



- The user input must fall into the min-max range for the selected crop! Otherwise, when the input number is smaller than the minimum number, the minimum number will be assumed. When the input number is greater than the maximum number, the maximum number will be assumed.
- The number of seeds required is calculated from settings for “Multi-locational phenotyping”. Say, when genotyping is conducted in F2, multi-locational phenotyping is only possible when each F2 plant can give enough seeds. Otherwise, phenotyping will be delayed until the required seeds are produced.

### 3.3 Modify parameters

Right click the MABC1 folder (Figure 3.4A) then choose **Modify Parameters**, or click **Task-> Modify Parameters** (Figure 3.4B).

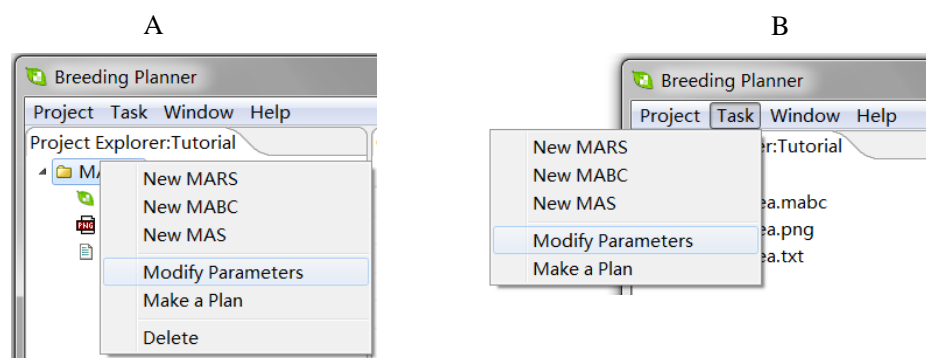


Figure 3.4 Button for modifying parameters

Window for modifying parameters is similar to the window for setting parameters (Figure 3.5).

The 'Modify Parameters' window is a dialog box with a title bar and a close button. It contains several sections of input fields:
 

- Input Information:** A text field for 'Import file' and a 'Browse' button.
- Researcher information:** 'Country' (dropdown menu set to 'China'), 'Researcher's name' (text field set to 'CAAS').
- Species information:** 'Crop species' (dropdown menu set to 'Cowpea'), 'Expected seeds per plant' (spin box set to '150').
- Field condition:** 'Seasons per year' (dropdown menu set to '1'), '1st season starts in' (dropdown menu set to 'Jan'), '2nd season starts in' (empty dropdown), '3rd season starts in' (empty dropdown).
- Growing condition:** 'Parental generation starts in' (calendar icon set to 'November, 2013'), 'Greenhouse/offseason' (radio button selected), 'Field condition' (radio button unselected).
- Backcross and self generations:** 'Backcrossing generations' (dropdown menu set to '1'), 'Repeated selfing generations' (dropdown menu set to '1').

 At the bottom right are 'Finish' and 'Cancel' buttons.

Figure 3.5 Window for modifying parameters

### 3.4 Breeding Scheme Window

The flowchart of MABC is shown in the Breeding Scheme Window (Figure 3.6).

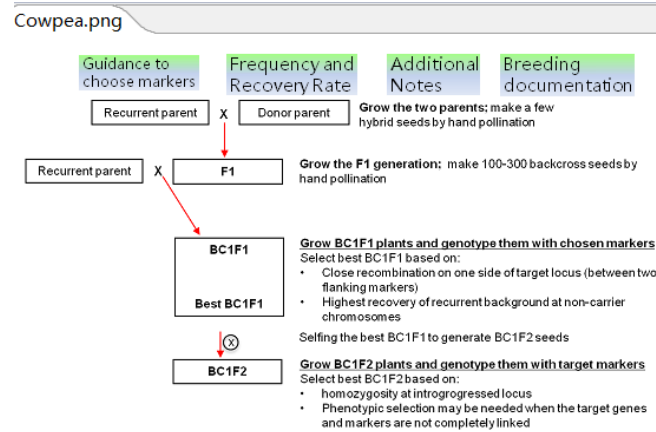


Figure 3.6 Flowchart in PMABC

There are also some documents and tables in this window:

- Breeding Documentation: The pure text describing the whole flow of the MABC program (Figure 3.7).
- Guidance to choose markers: help to choose markers to use in MABC process (Figure 3.8)
- Frequency and Recovery Rate: help to get the Rate (%) of homozygosity during repeated selfing (Figure 3.9)
- Additional Notes: help to conduct the MABC process (Figure 3.10)

