

**Supporting information for:**

**Cadmium distribution in mature durum wheat grains using dissection, laser ablation-ICP-MS and synchrotron techniques**

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### **Comment S1.** Details of the hydroponic culture

The hydroponic conditions were almost the same as those described in Yan et al. (2018). Briefly, germinated grains were pre-grown for 10 days in 50-mL Falcon<sup>®</sup> tubes before being transferred to polypropylene pots (height 16 cm, diameter 23 cm, one seedling per pot) filled with a modified Hoagland nutrient solution containing 0.25 mM KH<sub>2</sub>PO<sub>4</sub>, 1.25 mM KNO<sub>3</sub>, 1.25 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 0.5 mM MgSO<sub>4</sub>, 46.25 μM H<sub>3</sub>BO<sub>3</sub>, 1 μM MnCl<sub>2</sub>, 10 μM ZnSO<sub>4</sub>, 2 μM CuSO<sub>4</sub>, 0.03 μM (NH<sub>4</sub>)<sub>6</sub>MO<sub>7</sub>O<sub>24</sub>, 100 μM KCl, 71.63 μM FeNaEDTA, 25 μM HEDTA, 2 mM MES buffer and 50 mg L<sup>-1</sup> SiO<sub>2</sub>. The pH of the solution was adjusted to 6.0 with solid KOH and supplemented with 100 (± 10) nM Cd(NO<sub>3</sub>)<sub>2</sub>. The supply of HEDTA buffered free ion metal activities to environmentally appropriate values (hereafter referred to as -log<sub>10</sub> of concentrations in M): 17.15 for Fe<sup>3+</sup>, 7.30 for Mn<sup>2+</sup>, 12.89 for Cu<sup>2+</sup>, 9.41 for Zn<sup>2+</sup> and 10.78 for Cd<sup>2+</sup>, calculated using the Visual MINTEQ geochemical model. Before anthesis, the nutrient solution was renewed automatically using an overflow system. The turnover rate was gradually increased from 6% per day during the first two weeks to 26% per day at the end of the vegetative growth period. After anthesis, all the nutrient solution in each pot was manually replaced twice a week. From the start of stem elongation, only the first four tillers were allowed to develop, the others were removed during the early stage of emergence. Plants were harvested 70 days after transplanting (1250 growing degree days) which corresponded to grain physiological maturity. The daily mean temperature in the greenhouse ranged from 16 to 25 °C before anthesis (average 21 °C) and from 19 to 28 °C from anthesis to maturity (average 24 °C).

## Comment S2. Details of the grain dissection protocol

A first set of 25 grains (five replicates, each representing a pool made of five grains) were used to collect a subsample of the core of the endosperm and the crease (*CR*) fraction. Grains were soaked in ultrapure water at room temperature for 24 hours to soften the endosperm. The other parts and germ were not collected because the long soaking could result in losses of elements. The grains were cut longitudinally along the crease. The black strand part was collected as the *CR* fraction. The core of the endosperm of each grain (*SE*) was then separated, taking care to not sample the envelope. On a second set of 25 grains soaked in ultra-pure water for only four hours at 4 °C to minimize losses from periphery tissues, a longitudinal incision was made on the side opposite the crease (dorsal side) that made it possible to remove the outer periphery (*OP*) part and then the germ fraction (*GM*). The rest of the grain (*RP*) was sampled as remaining parts:  $RP = CR + SE + IP$ . All the fractions were freeze-dried at -60 °C overnight and weighed before wet digestion to determine their mineral contents (see below). For a given element, if  $C_i$ ,  $Q_i$ ,  $DW_i$  are the concentration, quantity and dry weight of the fraction  $i = \{\text{whole grain, } OP, GM, CR, SE, RP, IP\}$ , respectively:

$$Q_{\text{wholegrain}} = Q_{OP} + Q_{IP} + Q_{GM} + Q_{CR} + Q_{SE} = Q_{RP} \quad (1)$$

$$Q_{OP} = C_{OP} \cdot DW_{OP} \quad (2)$$

$$Q_{GM} = C_{GM} \cdot DW_{GM} \quad (3)$$

$$Q_{CR} = C_{CR} \cdot DW_{CR} \quad (4)$$

$$Q_{SE} = C_{SE} \cdot DW_{SE} \quad (5)$$

$$Q_{RP} = Q_{CR} + Q_{IP} + Q_{SE} = C_{RP} \cdot DW_{RP} \quad (6)$$

$$Q_{IP} = Q_{RP} - Q_{CR} - Q_{SE} \quad (7)$$

$$DW_{IP} = DW_{\text{wholegrain}} - DW_{GM} - DW_{OP} - DW_{CR} - DW_{SE} \quad (8)$$

$$C_{IP} = \frac{Q_{RP} - Q_{CR} - Q_{SE}}{DW_{IP}} \quad (9)$$

$$C_{\text{wholegrain}} = \frac{Q_{\text{wholegrain}}}{DW_{\text{wholegrain}}} \quad (10)$$

Except for  $DW_{SE}$ ,  $DW_{IP}$ ,  $C_{IP}$  and  $C_{\text{wholegrain}}$ , all dry weights and concentrations used in Eq. 1-10 were determined experimentally. The contribution of *SE* to total grain dry weight was taken from the literature (Pieczonka and Rosopulo 1985; Barron *et al.* 2007; Hemery *et al.* 2009) whereas  $DW_{IP}$ ,  $C_{IP}$  and  $C_{\text{wholegrain}}$  were calculated from Eq. 7, 8 and 10, respectively.

