Manipulation of the plant isoprenoid pathway enhances vitamin and lycopene contents in tomato

調控植物異戊二烯合成途徑促進番茄中維他命和番茄紅素含量

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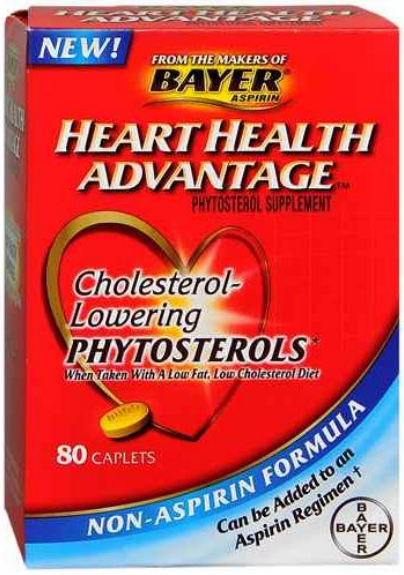
aab Association of Applied Biologists
SEB Society for Experimental Biology

Isoprenoids (異戊二烯化合物)

- Large, diverse, natural products
- **Vitamin E, provitamin A (β-carotene)** (β-胡蘿蔔素) **and lycopene** (番茄紅素): antioxidant activities
- **Squalene** (角鯊烯): skin protection; used in skin moisturizers
- **Phytosterols** (植物甾醇): lower cholesterols
- Gibberellic acid, abscisic acid (脫落酸,一種制止植物生長的有機物質，縮寫為 ABA) & cytokinins (細胞分裂素): growth & development



All figures from websites of google

Isoprenoid biosynthesis in plants (植物中異戊二烯化合物的生物合成)

- **2 Pathways**
- **Isoprenoids**

Large, diverse, natural products

Squalene (角鯊烯)

skin protection; used in skin moisturizers

Phytosterols (植物甾醇)

lower cholesterols

Vitamin E (α-tocopherol) (維他命E (α-生育酚))

Provitamin A (β-carotene, β-胡蘿蔔素)

Lycopene (番茄紅素)

antioxidant activities

Gibberellic acid (GA), abscisic acid (脫落酸,一種制止植物生長的有機物質，縮寫為 ABA) & cytokinins (細胞分裂素):

