Tutorial of the Breeding Planner (BP) for Marker Assisted Recurrent Selection (MARS)

BP system consists of three tools relevant to molecular breeding.

- MARS: Marker Assisted Recurrent Selection
- MABC: Marker Assisted Backcrossing
- MAS: Marker Assisted Selection for pyramiding multiple genes

- This tutorial is designed for MARS

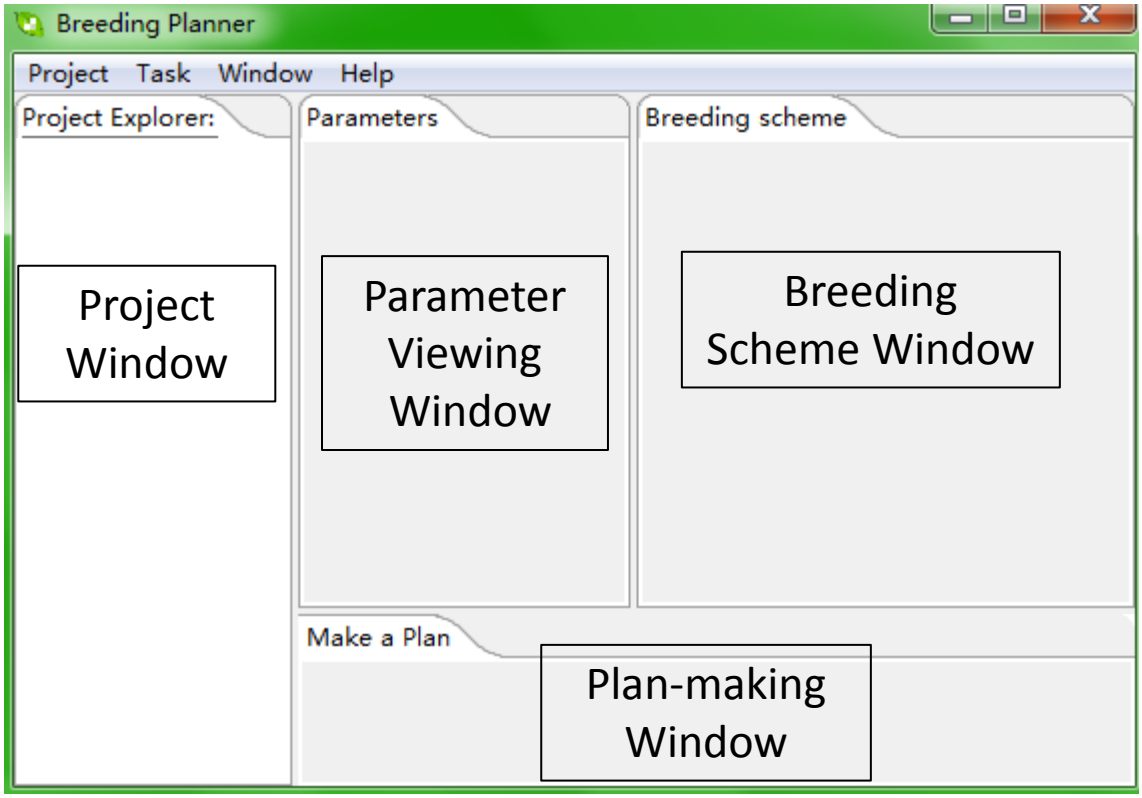
August 2013

Please make sure you have installed Java jdk 1.6 or 1.7 in your computer!

Open the Breeder Planner (BP) software

1. **Open the software:** The BP software can be opened by double click the **software icon** in your computer desktop

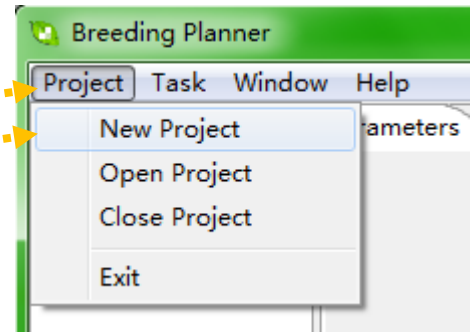
2. **Overview of the software.** The screen is split into four window.
   - **Project Window:** List all molecular breeding programs you have planned. Three distinct breeding programs can be considered: MARS, MABC and MAS.
   - **Parameter Viewing Window:** You can view your breeding parameters in this 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.