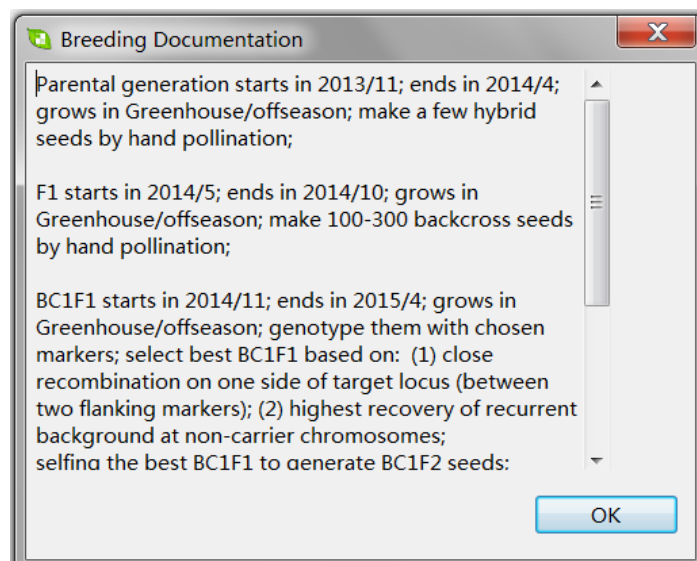


Figure 3.7 Breeding Documentation window

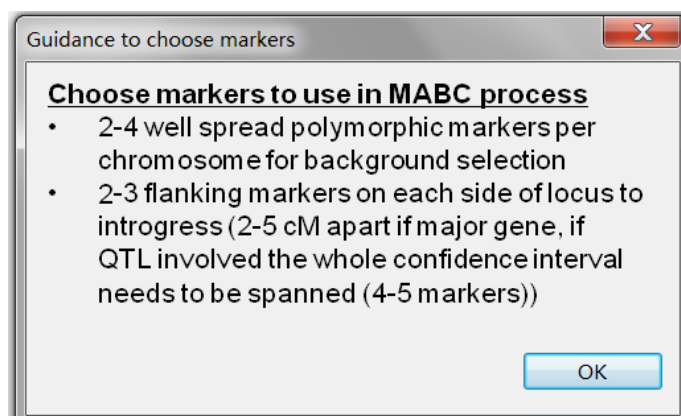
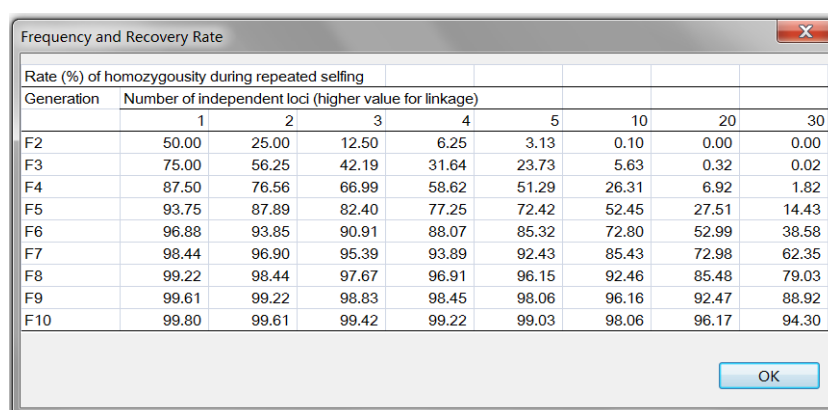


Figure 3.8 Guidance to choose markers window



Frequency and Recovery Rate

Rate (%) of homozygosity during repeated selfing								
Generation	Number of independent loci (higher value for linkage)							
	1	2	3	4	5	10	20	30
F2	50.00	25.00	12.50	6.25	3.13	0.10	0.00	0.00
F3	75.00	56.25	42.19	31.64	23.73	5.63	0.32	0.02
F4	87.50	76.56	66.99	58.62	51.29	26.31	6.92	1.82
F5	93.75	87.89	82.40	77.25	72.42	52.45	27.51	14.43
F6	96.88	93.85	90.91	88.07	85.32	72.80	52.99	38.58
F7	98.44	96.90	95.39	93.89	92.43	85.43	72.98	62.35
F8	99.22	98.44	97.67	96.91	96.15	92.46	85.48	79.03
F9	99.61	99.22	98.83	98.45	98.06	96.16	92.47	88.92
F10	99.80	99.61	99.42	99.22	99.03	98.06	96.17	94.30

