# Study on molecular mechanism of a new virescent mutant (*vsp*) in short season cotton (*Gossypium hirsutum* L.)

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# Background

- **Cotton is one of the most important economic crops**

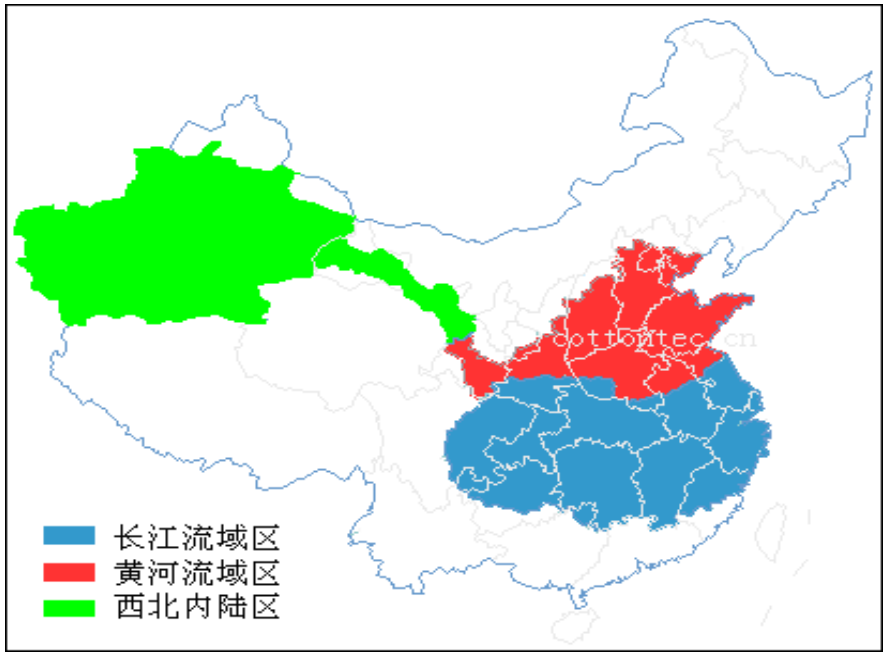
- **Over 80 cotton-cultivating countries located between 32° south and 47° north latitude on the globe**
- **About 33 million ha or 5% of the world's arable land is used for cotton planting annually**

# China is one of the major cotton-producing countries

**Cotton planting area:** 5-5.5 million ha

**Cotton yield:** 1350kg·ha$^{-1}$

**Three main produced regions:** Yangtze cotton region, Huanghe cotton region, Northwest inland cotton region




- **Virescent leaves are an important character of plant that can be inherited**

  **Many mutated methods: nature condition, physical treatment(γ-irradiation ), chemical treatment(EMS) and tissue culture.**

  **Genetic mutation: one recessive genes in nucleus**

- **Virescent mutations occur in a wide range of flowering plants**

  **cotton (Kohel, 1967, 1974, 1983), arabidopsis (Jitae et al. 2009), rice (Wu et al. 2007), maize (Gerald et al. 1988), tobacco (Archer et al. 1987), peanut (Benedict & Ketringd, 1972), beans (Grafton et al. 1983; Palmer et al. 1980) and tomato (Richard, 1954).**

- **about 26 virescent genes in nucleus that have been identified in cotton**

  **a single locus:** ***v1*, *v2*, *v3*, *v4*, *v7*, *v8*, *v9*, *v10*, *v11*, *v12*, *v13*, *v14*, *v15*, *v16*, *v17*, *v18*, *v19*, *v20*, *v22***

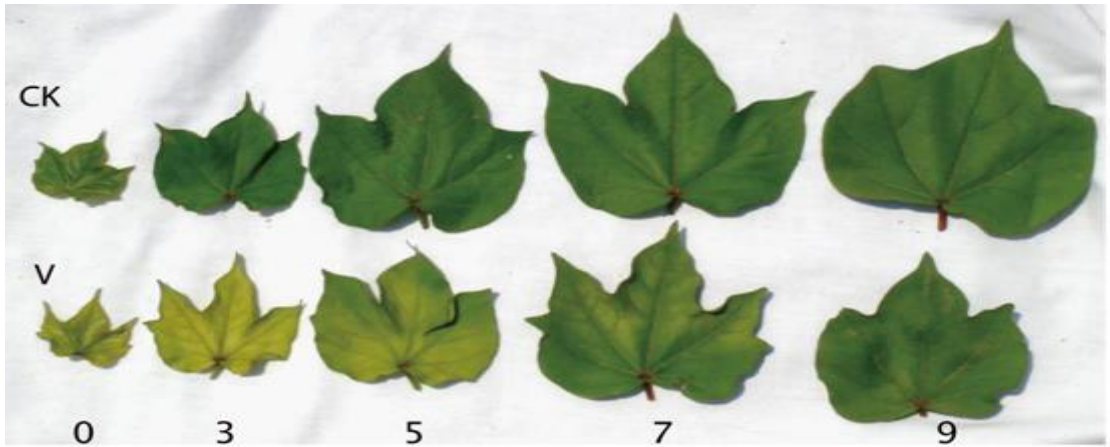
  **double recessive alleles:** ***v5v6***

# *Vsp* mutated by space

- **Shijian-8 seed-breeding satellite**
- **launched on Sep.9,2006, and landed in Sichuan Province at 10:43 am Beijing time Sep.24,2006 after a 15-day flight in space**
- **The satellite carried 215 kilograms of seeds of vegetables, fruits, grains and cotton**
- **being exposed to cosmic radiation and zero gravity**


# Morphology and agronomy of *Vsp*

- **Cotyledon color of *vsp* mutant was green and new young true leaves expressed virescence.**
- **Virescent yellow traits appeared from the first true leaf to anthesis.**
- **The virescent yellow color of one leaf lasted 9 days (216 hours), and divided into three periods**



## ➢ Agronomy traits of the *vsp* mutant

**Height, fruit branches, boll numbers, boll weight and yield of *Vsp* were lower.**

**Fiber length and fiber strength of Vsp were lower.**

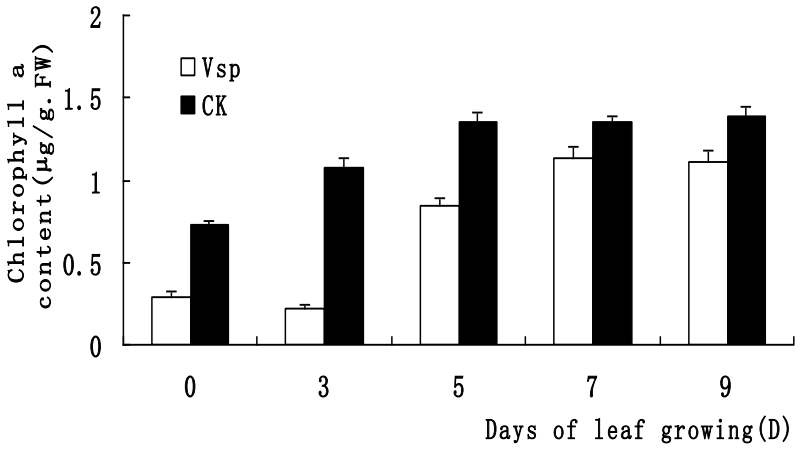
**Table1. Agronomic characters of *vsp* mutant**

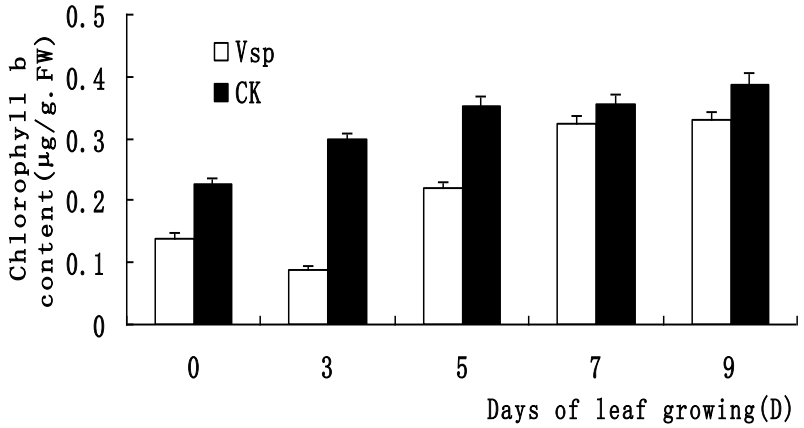
| traits | Plant height(cm) | No. of fruit branches | No. of bolls | No. of young bolls | No. of open bolls | Yield of plant (g) | Boll weight(g) | Fiber index(g) | Fiber length (mm) | Fiber strength (cN/tex) | Micro-naire |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ***vsp* mutant** | 41.36* | 11.58* | 7.37* | 1.19* | 0.54 | 38.05* | 4.81* | 35.27 | 27.95* | 26.68* | 4.81 |
| **Wild type** | 56.50 | 15.00 | 13.79 | 3.14 | 0.57 | 74.53 | 5.19 | 32.73 | 30.20 | 29.80 | 4.39 |

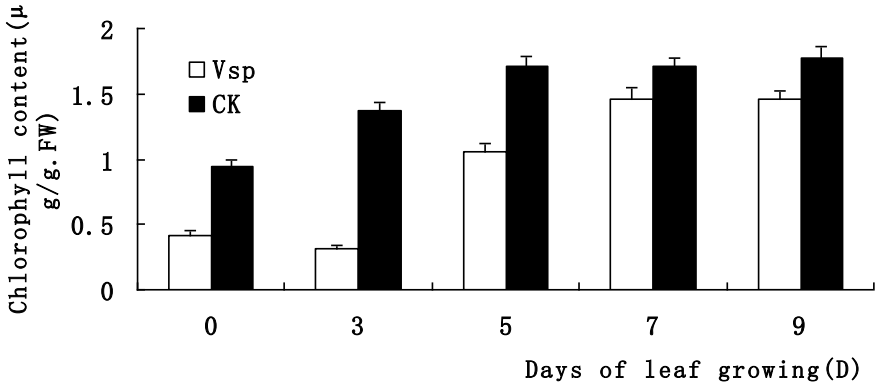
**Note**: * Different significance at 0.05 level.

