# Isolation and characterization of low- tolerant mutants of Arabidopsis

*Yu Wu, Qing Zhao, Lei Gao, Xiao-Min Yu, Ping Fang, David J Oliver, and Chengbin Xiang*

## Supplementary materials for a detailed description of the genetic screen for low-tolerance mutants

### Medium preparation

The medium with no added sulphur was designated as "sulphur-free". In fact, trace amounts of sulphur were inevitable due to contamination in the chemicals used in the medium. To decrease the sulphur to as low a level as possible, the sulphur-free medium was prepared using de-ionized water and was solidified with agarose (Cat. Number: BI0079, Sino-American Biotechnology Co., China). To minimize the trace sulphur level, the agarose could be washed with de-ionized water several times before prepared the sulphur-free medium. The stock solutions for both the sulphur-containing and sulphur-free media are shown in Supplemental Table 1 and only differed in the composition of solution 2. For 1 liter of medium, 50 ml/L of stock solution 1, 50 ml/L of the correct stock solution 2 for sulphur-containing or sulphur-free media, 5 ml/L of stock solution 3, and 5 ml/L of stock solution 4 were mixed and the volume was brought to 1 liter with deionized water. The pH was adjusted to 5.8 and 8.0 g/L of agarose was added before autoclaving for 15 minutes. When the medium was cooled to about 45°C, the appropriate antibiotic or herbicide was added if needed. The medium was poured into Petri dishes (75 ml/150 mm plate). All of the chemicals used for stock solutions were purchased from Sinopharm Chemical Reagent Beijing Co. (China).

MS medium was prepared by mixing the stock solutions in Supplemental Table 2 as follows: 100 ml/L of stock solution 1, 10 ml/L of stock solution 2， 10 ml/L of stock solution 3, and 20 g/L of sucrose. The volume was brought to 1 liter with de-ionized water and the pH was adjusted to 5.8 before 9.0 g/L of agar was added and the media autoclaving for 15 minutes. 1/2 X MS was prepared using half strength of the ingredients.

**Primary screen for mutants with improved sulphate utilization efficiency**

For the primary screen, about 15,000 seeds were used from each pool. Seeds were surfaced sterilized for 10 minutes with 50% commercial bleach and a few drops of Triton X100 and washed five times with sterile water. The surface sterilized seeds were suspended in 0.12 % agarose and an appropriate amount was poured onto the sulphate-free medium containing 50 mg/L of the herbicide glufosinate ammonium at a seed density of 3,500 to 4,000 seeds/150 mm plate. After the 0.12% agarose was air dried in a sterile hood, the plates were stored at 4°C for 2 days before being placed horizontally at 22°C with a 16 h photoperiod. After 12 days, plates were visually examined for putative mutants that were able to continue to grow under sulphate-free conditions. The standard for mutant selection was healthy seedlings with continued growth of true leaves and elongating roots. Under the selection condition, wildtype seeds germinated but failed to grow further without emergence of true leaves or elongating roots. The putative mutants were transferred to soil and grown to maturity. Seeds were collected from individual plants for further analysis.

**Secondary screen of the mutants**

To confirm the mutant phenotype, a secondary screen was carried out for each mutant using T3 seeds. The secondary screen was similar to the primary screen on the sulphate-free medium except the seeds density was 50 mutant seeds and 50 wild type seeds per 100 mm plate. After 12 days, mutant and wild type phenotypes were scored for $\chi^2$ analysis. The mutant seeds were also grown vertically on sulphur-free medium to examine their root growth and the primary root length was recorded for statistical analysis.

Mutant seedlings were transferred to MS medium containing the herbicide for T-DNA co-segregation analysis. The seedlings of genetically confirmed mutant lines were transferred to soil to grown to maturity and seeds were collected from individual plants and homozygous seeds were obtained for subsequent studies.