Pieczonka K, Rosopulo A. 1985. *Analytical and Bioanalytical Chemistry* 322, 697-699

Barron C et al. 2007. *Journal of Cereal Science* 45, 88-96

Hemery Y et al. 2009. *Journal of Cereal Science* 49, 55-64

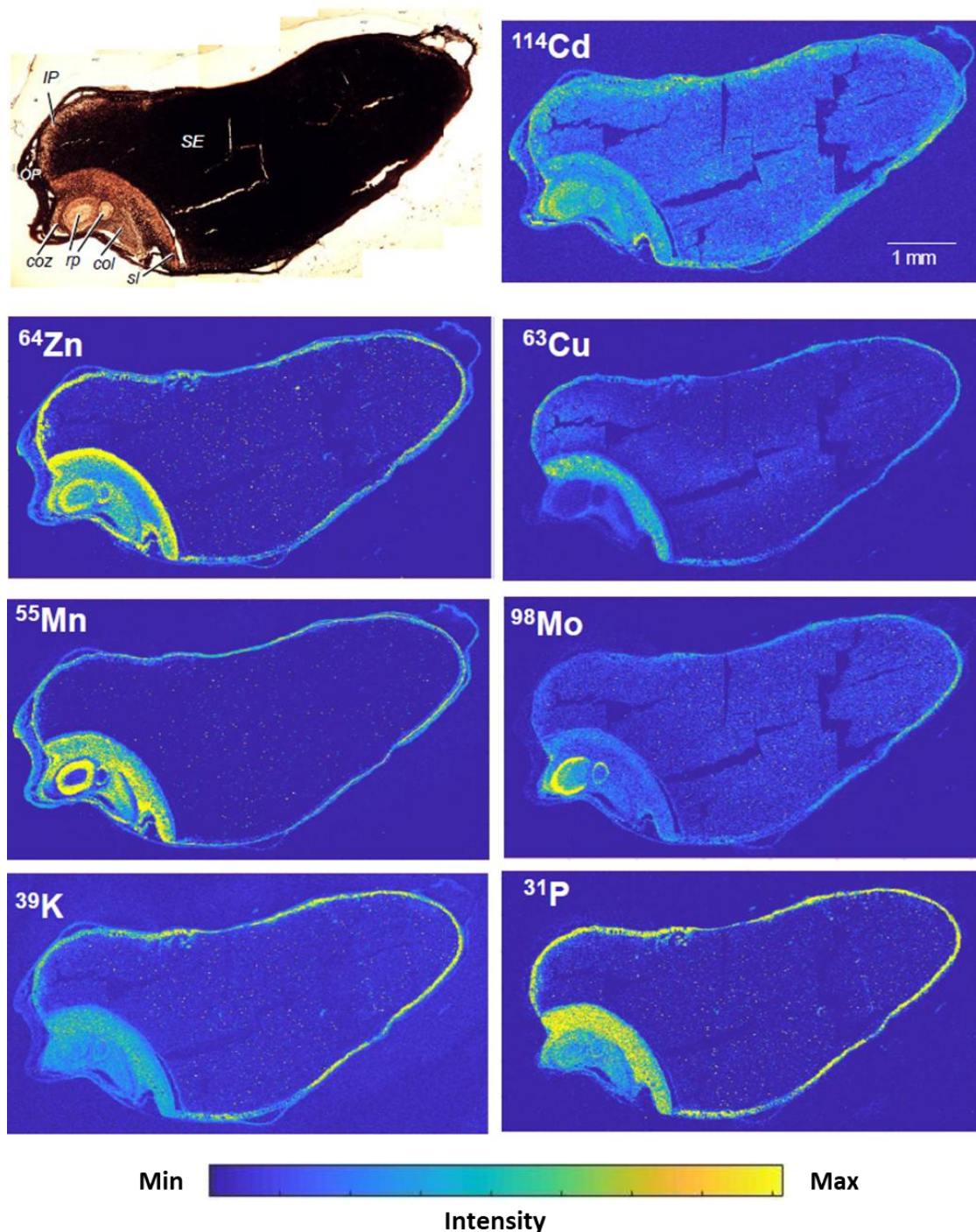
**Table S1.** Characteristics of the durum wheat grains used for LA-ICP-MS and  $\mu$ XRF elemental mappings as well as for grain dissection

	<b>DW</b>	<b>Ca</b>	<b>K</b>	<b>Mg</b>	<b>P</b>	<b>S</b>	<b>Cu</b>	<b>Fe</b>	<b>Mn</b>	<b>Mo</b>	<b>Zn</b>	<b>Cd</b>
	<b>mg</b>	<b>mg g<sup>-1</sup> DW</b>					<b><math>\mu</math>g g<sup>-1</sup> DW</b>					
<b>Mean</b>	69	0.47	5.97	1.52	2.61	2.22	9.8	45	50	2.9	131	2.5
<b>SD</b>	1	0.02	0.26	0.05	0.64	0.10	0.3	2	5	0.2	5	0.1

**Table S2.** Normalized concentrations (unitless) of macronutrients, micronutrients and Cd in durum wheat grain tissues and fractions