# Pigment content of *Vsp*

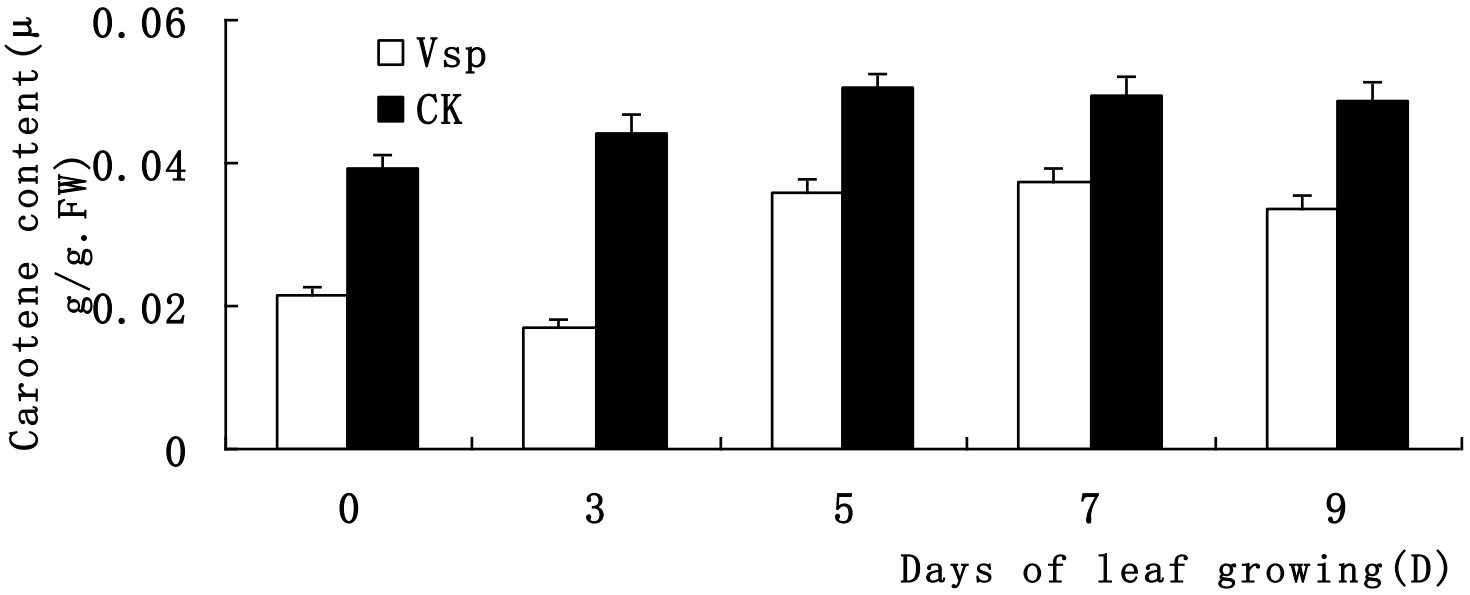
- **Chlorophyll a, b and total content**



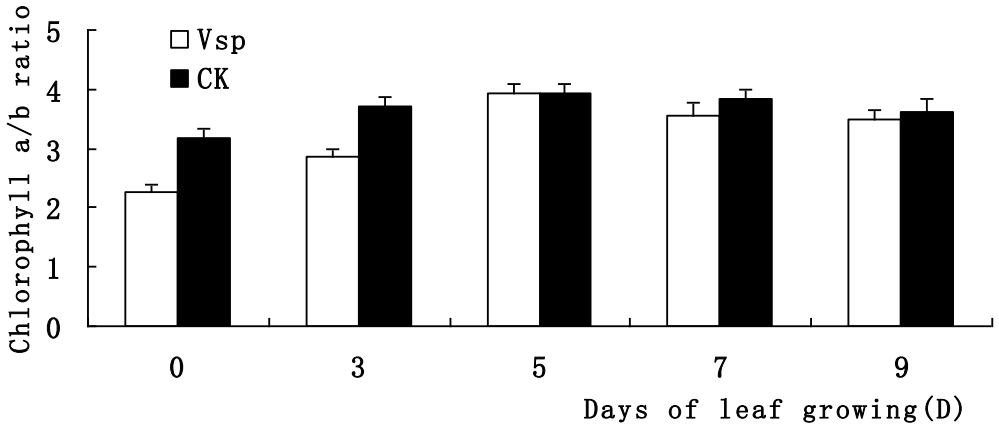



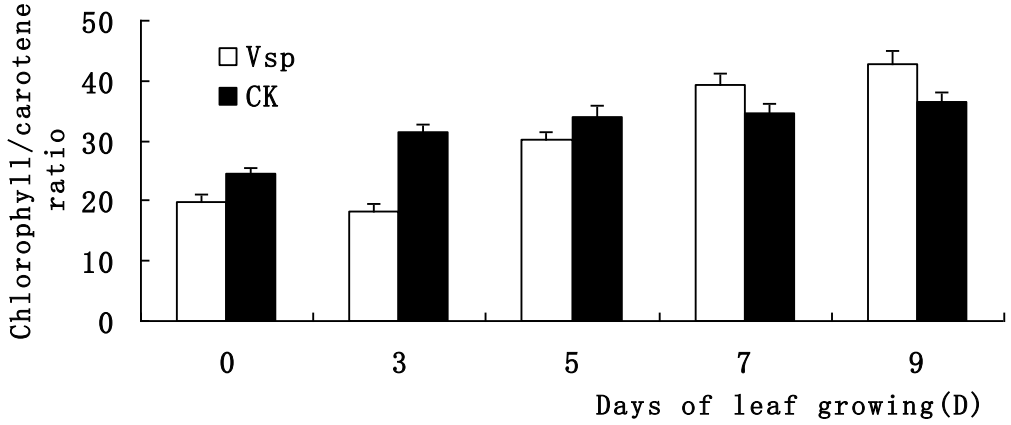
# Carotenoid Content



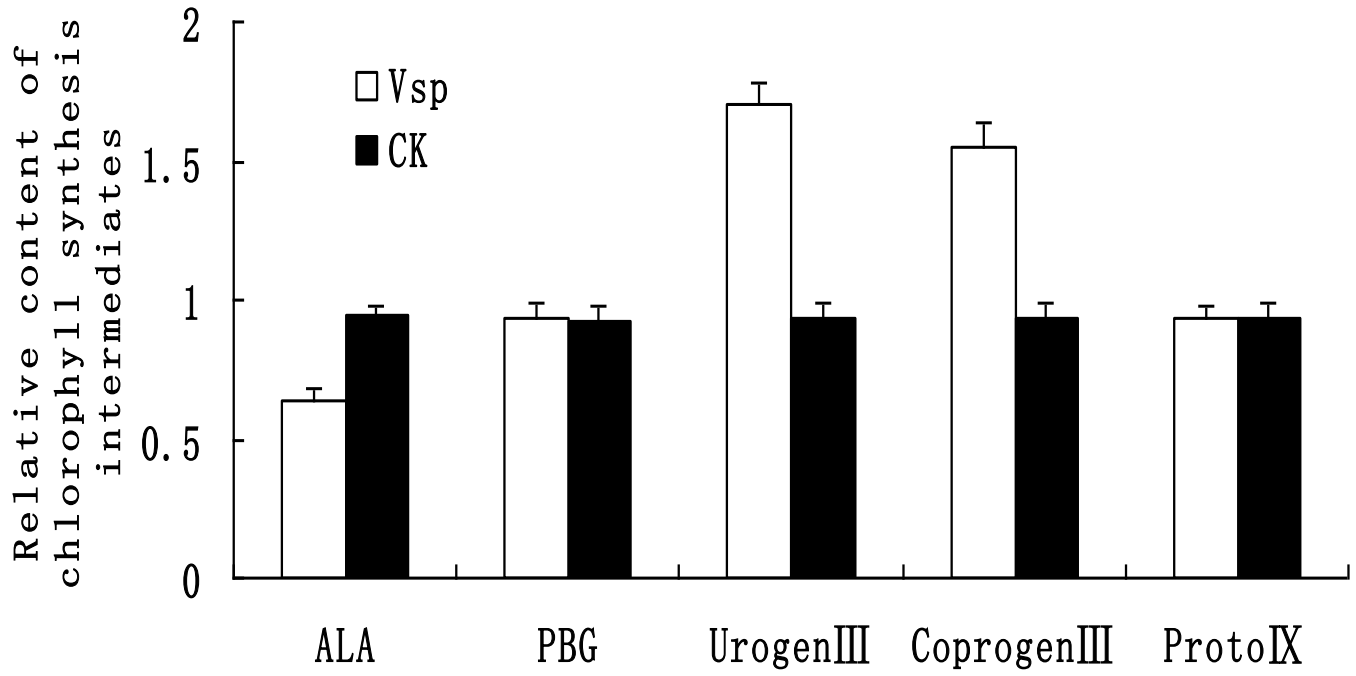
# Ratio of chlorophyll a/b




# Ratio of Chlorophyll / Carotenoid



# Intermediates of chlorophyll biosynthesis



# ➢Photochemical efficiency

Table 2. Photochemical efficiency of natural light on fluorescence parameters Fo, Fv/Fm, $PI_{ABS}$, $RC/CS_0$ , VJ and Wk in five leaves of wild type and the vsp mutant

