

Mapping of Two White Stem Genes in Tetraploid Tobacco (*Nicotiana tabacum* L.)

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Outline

- **Background:** leaf color and Chl metabolism
- **Results/methods**
- **conclusions**

Background

The roles of chlorophyll (Chl)

- Perform photosynthesis and maintain global ecosystem
- Indicator of leaf color (albino, light green, dark green *et al.*)



Gene mutations



Abnormal leaf color



Which pathways are these genes involved in?



molecular biological technology

Four pathways on Chl metabolism

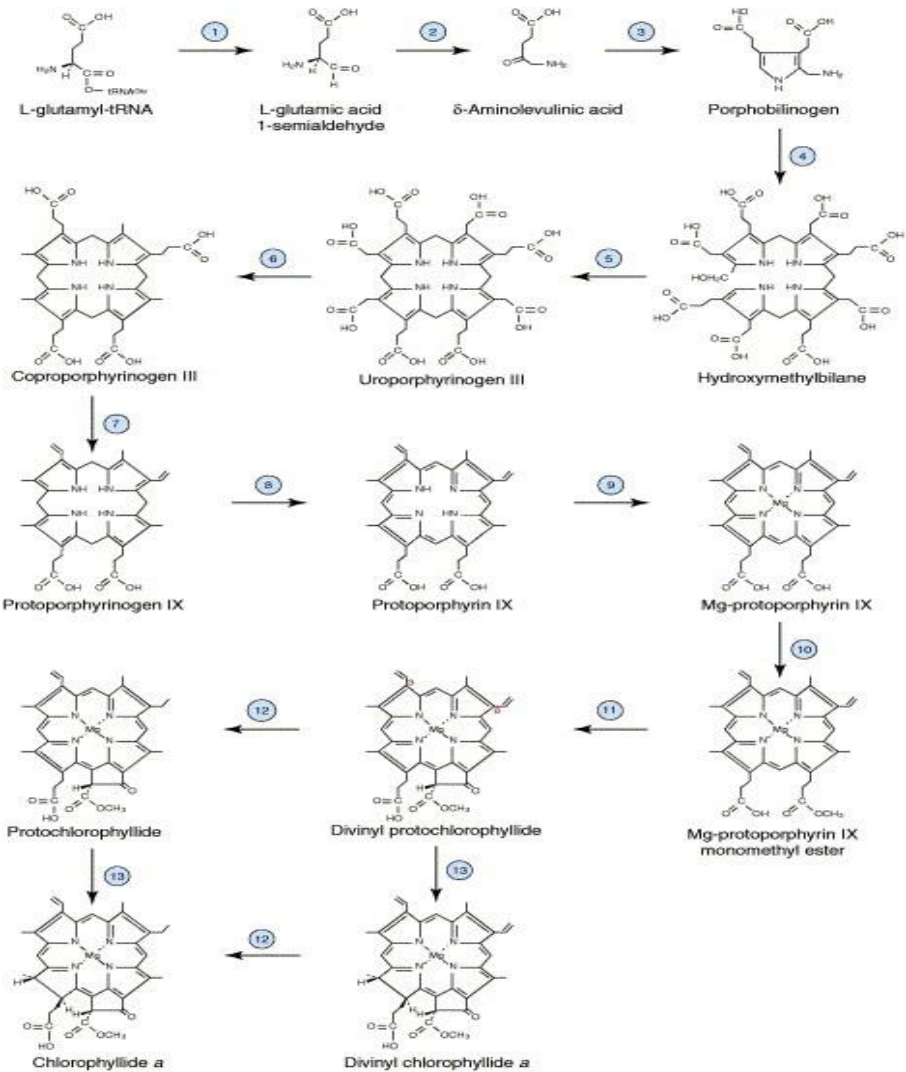
1. Chl biosynthesis

15 enzymatic steps

27 coding genes

Mutant phenotype:

