# The Related Research of a Chlorophyll-Deficient Mutant in Chlorophyll Biosynthesis

an example from rice

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# Outline

- Introduction

  Background、Significance、Research advance
- The Process of Chl Biosynthesis
- Chlorophyll-Deficient Mutant (Oryza sativa)
- Results and Discussion
- Acknowledgments

# Background

1.叶绿素分子广泛存在于进行光合作用的有机体中，起着吸收光能、传递电子的功能。

2.20世纪30年代，在叶绿素合成缺陷突变体方面就有所研究。我们在棉花中称这一突变性状为"芽黄"virescent (南京农业大学)。

3.腺体、蜜腺、卷曲叶、长柱头、芽黄等众多的棉花突变性状，在利用杂种优势育种时可以作为指示性状，尤其是芽黄这一性状易于观察。

# Significance

1. 棉花杂种优势利用中作为标记性状
2. 水稻等两系法杂交制种中做标记性状保证种子纯度
3. 转基因用于雄性不育制种

# Research advance

1.芽黄性状一般是由隐性基因控制的可遗传性状，并在拟南芥、水稻、玉米、烟草、番茄等开花植物中表现。

2.芽黄性状一般在苗期表现明显，花期或者盛花期时，子叶或者真叶在苗期表现出的不同程度的黄色将会慢慢变绿。

3.Wu等已培育出携带转绿型黄叶标记的水稻光敏雄性不育系“Xinguang S”，这种将叶色突变与雄性不育相结合的方法，为保证杂交种纯度提供了有效途径。

4.在陆地棉异源四倍体中已鉴定的芽黄基因有26个，其中有部分同源基因对存在于22个芽黄突变体。

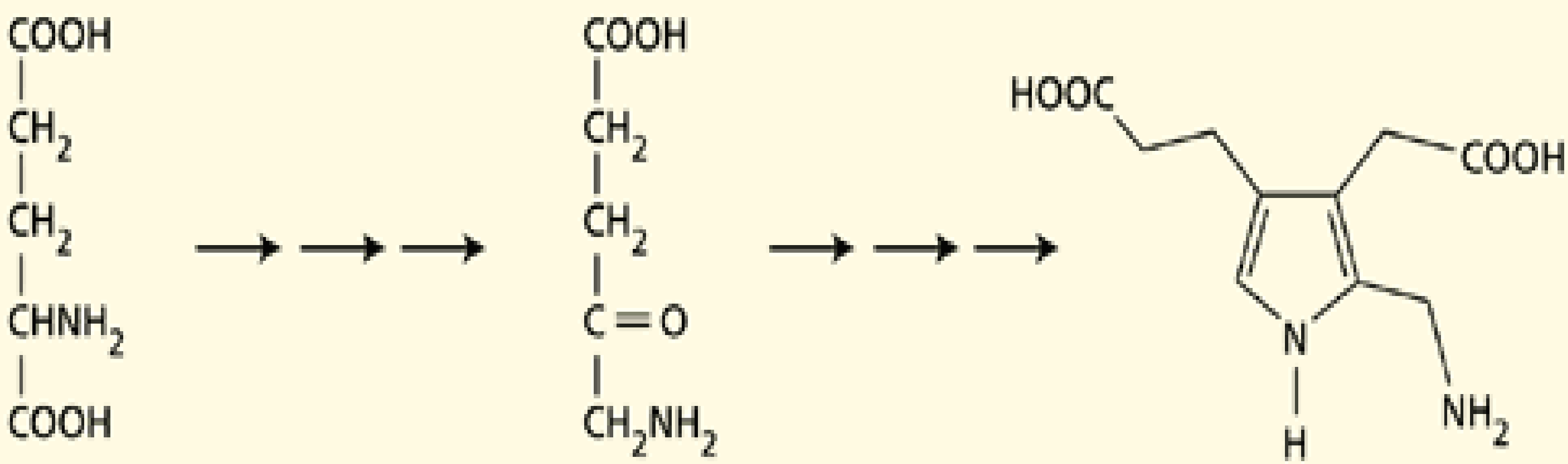
5.目前在棉花中，芽黄基因的核酸序列还没有报道。

6.水稻上的芽黄基因已有报道，以水稻为例，可为以后棉花芽黄基因的预测做准备。

# The process of Chl biosynthesis

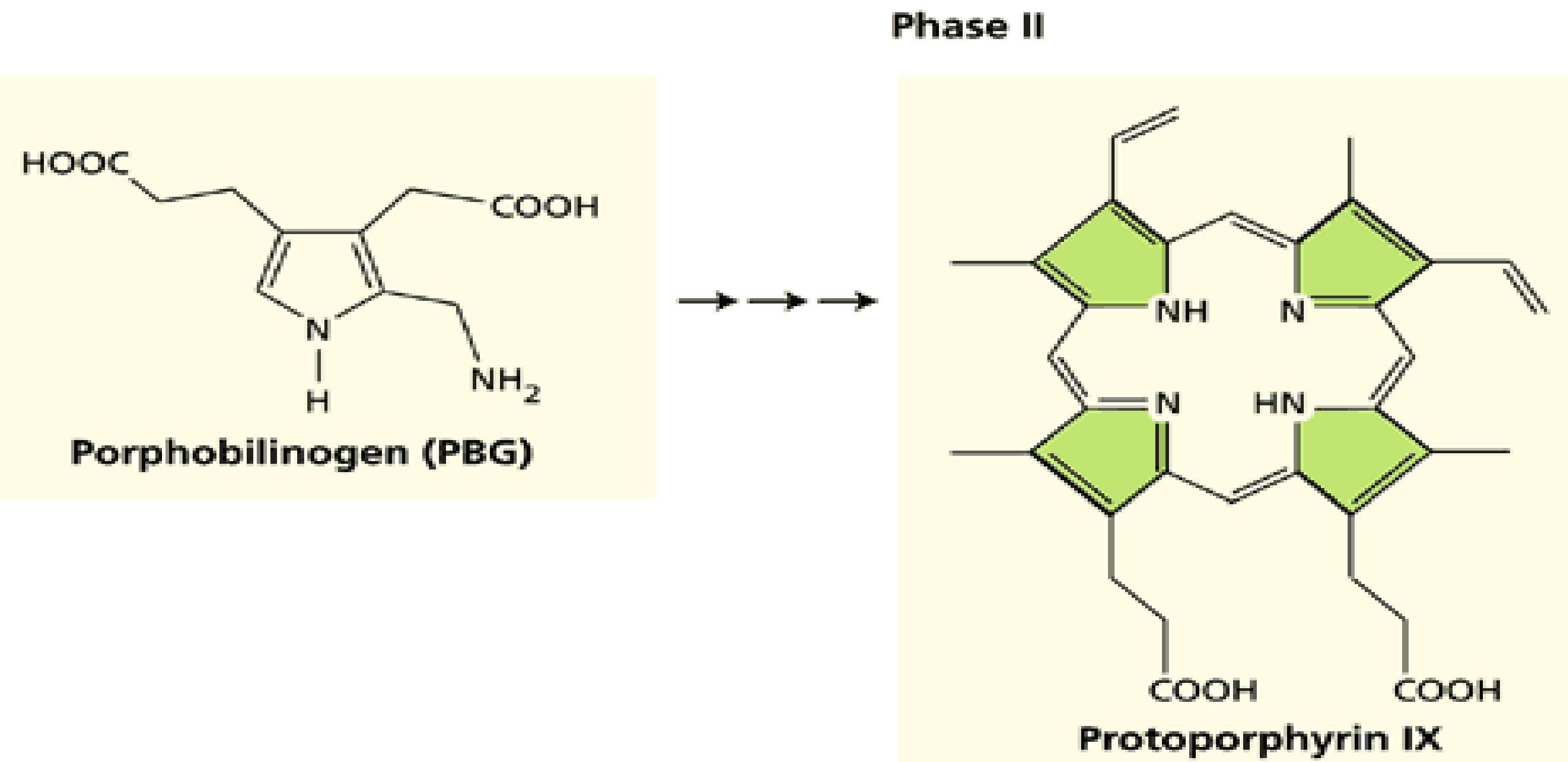
第一阶段： 谷氨酸 + tRNA$^{Glu}$ → 谷氨酰tRNA → → →
5-氨基乙酰丙酸（ALA) → → → 胆色素原（PBG）

