

# The Effect of Exogenous Fumarylacetate to *Arabidopsis thaliana*

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**Abstract:** We isolated a single recessive mutant *sscd1*, which exhibited phenotype of wilting and whitened under short-day condition (8h of light/16h of dark). The *SSCD1* gene was cloned via a map-based cloning approach and found to encode an *Arabidopsis* putative FAH (fumarylacetoacetate hydrolase, FAH), which is the last enzyme in Tyr degradation. We found low concentration of exogenous fumarylacetoacetate (FA) can benefits the growth of seedings under long-day condition, but high concentration would significantly suppress wild-type seedings germination, and the effect weakened when FAH gene was abolished. Moreover, high concentration of exogenous FA results in seeding whitened.

**Keywords:** Fumarylacetate, Fumarylacetoacetate Hydrolase, Tyr degradation, *Arabidopsis thaliana*

## 1. Introduction

The first steps of the Tyr degradation pathway result in the formation of homogentisate that then undergoes oxidative ring cleavage, mediated by homogentisate 1,2 dioxygenase (HGO) to produce maleylacetoacetate which is isomerized to fumarylacetoacetate by maleylacetoacetate isomerase (MAAI), and finally fumarylacetoacetate is hydrolyzed by fumarylacetoacetate hydrolase (FAH) to form fumarate and acetoacetate [1].

FAH plays an important role in Tyr degradation metabolic and blockage of it results in metabolic disorder diseases. For example, human FAH deficiency causes hereditary tyrosinemia type I (HT1), an inborn lethal disease [2]. However, the role of the Tyr degradation pathway in plants is still unknown

Amino acid play a important role in seedings germination, Ruthie Angelovici found significant elevation of Lysine levels in *Arabidopsis* seeds, by enhancing its synthesis and blocking its catabolism, causes a retardation of germination [3]. But, the relationship between Tyr degradation and germination need to be researched.

## 2. Materials and Methods

### 2.1 Plant Materials

The *Arabidopsis* (*Arabidopsis thaliana*) wild type (Col-0, WT) and mutant *sscd1* are both provided by our own laboratory—Hunan Provincial Key Laboratory of Crop Germplasm Innovation and Utilization.

### 2.2 Growth Conditions

Seeds were surface-sterilized with 20% chlorine bleach containing 0.1% Triton X-100 for 10 min, washed five times with sterile water, plated on plant growth medium (Murashige and Skoog medium supplemented with 1% Sucrose), chilled at 4°C for 3 d, and then transferred to a growth chamber under long-day condition (16h of light/8h of dark) at 150 mol m<sup>-2</sup> s<sup>-1</sup> and 22°C.

### 2.3 FA treatment

Col-0 and *sscd1* seedings were grown on medium without or with 1mM, 1.25 mM, 1.5 mM, 1.75 mM and 2 mM FA. FA solution was sterilized by pressure filtration.

## 3. Results and Discussion

### 3.1 high-concentration FA inhibit the germination of Col-0

Col-0 was germinated on medium without or with 1mM, 1.25 mM, 1.5 mM, 1.75 mM and 2 mM FA under long-day condition. 2 days later, we observed that most of Col-0 seedings were normally germinated on medium without or with 1mM and 1.25 mM FA. However, germination on medium with high concentration of FA were obvious inhibited. 4 days later, germination increased at all concentration but still demonstrated suppression trend under high-concentration FA. We calculated the rate of germination on the 10th day, the statics also showed exogenous FA inhibited Col-0 germination in a dose depend manner (Figure 1).

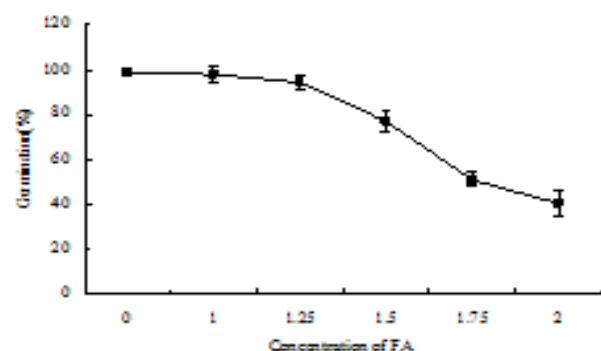
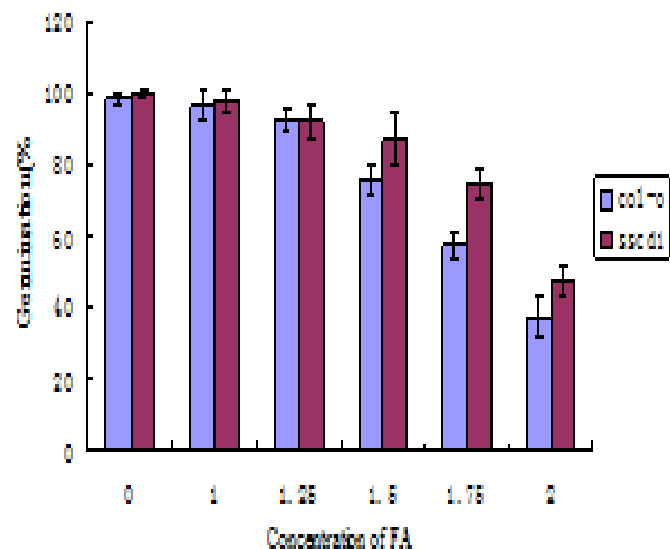


