

## Supporting Information

# Grain unloading of selenium species in rice (*Oryza sativa* L.).

Anne-Marie Carey<sup>1</sup>, Kirk G. Scheckel<sup>2</sup>, Enzo Lombi<sup>3</sup>, Matt Newville<sup>4</sup>, Yongseong Choi<sup>4</sup>, Gareth J. Norton<sup>1</sup>, Adam H. Price<sup>1</sup> and Andrew A. Meharg<sup>1</sup>

<sup>1</sup> Institute of Biological and Environmental Sciences, University of Aberdeen,  
Cruickshank Building, St Machar Drive, Aberdeen, AB24 3UU, UK

<sup>2</sup> National Risk Management Research Laboratory, US Environmental Protection  
Agency, 5995 Centre Hill Avenue, Cincinnati, Ohio 45224, USA

<sup>3</sup> Centre for Environmental Risk Assessment and Remediation, University of South  
Australia, Building X, Mawson Lakes Campus Mawson Lakes, South Australia, SA-  
5095 Australia

<sup>4</sup> Centre for Advanced Radiation Sources, The University of Chicago, Chicago, IL  
60637.

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## Detailed Methods

### Stem-girdling and panicle excision

Stem-girdled panicles were exposed to a 30 s jet of steam, applied immediately below the panicle head as described in Carey et al. (18,27), to prevent further phloem transport into the developing grain without damaging xylem transport (19,26,43).

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This technique interrupts phloem transport by destroying the living phloem cells. Since xylem vessels are already dead, xylem transport should remain functional. It should be noted that despite the inclusion of markers for phloem and xylem transport, stem-girdling panicles with steam and excising them from the main plant could yield unknown effects on physiological processes within the panicle. Rice plants were then placed in darkness at 10 d post-anthesis (DPA) for 2 h to reduce transpiration, limiting the formation of air bubbles in the xylem during excision, before being excised below the flag leaf node and transferred into autoclaved hydroponic treatment solutions as described in Carey et al. (18).

### Leaf feeding of selenium species – treatment solutions

Treatment stock solutions of 12.66 mM were prepared by dissolving the appropriate salt in Milli-Q deionised water, and 7 subsamples of each stock were frozen for subsequent removal and use on the relevant treatment day. Treatment vials were prepared by making 0.1 ml of the defrosted treatment stock up to 1 ml with Milli-Q deionized water and then pipetting 0.1 ml of this diluted stock into a weighed Eppendorf vial.

### Synchrotron XRF microtomography

The electron storage ring operated at 7 GeV with a top-up fill mode. The incident X-ray energy was 18 KeV. Fresh rice grain was suspended from a rotation-translation stage in the path of a 3  $\mu\text{m}$  x 3  $\mu\text{m}$  X-ray beam and translated across in 10  $\mu\text{m}$  steps with a dwell time of 0.25 s per step. The grain was then rotated 1° and the scan process repeated until a rotation of 0 to 180° was complete. Fluorescence data were collected using an energy dispersive detector and the resulting 2D sine wave plots were reconstructed as described in McNear et al. (44).

