# 非光合作用型硫鐵蛋白透過乙烯介導的路徑調控番茄植株生長及逆境耐受能力

# Non-photosynthetic Type Ferredoxin Regulated Plant Development and Stress Tolerance via Ethylene Associating Pathway in Tomato

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乙烯為植物逆境調控荷爾蒙之一，具有調控植株生長、花朵凋亡、坐果、果實成熟及逆境抗性等能力，而在乾旱及淹水逆境中植物會因乙烯及其前驅物的大量生成導致植株的生長抑制、上偏性生長及葉片脫落等，並因活性氧化物累積而導致植株死亡。但在另一方面，乙烯下游的 *ERF1* 轉錄因子也可以透過提高過氧化物的去除能力來幫助植株抵抗逆境，此外也有相關文獻指出乙烯抗性路徑可以幫助植株抵抗生物性逆境。除了荷爾蒙的調控，植物也需要改變多種酵素以調控基礎代謝反應進而抵抗逆境。其中非光合作用型硫鐵蛋白(NP-Fd)是其中一種只在植物儲藏性組織大量表現的電子傳遞蛋白，會在植物進入開花繁殖期或遭遇逆境時被大量誘導表現，並具有幫助植物抵抗逆境的效果。此外在柑橘上的研究發現處理乙烯後 NP-Fd 會有被誘導的情形，顯示兩者間對於植物的抗性誘導有所關連。因此為了更加了解乙烯與 NP-Fd 在調控植株抗性間的關聯，本實驗在番茄植株上利用35S啟動子增量表現阿拉伯芥NP-Fd (*Atfd3*)希望更進一步瞭解兩者間的關係。

## 在阿拉伯芥葉部處理乙烯可誘導 *AtFd3* 基因表現

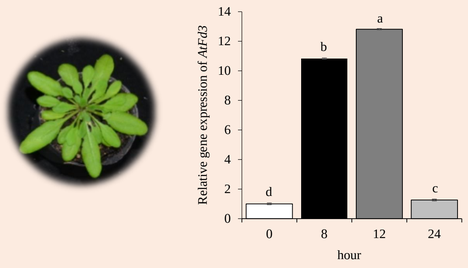


**Fig 1. Ethylene can induced *Atfd3* gene expression in *Arabidopsis*.** The seed of wild type *Arabidopsis* (Wt) were planted and grown to 112 days and injected by 0.1975 % Ethephon and then total RNA were extracted at 0, 8, 12, 24 hour and estimated by Quantitative RT-PCR with primer specificed to *AtFd3* genes. The primer specificed to *AtEf1a* was used as control. Error bars is the standard error of the mean (n=5). Means within each column follows by the different letter(s) are significantly different at P<0.05 by Fisher's protected LSD test.

## *AtFd3* 增量表現番茄植株建立與基因及蛋白質表現量檢測

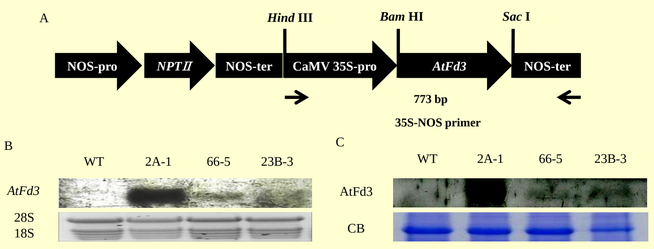


**Fig 3. The expressing level of foreign gene *AtFd3* in transgenic lines.** The plasmid pBI121 with the nopaline synthase promotor (NOS-pro), Nopaline synthase terminator (NOS-ter) and neomycin phosphotransferase gene (*npt II*) was used as the cloning vector. The cauliflower mosaic virus 35S promotor (CaMV 35S pro) and AtFd3 gene were cloned by restriction enzymes *Bam HI*, *Hind III* and *Sac1* individually. The Schematic Diagram of pBI121-*AtFd3* was Shown at (A).The expressing levels of transgenic lines *AtFd3* gene of wild type (WT) and *AtFd1-OE* lines (2A-1, 66-5 and 23B-3) were estimated by Northern blot with *Atfd3* probe at 68 ℃. The rRNA staining with ethidium bromide (rRNA) was used as loading control (B). The total protein (30 mg) was extracted for Western blot with polyclone antiserum against Atfd3. The staining of Comassie blue (CB) was used as loading control (C).

