MAPMAKER/EXP Version 3.0b: Though Used Since Time Immemorial Still Difficult for Beginners to Start with Map Construction for the Genetic Studies

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Abstract: With the advancement of molecular biology, interest in linkage map construction and QTL identification is growing among researchers day by day. Plant genome mapping and QTL analysis allows the identification of genes associated with economically important traits and the use of this information to further improve crops. So, we also attempted to construct a genetic linkage maps in our laboratory using more than one molecular marker systems and to analyze Quantitative Trait Loci (QTL) from the constructed linkage map. Every first time user finds many hurdles about the use of the software MAPMAKER. Though the problems were not very big but there was no one to tell us about the start of the software MAPMAKER and one by one commands to follow. So this article is written keeping in mind those beginners who are new to linkage map construction and QTL identification but it's their desire to find certain genes of interest in their test crop.

Keywords: Mapmaker, QTL, Trait, Genotyping, LOD.

1. INTRODUCTION

In vast majority of plants whose genomes are yet to be sequenced the genetic maps provide an important resource to understand the order and spacing of markers and to those crops where genome has been sequenced, these linkage maps provide a scaffold for genome sequence assembly and validation. The speed and precision of breeding can be improved by the development of genetic linkage maps based on molecular markers to locate discrete chromosomal regions viz., QTLs, which control a number of complex polygenic traits. In plant studies, a genetic map is estimated from a dataset derived from a mapping population of two contrasting parents.

Advances in computational biology have revolutionized the progress in DNA marker based linkage map construction and QTL identification. Various softwares viz., AntMap, Carthagene, DGMAP, Joinmap, MadMapper, MAPMAKER/EXP, Map Manager QTX, MST_{MAP}, Neighbour Mapping, RECORD and THREaD Mapper are available. For the present review, we have used MAPMAKER/EXP 3.0 as it has already been used in number of studies viz., in *Eucalyptus* [1], rice [2], *Tetramolopium* [3], water-melon [4], cowpea [5], Garlic [6], mulberry [7], *Sonchus alliance* [8] and *Dendrobium officinale* [9] and many more.

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Genetic mapping involves the calculation of pairwise recombination frequencies between markers, establishment of linkage groups, estimation of map distance and determination of the map order by statistical programs [10]. In the present review, following the scoring of each marker, segregation data was analyzed and linkage was detected using MAPMAKER/EXP version 3.0b. [11, 12] which is DOS based software. MAPMAKER/EXP version 3.0b performs full multipoint linkage analyses i.e., estimation of all recombination fractions from the marker data for dominant, recessive and co-dominant markers and uses a simple two-point approach to infer linkage groups, using a transitive procedure on two-point maximum likelihood distances and LOD scores.

2. USE OF THE SOFTWARE MAPMAKER/EXP

The manual of instruction is freely available in public domain. However, a simplified version is presented giving one by one command and output of mapmaker. This is an attempt to make the software usable for thousands of users.

First of all, create a 2003 excel sheet of your genotypic results and convert the excel file into .txt file in notepad. Mapmaker files are in the form of "matrices" of "A" i.e. individual similar to parent A, "B" i.e. individual similar to parent B and "H" i.e. heterozygote. The first row comprises of data type and the numbers in the second cell represents mapping population of **66**, **60** loci found to be polymorphic among parents and **1** suggests the number of traits in phenotyping. The excel data file shown in Figure **1**.

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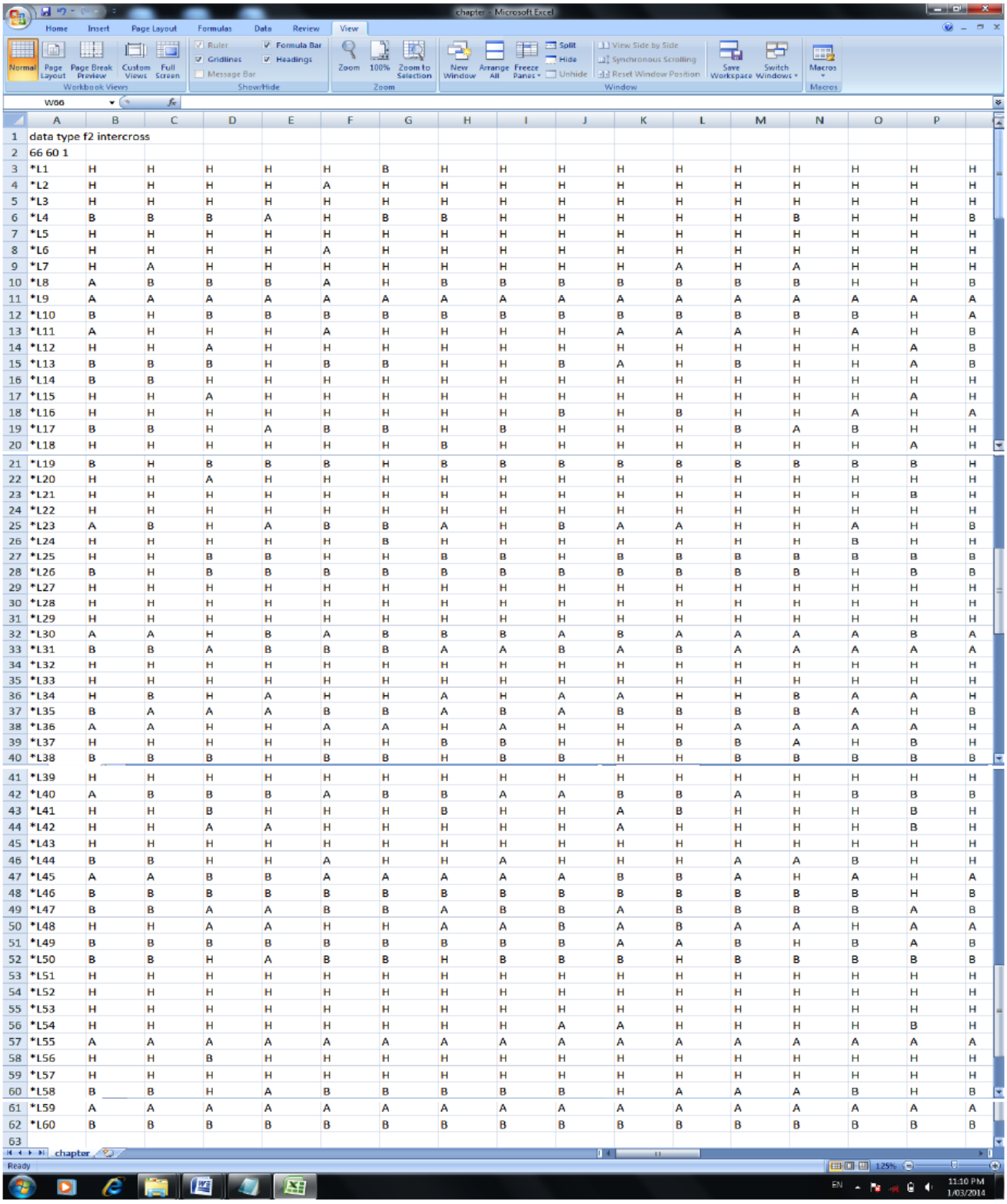