OK

Figure 3.9 Frequency and Recovery Rate window

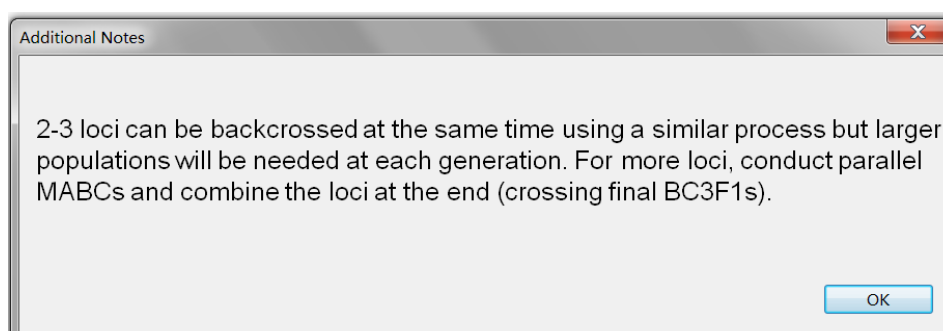
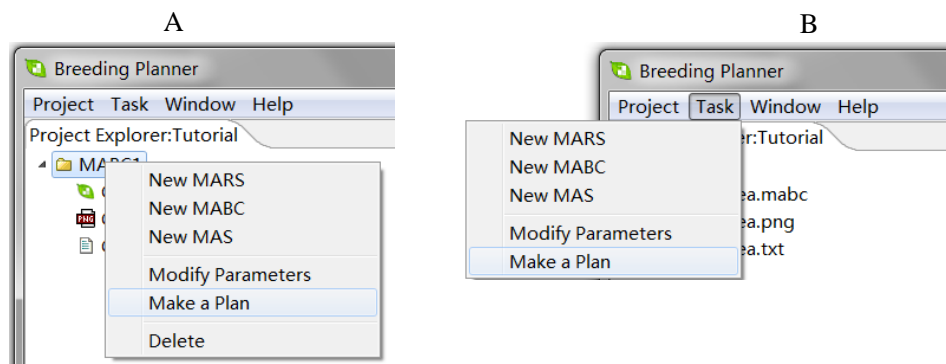


Figure 3.10 Additional Notes window

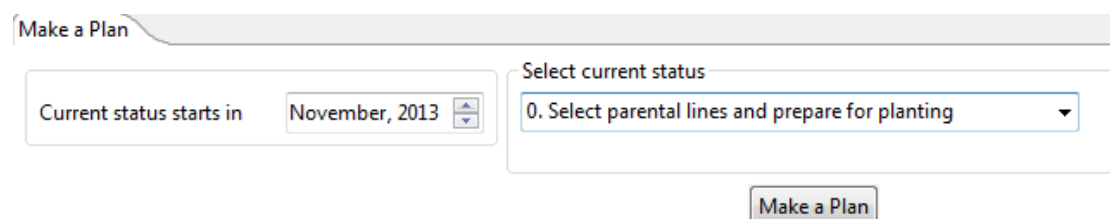
### 3.5 Plan-making Window

Right click the **MABC** folder in the **Project Window** (Figure 3.11A), then choose **Make a Plan**, or click **Task-> Make a Plan** (Figure 3.11B).



*Figure 3.11 Button for Make a Plan*

The window for making a plan will be initialized. Some dialog information will appear in the Plan-making Window (Figure 3.12).

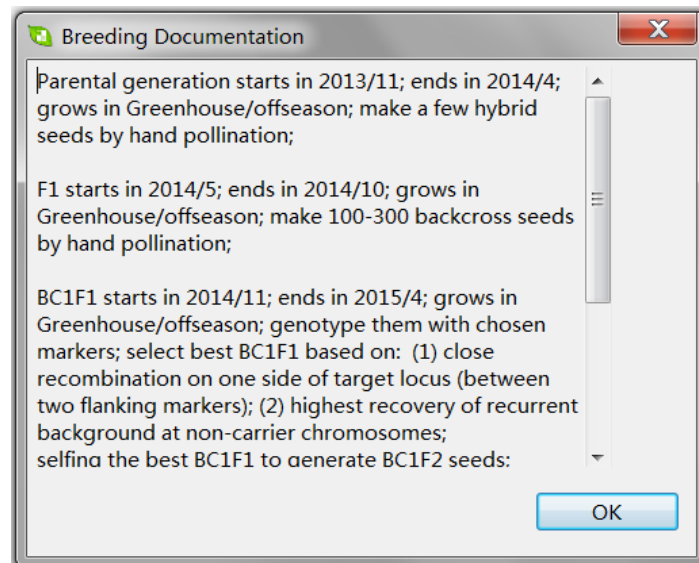


*Figure 3.12 Plan-making Window*

Two inputs are needed in this window:

- Tell the system when the current season starts. The system will know the current season is grown in Greenhouse or in Field from the breeding parameters you specified.
- Tell the system where you are by selecting:
  0. Select parental lines and prepare for planting
  1. Parental lines is growing
  2. F1 generation is growing
  3. BC1F1 generation is growing
  4. BC2F1 generation is growing
  5. BC3F1 generation is growing
  6. BC3F2 generation is growing
  7. BC3F3 generation is growing
  8. BC4F4 generation is growing

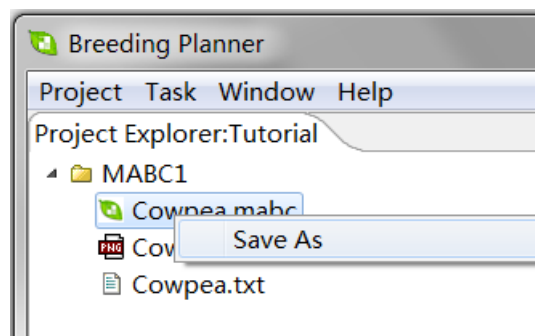
Then click the **Make a Plan** button to complete the on-going MABC breeding program. New window will show the **Breeding Documentation** for the remaining status, and the time to complete the MABC breeding program (Figure 3.13).