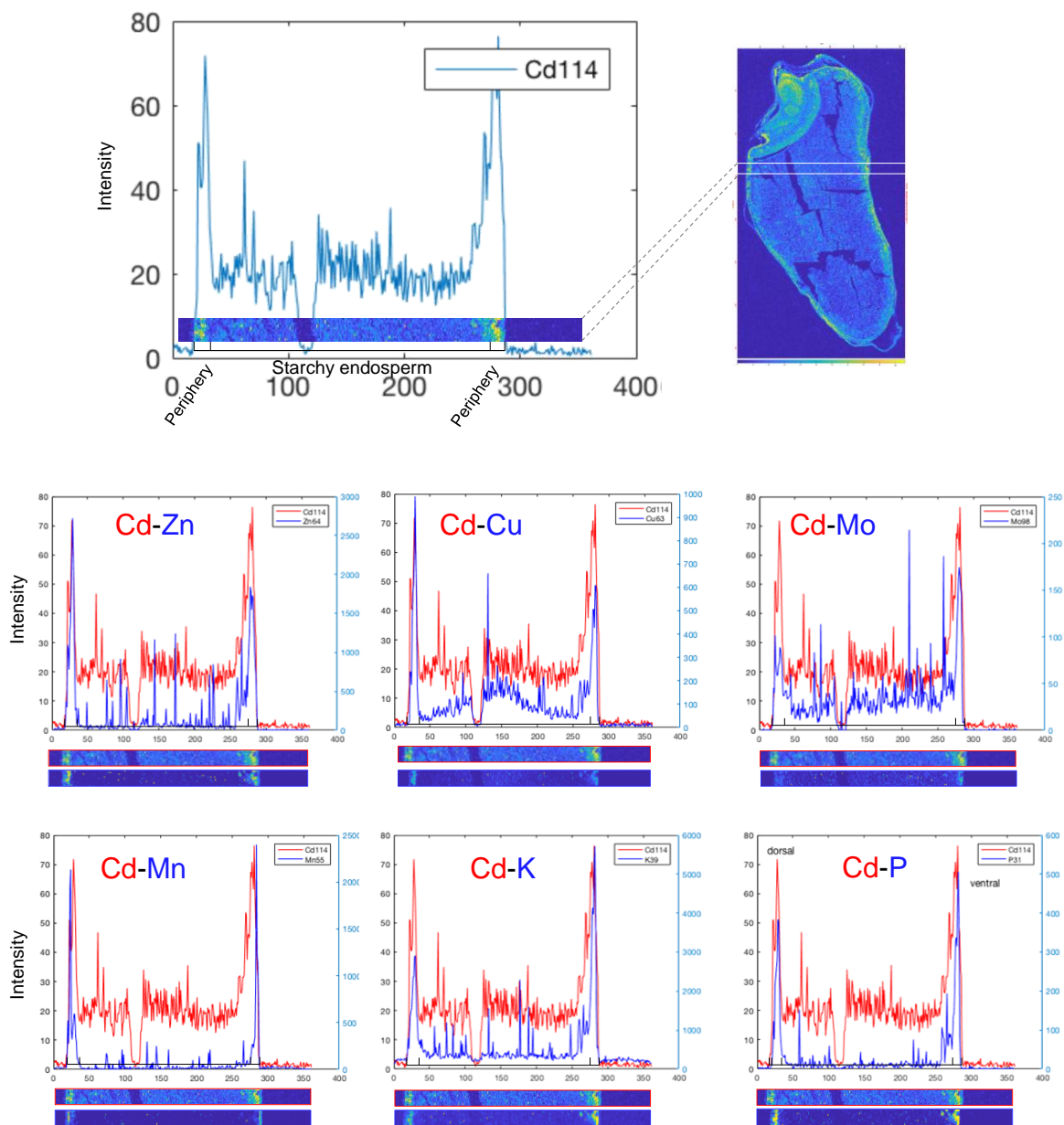
<b>Tissue / fraction</b>	<b>Ca</b>	<b>K</b>	<b>Mg</b>	<b>P</b>	<b>Cu</b>	<b>Fe</b>	<b>Mn</b>	<b>Mo</b>	<b>Zn</b>	<b>Cd</b>
<i>GM</i>	3.05	3.57	3.53	8.98	5.44	5.21	6.21	2.72	3.91	2.36
<i>OP</i>	2.11	0.72	0.16	0.04	0.16	0.21	3.24	0.25	1.27	2.43
<i>IP</i>	5.44	5.88	7.72	2.47	3.54	6.40	4.16	3.40	4.88	2.92
<i>SE</i>	0.30	0.33	0.13	0.56	0.55	0.16	0.13	0.65	0.22	0.48
<i>CR</i>	4.35	0.76	0.52	1.36	1.94	6.22	19.2	3.42	17.7	16.6
<i>IP+SE</i>	0.89	0.97	0.99	0.99	0.98	0.93	0.71	0.95	0.79	0.75
<i>IP+SE+GM</i>	0.89	0.97	0.99	0.99	0.98	0.93	0.71	0.96	0.79	0.76
<i>IP+SE+GM+CR</i>	0.84	0.87	0.89	0.72	0.83	0.83	0.70	0.92	0.84	0.86
<i>SE+CR</i>	0.29	0.28	0.11	0.47	0.47	0.19	0.29	0.57	0.35	0.55
<i>SE+CR+GM</i>	0.29	0.28	0.11	0.47	0.47	0.19	0.29	0.58	0.35	0.56

*GM*= germ; *OP*= outer periphery; *IP*= inner periphery; *SE*= starchy endosperm; *CR*= crease.