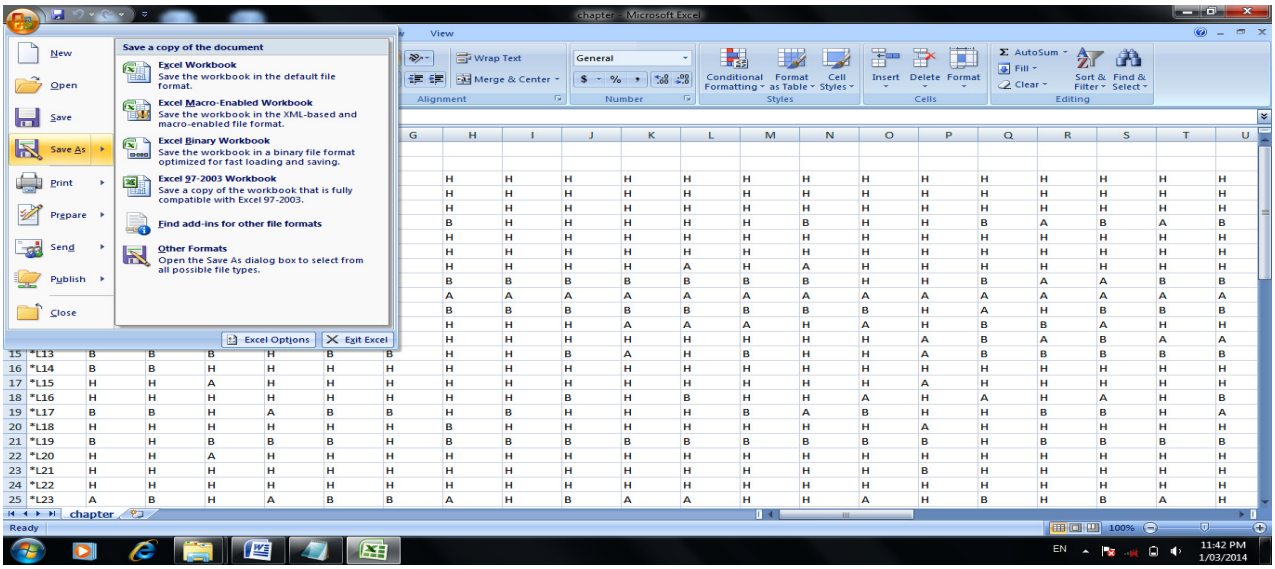
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	data type f2 intercross															
2	66 60 1															
3	*L1	H	H	H	H	H	B	H	H	H	H	H	H	H	H	H
4	*L2	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H
5	*L3	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
6	*L4	B	B	B	A	H	B	B	H	H	H	H	H	B	H	H
7	*L5	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
8	*L6	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H
9	*L7	H	A	H	H	H	H	H	H	H	H	A	H	A	H	H
10	*L8	A	B	B	B	A	H	B	B	B	B	B	B	B	H	H
11	*L9	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
12	*L10	B	H	B	B	B	B	B	B	B	B	B	B	B	B	H
13	*L11	A	H	H	H	A	H	H	H	H	A	A	A	H	A	H
14	*L12	H	H	A	H	H	H	H	H	H	H	H	H	H	H	A
15	*L13	B	B	B	H	B	B	H	H	B	A	H	B	H	H	A
16	*L14	B	B	H	H	H	H	H	H	H	H	H	H	H	H	H
17	*L15	H	H	A	H	H	H	H	H	H	H	H	H	H	H	A
18	*L16	H	H	H	H	H	H	H	H	B	H	B	H	H	A	H
19	*L17	B	B	H	A	B	B	H	B	H	H	H	B	A	B	H
20	*L18	H	H	H	H	H	H	B	H	H	H	H	H	H	A	H
21	*L19	B	H	B	B	B	H	B	B	B	B	B	B	B	B	H
22	*L20	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H
23	*L21	H	H	H	H	H	H	H	H	H	H	H	H	H	B	H
24	*L22	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
25	*L23	A	B	H	A	B	B	A	H	B	A	A	H	H	A	H
26	*L24	H	H	H	H	H	B	H	H	H	H	H	H	H	B	H
27	*L25	H	H	B	B	H	H	B	B	H	B	B	B	B	B	B
28	*L26	B	H	B	B	B	B	B	B	B	B	B	B	B	H	B
29	*L27	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
30	*L28	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
31	*L29	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
32	*L30	A	A	H	B	A	B	B	B	A	B	A	A	A	A	B
33	*L31	B	B	A	B	B	B	A	A	B	A	B	A	A	A	A
34	*L32	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
35	*L33	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
36	*L34	H	B	H	A	H	H	A	H	A	A	H	H	B	A	A
37	*L35	B	A	A	A	B	B	A	B	A	B	B	B	B	A	H
38	*L36	A	A	H	H	A	A	H	A	H	H	H	A	A	A	A
39	*L37	H	H	H	H	H	H	B	B	H	H	B	B	A	H	B
40	*L38	B	B	B	H	B	B	H	B	B	H	H	B	B	B	B
41	*L39	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
42	*L40	A	B	B	B	A	B	B	A	A	B	B	A	H	B	B
43	*L41	H	H	B	H	H	H	B	H	H	A	B	H	H	B	H
44	*L42	H	H	A	A	H	H	H	H	H	A	H	H	H	B	H
45	*L43	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
46	*L44	B	B	H	H	A	H	H	A	H	H	H	A	A	B	H
47	*L45	A	A	B	B	A	A	A	A	A	B	B	A	H	A	H
48	*L46	B	B	B	B	B	B	B	B	B	B	B	B	B	B	H
49	*L47	B	B	A	A	B	B	A	B	B	A	B	B	B	B	A
50	*L48	H	H	A	A	H	H	A	A	B	A	B	A	A	H	A
51	*L49	B	B	B	B	B	B	B	B	B	A	A	B	H	B	A
52	*L50	B	B	H	A	B	B	H	B	B	B	H	B	B	B	B
53	*L51	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
54	*L52	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
55	*L53	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
56	*L54	H	H	H	H	H	H	H	H	A	A	H	H	H	B	H
57	*L55	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
58	*L56	H	H	B	H	H	H	H	H	H	H	H	H	H	H	H
59	*L57	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
60	*L58	B	B	H	A	B	B	B	B	B	H	A	A	A	B	H
61	*L59	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
62	*L60	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
63																