growth & development

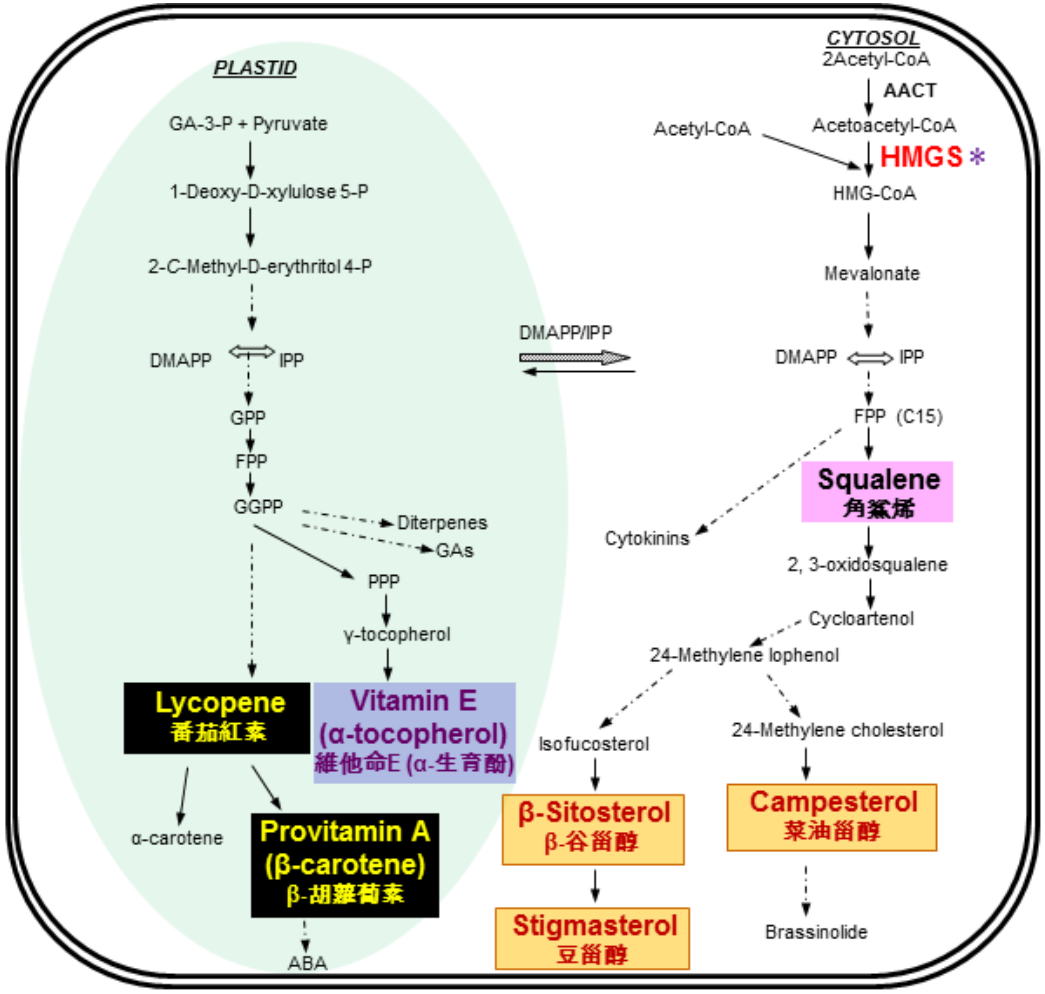
- **Hypothesis:**

Overexpression of HMGS

will increase phytosterols

(end products)

2 Pathways



Enzymes are shown in bold. Pathway inside the mitochondria and plastid are boxed. Arrows between cytosolic and plastid compartments represent metabolic flow between them (greater arrow for more flux). Abbreviations: ABA, abscisic acid; AACT, acetoacetyl-CoA thiolase; BR6OX2, brassinosteroid-6-oxidase 2; CYP710A1, sterol C-22 desaturase; CYP85A1, cytochrome P450 monooxygenase; DMAPP, dimethylallyl diphosphate; DWF1, delta-24 sterol reductase; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; FPP, farnesyl diphosphate; GA-3-P, glyceraldehyde-3-phosphate; FPPS, farnesyl diphosphate synthase; GAs, gibberellins; GGPP, geranylgeranyl diphosphate; GGPPS, geranylgeranyl diphosphate synthase; GPP, geranyl diphosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMGS, 3-hydroxy-3-methylglutaryl-CoA synthase; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; IPP, isopentenyl diphosphate; IPPI, isopentenyl/dimethylallyl diphosphate isomerase; Q_{10}, coenzyme Q_{10}; SMT, sterol methyltransferase; SQS, squalene synthase.

Isolated *BjHMGS* cDNA encoding 3-hydroxy-3-methylglutaryl-CoA synthase (BjHMGS)

from an edible plant, *Brassica juncea* (芸苔屬芥菜) (Brassicaceae family)

從可食用植物芸苔屬芥菜中分離得到*HMGS* 基因

Brassica juncea (芸苔屬芥菜)

Characterisation of BjHMGS

- **BjHMGS**

  is developmentally regulated and stress-responsive

  Alex et al. (2000) *Plant Journal* 22: 415-426

  Nagegowda et al. (2005) *Planta* 221: 844-856

- **Expression of recombinant (重組的) wild-type (野生型HMGS) & variants (HMGS 中個別氨基酸改變成其他氨基酸) in bacteria showed**

  variant S359A displayed a 10-fold higher enzyme activity

  Nagegowda et al. (2004) *Biochemical Journal* 383: 517-527

- **Crystal structure of BjHMGS**

  Pojer et al. (2006) *Proc Natl Acad Sci USA* 103: 11491-11496

Tested the hypothesis in Arabidopsis,
model plant with short generation time
Brassicaceae family,
closely-related to *Brassica*
模式植物擬南芥中驗證假設