*Figure 3.13 Breeding Documentation for Plan-making*

### 3.6 Save the results

Right click the file name, and then select **Save As** to save the breeding documentation on your computer (Figure 3.14), and then specify the path and rename of the output file.



*Figure 3.14 Button for Save As*

## Chapter 4. Planning tool of MAS (PMAS)

PMAS provides breeders efficient crossing and selection schemes when integrating marker-assisted selection into conventional breeding programs. Given the genes in pyramiding, and their distribution in parental lines, the tool will provide the optimum crossing and selection schemes maximizing the genetic gain and cost-efficiency.

### 4.1 Make a new MAS program to the newly-built project

Click **Task-> New MAS** (Figure 4.1A) or right click in the blank area of project explorer, then choose **New MAS** (Figure 4.1B).

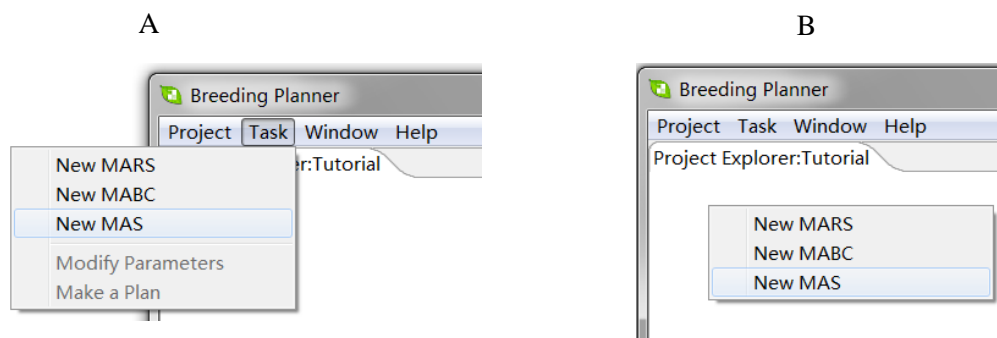
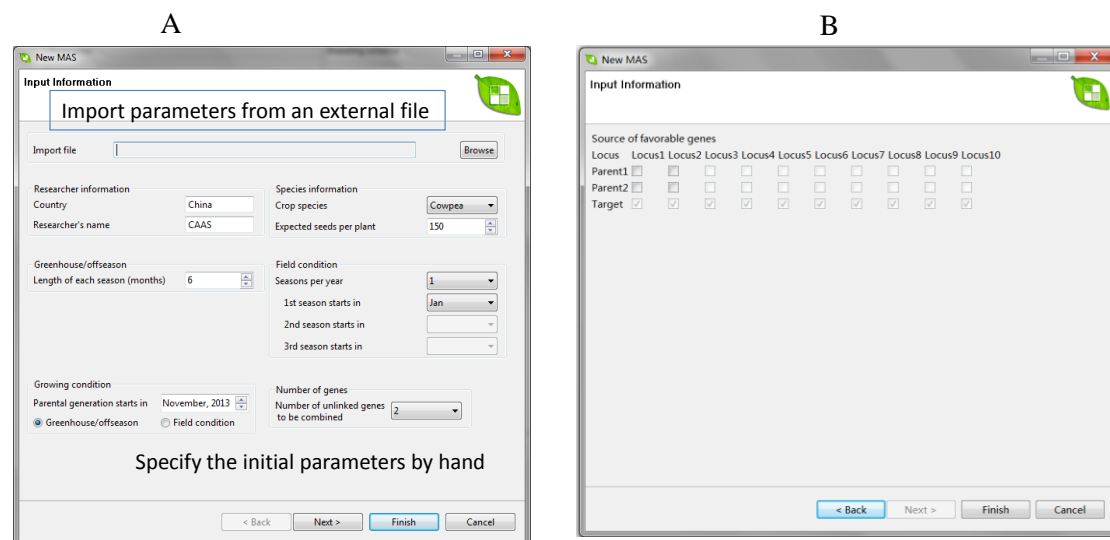


Figure 4.1 Window for making a new MAS program

### 4.2 Parameters required to define a MAS program

There are two pages of input parameters (Figure 4.2A and B). Users can use either way to set parameters for a MAS program:

- Import parameters from an external file
- Specify the initial parameters by hand



*Figure 4.2 Window for setting parameters for a new MAS program*

Below are more details on the required parameters.