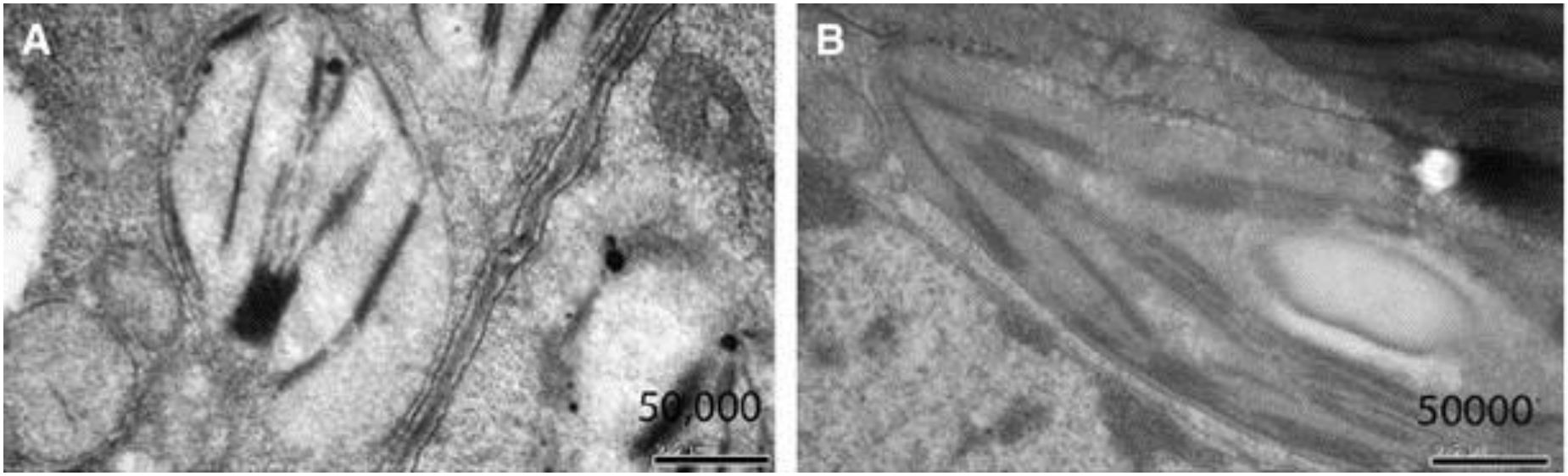
| Treatment | Leave age | $F_0$ | Fv/Fm | $PI_{ABS}$ | RC/CS | $V_J$ | $W_K$ |
|---|---|---|---|---|---|---|---|
| Ck | 0 | 511±50 | 0.61±0.02 | 0.12±0.01 | 131±11 | 0.78±0.02 | 0.54±0.04 |
| Vsp | | 818±37 | 0.44±0.03 | 0.06±0.05 | 123±14 | 0.68±0.03 | 0.68±0.02 |
| Ck | 3 | 536±69 | 0.72±0.03 | 0.80±0.01 | 203±14 | 0.55±0.02 | 0.42±0.02 |
| Vsp | | 1122±23 | 0.28±0.01 | 0.02±0.20 | 104±14 | 0.66±0.05 | 0.70±0.02 |
| Ck | 5 | 499±56 | 0.75±0.03 | 1.57±0.09 | 231±13 | 0.48±0.02 | 0.36±0.04 |
| Vsp | | 730±17 | 0.57±0.01 | 0.27±0.23 | 173±24 | 0.55±0.04 | 0.55±0.03 |
| Ck | 7 | 486±28 | 0.78±0.01 | 2.27±0.20 | 264±21 | 0.46±0.03 | 0.32±0.02 |
| Vsp | | 511±16 | 0.75±0.01 | 1.26±0.27 | 233±14 | 0.52±0.03 | 0.37±0.02 |
| Ck | 9 | 476±18 | 0.81±0.01 | 4.25±0.35 | 280±13 | 0.37±0.02 | 0.30±0.01 |
| Vsp | | 407±60 | 0.80±0.01 | 3.15±1.20 | 216±37 | 0.40±0.04 | 0.33±0.02 |

# Cytological change of *Vsp*

- **Day 0 leaves**

**Vsp mutant:** **The boundary of cell membrane was not clear and the structure of cell wall was blurred.**

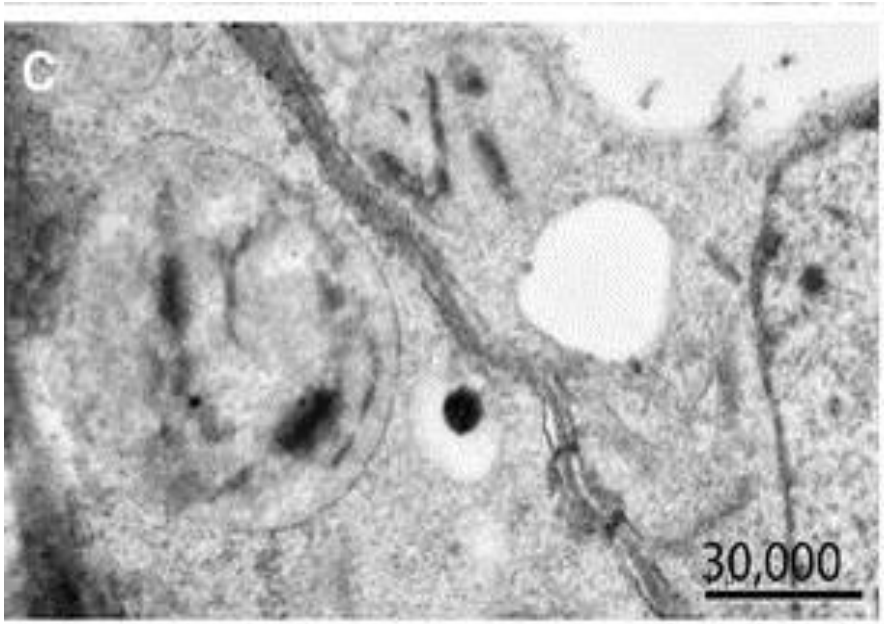
**Matrix granule and starch grains were not seen in chloroplasts.**



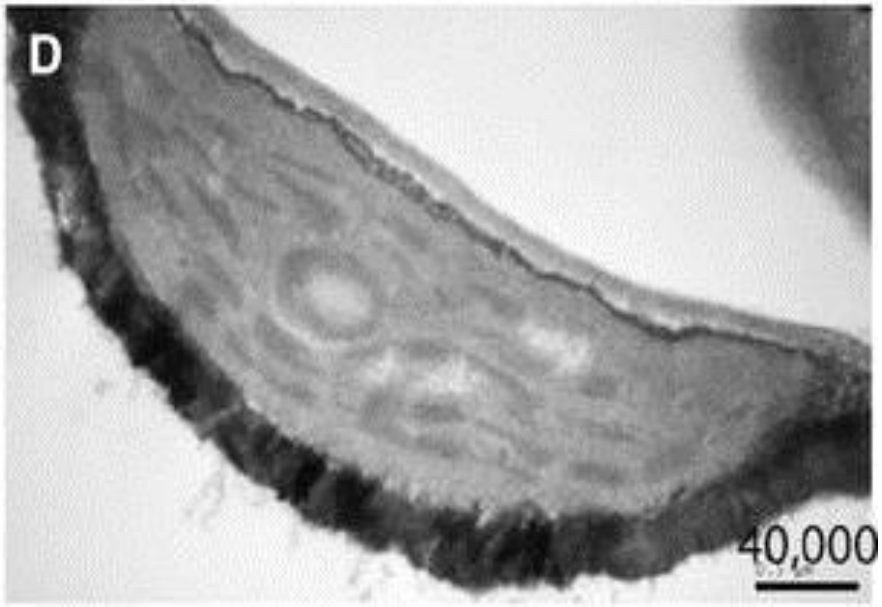

**vsp mutant** **wild type**

- **Day 3 leaves**

**Vsp mutant:** **The boundary of cell wall and cell membrane was not clear. Grana and stroma lamellas were decreased and arranged irregularly. Thylakoids decreased and there was hollow and no inclusion.**