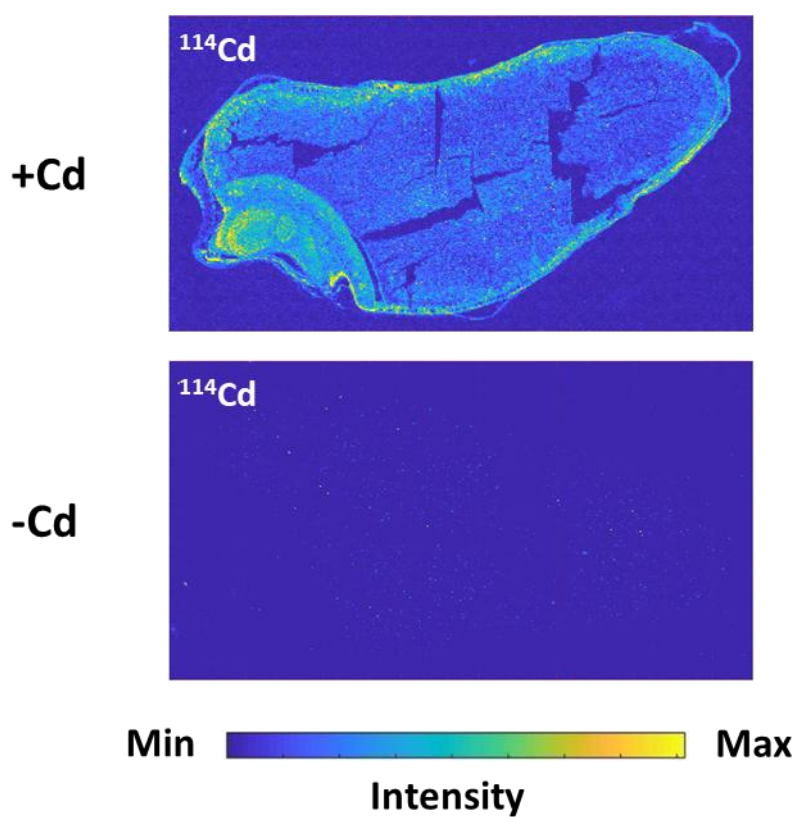
**Figure S1.** LA-ICP-MS distribution maps of  $^{114}\text{Cd}$ ,  $^{64}\text{Zn}$ ,  $^{63}\text{Cu}$ ,  $^{55}\text{Mn}$ ,  $^{98}\text{Mo}$ ,  $^{39}\text{K}$  and  $^{31}\text{P}$  in mature durum wheat grains sectioned longitudinally alongside the crease. The color scale represents different signal intensities, blue corresponds to the lowest relative intensity and yellow to the highest. The optical image (upper left) shows the different grain tissues visible on this longitudinal section: *OP*, outer periphery (likely the outer pericarp); *IP*, inner periphery (likely the aleurone layer, the seed coat, the inner pericarp and other maternal peripheral layers); *SE*, starchy endosperm; *col*, coleoptile; *coz*, coleorhiza; *rp*, root primordia;

*sl*, scutellum.