low Chl level & light green



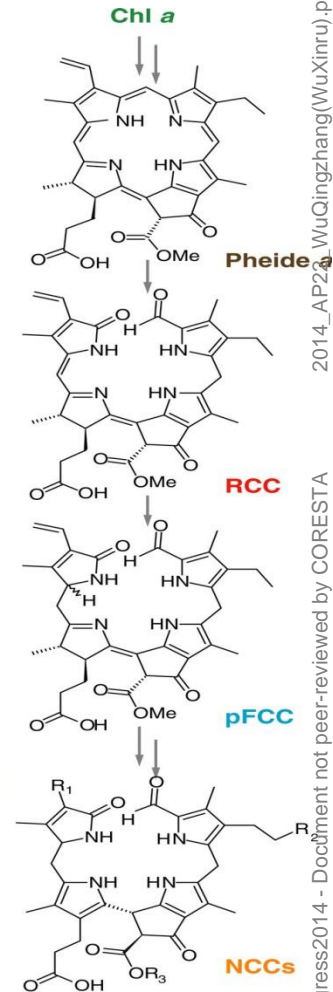
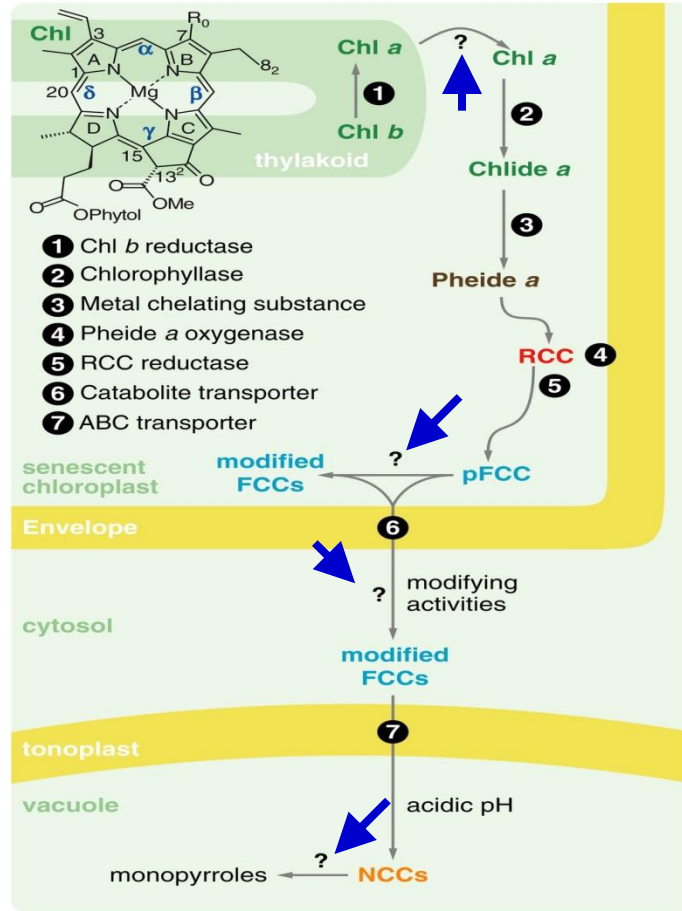
2. Chl degradation

Situation:

Has not completely been clarified

Mutant phenotype:

high Chl level & stay green



3. Chromophore biosynthesis

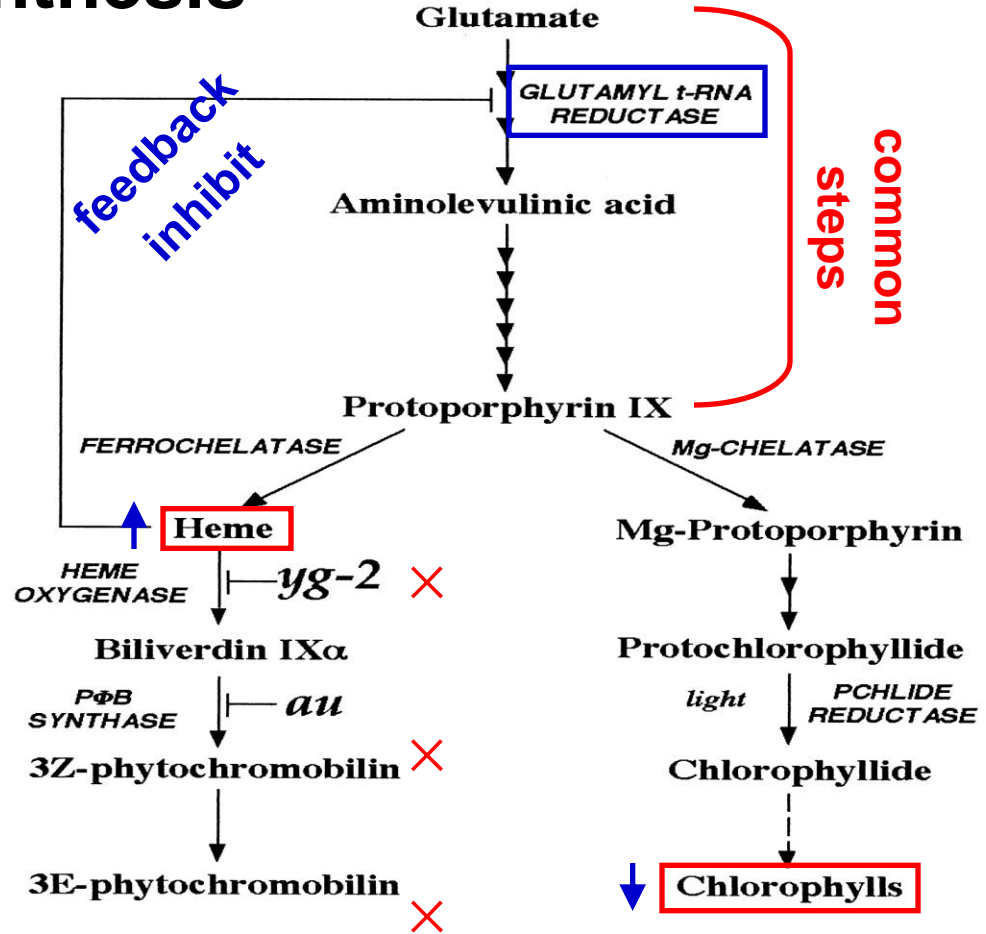
Reason:

Heme feedback inhibits

Chl biosynthesis

Mutant phenotype:

low Chl level & light green



4. Chloroplast biogenesis

Reason:

Disrupt Chl metabolism

Mutant phenotype:

More albino



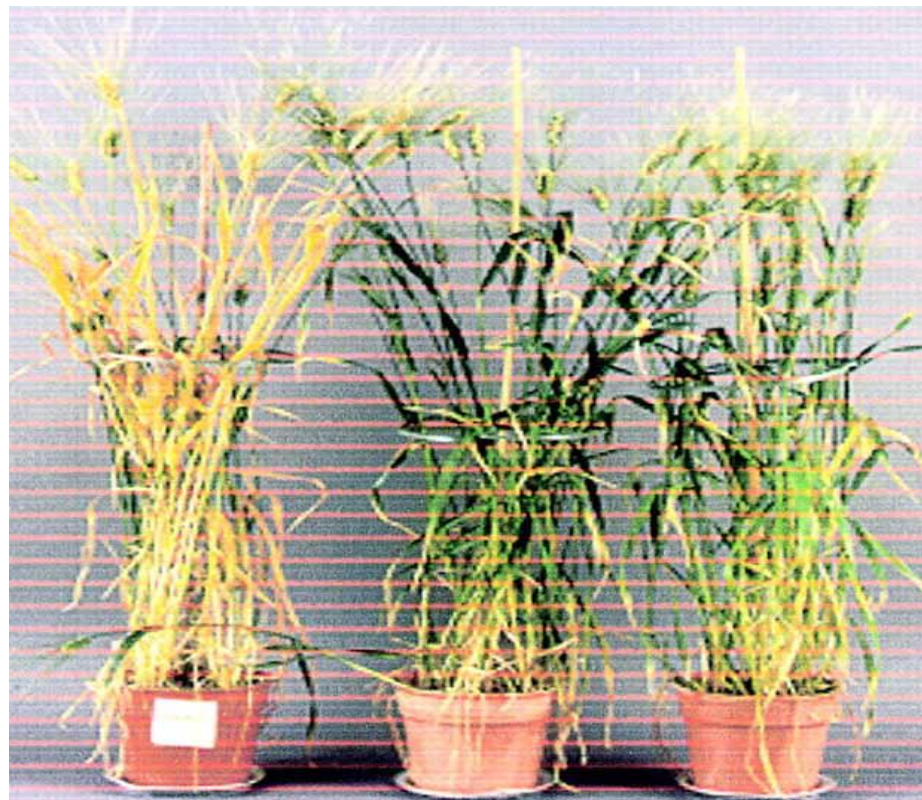
Leaf color mutant utilization

Most useless: adverse to plant growth, **however:**

- **cotton virescent & rice green-revertible albino mutant**--- cost-efficient phenotypic markers for the hybrid seed purity
- **durum wheat stay green mutants**---delay Chl breakdown, prolong photosynthetic competence, increase grain yields
- **ornamental plants**---lower ratios between Chl and other pigments, mainly carotenoids and anthocyanins, cause color variation of leaves