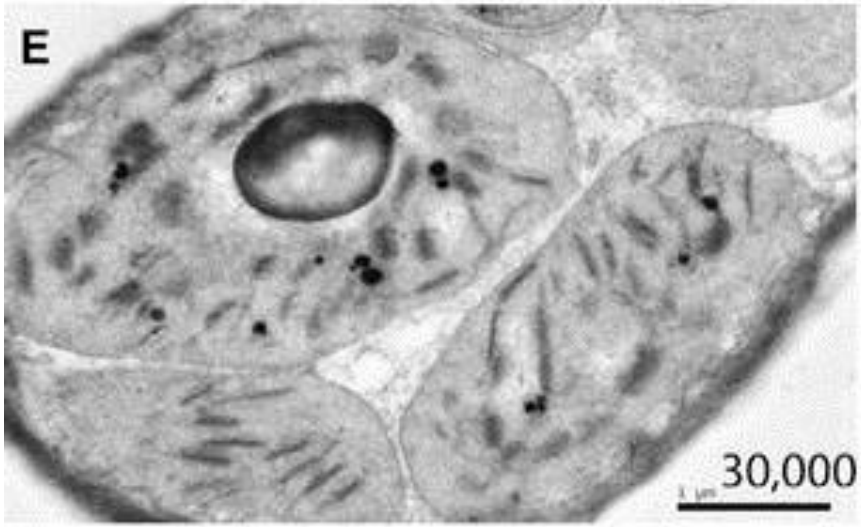

**vsp mutant**

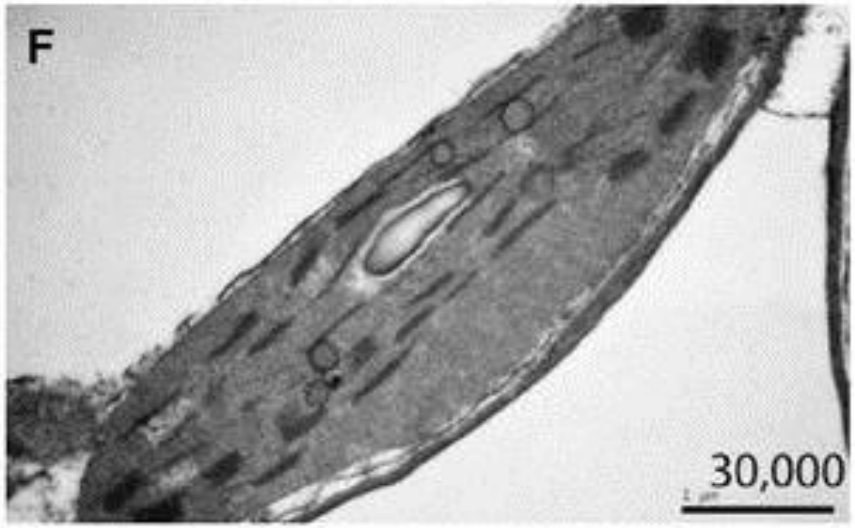



**wild type**

- **Day 5 leaves**

**Vsp mutant: The boundary of cell wall and cell membrane was relatively clear, the numbers of chloroplasts increased. the shape like spindle, matrix granules appeared. inclusion appeared and starch grains were large.**

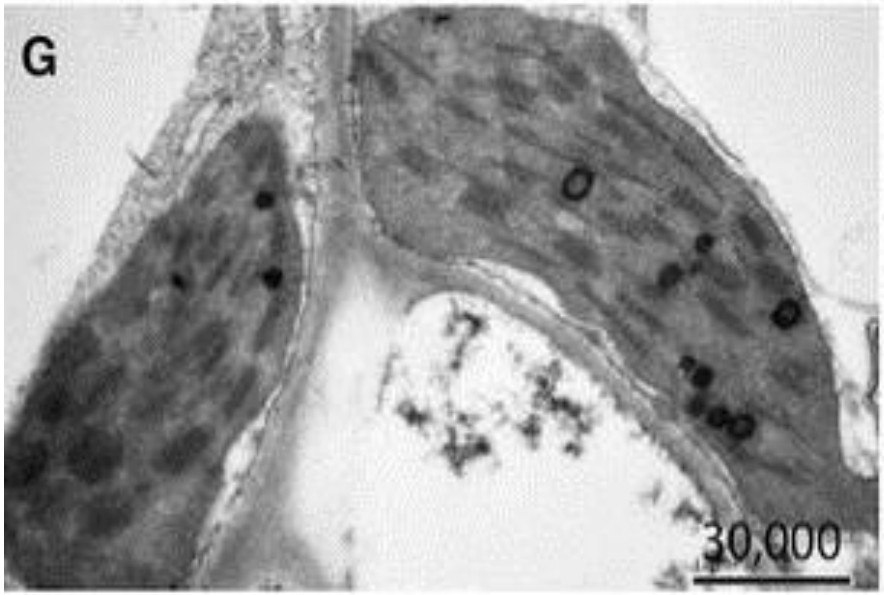


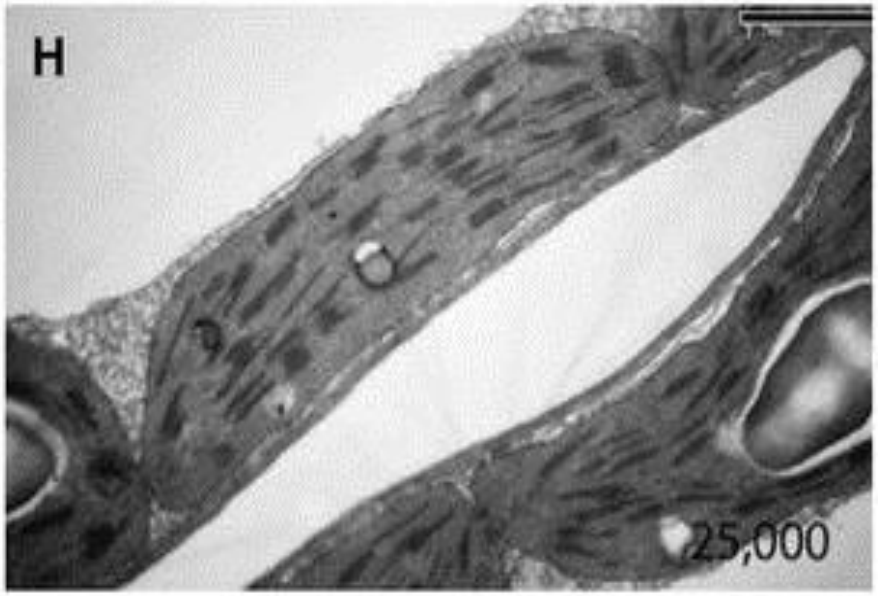




**vsp mutant** **wild type**

- **Day 7 leaves**

**Vsp mutant: the structure of cell wall and membrane were clear and bilayer was intact. Grana and stroma lamella were thickened. Numbers of chloroplasts increased and the shape of chloroplasts looked like spindle. There were more inclusion.**

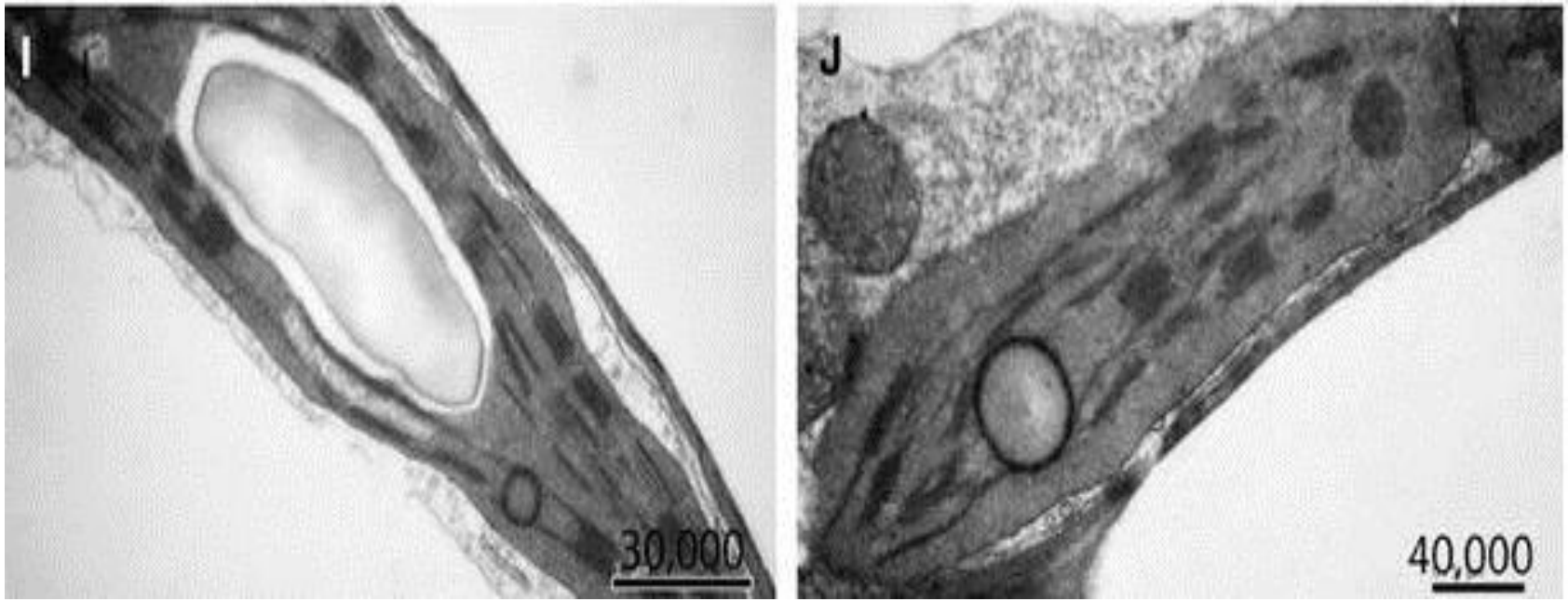



**vsp mutant** **wild type**

- **Day 9 leaves**

**Vsp mutant: Chloroplasts developed perfectly. The structure of chloroplasts was the same as the chloroplasts of the wild type.**




**vsp mutant** **wild type**

# Genetic character of *Vsp*

**Reciprocal crosses were made between the *vsp* mutant and wild type**

Table 3. Segregation of $F_2$ population from two crosses of wild type and the *vsp* mutant

| $F_2$ progeny segregation | $F_2$ (CRRI 58×Vsp) | $F_2$ (Vsp× CRRI 58) |
|---|---|---|
| Green plants | 1120 | 1243 |
| Virescent plants | 351 | 401 |
| Total plants | 1471 | 1644 |
| $\chi^2$ | 0.96 | 0.29 |

Note: $\chi^2_{0.05,1}=3.84$， $\chi^2_{c\ 0.05,1}<\chi^2_{0.05,1}$ Difference was not reached 0.05 significance level. The result met with theory ratio 3 : 1. Vsp represent the *vsp* mutant.