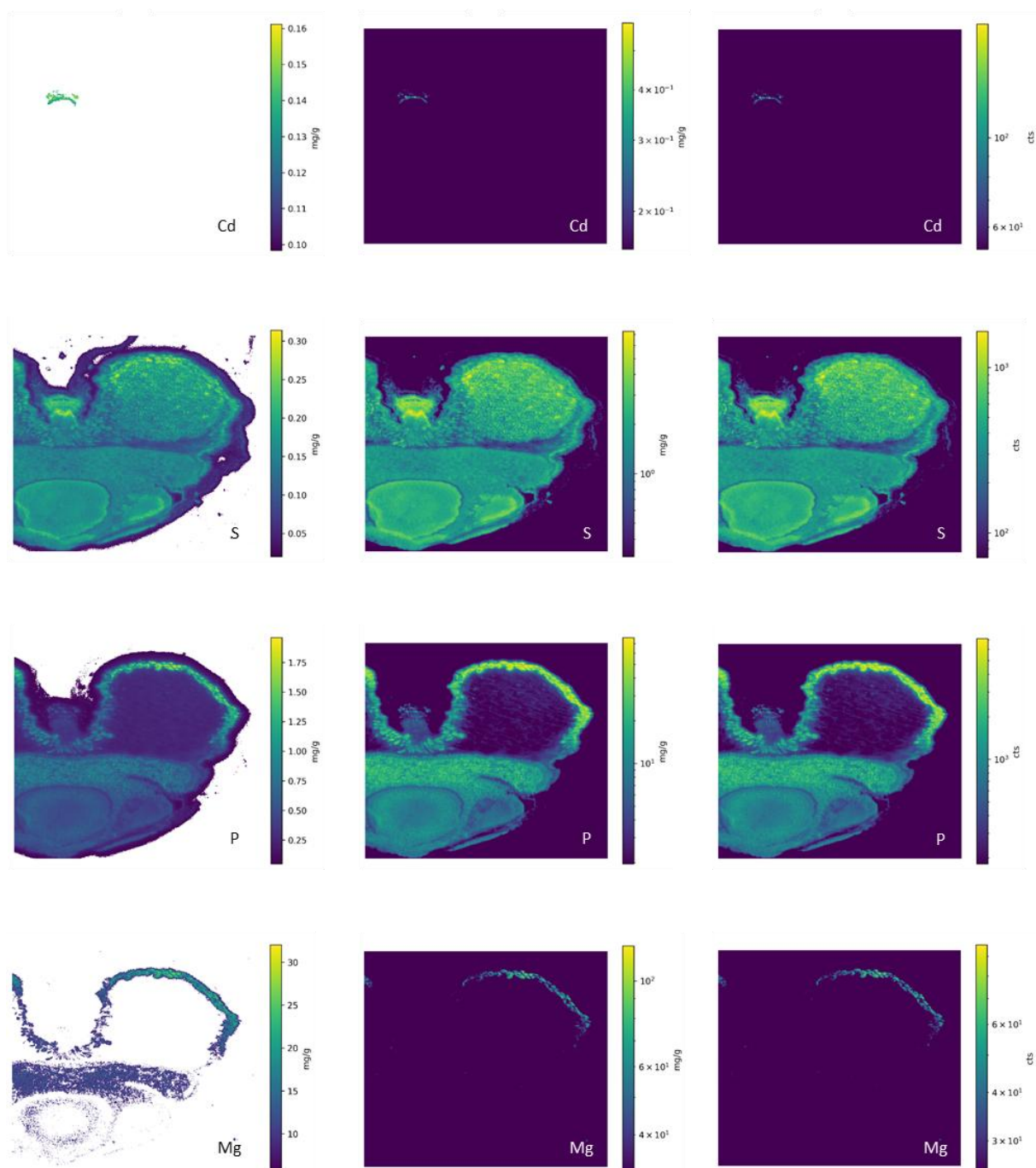


**Figure S2.** LA-ICP-MS line scan of  $^{114}\text{Cd}$  for the area shown in the optical map (upper right). The line scan of  $^{114}\text{Cd}$  was compared to the line scans of  $^{64}\text{Zn}$ ,  $^{63}\text{Cu}$ ,  $^{98}\text{Mo}$ ,  $^{55}\text{Mn}$ ,  $^{39}\text{K}$  and  $^{31}\text{P}$  in the same area. The images below each plot were extracted from Fig. 1, the red line frames Cd and the blue line frames the other elements. The values on the X-axis are the number of lines in the LA-ICP-MS, which correspond to a distance of  $2654\ \mu\text{m}$  between the two sides of the grain. The black scale on the Y-axis corresponds to the intensity of Cd and the blue scale to the intensity of the element to which Cd is being compared.