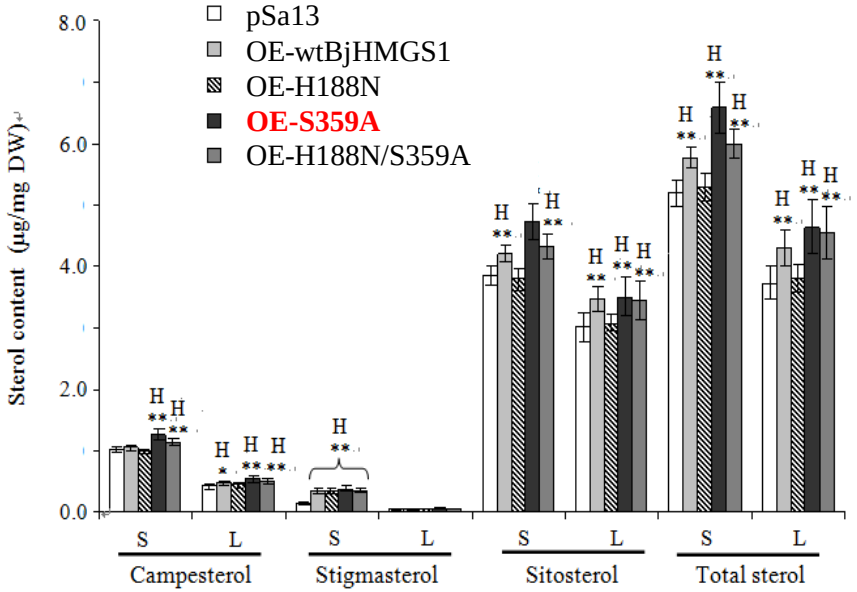
Wang et al. (2012) *Plant Biotechnology Journal* 10: 31-42

Plant Biotechnology Journal

Plant Biotechnology Journal (2012) **10**, pp. 31–42 doi: 10.1111/j.1467-7652.2011.00631.x

Overexpression of *Brassica juncea* wild-type and mutant HMG-CoA synthase 1 in Arabidopsis up-regulates genes in sterol biosynthesis and enhances sterol production and stress tolerance

Hui Wang[1], Dinesh A. Nagegowda[1,2], Reetika Rawat[1], Pierrette Bouvier-Navé[3], Dianjing Guo[4], Thomas J. Bach[3] and Mee-Len Chye[1,*]



Tested the hypothesis in tomato, *(Solanum lycopersicum)* a crop plant

農作物番茄中驗證假設

- Popular
- Edible fruit
- Technology mature and stable

Control **S359A**

Liao et al. (2017) *Plant Biotechnology Journal* DOI: 10.1111/pbi.12828

Plant Biotechnology Journal

Plant Biotechnology Journal (2017) doi: 10.1111/pbi.12828

Improved fruit α-tocopherol, carotenoid, squalene and phytosterol contents through manipulation of *Brassica juncea* 3-HYDROXY-3-METHYLGLUTARYL-COA SYNTHASE1 in transgenic tomato

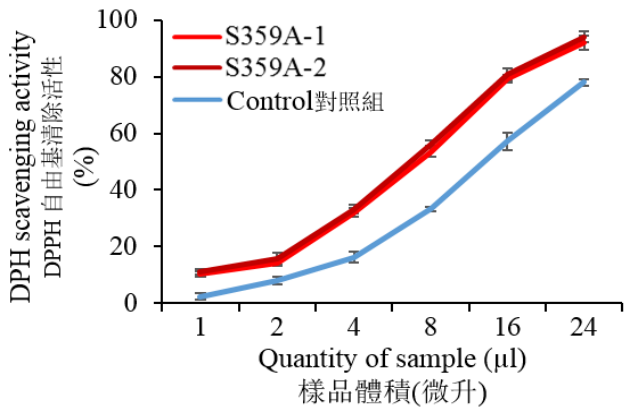
Pan Liao[1,2], Xinjian Chen[1], Mingfu Wang[1], Thomas J. Bach[3], Mee-Len Chye[1,2]*

Control **S359A-1 S359A-2**

Carotenoid extracts from transformed **fruits** overexpressing S359A contain more carotenoids (provitamin A and lycopene) than the control.