### **1. Researcher information**

- Country;
- Researcher's name;

### **2. Species information**

- Crop species: Select one crop species from Cowpea, Rice, Wheat, Maize, Groundnuts, and Cassava
- Expected seeds per plant (or propagation rate) (the suggested values for different crops are given in Table 2.1)

### **3. Greenhouse/offseason**

- Length of each season (months).

**Note:** We assume the crop can be grown across the whole year under the Greenhouse condition. That is, the next season can start in the same month when the previous season is harvested. So the planting time for each season is not needed.

### **4. Field condition**

- Seasons per year: the number of seasons per year, select from 1-3
- 1st season starts in: select a month
- 2nd season starts in: select a month, after the end of the 1st season
- 3rd season starts in: select a month, after the end of the 2nd season

**Note:** The crop cannot be grown across the whole year under the Field condition. So if multiple seasons are possible, the system asks for the planting time for each season.

### **5. Growing condition**

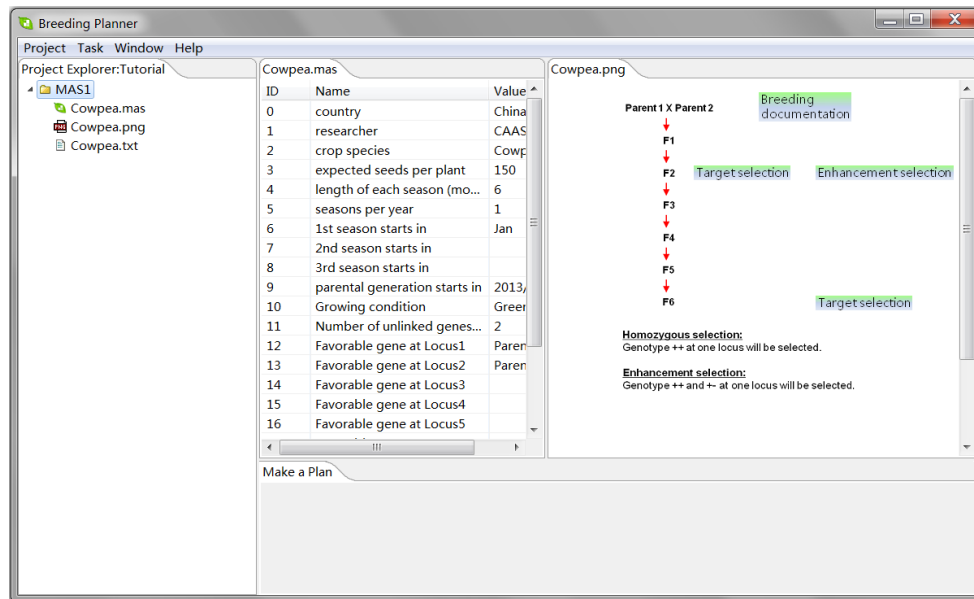
- Parental generation starts in: select the start time of the parental generation
- Early generation growing condition: select one case "Greenhouse/offseason" or "Field condition"

### **6. Number of genes**

- Number of unlinked genes to be combined: 2-10

### **7. Source of favorable genes:** select the favorable genes in the two parents

When all parameters are set, the parameters will appear in the Parameter Viewing Window and the flowchart will appear in the Breeding Scheme Window (Figure 4.3).



*Figure 4.3 Interface of PMAS*

It should be noted that:

- Expected seeds per plant in Breeding Planner will be used to calculate if there are enough seeds for phenotyping. If not, additional seed increase (by selfing) will be requested.
- The user input must fall into the min-max range for the selected crop! Otherwise, when the input number is smaller than the minimum number, the minimum number will be assumed. When the input number is greater than the maximum number, the maximum number will be assumed.
- The number of seeds required is calculated from settings for “Multi-locational phenotyping”. Say, when genotyping is conducted in F2, multi-locational phenotyping is only possible when each F2 plant can give enough seeds. Otherwise, phenotyping will be delayed until the required seeds are produced.

### 4.3 Modify parameters

Right click the MAS1 folder (Figure 4.4A) then choose **Modify Parameters**, or click **Task-> Modify Parameters** (Figure 4.4B).



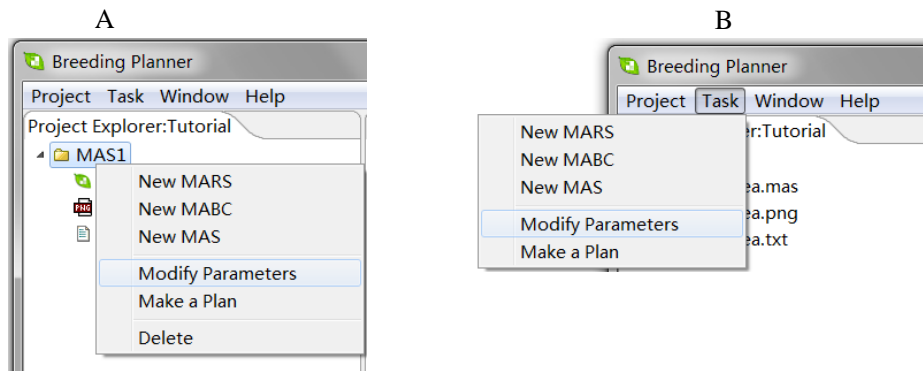


Figure 4.4 Button for modifying parameters

Window for modifying parameters is similar to the window for setting parameters (Figure 4.5).