Tobacco virescent



T. durum stay green



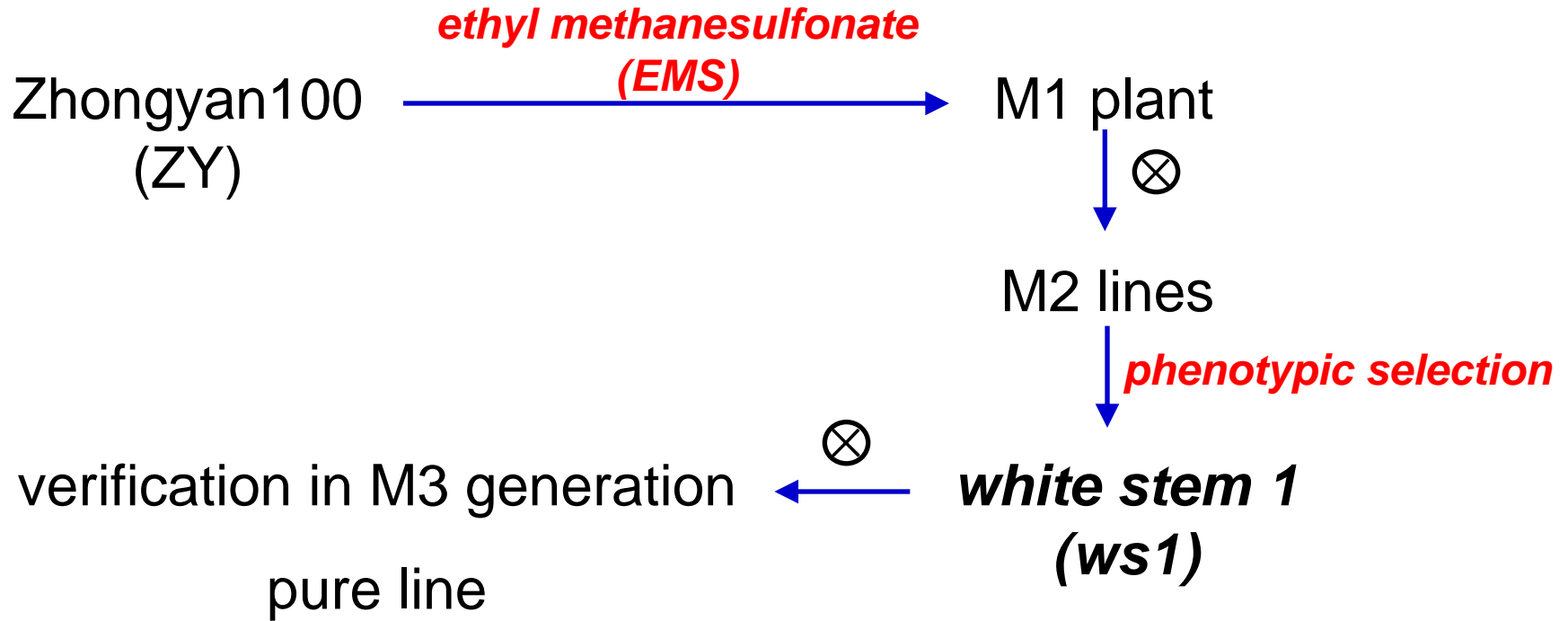
Some ornamental plants

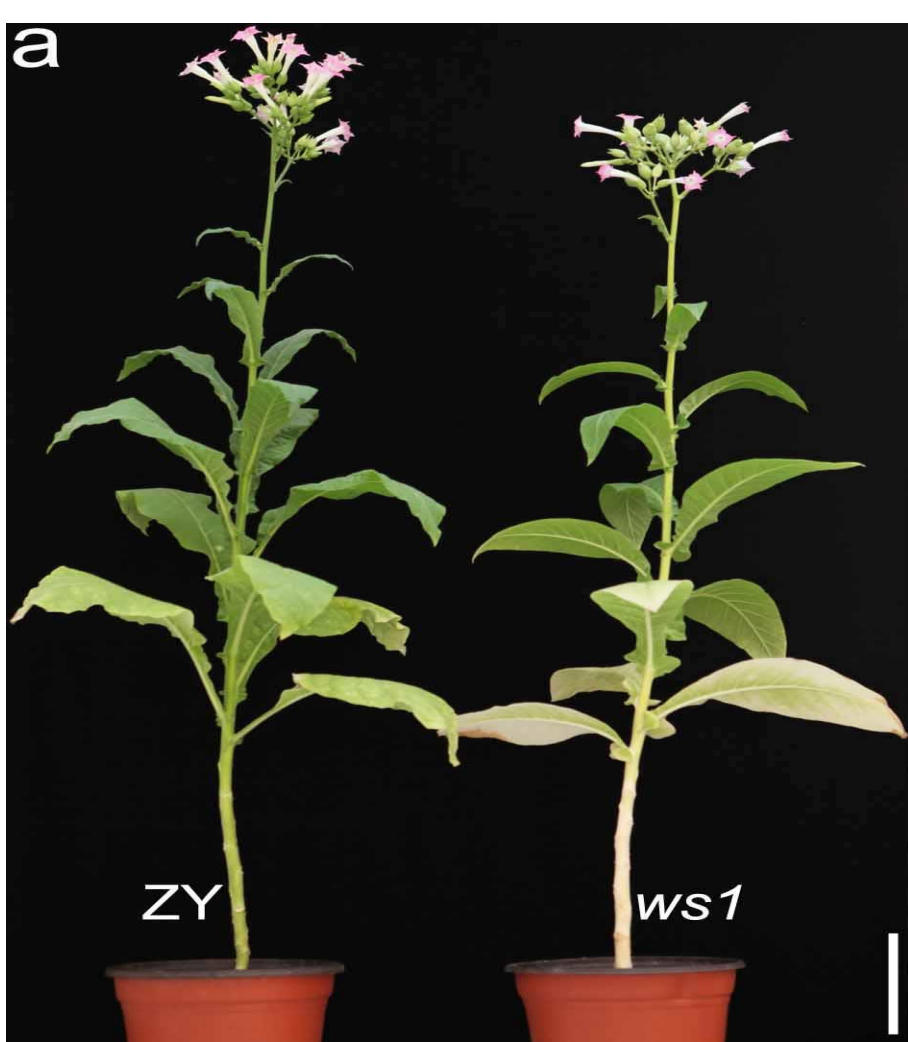
white tea and burley tobacco

with better taste and quality due to the reduction of Chl level



Results





stem color

upper

lower

ZY: green

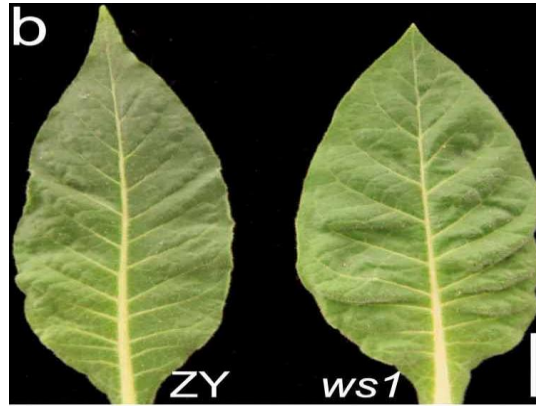
light green

ws1: light green

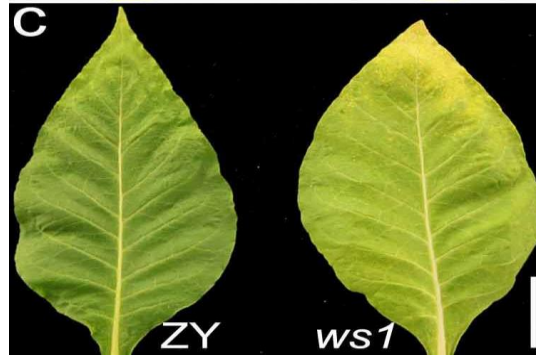
white

significant difference

upper leaves

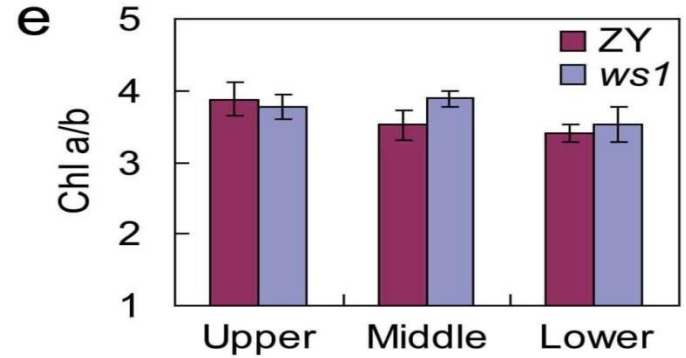
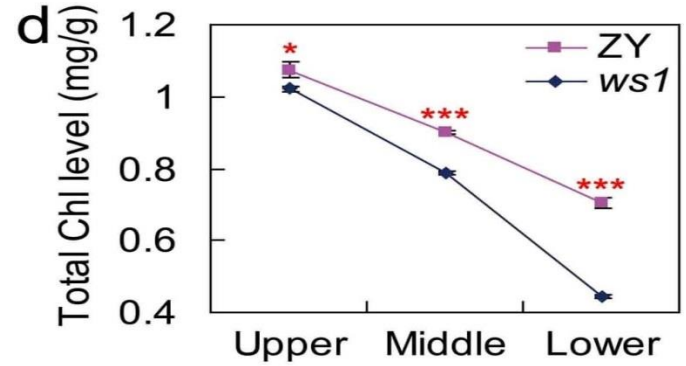


lower leaves



leaf color: *upper* *lower*

ZY:	green	light green
ws1:	green	yellow green



a defect in Chl metabolism

Table 1. Phenotypic segregations of four crosses derived from HD and ws1

Cross	Total	Green	White	Ratio	χ^2	
F2 (HD \times ws1)	234	221	13	17.00:1	0.193	15:1
RF2 (ws1 \times HD)	285	273	12	22.75:1	2.023	15:1
BC1F1 ((HD \times ws1) \times ws1)	52	40	12	3.33:1	0.103	3:1
RBC1F1 ((ws1 \times HD) \times ws1)	134	106	28	3.79:1	1.204	3:1

Honghua Dajinyuan (HD): a green-colored WT cultivar, similar to ZY

$$\chi^2 < \chi^2_{0.05} = 3.841$$

mutant phenotype is controlled by two recessive nuclear genes and neither gene alone can lead to the phenotype

the burley tobacco:

similar mutant phenotype to *ws1*
two recessive genes control

↓
allelic?

↓ *ws1* crossed with burley
tobacco cultivar BL21

ws1 phenotype in hybrid
F1 & selfed F2 plants

↓
allelic!