## AtFd3-OE番茄乙烯基因 *ACO1* 及 *ERF1* 較低 但對乙烯敏感度較高

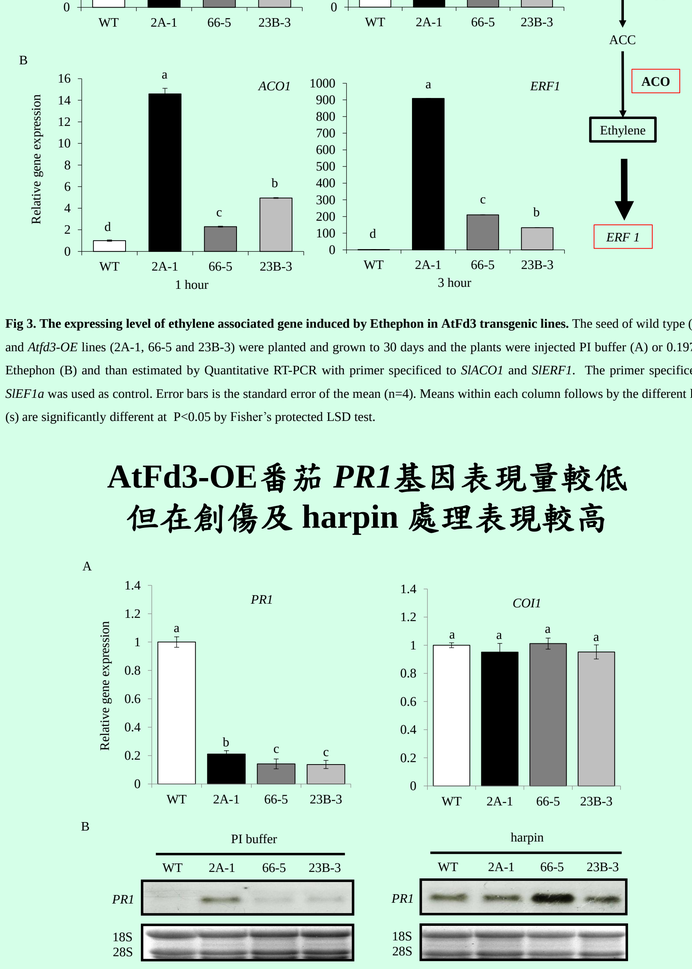


**Fig 3. The expressing level of ethylene associated gene induced by Ethephon in AtFd3 transgenic lines.** The seed of wild type (WT) and *Atfd3-OE* lines (2A-1, 66-5 and 23B-3) were planted and grown to 30 days and the plants were injected PI buffer (A) or 0.1975 % Ethephon (B) and than estimated by Quantitative RT-PCR with primer specificed to *SlACO1* and *SlERF1*. The primer specificed to *SlEF1a* was used as control. Error bars is the standard error of the mean (n=4). Means within each column follows by the different letter (s) are significantly different at P<0.05 by Fisher's protected LSD test.

## AtFd3-OE番茄 *PR1*基因表現量較低 但在創傷及 harpin 處理表現較高




**Fig 4. The expressing level of defense associated genes in AtFd3 transgenic lines.** The seed of wild type (WT) and *Atfd3-OE* lines (2A-1, 66-5 and 23B-3) were planted and grown to 30 days. The plants were estimated by Quantitative RT-PCR with primer specificed to *SlPR1* and *SlCOI1*. The primer specificed to *Sl-EF1a* was used as control. Error bars is the standard error of the mean (n=4). Means within each column follows by the different letter (s) are significantly different at P<0.05 by Fisher's protected LSD test (A). The plants were treated with 0.1 μg/μL Harpin and estimated by Nrothern blot with probe specificed to Sl-PR1. The rRNA staining with ethidium bromide was used as loading control. The plants were treated with 0.1 μg/μL Harpin and estimated by Nrothern blot with probe specificed to *SlPR1*. The rRNA staining with ethidium bromide was used as loading control (B).