Phase I

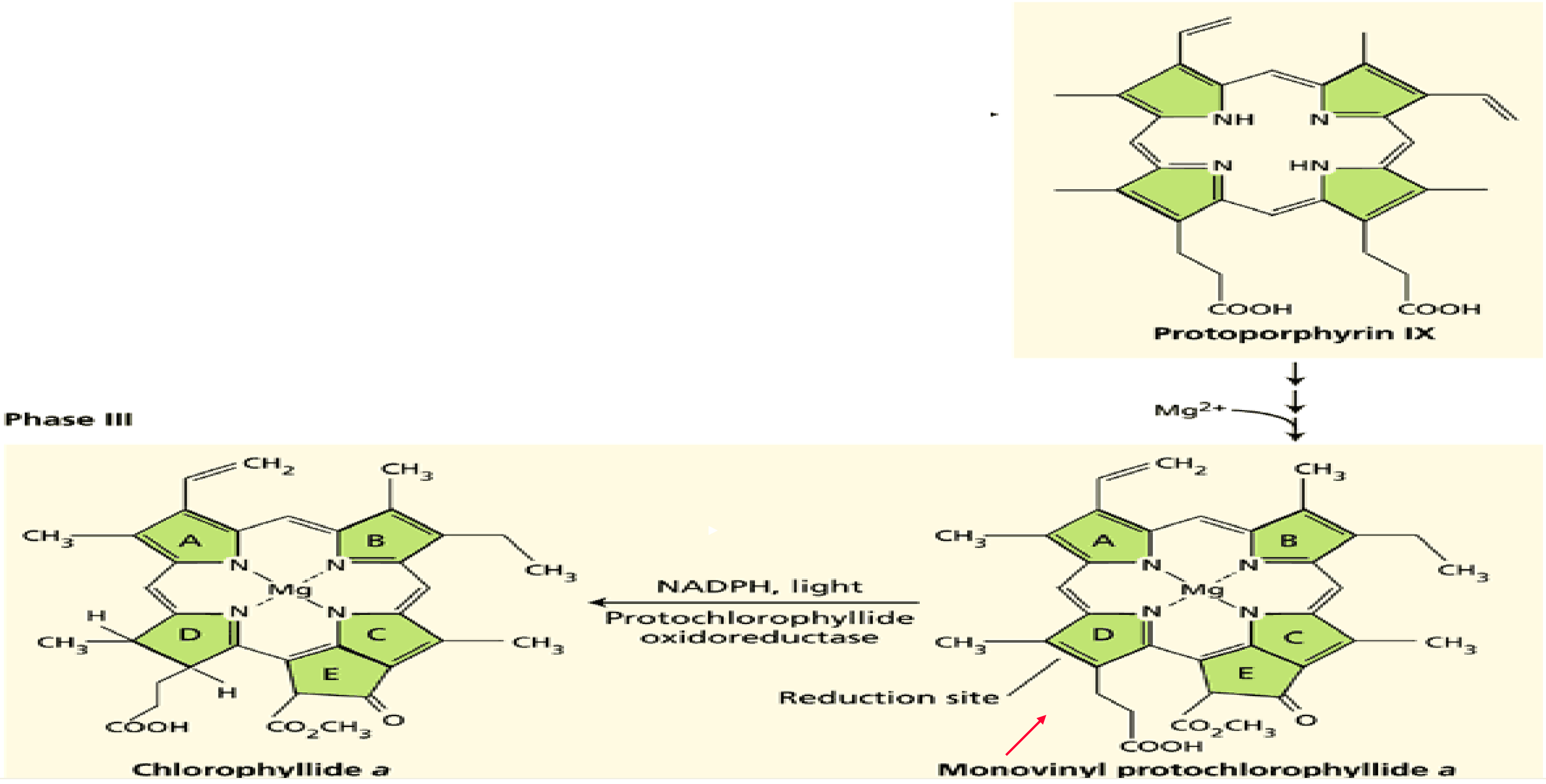


Glutamic acid　5-Aminolevulinic acid (ALA)　Porphobilinogen (PBG)