How to map two recessive genes

<i>Strategy</i>	<i>population</i>	<i>advantage</i>	<i>disadvantage</i>
QTL	F2/BC1	quick	rough
AB	≥BC3	fine mapping	more time & labour
(advanced backcross)			

Oryza sativa: fertility restorer (*Rf*) F2 rough

Triticum durum: yellow-green leaf durum (*ygld*) F2 rough

Brassica napus: recessive genic male sterile (*ms*) BC5 fine

Brassica juncea: trilocular silique (*mc*) BC4 fine

Fig1. Genotypic and phenotypic segregations of populations

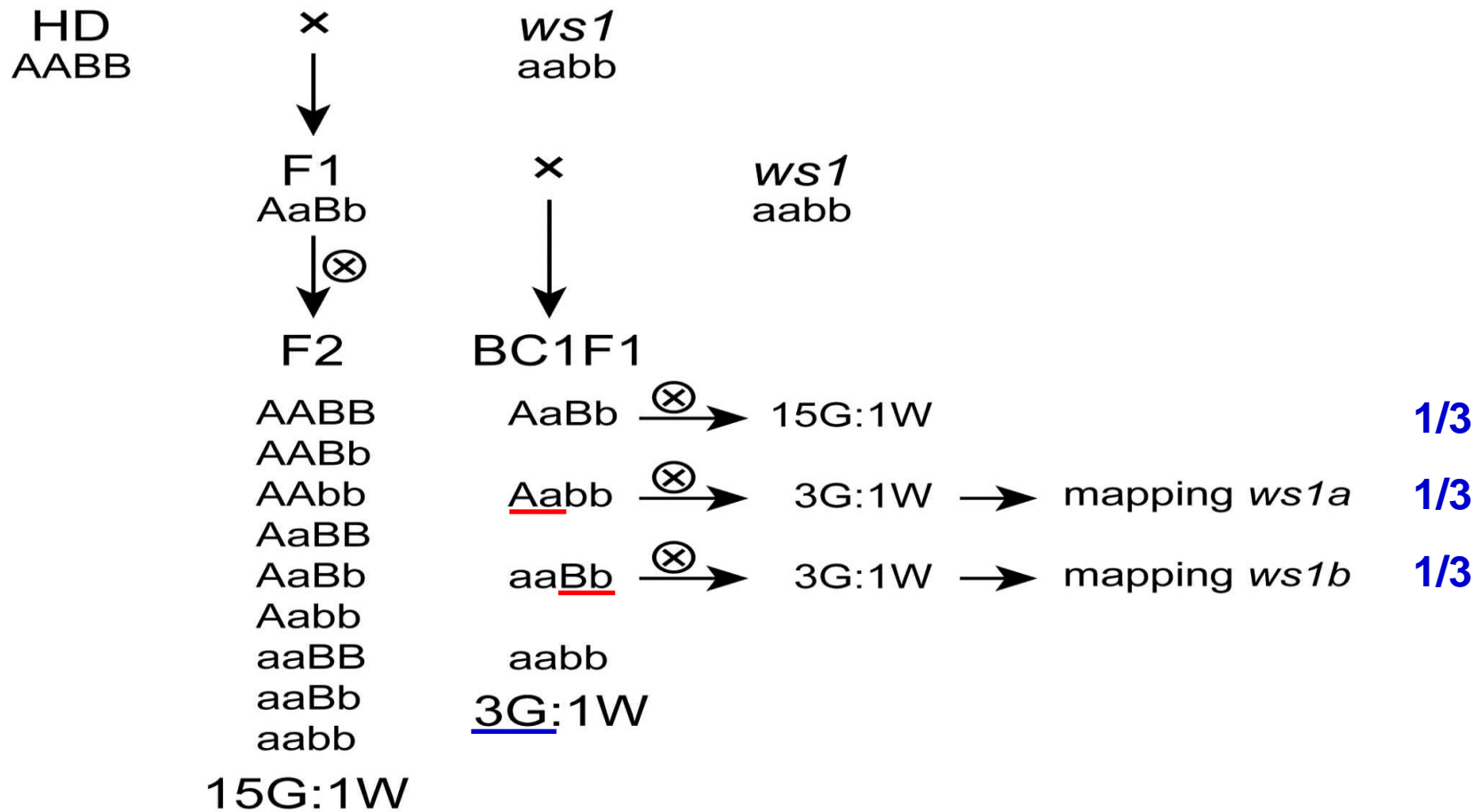


Table2. Phenotypic segregations of nine BC1F2 populations and their BC1F1 genotypes

No.	Total	Green	White	Ratio	χ^2	BC1F1 genotype
1	93	88	5	17.60:1	0.121	AaBb
2	94	89	5	17.80:1	0.139	AaBb
3	134	128	6	21.33:1	0.718	AaBb
4	91	87	4	21.75:1	0.534	AaBb
5	241	190	51	3.73:1	1.893	Aabb/ aaBb
6	130	101	29	3.48:1	0.503	Aabb/ aaBb
7	91	73	18	4.06:1	1.322	Aabb/ aaBb
8	235	187	48	3.90:1	2.623	Aabb/ aaBb
9	97	80	17	4.71:1	2.890	Aabb/ aaBb