## AtFd3-OE番茄對巴拉刈較敏感




**Fig 6. The responding to MV in AtFd3 transgenic lines.** The seed of wild type (WT) and *Atfd3-OE* lines (2A-1, 66-5 and 23B-3) were planted and grown to 50 days. The 0.6 dimeter leaf disc was cut and immersed in 0.025% Silwet L-77 containing 0.1 μM MV. The photograph was taken at hour after treatment (A) and the level of ion leakage were estimated (B). Error bars is the standard error of the mean (n=4). Means within each column follows by the different letter(s) are significantly different at P<0.05 by Fisher's protected LSD test.

## AtFd3-OE 番茄植株高度較矮且葉片捲曲 但具有較多的根系

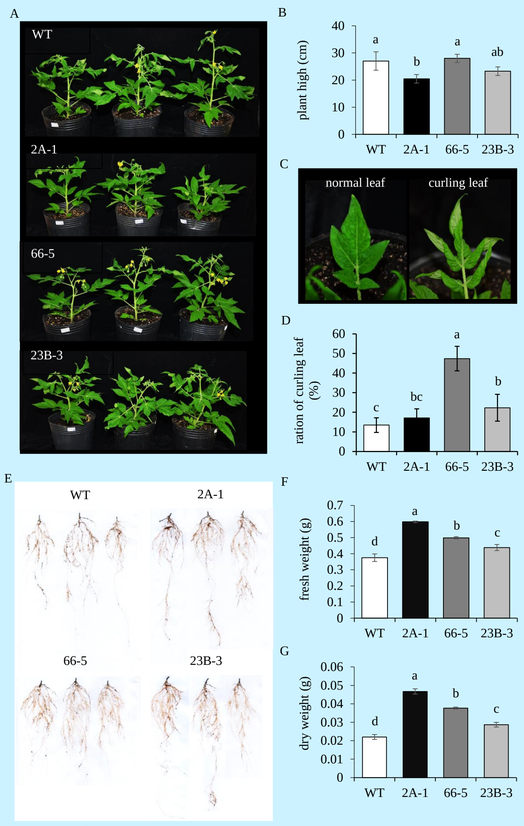


**Fig 9. The developments of *AtFd3-OE* lines transgenic tomato.** The seed of wild type (WT) and *Atfd3-OE* lines (2A-1, 66-5 and 23B-3) were planted and grown to 60 days. The photograph was taken (A) and the plant high was estimated in the growth period (B). The leaf appearance was distinguished as normal leaf and curling leaf as demonstrated in (C). The ration of curling leaf was estimated (D). The photograph of root tissue was taken (E) and the fresh weight (F) and dry weight (G) were estimated. Error bars is the standard error of the mean (n=5). Means within each column follows by the different letter(s) are significantly different at P<0.05 by Fisher's protected LSD test.

## AtFd3-OE 番茄花多青果少紅果不變

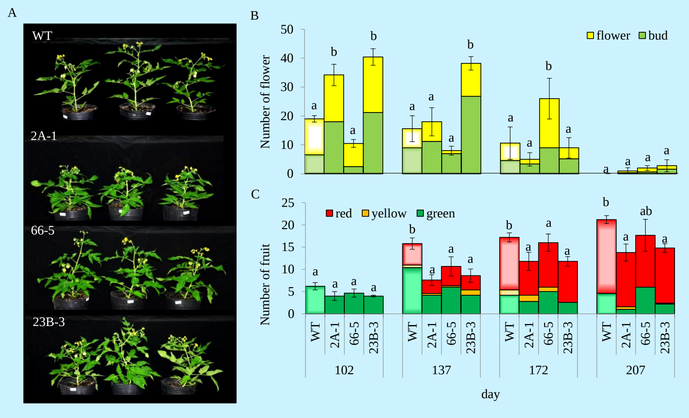


**Fig 11. The reproductive tissue development of *Atfd3-OE* lines.** The seed of wild type (WT) and *Atfd3-OE* lines (2A-1, 66-5 and 23B-3) were planted and grown to 102 days and the photograph was taken (A). The number of flower (B) and fruit (C) were estimated. Error bars is the standard error of the mean (n=5). Means within each column follows by the different letter(s) are significantly different at P<0.05 by Fisher's protected LSD test.