**Allelic test:** The *vsp* mutant was reciprocal crossed with yellow mutant germplasms (*v1v1*, *v3v3*, *v5v5v6v6*, *v8v8*, *v9v9*, *v10v10*, *v11v11*, *v13v13*, *v14v14*, *v18v18*, *v19v19* and *vgvg*), all obtained plants of $F_1$ showed green leaves.

Table 4. Phenotype of $F_1$ progenies from crosses of *vsp* mutant with known virescent mutants

| $F_1$ progeny phenotype | $v_1v_1$ | $v_3v_3$ | $v_5v_5$ $v_6v_6$ | $v_8v_8$ | $v_9v_9$ | $v_{10}v_{10}$ | $v_{11}v_{11}$ | $v_{13}v_{13}$ | $v_{14}v_{14}$ | $v_{18}v_{18}$ | $v_{19}v_{19}$ | $v_gv_g$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year 2008 (*vspvsp* ♂) | 68:0 | 23:0 | 49:0 | 72:0 | 164:0 | 25:0 | 17:0 | 7:0 | 142:0 | 93:0 | 36:0 | 10:0 |
| Year 2008 (*vspvsp* ♀) | — | 107:0 | — | 171:0 | 44:0 | — | — | — | 154:0 | 171:0 | 44:0 | — |
| Year 2009 (*vsp vsp* ♂) | — | 131:0 | 122:0 | — | — | — | — | — | — | — | — | 75:0 |

Note: "—" express no this data. The ratio represent number of green leaf plants to number of yellow leaf plants.

# Molecular mapping of *Vsp*

## Bulked segregate analysis (BSA)

**Molecular marker technology provides the opportunity to map a gene of agronomic importance in segregating generations (Michelmore et al. 1991). Numerous genes had been mapped using this method.**

**SSR markers and BSA were combined for molecular mapping of *Vsp* gene.**

**Parents:** vsp mutant and TM-1

**$F_2$ population :** 186 plants

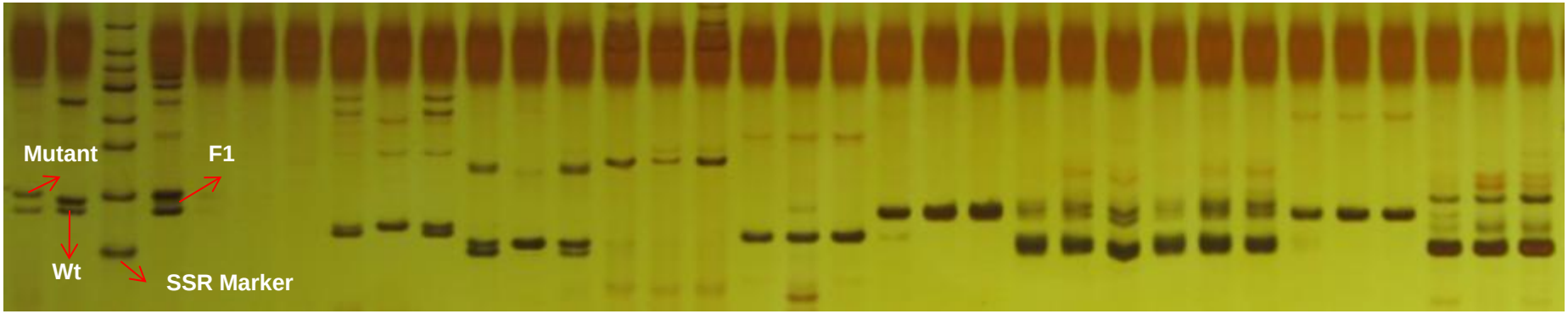
**16000** primers from CMD (Cotton Marker Database) were used to identify polymorphisms between the parents.

Finally, **400 primers** could produce polymorphism between the parents.

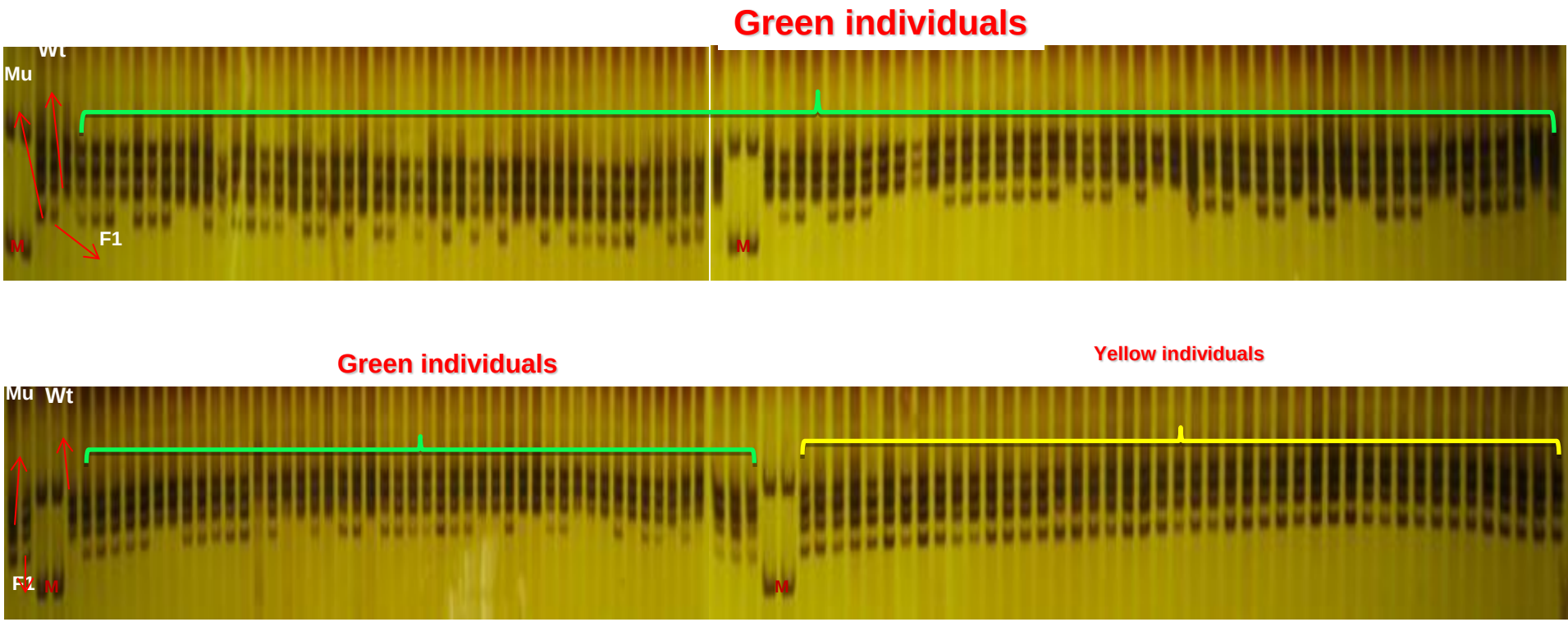
**Table 5. SSR primers used in this study**

| SSR prefix | BNL | C2 | CIR | CM | DPL | Gh | HAU | JESPR | MGHES | MUSS | NAU | PGML | STV | SWU | TMB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of SSR pair | 379 | 187 | 392 | 53 | 200 | 700 | 3382 | 309 | 84 | 554 | 3928 | 3997 | 192 | 1410 | 750 |
| Total No.=16544 | | | | | | | | | | | | | | | |