S359A, transgenic tomato overexpressing HMGS variant S359A. S359A-1 and S359A-2, two independent lines

Components	% increase in OE-S359A over vector control in tomato fruits
Provitamin A (β-carotene)	**169**
Lycopene	**111**



Enhanced antioxidant activity

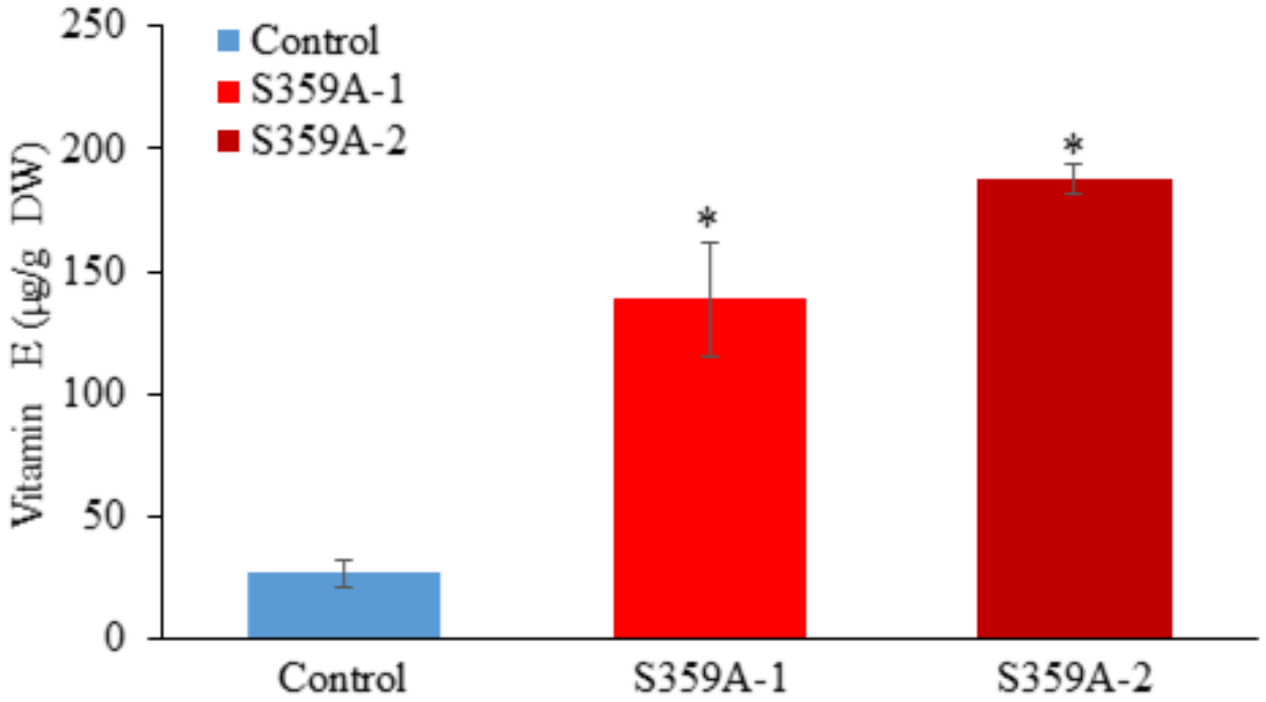
提升抗氧化活性

Antioxidant activity was defined by the ability to scavenge free radicals in DPPH (1,1-diphenyl-2-picrylhydrazyl).

Vitamin E (維生素E) (α-tocopherol, α-生育酚)