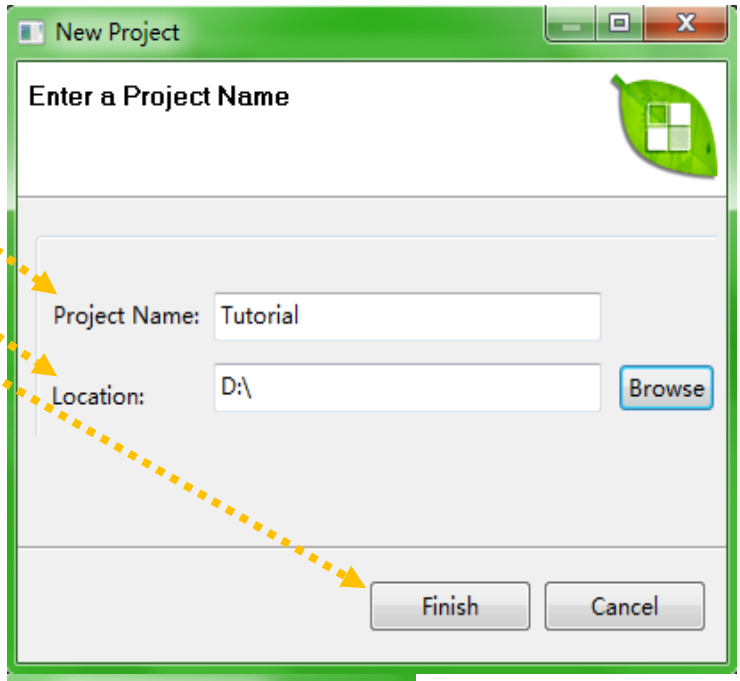
Build a new project

3. Build a new project

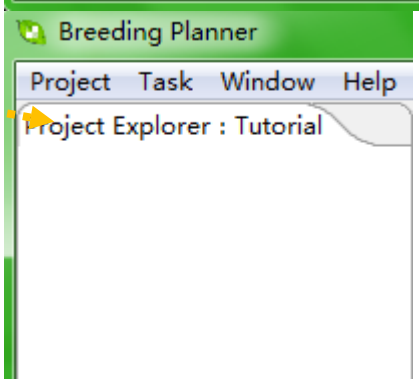
- Click **Project** menu
- Choose **New Project**



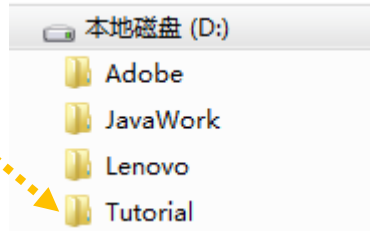
- Specify Project Name (Tutorial for example) and Project Path (D:\ for example)
- Click **Finish** to complete



New project is displayed in the **Project Window**



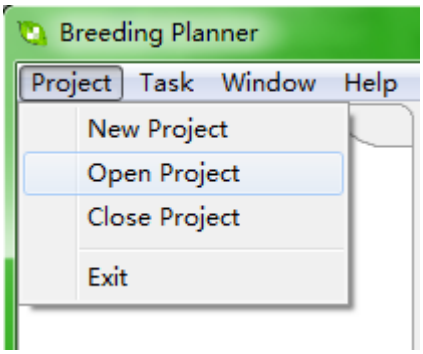
All inputs and outputs will be automatically saved in path of the project **(D:\Tutorial\... for example)**