Figure 1: Excel file format data file as a result of genotyping in mapping population.

Below the genotypic data, phenotypic data has to be written that is the data of trait of interest for QTL identification. In this review we have denoted trait as R.

Now Go to save As from file

A new dialogue box will appear from save as type, choose text (MS DOS) and now name the file as xyz. raw and the input file has now created. Then copy this input file to the place where application files of software are located or save this .raw file in Mapmaker folder.



For linkage mapping analysis of marker data in MAPMAKER, raw file containing information on mapping population type, genotypic data of number of markers, number of phenotypic data of quantitative traits, coding scheme of your data set was prepared.

3. RUNNING MAPMAKER

3.1. Step1. Open the MAPMAKER Folder, Double Click Mapmaker Application

A dialogue box will appear, click on Run and finally a window as shown in Figure **2** will appear

4. ONE BY ONE COMMANDS AND THEIR OUTPUT (COMMANDS IN BOLD AND OUTPUT NORMAL)

1> prepare xyz.raw (This command uploads the data to the software)

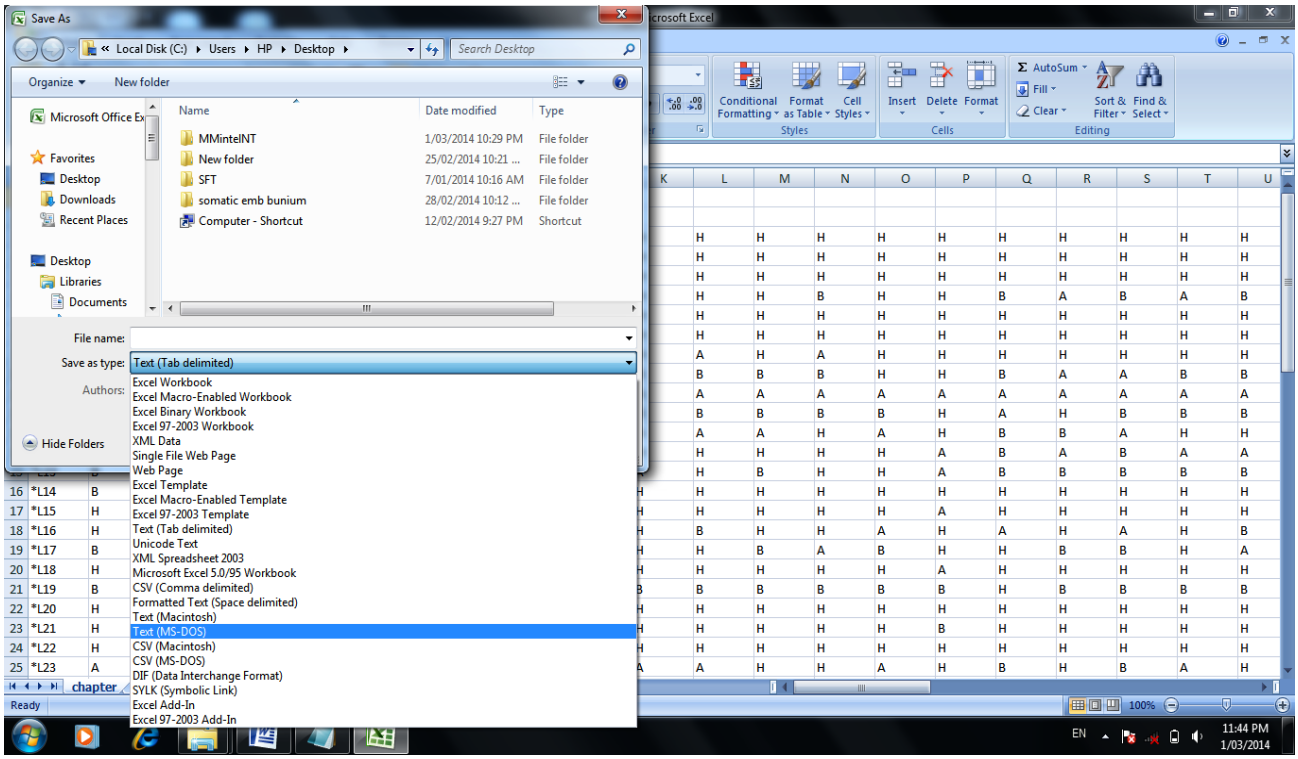
preparing data file 'xyz.raw'… ok

F2 intercross data <66 individuals, 60 loci>… ok

unable to run file 'xyz.prep'… skipping initialization

saving genotype data in file 'xyz.data'… ok

saving map data in file 'xyz.maps'… ok



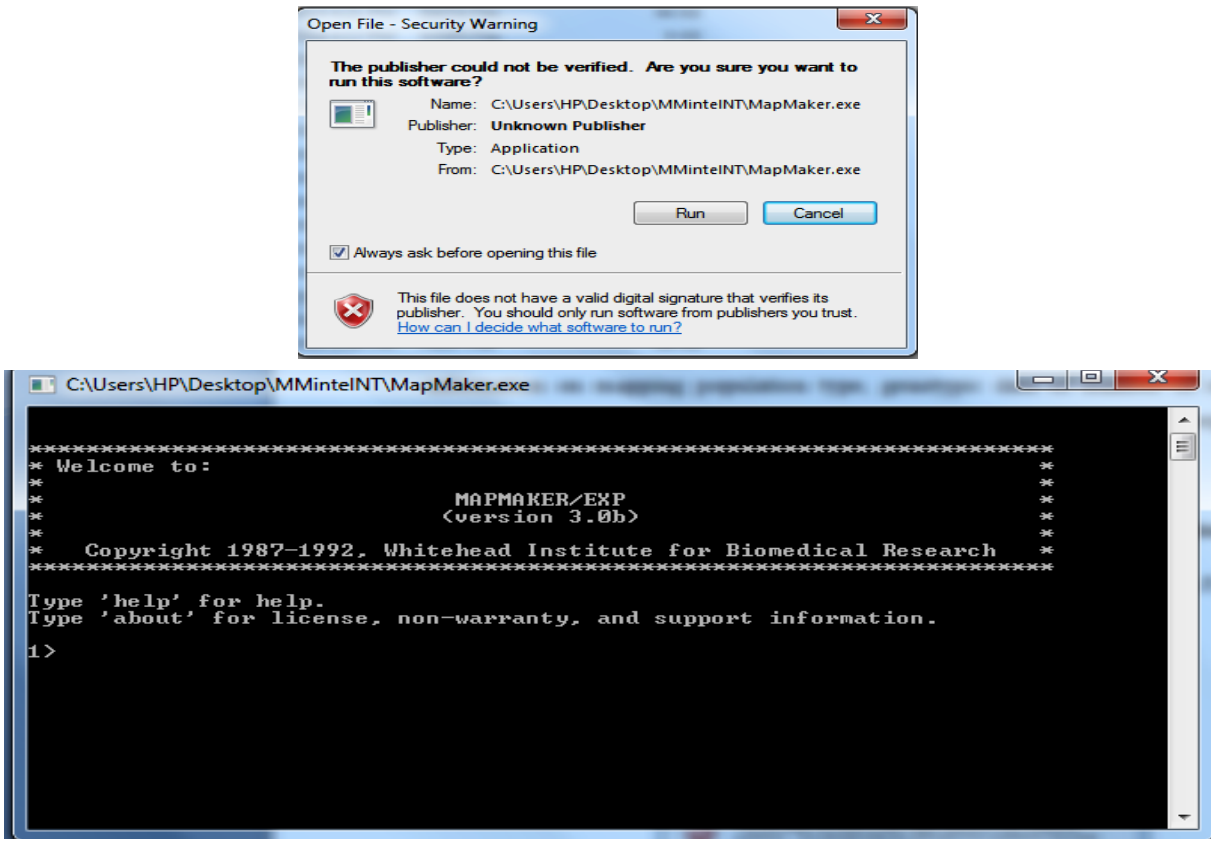


Figure 2: Mapmaker DOS screen to input one by one commands.

saving traits data in file 'xyz.traits'… ok

2> photo xyz. raw

'photo' is on: file is 'xyz.raw'

3> units

the 'units' are currently (Haldane) centimorgans

4> cent func k

centimorgan function: Kosambi