↑ 494% in S359A-transformed tomato

過量表達S359A的番茄中維生素E（ α-生育酚）含量提高494%



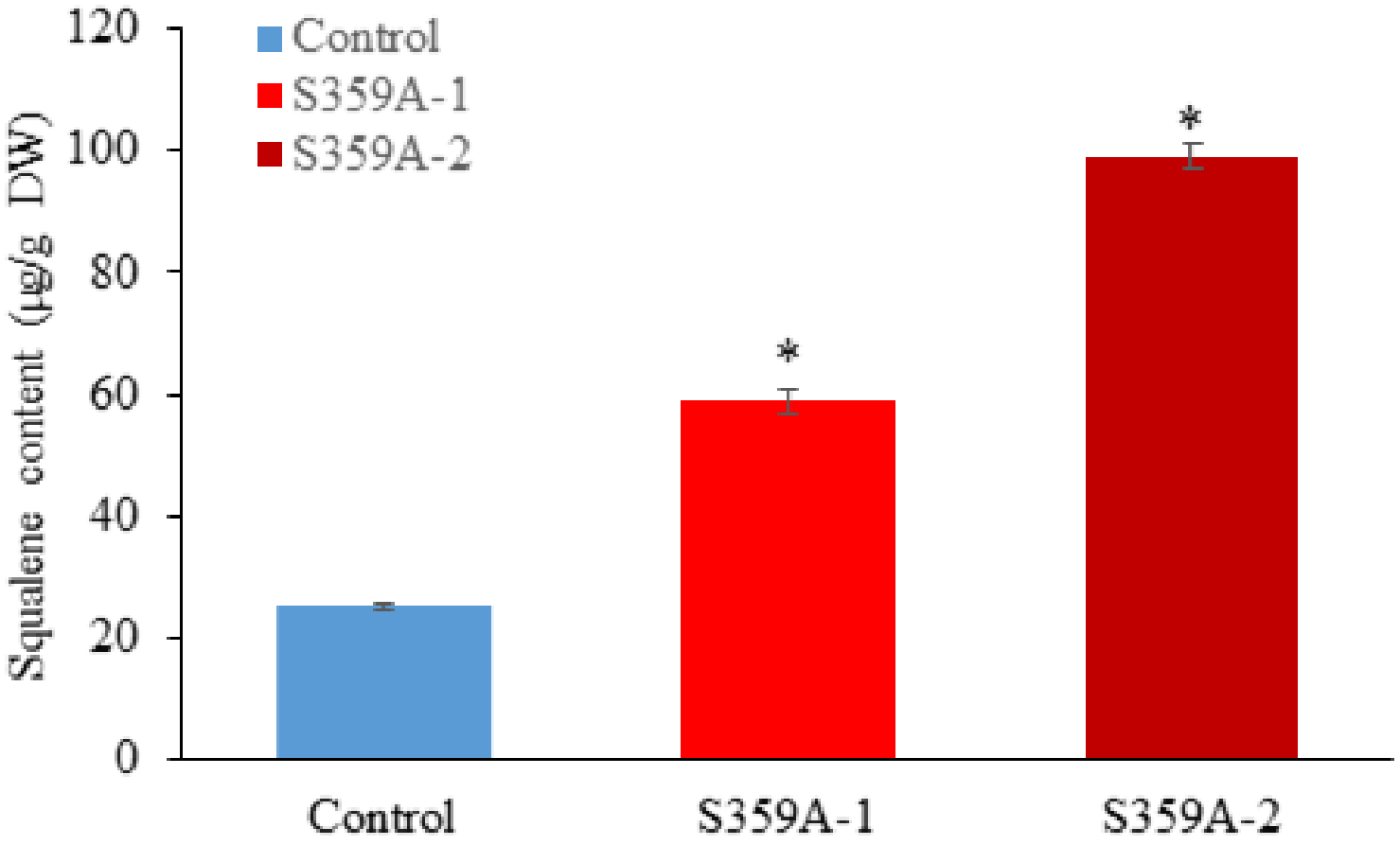
S359A-1 and S359A-2 represent two independent S359A tomato lines