## AtFd3-OE番茄光合作用效率不受影響

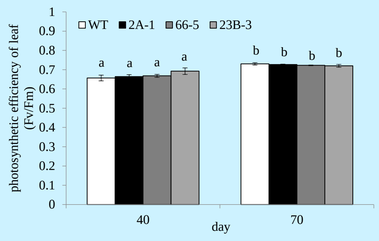


**Fig 12. The photosynthetic efficiency of *Atfd3-OE* lines.** The seed of wild type (WT) and *Atfd3-OE* lines (2A-1, 66-5 and 23B-3) were planted and grown to 40 and 70 days. The photosynthetic efficiency of leaf (Fv/Fm) were estimated by JUNIOR-PAM Teaching Chlorophyll Flourometer (WALZ). Error bars is the standard error of the mean (n=4). Means within each column follows by the different letter(s) are significantly different at P<0.05 by Fisher's protected LSD test.

## AtFd3-OE番茄對淹水逆境耐受性較高

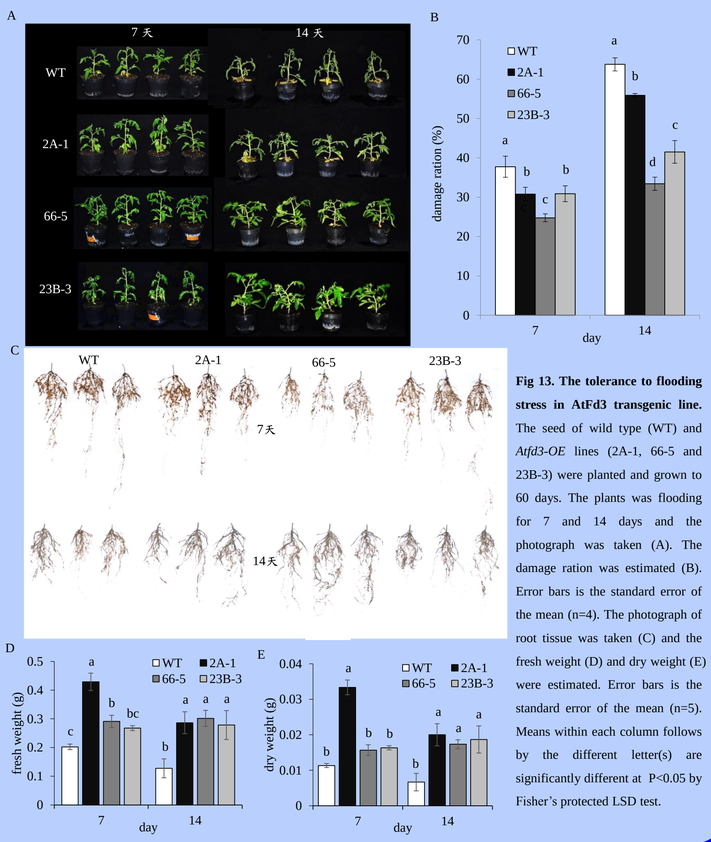


**Fig 13. The tolerance to flooding stress in AtFd3 transgenic line.** The seed of wild type (WT) and *Atfd3-OE* lines (2A-1, 66-5 and 23B-3) were planted and grown to 60 days. The plants was flooding for 7 and 14 days and the photograph was taken (A). The damage ration was estimated (B). Error bars is the standard error of the mean (n=4). The photograph of root tissue was taken (C) and the fresh weight (D) and dry weight (E) were estimated. Error bars is the standard error of the mean (n=5). Means within each column follows by the different letter(s) are significantly different at P<0.05 by Fisher's protected LSD test.