After loading this .raw file to MAPMAKER and setting map function as kosambi, triple error detection was set on to know error probabilities and logarithm of the odds (LOD) error values. Hence, recombination values were converted to genetic distances using the Kosambi mapping function.

5> print names on

'print names' is on.

6> triple error detection on

'triple error detection' is on.

Then, minimum logarithm of the odds (LOD) and maximum centiMorgan (cM) distance to declare linkage between markers was set. In the present review, LOD was 6 and maximum cM distance was set 30. Then by using "GROUP" command markers were separated in sequence into linkage groups. After that by using "ORDER" command it automatically builds map orders. "LOD" command was used to print all the two point data, the results obtained were LOD score and cM distance.

7> default linkage 6 30

default LOD score threshold is 6.00

default centimorgan distance threshold is 30.00

8> sequence L1-L60 (This command consists of range of total number of loci)

sequence #1= L1-L60

9> group (This command tells the number of linkage groups to be obtained)

Linkage Groups at min LOD 6.00, max Distance 30.0

group1= L1 L2 L3 L5 L6 L7 L14 L15 L18 L20 L22 L24 L27 L28 L29 L32 L33 L39 L41

L43 L51 L52 L53 L56 L57

group2= L9 L55 L59

group3= L10 L13 L19 L25 L26 L38 L40 L46 L47 L49 L58 L60

unlinked= L4 L8 L11 L12 L16 L17 L21 L23 L30 L31 L34 L35 L36 L37 L42 L44 L45 L48

L50 L54

10> order (This command tells the order of markers on the linkage group) Long output will be obtained in this case we are just presenting few lines

Linkage Groups at min LOD 6.00, max Distance 30.0

Starting Orders: Size 5, Log-Likelihood 3.00, Searching up to 50 subsets

Informativeness: min #Individuals 1, min Distance 0.9

Placement Threshold-1 3.00, Threshold-2 2.00, Npt-Window 7

Linkage group 1, 25 Markers:

Linkage group 2, 3 Markers:

Linkage group 3, 12 Markers: … and so on.