S359A-1 和S359A-2 是兩個獨立的過量表達S359A 的番茄株系

Liao et al. (2017) *Plant Biotechnology Journal* DOI: 10.1111/pbi.12828

Squalene (角鯊烯) ↑210% in S359A-transformed tomato

過量表達S359A的番茄中角鯊烯含量提高210%



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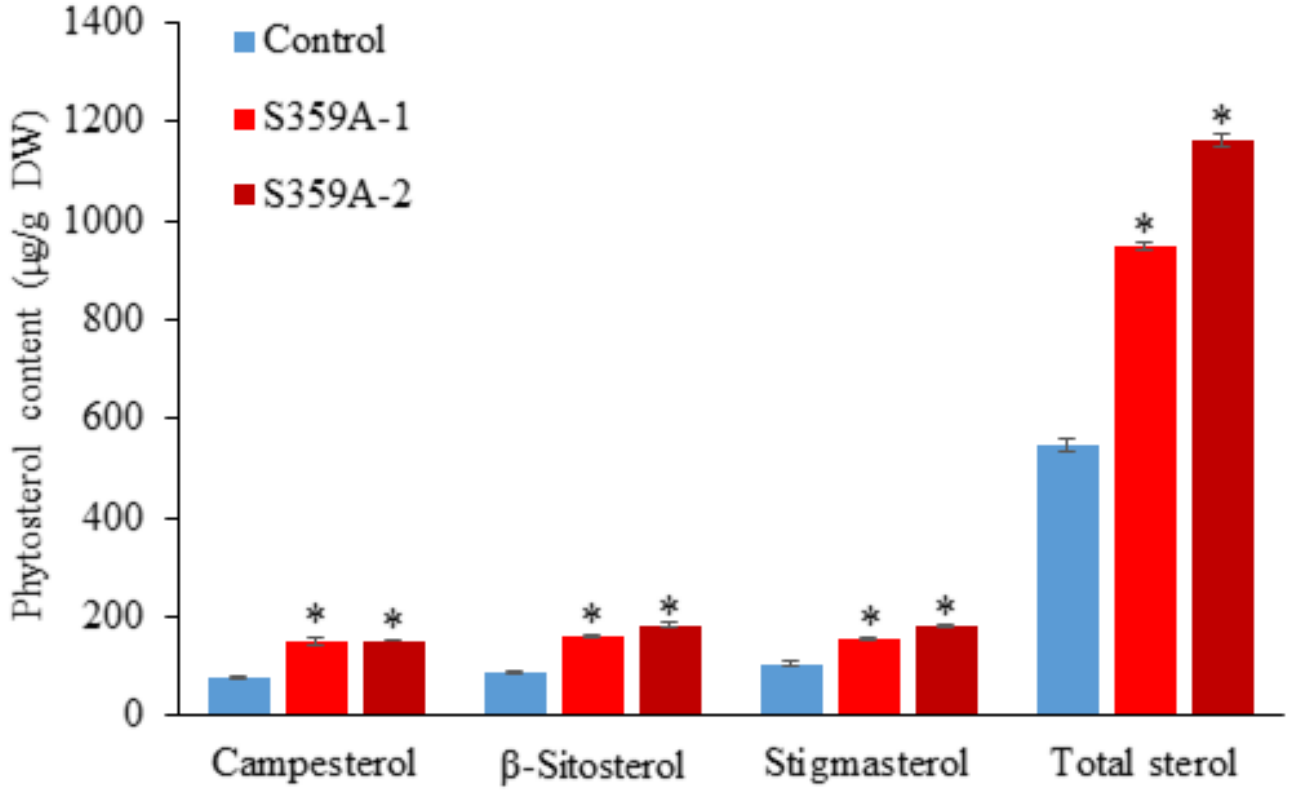
Liao et al. (2017) *Plant Biotechnology Journal* DOI: 10.1111/pbi.12828