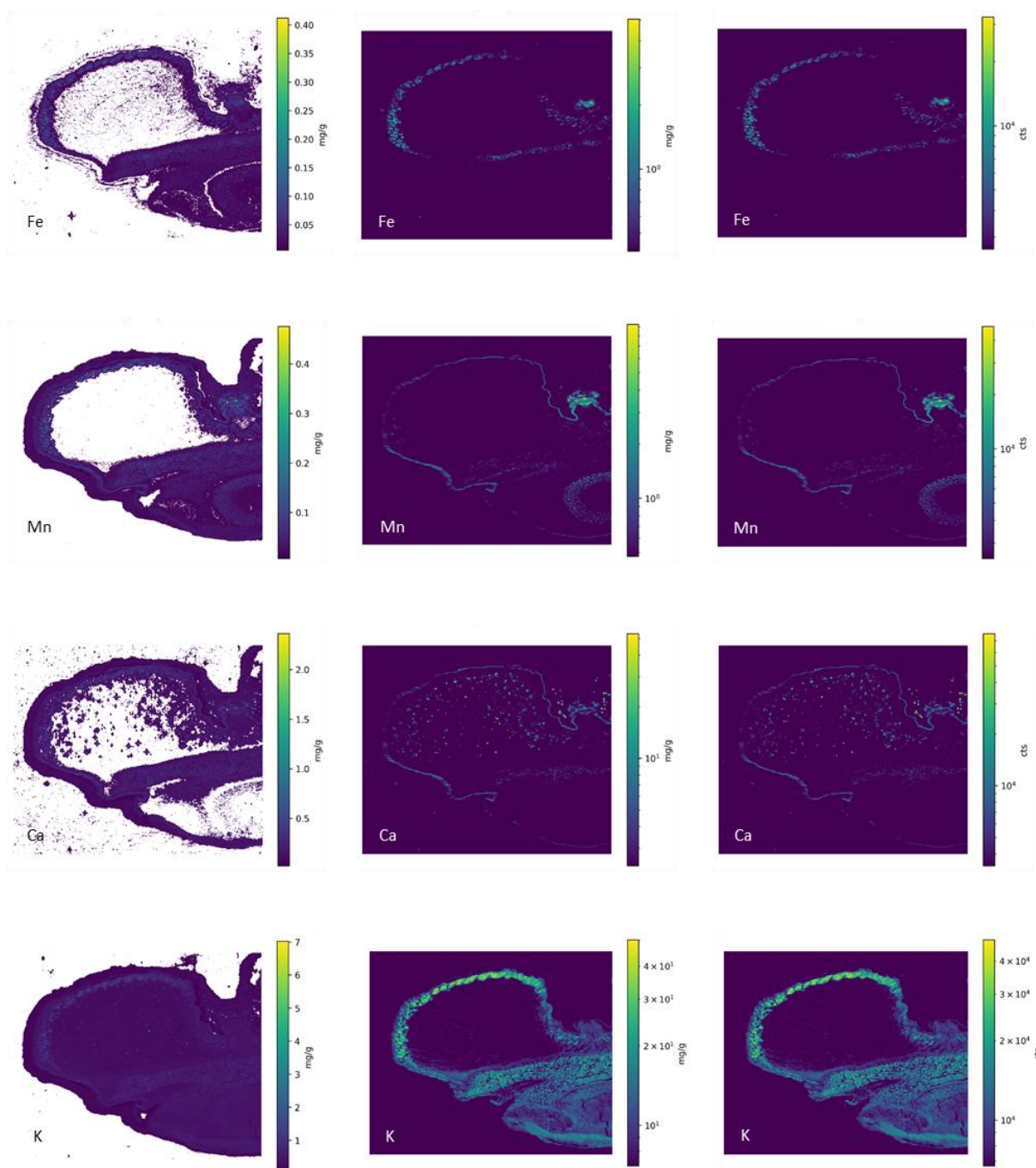




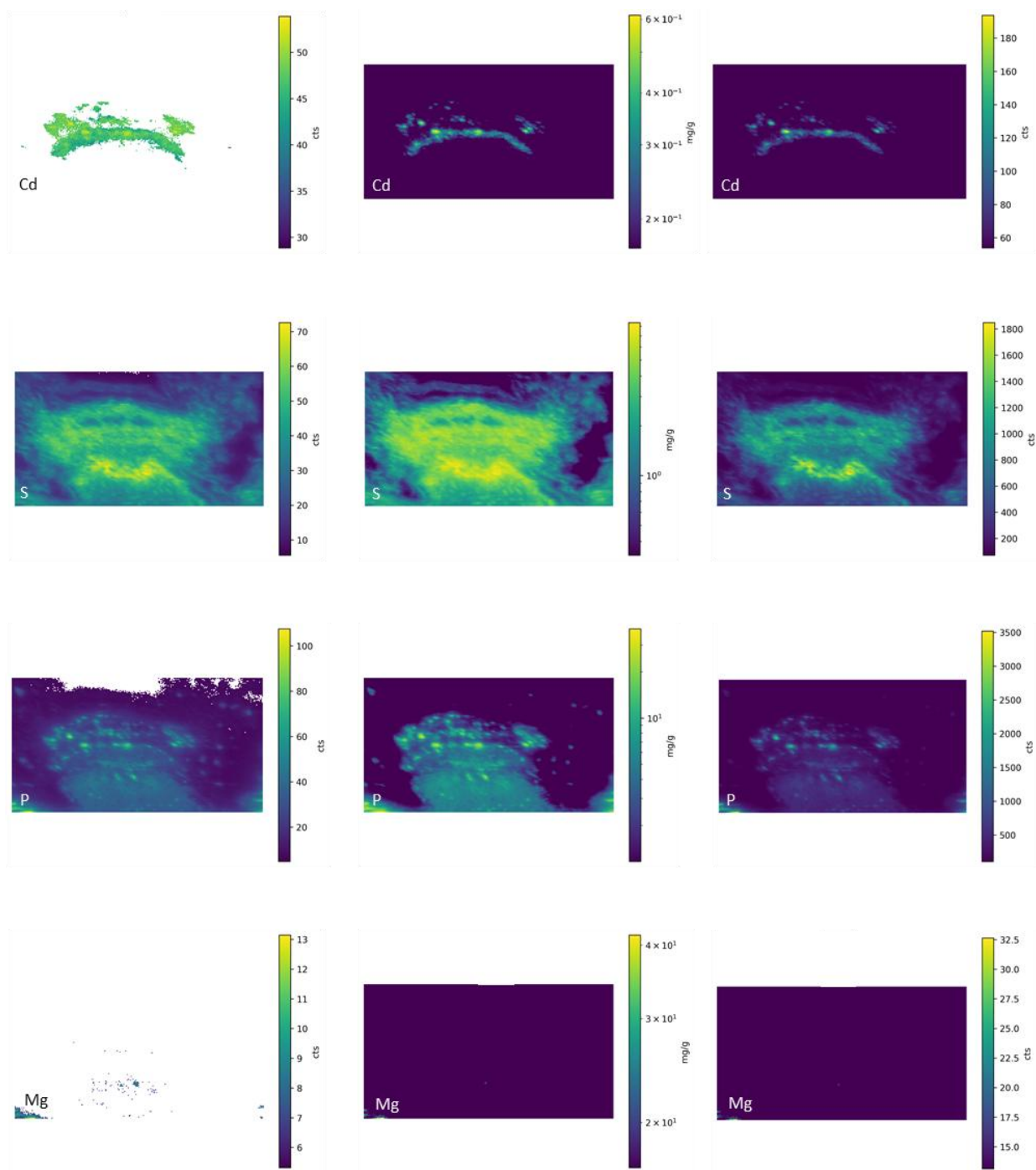
**Figure S3.** LA-ICP-MS distribution maps of  $^{114}\text{Cd}$  in mature durum wheat grains sectioned longitudinally alongside the crease and contaminated (+Cd) or not (-Cd) with Cd. The total Cd concentration was  $2.5 \mu\text{g g}^{-1}$  DW in +Cd grains and  $0.0024 \mu\text{g g}^{-1}$  in -Cd grains. The color scale represents the different signal intensities, blue corresponds to the lowest relative intensity and yellow to the highest.