**The polymorphic markers between the parents**

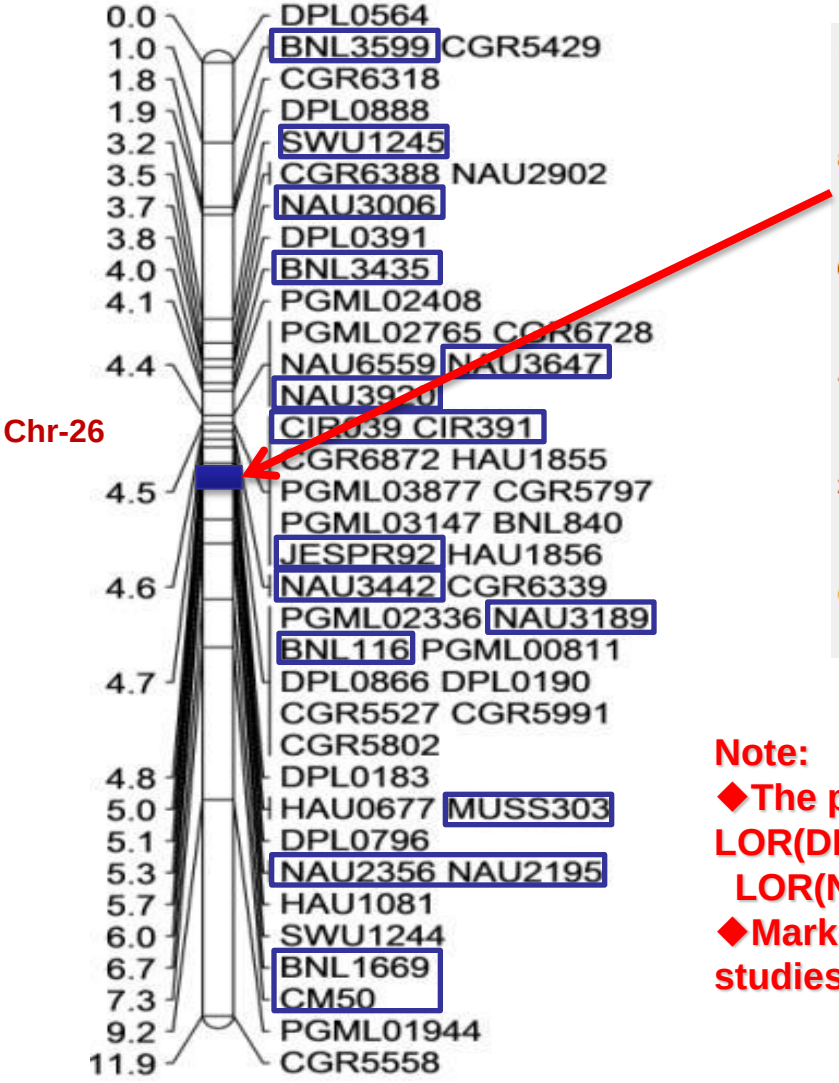


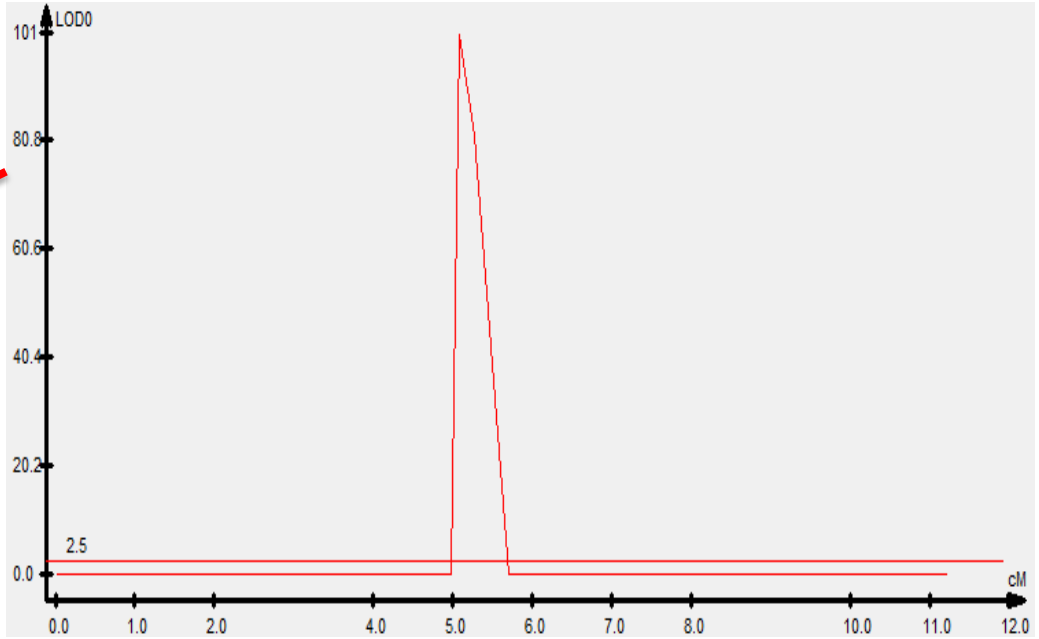
**These primers were selected by BSA using the two pools, 54 primers linked with the *Vsp* gene were identified. They were used for mapping the *Vsp* gene.**




**The PCR products of PGML01944**

**The final linkage map consists of 50 markers, the other 4 were removed because of distortion segregation. Then, we used the WinQTLCart software to search for *Vsp* loci. The *Vsp* gene was predicted between marker DPL0796(5.1cM) and NAU2356/2198(5.3cM) on Chr-26.**




**Note:**

**◆The predicted region of Vsp by WinQTLCart, LOR(DPL0796)=464.18**
**LOR(NAU2356/2198)=376.75**

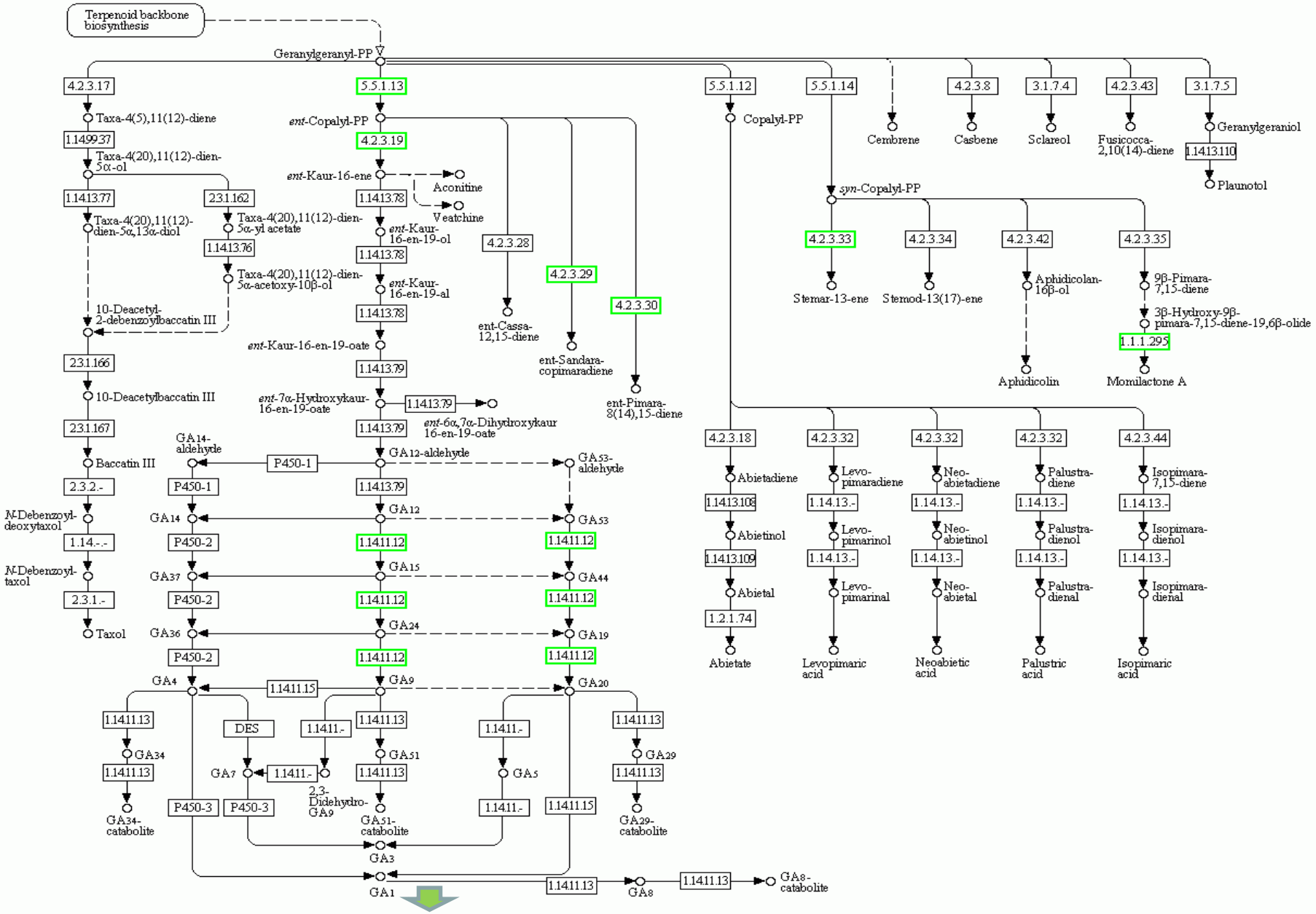
**◆Markers indicated by boxes are on Chr-26 identified in other studies.**