Total phytosterols ↑94% in S359A-transformed tomato

過量表達S359A的番茄中總植物甾醇含量提高94%



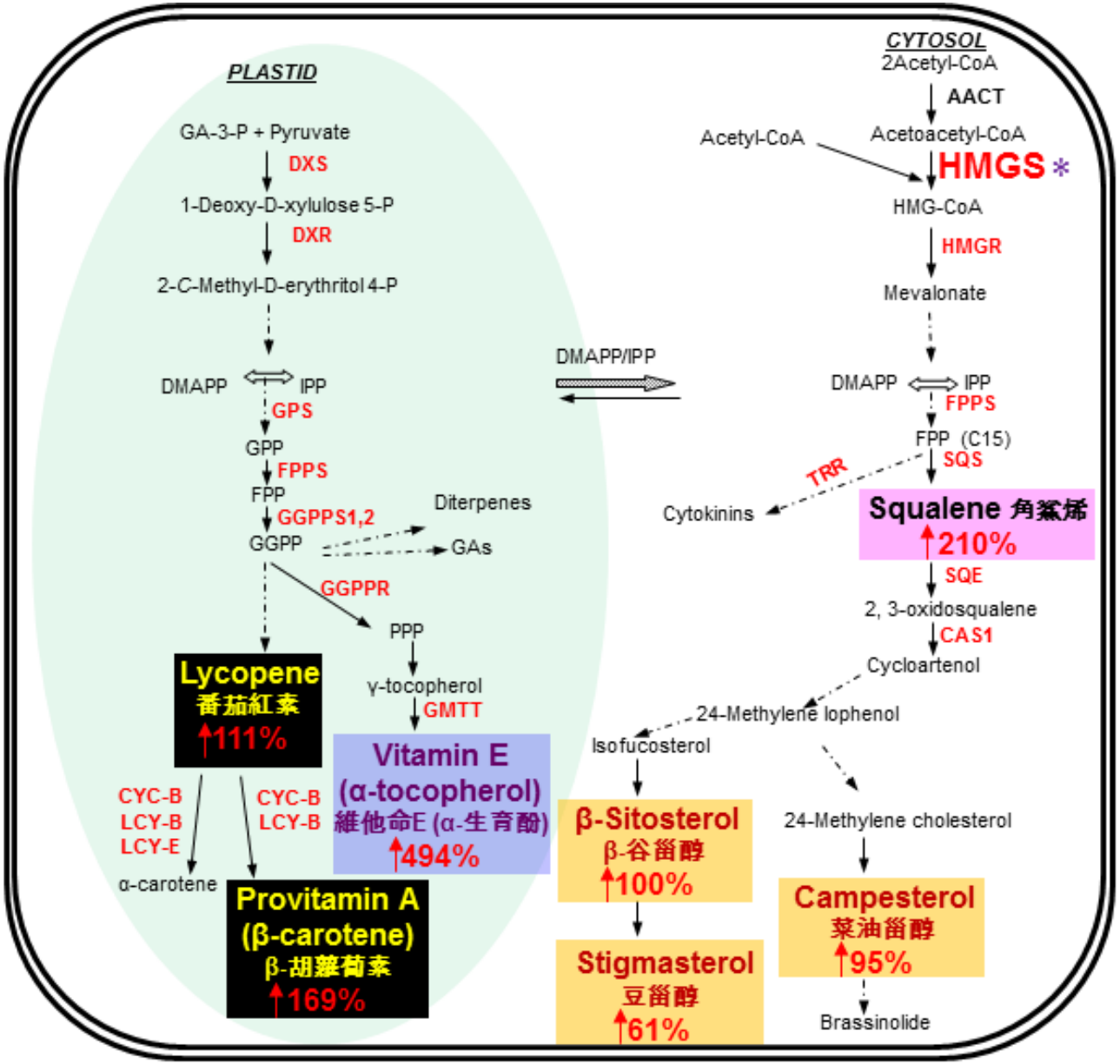
Phytosterol: campesterol (菜油甾醇), β-sitosterol (β-谷甾醇) and stigmasterol (豆甾醇)