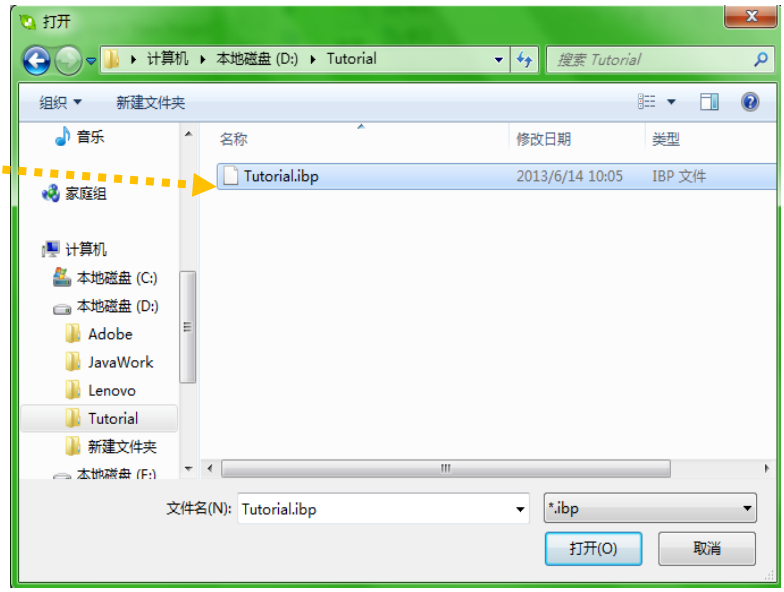
Open an existing project

4. Open an existing project

- Click **Project** menu
- Choose **Open Project**



- Choose an existing project in your computer

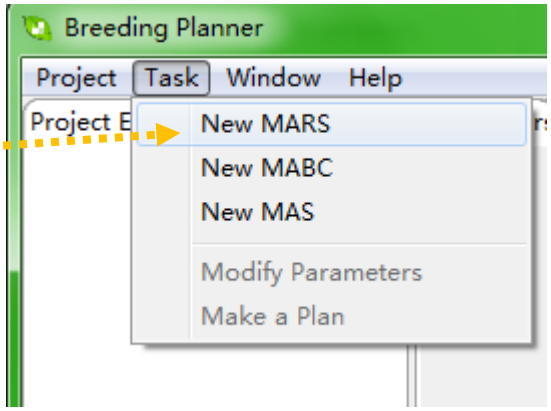


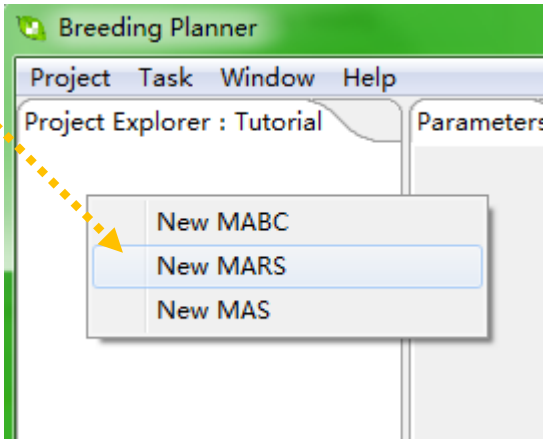
- **Please note:** The BP system is project-based. When you first use the system, you need to build a new project first. Then you can make various breeding programs. When you leave the system, the system automatically save the jobs you have done. The next time, you can start from a new project, or from an existing project.

PMARS Tutorial: Start a new MARS breeding program

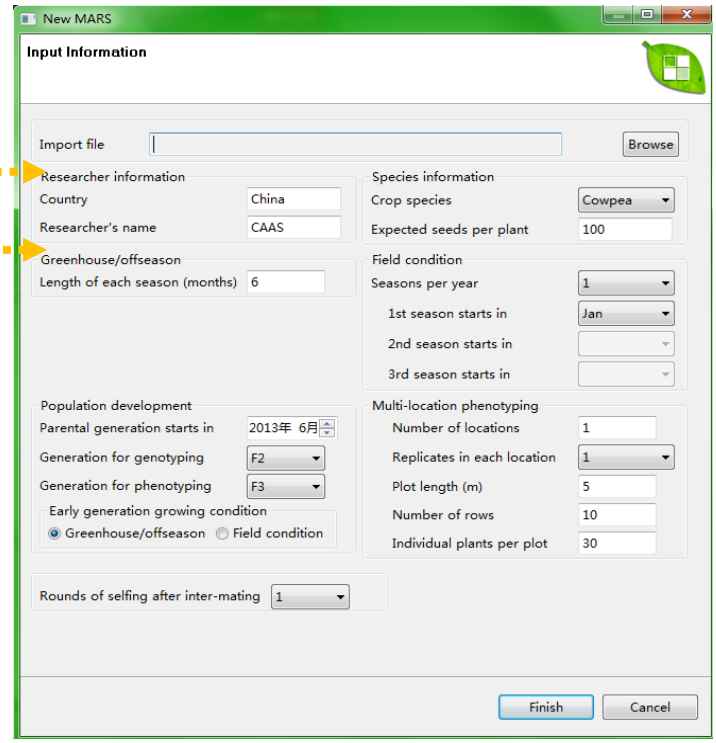
5. Make a new MARS program to the newly-built project

- Click **Task-> New MARS**
- Or right click in the blank area of project explorer, then choose **New MARS**





- You can use either way to make a MARS program
  - Import parameters from an external file
  - Or specify the initial parameters by hand



PMARS Tutorial: Parameters required to define a MARS program

Users have to specify a set of parameters before a MARS scheme can be given by the system. 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) **(more on the next page)**

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

Minimum and maximum numbers of seeds per plant for each crop under optimum or normal conditions

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

Notes:

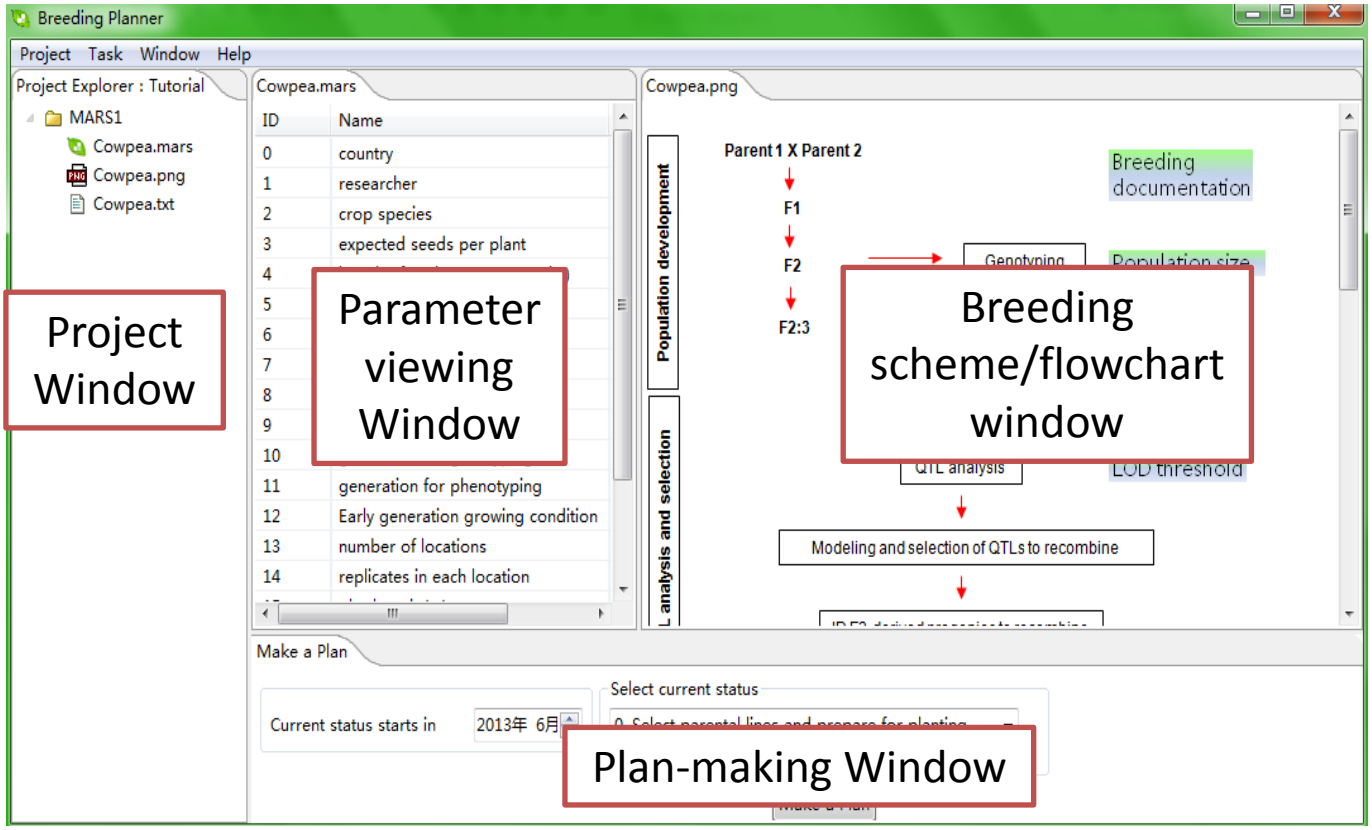
- Expected seeds per plant in Breeding Planner will be used to calculate if there are enough seeds for phenotyping. If not, additional seed increase (by selfing) will be requested.
- The user input must fall into the min-max range for the selected crop! Otherwise, when the input number is smaller than 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.

PMARS Tutorial: The interface

6. **Overview of the MARS functionality:** There are four windows in MARS functionality

- **Project Window:** List all molecular breeding programs you have planned. Three distinct MB 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.

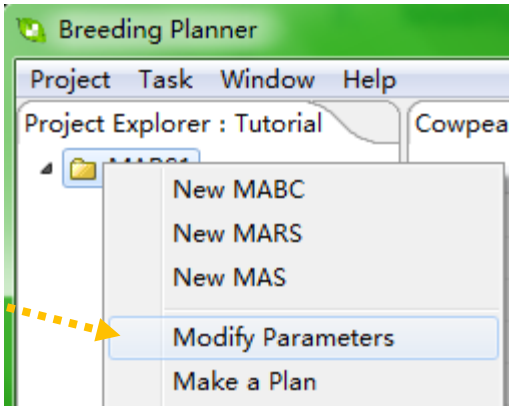
When the required parameters are set in the Parameter setting/viewing Window, the defined MARS breeding program is graphed in the Breeding Scheme/flowchart Window. A set of output files are listed in the Project Window.