## AtFd3-OE番茄對乾旱逆境抵抗與回復能力較高

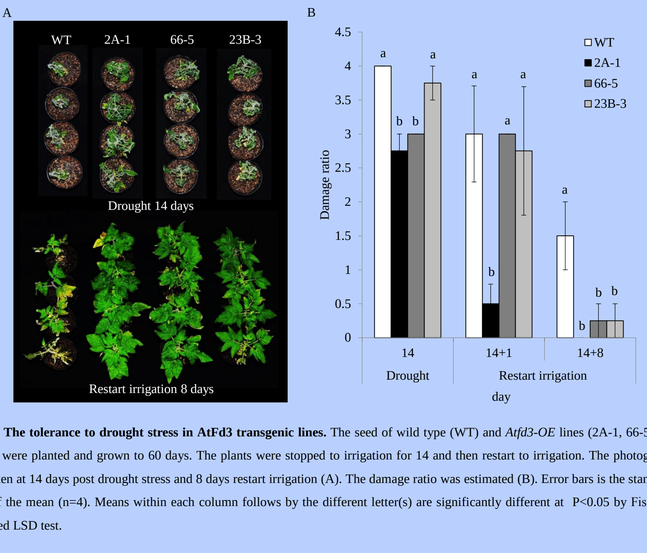


**Fig 15. The tolerance to drought stress in AtFd3 transgenic lines.** The seed of wild type (WT) and *Atfd3-OE* lines (2A-1, 66-5 and 23B-3) were planted and grown to 60 days. The plants were stopped to irrigation for 14 and then restart to irrigation. The photograph was taken at 14 days post drought stress and 8 days restart irrigation (A). The damage ratio was estimated (B). Error bars is the standard error of the mean (n=4). Means within each column follows by the different letter(s) are significantly different at P<0.05 by Fisher's protected LSD test.

## AtFd3-OE 番茄處理在乾旱及淹水後氧化損傷較低

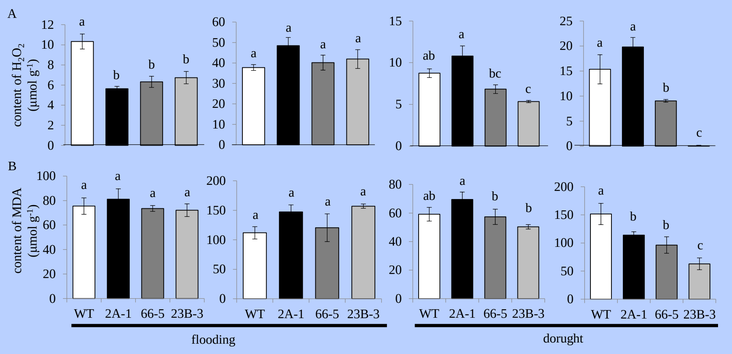


**Fig 16. The tolerance to flooding and dorught stress in AtFd3 transgenic line.** The seed of wild type (WT) and *Atfd3-OE* lines (2A-1, 66-5 and 23B-3) were planted and grown to 60 days. The plants was flooding or irrigation for 7 and 14 days and the leaf tissue was taken to estimate the content of $H_2O_2$ (A) and MDA (B). Error bars is the standard error of the mean (n=5). Means within each column follows by the different letter (s) are significantly different at P<0.05 by Fisher's protected LSD test.

## AtFd3-OE 番茄淹水後 *ERF1* 較高

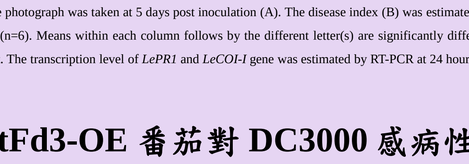


**Fig 18. The tolerance to flooding stress in AtFd3 transgenic line.** The seed of wild type (WT) and *Atfd3-OE* lines (2A-1, 66-5 and 23B-3) were planted and grown to 60 days. The total RNA were extracted and estimated by Quantitative RT-PCR with primer specificed to *SlERF1*. Error bars is the standard error of the mean (n=5). Means within each column follows by the different letter (s) are significantly different at P<0.05 by Fisher's protected LSD test.