**第二阶段：**4 分子的PBG聚合形成卟啉结构，经过6步酶促反应，最终形成原卟啉IX。以上反应是合成叶绿素和血红素共有的。



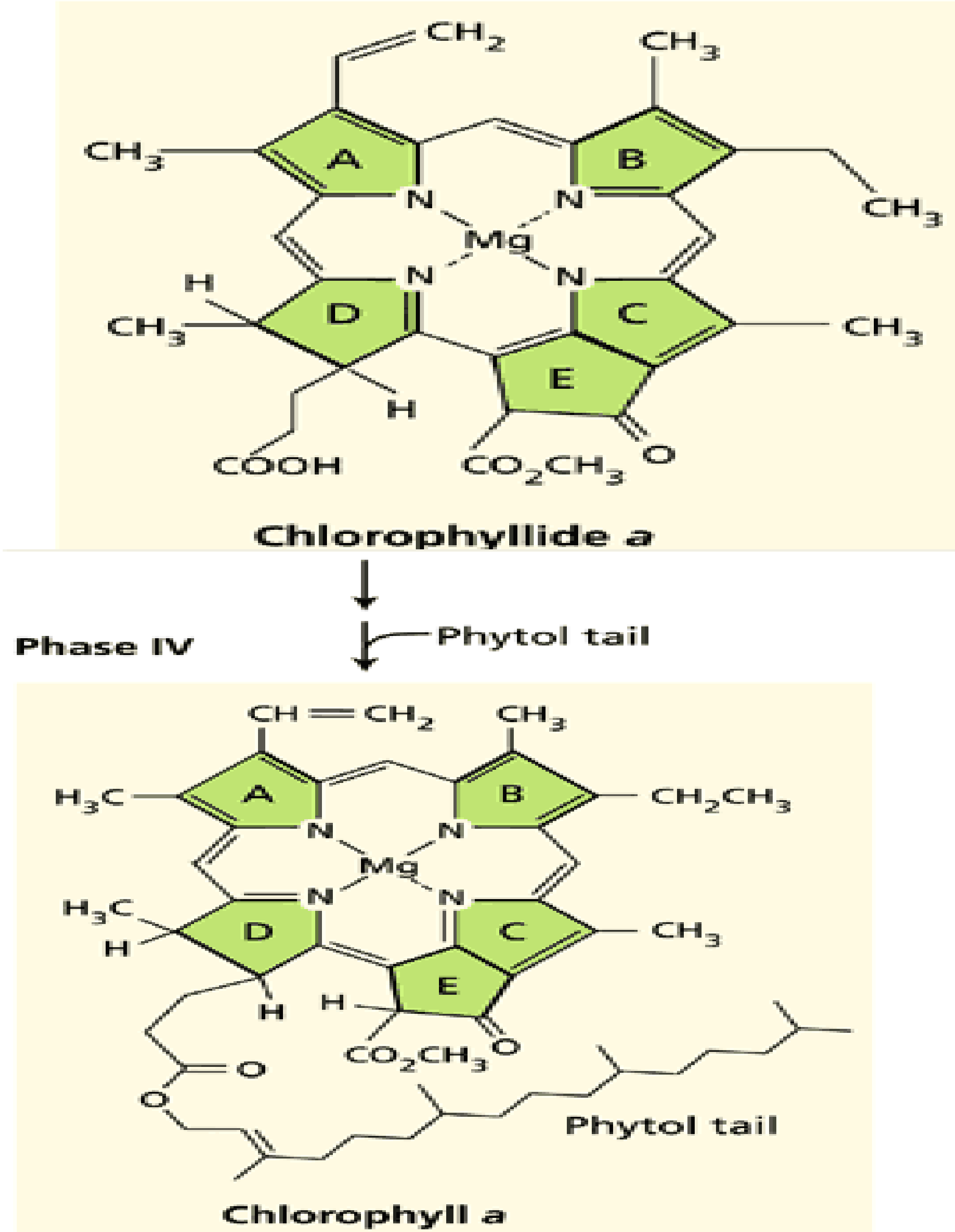
第三阶段： 原卟林IX 螯合镁; 环化形成 E环, 甲酯化; D环被还原.

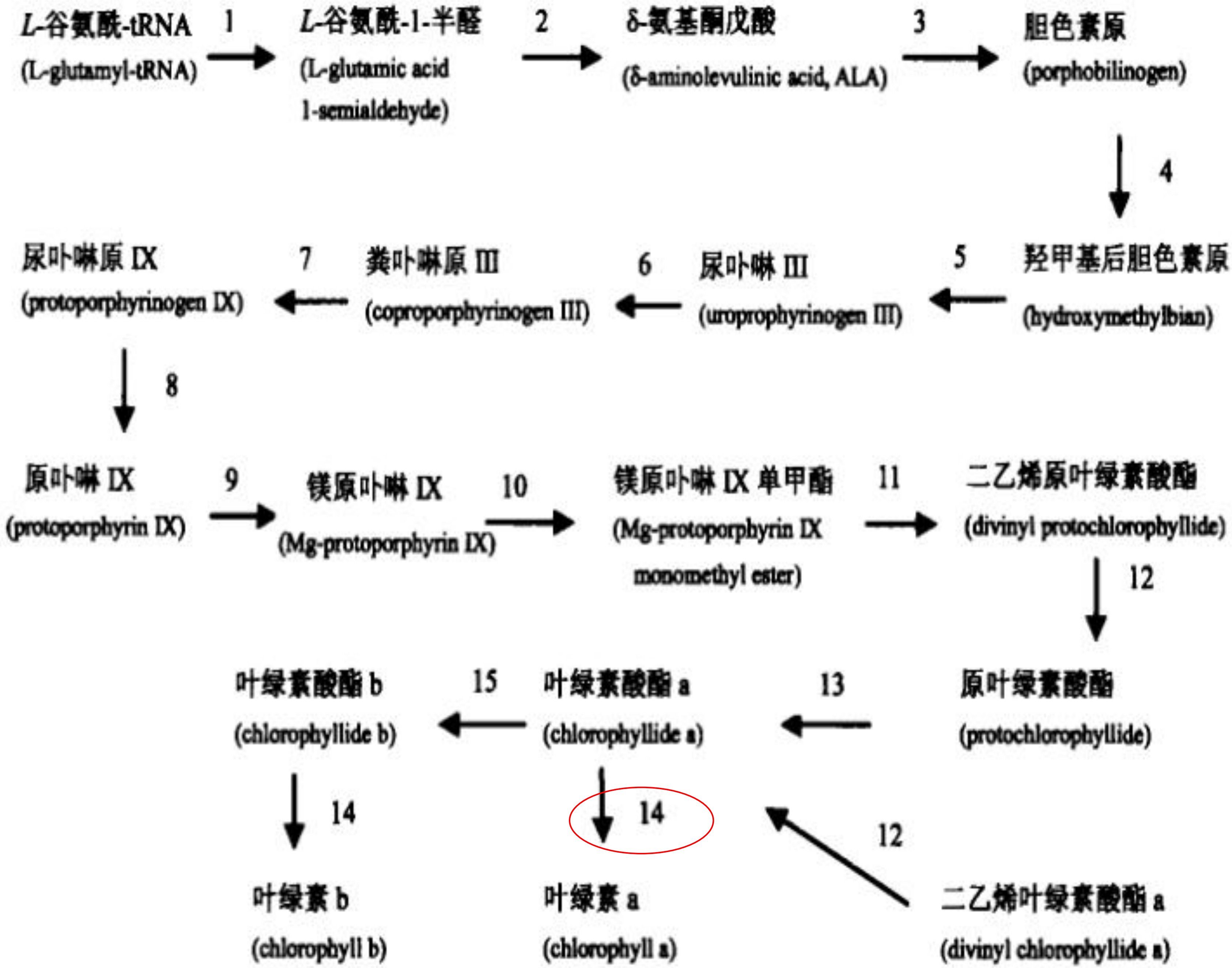



叶绿酸酯a

原叶绿酸酯a

**最后阶段:** 叶绿酸酯a在叶绿素合成酶作用下被叶醇酯化形成叶绿素a





L-谷氨酰-tRNA
(L-glutamyl-tRNA)
1
L-谷氨酰-1-半醛
(L-glutamic acid 1-semialdehyde)
2
δ-氨基酮戊酸
(δ-aminolevulinic acid, ALA)
3
胆色素原
(porphobilinogen)
4
羟甲基后胆色素原
(hydroxymethylbian)
5
尿卟啉 III
(uroprophyrinogen III)
6
粪卟啉原 III
(coproporphyrinogen III)
7
尿卟啉原 IX
(protoporphyrinogen IX)
8
原卟啉 IX
(protoporphyrin IX)
9
镁原卟啉 IX
(Mg-protoporphyrin IX)
10
镁原卟啉 IX 单甲酯
(Mg-protoporphyrin IX monomethyl ester)
11
二乙烯原叶绿素酸酯
(divinyl protochlorophyllide)
12
原叶绿素酸酯
(protochlorophyllide)
13
叶绿素酸酯 a
(chlorophyllide a)
15
叶绿素酸酯 b
(chlorophyllide b)
14
叶绿素 b
(chlorophyll b)
14
叶绿素 a
(chlorophyll a)
12
二乙烯叶绿素酸酯 a
(divinyl chlorophyllide a)