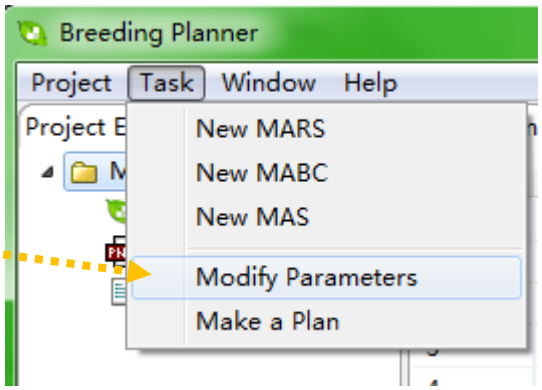
PMARS Tutorial: Modify your parameters

7. Modify Parameters

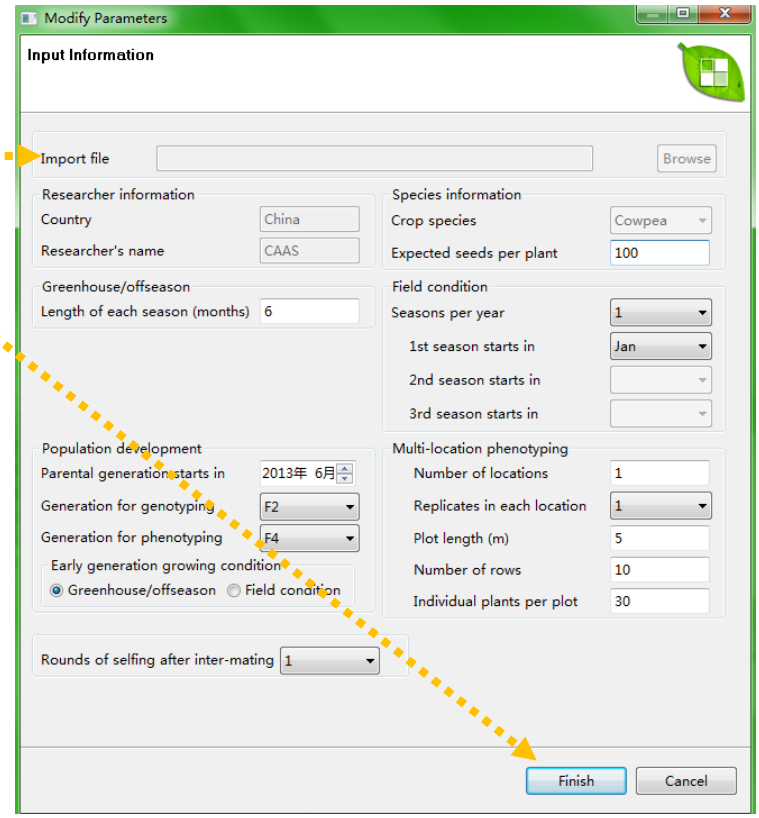
- Right click the MARS1 folder, then choose **Modify Parameters**



- Or click **Task-> Modify Parameters**



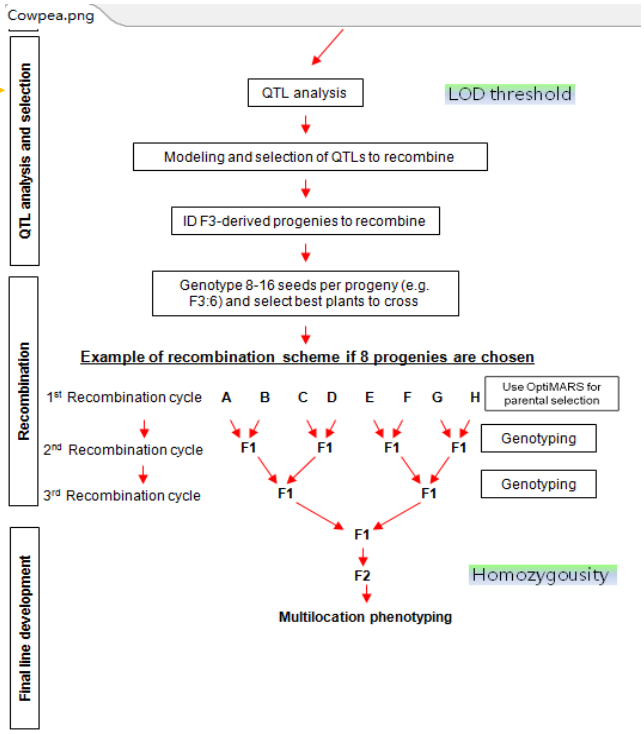
- You may modify the parameters in the Dialog Window, and then click Finish button to save your modifications. The breeding scheme will be automatically modified.