## **Results**

### **Total rubidium and strontium in grain and flag leaves for leaf fed panicles**

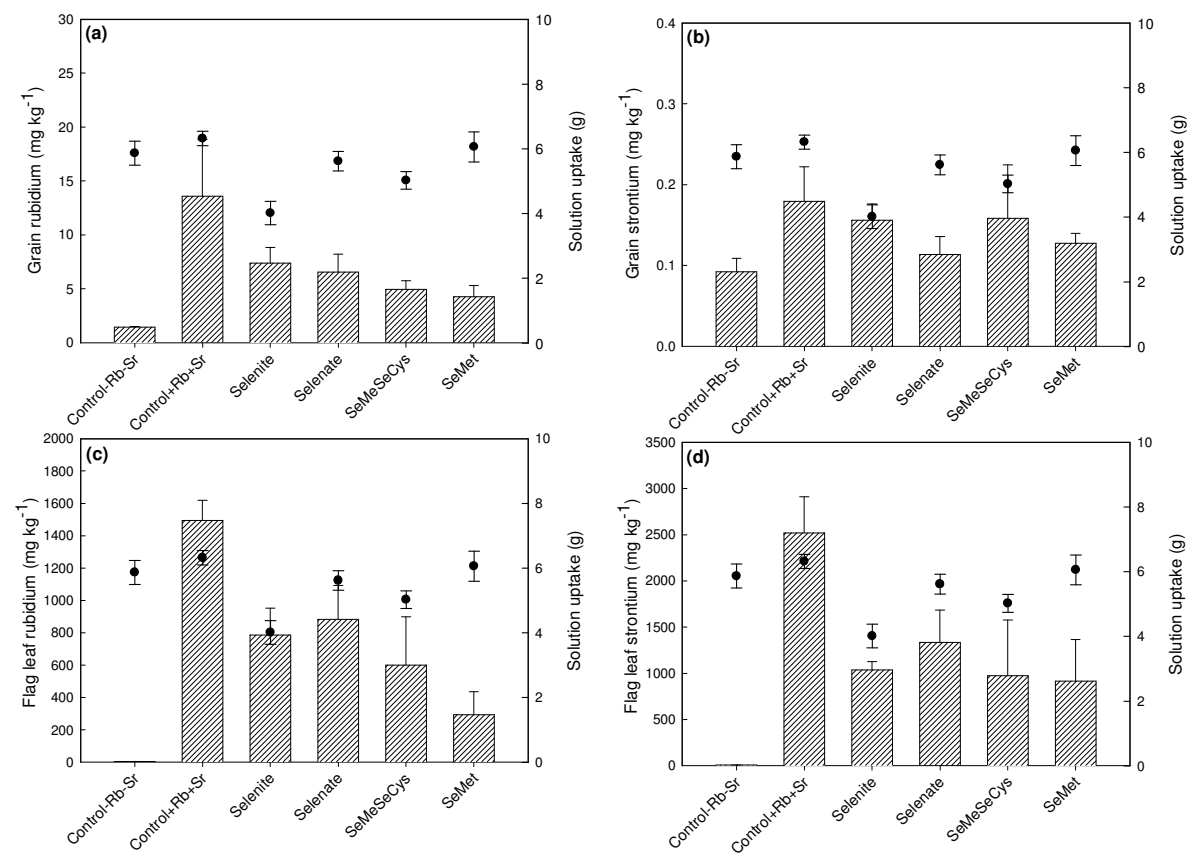
Total Rb concentrations in grains and flag leaves, together with solution uptake, for plants pulsed via a cut flag leaf on intact plants are shown in Figure S1a and S1c, respectively. All Se treatments and controls pulsed with Rb yielded significantly higher flag leaf Rb compared with zero exposure controls (one-way ANOVA,  $P < 0.001$ , Figure S1c) and there were no significant differences in flag leaf Rb levels between the Se treatments, although Fisher's pairwise comparisons showed that the Se controls did yield significantly greater flag leaf Rb than the two organic Se treatments.

All Se treatments and controls pulsed with Rb led to significantly increased grain Rb levels compared to zero exposure controls (one-way ANOVA,  $P < 0.001$ , Figure S1a), however, pairwise comparisons showed that there were no significant differences in grain Rb between the Se treatments.

Total Sr concentrations in grains and flag leaves, together with solution uptake, for plants pulsed via a cut flag leaf on intact plants are shown in Figure S1b and S1d, respectively. One-way ANOVA determined that exposure to Sr led to significantly higher Sr in flag leaves ( $P<0.001$ , Figure S1d) and that there were no significant differences in flag leaf Sr levels between Se treatments. There was, however, no significant Sr transport to the grain, over the exposure period, for any of the Se treatments; with no significant differences in grain Sr levels between all treatments and the zero exposure controls (Figure S1b).

### **Background concentrations of Se in grain and flag leaves**

Se control and zero exposure control panicles and plants were not exposed to any Se. For both leaf fed and excised stem fed panicles, background concentrations of Se were extremely low in Se control grain and zero exposure control grain, ranging between 0.02 and 0.05 mg kg<sup>-1</sup>. Feeding the cut flag leaves of intact plants with Se led to grain concentrations of Se that were between 50 and 500-fold greater than the Se and zero exposure controls. Treating excised panicles with Se led to grain Se concentrations up to 10,000 times those of the Se and zero exposure controls. Background flag leaf concentrations of Se ranged between 0.2 and 1 mg kg<sup>-1</sup> and were approximately 10 to 500-fold lower than flag leaf Se concentrations in those leaves treated with Se via the cut flag leaf of intact plants, and up to 3000-fold lower than Se in the flag leaves of excised panicles treated with Se.



**Figure S1.** Mean total Rb and Sr concentrations (bars) in grain (a and b) and flag leaf (c and d) for rice fed either 126.6  $\mu\text{M}$  selenate, selenite, selenomethionine (SeMet) or selenomethylcysteine (SeMeSeCys), together with 1 mM Rb and Sr, via the flag leaf of intact plants for a 7 d period during grain fill. Total solution uptake is also shown for each treatment (circles). Error bars represent  $\pm$  SE of four replicates.