1 谷氨酰-tRNA还原酶(glutamyl tRNA reductase)

2 谷氨酸-1-半醛转氨酶(glutamate 1-semialdehyde aminotransferase)

3 胆色素原合酶(porphobilinogen synthase) [5-氨基酮戊酸脱水酶(5-aminolevulinate dehydratase)]

4 羟甲基后胆色素原合酶(hydroxymethylbilane synthase) [胆色素原合酶脱氨酶(porphobilinogen deaminase)]

5 尿卟啉原III合酶(uroporphyrinogen III synthase) [尿卟啉原III共合酶(uroporphyrinogen III co-synthase)]

6 尿卟啉原脱羧酶(uroporphyrinogen decarboxylase)

7 粪卟啉原氧化脱羧酶(coproporphyrinogen oxidative decarboxylase)

8 原卟啉原氧化酶(protoporphyrinogen oxidase)

9 镁螯合酶D亚基(Mg chelatase D subunit)
镁螯合酶H亚基(Mg chelatase H subunit)
镁螯合酶I亚基(Mg chelatase I subunit)

10 镁原卟啉IX甲基转移酶(Mg-protoporphyrin IX methyltransferase)

11 镁原卟啉原IX单甲酯环化酶(Mg-protoporphyrinogen IX monomethylester cyclase)

12 二乙烯还原酶(divinyl reductase)

13 NADPH原叶绿素酸酯氧化还原酶(NADPH:protochlorophyllide oxidoreductase)

14 叶绿素合酶(chlorophyll synthase)

15 叶绿素酸酯a氧化酶(chlorophyllide a oxygenase)

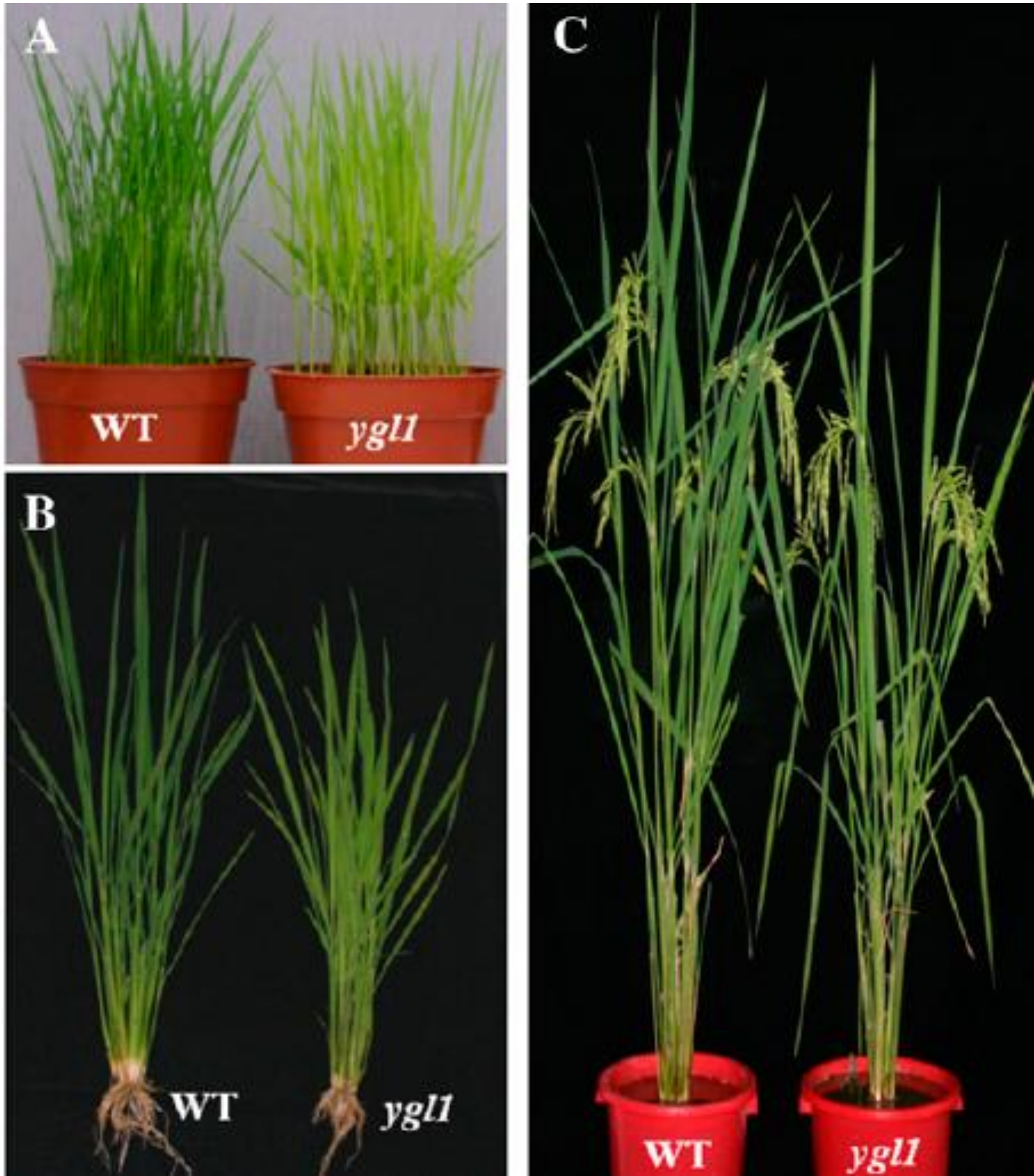
# Chlorophyll-Deficient Mutant

## The ygl1 Mutant

A, Four-week-old plants.

B,Ten-week-old plants.

C, Fifteen-week-old plants

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- The ygl1 Mutant showed yellow-green leaves in young plants with decreased Chl synthesis, increased level of tetrapyrrole intermediates, and delayed chloroplast development.

- Leaves of the ygl1 mutant had 20% to 70% reduction of Chl, and 30% to 40% reduction of Car levels compared to those in wild type at different stages, with the most significant differences detectable in 4-week-old plants.