11> three point (Very long output will be obtained in this case we are just presenting few lines)

Linkage Groups at min LOD 6.00, max Distance 30.0

Triplet criteria: LOD 3.00, Max-Dist 37.2, #Linkages 2

'triple error detection' is on.

counting...2450 linked triplets in 3 linkage groups

	log-likelihood differences			
count markers	a-b-c	b-a-c	a-c-b	
1: L1 L2 L3	-4.45	-0.91	0.00	
2: L1 L2 L5	-3.74	-0.97	0.00	
3: L1 L2 L6	-3.67	-1.23	0.00	
4: L1 L2 L7	-1.25	-0.26	0.00	… and so on.

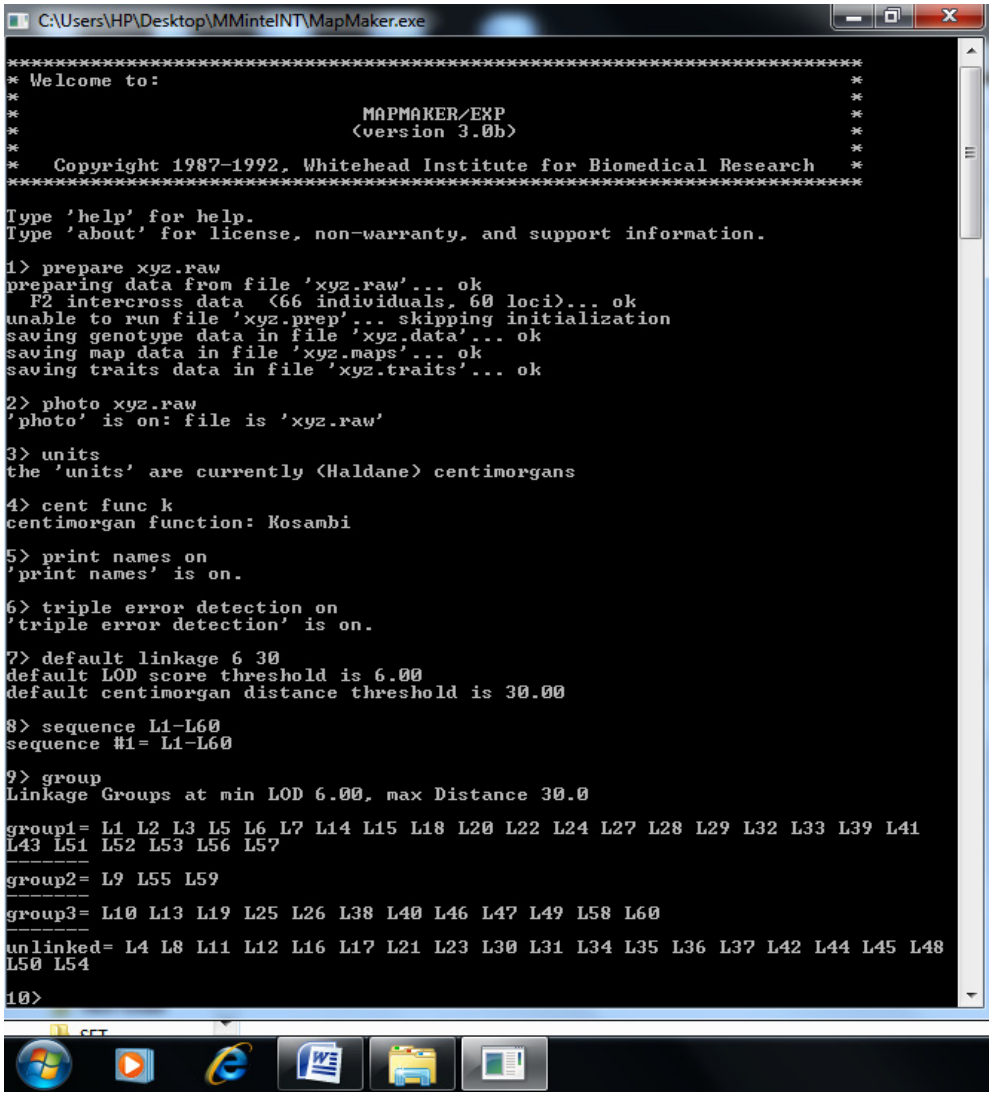


Figure 3: Pictorial presentation of all the above mentioned commands.

12> lod (Very long output will be obtained in this case we are just presenting few lines)

Bottom number is LOD score, top number is centimorgan distance:

13> sequence L1 L2 L3 L5 L6 L7 L14 L15 L18 L20 L22 L24 L27 L28 L29 L32 L33 L39 L41 L43 L51 L52 L53 L56 L57 (In this command one has to write all those loci that were in group 1, in group command)

sequence #2= L1 L2 L3 L5 L6 L7 L14 L15 L18 L20 L22 L24 L27 L28 L29 L32 L33 L39

L41 L43 L51 L52 L53 L56 L57

14> map ("MAP" command calculated and displayed the maximum likelihood map for the order of markers specified).