PMARS Tutorial: The breeding scheme and documentation

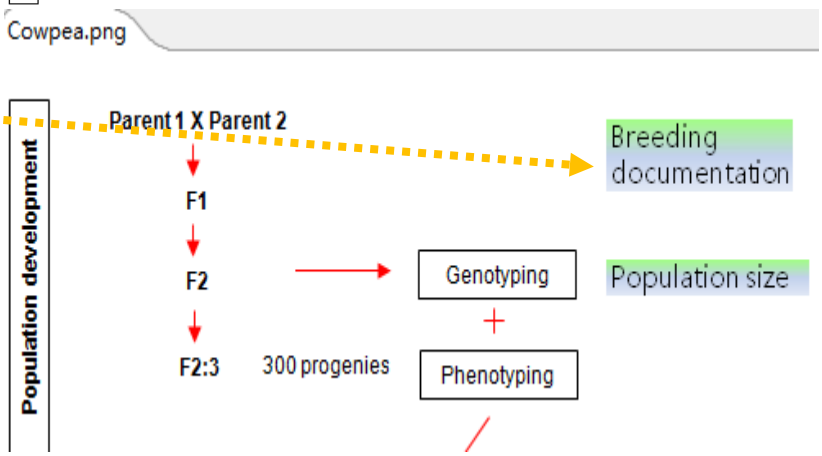
8. View the flowchart

- It is in the right window

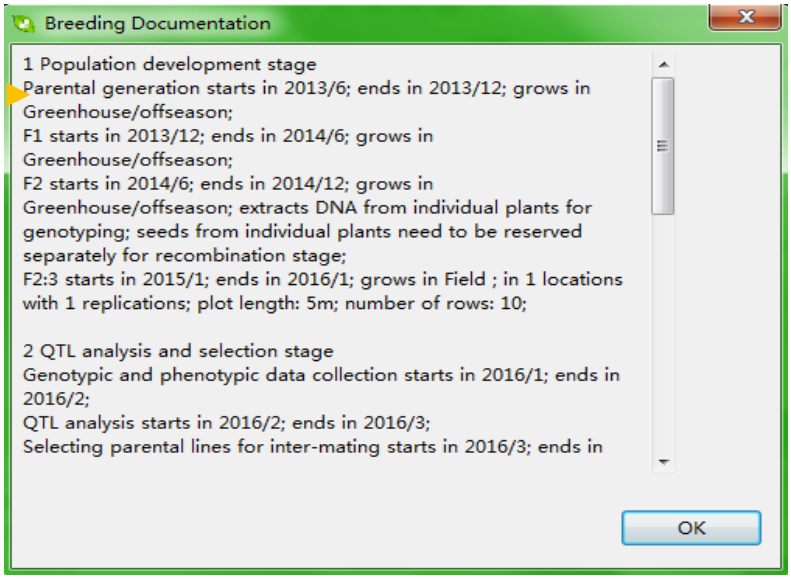


9. View breeding documentation

- Click the **Breeding Documentation** button



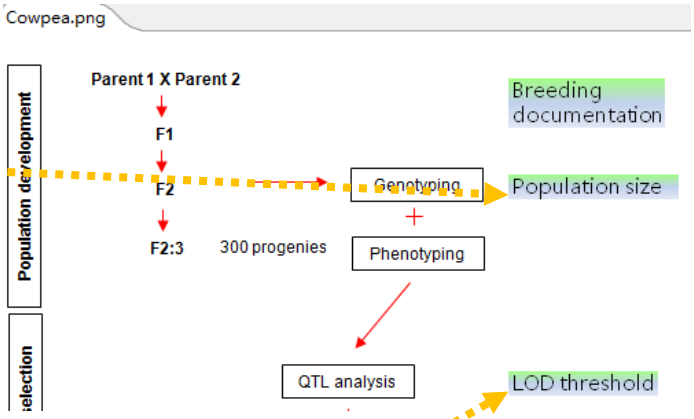
- The **Breeding Documentation** is in the pure text describing the whole flow of the MARS program. There are four major stages, each consisting of several steps.



PMARS Tutorial: Additional information

10. Additional information on the flowchart

- Click **Population size**



- The table for population size will be popped up to help to decide on a suitable population size
-
- Click **LOD threshold**

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

- The table for LOD threshold will be popped up to help to decide on a suitable threshold value in QTL mapping

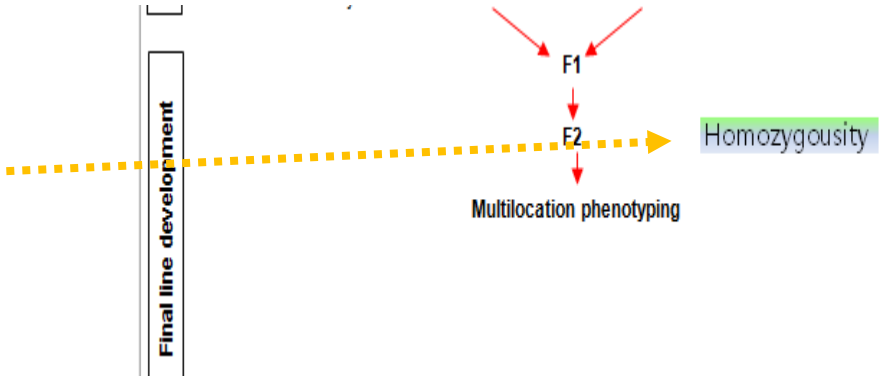
LOD threshold

LOD threshold required to achieve an overall significance of α 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	
	α=0.05	α=0.01	α=0.05	α=0.01	α=0.05	α=0.01	α=0.05	α=0.01	α=0.05	α=0.01	α=0.05	α=0.0
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

OK

PMARS Tutorial: Additional information

- Click **Homozygousity**



- The table homozygousity will be popped up to help to learn the rate of homozygousity during repeated selfing.