- The Chl a/b ratio appeared highest at the seedling stage, due likely to the potential of Chl b synthesis in suffering a more severe decline than Chla . The Chl a/b ratio then declined to eventually reach the wild-type level.

- this suggests that the ygl1 mutant exhibited delayed greening during photomorphogenesis because of slow rates of Chl accumulation.

●Granal stacks in the ygl1 mutant appeared less dense and lacked granal membranes compared to those of wild type in developing leaves.

●Granal development in the ygl1 mutant was slower than that of wild type, and granal membranes in the ygl1 mutant increased when the leaf became mature.

# How the ygl1 mutation affects chloroplast  development ?

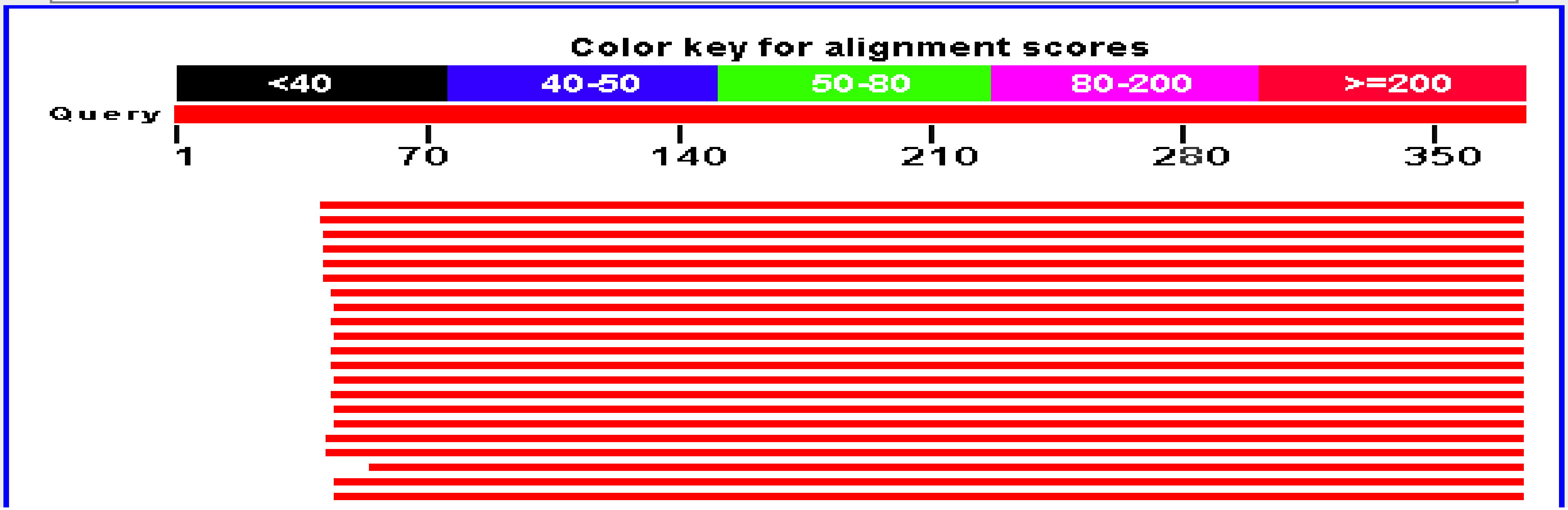
# Protein BLAST

**Sequences producing significant alignments:**

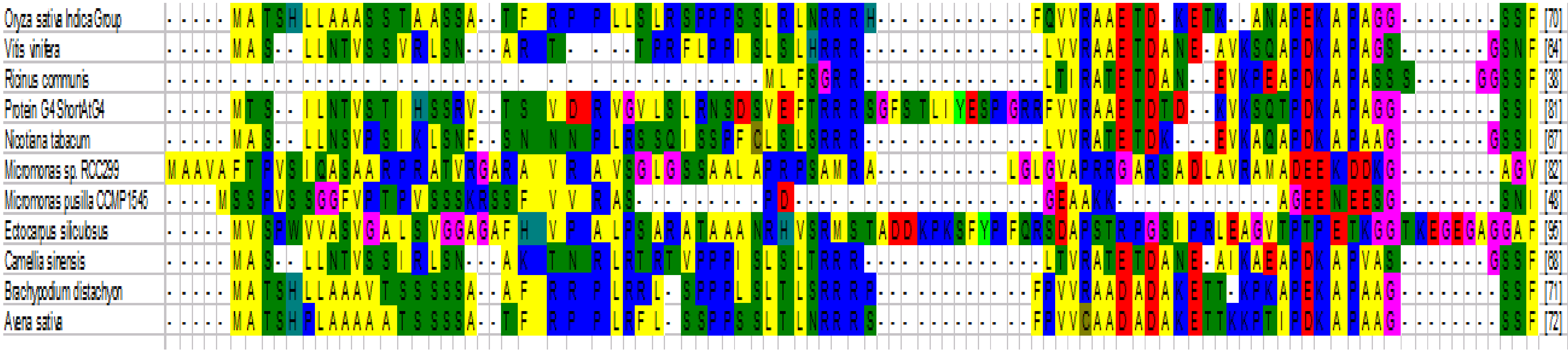
| Accession | Description | Max score |
|---|---|---|
| EEC79019.1 | hypothetical protein OsI_19554 [Oryza sativa Indica Group] | 655 |
| NP_001055272.1 | Os05g0349700 [Oryza sativa Japonica Group] >sp\|Q5W6H5.1\|CHLG_ | 655 |
| XP_002440929.1 | hypothetical protein SORBIDRAFT_09g016840 [Sorghum bicolor] >gb | 611 |
| NP_001142204.1 | uncharacterized protein LOC100274372 [Zea mays] >gb\|ACF87878.1 | 608 |
| XP_003568641.1 | PREDICTED: chlorophyll synthase, chloroplastic-like [Brachypodium d | 604 |
| Q9M3W5.1 | RecName: Full=Chlorophyll synthase, chloroplastic; AltName: Full=Po | 599 |
| XP_002263271.1 | PREDICTED: chlorophyll synthase, chloroplastic [Vitis vinifera] >emb\| | 588 |
| AEI83422.1 | chlorophyll synthase [Camellia sinensis] | 585 |
| ACQ44245.1 | chlorophyll synthase [Nicotiana tabacum] | 580 |
| XP_002530507.1 | bacteriochlorophyll synthase, putative [Ricinus communis] >gb\|EEF3 | 577 |
| ACQ44244.1 | chlorophyll synthase [Nicotiana tabacum] | 577 |
| XP_002877826.1 | ATG4/CHLG/G4 [Arabidopsis lyrata subsp. lyrata] >gb\|EFH54085.1\| A | 578 |
| XP_002308227.1 | predicted protein [Populus trichocarpa] >gb\|ABK95029.1\| unknown [ | 577 |
| NP_190750.1 | chlorophyll synthase [Arabidopsis thaliana] >sp\|Q38833.1\|CHLG_ARA | 576 |