1376 pairs of tobacco SSR primers
(Bindler et al., 2011)

amplification between HD and ws1

183 polymorphic SSR markers

selection

96 markers evenly distributed on the 24 linkage groups (LG)

amplification

10 green-colored and 10 white-colored individuals of a BC1F2 population

identification

the linked SSR marker and its location in genetic map

amplification using more adjacent markers

The remaining recessive individuals of population

*construction of linkage map with
QTL IciMapping software*

the restricted mapping interval of *ws1a/b* gene

Fig2. Linkage relationships between SSR markers and *ws1a*

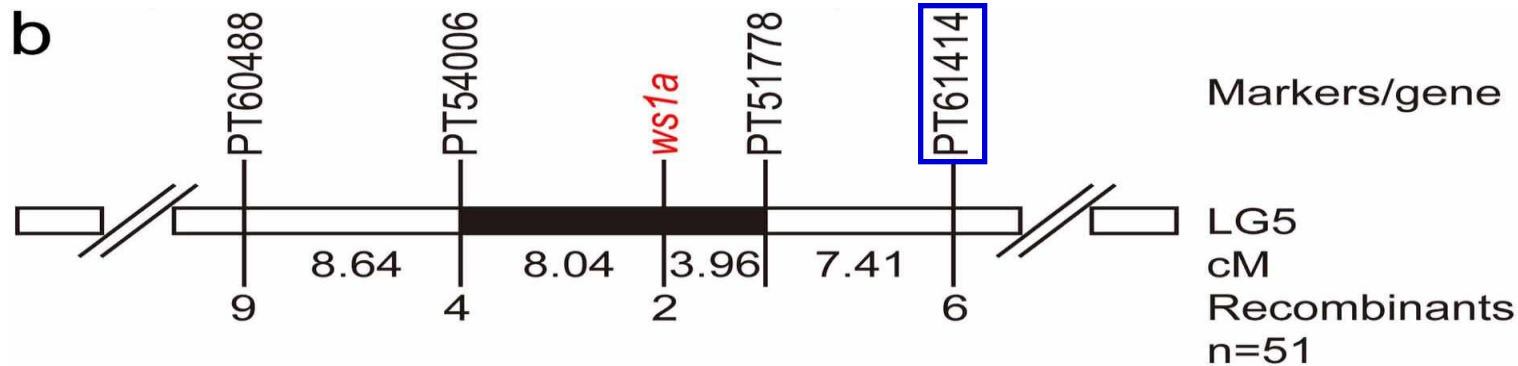
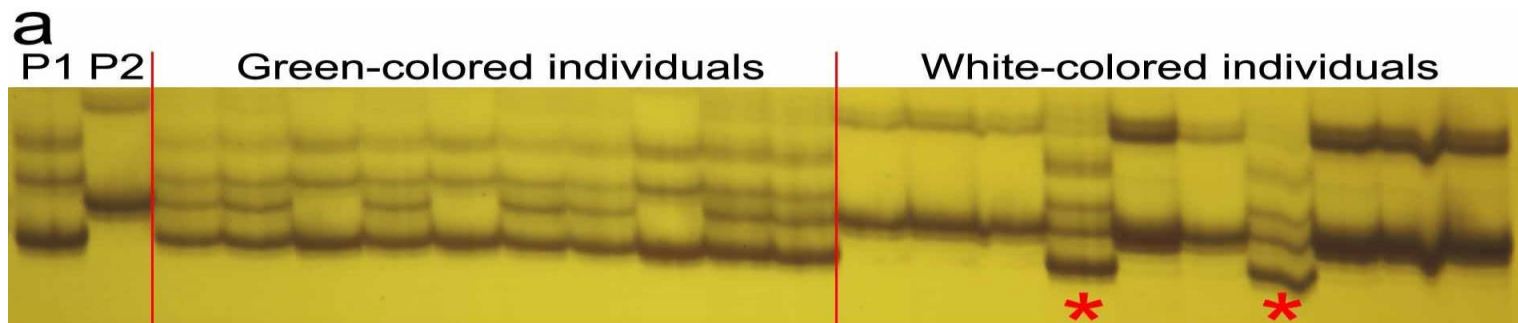
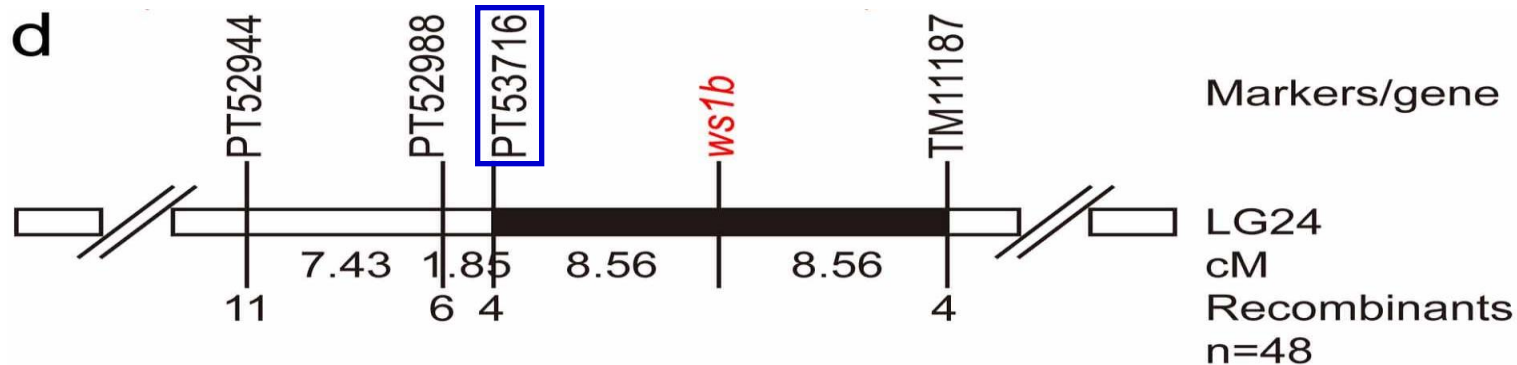
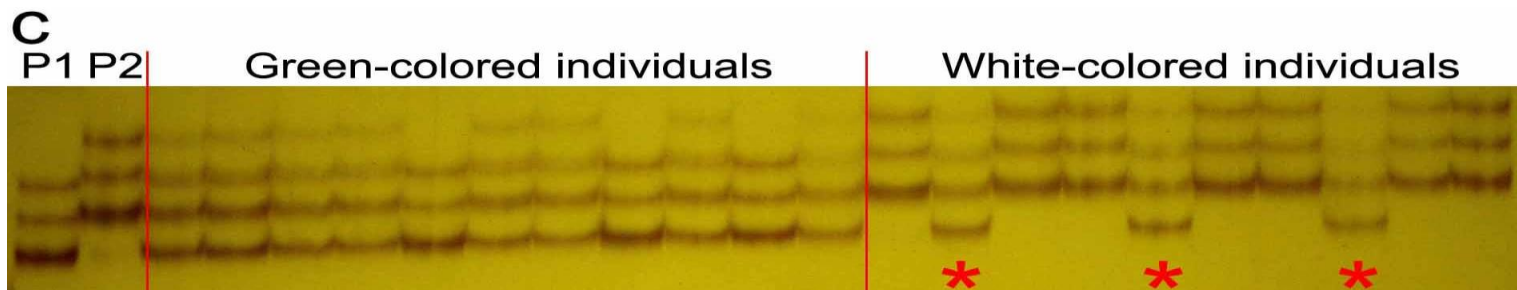