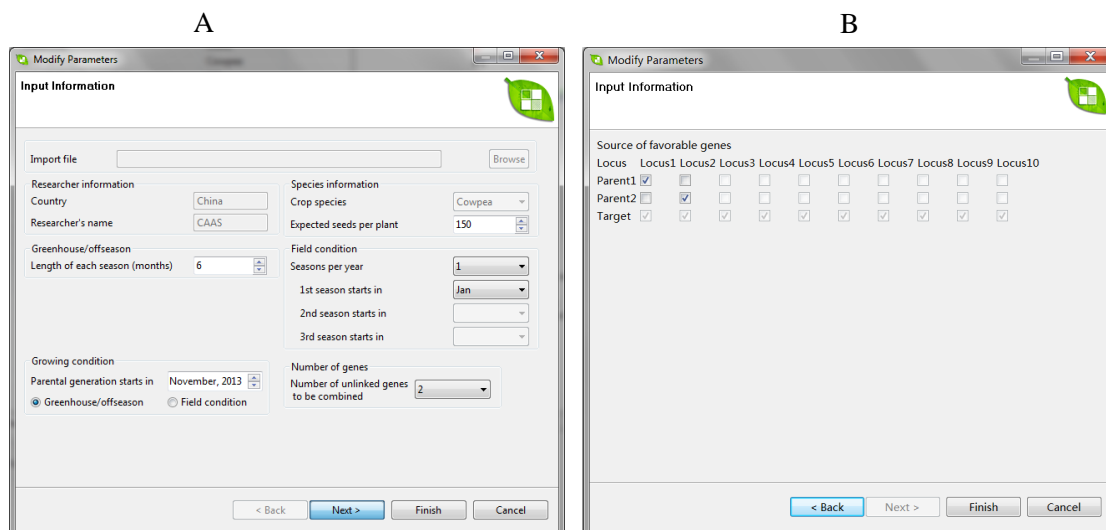
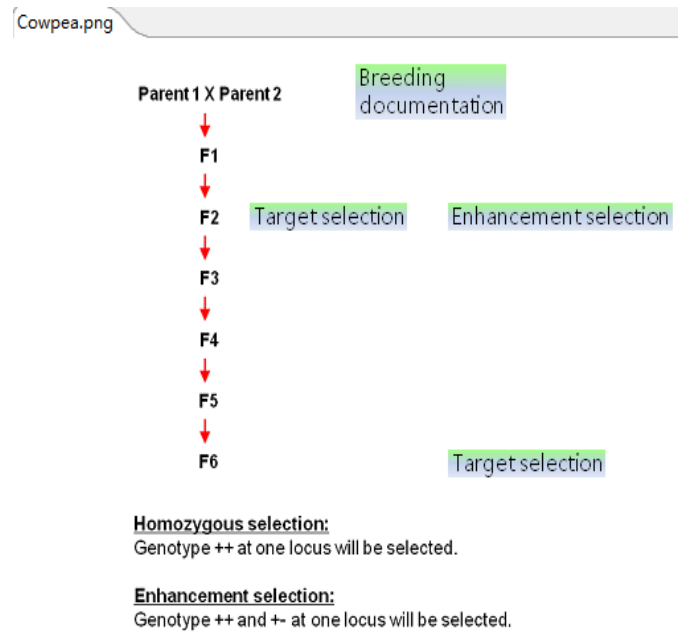


Figure 4.5 Window for modifying parameters

#### 4.4 Breeding Scheme Window

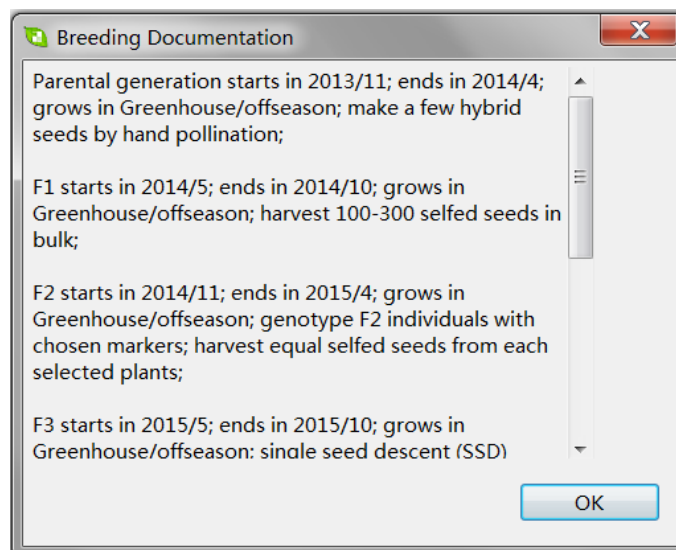
The flowchart of MAS is shown in the Breeding Scheme Window (Figure 4.6).