# The results of transcriptome sequencing

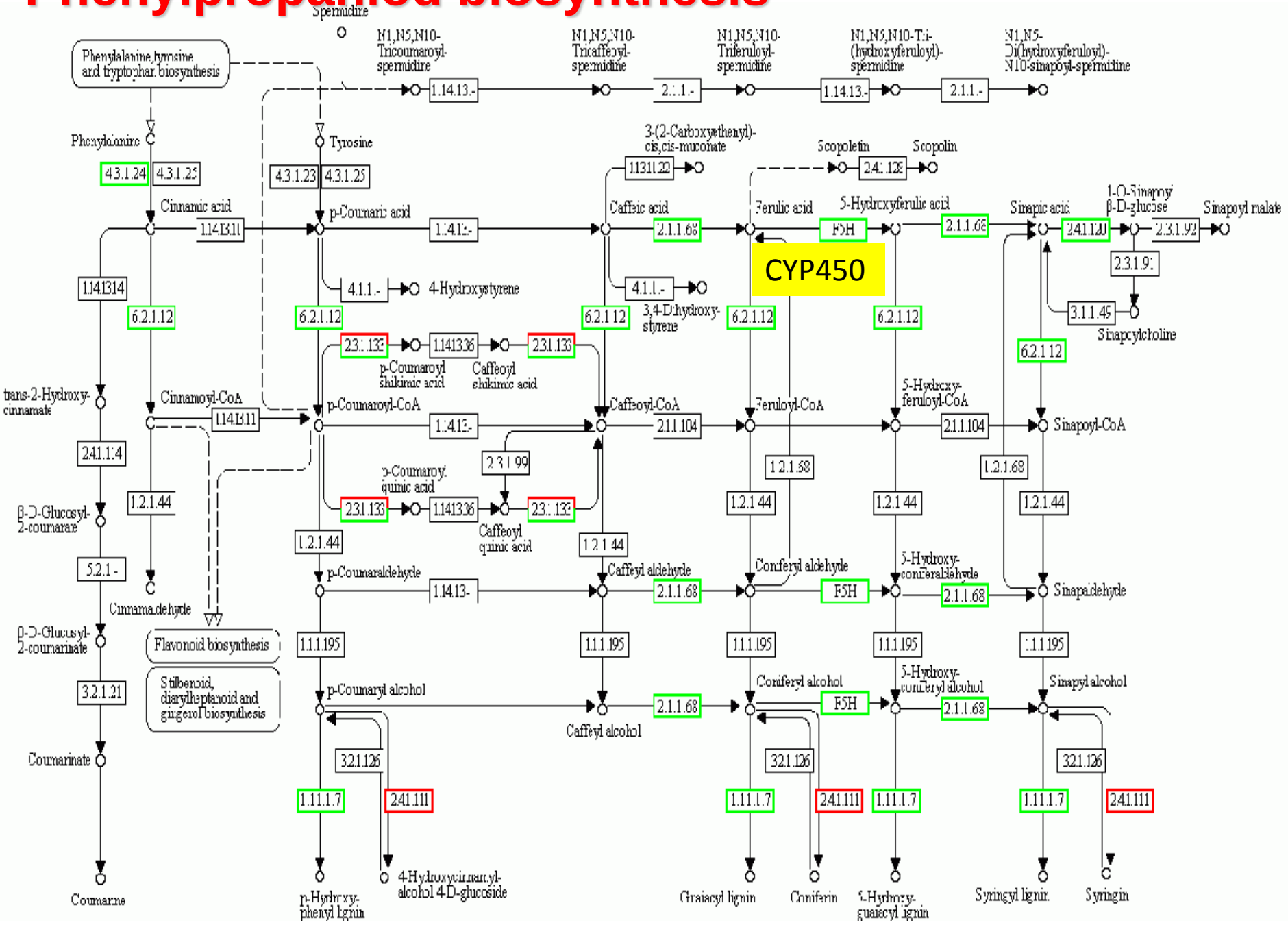
- **Pathway analysis**

| pathway | Wild and vsp | All-Unigene | Pvalue | Qvalue |
|---|---|---|---|---|
| Amino sugar and nucleotide sugar metabolism | 28 | 446 | 9.5e-10 | 7.8e-08 |
| Diterpenoid biosynthsis | 9 | 124 | 0.0001 | 6.9e-03 |
| Galactose metabolism | 12 | 229 | 0.0003 | 9.0e-03 |
| Glycerophospholipid metabolism | 27 | 930 | 0.0018 | 2.9e-02 |
| Spliceosome | 31 | 117 | 0.0021 | 2.9e-02 |
| Phenylpropaniod biosynthesis | 17 | 485 | 0.0021 | 2.9e-02 |
| Plagosome | 15 | 434 | 0.0043 | 4.3e-02 |
| Plant-pathogen interaction | 53 | 2909 | 0.0047 | 4.3e-02 |
| Photosynthesis antenna proteins | 4 | 43 | 0.0047 | 4.3e-02 |

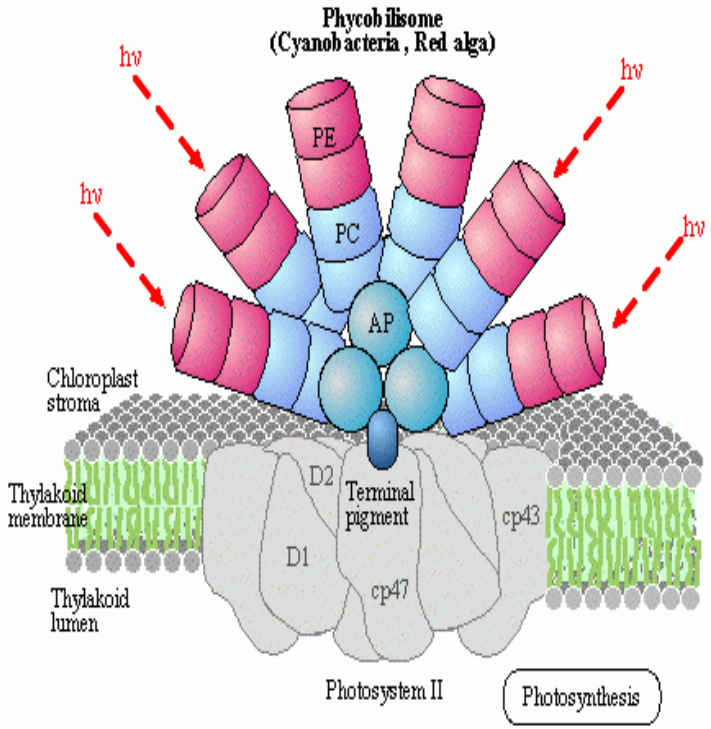
# Diterpenoid biosynthsis

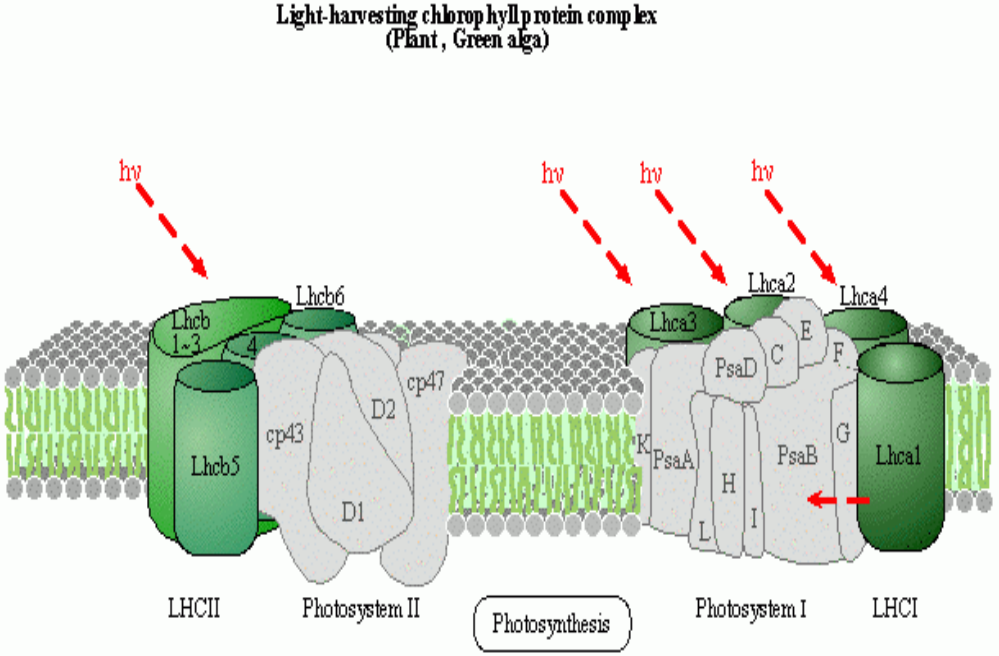


# Phenylpropaniod biosynthesis



# Photosynthesis antenna proteins




Allophycocyanin(AP)

| ApcA | ApcB | ApcC | ApcD | ApcE | ApcF |
|---|---|---|---|---|---|

Phycocyanin(PC) / Phycoerythrocyanin(PEC)

| CpcA | CpcB | CpcC | CpcD | CpcE | CpcF | CpcG |
|---|---|---|---|---|---|---|

Phycoerythrin(PE)