**Supplementary Table 1. Composition of stock solutions for sulphur-containing and sulphur-free medium**

| | Chemical | Stock concentration (mmol/L) | Final concentration (mmol/L) |
|---|---|---|---|
| Stock solution 1 (20×) | $Ca(NO_3)_2.4H_2O$ | 40.000 | 2.00 |
| | $KNO_3$ | 100.000 | 5.00 |
| Sulphur-free Stock solution 2 (20×) | $KH_2PO_4$ | 50.000 | 2.50 |
| | $MgCl_2.6H_2O$ | 40.000 | 2.00 |
| Sulphur-containing stock solution 2 (20×) | $KH_2PO_4$ | 50.000 | 2.50 |
| | $MgSO_4.7H_2O$ | 40.000 | 2.00 |
| Stock solution 3 (200×) | $Na_4EDTA$ | 10.000 | 0.05 |
| | $FeCl_2.4H_2O$ | 10.000 | 0.05 |
| Stock solution 4 (200×) | $H_3BO_3$ | 14.000 | 0.07 |
| | $MnCl_2$ | 2.000 | 0.01 |
| | $ZnCl_2$ | 0.200 | $1\times10^{-3}$ |
| | $Na_2MoO_4.2H_2O$ | 0.060 | $3\times10^{-4}$ |
| | $CuCl_2.2H_2O$ | 0.100 | $5\times10^{-4}$ |
| | $CoCl_2.6H_2O$ | 0.002 | $1\times10^{-5}$ |

**Supplementary Table 2. Composition of stock solutions for MS medium**

| | Chemical | Stock concentration (mM) | Final concentration (mM) |
|---|---|---|---|
| Stock solution 1 (10×) | $NH_4NO_3$ | 200.00 | 20.00 |
| | $KNO_3$ | 200.00 | 20.00 |
| | $KH_2PO_4$ | 12.50 | 1.25 |
| | $MgSO_4.7H_2O$ | 15.00 | 1.50 |
| | $CaCl_2.2H_2O$ | 30.00 | 3.00 |
| Stock solution 2 (100×) | KI | 5.00 | 0.05 |
| | $H_3BO_3$ | 10.00 | 0.10 |
| | $MnSO_4.4H_2O$ | 10.00 | 0.10 |
| | $ZnSO_4.7H_2O$ | 3.00 | 0.03 |
| | $Na_2MoO_4.2H_2O$ | 0.10 | $1\times10^{-3}$ |
| | $CuSO_4.5H_2O$ | 0.01 | $1\times10^{-4}$ |
| | $CoCl_2.6H_2O$ | 0.01 | $1\times10^{-4}$ |
| Stock solution 3 (100×) | $Na_4EDTA$ | 100.00 | 1.00 |
| | $FeSO_4.7H_2O$ | 100.00 | 1.00 |

**Supplementary Figure 1. T-DNA-tagged loci in *sue3* and isolation of Salk_001014 homozygous lines.**

A. Locations of two T-DNA insertion sites in *sue3*. Southern blot analysis indicated that there were two T-DNA insertion sites in *sue3* (data not shown). These sites were identified by TAIL-PCR and sequencing. One T-DNA was inserted on chromosome 1 in the second intron of the At1g43700 and the other on chromosome 2 at 382bp upstream the ATG codon of At52g62200.

B. RT- PCR analysis of transcript level of At5g62200 and At5g62210 in *sue3*. RNA was isolated from 2-week-old seedlings. The transcript level of At5g62200 and At5g62210 surrounding the T-DNA insertion was analyzed via RT-PCR. The results show that there were no significant difference between the wild type and mutant, indicating these two genes were not affected by the T-DNA insertion. Tubulin was used as loading control.

C. Screen for homozygous mutant plants of Salk_001014 by genomic PCR. Lane 2 and 4 were homozygous lines. The rest were wild type lines. The wild type was used as control

D. RT-PCR analysis of Atag43700 and At3g55880 expression in the wild type under normal condition and low sulphur stress. 7-day-old wild type was transplanted from MS medium (750μM sulphate) to low-sulphur (75μM sulphate) and sulphate-free medium (0μM sulphate) for 48 hours before RNA extracts were prepared for RT-PCR. Neither At1g43700 nor At3g55880 was responsive to low-sulphur stress.