**Distribution of 100 Blast Hits on the Query Sequence**

Mouse-over to show defline and scores, click to show alignments



# 建进化树



Oryza sativa Indica Group
Vitis vinifera
Ricinus communis
Protein G4 ShortAtG4
Nicotiana tabacum
Micromonas sp. RCC299
Micromonas pusilla CCMP1545
Ectocarpus siliculosus
Camellia sinensis
Brachypodium distachyon
Avena sativa
[70]
[84]
[38]
[81]
[67]
[82]
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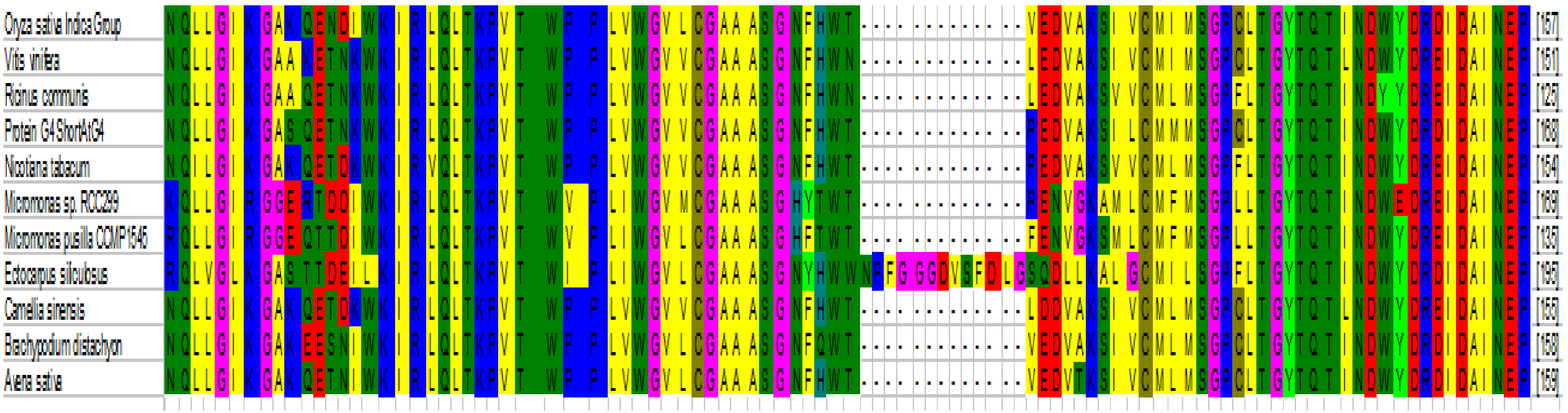
[56]
[68]
[71]
[72]

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Micromonas pusilla CCMP1545
Ectocarpus siliculosus
Camellia sinensis
Brachypodium distachyon
Avena sativa
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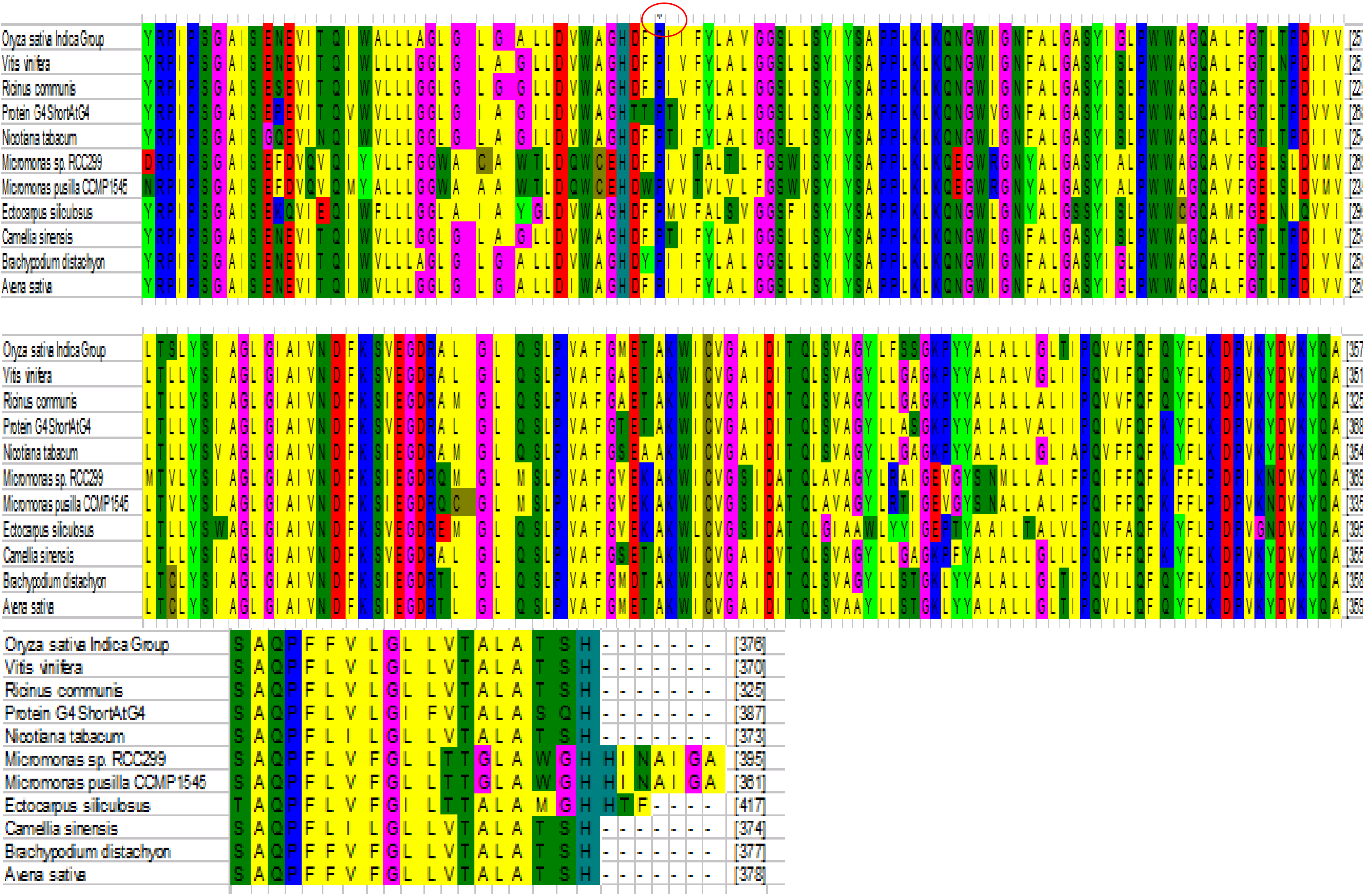
[159]


Oryza sativa Indica Group
Vitis vinifera
Ricinus communis
Protein G4 ShortAtG4
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Micromonas sp. RCC299
Micromonas pusilla CCMP1545
Ectocarpus siliculosus
Camellia sinensis
Brachypodium distachyon
Avena sativa