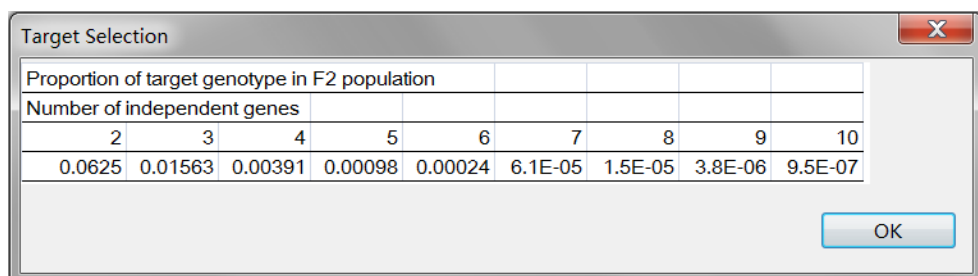
*Figure 4.6 Flowchart in PMAS*

There are also some documents and tables in this window:

- Breeding Documentation: The pure text describing the whole flow of the MAS program (Figure 4.7).
- Target Selection: Show the proportion of target genotype in F2 population (Figure 4.8)
- Enhancement Selection: show the proportion for enhancement selection (Figure 4.9)
- Target Selection 2: show the proportion of target genotype when enhancement selection is applied in F2 population (Figure 4.10)



*Figure 4.7 Breeding Documentation window*

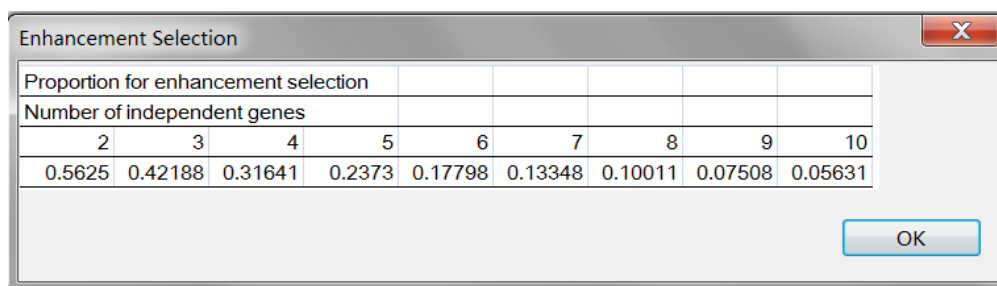


Target Selection

Proportion of target genotype in F2 population									
Number of independent genes									
2	3	4	5	6	7	8	9	10	
0.0625	0.01563	0.00391	0.00098	0.00024	6.1E-05	1.5E-05	3.8E-06	9.5E-07	

OK

Figure 4.8 The first Target Selection window

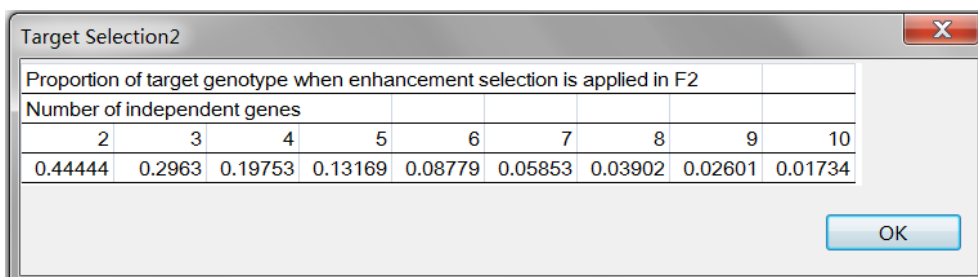


Enhancement Selection

Proportion for enhancement selection									
Number of independent genes									
2	3	4	5	6	7	8	9	10	
0.5625	0.42188	0.31641	0.2373	0.17798	0.13348	0.10011	0.07508	0.05631	