Map:

Markers	Distance
1 L1	8.3 cM
2 L2	5.6 cM
3 L3	0.8 cM
5 L5	2.3 cM
6 L6	5.6 cM
7 L7	5.6 cM
14 L14	4.8 cM
15 L15	3.1 cM
18 L18	9.3 cM
20 L20	7.4 cM
22 L22	12.3 cM
24 L24	12.3 cM
27 L27	2.3 cM
28 L28	2.3 cM
29 L29	0.0 cM
32 L32	0.0 cM
33 L33	0.0 cM
39 L39	5.6 cM
41 L41	5.6 cM
43 L43	0.8 cM
51 L51	0.0 cM
52 L52	0.8 cM
53 L53	5.6 cM
56 L56	5.6 cM
57 L57	----------

106.5 cM 25 markers log-likelihood= -212.37

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15> sequence L9 L55 L59 (Similarly this command for those in group 2)

sequence #3= L9 L55 L59

16> map

Map:

Markers	Distance
9 L9	11.6 cM
55 L55	2.3 cM
59 L59	----------

13.8 cM 3 markers log-likelihood= -62.64

17> sequence L10 L13 L19 L25 L26 L38 L40 L46 L47 L49 L58 L60 (Similarly this command for those in group 3)

sequence #4= L10 L13 L19 L25 L26 L38 L40 L46 L47 L49 L58 L60

18> map

Map:

Markers	Distance
10 L10	35.9 cM
13 L13	20.8 cM
19 L19	13.3 cM
25 L25	20.7 cM
26 L26	33.5 cM
38 L38	72.1 cM
40 L40	35.1 cM … and so on.

19> sequence L1

sequence #5= L1

20> make chromosome chro1

chromosomes defined: chro1

21> anchor chro1

L1 - anchor locus on chro1

chromosome chro1 anchor(s): L1

22> sequence L1 L2 L3 L5 L6 L7 L14 L15 L18 L20 L22 L24 L27 L28 L29 L32 L33 L39 L41 L43 L51 L52 L53 L56 L57 (those in group 1)

sequence #6= L1 L2 L3 L5 L6 L7 L14 L15 L18 L20 L22 L24 L27 L28 L29 L32 L33 L39

L41 L43 L51 L52 L53 L56 L57

23> assign

L1 - anchor locus on chro1...cannot re-assign

L2 - assigned to chro1 at LOD 7.4

L3 - assigned to chro1 at LOD 14.6

L5 - assigned to chro1 at LOD 17.6

L6 - assigned to chro1 at LOD 16.0

L7 - assigned to chro1 at LOD 12.2 …and so on.

24> frame chro1

setting framework for chromosome chro1...

chro1 framework:

Markers	Distance
1 L1	8.3 cM
2 L2	5.6 cM
3 L3	0.8 cM
5 L5	2.3 cM
6 L6	5.6 cM … and so on.

25> draw chromosome chro1

Drawing chromosome chro1 in PostScript file 'chro1.ps'...

ok

26> draw map

Drawing map in PostScript file 'map.ps'...

ok

27> sequence L9

sequence #7= L9

28> make chromosome chro2

chromosomes defined: chro1 chro2

29> anchor chro2

L9 - anchor locus on chro2

chromosome chro2 anchor(s): L9

30> sequence L9 L55 L59

sequence #8= L9 L55 L59

31> assign

L9 - anchor locus on chro2...cannot re-assign

L55 - assigned to chro2 at LOD 19.4

L59 - assigned to chro2 at LOD 33.5

32> frame chro2

setting framework for chromosome chro2...

chro2 framework:

Markers	Distance
9 L9	11.6 cM
55 L55	2.3 cM
59 L59	----------
	13.8 cM 3 markers log-likelihood= -62.64

33> draw chromosome chro2

Drawing chromosome chro2 in PostScript file 'chro2.ps'...

ok

34> draw map

Drawing map in PostScript file 'map.ps'...

ok

35> sequence L10

sequence #9= L10

36> make chromosome chro3

chromosomes defined: chro1 chro2 chro3

37> anchor chro3

L10 - anchor locus on chro3

chromosome chro3 anchor(s): L10

38> sequence L10 L13 L19 L25 L26 L38 L40 L46 L47 L49 L58 L60

sequence #10= L10 L13 L19 L25 L26 L38 L40 L46 L47 L49 L58 L60

39> assign

L10 - anchor locus on chro3...cannot re-assign

L26 - assigned to chro3 at LOD 8.0

L46 - assigned to chro3 at LOD 10.1

L49 - assigned to chro3 at LOD 12.9

L58 - assigned to chro3 at LOD 11.2

L60 - assigned to chro3 at LOD 23.3 … and so on.

40> frame chro3

setting framework for chromosome chro3...

chro3 framework:

Markers	Distance

10 L10 35.9 cM

13 L13 20.8 cM … and so on

41> draw chromosome chro3

Drawing chromosome chro3 in PostScript file 'chro3.ps'...

42> draw map

Drawing map in PostScript file 'map.ps'...

43> quit

save data before quitting? [yes] y

saving map data in file 'xyz.maps'... ok

saving two-point data in file 'xyz.2pt'... ok

saving three-point data in file 'xyz.3pt'... ok

At the end .data and .traits output was obtained and these output files would serve as an input file for QTLmap application of MAPMAKER.

5. RUNNING QTL APPLICATION OF MAPMAKER

Double click on QTL map application a dialogue box will appear, click on Run and finally a window as shown in Figure **4** will appear

1> load xyz.data

data files ‘xyz.data’ and ‘xyz.traits’ are loaded.

<66 intercross progeny, 41 loci, 1 trait>

QTL map data in file ‘xyz.qtls’ have been loaded.