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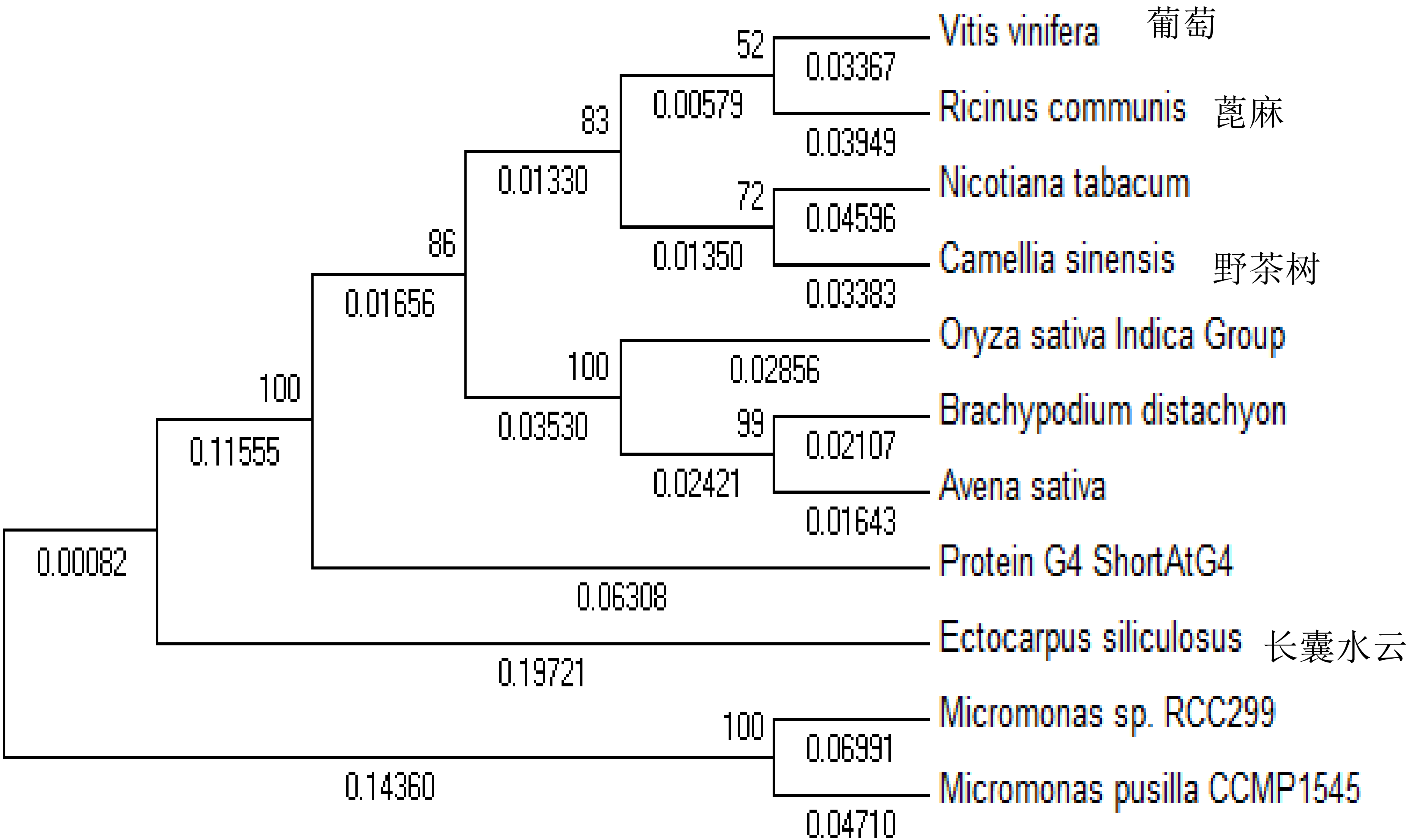
Vitis vinifera 葡萄
Ricinus communis 蓖麻
Nicotiana tabacum
Camellia sinensis 野茶树
Oryza sativa Indica Group
Brachypodium distachyon
Avena sativa
Protein G4 ShortAtG4
Ectocarpus siliculosus 长囊水云
Micromonas sp. RCC299
Micromonas pusilla CCMP1545
52
83
72
86
100
100
99
100
0.03367
0.00579
0.03949
0.01330
0.04596
0.01350
0.03383
0.01656
0.02856
0.03530
0.02107
0.02421
0.01643
0.11555
0.00082
0.06308
0.19721
0.06991
0.14360
0.04710

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PEPSTATS of [Oryza from 1 to 376

Molecular weight = 40578.88 Residues = 376

Average Residue Weight = 107.923 Charge = 4.5

Isoelectric Point = 8.2793

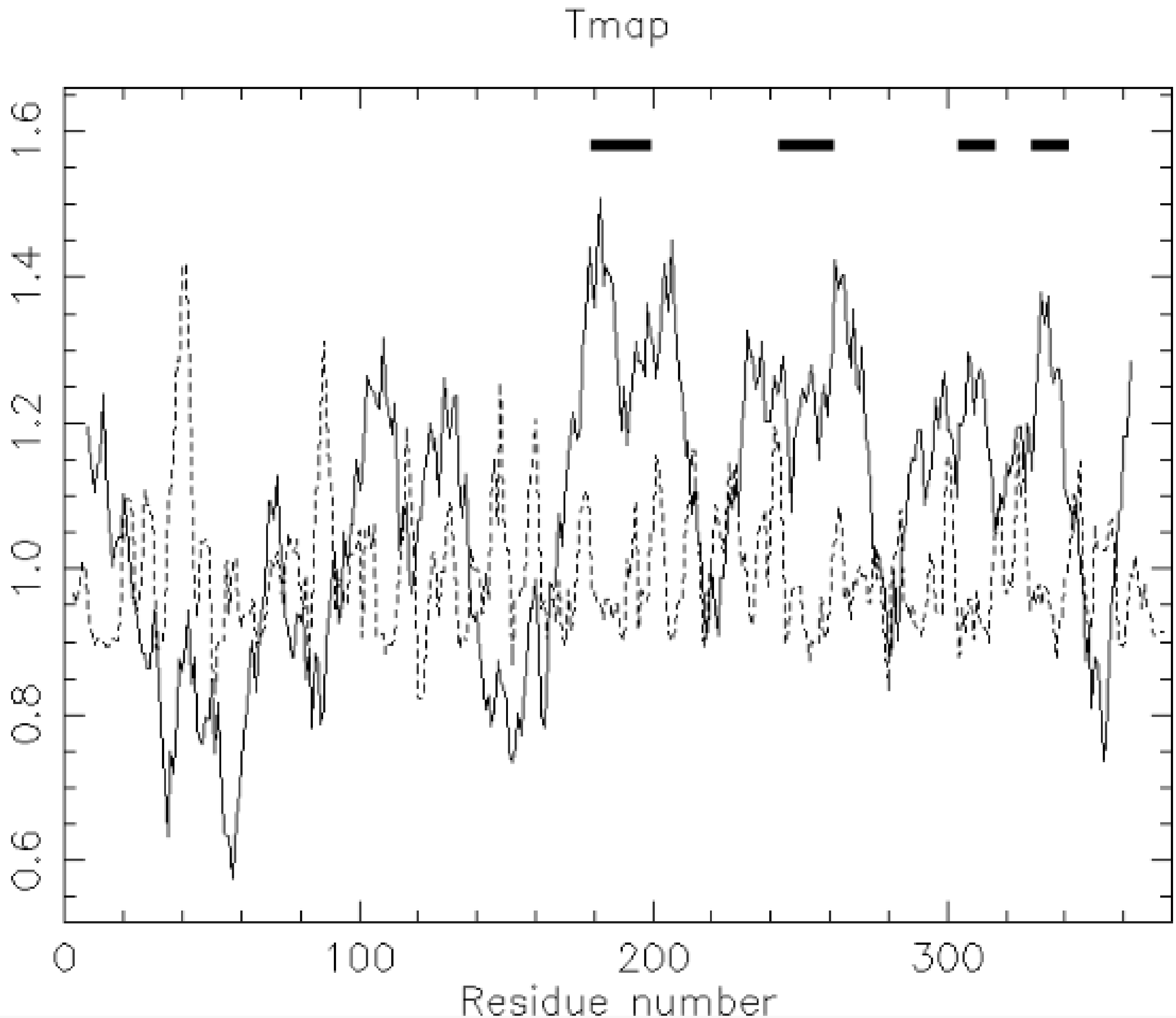
A280 Molar Extinction Coefficient = 80510