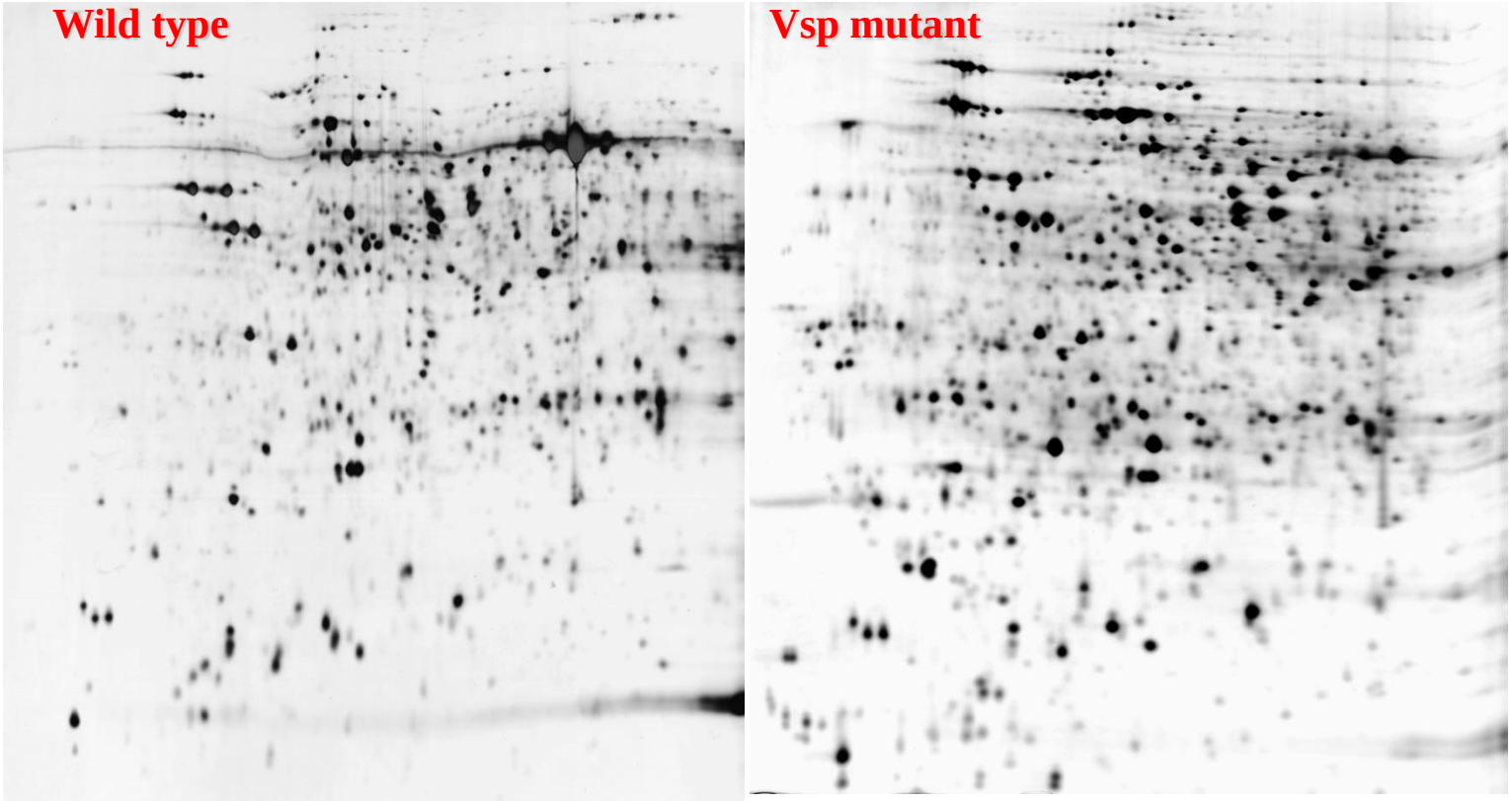
| CpeA | CpeB | CpeC | CpeD | CpeE | CpeR | CpeS | CpeT | CpeU | CpeY | CpeZ |
|---|---|---|---|---|---|---|---|---|---|---|

Light-harvesting chlorophyll protein complex(LHC)

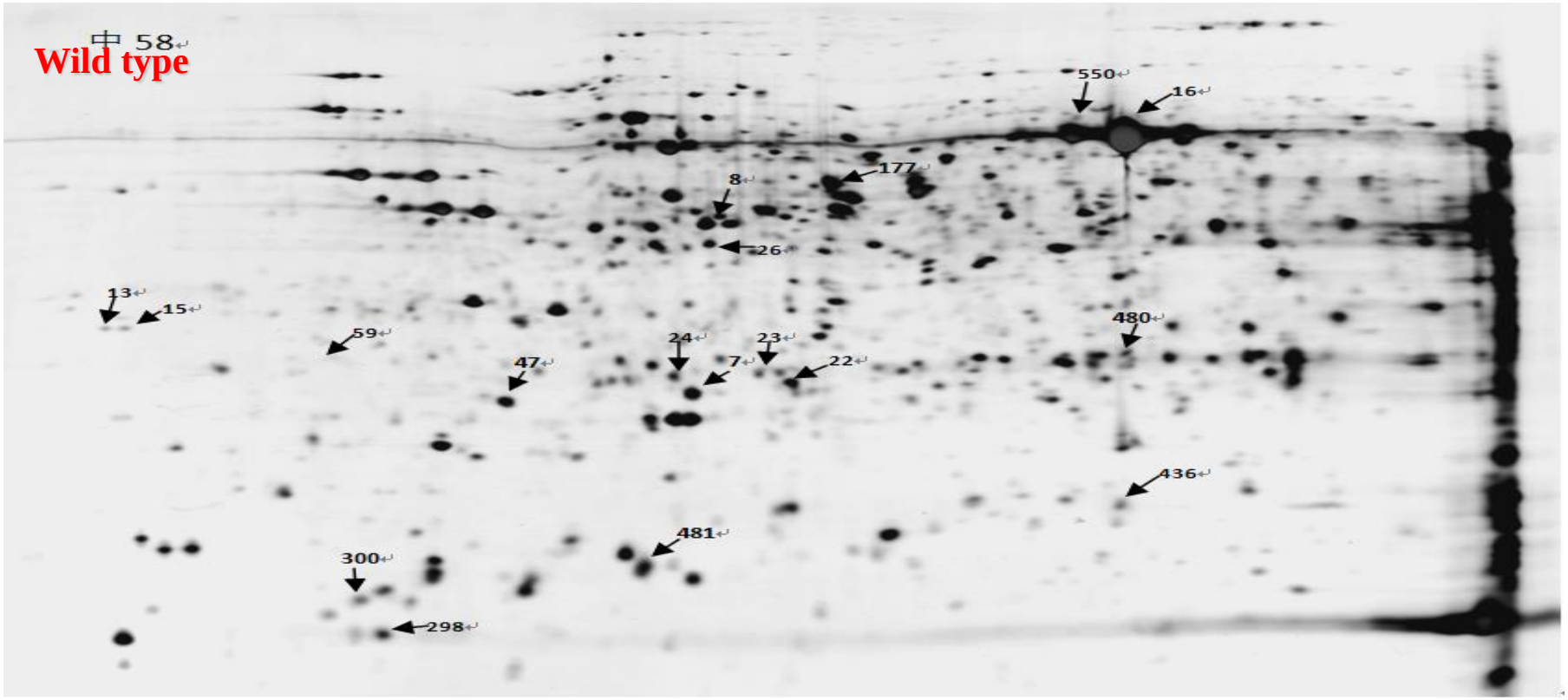
| Lhca1 | Lhca2 | Lhca3 | Lhca4 | Lhca5 |
|---|---|---|---|---|

| Lhcb1 | Lhcb2 | Lhcb3 | Lhcb4 | Lhcb5 | Lhcb6 | Lhcb7 |
|---|---|---|---|---|---|---|

# Proteome research of vsp mutant



# Different Protein spots between vsp and wild




**48 protein spots were discovered and 18 were identified as significant, with 10 expressed or upregulated and 8 expressed or down-regulated in vsp with MALDI-TOF MS.**

# 8 down-regulate proteins in vsp mutant

| Spot NO | Protein name | Accession | Species | Ratio CK/V |
|---|---|---|---|---|
| 16 | Rubisco large subunit | gi\|1771188 | Leonia glycycarpa | 30.03 |
| 22 | Unknown | gi\|255634578 | Glycine max | 4.11 |
| 298 | Rubisco large subunit | gi\|323690315 | Priva cordifolia | 2.86 |
| 481 | Rubisco large subunit | gi\|30313565 | Leptolaena multiflora | 2.36 |
| 550 | Rubisco large subunit | gi\|2342972 | Lespedeza cuneata | 2.86 |
| 8 | gibberellin 20-oxidase 1 | gi\|74273629 | Gossypium hirsutum | 10.31 |
| 59 | Rubisco large subunit-binding protein | gi\|2506277 | Gossypium hirsutum | 4.87 |
| 300 | Rubisco large subunit | gi\|326535361 | Gossypium arboreum | 5.50 |

**Rubisco large subunit, GA20-oxidase.**

# 10 up-regulate proteins in vsp mutant

| Spot NO | Protein name | Accession | Species | Ratio CK/V |
|---|---|---|---|---|
| 7 | groes chaperonin | gi\|255550363 | Ricinus communis | 0.29 |
| 23 | triosephosphate isomerase | gi\|295687231 | Gossypium hirsutum | 0.31 |
| 26 | receptor kinase-like protein | gi\|1122443 | Oryza sativa | 0.20 |
| 480 | 20S proteasome subunit alpha-1 | gi\|211906468 | Gossypium hirsutum | 0.20 |
| 47 | cp10-like protein | gi\|21780187 | Gossypium hirsutum | 0.35 |
| 15 | Unknown | gi\|224286559 | Picea sitchensis | 0.46 |
| 177 | S-adenosylmethionine synthetase | gi\|307948774 | Gossypium hirsutum | 0.22 |
| 24 | chloroplast triose phosphate isomerase | gi\|88770718 | Rhodomonas salina | 0.31 |
| 13 | nascent polypeptide associated complex alpha chain | gi\|1658271 | Nicotiana tabacum | 0.49 |
| 436 | phosphoglycerate kinase | gi\|211906450 | Gossypium arboreum | 0.43 |

**Receptor kinase, S-adenosyl-L-methionine synthetase, 20S proteasome subunit.**

# Summary

- *vsp* mutant is an new one and show earlier virescence and specific only to true leaves.
- plant height, number of bolls, boll weight, yield and fiber quality were significantly lower than wild type.
- Chlorophyll, carotenoid level and photo-chemical efficiency of vsp mutant true leaves were significantly lower.
- vsp mutant lacked grana in the thylakoids of the mesophyll cells at young leave stage.
- This indicated that chlorophyll and carotenoid levels were related with chloroplast structure.
- Down-regulate Rubisco large subunit and up-regulate protein kinases could cause to delay the chlorophyll synthosis.

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# Thank you for your attention

# Welcome to CCRI