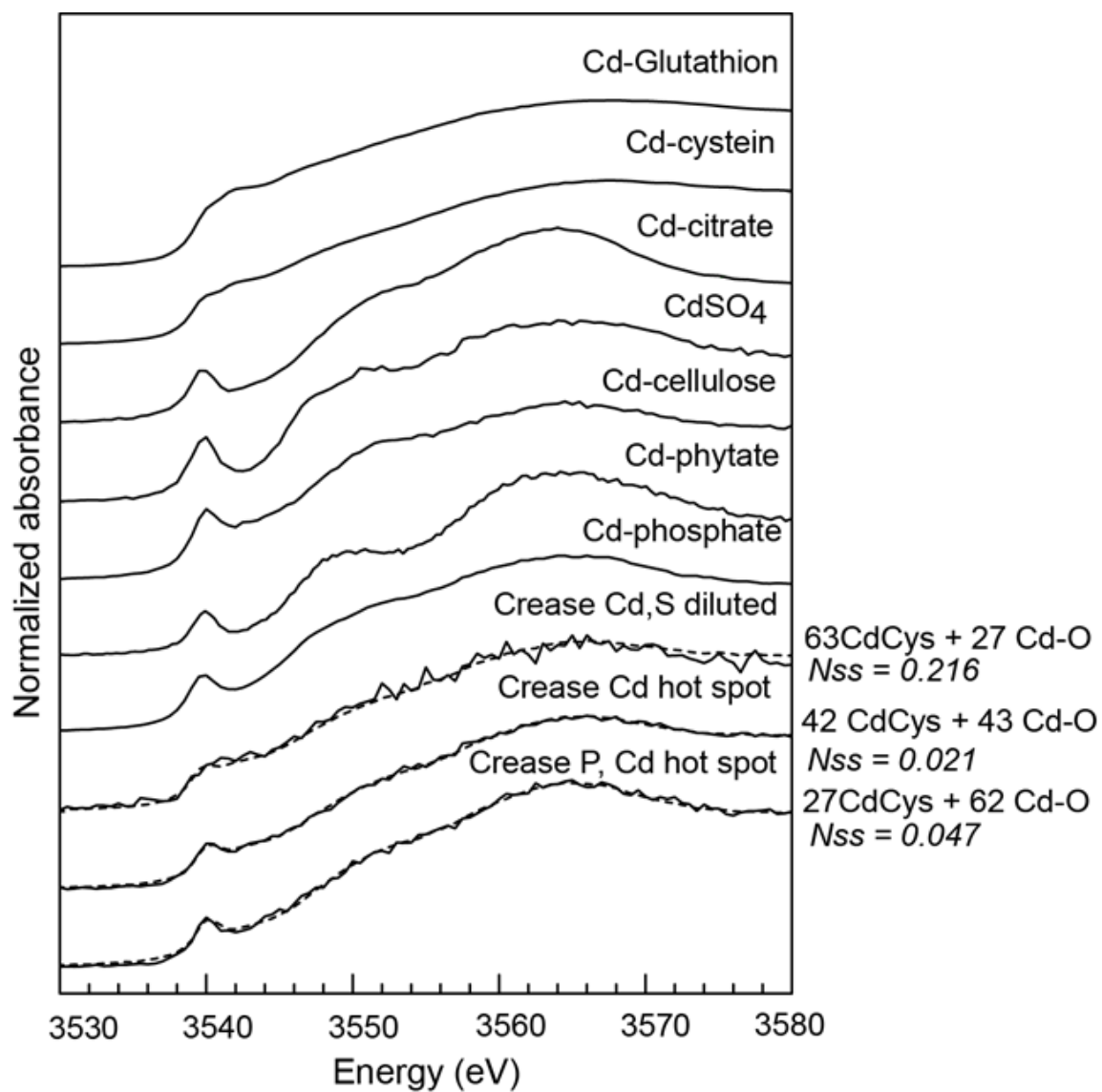
**Figure S4.** Synchrotron XRF distribution maps of Cd, S, P, and Mg in mature durum wheat grains sectioned transversally at the position of the root primordia. Chemical mapping was performed at 3570 eV, with a 2  $\mu\text{m}$  step and a counting time of 100 ms per point. The maps display the results after data processing. The first column shows the limit of detection (LOD) maps (in mass fraction) where white pixels are below the detection limit. The second and third column show the elemental maps (in mass fraction and fluorescence intensity, respectively) with the lowest threshold set at the max of the LOD.