## Atfd3-OE番茄對青枯病菌抗性較高

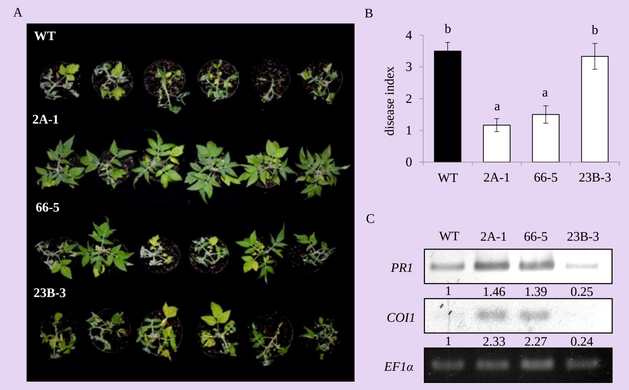


**Fig 19. The inoculation of *R. solanacearum* Rd4 in the AtFd3 transgenic lines.** The seed of wild type (WT) and *Atfd3-OE* lines (2A-1, 66-5 and 23B-3) were planted and grown to 60 days. The plants were injected by 100 μl bacterial suspension of *R. solanacearum* Rd4 ($10^7$ CFU/ml). The photograph was taken at 5 days post inoculation (A). The disease index (B) was estimated. Error bars is the standard error of the mean (n=6). Means within each column follows by the different letter(s) are significantly different at P<0.05 by Fisher's protected LSD test. The transcription level of *LePR1* and *LeCOI-I* gene was estimated by RT-PCR at 24 hours post infiltration (C).

## AtFd3-OE 番茄對 DC3000 感病性較高

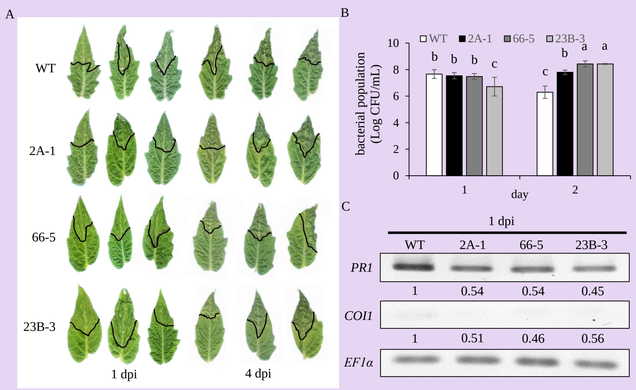


**Fig 20. The inoculation of *P. syringae* pv. tomato DC3000 in the AtFd3 transgenic lines.** The seed of wild type (WT) and *Atfd3-OE* lines (2A-1, 66-5 and 23B-3) were planted and grown to 60 days. The plants were injected by bacterial suspension of *Pseudomonas syringae* pv. tomato DC3000 ($10^7$ CFU/ml). The photograph was taken at 1 and 4 days post inoculation (A). The bacterial population (B) was estimated. The total RNA was extracted and used the primer specificed to *SlPR1* and *SlCOI1* for RT-PCR. The *SlEF1a* was used as control (C). Error bars is the standard error of the mean (n=6). Means within each column follows by the different letter(s) are significantly different at P<0.05 by Fisher's protected LSD test.

過去研究指出，乙烯與 NP-Fd 皆具有同時調控多種逆境抗性的能力，且乙烯具有誘導 NP-Fd 的能力。

在本研究中發現增量表現 NP-Fd 可以降低番茄植株乙烯生成基因並提高對於乙烯訊號路徑的敏感度，進而讓植株出現高度矮化、葉片捲曲、根系較多及坐果減少的情況，並在遭遇淹水及乾旱逆境時可以具有較高的 *ERF1* 基因表現，並降低對於植株的氧化損傷。

而生物逆境的部分在青枯病與葉斑病的抗性顯示出相反的結果，這部份則需要更詳細的實驗來瞭解相關的機制。