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9	97	80	17	4.71:1	2.890	aaBb

Fig3. Linkage relationships between SSR markers and *ws1b*



Conclusions

1. The close and accurate mapping result

Pre: monosome rough and less confident

Now: molecular marker larger interval but definite

2. The connection between chromosomes and linkage groups

Chromosome: Chr B/O

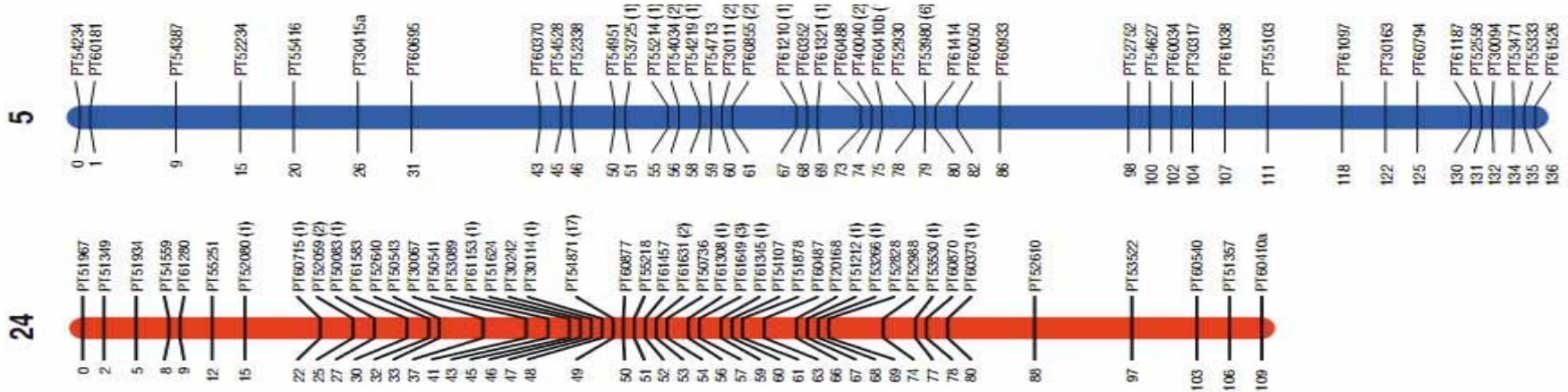
Linkage group: LG 5/24



fine mapping

3. *ws1a* and *ws1b* are probable paralogs of each other

- *N. tabacum* (*N. sylvestris* × *N. tomentosiformis*)---2 copies
- *ws1a* & *ws1b*: function compensation for each other
- LG5: *N. sylvestris* LG24: *N. tomentosiformis*



4. *ws1a* & *ws1b* may play key roles in Chl biosynthesis

- similar mutant phenotype to Chl-deficient mutant (light color)
- no loss-of-function gene has yet been reported to promote the breakdown of Chl

Thank You