2> photo xyz.data

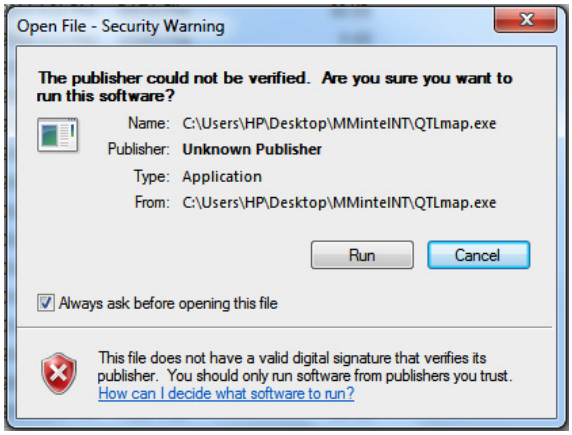
‘photo’ is on: file is ‘xyz.data’

3> trait 1

The current trait is now: 1 (R)

4> show trait

Trait 1 (R):



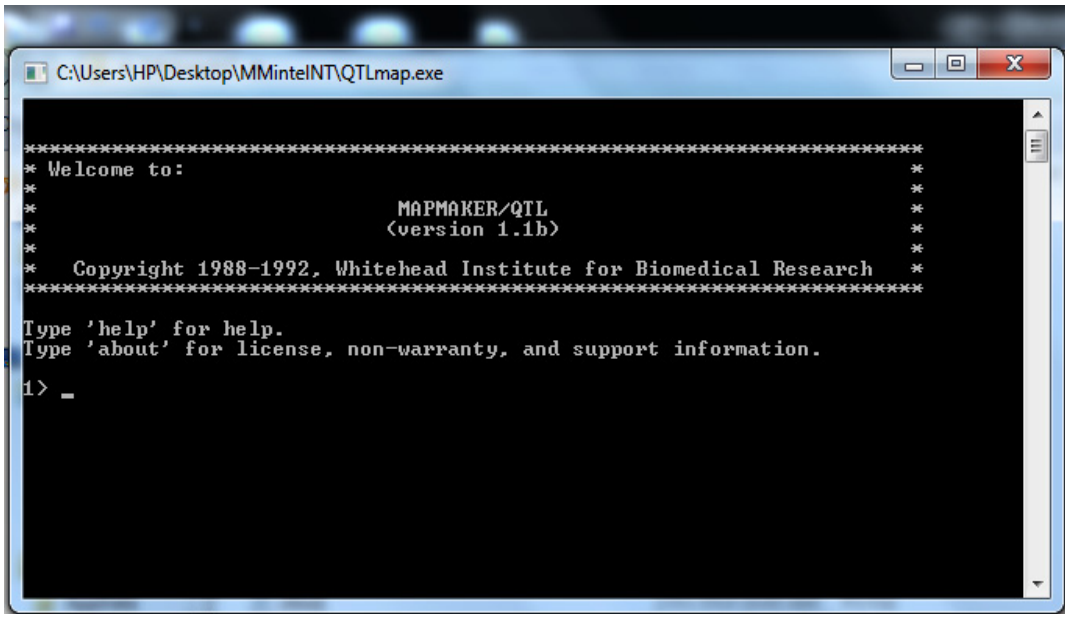


Figure 4: QTL map application of Mapmaker DOS screen to input one by one commands.

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distribution: quartile | fraction within n deviations:

mean sigma skewness kurtosis ratio | 1/4 1/2 1 2 3

3.56 2.09 0.52 -0.12 0.77 | 0.22 0.46 0.62 0.97 1.00

-0.62 |

0.42 |*******

1.47 |**

2.52 |*************************

3.56 |**

4.61 |**

5.66 |***********

6.71 |*****************

7.75 |*****************

8.80 |***

5> seq [all]

The sequence is now '[all]'\

6> show linkage maps (Very long output will be obtained in this case we are just presenting few lines)

linkage maps:

1-2 9.0 cM 8.3 %

2-3 6.0 cM 5.6 %

3-5 0.8 cM 0.8 %

5-6 2.4 cM 2.3 %

6-7 6.0 cM 5.6 %

7-14 6.0 cM 5.6 %

14-15 5.0 cM 4.8 % … and so on.

7> trait 1

The current trait is now: 1 (R)

8> scan

QTL maps for trait 1 (R):

Sequence: [all]

LOD threshold: 2.00 Scale: 0.25 per '*' Scanned QTL genetics are free.

POS WEIGHT DOM %VAR LOG-LIKE |

----------| 1-2 9.0 cM

0.0 0.231 -0.786 0.6% 0.078 |

2.0 -0.371 -0.685 1.0% 0.089 | … and so on.

9> show peaks

LOD score peaks for scan 1.1 of trait 1 (R).

Sequence: [all]

Scanned QTL genetics are free.

Peak Threshold: 2.00 Falloff: -2.00

QTL-Map for peak 1:

Confidence Interval: Left Boundary= 38-40 + 66.0, Right Boundary= 40-46 + 22.0

INTERVAL LENGTH QTL-POS GENETICS WEIGHT DOMINANCE

38-40 112.2 102.0 free 0.3801 4.0995

chi^2= 10.260 (2 D.F.) log-likelihood= 2.23

mean= 2.302 sigma^2= 2.073 variance-explained= 52.7%

==========

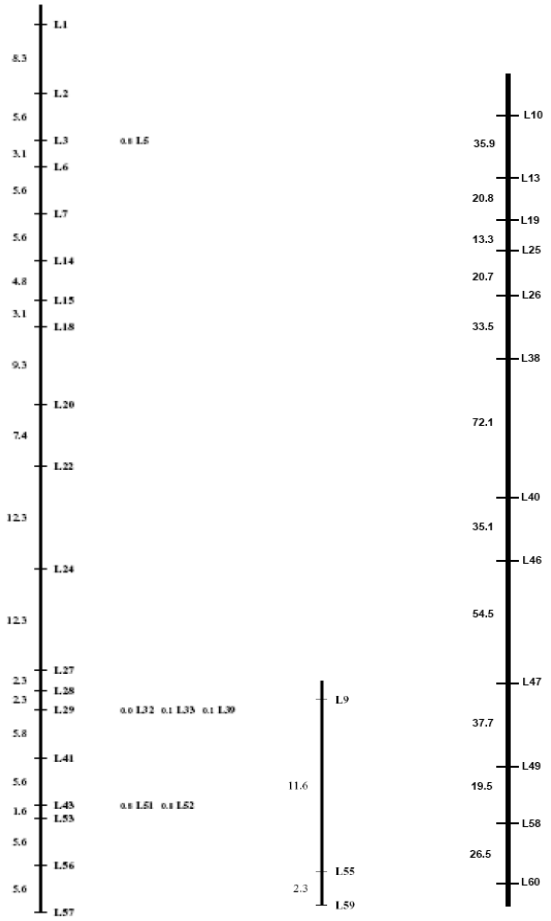

Figure 5: Linkage maps constructed as an output.