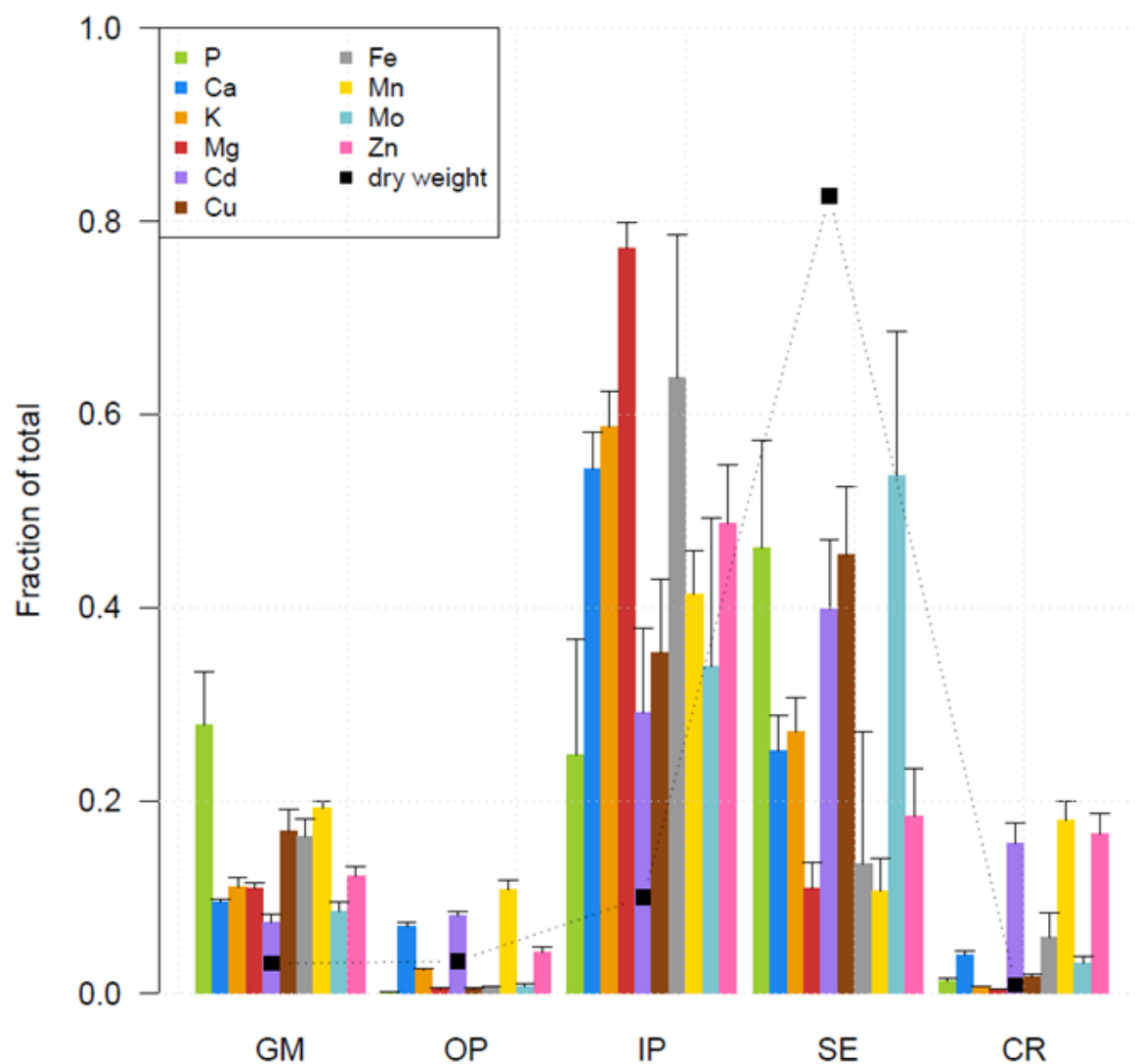
**Figure S5.** Synchrotron XRF distribution maps of Fe, Mn, Ca and K in mature durum wheat grains sectioned transversally at the position of the root primordia. Chemical mapping was performed at 7200 eV, with a 2  $\mu\text{m}$  step and a counting time of 100 ms per point. The maps display the results after data processing. The first column shows the limit of detection (LOD) maps (in mass fraction) where white pixels are below the detection limit. The second and third column show the elemental maps (in mass fraction and fluorescence intensity, respectively) with the lowest threshold set at the max of the LOD.



**Figure S6.** Synchrotron XRF distribution maps of Cd, S, P, and Mg in the crease. Chemical mapping was performed at 3570 eV, with a 2  $\mu\text{m}$  step and a counting time of 100 ms per point. The maps display the results after data processing. The first column shows the limit of detection (LOD) maps (in fluorescence intensity) where white pixels are below the detection limit. The second and third column show the elemental maps (in mass fraction and fluorescence intensity, respectively) with the lowest threshold set at the max of the LOD.



**Figure S7.** Cadmium L<sub>III</sub>-edge  $\mu$ XANES spectra of Cd references and of various areas of the crease with their linear combination fits (dotted line). The fit criteria are expressed as normalized residual sums of squares  $Nss$  ( $Nss = \frac{\sum [\mu_{\text{exp}} - \mu_{\text{fit}}]^2}{\sum [\mu_{\text{exp}}]^2} \times 100$ ).



**Figure S8.** Amounts of P, Ca, K, Mg, Cd, Cu, Fe, Mn, Mo, and Zn measured in the following dissected grain tissues: germ (*GM*), outer periphery (*OP*), inner periphery (*IP*), starchy endosperm (*SE*) and crease (*CR*). The amount of a given element in a tissue is expressed as a fraction of the total amount of the element in the whole grain.