A280 Extinction Coefficient 1mg/ml = 1.98

Improbability of expression in inclusion bodies = 0.819

| Residue | Number | Mole% | DayhoffStat |
|---|---|---|---|
| A = Ala | 43 | 11.436 | 1.330 |
| B = Asx | 0 | 0.000 | 0.000 |
| C = Cys | 4 | 1.064 | 0.367 |
| D = Asp | 15 | 3.989 | 0.725 |
| E = Glu | 10 | 2.660 | 0.443 |
| F = Phe | 16 | 4.255 | 1.182 |
| G = Gly | 32 | 8.511 | 1.013 |
| H = His | 5 | 1.330 | 0.665 |
| I = Ile | 25 | 6.649 | 1.478 |
| J = --- | 0 | 0.000 | 0.000 |
| K = Lys | 16 | 4.255 | 0.645 |
| L = Leu | 47 | 12.500 | 1.689 |
| M = Met | 4 | 1.064 | 0.626 |
| N = Asn | 11 | 2.926 | 0.680 |
| O = --- | 0 | 0.000 | 0.000 |
| P = Pro | 24 | 6.383 | 1.227 |
| Q = Gln | 15 | 3.989 | 1.023 |
| R = Arg | 11 | 2.926 | 0.597 |
| S = Ser | 29 | 7.713 | 1.102 |
| T = Thr | 20 | 5.319 | 0.872 |
| U = --- | 0 | 0.000 | 0.000 |
| V = Val | 24 | 6.383 | 0.967 |
| W = Trp | 11 | 2.926 | 2.250 |
| X = Xaa | 0 | 0.000 | 0.000 |
| Y = Tyr | 14 | 3.723 | 1.095 |
| Z = Glx | 0 | 0.000 | 0.000 |

| Property | Residues | Number | Mole% |
|---|---|---|---|
| Tiny | (A+C+G+S+T) | 128 | 34.043 |
| Small | (A+B+C+D+G+N+P+S+T+V) | 202 | 53.723 |


Tmap
1.6
1.4
1.2
1.0
0.8
0.6
0
100
200
300
Residue number

```
########################################
# Program: tmap
# Rundate: Tue 21 Feb 2012 04:12:57
# Commandline: tmap
#     -auto
#     -graph png
#     -sequences 509968
#     -goutfile 509969
#     -outfile 509970
# Report_format: seqtable
# Report_file: 509970
########################################
#=======================================
#
# Sequence: Consensus     from: 1    to: 376
# HitCount: 4
#=======================================
  Start     End TransMem Sequence
    175     203        1 IWALLLAGLGLGALLDVWAG
    239     265        2 LPWWAGQALFGTLTPDIVVL
    300     320        3 WICVGAIDITQLSVAGYLFS
    325     345        4 YALALLGLTIPQVVFQFQYF
#---------------------------------------
#---------------------------------------
#=======================================
#
# Sequence: [Oryza     from: 1    to: 376
# HitCount: 4
#=======================================
  Start     End TransMem Sequence
    175     203        1 IWALLLAGLGLGALLDVWAG
    239     265        2 LPWWAGQALFGTLTPDIVVL
    300     320        3 WICVGAIDITQLSVAGYLFS
    325     345        4 YALALLGLTIPQVVFQFQYF
#---------------------------------------
#---------------------------------------
#---------------------------------------
# Total_sequences: 2
# Total_hitcount: 8
#---------------------------------------
```

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# Results

The ygl1 mutant was a spontaneous mutant which has reduced chl accumulation and delayed chloroplast development

1. Genetic analysis demonstrated that the phenotype of ygl1 was caused by a recessive mutation in a nuclear gene. The ygl1 locus was mapped to chromosome 5 and isolated by map-based cloning.

2. A point mutation (Pro-198 to Ser) in YGL1 was found at the highly conserved Pro-198, which compromised the esterification activity of Chl synthase.

- 四个跨膜区
- 位于类囊体膜上
- 突变位点（Pro-198 to Ser）几乎位于第一个跨膜螺旋的终点
- 突变位于保守位点

# DISCUSSION

Why the ygl1 mutation affects Chl biosynthesis most dramatically in the early developmental stage but is restored in later stages is not yet completely understood?

1. One possible explanation is that there might be other Chl synthase homologs with redundant functional activities in later stages

   However, no other rice Chl synthase genes were identified from a survey of the rice genome database.

2. Since there are not find significant differences in transcription level of the YGL1 gene at the different development stages, one possibility is that the enzyme is regulated at the translational level.

Why the Pro-198 residue is important in YGL1 ?

It could be attributed to its location in or proximity to the binding site of Chlide

The Car content was significantly lower in the mutant plants compared to wild type, even in older leaves in which the Chl content was the same as wild type?

This result might be related to the parallel degradation of pigments and pigment-binding proteins of the photosynthetic apparatus.

# Literature cited

1.Ziming Wu etal. A Chlorophyll-Deficient Rice Mutant with Impaired Chlorophyllide Esterification in Chlorophyll Biosynthesis

2.宋美珍等.  一个短季棉芽黄基因型的鉴定及生理生化分析

3.黄晓群等. 水稻叶绿素合成缺陷突变体及其生物学研究进展

4.吴自明等.叶绿素生物合成的分子调控

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