Liao et al. (2017) *Plant Biotechnology Journal* DOI: 10.1111/pbi.12828

Conclusion（結論）

HMGS overexpression affects plastid & cytosolic pathways in tomato

HMGS
調控番茄中
兩條異戊二
烯生物合成
途徑的基因



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campesterol
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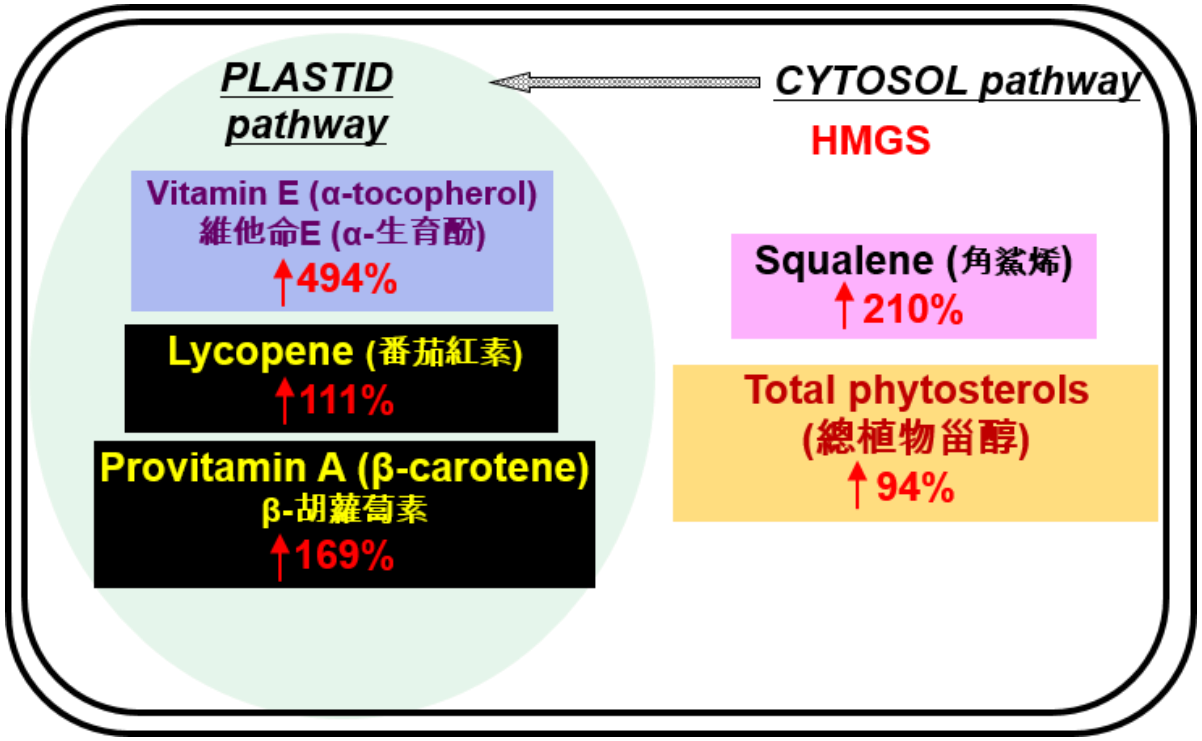
and
stigmasterol
(豆甾醇)

Significance of findings（研究意義）

Study demonstrated that the isoprenoid pathway can be engineered using **HMGS S359A** to simultaneously enhance **health-promoting** **vitamin E,** **provitamin A, lycopene,** **squalene** **and** **phytosterol** contents in edible fruits which would benefit human health.

本研究證明HMGS S359A可用於在可食用果實中調控異戊二烯途徑，從而同時促進對人體健康有益的維他命E、β-胡蘿蔔素、番茄紅素、角鯊烯和植物甾醇含量的提高。



Potential applications（潜在应用价值）

1. Provide added-value to fruits and vegetables in the human diet, as well as enrich feed for livestock and aquaculture
2. Use in production of anti-ageing cream and sun-care lotion

1. 可用於提升人類、家畜、水產養殖生物日常飲食的營養價值
2. 可用於生產抗衰老面霜和防曬乳液

Research Output (研究成果)

P Liao, X Chen, M Wang, TJ Bach, ML Chye* (2017)

Improved fruit α-tocopherol, carotenoid, squalene and phytosterol content through manipulation of *Brassica juncea* 3-HYDROXY-3-METHYLGLUTARYL-COA SYNTHASE1 in transgenic tomato.

Plant Biotechnology Journal (DOI: 10.1111/pbi.12828)

http://onlinelibrary.wiley.com/doi/10.1111/pbi.12828/full

(Ranking 7/211 in Plant Sciences, 10/158 in Biotechnology & Applied Microbiology, ***impact factor: 7.443****).*

P Liao, A Hemmerlin, TJ Bach, ML Chye* (2016)

The potential of the mevalonate pathway for enhanced isoprenoid production.

Biotechnology Advances 34, 697-713

(Ranking 6/158 in Biotechnology & Applied Microbiology, ***impact factor: 10.597****).*

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Thank you for your attention