OK

Figure 4.9 Enhancement Selection window



Target Selection2

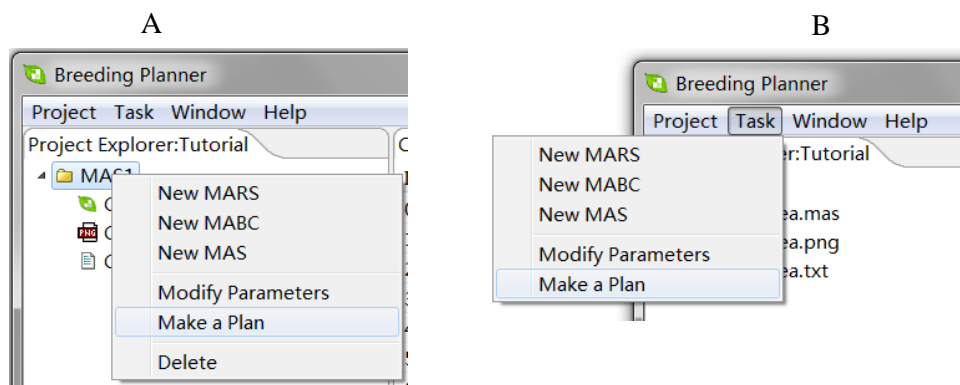
Proportion of target genotype when enhancement selection is applied in F2									
Number of independent genes									
2	3	4	5	6	7	8	9	10	
0.44444	0.2963	0.19753	0.13169	0.08779	0.05853	0.03902	0.02601	0.01734	

OK

Figure 4.10 The second Target Selection window

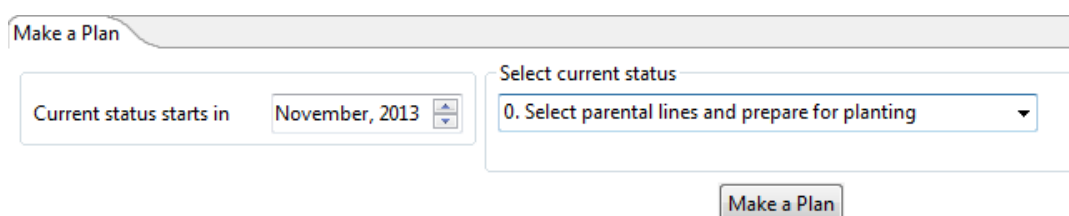
## 4.5 Plan-making Window

Right click the **MAS** folder in the **Project Window** (Figure 4.11A), then choose **Make a Plan**, or click **Task-> Make a Plan** (Figure 4.11B).



*Figure 4.11 Button for Make a Plan*

The window for making a plan will be initialized. Some dialog information will appear in the Plan-making Window (Figure 4.12).

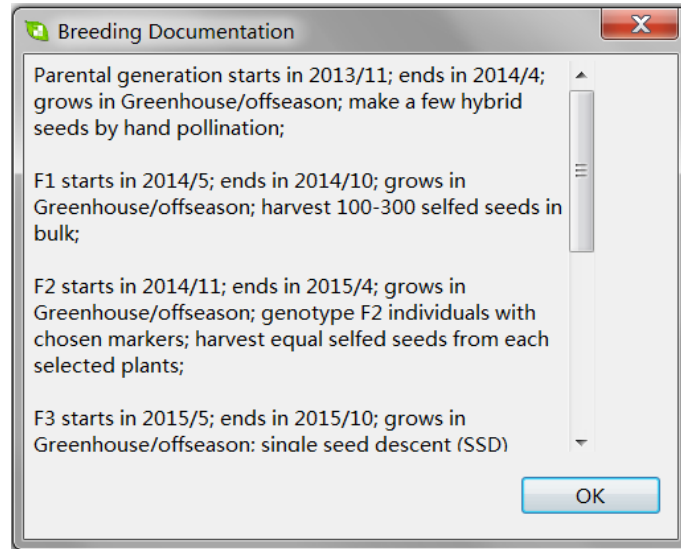


*Figure 4.12 Plan-making Window*

Two inputs are needed in this window:

- Tell the system when the current season starts. The system will know the current season is grown in Greenhouse or in Field from the breeding parameters you specified.
- Tell the system where you are by selecting:
  0. Select parental lines and prepare for planting
  1. Parental lines is growing
  2. F1 generation is growing
  3. F2 generation is growing
  4. F3 generation is growing
  5. F4 generation is growing
  6. F5 generation is growing
  7. F6 generation is growing

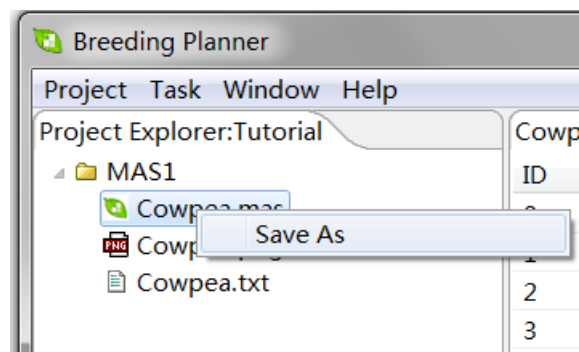
Then click the **Make a Plan** button to complete the on-going MAS breeding program. New window will show the **Breeding Documentation** for the remaining status, and the time to complete the MAS breeding program (Figure 4.13).



*Figure 4.13 Breeding Documentation for Plan-making*

#### 4.6 Save the results

Right click the file name, and then select **Save As** to save the breeding documentation on your computer (Figure 4.14), and then specify the path and rename of the output file.



*Figure 4.14 Button for Save As*