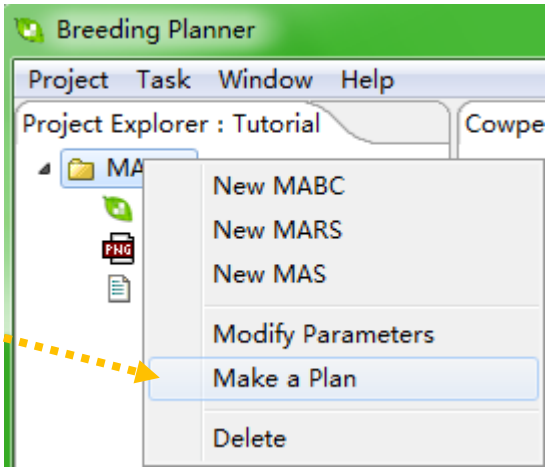
Homzygousity

Rate (%) of homozygousity 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

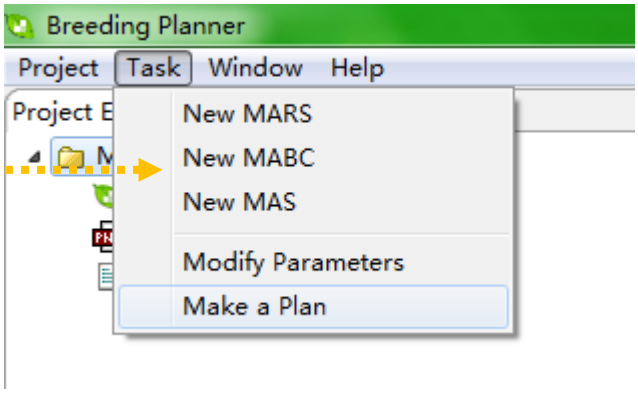
OK

11. Make a plan

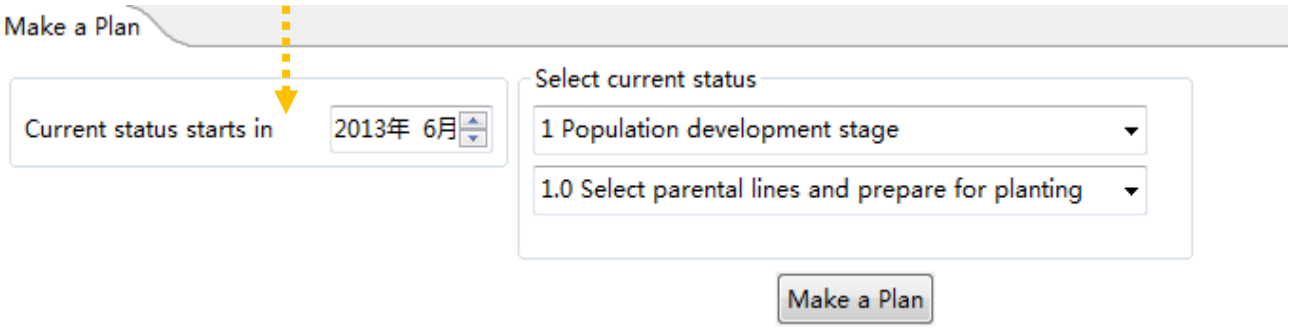
- Right click the **MARS** folder in the **Project Window**, then choose **Make a Plan**



- Or click **Task-> Make a Plan**



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

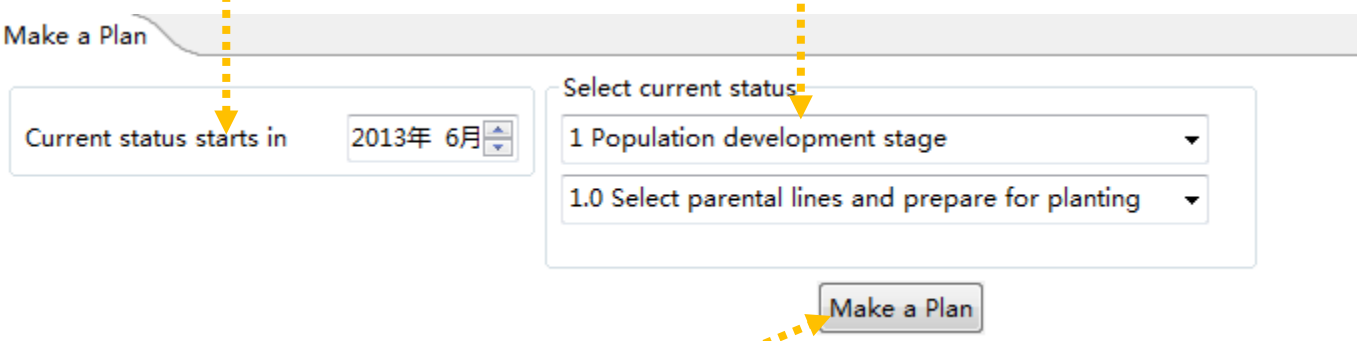


PMARS Tutorial: Tell the system where you are

- 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:

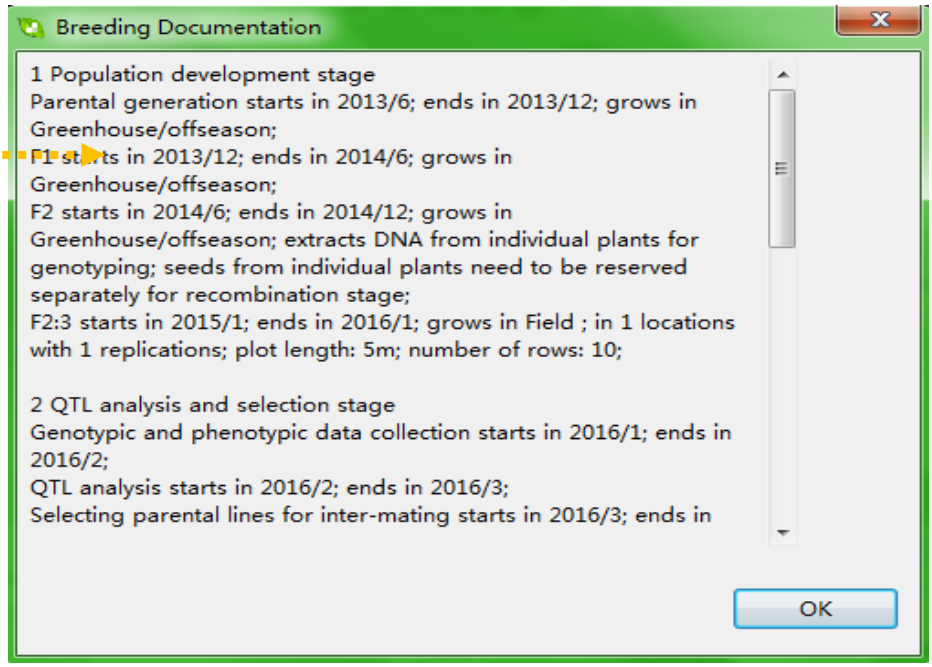
1. Population development stage
    - 1.0 Select parental lines and prepare for planting
    - 1.1 Parental lines is growing
    - 1.2 F1 generation is growing
    - 1.3 F2 generation is growing
    - 1.4 F3 generation is growing
    - 1.5 F4 generation is growing
2. QTL analysis and selection stage
    - 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
    - ……



- Click the **Make a Plan** button to complete the on-going MARS breeding program.

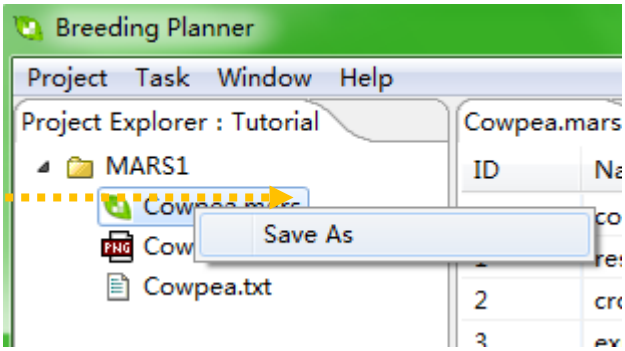
PMARS Tutorial: See the breeding plan the system makes

- New window will show the **Breeding Documentation** for the remaining status, and the time to complete the MARS breeding program

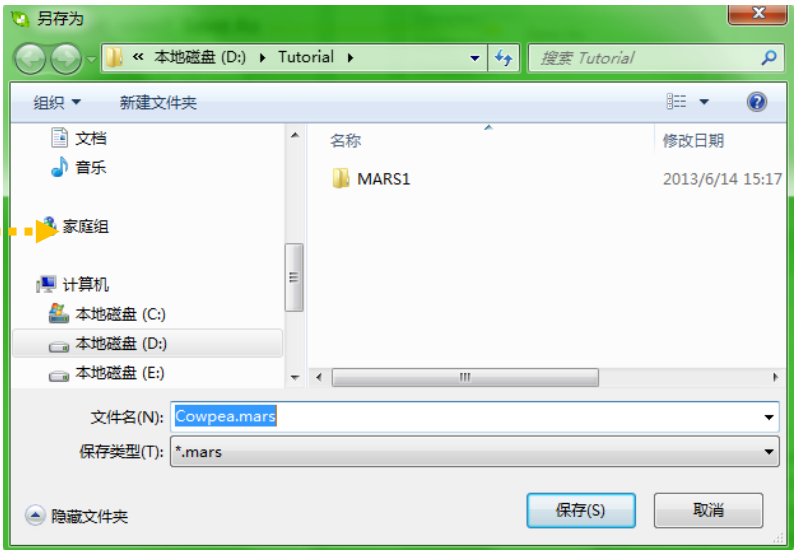


12. Save the result files

- Right click the file name, and then select **Save As** to save the breeding documentation on your computer



- Specify the path and rename of the output file



BP-MARS Tutorial: The contact information

- Any comments or suggestions? You may contact any one on the BP development team
  - Dr. Jiankang Wang, CIMMYT China and CAAS, wangjiankang@caas.cn or jkwang@cgiar.org
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