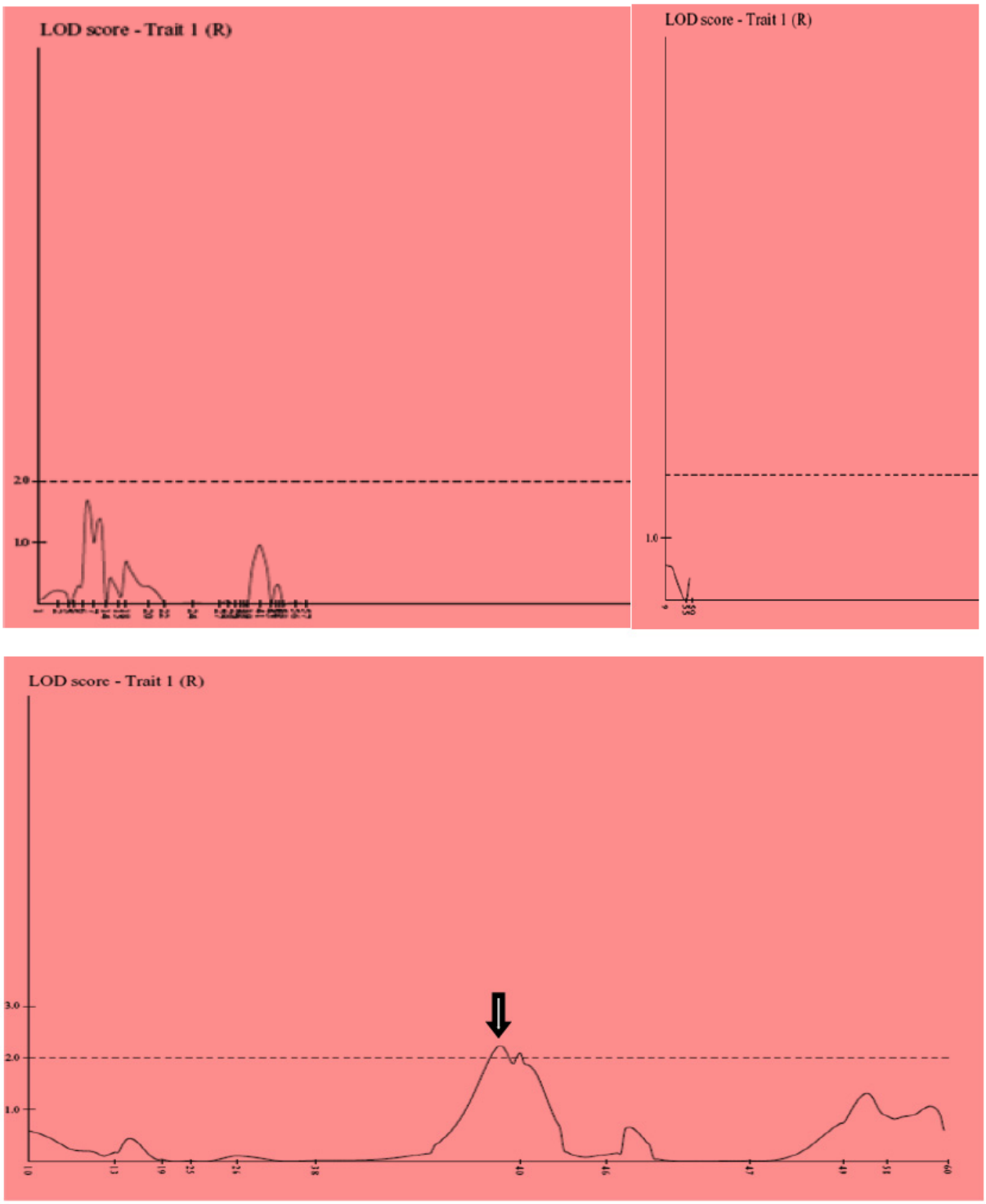

Figure 6: QTL obtained as an output of QTLmap application (there is one major QTL denoted as ⇩).

10> draw scan

scan 1.1 saved in PostScript file 'scan1_1.ps'

11> map (Very long output will be obtained in this case we are just presenting few lines)

QTL map for trait 1 (R):

INTERVAL LENGTH QTL-POS WEIGHT DOMINANCE

1-2 9.0 6.3 -0.8796 -0.4088

chi^2= 0.936 (2 D.F.) log-likelihood= 0.20

mean= 4.803 sigma^2= 4.294 variance-explained= 2.1 % … and so on.

12> quit

save data before quitting? [yes] y

Now saving xyz.qtls...

Now saving xyz.traits...

Note: If there are two traits for phenotyping then at 5th command in QTL map application (if we assume other trait as S)

5> make trait log S = log (S) and then follow all other commands afterwards

The linkage maps and QTL were obtained in .ps files that could be viewed with .psviewer. Linkage was detected and majority of loci were ordered into a linear map.

Linkage map of arbitrary data based on F_2 population using MAPMAKER/EXP version 3.0 b. Locus names are listed on the right of the linkage groups and map distances between markers in centiMorgan (cM) on the left.

CONFLICT OF INTEREST

Both the authors declare that they have no conflict of interest.

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