Figure 1: Germination of wild-type (Col-0, WT) grown on medium without or with FA

### 3.2 The effect of FA to Col-0 germination is weakened when FAH gene was abolished

Col-0 and *sscd1* seedlings were germinated on medium without or with 1mM, 1.25 mM, 1.5 mM, 1.75 mM and 2 mM FA under long-day condition. 2 days later, we obvious observed Col-0 germination were inhibited by FA, while most of the *sscd1* germinated normally even on the medium with 1.5mM FA. 4 days later, the rate of *sscd1* germination is up to  $\geq 70\%$  on the medium with highest FA. However, Col-0 only has 50% (Figure 2).



.Figure 2: Germination of wild-type (Col-0, WT) and *sscd1* grown on medium without or with FA.

### 3.3 low-concentration of exogenous FA benefits the growth of seedlings

We accidently found low concentration of exogenous FA was beneficial to the growth of Col-0 and *sscd1*. It was apparent the seedlings grown on medium with 1mM FA were bigger and stronger than the seedlings grown on medium without FA. But, this phenomenon couldn't happen when the concentration higher than 1mM (Figure 3).

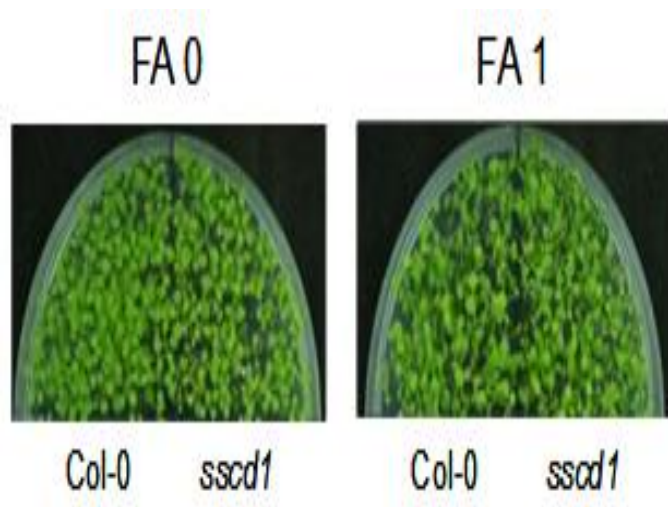


Figure 3: The seedlings of Col-0 and *sscd1* grown on medium without and with 1mM FA for 10 days under long-day condition.

### 3.4 Exogenous FA results in the leaves of seedlings are whitened

We isolated *sscd1* mutant, which became wilted and whitened under short-day condition. However, under long-day condition, high concentration of exogenous FA results in the leaves of both Col-0 and *sscd1* are whitened (Figure 4).

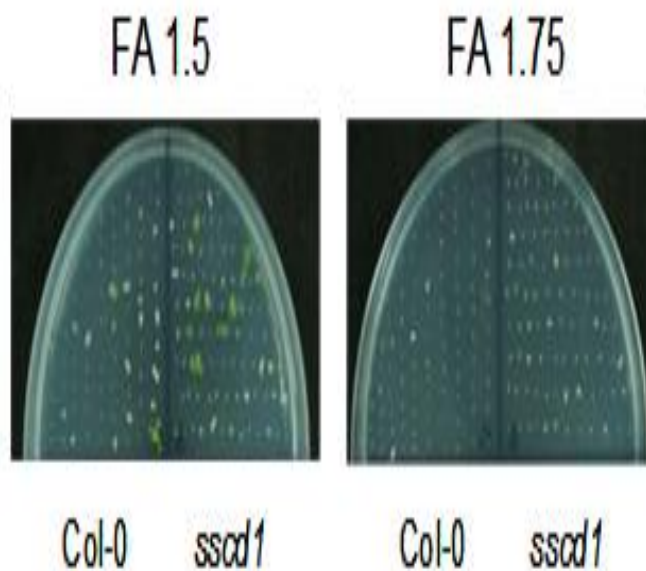


Figure 4: The seedlings of Col-0 and *sscd1* grown on medium with 1.5mM and 1.75mM FA for 10 days under long-day condition.

## 4. Conclusion

The results showed that under long-day condition, Col-0 seedlings germination were inhibited by high concentration of exogenous FA, but the effect became weakened when *FAH* gene was abolished—*sscd1*; The growth of Col-0 and *sscd1* were promoted by low concentration of FA, but this phenomenon was no longer exist when concentration became high; high concentration of exogenous FA results in the leaves of both Col-0 and *sscd1* are whitened.

## Reference

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